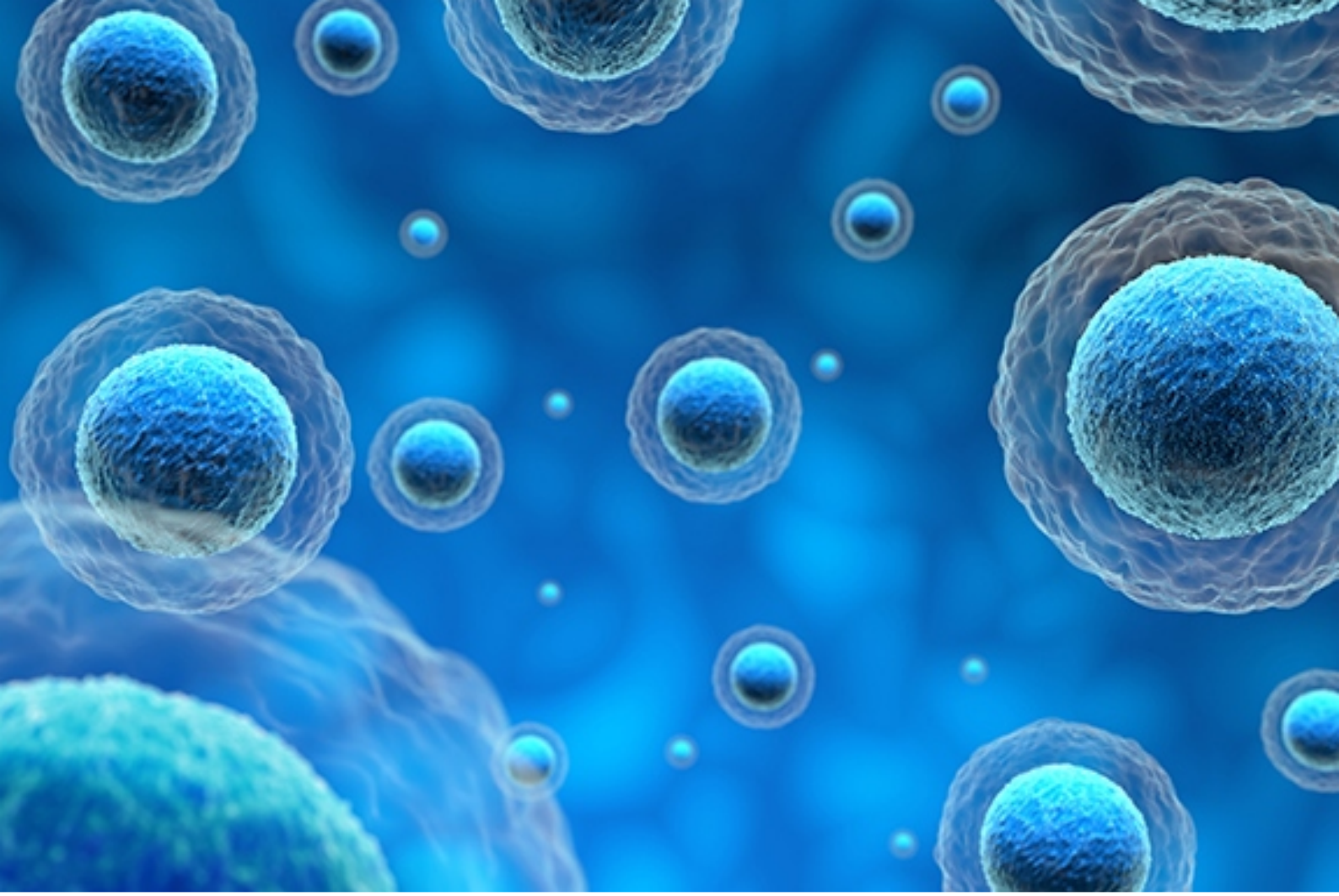

Biosynthetic Pathways

Alan Ellison

BIOSYNTHETIC PATHWAYS

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by Alan Ellison

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Contents

Chapter 1 Biosynthesis 1

Chapter 2 Enzyme-catalyzed Process 24

Chapter 3 Biofuel 64

Chapter 4 Industrial Applications 82

Chapter 5 Molecular Biology and Food Processing 149

Chapter 6 Protein Biosynthesis 169

Chapter 1

Biosynthesis

Biosynthesis is a multi-step, enzyme-catalyzed process where substratesare converted into more complex products in living organisms. In biosynthesis, simple compounds are modified, converted into other compounds, or joined together to form macromolecules. This process often consists of metabolic pathways. Some of these biosynthetic pathways are located within a single cellular organelle, while others involve enzymes that are located within multiple cellular organelles. Examples of these biosynthetic pathways include the production of lipid membrane components and nucleotides. Biosynthesis is usually synonymous with anabolism.

The prerequisite elements for biosynthesis include:precursor compounds, chemical energy (e.g. ATP), and catalytic enzymes which may require coenzymes (e.g.NADH, NADPH). These elements create monomers, the building blocks for macromolecules. Some important biological macromolecules include:proteins, which are composed of amino acid monomers joined via peptide bonds, and DNA molecules, which are composed of nucleotides joined via phosphodiester bonds.

Properties of chemical reactions

Biosynthesis occurs due to a series of chemical reactions. For these reactions to take place, the following elements are necessary:

- Precursor compounds: these compounds are the starting molecules or substrates in a reaction. These may also be viewed as the reactants in a given chemical process.
- Chemical energy: chemical energy can be found in the form of high energy molecules. These molecules are required for energetically unfavorable reactions. Furthermore, the hydrolysis of these compounds drives a reaction forward. High energy molecules, such as ATP, have three phosphates. Often, the terminal phosphate is split off during hydrolysis and transferred to another molecule.
- Catalytic enzymes: these molecules are special proteins that catalyze a reaction by increasing the rate of the reaction and lowering the activation energy.
- Coenzymes or cofactors: cofactors are molecules that assist in chemical reactions. These may be metal ions, vitamin derivatives such as NADH and acetyl CoA, or non-vitamin derivatives such as ATP. In the case of NADH, the molecule transfers a hydrogen, whereas acetyl CoA transfers an acetyl group, and ATP transfers a phosphate.

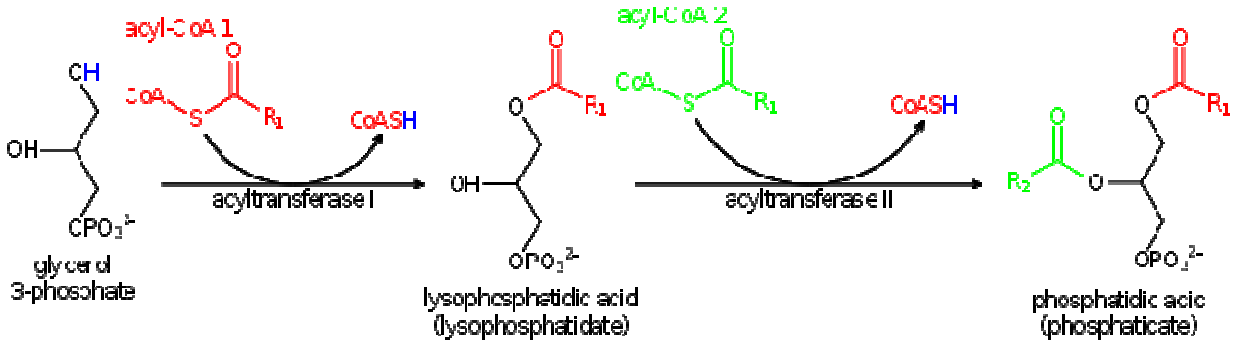
Lipid

Many intricate macromolecules are synthesized in a pattern of simple, repeated structures. For example, the simplest structures of lipids are fatty acids. Fatty acids are hydrocarbon derivatives; they contain a carboxyl group "head" and a hydrocarbon chain "tail". These fatty acids create larger

components, which in turn incorporate noncovalent interactions to form the lipid bilayer. Fatty acid chains are found in two major components of membrane lipids: phospholipids and sphingolipids. A third major membrane component, cholesterol, does not contain these fatty acid units.

Phospholipids

The foundation of all biomembranes consists of a bilayer structure of phospholipids. The phospholipid molecule is amphipathic; it contains a hydrophilic polar head and a hydrophobic nonpolar tail. The phospholipid heads interact with each other and aqueous media, while the hydrocarbon tails orient themselves in the center, away from water. These latter interactions drive the bilayer structure that acts as a barrier for ions and molecules. There are various types of phospholipids; consequently, their synthesis pathways differ. However, the first step in phospholipid synthesis involves the formation of phosphatidate or diacylglycerol 3-phosphate at the endoplasmic reticulum and outer mitochondrial membrane. The synthesis pathway is found below:

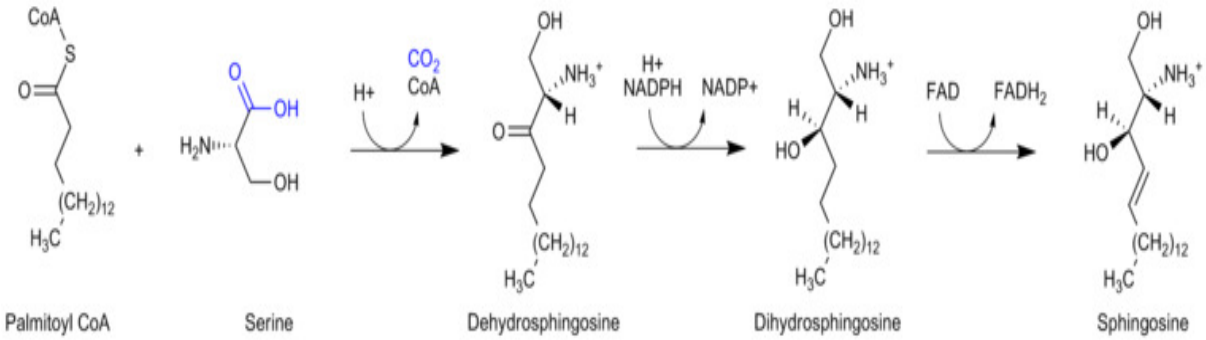


The pathway starts with glycerol 3-phosphate, which gets converted to lysophosphatidate via the addition of a fatty acid

chain provided by acyl coenzyme A. Then, lysophosphatidate is converted to phosphatidate via the addition of another fatty acid chain contributed by a second acyl CoA; all of these steps are catalyzed by the glycerol phosphate acyltransferase enzyme. Phospholipid synthesis continues in the endoplasmic reticulum, and the biosynthesis pathway diverges depending on the components of the particular phospholipid.

Sphingolipids

Like phospholipids, these fatty acid derivatives have a polar head and nonpolar tails. Unlike phospholipids, sphingolipids have a sphingosine backbone. Sphingolipids exist in eukaryotic cells and are particularly abundant in the central nervous system. For example, sphingomyelin is part of the myelin sheath of nerve fibers. Sphingolipids are formed from ceramides that consist of a fatty acid chain attached to the amino group of a sphingosine backbone. These ceramides are synthesized from the acylation of sphingosine. The biosynthetic pathway for sphingosine is found below:

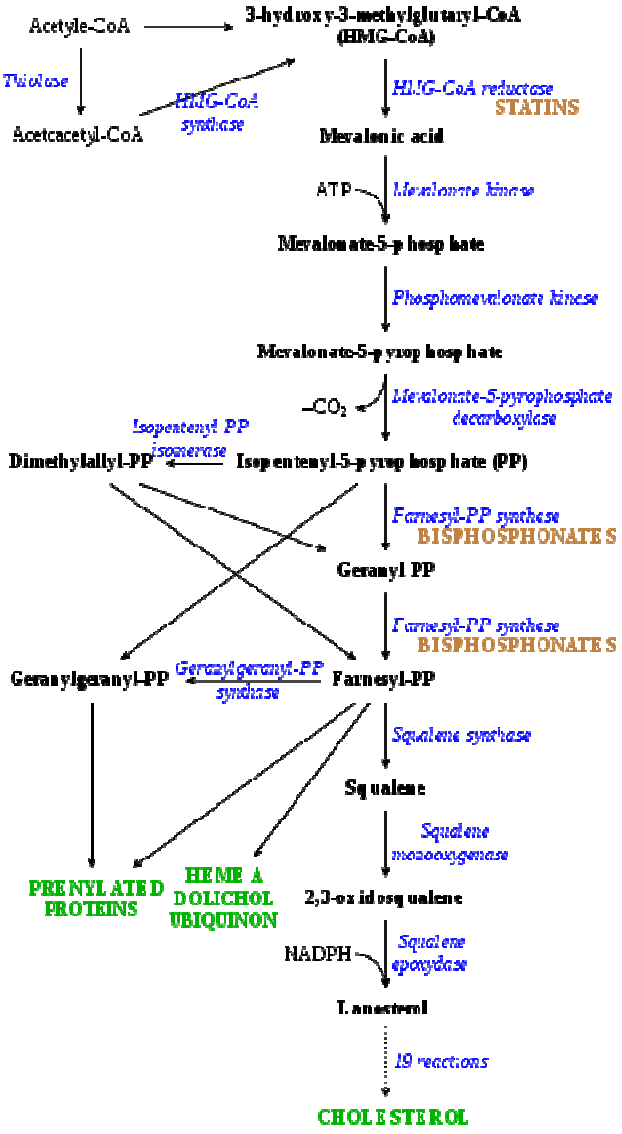


As the image denotes, during sphingosine synthesis, palmitoyl CoA and serine undergo a condensation reaction which results in the formation of dehydrosphingosine. This product is then

reduced to form dihydrospingosine, which is converted to sphingosine via the oxidation reaction by FAD.

Cholesterol

This lipid belongs to a class of molecules called sterols. Sterols have four fused rings and a hydroxyl group. Cholesterol is a particularly important molecule. Not only does it serve as a component of lipid membranes, it is also a precursor to several steroid hormones, including cortisol, testosterone, and estrogen. Cholesterol is synthesized from acetyl CoA. The pathway is shown below:



More generally, this synthesis occurs in three stages, with the first stage taking place in the cytoplasm and the second and third stages occurring in the endoplasmic reticulum. The stages are as follows:

- 1. The synthesis of isopentenyl pyrophosphate, the "building block" of cholesterol
- 2. The formation of squalene via the condensation of six molecules of isopentenyl phosphate
- 3. The conversion of squalene into cholesterol via several enzymatic reactions

Nucleotides

The biosynthesis of nucleotides involves enzyme-catalyzed reactions that convert substrates into more complex products. Nucleotides are the building blocks of DNA and RNA. Nucleotides are composed of a five-membered ring formed from ribose sugar in RNA, and deoxyribose sugar in DNA; these sugars are linked to a purine or pyrimidine base with a glycosidic bond and a phosphate group at the 5' location of the sugar.

Purine nucleotides

The DNA nucleotides adenosine and guanosine consist of a purine base attached to a ribose sugar with a glycosidic bond. In the case of RNA nucleotides deoxyadenosine and deoxyguanosine, the purine bases are attached to a deoxyribose sugar with a glycosidic bond. The purine bases on DNA and RNA nucleotides are synthesized in a twelve-step reaction mechanism present in most single-celled organisms.

Higher eukaryotes employ a similar reaction mechanism in ten reaction steps. Purine bases are synthesized by converting phosphoribosyl pyrophosphate (PRPP) to inosine monophosphate (IMP), which is the first key intermediate in purine base biosynthesis. Further enzymatic modification of IMP produces the adenosine and guanosine bases of nucleotides.

- The first step in purine biosynthesis is a condensation reaction, performed by glutamine-PRPP amidotransferase. This enzyme transfers the amino group from glutamine to PRPP, forming 5-phosphoribosylamine. The following step requires the activation of glycine by the addition of a phosphate group from ATP.
- GAR synthetase performs the condensation of activated glycine onto PRPP, forming glycineamide ribonucleotide (GAR).
- GAR transformylase adds a formyl group onto the amino group of GAR, forming formylglycinamide ribonucleotide (FGAR).
- FGAR amidotransferase catalyzes the addition of a nitrogen group to FGAR, forming formylglycinamidine ribonucleotide (FGAM).
- FGAM cyclase catalyzes ring closure, which involves removal of a water molecule, forming the 5-membered imidazole ring 5-aminoimidazole ribonucleotide (AIR).
- N5-CAIR synthetase transfers a carboxyl group, forming the intermediate N5-carboxyaminoimidazole ribonucleotide (N5-CAIR).

- N5-CAIR mutase rearranges the carboxyl functional group and transfers it onto the imidazole ring, forming carboxyamino- imidazole ribonucleotide (CAIR). The two step mechanism of CAIR formation from AIR is mostly found in single celled organisms. Higher eukaryotes contain the enzyme AIR carboxylase, which transfers a carboxyl group directly to AIR imidazole ring, forming CAIR.
- SAICAR synthetase forms a peptide bond between aspartate and the added carboxyl group of the imidazole ring, forming N-succinyl-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR).
- SAICAR lyase removes the carbon skeleton of the added aspartate, leaving the amino group and forming 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR).
- AICAR transformylase transfers a carbonyl group to AICAR, forming N-formylaminoimidazole- 4-carboxamide ribonucleotide (FAICAR).
- The final step involves the enzyme IMP synthase, which performs the purine ring closure and forms the inosine monophosphate intermediate.

Pyrimidine nucleotides

Other DNA and RNA nucleotide bases that are linked to the ribose sugar via a glycosidic bond are thymine, cytosine and uracil (which is only found in RNA). Uridine monophosphate biosynthesis involves an enzyme that is located in the mitochondrial inner membrane and multifunctional enzymes that are located in the cytosol.

The first step involves the enzyme carbamoyl phosphate synthase combining glutamine with CO_2 in an ATP dependent reaction to form carbamoyl phosphate.

Aspartate carbamoyltransferase condenses carbamoyl phosphate with aspartate to form uridosuccinate.

Dihydroorotase performs ring closure, a reaction that loses water, to form dihydroorotate.

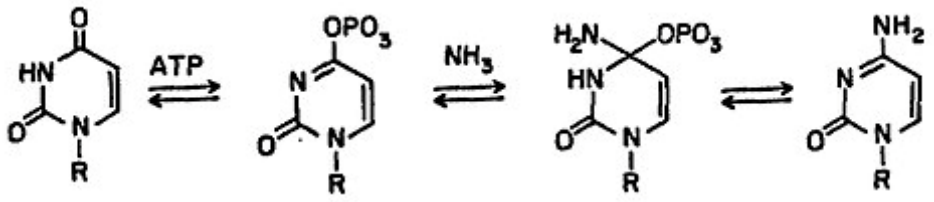
Dihydroorotate dehydrogenase, located within the mitochondrial inner membrane, oxidizes dihydroorotate to orotate.

Orotate phosphoribosyl hydrolase (OMP pyrophosphorylase) condenses orotate with PRPP to form orotidine-5'-phosphate.

OMP decarboxylase catalyzes the conversion of orotidine-5'-phosphate to UMP.

After the uridine nucleotide base is synthesized, the other bases, cytosine and thymine are synthesized. Cytosine biosynthesis is a two-step reaction which involves the conversion of UMP to UTP. Phosphate addition to UMP is catalyzed by a kinase enzyme.

The enzyme CTP synthase catalyzes the next reaction step: the conversion of UTP to CTP by transferring an amino group from glutamine to uridine; this forms the cytosine base of CTP. The mechanism, which depicts the reaction UTP + ATP + glutamine ⇔ CTP + ADP + glutamate, is below:



Cytosine is a nucleotide that is present in both DNA and RNA. However, uracil is only found in RNA. Therefore, after UTP is synthesized, it is must be converted into a deoxy form to be incorporated into DNA. This conversion involves the enzyme ribonucleoside triphosphate reductase. This reaction that removes the 2'-OH of the ribose sugar to generate deoxyribose is not affected by the bases attached to the sugar. This non-specificity allows ribonucleoside triphosphate reductase to convert all nucleotide triphosphates to deoxyribonucleotide by a similar mechanism. In contrast to uracil, thymine bases are found mostly in DNA, not RNA. Cells do not normally contain thymine bases that are linked to ribose sugars in RNA, thus indicating that cells only synthesize deoxyribose-linked thymine. The enzyme thymidylate synthetase is responsible for synthesizing thymine residues from dUMP to dTMP. This reaction transfers a methyl group onto the uracil base of dUMP to generate dTMP. The thymidylate synthase reaction, dUMP + 5,10-methylenetetrahydrofolate ⇔ dTMP + dihydrofolate, is shown to the right.

DNA

Although there are differences between eukaryotic and prokaryotic DNA synthesis, the following section denotes key characteristics of DNA replication shared by both organisms.

DNA is composed of nucleotides that are joined by phosphodiester bonds.DNA synthesis, which takes place in the nucleus, is a semiconservative process, which means that the resulting DNA molecule contains an original strand from the parent structure and a new strand. DNA synthesis is catalyzed by a family of DNA polymerases that require four deoxynucleoside triphosphates, a template strand, and a primer with a free 3'OH in which to incorporate nucleotides.

In order for DNA replication to occur, a replication fork is created by enzymes called helicases which unwind the DNA helix.Topoisomerases at the replication fork remove supercoils caused by DNA unwinding, and single-stranded DNA binding proteins maintain the two single-stranded DNA templates stabilized prior to replication.

DNA synthesis is initiated by the RNA polymeraseprimase, which makes an RNA primer with a free 3'OH. This primer is attached to the single-stranded DNA template, and DNA polymerase elongates the chain by incorporating nucleotides; DNA polymerase also proofreads the newly synthesized DNA strand.

During the polymerization reaction catalyzed by DNA polymerase, a nucleophilic attack occurs by the 3'OH of the growing chain on the innermost phosphorus atom of a deoxynucleoside triphosphate; this yields the formation of a phosphodiester bridge that attaches a new nucleotide and releases pyrophosphate.

Two types of strands are created simultaneously during replication: the leading strand, which is synthesized continuously and grows towards the replication fork, and the

lagging strand, which is made discontinuously in Okazaki fragments and grows away from the replication fork. Okazaki fragments are covalently joined by DNA ligase to form a continuous strand. Then, to complete DNA replication, RNA primers are removed, and the resulting gaps are replaced with DNA and joined via DNA ligase.

Amino acids

A protein is a polymer that is composed from amino acids that are linked by peptide bonds. There are more than 300 amino acids found in nature of which only twenty, known as the standard amino acids, are the building blocks for protein. Only green plants and most microbes are able to synthesize all of the 20 standard amino acids that are needed by all living species. Mammals can only synthesize ten of the twenty standard amino acids. The other amino acids, valine, methionine, leucine, isoleucine, phenylalanine, lysine, threonine and tryptophan for adults and histidine, and arginine for babies are obtained through diet.

Amino acid basic structure

The general structure of the standard amino acids includes a primary amino group, a carboxyl group and the functional group attached to the α-carbon. The different amino acids are identified by the functional group. As a result of the three different groups attached to the α-carbon, amino acids are asymmetrical molecules. For all standard amino acids, except glycine, the α-carbon is a chiral center. In the case of glycine,

the α-carbon has two hydrogen atoms, thus adding symmetry to this molecule. With the exception of proline, all of the amino acids found in life have the L-isoform conformation. Proline has a functional group on the α-carbon that forms a ring with the amino group.Nitrogen source

One major step in amino acid biosynthesis involves incorporating a nitrogen group onto the α-carbon. In cells, there are two major pathways of incorporating nitrogen groups. One pathway involves the enzyme glutamine oxoglutarate aminotransferase (GOGAT) which removes the amide amino group of glutamine and transfers it onto 2-oxoglutarate, producing two glutamate molecules. In this catalysis reaction, glutamine serves as the nitrogen source. An image illustrating this reaction is found to the right.

The other pathway for incorporating nitrogen onto the α-carbon of amino acids involves the enzyme glutamate dehydrogenase (GDH). GDH is able to transfer ammonia onto 2-oxoglutarate and form glutamate. Furthermore, the enzyme glutamine synthetase (GS) is able to transfer ammonia onto glutamate and synthesize glutamine, replenishing glutamine.

The glutamate family of amino acids

The glutamate family of amino acids includes the amino acids that derive from the amino acid glutamate. This family includes: glutamate, glutamine, proline, and arginine. This family also includes the amino acid lysine, which is derived from α-ketoglutarate.

The biosynthesis of glutamate and glutamine is a key step in the nitrogen assimilation discussed above. The enzymes GOGAT and GDH catalyze the nitrogen assimilation reactions.

In bacteria, the enzyme glutamate 5-kinase initiates the biosynthesis of proline by transferring a phosphate group from ATP onto glutamate. The next reaction is catalyzed by the enzyme pyrroline-5-carboxylate synthase (P5CS), which catalyzes the reduction of the ϒ-carboxyl group of L-glutamate 5-phosphate. This results in the formation of glutamate semialdehyde, which spontaneously cyclizes to pyrroline-5-carboxylate. Pyrroline-5-carboxylate is further reduced by the enzyme pyrroline-5-carboxylate reductase (P5CR) to yield a proline amino acid.

In the first step of arginine biosynthesis in bacteria, glutamate is acetylated by transferring the acetyl group from acetyl-CoA at the N-α position; this prevents spontaneous cyclization. The enzyme N-acetylglutamate synthase (glutamate N-acetyltransferase) is responsible for catalyzing the acetylation step. Subsequent steps are catalyzed by the enzymes N-acetylglutamate kinase, N-acetyl-gamma-glutamyl-phosphate reductase, and acetylornithine/succinyldiaminopimelate aminotransferase and yield the N-acetyl-L-ornithine. The acetyl group of acetylornithineis removed by the enzyme acetylornithinase (AO) or ornithine acetyltransferase (OAT), and this yields ornithine. Then, the enzymes citrulline and argininosuccinate convert ornithine to arginine.

There are two distinct lysine biosynthetic pathways: the diaminopimelic acid pathway and the α-aminoadipate pathway. The most common of the two synthetic pathways is the

diaminopimelic acid pathway; it consists of several enzymatic reactions that add carbon groups to aspartate to yield lysine:

- Aspartate kinase initiates the diaminopimelic acid pathway by phosphorylating aspartate and producing aspartyl phosphate.
- Aspartate semialdehyde dehydrogenase catalyzes the NADPH-dependent reduction of aspartyl phosphate to yield aspartate semialdehyde.
- 4-hydroxy-tetrahydrodipicolinate synthase adds a pyruvate group to the β-aspartyl-4-semialdehyde, and a water molecule is removed. This causes cyclization and gives rise to (2S,4S)-4-hydroxy-2,3,4,5-tetrahydrodipicolinate.
- 4-hydroxy-tetrahydrodipicolinate reductase catalyzes the reduction of (2S,4S)-4-hydroxy-2,3,4,5-tetrahydrodipicolinate by NADPH to yield Δ'-piperideine-2,6-dicarboxylate (2,3,4,5-tetrahydro-dipicolinate) and H_2O.
- Tetrahydrodipicolinate acyltransferase catalyzes the acetylation reaction that results in ring opening and yields N-acetyl α-amino-ε-ketopimelate.
- N-succinyl-α-amino-ε-ketopimelate-glutamate aminotransaminase catalyzes the transamination reaction that removes the keto group of N-acetyl α-amino-ε-ketopimelate and replaces it with an amino group to yield N-succinyl-L-diaminopimelate.
- N-acyldiaminopimelatedeacylase catalyzes the deacylation of N-succinyl-L-diaminopimelate to yield L,L-diaminopimelate.

- DAP epimerase catalyzes the conversion of L,L-diaminopimelate to the meso form of L,L-diaminopimelate.
- DAP decarboxylase catalyzes the removal of the carboxyl group, yielding L-lysine.

The serine family of amino acids

The serine family of amino acid includes: serine, cysteine, and glycine. Most microorganisms and plants obtain the sulfur for synthesizing methionine from the amino acid cysteine. Furthermore, the conversion of serine to glycine provides the carbons needed for the biosynthesis of the methionine and histidine.

During serine biosynthesis, the enzyme phosphoglycerate dehydrogenase catalyzes the initial reaction that oxidizes3-phospho-D-glycerate to yield 3-phosphonooxypyruvate. The following reaction is catalyzed by the enzyme phosphoserine aminotransferase, which transfers an amino group from glutamate onto 3-phosphonooxypyruvate to yield L-phosphoserine. The final step is catalyzed by the enzyme phosphoserine phosphatase, which dephosphorylates L-phosphoserine to yield L-serine.

There are two known pathways for the biosynthesis of glycine. Organisms that use ethanol and acetate as the major carbon source utilize the glyconeogenic pathway to synthesize glycine. The other pathway of glycine biosynthesis is known as the glycolytic pathway. This pathway converts serine synthesized from the intermediates of glycolysis to glycine. In the glycolytic pathway, the enzyme serine hydroxymethyltransferase

catalyzes the cleavage of serine to yield glycine and transfers the cleaved carbon group of serine onto tetrahydrofolate, forming 5,10-methylene-tetrahydrofolate.

Cysteine biosynthesis is a two-step reaction that involves the incorporation of inorganic sulfur. In microorganisms and plants, the enzyme serine acetyltransferase catalyzes the transfer of acetyl group from acetyl-CoA onto L-serine to yield O-acetyl-L-serine. The following reaction step, catalyzed by the enzyme O-acetyl serine (thiol) lyase, replaces the acetyl group of O-acetyl-L-serine with sulfide to yield cysteine.

The aspartate family of amino acids

The aspartate family of amino acids includes:threonine, lysine, methionine, isoleucine, and aspartate. Lysine and isoleucine are considered part of the aspartate family even though part of their carbon skeleton is derived from pyruvate. In the case of methionine, the methyl carbon is derived from serine and the sulfur group, but in most organisms, it is derived from cysteine.

The biosynthesis of aspartate is a one step reaction that is catalyzed by a single enzyme. The enzyme aspartate aminotransferase catalyzes the transfer of an amino group from aspartate onto α-ketoglutarate to yield glutamate and oxaloacetate. Asparagine is synthesized by an ATP-dependent addition of an amino group onto aspartate; asparagine synthetase catalyzes the addition of nitrogen from glutamine or soluble ammonia to aspartate to yield asparagine.

The diaminopimelic acid biosynthetic pathway of lysine belongs to the aspartate family of amino acids. This pathway involves

nine enzyme-catalyzed reactions that convert aspartate to lysine.

- Aspartate kinase catalyzes the initial step in the diaminopimelic acid pathway by transferring a phosphoryl from ATP onto the carboxylate group of aspartate, which yields aspartyl-β-phosphate.
- Aspartate-semialdehyde dehydrogenase catalyzes the reduction reaction by dephosphorylation of aspartyl-β-phosphate to yield aspartate-β-semialdehyde.
- Dihydrodipicolinate synthase catalyzes the condensation reaction of aspartate-β-semialdehyde with pyruvate to yield dihydrodipicolinic acid.
- 4-hydroxy-tetrahydrodipicolinate reductase catalyzes the reduction of dihydrodipicolinic acid to yield tetrahydrodipicolinic acid.
- Tetrahydrodipicolinate N-succinyltransferase catalyzes the transfer of a succinyl group from succinyl-CoA on to tetrahydrodipicolinic acid to yield N-succinyl-L-2,6-diaminoheptanedioate.
- N-succinyldiaminopimelate aminotransferase catalyzes the transfer of an amino group from glutamate onto N-succinyl-L-2,6-diaminoheptanedioate to yield N-succinyl-L,L-diaminopimelic acid.
- Succinyl-diaminopimelatedesuccinylase catalyzes the removal of acyl group from N-succinyl-L,L-diaminopimelic acid to yield L,L-diaminopimelic acid.
- Diaminopimelate epimerase catalyzes the inversion of the α-carbon of L,L-diaminopimelic acid to yield meso-diaminopimelic acid.

- Siaminopimelate decarboxylase catalyzes the final step in lysine biosynthesis that removes the carbon dioxide group from meso-diaminopimelic acid to yield L-lysine.

Proteins

Protein synthesis occurs via a process called translation. During translation, genetic material called mRNA is read by ribosomes to generate a protein polypeptide chain. This process requires transfer RNA (tRNA) which serves as an adaptor by binding amino acids on one end and interacting with mRNA at the other end; the latter pairing between the tRNA and mRNA ensures that the correct amino acid is added to the chain. Protein synthesis occurs in three phases: initiation, elongation, and termination. Prokaryotic (archaeal and bacterial) translation differs from eukaryotic translation; however, this section will mostly focus on the commonalities between the two organisms.

Additional background

- Before translation can begin, the process of binding a specific amino acid to its corresponding tRNA must occur. This reaction, called tRNA charging, is catalyzed by aminoacyl tRNAsynthetase. A specific tRNAsynthetase is responsible for recognizing and charging a particular amino acid. Furthermore, this enzyme has special discriminator regions to ensure the correct binding between tRNA and its cognate amino acid.

The combination of these two steps, both of which are catalyzed by aminoacyl tRNAsynthetase, produces a charged tRNA that is ready to add amino acids to the growing polypeptide chain.

In addition to binding an amino acid, tRNA has a three nucleotide unit called an anticodon that base pairs with specific nucleotide triplets on the mRNA called codons; codons encode a specific amino acid. This interaction is possible thanks to the ribosome, which serves as the site for protein synthesis. The ribosome possesses three tRNA binding sites: the aminoacyl site (A site), the peptidyl site (P site), and the exit site (E site).

There are numerous codons within an mRNA transcript, and it is very common for an amino acid to be specified by more than one codon; this phenomenon is called degeneracy. In all, there are 64 codons, 61 of each code for one of the 20 amino acids, while the remaining codons specify chain termination.

Translation in steps

- As previously mentioned, translation occurs in three phases: initiation, elongation, and termination.

Step 1: Initiation

The completion of the initiation phase is dependent on the following three events:

1. The recruitment of the ribosome to mRNA

2. The binding of a charged initiator tRNA into the P site of the ribosome

3. The proper alignment of the ribosome with mRNA's start codon

Step 2: Elongation

Following initiation, the polypeptide chain is extended via anticodon:codon interactions, with the ribosome adding amino acids to the polypeptide chain one at a time. The following steps must occur to ensure the correct addition of amino acids:

1. The binding of the correct tRNA into the A site of the ribosome

2. The formation of a peptide bond between the tRNA in the A site and the polypeptide chain attached to the tRNA in the P site

3. Translocation or advancement of the tRNA-mRNA complex by three nucleotides

Translocation "kicks off" the tRNA at the E site and shifts the tRNA from the A site into the P site, leaving the A site free for an incoming tRNA to add another amino acid.

Step 3: Termination

The last stage of translation occurs when a stop codon enters the A site. Then, the following steps occur:

1. The recognition of codons by release factors, which causes the hydrolysis of the polypeptide chain from the tRNA located in the P site

2. The release of the polypeptide chain

3. The dissociation and "recycling" of the ribosome for future translation processes

Diseases associated with macromolecule deficiency

Errors in biosynthetic pathways can have deleterious consequences including the malformation of macromolecules or the underproduction of functional molecules. Below are examples that illustrate the disruptions that occur due to these inefficiencies.

- Familial hypercholesterolemia: this disorder is characterized by the absence of functional receptors for LDL. Deficiencies in the formation of LDL receptors may cause faulty receptors which disrupt the endocytic pathway, inhibiting the entry of LDL into the liver and other cells. This causes a buildup of LDL in the blood plasma, which results in atherosclerotic plaques that narrow arteries and increase the risk of heart attacks.
- Lesch–Nyhan syndrome: this genetic disease is characterized by self- mutilation, mental deficiency, and gout. It is caused by the absence of hypoxanthine-guanine phosphoribosyltransferase,

which is a necessary enzyme for purine nucleotide formation. The lack of enzyme reduces the level of necessary nucleotides and causes the accumulation of biosynthesis intermediates, which results in the aforementioned unusual behavior.

- Severe combined immunodeficiency (SCID): SCID is characterized by a loss of T cells. Shortage of these immune system components increases the susceptibility to infectious agents because the affected individuals cannot develop immunological memory. This immunological disorder results from a deficiency in adenosine deanimase activity, which causes a buildup of dATP. These dATP molecules then inhibit ribonucleotide reductase, which prevents of DNA synthesis.
- Huntington's disease: this neurological disease is caused from errors that occur during DNA synthesis. These errors or mutations lead to the expression of a mutant huntingtin protein, which contains repetitive glutamine residues that are encoded by expanding CAG trinucleotide repeats in the gene.Huntington's disease is characterized by neuronal loss and gliosis. Symptoms of the disease include: movement disorder, cognitive decline, and behavioral disorder.

Chapter 2

Enzyme-catalyzedProcess

Enzyme

Enzymes (/ˈɛnzaɪmz/) are proteins that act as biological catalysts (biocatalysts). Catalysts accelerate chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life. Metabolic pathways depend upon enzymes to catalyze individual steps. The study of enzymes is called *enzymology* and the field of pseudoenzyme analysis recognizes that during evolution, some enzymes have lost the ability to carry out biological catalysis, which is often reflected in their amino acid sequences and unusual 'pseudocatalytic' properties.

Enzymes are known to catalyze more than 5,000 biochemical reaction types. Other biocatalysts are catalytic RNA molecules, called ribozymes. Enzymes' specificity comes from their unique three-dimensional structures.

Like all catalysts, enzymes increase the reaction rate by lowering its activation energy. Some enzymes can make their conversion of substrate to product occur many millions of times faster. An extreme example is orotidine 5'-phosphate decarboxylase, which allows a reaction that would otherwise take millions of years to occur in milliseconds. Chemically,

enzymes are like any catalyst and are not consumed in chemical reactions, nor do they alter the equilibrium of a reaction. Enzymes differ from most other catalysts by being much more specific. Enzyme activity can be affected by other molecules: inhibitors are molecules that decrease enzyme activity, and activators are molecules that increase activity. Many therapeutic drugs and poisons are enzyme inhibitors. An enzyme's activity decreases markedly outside its optimal temperature and pH, and many enzymes are (permanently) denatured when exposed to excessive heat, losing their structure and catalytic properties.

Some enzymes are used commercially, for example, in the synthesis of antibiotics. Some household products use enzymes to speed up chemical reactions: enzymes in biological washing powders break down protein, starch or fat stains on clothes, and enzymes in meat tenderizer break down proteins into smaller molecules, making the meat easier to chew.

Etymology and history

By the late 17th and early 18th centuries, the digestion of meat by stomach secretions and the conversion of starch to sugars by plant extracts and saliva were known but the mechanisms by which these occurred had not been identified.

French chemist AnselmePayen was the first to discover an enzyme, diastase, in 1833. A few decades later, when studying the fermentation of sugar to alcohol by yeast, Louis Pasteur concluded that this fermentation was caused by a vital force contained within the yeast cells called "ferments", which were thought to function only within living organisms. He wrote that

"alcoholic fermentation is an act correlated with the life and organization of the yeast cells, not with the death or putrefaction of the cells."

In 1877, German physiologist Wilhelm Kühne (1837–1900) first used the term *enzyme*, which comes from Greekἔνζυμον, "leavened" or "in yeast", to describe this process. The word *enzyme* was used later to refer to nonliving substances such as pepsin, and the word *ferment* was used to refer to chemical activity produced by living organisms.

Eduard Buchner submitted his first paper on the study of yeast extracts in 1897. In a series of experiments at the University of Berlin, he found that sugar was fermented by yeast extracts even when there were no living yeast cells in the mixture. He named the enzyme that brought about the fermentation of sucrose "zymase". In 1907, he received the Nobel Prize in Chemistry for "his discovery of cell-free fermentation". Following Buchner's example, enzymes are usually named according to the reaction they carry out: the suffix *-ase* is combined with the name of the substrate (e.g., lactase is the enzyme that cleaves lactose) or to the type of reaction (e.g., DNA polymerase forms DNA polymers).

The biochemical identity of enzymes was still unknown in the early 1900s. Many scientists observed that enzymatic activity was associated with proteins, but others (such as Nobel laureate Richard Willstätter) argued that proteins were merely carriers for the true enzymes and that proteins *per se* were incapable of catalysis. In 1926, James B. Sumner showed that the enzyme urease was a pure protein and crystallized it; he did likewise for the enzyme catalase in 1937.The conclusion

that pure proteins can be enzymes was definitively demonstrated by John Howard Northrop and Wendell Meredith Stanley, who worked on the digestive enzymes pepsin (1930), trypsin and chymotrypsin. These three scientists were awarded the 1946 Nobel Prize in Chemistry.

The discovery that enzymes could be crystallized eventually allowed their structures to be solved by x-ray crystallography. This was first done for lysozyme, an enzyme found in tears, saliva and egg whites that digests the coating of some bacteria; the structure was solved by a group led by David Chilton Phillips and published in 1965. This high-resolution structure of lysozyme marked the beginning of the field of structural biology and the effort to understand how enzymes work at an atomic level of detail.

Classification and nomenclature

Enzymes can be classified by two main criteria: either amino acid sequence similarity (and thus evolutionary relationship) or enzymatic activity.

Enzyme activity. An enzyme's name is often derived from its substrate or the chemical reaction it catalyzes, with the word ending in *-ase*. Examples are lactase, alcohol dehydrogenase and DNA polymerase. Different enzymes that catalyze the same chemical reaction are called isozymes.

The International Union of Biochemistry and Molecular Biology have developed a nomenclature for enzymes, the EC numbers (for "Enzyme Commission"). Each enzyme is described by "EC" followed by a sequence of four numbers which represent the

hierarchy of enzymatic activity (from very general to very specific). That is, the first number broadly classifies the enzyme based on its mechanism while the other digits add more and more specificity.

The top-level classification is:

- EC 1, Oxidoreductases: catalyze oxidation/reduction reactions
- EC 2, Transferases: transfer a functional group (*e.g.* a methyl or phosphate group)
- EC 3, Hydrolases: catalyze the hydrolysis of various bonds
- EC 4, Lyases: cleave various bonds by means other than hydrolysis and oxidation
- EC 5, Isomerases: catalyze isomerization changes within a single molecule
- EC 6, Ligases: join two molecules with covalent bonds.
- EC 7, Translocases: catalyze the movement of ions or molecules across membranes, or their separation within membranes.

These sections are subdivided by other features such as the substrate, products, and chemical mechanism. An enzyme is fully specified by four numerical designations. For example, hexokinase (EC 2.7.1.1) is a transferase (EC 2) that adds a phosphate group (EC 2.7) to a hexose sugar, a molecule containing an alcohol group (EC 2.7.1).

Sequence similarity. EC categories do **not** reflect sequence similarity. For instance, two ligases of the same EC number that catalyze exactly the same reaction can have completely

different sequences. Independent of their function, enzymes, like any other proteins, have been classified by their sequence similarity into numerous families. These families have been documented in dozens of different protein and protein family databases such as Pfam.

Structure

Enzymes are generally globular proteins, acting alone or in larger complexes. The sequence of the amino acids specifies the structure which in turn determines the catalytic activity of the enzyme. Although structure determines function, a novel enzymatic activity cannot yet be predicted from structure alone. Enzyme structures unfold (denature) when heated or exposed to chemical denaturants and this disruption to the structure typically causes a loss of activity. Enzyme denaturation is normally linked to temperatures above a species' normal level; as a result, enzymes from bacteria living in volcanic environments such as hot springs are prized by industrial users for their ability to function at high temperatures, allowing enzyme-catalysed reactions to be operated at a very high rate.

Enzymes are usually much larger than their substrates. Sizes range from just 62 amino acid residues, for the monomer of 4-oxalocrotonate tautomerase, to over 2,500 residues in the animal fatty acid synthase. Only a small portion of their structure (around 2–4 amino acids) is directly involved in catalysis: the catalytic site. This catalytic site is located next to one or more binding sites where residues orient the substrates. The catalytic site and binding site together compose the enzyme's active site. The remaining majority of

the enzyme structure serves to maintain the precise orientation and dynamics of the active site.

In some enzymes, no amino acids are directly involved in catalysis; instead, the enzyme contains sites to bind and orient catalytic cofactors. Enzyme structures may also contain allosteric sites where the binding of a small molecule causes a conformational change that increases or decreases activity.

A small number of RNA-based biological catalysts called ribozymes exist, which again can act alone or in complex with proteins. The most common of these is the ribosome which is a complex of protein and catalytic RNA components.

Mechanism

Substrate binding

Enzymes must bind their substrates before they can catalyse any chemical reaction. Enzymes are usually very specific as to what substrates they bind and then the chemical reaction catalysed. Specificity is achieved by binding pockets with complementary shape, charge and hydrophilic/hydrophobic characteristics to the substrates. Enzymes can therefore distinguish between very similar substrate molecules to be chemoselective, regioselective and stereospecific.

Some of the enzymes showing the highest specificity and accuracy are involved in the copying and expression of the genome. Some of these enzymes have "proof-reading" mechanisms. Here, an enzyme such as DNA polymerase catalyzes a reaction in a first step and then checks that the

product is correct in a second step. This two-step process results in average error rates of less than 1 error in 100 million reactions in high-fidelity mammalian polymerases. Similar proofreading mechanisms are also found in RNA polymerase,aminoacyl tRNAsynthetases and ribosomes.

Conversely, some enzymes display enzyme promiscuity, having broad specificity and acting on a range of different physiologically relevant substrates. Many enzymes possess small side activities which arose fortuitously (i.e. neutrally), which may be the starting point for the evolutionary selection of a new function.

"Lock and key" model

To explain the observed specificity of enzymes, in 1894 Emil Fischer proposed that both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. This early model explains enzyme specificity, but fails to explain the stabilization of the transition state that enzymes achieve.

Induced fit model

In 1958, Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to

perform its catalytic function.In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge distribution is determined. Induced fit may enhance the fidelity of molecular recognition in the presence of competition and noise via the conformational proofreading mechanism.

Catalysis

Enzymes can accelerate reactions in several ways, all of which lower the activation energy (ΔG, Gibbs free energy)

- By stabilizing the transition state:
- Creating an environment with a charge distribution complementary to that of the transition state to lower its energy
- By providing an alternative reaction pathway:
- Temporarily reacting with the substrate, forming a covalent intermediate to provide a lower energy transition state
- By destabilising the substrate ground state:
- Distorting bound substrate(s) into their transition state form to reduce the energy required to reach the transition state
- By orienting the substrates into a productive arrangement to reduce the reaction entropy change (the contribution of this mechanism to catalysis is relatively small)

Enzymes may use several of these mechanisms simultaneously. For example, proteases such as trypsin perform covalent catalysis using a catalytic triad, stabilise charge build-up on the transition states using an oxyanion hole, complete hydrolysis using an oriented water substrate.

Dynamics

Enzymes are not rigid, static structures; instead they have complex internal dynamic motions – that is, movements of parts of the enzyme's structure such as individual amino acid residues, groups of residues forming a protein loop or unit of secondary structure, or even an entire protein domain. These motions give rise to a conformational ensemble of slightly different structures that interconvert with one another at equilibrium. Different states within this ensemble may be associated with different aspects of an enzyme's function. For example, different conformations of the enzyme dihydrofolate reductase are associated with the substrate binding, catalysis, cofactor release, and product release steps of the catalytic cycle, consistent with catalytic resonance theory.

Substrate presentation

Substrate presentation is a process where the enzyme is sequestered away from its substrate. Enzymes can be sequestered to the plasma membrane away from a substrate in the nucleus or cytosol. Or within the membrane, an enzyme can be sequestered into lipid rafts away from its substrate in the disordered region. When the enzyme is released it mixes with its substrate. Alternatively, the enzyme can be sequestered near its substrate to activate the enzyme. For

example, the enzyme can be soluble and upon activation bind to a lipid in the plasma membrane and then act upon molecules in the plasma membrane.

Allosteric modulation

Allosteric sites are pockets on the enzyme, distinct from the active site, that bind to molecules in the cellular environment. These molecules then cause a change in the conformation or dynamics of the enzyme that is transduced to the active site and thus affects the reaction rate of the enzyme. In this way, allosteric interactions can either inhibit or activate enzymes. Allosteric interactions with metabolites upstream or downstream in an enzyme's metabolic pathway cause feedback regulation, altering the activity of the enzyme according to the flux through the rest of the pathway.

Cofactors

Some enzymes do not need additional components to show full activity. Others require non-protein molecules called cofactors to be bound for activity. Cofactors can be either inorganic (e.g., metal ions and iron-sulfur clusters) or organic compounds (e.g., flavin and heme). These cofactors serve many purposes; for instance, metal ions can help in stabilizing nucleophilic species within the active site. Organic cofactors can be either coenzymes, which are released from the enzyme's active site during the reaction, or prosthetic groups, which are tightly bound to an enzyme. Organic prosthetic groups can be covalently bound (e.g., biotin in enzymes such as pyruvate carboxylase).

An example of an enzyme that contains a cofactor is carbonic anhydrase, which uses a zinc cofactor bound as part of its active site. These tightly bound ions or molecules are usually found in the active site and are involved in catalysis. For example, flavin and heme cofactors are often involved in redox reactions.

Enzymes that require a cofactor but do not have one bound are called*apoenzymes* or *apoproteins*. An enzyme together with the cofactor(s) required for activity is called a *holoenzyme* (or haloenzyme). The term *holoenzyme* can also be applied to enzymes that contain multiple protein subunits, such as the DNA polymerases; here the holoenzyme is the complete complex containing all the subunits needed for activity.

Coenzymes

Coenzymes are small organic molecules that can be loosely or tightly bound to an enzyme. Coenzymes transport chemical groups from one enzyme to another. Examples include NADH, NADPH and adenosine triphosphate (ATP). Some coenzymes, such as flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), thiamine pyrophosphate (TPP), and tetrahydrofolate (THF), are derived from vitamins. These coenzymes cannot be synthesized by the body *de novo* and closely related compounds (vitamins) must be acquired from the diet. The chemical groups carried include:

- the hydride ion (H^-), carried by NAD or $NADP^+$
- the phosphate group, carried by adenosine triphosphate
- the acetyl group, carried by coenzyme A

- formyl, methenyl or methyl groups, carried by folic acid and
- the methyl group, carried by S-adenosylmethionine

Since coenzymes are chemically changed as a consequence of enzyme action, it is useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different enzymes. For example, about 1000 enzymes are known to use the coenzyme NADH.

Coenzymes are usually continuously regenerated and their concentrations maintained at a steady level inside the cell. For example, NADPH is regenerated through the pentose phosphate pathway and S-adenosylmethionine by methionine adenosyltransferase. This continuous regeneration means that small amounts of coenzymes can be used very intensively. For example, the human body turns over its own weight in ATP each day.

Kinetics

Enzyme kinetics is the investigation of how enzymes bind substrates and turn them into products. The rate data used in kinetic analyses are commonly obtained from enzyme assays. In 1913 Leonor Michaelis and Maud Leonora Menten proposed a quantitative theory of enzyme kinetics, which is referred to as Michaelis–Menten kinetics. The major contribution of Michaelis and Menten was to think of enzyme reactions in two stages. In the first, the substrate binds reversibly to the enzyme, forming the enzyme-substrate complex. This is sometimes called the Michaelis–Menten complex in their honor. The enzyme then catalyzes the chemical step in the reaction

and releases the product. This work was further developed by G. E. Briggs and J. B. S. Haldane, who derived kinetic equations that are still widely used today.

Enzyme rates depend on solution conditions and substrate concentration. To find the maximum speed of an enzymatic reaction, the substrate concentration is increased until a constant rate of product formation is seen. This is shown in the saturation curve on the right. Saturation happens because, as substrate concentration increases, more and more of the free enzyme is converted into the substrate-bound ES complex. At the maximum reaction rate (V_{max}) of the enzyme, all the enzyme active sites are bound to substrate, and the amount of ES complex is the same as the total amount of enzyme.

V_{max} is only one of several important kinetic parameters. The amount of substrate needed to achieve a given rate of reaction is also important. This is given by the Michaelis–Menten constant (K_m), which is the substrate concentration required for an enzyme to reach one-half its maximum reaction rate; generally, each enzyme has a characteristic K_M for a given substrate. Another useful constant is k_{cat}, also called the *turnover number*, which is the number of substrate molecules handled by one active site per second.

The efficiency of an enzyme can be expressed in terms of k_{cat}/K_m. This is also called the specificity constant and incorporates the rate constants for all steps in the reaction up to and including the first irreversible step. Because the specificity constant reflects both affinity and catalytic ability, it is useful for comparing different enzymes against each other, or the same enzyme with different substrates. The theoretical

maximum for the specificity constant is called the diffusion limit and is about 10^8 to 10^9 (M^{-1} s^{-1}). At this point every collision of the enzyme with its substrate will result in catalysis, and the rate of product formation is not limited by the reaction rate but by the diffusion rate. Enzymes with this property are called*catalytically perfect* or *kinetically perfect*. Example of such enzymes are triose-phosphate isomerase, carbonic anhydrase, acetylcholinesterase, catalase, fumarase, β-lactamase, and superoxide dismutase. The turnover of such enzymes can reach several million reactions per second.Michaelis–Menten kinetics relies on the law of mass action, which is derived from the assumptions of free diffusion and thermodynamically driven random collision. Many biochemical or cellular processes deviate significantly from these conditions, because of macromolecular crowding and constrained molecular movement. More recent, complex extensions of the model attempt to correct for these effects.

Inhibition

Enzyme reaction rates can be decreased by various types of enzyme inhibitors.

Types of inhibition

Competitive

A competitive inhibitor and substrate cannot bind to the enzyme at the same time. Often competitive inhibitors strongly resemble the real substrate of the enzyme. For example, the drug methotrexate is a competitive inhibitor of the enzyme

dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate. The similarity between the structures of dihydrofolate and this drug are shown in the accompanying figure. This type of inhibition can be overcome with high substrate concentration. In some cases, the inhibitor can bind to a site other than the binding-site of the usual substrate and exert an allosteric effect to change the shape of the usual binding-site.

Non-competitive

A non-competitive inhibitor binds to a site other than where the substrate binds. The substrate still binds with its usual affinity and hence K_m remains the same. However the inhibitor reduces the catalytic efficiency of the enzyme so that V_{max} is reduced. In contrast to competitive inhibition, non-competitive inhibition cannot be overcome with high substrate concentration.

Uncompetitive

An uncompetitive inhibitor cannot bind to the free enzyme, only to the enzyme-substrate complex; hence, these types of inhibitors are most effective at high substrate concentration. In the presence of the inhibitor, the enzyme-substrate complex is inactive. This type of inhibition is rare.

Mixed

A mixed inhibitor binds to an allosteric site and the binding of the substrate and the inhibitor affect each other. The enzyme's function is reduced but not eliminated when bound to the

inhibitor. This type of inhibitor does not follow the Michaelis–Menten equation.

Irreversible

An irreversible inhibitor permanently inactivates the enzyme, usually by forming a covalent bond to the protein.Penicillin and aspirin are common drugs that act in this manner.

Functions of inhibitors

In many organisms, inhibitors may act as part of a feedback mechanism. If an enzyme produces too much of one substance in the organism, that substance may act as an inhibitor for the enzyme at the beginning of the pathway that produces it, causing production of the substance to slow down or stop when there is sufficient amount. This is a form of negative feedback. Major metabolic pathways such as the citric acid cycle make use of this mechanism.

Since inhibitors modulate the function of enzymes they are often used as drugs. Many such drugs are reversible competitive inhibitors that resemble the enzyme's native substrate, similar to methotrexate above; other well-known examples include statins used to treat high cholesterol, and protease inhibitors used to treat retroviral infections such as HIV. A common example of an irreversible inhibitor that is used as a drug is aspirin, which inhibits the COX-1 and COX-2 enzymes that produce the inflammation messenger prostaglandin. Other enzyme inhibitors are poisons. For example, the poison cyanide is an irreversible enzyme inhibitor

that combines with the copper and iron in the active site of the enzyme cytochrome c oxidase and blocks cellular respiration.

Biological function

Enzymes serve a wide variety of functions inside living organisms. They are indispensable for signal transduction and cell regulation, often via kinases and phosphatases. They also generate movement, with myosin hydrolyzing ATP to generate muscle contraction, and also transport cargo around the cell as part of the cytoskeleton. Other ATPases in the cell membrane are ion pumps involved in active transport. Enzymes are also involved in more exotic functions, such as luciferase generating light in fireflies.Viruses can also contain enzymes for infecting cells, such as the HIV integrase and reverse transcriptase, or for viral release from cells, like the influenza virus neuraminidase.

An important function of enzymes is in the digestive systems of animals. Enzymes such as amylases and proteases break down large molecules (starch or proteins, respectively) into smaller ones, so they can be absorbed by the intestines. Starch molecules, for example, are too large to be absorbed from the intestine, but enzymes hydrolyze the starch chains into smaller molecules such as maltose and eventually glucose, which can then be absorbed. Different enzymes digest different food substances. In ruminants, which have herbivorous diets, microorganisms in the gut produce another enzyme, cellulase, to break down the cellulose cell walls of plant fiber.

Metabolism

Several enzymes can work together in a specific order, creating metabolic pathways. In a metabolic pathway, one enzyme takes the product of another enzyme as a substrate. After the catalytic reaction, the product is then passed on to another enzyme. Sometimes more than one enzyme can catalyze the same reaction in parallel; this can allow more complex regulation: with, for example, a low constant activity provided by one enzyme but an inducible high activity from a second enzyme.

Enzymes determine what steps occur in these pathways. Without enzymes, metabolism would neither progress through the same steps and could not be regulated to serve the needs of the cell. Most central metabolic pathways are regulated at a few key steps, typically through enzymes whose activity involves the hydrolysis of ATP. Because this reaction releases so much energy, other reactions that are thermodynamically unfavorable can be coupled to ATP hydrolysis, driving the overall series of linked metabolic reactions.

Control of activity

There are five main ways that enzyme activity is controlled in the cell.

Regulation

Enzymes can be either activated or inhibited by other molecules. For example, the end product(s) of a metabolic pathway are often inhibitors for one of the first enzymes of the

pathway (usually the first irreversible step, called committed step), thus regulating the amount of end product made by the pathways. Such a regulatory mechanism is called a negative feedback mechanism, because the amount of the end product produced is regulated by its own concentration. Negative feedback mechanism can effectively adjust the rate of synthesis of intermediate metabolites according to the demands of the cells. This helps with effective allocations of materials and energy economy, and it prevents the excess manufacture of end products. Like other homeostatic devices, the control of enzymatic action helps to maintain a stable internal environment in living organisms.

Post-translational modification

Examples of post-translational modification include phosphorylation, myristoylation and glycosylation. For example, in the response to insulin, the phosphorylation of multiple enzymes, including glycogen synthase, helps control the synthesis or degradation of glycogen and allows the cell to respond to changes in blood sugar. Another example of post-translational modification is the cleavage of the polypeptide chain.

Chymotrypsin, a digestive protease, is produced in inactive form as chymotrypsinogen in the pancreas and transported in this form to the stomach where it is activated. This stops the enzyme from digesting the pancreas or other tissues before it enters the gut. This type of inactive precursor to an enzyme is known as a zymogen or proenzyme.

Quantity

Enzyme production (transcription and translation of enzyme genes) can be enhanced or diminished by a cell in response to changes in the cell's environment. This form of gene regulationis calledenzyme induction. For example, bacteria may become resistant to antibiotics such as penicillin because enzymes called beta-lactamases are induced that hydrolyse the crucial beta-lactam ring within the penicillin molecule. Another example comes from enzymes in the liver called cytochrome P450 oxidases, which are important in drug metabolism. Induction or inhibition of these enzymes can cause drug interactions. Enzyme levels can also be regulated by changing the rate of enzyme degradation. The opposite of enzyme induction is enzyme repression.

Subcellular distribution

Enzymes can be compartmentalized, with different metabolic pathways occurring in different cellular compartments. For example, fatty acids are synthesized by one set of enzymes in the cytosol, endoplasmic reticulum and Golgi and used by a different set of enzymes as a source of energy in the mitochondrion, through β-oxidation. In addition, trafficking of the enzyme to different compartments may change the degree of protonation (e.g., the neutral cytoplasm and the acidic lysosome) or oxidative state (e.g., oxidizing periplasm or reducing cytoplasm) which in turn affects enzyme activity. In contrast to partitioning into membrane bound organelles, enzyme subcellular localisation may also be altered through polymerisation of enzymes into macromolecular cytoplasmic filaments.

Organ specialization

In multicellulareukaryotes, cells in different organs and tissues have different patterns of gene expression and therefore have different sets of enzymes (known as isozymes) available for metabolic reactions. This provides a mechanism for regulating the overall metabolism of the organism. For example, hexokinase, the first enzyme in the glycolysis pathway, has a specialized form called glucokinase expressed in the liver and pancreas that has a lower affinity for glucose yet is more sensitive to glucose concentration. This enzyme is involved in sensing blood sugar and regulating insulin production.

Involvement in disease

Since the tight control of enzyme activity is essential for homeostasis, any malfunction (mutation, overproduction, underproduction or deletion) of a single critical enzyme can lead to a genetic disease. The malfunction of just one type of enzyme out of the thousands of types present in the human body can be fatal. An example of a fatal genetic disease due to enzyme insufficiency is Tay–Sachs disease, in which patients lack the enzyme hexosaminidase.

One example of enzyme deficiency is the most common type of phenylketonuria. Many different single amino acid mutations in the enzyme phenylalanine hydroxylase, which catalyzes the first step in the degradation of phenylalanine, result in build-up of phenylalanine and related products. Some mutations are in the active site, directly disrupting binding and catalysis, but many are far from the active site and reduce activity by

destabilising the protein structure, or affecting correct oligomerisation. This can lead to intellectual disability if the disease is untreated. Another example is pseudocholinesterase deficiency, in which the body's ability to break down choline ester drugs is impaired. Oral administration of enzymes can be used to treat some functional enzyme deficiencies, such as pancreatic insufficiency and lactose intolerance.

Another way enzyme malfunctions can cause disease comes from germline mutations in genes coding for DNA repair enzymes. Defects in these enzymes cause cancer because cells are less able to repair mutations in their genomes. This causes a slow accumulation of mutations and results in the development of cancers. An example of such a hereditary cancer syndrome is xerodermapigmentosum, which causes the development of skin cancers in response to even minimal exposure to ultraviolet light.

Evolution

Similar to any other protein, enzymes change over time through mutations and sequence divergence. Given their central role in metabolism, enzyme evolution plays a critical role in adaptation. A key question is therefore whether and how enzymes can change their enzymatic activities alongside. It is generally accepted that many new enzyme activities have evolved through gene duplication and mutation of the duplicate copies although evolution can also happen without duplication. One example of an enzyme that has changed its activity is the ancestor of methionyl amino peptidase (MAP) and creatineamidinohydrolase (creatinase) which are clearly homologous but catalyze very different reactions (MAP removes

the amino-terminal methionine in new proteins while creatinase hydrolyses creatine to sarcosine and urea). In addition, MAP is metal-ion dependent while creatinase is not, hence this property was also lost over time. Small changes of enzymatic activity are extremely common among enzymes. In particular, substrate binding specificity (see above) can easily and quickly change with single amino acid changes in their substrate binding pockets. This is frequently seen in the main enzyme classes such as kinases.

Artificial (in vitro) evolution is now commonly used to modify enzyme activity or specificity for industrial applications (see below).

Catalysis

Catalysis (/kəˈtæləsɪs/) is the process of increasing the rate of a chemical reaction by adding a substance known as a **catalyst** (/ˈkætəlɪst/). Catalysts are not consumed in the reaction and remain unchanged after it. If the reaction is rapid and the catalyst recycles quickly, very small amounts of catalyst often suffice; mixing, surface area, and temperature are important factors in reaction rate. Catalysts generally react with one or more reactants to form intermediates that subsequently give the final reaction product, in the process regenerating the catalyst.

Catalysis may be classified as either homogeneous, whose components are dispersed in the same phase (usually gaseous or liquid) as the reactant, or heterogeneous, whose components are not in the same phase. Enzymes and other biocatalysts are often considered as a third category.

Catalysis is ubiquitous in chemical industry of all kinds. Estimates are that 90% of all commercially produced chemical products involve catalysts at some stage in the process of their manufacture.

The term "catalyst" is derived from Greekκαταλύειν, *katalúō*, meaning "loosen" or "untie". The concept of catalysis was invented by chemist Elizabeth Fulhame, based on her novel work in oxidation-reduction experiments.

General principles

Illustration

Illustrative is the disproportionation of hydrogen peroxide to water and oxygen:

- $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

This reaction proceeds because the reaction products are more stable than the starting material. The uncatalysed reaction is slow. In fact, the decomposition of hydrogen peroxide is so slow that hydrogen peroxide solutions are commercially available. This reaction is strongly affected by catalysts such as manganese dioxide, or the enzyme peroxidase in organisms. Upon the addition of a small amount of manganese dioxide, the hydrogen peroxide reacts rapidly. This effect is readily seen by the effervescence of oxygen. The manganese dioxide is not consumed in the reaction, and thus may be recovered unchanged, and re-used indefinitely. Accordingly, manganese dioxide *catalyses* this reaction.

Units

The SI derived unit for measuring the **catalytic activity** of a catalyst is the katal, which is quantified in moles per second. The productivity of a catalyst can be described by the turnover number (or TON) and the catalytic activity by the *turn over frequency* (TOF), which is the TON per time unit. The biochemical equivalent is the enzyme unit. For more information on the efficiency of enzymatic catalysis, see the article on *enzymes*.

Catalytic reaction mechanisms

In general, chemical reactions occur faster in the presence of a catalyst because the catalyst provides an alternative reaction pathway - or mechanism - with a lower activation energy than the non-catalyzed mechanism. In catalyzed mechanisms, the catalyst usually reacts to form an intermediate, which then regenerates the original catalyst in the process.

Catalysts generally react with one or more reactants to form intermediates that subsequently give the final reaction product. In the process, the catalyst is regenerated. In principle, often only small amounts of catalysts are required to increase the rate of the reaction. In practice, however, catalysts are sometimes slow or are even consumed in secondary processes. The catalyst does often appear in the rate equation.

As a simple example in the gas phase, the reaction $2\ SO_2 + O_2 \rightarrow 2\ SO_3$can be catalyzed by adding nitric oxide. The reaction mechanism occurs in two steps:

- $2\ NO + O_2 \rightarrow 2\ NO_2$ (slow)
- $NO_2 + SO_2 \rightarrow NO + SO_3$ (fast, twice)

The overall reaction is the first equation plus twice the second reaction, so that the NO catalyst is regenerated and does not participate in the net reaction. The overall rate is the rate of the slow step

- $v = 2k_1[NO]^2[O_2]$.

As an example of a detailed mechanism for heterogeneous catalysis at a surface, when oxygen and hydrogen combine on the surface of titanium dioxide (TiO_2, or *titania*) to produce water. Scanning tunneling microscopyshowed that the molecules undergo adsorption, dissociation, and diffusion before reacting. The intermediate reaction states were: HO_2, H_2O_2, then H_3O_2 and the final reaction product (water molecule dimers), after which the water molecule desorbs from the catalyst surface.

Reaction energetics

Catalysts work by providing an (alternative) mechanism involving a different transition state and lower activation energy. Consequently, more molecular collisions have the energy needed to reach the transition state. Hence, catalysts can enable reactions that would otherwise be blocked or slowed by a kinetic barrier. The catalyst may increase reaction rate or selectivity, or enable the reaction at lower temperatures. This effect can be illustrated with an energy profile diagram.

In the catalyzed elementary reaction, catalysts do **not** change the extent of a reaction: they have **no** effect on the chemical

equilibrium of a reaction because the rate of both the forward and the reverse reaction are both affected (see also thermodynamics). The second law of thermodynamics describes why a catalyst does not change the chemical equilibrium of a reaction. Suppose there was such a catalyst that shifted an equilibrium. Introducing the catalyst to the system would result in a reaction to move to the new equilibrium, producing energy. Production of energy is a necessary result since reactions are spontaneous only if Gibbs free energyis produced, and if there is no energy barrier, there is no need for a catalyst. Then, removing the catalyst would also result in reaction, producing energy; i.e. the addition and its reverse process, removal, would both produce energy. Thus, a catalyst that could change the equilibrium would be a perpetual motion machine, a contradiction to the laws of thermodynamics. Thus, catalyst **does not** alter the equilibrium constant. (A catalyst can however change the equilibrium concentrations by reacting in a subsequent step. It is then consumed as the reaction proceeds, and thus it is also a reactant. Illustrative is the base-catalysedhydrolysis of esters, where the produced carboxylic acid immediately reacts with the base catalyst and thus the reaction equilibrium is shifted towards hydrolysis.)

The catalyst stabilizes the transition state more than it stabilizes the starting material. It decreases the kinetic barrier by decreasing the *difference* in energy between starting material and transition state. It **does not** change the energy difference between starting materials and products (thermodynamic barrier), or the available energy (this is provided by the environment as heat or light).

Related concepts

Some so-called catalysts are really **precatalysts**. Precatalysts convert to catalysts in the reaction. For example, Wilkinson's catalyst$RhCl(PPh_3)_3$ loses one triphenylphosphine ligand before entering the true catalytic cycle. Precatalysts are easier to store but are easily activatedin situ. Because of this preactivation step, many catalytic reactions involve an induction period.

Chemical species that improve catalytic activity are called**co-catalysts** (**cocatalysts**) or **promoters** in **cooperative catalysis**.

In tandem catalysis two or more different catalysts are coupled in a one-pot reaction.

In autocatalysis, the catalyst *is* a product of the overall reaction, in contrast to all other types of catalysis considered in this article. The simplest example of autocatalysis is a reaction of type A + B → 2 B, in one or in several steps. The overall reaction is just A→ B, so that B is a product. But since B is also a reactant, it may be present in the rate equation and affect the reaction rate. As the reaction proceeds, the concentration of B increases and can accelerate the reaction as a catalyst. In effect, the reaction accelerates itself or is autocatalyzed. An example is the hydrolysis of an ester such as aspirin to a carboxylic acid and an alcohol. In the absence of added acid catalysts, the carboxylic acid product catalyzes the hydrolysis.

Classification

Catalysis may be classified as either homogeneous or heterogeneous. A homogeneous catalysis is one whose components are dispersed in the same phase (usually gaseous or liquid) as the reactant's molecules. A heterogeneous catalysis is one where the reaction components are not in the same phase. Enzymes and other biocatalysts are often considered as a third category. Similar mechanistic principles apply to heterogeneous, homogeneous, and biocatalysis.

Heterogeneous catalysis

Heterogeneous catalysts act in a different phase than the reactants. Most heterogeneous catalysts are solids that act on substrates in a liquid or gaseous reaction mixture. Important heterogeneous catalysts include zeolites, alumina, higher-order oxides, graphitic carbon, transition metaloxides, metals such as Raney nickel for hydrogenation, and vanadium(V) oxide for oxidation of sulfur dioxide into sulfur trioxide by the so-called contact process.

Diverse mechanisms for reactions on surfaces are known, depending on how the adsorption takes place (Langmuir-Hinshelwood, Eley-Rideal, and Mars-van Krevelen). The total surface area of solid has an important effect on the reaction rate. The smaller the catalyst particle size, the larger the surface area for a given mass of particles.

A heterogeneous catalyst has **active sites**, which are the atoms or crystal faces where the reaction actually occurs. Depending on the mechanism, the active site may be either a planar

exposed metal surface, a crystal edge with imperfect metal valence, or a complicated combination of the two. Thus, not only most of the volume but also most of the surface of a heterogeneous catalyst may be catalytically inactive. Finding out the nature of the active site requires technically challenging research. Thus, empirical research for finding out new metal combinations for catalysis continues.

For example, in the Haber process, finely divided iron serves as a catalyst for the synthesis of ammonia from nitrogen and hydrogen. The reacting gasesadsorb onto active sites on the iron particles. Once physically adsorbed, the reagents undergo chemisorption that results in dissociation into adsorbed atomic species, and new bonds between the resulting fragments form in part due to their close proximity. In this way the particularly strong triple bond in nitrogen is broken, which would be extremely uncommon in the gas phase due to its high activation energy. Thus, the activation energy of the overall reaction is lowered, and the rate of reaction increases. Another place where a heterogeneous catalyst is applied is in the oxidation of sulfur dioxide on vanadium(V) oxide for the production of sulfuric acid.

Heterogeneous catalysts are typically "supported," which means that the catalyst is dispersed on a second material that enhances the effectiveness or minimizes their cost. Supports prevent or reduce agglomeration and sintering small catalyst particles, exposing more surface area, thus catalysts have a higher specific activity (per gram) on a support. Sometimes the support is merely a surface on which the catalyst is spread to increase the surface area. More often, the support and the catalyst interact, affecting the catalytic reaction. Supports can

also be used in nanoparticle synthesis by providing sites for individual molecules of catalyst to chemically bind. Supports are porous materials with a high surface area, most commonly alumina, zeolites or various kinds of activated carbon. Specialized supports include silicon dioxide, titanium dioxide, calcium carbonate, and barium sulfate.

In slurry reactions, heterogeneous catalysts can be lost by dissolving.

Many heterogeneous catalysts are in fact nanomaterials. Nanomaterial-based catalysts with enzyme-mimicking activities are collectively called as nanozymes.

Electrocatalysts

In the context of electrochemistry, specifically in fuel cell engineering, various metal-containing catalysts are used to enhance the rates of the half reactions that comprise the fuel cell. One common type of fuel cell electrocatalystis based upon nanoparticles of platinum that are supported on slightly larger carbon particles. When in contact with one of the electrodes in a fuel cell, this platinum increases the rate of oxygen reduction either to water, or to hydroxide or hydrogen peroxide.

Homogeneous catalysis

Homogeneous catalysts function in the same phase as the reactants. Typically homogeneous catalysts are dissolved in a solvent with the substrates. One example of homogeneous catalysis involves the influence of H^+ on the esterification of carboxylic acids, such as the formation of methyl acetate from

acetic acid and methanol. High-volume processes requiring a homogeneous catalyst include hydroformylation, hydrosilylation, hydrocyanation. For inorganic chemists, homogeneous catalysis is often synonymous with organometallic catalysts. Many homogeneous catalysts are however not organometallic, illustrated by the use of cobalt salts that catalyze the oxidation of p-xylene to terephthalic acid.

Organocatalysis

Whereas transition metals sometimes attract most of the attention in the study of catalysis, small organic molecules without metals can also exhibit catalytic properties, as is apparent from the fact that many enzymes lack transition metals. Typically, organic catalysts require a higher loading (amount of catalyst per unit amount of reactant, expressed in mol%amount of substance) than transition metal(-ion)-based catalysts, but these catalysts are usually commercially available in bulk, helping to reduce costs. In the early 2000s, these organocatalysts were considered "new generation" and are competitive to traditional metal(-ion)-containing catalysts. Organocatalysts are supposed to operate akin to metal-free enzymes utilizing, e.g., non-covalent interactions such as hydrogen bonding. The discipline organocatalysis is divided in the application of covalent (e.g., proline, DMAP) and non-covalent (e.g., thioureaorganocatalysis) organocatalysts referring to the preferred catalyst-substratebinding and interaction, respectively.

Photocatalysts

Photocatalysis is the phenomenon where the catalyst can receive light (such as visible light), be promoted to an excited state, and then undergo intersystem crossing with the starting material, returning to ground state without being consumed. The excited state of the starting material will then undergo reactions it ordinarily could not if directly illuminated. For example, singlet oxygenis usually produced by photocatalysis. Photocatalysts are also the main ingredient in dye-sensitized solar cells.

Enzymes and biocatalysts

In biology, enzymes are protein-based catalysts in metabolism and catabolism. Most biocatalysts are enzymes, but other non-protein-based classes of biomolecules also exhibit catalytic properties including ribozymes, and synthetic deoxyribozymes.

Biocatalysts can be thought of as intermediate between homogeneous and heterogeneous catalysts, although strictly speaking soluble enzymes are homogeneous catalysts and membrane-bound enzymes are heterogeneous. Several factors affect the activity of enzymes (and other catalysts) including temperature, pH, concentration of enzyme, substrate, and products. A particularly important reagent in enzymatic reactions is water, which is the product of many bond-forming reactions and a reactant in many bond-breaking processes.

In biocatalysis, enzymes are employed to prepare many commodity chemicals including high-fructose corn syrup and acrylamide.

Some monoclonal antibodies whose binding target is a stable molecule which resembles the transition state of a chemical reaction can function as weak catalysts for that chemical reaction by lowering its activation energy. Such catalytic antibodies are sometimes called "abzymes".

Significance

Estimates are that 90% of all commercially produced chemical products involve catalysts at some stage in the process of their manufacture. In 2005, catalytic processes generated about $900 billion in products worldwide. The global demand for catalysts in 2014 was estimated at approximately US$33.5 billion. Catalysis is so pervasive that subareas are not readily classified. Some areas of particular concentration are surveyed below.

Energy processing

Petroleum refining makes intensive use of catalysis for alkylation, catalytic cracking (breaking long-chain hydrocarbons into smaller pieces), naphtha reforming and steam reforming (conversion of hydrocarbons into synthesis gas). Even the exhaust from the burning of fossil fuels is treated via catalysis: Catalytic converters, typically composed of platinum and rhodium, break down some of the more harmful byproducts of automobile exhaust.

- $2\ CO + 2\ NO \rightarrow 2\ CO_2 + N_2$

With regard to synthetic fuels, an old but still important process is the Fischer-Tropsch synthesis of hydrocarbons from

synthesis gas, which itself is processed via water-gas shift reactions, catalysed by iron. Biodiesel and related biofuels require processing via both inorganic and biocatalysts.

Fuel cells rely on catalysts for both the anodic and cathodic reactions.

Catalytic heaters generate flameless heat from a supply of combustible fuel.

Bulk chemicals

Some of the largest-scale chemicals are produced via catalytic oxidation, often using oxygen. Examples include nitric acid (from ammonia), sulfuric acid (from sulfur dioxide to sulfur trioxide by the contact process), terephthalic acid from p-xylene, acrylic acid from propylene or propane and acrylonitrile from propane and ammonia.

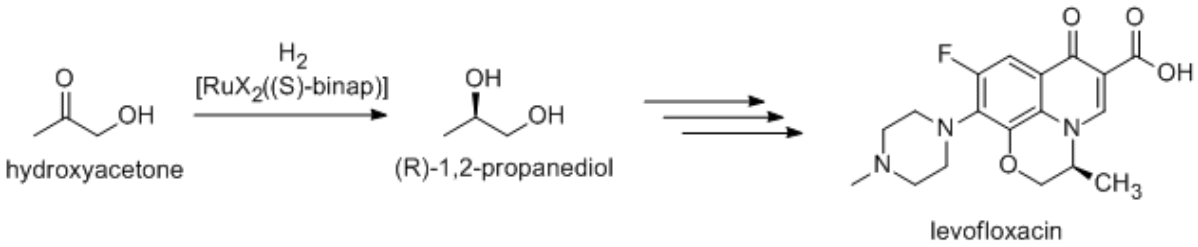
The production of ammonia is one of the largest-scale and most energy-intensive processes. In the Haber processnitrogen is combined with hydrogen over an iron oxide catalyst.Methanol is prepared from carbon monoxide or carbon dioxide but using copper-zinc catalysts.

Bulk polymers derived from ethylene and propylene are often prepared via Ziegler-Natta catalysis. Polyesters, polyamides, and isocyanatesare derived via acid-base catalysis.

Most carbonylation processes require metal catalysts, examples include the Monsanto acetic acid process and hydroformylation.

Fine chemicals

Many fine chemicals are prepared via catalysis; methods include those of heavy industry as well as more specialized processes that would be prohibitively expensive on a large scale. Examples include the Heck reaction, and Friedel–Crafts reactions. Because most bioactive compounds are chiral, many pharmaceuticals are produced by enantioselective catalysis (catalytic asymmetric synthesis).(R)-1,2-Propandiol, precursor to the antibacterial levofloxacin, can be efficiently synthesized from hydroxyacetone using Noyori asymmetric hydrogenation:



Food processing

One of the most obvious applications of catalysis is the hydrogenation (reaction with hydrogen gas) of fats using nickel catalyst to produce margarine. Many other foodstuffs are prepared via biocatalysis (see below).

Environment

Catalysis impacts the environment by increasing the efficiency of industrial processes, but catalysis also plays a direct role in the environment. A notable example is the catalytic role of chlorinefree radicals in the breakdown of ozone. These radicals

are formed by the action of ultravioletradiation on chlorofluorocarbons (CFCs).

- $Cl^{\cdot} + O_3 \rightarrow ClO^{\cdot} + O_2$
- $ClO^{\cdot} + O^{\cdot} \rightarrow Cl^{\cdot} + O_2$

History

Generally speaking, anything that increases the rate of a process is a "catalyst", a term derived from Greekκαταλύειν, meaning "to annul," or "to untie," or "to pick up." The concept of catalysis was invented by chemist Elizabeth Fulhame and described in a 1794 book, based on her novel work in oxidation-reduction experiments. The first chemical reaction in organic chemistry that utilized a catalyst was studied in 1811 by Gottlieb Kirchhoff who discovered the acid-catalyzed conversion of starch to glucose. The term *catalysis* was later used by JönsJakob Berzelius in 1835 to describe reactions that are accelerated by substances that remain unchanged after the reaction. Fulhame, who predated Berzelius, did work with water as opposed to metals in her reduction experiments. Other 18th century chemists who worked in catalysis were EilhardMitscherlich who referred to it as *contact* processes, and Johann Wolfgang Döbereiner who spoke of *contact action.* He developed Döbereiner's lamp, a lighter based on hydrogen and a platinum sponge, which became a commercial success in the 1820s that lives on today. Humphry Davy discovered the use of platinum in catalysis. In the 1880s, Wilhelm Ostwald at Leipzig University started a systematic investigation into reactions that were catalyzed by the presence of acids and bases, and found that chemical reactions occur at finite rates

and that these rates can be used to determine the strengths of acids and bases. For this work, Ostwald was awarded the 1909 Nobel Prize in Chemistry.Vladimir Ipatieff performed some of the earliest industrial scale reactions, including the discovery and commercialization of oligomerization and the development of catalysts for hydrogenation.

Inhibitors, poisons, and promoters

An added substance which does reduce the reaction rate is a reaction inhibitor if reversible and catalyst poisons if irreversible. Promoters are substances that increase the catalytic activity, even though they are not catalysts by themselves.

Inhibitors are sometimes referred to as "negative catalysts" since they decrease the reaction rate. However the term inhibitor is preferred since they do not work by introducing a reaction path with higher activation energy; this would not reduce the rate since the reaction would continue to occur by the non-catalyzed path. Instead, they act either by deactivating catalysts, or by removing reaction intermediates such as free radicals. In heterogeneous catalysis, coking inhibits the catalyst, which becomes covered by polymeric side products.

The inhibitor may modify selectivity in addition to rate. For instance, in the reduction of alkynes to alkenes, a palladium (Pd) catalyst partly "poisoned" with lead(II) acetate ($Pb(CH_3CO_2)_2$) can be used. Without the deactivation of the catalyst, the alkene produced would be further reduced to alkane.

The inhibitor can produce this effect by, e.g., selectively poisoning only certain types of active sites. Another mechanism is the modification of surface geometry. For instance, in hydrogenation operations, large planes of metal surface function as sites of hydrogenolysis catalysis while sites catalyzing hydrogenation of unsaturates are smaller. Thus, a poison that covers the surface randomly will tend to reduce the number of uncontaminated large planes but leave proportionally more smaller sites free, thus changing the hydrogenation vs. hydrogenolysis selectivity. Many other mechanisms are also possible.

Promoters can cover up the surface to prevent production of a mat of coke, or even actively remove such material (e.g., rhenium on platinum in platforming). They can aid the dispersion of the catalytic material or bind to reagents.

Chapter 3

Biofuel

Biofuel is fuel that is produced through contemporary processes from biomass, rather than by the very slow geological processes involved in the formation of fossil fuels, such as oil. Since biomass technically can be used as a fuel directly (e.g. wood logs), some people use the terms biomass and biofuel interchangeably. More often than not, however, the word biomass simply denotes the biological raw material the fuel is made of, or some form of thermally/chemically altered *solid*end product, like torrefied pellets or briquettes.

The word biofuel is usually reserved for *liquid* or *gaseous* fuels, used for transportation. The U.S. Energy Information Administration (EIA) follows this naming practice. Drop-in biofuels are functionally equivalent to petroleum fuels and fully compatible with the existing petroleum infrastructure. They require no engine modification of the vehicle.

Biofuel can be produced from plants (i.e. energy crops), or from agricultural, commercial, domestic, and/or industrial wastes (if the waste has a biological origin). Biofuel generally involve contemporary carbon fixation, such as those that occur in plants or microalgae through the process of photosynthesis. The greenhouse gas mitigation potential of biofuel varies considerably, from emission levels comparable to fossil fuels in some scenarios to negative emissions in others. The IPCC (Intergovernmental Panel on Climate Change) defines bioenergy as a renewable form of energy.

The two most common types of biofuel are bioethanol and biodiesel.

- Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn, sugarcane, or sweet sorghum. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form (E100), but it is usually used as a gasolineadditive to increase octane and improve vehicle emissions. Bioethanol is widely used in the United States and in Brazil.
- Biodieselis produced from oils or fats using transesterification and is the most common biofuel in Europe. It can be used as a fuel for vehicles in its pure form (B100), but it is usually used as a diesel additive to reduce levels of particulates, carbon monoxide, and hydrocarbons from diesel-powered vehicles.

In 2019, worldwide biofuel production reached 161 billion liters (43 billion gallons US), up 6% from 2018, and biofuels provided 3% of the world's fuels for road transport. The International Energy Agency want biofuels to meet more than a quarter of world demand for transportation fuels by 2050, in order to reduce dependency on petroleum.

However, the production and consumption of biofuels are not on track to meet the IEA's sustainable development scenario. From 2020 to 2030 global biofuel output has to increase by

10% each year to reach IEA's goal. Only 3% growth annually is expected in the next 5 years.

Generations

First-generation biofuels

First-generation biofuels are fuels made from food crops grown on arable land. The crop's sugar, starch, or oil content is converted into biodiesel or ethanol, using transesterification, or yeast fermentation.

Second-generation biofuels

Second-generation biofuels are fuels made from lignocellulosic or woody biomass, or agricultural residues/waste. The feedstock used to make the fuels either grow on arable land but are byproducts of the main crop, or they are grown on marginal land. Second-generation feedstocks include straw, bagasse, perennial grasses, jatropha, waste vegetable oil, municipal solid waste and so forth.

Third-generation biofuels

Algae can be produced in ponds or tanks on land, and out at sea. Algal fuels have high yields, can be grown with minimal impact on fresh water resources, can be produced using saline water and wastewater, have a high ignition point, and are biodegradable and relatively harmless to the environment if spilled. Production requires large amounts of energy and fertilizer, the produced fuel degrades faster than other biofuels, and it does not flow well in cold temperatures. By

2017, due to economic considerations, most efforts to produce fuel from algae have been abandoned or changed to other applications.

Fourth-generation biofuels

This class of biofuels includes electrofuels and solar fuels. Electrofuelsare made by storing electrical energy in the chemical bonds of liquids and gases. The primary targets are butanol, biodiesel, and hydrogen, but include other alcohols and carbon-containing gases such as methane and butane. A solar fuel is a synthetic chemical fuel produced from solar energy. Light is converted to chemical energy, typically by reducing protons to hydrogen, or carbon dioxide to organic compounds.

Types

The following fuels can be produced using first, second, third or fourth-generation biofuel production procedures. Most of these can be produced using two or three of the different biofuel generation procedures.

Gaseous biofuel

Biogas and biomethane

Biogas is methane produced by the process of anaerobic digestion of organic material by anaerobes. It can be produced either from biodegradable waste materials or by the use of energy crops fed into anaerobic digesters to supplement gas

yields. The solid byproduct, digestate, can be used as a biofuel or a fertilizer. When CO_2 and other impurities are removed from biogas, it is called biomethane.

Biogas can be recovered from mechanical biological treatment waste processing systems. Landfill gas, a less clean form of biogas, is produced in landfills through naturally occurring anaerobic digestion. If it escapes into the atmosphere, it acts as a greenhouse gas.

Farmers can produce biogas from manure from their cattle by using anaerobic digesters.

Syngas

Syngas, a mixture of carbon monoxide, hydrogen and other hydrocarbons, is produced by partial combustion of biomass, that is, combustion with an amount of oxygen that is not sufficient to convert the biomass completely to carbon dioxide and water. Before partial combustion, the biomass is dried, and sometimes pyrolysed. The resulting gas mixture, syngas, is more efficient than direct combustion of the original biofuel; more of the energy contained in the fuel is extracted.

Syngas may be burned directly in internal combustion engines, turbines or high-temperature fuel cells. The wood gas generator, a wood-fueled gasification reactor, can be connected to an internal combustion engine.

Syngas can be used to produce methanol, DME and hydrogen, or converted via the Fischer–Tropsch process to produce a diesel substitute, or a mixture of alcohols that can be blended

into gasoline. Gasification normally relies on temperatures greater than 700 °C.

Lower-temperature gasification is desirable when co-producing biochar, but results in syngas polluted with tar.

Liquid biofuel

Ethanol

Biologically produced alcohols, most commonly ethanol, and less commonly propanol and butanol, are produced by the action of microorganisms and enzymes through the fermentation of sugars or starches (easiest), or cellulose (which is more difficult). Biobutanol (also called biogasoline) is often claimed to provide a direct replacement for gasoline, because it can be used directly in a gasoline engine.

Ethanol fuel is the most common biofuel worldwide, particularly in Brazil. Alcohol fuels are produced by fermentation of sugars derived from wheat, corn, sugar beets, sugar cane, molasses and any sugar or starch from which alcoholic beverages such as whiskey, can be made (such as potato and fruit waste, etc.). The ethanol production methods used are enzyme digestion (to release sugars from stored starches), fermentation of the sugars, distillation and drying. The distillation process requires significant energy input for heat (sometimes unsustainable natural gas fossil fuel, but cellulosic biomass such as bagasse, the waste left after sugar cane is pressed to extract its juice, is the most common fuel in Brazil, while pellets, wood chips and also waste heat are more common in Europe) Waste steam fuels ethanol factory – where

waste heat from the factories also is used in the district heating grid. Ethanol can be used in petrol engines as a replacement for gasoline; it can be mixed with gasoline to any percentage. Most existing car petrol engines can run on blends of up to 15% bioethanol with petroleum/gasoline. Ethanol has a smaller energy density than that of gasoline; this means it takes more fuel (volume and mass) to produce the same amount of work. An advantage of ethanol (CH

$_3$CH

$_2$OH) is that it has a higher octane rating than ethanol-free gasoline available at roadside gas stations, which allows an increase of an engine's compression ratio for increased thermal efficiency. In high-altitude (thin air) locations, some states mandate a mix of gasoline and ethanol as a winter oxidizer to reduce atmospheric pollution emissions.

Ethanol is also used to fuel bioethanol fireplaces. As they do not require a chimney and are "flueless", bioethanol fires are extremely useful for newly built homes and apartments without a flue. The downsides to these fireplaces is that their heat output is slightly less than electric heat or gas fires, and precautions must be taken to avoid carbon monoxide poisoning.

Corn-to-ethanol and other food stocks has led to the development of cellulosic ethanol. According to a joint research agenda conducted through the US Department of Energy, the fossil energy ratios (FER) for cellulosic ethanol, corn ethanol, and gasoline are 10.3, 1.36, and 0.81, respectively.

Ethanol has roughly one-third lower energy content per unit of volume compared to gasoline. This is partly counteracted by the better efficiency when using ethanol (in a long-term test of more than 2.1 million km, the BEST project found FFV vehicles to be 1–26% more energy efficient than petrol cars, but the volumetric consumption increases by approximately 30%, so more fuel stops are required).

Other bioalcohols

Methanolis currently produced from natural gas, a non-renewable fossil fuel. In the future it is hoped to be produced from biomass as biomethanol. This is technically feasible, but the production is currently being postponed for concerns that the economic viability is still pending. The methanol economy is an alternative to the hydrogen economy to be contrasted with today's hydrogen production from natural gas.

Butanol (C_4H_9OH) is formed by ABE fermentation (acetone, butanol, ethanol) and experimental modifications of the process show potentially high net energy gains with butanol as the only liquid product. Butanol will produce more energy than ethanol because of its lower oxygen content and allegedly can be burned "straight" in existing gasoline engines (without modification to the engine or car), and is less corrosive and less water-soluble than ethanol, and could be distributed via existing infrastructures. DuPont and BP are working together to help develop butanol.*Escherichia coli* strains have also been successfully engineered to produce butanol by modifying their amino acid metabolism. One drawback to butanol production in E. coli remains the high cost of nutrient rich media,

however, recent work has demonstrated E. coli can produce butanol with minimal nutritional supplementation.

Biodiesel

Biodiesel is the most common biofuel in Europe. It is produced from oils or fats using transesterification and is a liquid similar in composition to fossil/mineral diesel. Chemically, it consists mostly of fatty acid methyl (or ethyl) esters (FAMEs). Feedstocks for biodiesel include animal fats, vegetable oils, soy, rapeseed, jatropha, mahua, mustard, flax, sunflower, palm oil, hemp, field pennycress, *Pongamiapinnata* and algae. Pure biodiesel (B100, also known as "neat" biodiesel) currently reduces emissions with up to 60% compared to diesel Second generation B100. As of 2020, researchers at Australia's CSIRO have been studying safflower oil as an engine lubricant, and researchers at Montana State University's Advanced Fuels Center in the US have been studying the oil's performance in a large diesel engine, with results described as a "game-changer".

Biodiesel can be used in any diesel engine when mixed with mineral diesel. It can also be used in its pure form (B100) in diesel engines, but some maintenance and performance problems may then occur during wintertime utilization, since the fuel becomes somewhat more viscous at lower temperatures, depending on the feedstock used.

In some countries, manufacturers cover their diesel engines under warranty for B100 use, although Volkswagen of Germany, for example, asks drivers to check by telephone with the VW environmental services department before switching to

B100. In most cases, biodiesel is compatible with diesel engines from 1994 onwards, which use 'Viton' (by DuPont) synthetic rubber in their mechanical fuel injection systems. Note however, that no vehicles are certified for using pure biodiesel before 2014, as there was no emission control protocol available for biodiesel before this date.

Electronically controlled 'common rail' and 'Unit Injector' type systems from the late 1990s onwards may only use biodiesel blended with conventional diesel fuel. These engines have finely metered and atomized multiple-stage injection systems that are very sensitive to the viscosity of the fuel. Many current-generation diesel engines are made so that they can run on B100 without altering the engine itself, although this depends on the fuel rail design. Since biodiesel is an effective solvent and cleans residues deposited by mineral diesel, engine filters may need to be replaced more often, as the biofuel dissolves old deposits in the fuel tank and pipes. It also effectively cleans the engine combustion chamber of carbon deposits, helping to maintain efficiency. In many European countries, a 5% biodiesel blend is widely used and is available at thousands of gas stations. Biodiesel is also an oxygenated fuel, meaning it contains a reduced amount of carbon and higher hydrogen and oxygen content than fossil diesel. This improves the combustion of biodiesel and reduces the particulate emissions from unburnt carbon. However, using pure biodiesel may increase NO_x-emissions

Biodiesel is also safe to handle and transport because it is non-toxic and biodegradable, and has a high flash point of about 300 °F (148 °C) compared to petroleum diesel fuel, which has a flash point of 125 °F (52 °C).

In the US, more than 80% of commercial trucks and city buses run on diesel. The emerging US biodiesel market is estimated to have grown 200% from 2004 to 2005. "By the end of 2006 biodiesel production was estimated to increase fourfold [from 2004] to more than" 1 billion US gallons (3,800,000 m^3).

In France, biodiesel is incorporated at a rate of 8% in the fuel used by all French diesel vehicles.Avril Group produces under the brand Diester, a fifth of 11 million tons of biodiesel consumed annually by the European Union. It is the leading European producer of biodiesel.

Green diesel

Green diesel is produced through hydrocracking biological oil feedstocks, such as vegetable oils and animal fats. Hydrocracking is a refinery method that uses elevated temperatures and pressure in the presence of a catalyst to break down larger molecules, such as those found in vegetable oils, into shorter hydrocarbon chains used in diesel engines. It may also be called renewable diesel, hydrotreated vegetable oil (HVO fuel) or hydrogen-derived renewable diesel. Unlike biodiesel, green diesel has exactly the same chemical properties as petroleum-based diesel. It does not require new engines, pipelines or infrastructure to distribute and use, but has not been produced at a cost that is competitive with petroleum. Gasoline versions are also being developed. Green diesel is being developed in Louisiana and Singapore by ConocoPhillips, Neste Oil, Valero, Dynamic Fuels, and Honeywell UOP as well as Preem in Gothenburg, Sweden, creating what is known as Evolution Diesel.

Straight vegetable oil

Straight unmodified edible vegetable oil is generally not used as fuel, but lower-quality oil has been used for this purpose. Used vegetable oil is increasingly being processed into biodiesel, or (more rarely) cleaned of water and particulates and then used as a fuel.

As with 100% biodiesel (B100), to ensure the fuel injectors atomize the vegetable oil in the correct pattern for efficient combustion, vegetable oil fuelmust be heated to reduce its viscosity to that of diesel, either by electric coils or heat exchangers. This is easier in warm or temperate climates. MAN B&W Diesel, Wärtsilä, and Deutz AG, as well as a number of smaller companies, such as Elsbett, offer engines that are compatible with straight vegetable oil, without the need for after-market modifications.

Vegetable oil can also be used in many older diesel engines that do not use common rail or unit injection electronic diesel injection systems. Due to the design of the combustion chambers in indirect injection engines, these are the best engines for use with vegetable oil. This system allows the relatively larger oil molecules more time to burn. Some older engines, especially Mercedes, are driven experimentally by enthusiasts without any conversion. A handful of drivers have experienced limited success with earlier pre-"Pumpe Duse" VW TDI engines and other similar engines with direct injection. Several companies, such as Elsbett or Wolf, have developed professional conversion kits and successfully installed hundreds of them over the last decades.

Oils and fats can be hydrogenated to give a diesel substitute. The resulting product is a straight-chain hydrocarbon with a high cetane number, low in aromatics and sulfur and does not contain oxygen. Hydrogenated oilscan be blended with diesel in all proportions. They have several advantages over biodiesel, including good performance at low temperatures, no storage stability problems and no susceptibility to microbial attack.

Bioethers

Bioethers (also referred to as fuel ethers or oxygenated fuels) are cost-effective compounds that act as octane rating enhancers."Bioethers are produced by the reaction of reactive iso-olefins, such as iso-butylene, with bioethanol."Bioethers are created from wheat or sugar beets. They also enhance engine performance, while significantly reducing engine wear and toxicexhaust emissions. Although bioethers are likely to replace petroethers in the UK, it is highly unlikely they will become a fuel in and of itself due to the low energy density. By greatly reducing the amount of ground-level ozone emissions, they contribute to air quality.

When it comes to transportation fuel there are six ether additives: dimethyl ether (DME), diethyl ether (DEE), methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), and *tert*-amyl ethyl ether (TAEE).

The European Fuel Oxygenates Association (EFOA) identifies methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE) as the most commonly used ethers in fuel to replace lead. Ethers were introduced in Europe in the 1970s to replace the highly toxic compound. Although Europeans still use

bioether additives, the US no longer has an oxygenate requirement therefore bioethers are no longer used as the main fuel additive.

Biofuels and the environment

Carbon neutrality

A biofuel project is said to be carbon-neutral if the CO_2 absorbed by the crop compensate for the greenhouse gas (GHG) emissions related to the project. CO_2 is the most important of the greenhouse gases, and there is approximately 27% carbon in CO_2 (12/44). This includes any emissions caused by direct or indirect land use change. Many first generation biofuel projects are not carbon neutral given this definition. Some have even higher emissions than some fossil based alternatives.

It is the total amount of absorption and emissions that together determines if the GHG life cycle cost of a biofuel project is positive, neutral or negative. If emissions during production, processing, transport and combustion are higher than what is absorbed, both above and below ground during crop growth, the GHG life cycle cost is positive. Likewise, if total absorption is higher than total emissions, the life cycle cost is negative.

Whitaker et al. argue that a miscanthus crop with a yield of 10 tonnes per hectare per year sequesters so much carbon that the crop more than compensates for both farm operations emissions and transport emissions. (The emissions originating from *combustion* are fully absorbed by next seasons' above-

ground plant growth.) The top chart on the right displays two CO_2 negative miscanthus production pathways, and two CO_2 positive poplar production pathways, represented in gram CO_2-equivalents per megajoule. The bars are sequential and move up and down as atmospheric $CO_2$is estimated to increase and decrease. The grey/blue bars represent agriculture, processing and transport related emissions, the green bars represents soil carbon change, and the yellow diamonds represent total final emissions.

Successful sequestration is dependent on planting sites, as the best soils for sequestration are those that are currently low in carbon. The varied results displayed in the graph highlights this fact. For the UK, successful sequestration is expected for arable land over most of England and Wales, with unsuccessful sequestration expected in parts of Scotland, due to already carbon rich soils (existing woodland) plus lower yields. Soils already rich in carbon includes peatland and mature forest. Grassland can also be carbon rich, however Milner et al. argue that the most successful carbon sequestration in the UK takes place below improved grasslands. The bottom chart displays the estimated yield necessary to achieve CO_2 negativity for different levels of existing soil carbon saturation. The higher the yield, the more likely CO_2 negativity becomes.

Air pollution

In general, substance or energy is considered pollution when released into the environment at a rate faster than the environment can disperse, dilute, decompose, recycle, or store it in some harmless form. Based on this definition, both fossil fuels and some traditional biofuels are polluting the

environment. For instance, the IPCC argues that the traditional use of wood in cook stoves and open fires produces pollutants, which can lead to severe health and environmental consequences. However, a shift to modern bioenergy contribute to improved livelihoods and can reduce land degradation and impacts on ecosystem services. According to the IPCC, there is strong evidence that modern bioenergy has "large positive impacts" on air quality. When combusted in industrial facilities, most of the pollutants originating from woody biomass reduce by 97-99%, compared to open burning. A study of the giant brown haze that periodically covers large areas in South Asia determined that two thirds of it had been principally produced by residential cooking and agricultural burning, and one third by fossil-fuel burning.

Power production compared to other renewables

To calculate land use requirements for different kinds of power production, it is essential to know the relevant area-specific power densities. Smil estimates that the average area-specific power densities for biofuels, wind, hydro and solar power production are 0.30 W/m^2, 1 W/m^2, 3 W/m^2 and 5 W/m^2, respectively (power in the form of heat for biofuels, and electricity for wind, hydro and solar). The average human power consumption on ice-free land is 0.125 W/m^2 (heat and electricity combined), although rising to 20 W/m^2 in urban and industrial areas. The reason for the low area-specific power density for biofuels is a combination of low yields and only partial utilization of the plant when making liquid fuels (for instance, ethanol is typically made from sugarcane's sugar content or corn's starch content, while biodiesel is often made from rapeseed and soybean's oil content).

Smil estimates the following densities:

Ethanol

- Winter wheat (USA) 0.08 W/m^2
- Corn 0.26 W/m^2 (yield 10 t/ha) [77]
- Wheat (Germany) 0.30 W/m^2
- *Miscanthus x giganteus* 0.40 W/m^2 (yield 15 t/ha)
- Sugarcane (Brazil) 0.50 W/m^2 (yield 80 t/ha wet)

Jet fuel

- Soybean 0.06 W/m^2
- Jathropa (marginal land) 0.20 W/m^2
- Palm oil 0.65 W/m^2

Biodiesel

- Rapeseed 0.12 W/m^2 (EU average)
- Rapeseed (adjusted for energy input, the Netherlands) 0.08 W/m^2
- Sugar beets (adjusted for energy input, Spain) 0.02 W/m^2

Combusting solid biomass is more energy efficient than combusting biofuel (liquids), as the whole plant is utilized. For instance, corn plantations producing solid biomass for combustion generate more than double the amount of power per square metre compared to corn plantations producing for ethanol, when the yield is the same: 10 t/ha generates 0.60 W/m^2 and 0.26 W/m^2 respectively. Oven dry biomass in general have a calorific content of roughly 18 GJ/t, and every t/ha of

dry biomass yield increases a plantation's power production by 0.06 W/m^2.

As mentioned above, Smil estimates that the world average for wind, hydro and solar power production is 1 W/m^2, 3 W/m^2 and 5 W/m^2 respectively. In order to match these power densities, plantation yields must reach 17 t/ha, 50 t/ha and 83 t/ha for wind, hydro and solar respectively. This seems achievable for tropical plantations – Smil estimate that large scale plantations with eucalyptus, acacia, leucaena, pinus and dalbergia in tropical and subtropical regions yield 20–25 t/ha, equivalent to 1.20–1.50 W/m^2. It also seems achievable for elephant grasses, e.g. miscanthus (10–40 t/ha, or 0.6–2.4 W/m^2), and napier (15–80 t/ha, or 0.9–4.8 W/m^2), but unlikely for forest and many other types of biomass crops – Smil'sestimate for natural temperate mixed forests is 1.5–2 dry tonnes per hectare (2–2,5 m^3, equivalent to 0.1 W/m^2), ranging from 0.9 m3 in Greece to 6 m^3 in France).

Chapter 4

Industrial Applications

Detergent enzymes

Detergent enzymes are biological enzymes that are used with detergents. They catalyze the reaction between stains and the water solution, thus aiding stain removal and improving efficiency.Laundry detergent enzymes are the largest application of industrial enzymes.

They can be a part of both liquid and powder detergents.

History

Otto Röhm introduced the use of enzymes in detergent by using trypsin extracted from the tissues of slaughtered animals. Röhm's formula, though more successful than German household cleaning methods, was considered unstable when used with alkali and bleach. In 1959 yields were improved by microbial synthesis of proteases.

Properties

Laundry enzymes must be able to function normally in a wide array of conditions: water temperatures ranging from 0 to 60 °C; alkaline and acidic environments; solutions with high ionic strength; and the presence of surfactants or oxidizing agents.

Types

The five classes of enzymes found in laundry detergent include proteases, amylases, lipases, cellulases, and mannanases. They break down proteins (e.g. in blood and egg stains), starch, fats, cellulose (e.g. in vegetable puree), and mannans (e.g. in bean gum stains) respectively.

Merits

Household energy savings

For stain removal, conventional household washing machines use heated water, as this increases the solubility of stains. However, heating the water to the required temperature uses a considerable amount of energy; energy usage can be reduced by using detergent enzymes which perform well in cold water, allowing low-temperature washes and removing the need for heated water.

Delicate materials

Clothes made of delicate materials such as wool and silk can be damaged in high-temperature washes, and jeans and denim can fade due to their dark dyes. Low-temperature washes with detergent enzymes can prevent this damage, meaning that consumers can buy clothes from a wider range of materials without worrying about damaging them during washing.

Leather manufacturing

The leather industry was historically considered noxious due to the leather-making process. The traditional procedure involved soaking animal hides in a mixture of urine and lime to remove unwanted hairs, flesh and fat, then kneading them in dog or pigeon feces with bare feet. The subsequent discharge and refuse disposal was severely hazardous to both human health and the environment because of the high amounts of concentrated sulfide and chromium in the effluence.

This method was eventually discarded by the industry in the early 20th century following Röhm's discovery, replaced by a more eco-friendly process involving detergent enzymes. Consequently, hazardous sodium sulfide (used to remove animal hair from hides) usage is lessened by 60%, while water usage for soaking and hair cutting is lowered by 25%. Additionally, toxic pollution and emissions have been reduced by 30%. These enzymes have never completely substituted the industrial chemicals. Nevertheless, the working conditions, wastewater quality, and processing times have been greatly improved.

Replacement for phosphate and synthetic surfactants

Increased legislation has led to a limit on the laundry detergent industry's use of environmentally-unfriendly synthetic surfactants and phosphate salts. In a bid to produce more environmentally-friendly products, several detergent manufacturers have increased their use of enzymes in the production process in combination with lower concentrations of the sufactants and phosphates. These biologically active

enzymes include bacteria, yeast, and mushrooms, which produce less chemical pollution and decompose certain toxicants.

Public concerns

Damage to delicate materials

In contrast to the benefits of low-temperature washing, a study of the effects of detergent enzymes on untreated knit and woolen fabrics showed damage proportional to both soaking time and the enzyme concentration.

Skin allergy and testing

Consumers' responses to detergent enzymes have varied. It is reported that some Philippine consumers who are used to laundering by hand slightly suffered from powder detergents, which mainly consisted of laundry enzyme formulations. As a result, it was thought that laundry enzymes have the potential to increase the likelihood of getting occupational type 1 allergic responses. However, a large-scale skin prick test (SPT) containing 15,765 volunteers with 8 different types of detergent enzymes found that the allergy reaction is extremely rare among the public, with only 0.23% showing a reaction. The issue in Filipino consumers is believed to be the rushed hand-laundering method. After various tests with several volunteers worldwide, it is found that exposure to laundry enzymes leads to neither skin allergy (Type I sensitization) nor skin erosion.

Brewing

Brewing is the production of beer by steeping a starch source (commonly cereal grains, the most popular of which is barley) in water and fermenting the resulting sweet liquid with yeast. It may be done in a brewery by a commercial brewer, at home by a homebrewer, or communally. Brewing has taken place since around the 6th millennium BC, and archaeological evidence suggests that emerging civilizations, including ancient Egypt and Mesopotamia, brewed beer. Since the nineteenth century the brewing industry has been part of most western economies.

The basic ingredients of beer are water and a fermentable starch source such as malted barley. Most beer is fermented with a brewer's yeast and flavoured with hops. Less widely used starch sources include millet, sorghum and cassava. Secondary sources (adjuncts), such as maize (corn), rice, or sugar, may also be used, sometimes to reduce cost, or to add a feature, such as adding wheat to aid in retaining the foamy head of the beer. The most common starch source is ground cereal or "grist" - the proportion of the starch or cereal ingredients in a beer recipe may be called grist, grain bill, or simply mash ingredients.

Steps in the brewing process include malting, milling, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging. There are three main fermentation methods, warm, cool and spontaneous. Fermentation may take place in an open or closed fermenting vessel; a secondary fermentation may also occur in the cask or bottle. There are several

additional brewing methods, such as Burtonisation, barrel-ageing, double dropping, and Yorkshire Square.

History

Brewing has taken place since around the 6th millennium BC, and archaeological evidence suggests emerging civilizations including ancient Egypt and Mesopotamia brewed beer. Descriptions of various beer recipes can be found in cuneiform (the oldest known writing) from ancient Mesopotamia. In Mesopotamia the brewer's craft was the only profession which derived social sanction and divine protection from female deities/goddesses, specifically: Ninkasi, who covered the production of beer, Siris, who was used in a metonymic way to refer to beer, and Siduri, who covered the enjoyment of beer. In pre-industrial times, and in developing countries, women are frequently the main brewers.

As almost any cereal containing certain sugars can undergo spontaneous fermentation due to wild yeasts in the air, it is possible that beer-like beverages were independently developed throughout the world soon after a tribe or culture had domesticated cereal. Chemical tests of ancient pottery jars reveal that beer was produced as far back as about 7,000 years ago in what is today Iran. This discovery reveals one of the earliest known uses of fermentation and is the earliest evidence of brewing to date. In Mesopotamia, the oldest evidence of beer is believed to be a 6,000-year-old Sumerian tablet depicting people drinking a beverage through reed straws from a communal bowl. A 3900-year-old Sumerian poem honouringNinkasi, the patron goddess of brewing, contains the oldest surviving beer recipe, describing the production of beer

from barley via bread. The invention of bread and beer has been argued to be responsible for humanity's ability to develop technology and build civilization. The earliest chemically confirmed barley beer to date was discovered at Godin Tepe in the central Zagros Mountains of Iran, where fragments of a jug, at least 5,000 years old was found to be coated with beerstone, a by-product of the brewing process. Beer may have been known in Neolithic Europeas far back as 5,000 years ago, and was mainly brewed on a domestic scale.

Ale produced before the Industrial Revolution continued to be made and sold on a domestic scale, although by the 7th century AD beer was also being produced and sold by European monasteries. During the Industrial Revolution, the production of beer moved from artisanal manufacture to industrial manufacture, and domestic manufacture ceased to be significant by the end of the 19th century. The development of hydrometers and thermometers changed brewing by allowing the brewer more control of the process, and greater knowledge of the results. Today, the brewing industry is a global business, consisting of several dominant multinational companies and many thousands of smaller producers ranging from brewpubs to regional breweries. More than 133 billion litres (35 billion gallons) are sold per year—producing total global revenues of $294.5 billion (£147.7 billion) in 2006.

Ingredients

The basic ingredients of beer are water; a starch source, such as malted barley, able to be fermented (converted into alcohol); a brewer's yeast to produce the fermentation; and a flavouring, such as hops, to offset the sweetness of the malt. A mixture of

starch sources may be used, with a secondary saccharide, such as maize (corn), rice, or sugar, these often being termed adjuncts, especially when used as a lower-cost substitute for malted barley. Less widely used starch sources include millet, sorghum, and cassava root in Africa, potato in Brazil, and agave in Mexico, among others. The most common starch source is ground cereal or "grist" - the proportion of the starch or cereal ingredients in a beer recipe may be called grist, grain bill, or simply mash ingredients.

Water

Beer is composed mostly of water. Regions have water with different mineral components; as a result, different regions were originally better suited to making certain types of beer, thus giving them a regional character. For example, Dublin has hard water well suited to making stout, such as Guinness; while Pilsen has soft water well suited to making pale lager, such as Pilsner Urquell. The waters of Burton in England contain gypsum, which benefits making pale ale to such a degree that brewers of pale ales will add gypsum to the local water in a process known as Burtonisation.

Starch source

The starch source in a beer provides the fermentable material and is a key determinant of the strength and flavour of the beer. The most common starch source used in beer is malted grain. Grain is malted by soaking it in water, allowing it to begin germination, and then drying the partially germinated grain in a kiln. Malting grain produces enzymes that will allow conversion from starches in the grain into fermentable sugars

during the mash process. Different roasting times and temperatures are used to produce different colours of malt from the same grain. Darker malts will produce darker beers.

Nearly all beer includes barley malt as the majority of the starch. This is because of its fibrous husk, which is important not only in the sparging stage of brewing (in which water is washed over the mashed barley grains to form the wort) but also as a rich source of amylase, a digestive enzyme that facilitates conversion of starch into sugars. Other malted and unmalted grains (including wheat, rice, oats, and rye, and, less frequently, maize (corn) and sorghum) may be used. In recent years, a few brewers have produced gluten-free beer made with sorghum with no barley malt for people who cannot digest gluten-containing grains like wheat, barley, and rye.

Hops

Hops are the female flower clusters or seed cones of the hop vine *Humuluslupulus*, which are used as a flavouring and preservative agent in nearly all beer made today. Hops had been used for medicinal and food flavouring purposes since Roman times; by the 7th century in Carolingian monasteries in what is now Germany, beer was being made with hops, though it isn't until the thirteenth century that widespread cultivation of hops for use in beer is recorded. Before the thirteenth century, beer was flavoured with plants such as yarrow, wild rosemary, and bog myrtle, and other ingredients such as juniper berries, aniseed and ginger, which would be combined into a mixture known as gruit and used as hops are now used; between the thirteenth and the sixteenth century, during which hops took over as the dominant flavouring, beer

flavoured with gruit was known as ale, while beer flavoured with hops was known as beer. Some beers today, such as *Fraoch* by the Scottish Heather Ales company and *Cervoise Lancelot* by the French Brasserie-Lancelot company, use plants other than hops for flavouring.

Hops contain several characteristics that brewers desire in beer: they contribute a bitterness that balances the sweetness of the malt; they provide floral, citrus, and herbal aromas and flavours; they have an antibiotic effect that favours the activity of brewer's yeast over less desirable microorganisms; and they aid in "head retention", the length of time that the foam on top of the beer (the beer head) will last. The preservative in hops comes from the lupulin glands which contain soft resins with alpha and beta acids. Though much studied, the preservative nature of the soft resins is not yet fully understood, though it has been observed that unless stored at a cool temperature, the preservative nature will decrease. Brewing is the sole major commercial use of hops.

Yeast

Yeast is the microorganism that is responsible for fermentation in beer. Yeast metabolises the sugars extracted from grains, which produces alcohol and carbon dioxide, and thereby turns wort into beer. In addition to fermenting the beer, yeast influences the character and flavour. The dominant types of yeast used to make beer are *Saccharomyces cerevisiae*, known as ale yeast, and *Saccharomyces pastorianus*, known as lager yeast; *Brettanomyces* ferments lambics, and *Torulasporadelbrueckii* ferments Bavarian weissbier. Before the role of yeast in fermentation was understood, fermentation

involved wild or airborne yeasts, and a few styles such as lambics still use this method today.Emil Christian Hansen, a Danish biochemist employed by the Carlsberg Laboratory, developed pure yeast cultures which were introduced into the Carlsberg brewery in 1883, and pure yeast strains are now the main fermenting source used worldwide.

- Clarifying agent

Some brewers add one or more clarifying agents to beer, which typically precipitate (collect as a solid) out of the beer along with protein solids and are found only in trace amounts in the finished product. This process makes the beer appear bright and clean, rather than the cloudy appearance of ethnic and older styles of beer such as wheat beers.

Examples of clarifying agents include isinglass, obtained from swim bladders of fish; Irish moss, a seaweed; kappa carrageenan, from the seaweed *kappaphycus*; polyclar (a commercial brand of clarifier); and gelatin. If a beer is marked "suitable for Vegans", it was generally clarified either with seaweed or with artificial agents, although the "Fast Cask" method invented by Marston's in 2009 may provide another method.

Brewing process

There are several steps in the brewing process, which may include malting, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging. The brewing equipment needed to make beer has grown more sophisticated over time, and now covers most aspects of the brewing process.

Malting is the process where barley grain is made ready for brewing. Malting is broken down into three steps in order to help to release the starches in the barley. First, during steeping, the grain is added to a vat with water and allowed to soak for approximately 40 hours. During germination, the grain is spread out on the floor of the germination room for around 5 days. The final part of malting is kilning when the malt goes through a very high temperature drying in a kiln; with gradual temperature increase over several hours. When kilning is complete, the grains are now termed malt, and they will be milled or crushed to break apart the kernels and expose the cotyledon, which contains the majority of the carbohydrates and sugars; this makes it easier to extract the sugars during mashing.

Mashingconverts the starches released during the malting stage into sugars that can be fermented. The milled grain is mixed with hot water in a large vessel known as a mash tun. In this vessel, the grain and water are mixed together to create a cereal mash. During the mash, naturally occurring enzymes present in the malt convert the starches (long chain carbohydrates) in the grain into smaller molecules or simple sugars (mono-, di-, and tri-saccharides). This "conversion" is called saccharificationwhich occurs between the temperatures 60–70 °C (140–158 °F). The result of the mashing process is a sugar-rich liquid or "wort", which is then strained through the bottom of the mash tun in a process known as lautering. Prior to lautering, the mash temperature may be raised to about 75–78 °C (167–172 °F) (known as a mashout) to free up more starch and reduce mash viscosity. Additional water may be sprinkled on the grains to extract additional sugars (a process known as sparging).

The wort is moved into a large tank known as a "copper" or kettle where it is boiled with hops and sometimes other ingredients such as herbs or sugars. This stage is where many chemical reactions take place, and where important decisions about the flavour, colour, and aroma of the beer are made. The boiling process serves to terminate enzymatic processes, precipitate proteins, isomerize hop resins, and concentrate and sterilize the wort. Hops add flavour, aroma and bitterness to the beer. At the end of the boil, the hopped wort settles to clarify in a vessel called a "whirlpool", where the more solid particles in the wort are separated out.

After the whirlpool, the wort is drawn away from the compacted hop trub, and rapidly cooled via a heat exchanger to a temperature where yeast can be added. A variety of heat exchanger designs are used in breweries, with the most common a plate-style. Water or glycol run in channels in the opposite direction of the wort, causing a rapid drop in temperature. It is very important to quickly cool the wort to a level where yeast can be added safely as yeast is unable to grow in very high temperatures, and will start to die in temperatures above 60 °C (140 °F). After the wort goes through the heat exchanger, the cooled wort goes into a fermentation tank. A type of yeast is selected and added, or "pitched", to the fermentation tank. When the yeast is added to the wort, the fermenting process begins, where the sugars turn into alcohol, carbon dioxide and other components. When the fermentation is complete the brewer may rack the beer into a new tank, called a conditioning tank. Conditioning of the beer is the process in which the beer ages, the flavour becomes smoother, and flavours that are unwanted dissipate. After conditioning

for a week to several months, the beer may be filtered and force carbonated for bottling, or fined in the cask.

Mashing

Mashing is the process of combining a mix of milled grain (typically maltedbarley with supplementary grains such as corn, sorghum, rye or wheat), known as the "grist" or "grain bill", and water, known as "liquor", and heating this mixture in a vessel called a "mash tun". Mashing is a form of steeping, and defines the act of brewing, such as with making tea, sake, and soy sauce. Technically, wine, cider and mead are not brewed but rather vinified, as there is no steeping process involving solids. Mashing allows the enzymes in the malt to break down the starch in the grain into sugars, typically maltose to create a malty liquid called wort. There are two main methods – infusion mashing, in which the grains are heated in one vessel; and decoction mashing, in which a proportion of the grains are boiled and then returned to the mash, raising the temperature. Mashing involves pauses at certain temperatures (notably 45–62–73 °C or 113–144–163 °F), and takes place in a "mash tun" – an insulated brewing vessel with a false bottom. The end product of mashing is called a "mash".

Mashing usually takes 1 to 2 hours, and during this time the various temperature rests activate different enzymes depending upon the type of malt being used, its modification level, and the intention of the brewer. The activity of these enzymes convert the starches of the grains to dextrins and then to fermentable sugars such as maltose. A mash rest from 49–55 °C (120–131 °F) activates various proteases, which break

down proteins that might otherwise cause the beer to be hazy. This rest is generally used only with undermodified (i.e. undermalted) malts which are decreasingly popular in Germany and the Czech Republic, or non-malted grains such as corn and rice, which are widely used in North American beers. A mash rest at 60 °C (140 °F) activates β-glucanase, which breaks down gummy β-glucans in the mash, making the sugars flow out more freely later in the process. In the modern mashing process, commercial fungal basedβ-glucanase may be added as a supplement. Finally, a mash rest temperature of 65–71 °C (149–160 °F) is used to convert the starches in the malt to sugar, which is then usable by the yeast later in the brewing process. Doing the latter rest at the lower end of the range favoursβ-amylase enzymes, producing more low-order sugars like maltotriose, maltose, and glucose which are more fermentable by the yeast. This in turn creates a beer lower in body and higher in alcohol. A rest closer to the higher end of the range favoursα-amylase enzymes, creating more higher-order sugars and dextrins which are less fermentable by the yeast, so a fuller-bodied beer with less alcohol is the result. Duration and pH variances also affect the sugar composition of the resulting wort.

Lautering

Lautering is the separation of the wort (the liquid containing the sugar extracted during mashing) from the grains. This is done either in a mash tun outfitted with a false bottom, in a lautertun, or in a mash filter. Most separation processes have two stages: first wort run-off, during which the extract is separated in an undiluted state from the spent grains, and sparging, in which extract which remains with the grains is

rinsed off with hot water. The lautertun is a tank with holes in the bottom small enough to hold back the large bits of grist and hulls (the ground or milled cereal). The bed of grist that settles on it is the actual filter. Some lautertuns have provision for rotating rakes or knives to cut into the bed of grist to maintain good flow. The knives can be turned so they push the grain, a feature used to drive the spent grain out of the vessel. The mash filter is a plate-and-frame filter. The empty frames contain the mash, including the spent grains, and have a capacity of around one hectoliter. The plates contain a support structure for the filter cloth. The plates, frames, and filter cloths are arranged in a carrier frame like so: frame, cloth, plate, cloth, with plates at each end of the structure. Newer mash filters have bladders that can press the liquid out of the grains between spargings. The grain does not act like a filtration medium in a mash filter.

Boiling

After mashing, the beer wort is boiled with hops (and other flavourings if used) in a large tank known as a "copper" or brew kettle – though historically the mash vessel was used and is still in some small breweries. The boiling process is where chemical reactions take place, including sterilization of the wort to remove unwanted bacteria, releasing of hop flavours, bitterness and aroma compounds through isomerization, stopping of enzymatic processes, precipitation of proteins, and concentration of the wort. Finally, the vapours produced during the boil volatiliseoff-flavours, including dimethyl sulfide precursors. The boil is conducted so that it is even and intense – a continuous "rolling boil". The boil on average lasts between

45 and 90 minutes, depending on its intensity, the hop addition schedule, and volume of water the brewer expects to evaporate. At the end of the boil, solid particles in the hopped wort are separated out, usually in a vessel called a "whirlpool".

Brew kettle or copper

Copper is the traditional material for the boiling vessel, because copper transfers heat quickly and evenly, and because the bubbles produced during boiling, and which would act as an insulator against the heat, do not cling to the surface of copper, so the wort is heated in a consistent manner. The simplest boil kettles are direct-fired, with a burner underneath. These can produce a vigorous and favourable boil, but are also apt to scorch the wort where the flame touches the kettle, causing caramelisation and making cleanup difficult. Most breweries use a steam-fired kettle, which uses steam jackets in the kettle to boil the wort. Breweries usually have a boiling unit either inside or outside of the kettle, usually a tall, thin cylinder with vertical tubes, called a calandria, through which wort is pumped.

Whirlpool

At the end of the boil, solid particles in the hopped wort are separated out, usually in a vessel called a "whirlpool" or "settling tank". The whirlpool was devised by Henry RanulphHudston while working for the Molson Brewery in 1960 to utilise the so-called tea leaf paradox to force the denser solids known as "trub" (coagulated proteins, vegetable matter from hops) into a cone in the centre of the whirlpool tank. Whirlpool systems vary: smaller breweries tend to use the brew

kettle, larger breweries use a separate tank, and design will differ, with tank floors either flat, sloped, conical or with a cup in the centre. The principle in all is that by swirling the wort the centripetal force will push the trub into a cone at the centre of the bottom of the tank, where it can be easily removed.

Hopback

A hopback is a traditional additional chamber that acts as a sieve or filter by using whole hops to clear debris (or "trub") from the unfermented (or "green") wort, as the whirlpool does, and also to increase hop aroma in the finished beer.

It is a chamber between the brewing kettle and wort chiller. Hops are added to the chamber, the hot wort from the kettle is run through it, and then immediately cooled in the wort chiller before entering the fermentation chamber.

Hopbacks utilizing a sealed chamber facilitate maximum retention of volatile hop aroma compounds that would normally be driven off when the hops contact the hot wort. While a hopback has a similar filtering effect as a whirlpool, it operates differently: a whirlpool uses centrifugal forces, a hopback uses a layer of whole hops to act as a filter bed. Furthermore, while a whirlpool is useful only for the removal of pelleted hops (as flowers do not tend to separate as easily), in general hopbacks are used only for the removal of whole flower hops (as the particles left by pellets tend to make it through the hopback). The hopback has mainly been substituted in modern breweries by the whirlpool.

Wort cooling

After the whirlpool, the wort must be brought down to fermentation temperatures 20–26 °C (68–79 °F) before yeast is added. In modern breweries this is achieved through a plate heat exchanger. A plate heat exchanger has many ridged plates, which form two separate paths. The wortis pumped into the heat exchanger, and goes through every other gap between the plates. The cooling medium, usually water, goes through the other gaps. The ridges in the plates ensure turbulent flow. A good heat exchanger can drop 95 °C (203 °F) wort to 20 °C (68 °F) while warming the cooling medium from about 10 °C (50 °F) to 80 °C (176 °F). The last few plates often use a cooling medium which can be cooled to below the freezing point, which allows a finer control over the wort-out temperature, and also enables cooling to around 10 °C (50 °F). After cooling, oxygen is often dissolved into the wort to revitalize the yeast and aid its reproduction. Some of the craft brewery, particularly those wanting to create steam beer, utilize coolship instead.

While boiling, it is useful to recover some of the energy used to boil the wort. On its way out of the brewery, the steam created during the boil is passed over a coil through which unheated water flows. By adjusting the rate of flow, the output temperature of the water can be controlled. This is also often done using a plate heat exchanger. The water is then stored for later use in the next mash, in equipment cleaning, or wherever necessary. Another common method of energy recovery takes place during the wort cooling. When cold water is used to cool the wort in a heat exchanger, the water is significantly warmed. In an efficient brewery, cold water is passed through the heat exchanger at a rate set to maximize the water's

temperature upon exiting. This now-hot water is then stored in a hot water tank.

Fermenting

Fermentation takes place in fermentation vessels which come in various forms, from enormous cylindroconical vessels, through open stone vessels, to wooden vats. After the wort is cooled and aerated – usually with sterile air – yeast is added to it, and it begins to ferment. It is during this stage that sugars won from the maltare converted into alcohol and carbon dioxide, and the product can be called beer for the first time.

Most breweries today use cylindroconical vessels, or CCVs, which have a conical bottom and a cylindrical top. The cone's angle is typically around 60°, an angle that will allow the yeast to flow towards the cone's apex, but is not so steep as to take up too much vertical space. CCVs can handle both fermenting and conditioning in the same tank. At the end of fermentation, the yeast and other solids which have fallen to the cone's apex can be simply flushed out of a port at the apex. Open fermentation vessels are also used, often for show in brewpubs, and in Europe in wheat beer fermentation. These vessels have no tops, which makes harvesting top-fermenting yeasts very easy. The open tops of the vessels make the risk of infection greater, but with proper cleaning procedures and careful protocol about who enters fermentation chambers, the risk can be well controlled. Fermentation tanks are typically made of stainless steel. If they are simple cylindrical tanks with beveled ends, they are arranged vertically, as opposed to conditioning tanks which are usually laid out horizontally. Only a very few breweries still use wooden vats for

fermentation as wood is difficult to keep clean and infection-free and must be repitched more or less yearly.

Fermentation methods

There are three main fermentation methods, warm, cool and wild or spontaneous. Fermentation may take place in open or closed vessels. There may be a secondary fermentation which can take place in the brewery, in the cask or in the bottle.

Brewing yeasts are traditionally classed as "top-cropping" (or "top-fermenting") and "bottom-cropping" (or "bottom-fermenting"); the yeasts classed as top-fermenting are generally used in warm fermentations, where they ferment quickly, and the yeasts classed as bottom-fermenting are used in cooler fermentations where they ferment more slowly. Yeast were termed top or bottom cropping, because the yeast was collected from the top or bottom of the fermenting wort to be reused for the next brew. This terminology is somewhat inappropriate in the modern era; after the widespread application of brewing mycology it was discovered that the two separate collecting methods involved two different yeast species that favoured different temperature regimes, namely *Saccharomyces cerevisiae* in top-cropping at warmer temperatures and *Saccharomyces pastorianus* in bottom-cropping at cooler temperatures. As brewing methods changed in the 20th century, cylindro-conical fermenting vessels became the norm and the collection of yeast for both *Saccharomyces* species is done from the bottom of the fermenter.Thus the method of collection no longer implies a species association. There are a few remaining breweries who collect yeast in the top-cropping method, such as Samuel

Smiths brewery in Yorkshire, Marstons in Staffordshire and several German hefeweizen producers.

For both types, yeast is fully distributed through the beer while it is fermenting, and both equally flocculate (clump together and precipitate to the bottom of the vessel) when fermentation is finished. By no means do all top-cropping yeasts demonstrate this behaviour, but it features strongly in many English yeasts that may also exhibit chain forming (the failure of budded cells to break from the mother cell), which is in the technical sense different from true flocculation. The most common top-cropping brewer's yeast, *Saccharomyces cerevisiae*, is the same species as the common baking yeast. However, baking and brewing yeasts typically belong to different strains, cultivated to favour different characteristics: baking yeast strains are more aggressive, in order to carbonate dough in the shortest amount of time; brewing yeast strains act slower, but tend to tolerate higher alcohol concentrations (normally 12–15% abv is the maximum, though under special treatment some ethanol-tolerant strains can be coaxed up to around 20%). Modern quantitative genomics has revealed the complexity of *Saccharomyces* species to the extent that yeasts involved in beer and wine production commonly involve hybrids of so-called pure species. As such, the yeasts involved in what has been typically called top-cropping or top-fermenting ale may be both *Saccharomyces cerevisiae* and complex hybrids of *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*. Three notable ales, Chimay, Orval and Westmalle, are fermented with these hybrid strains, which are identical to wine yeasts from Switzerland.

Warm fermentation

In general, yeasts such as *Saccharomyces cerevisiae* are fermented at warm temperatures between 15 and 20 °C (59 and 68 °F), occasionally as high as 24 °C (75 °F), while the yeast used by Brasserie Dupont for saison ferments even higher at 29 to 35 °C (84 to 95 °F). They generally form a foam on the surface of the fermenting beer, which is called barm, as during the fermentation process its hydrophobic surface causes the flocs to adhere to CO_2 and rise; because of this, they are often referred to as "top-cropping" or "top-fermenting" – though this distinction is less clear in modern brewing with the use of cylindro-conical tanks. Generally, warm-fermented beers, which are usually termed ale, are ready to drink within three weeks after the beginning of fermentation, although some brewers will condition or mature them for several months.

Cool fermentation

When a beer has been brewed using a cool fermentation of around 10 °C (50 °F), compared to typical warm fermentation temperatures of 18 °C (64 °F), then stored (or lagered) for typically several weeks (or months) at temperatures close to freezing point, it is termed a "lager". During the lagering or storage phase several flavour components developed during fermentation dissipate, resulting in a "cleaner" flavour. Though it is the slow, cool fermentation and cold conditioning (or lagering) that defines the character of lager, the main technical difference is with the yeast generally used, which is *Saccharomyces pastorianus*. Technical differences include the ability of lager yeast to metabolize melibiose, and the tendency to settle at the bottom of the fermenter (though ales yeasts can

also become bottom settling by selection); though these technical differences are not considered by scientists to be influential in the character or flavour of the finished beer, brewers feel otherwise - sometimes cultivating their own yeast strains which may suit their brewing equipment or for a particular purpose, such as brewing beers with a high abv.

Brewers in Bavariahad for centuries been selecting cold-fermenting yeasts by storing ("lagern") their beers in cold alpine caves. The process of natural selection meant that the wild yeasts that were most cold tolerant would be the ones that would remain actively fermenting in the beer that was stored in the caves. A sample of these Bavarian yeasts was sent from the Spaten brewery in Munich to the Carlsberg brewery in Copenhagen in 1845 who began brewing with it. In 1883 Emile Hansen completed a study on pure yeast culture isolation and the pure strain obtained from Spaten went into industrial production in 1884 as Carlsberg yeast No 1. Another specialized pure yeast production plant was installed at the Heineken Brewery in Rotterdam the following year and together they began the supply of pure cultured yeast to brewers across Europe. This yeast strain was originally classified as *Saccharomyces carlsbergensis*, a now defunct species name which has been superseded by the currently accepted taxonomic classification *Saccharomyces pastorianus*.

Spontaneous fermentation

Lambic beers are historically brewed in Brussels and the nearby Pajottenland region of Belgium without any yeast inoculation. The wort is cooled in open vats (called "coolships"), where the yeasts and microbiota present in the

brewery (such as *Brettanomyces*) are allowed to settle to create a spontaneous fermentation, and are then conditioned or matured in oak barrels for typically one to three years.

Conditioning

After an initial or primary fermentation, beer is *conditioned*, matured or aged, in one of several ways, which can take from 2 to 4 weeks, several months, or several years, depending on the brewer's intention for the beer. The beer is usually transferred into a second container, so that it is no longer exposed to the dead yeast and other debris (also known as "trub") that have settled to the bottom of the primary fermenter. This prevents the formation of unwanted flavours and harmful compounds such as acetaldehyde.

- Kräusening

Kräusening is a conditioning method in which fermenting wort is added to the finished beer. The active yeast will restart fermentation in the finished beer, and so introduce fresh carbon dioxide; the conditioning tank will be then sealed so that the carbon dioxide is dissolved into the beer producing a lively "condition" or level of carbonation. The kräusening method may also be used to condition bottled beer.

- Lagering

Lagers are stored at cellar temperature or below for 1–6 months while still on the yeast. The process of storing, or conditioning, or maturing, or aging a beer at a low temperature for a long period is called "lagering", and while it is associated

with lagers, the process may also be done with ales, with the same result – that of cleaning up various chemicals, acids and compounds.

- Secondary fermentation

During secondary fermentation, most of the remaining yeast will settle to the bottom of the second fermenter, yielding a less hazy product.

- Bottle fermentation

Some beers undergo an additional fermentation in the bottle giving natural carbonation. This may be a second and/or third fermentation. They are bottled with a viable yeast population in suspension. If there is no residual fermentable sugar left, sugar or wort or both may be added in a process known as priming. The resulting fermentation generates CO_2 that is trapped in the bottle, remaining in solution and providing natural carbonation. Bottle-conditioned beers may be either filled unfiltered direct from the fermentation or conditioning tank, or filtered and then reseeded with yeast.

- Cask conditioning

Cask ale (or cask-conditioned beer) is unfiltered, unpasteurised beer that is conditioned by a secondary fermentation in a metal, plastic or wooden cask. It is dispensed from the cask by being either poured from a tap by gravity, or pumped up from a cellar via a beer engine (hand pump). Sometimes a cask breather is used to keep the beer fresh by allowing carbon dioxide to replace oxygen as the beer is drawn off the cask. Until 2018, the Campaign for Real Ale (CAMRA)

defined real ale as beer "served without the use of extraneous carbon dioxide", which would disallow the use of a cask breather, a policy which was reversed in April 2018 to allow beer served with the use of cask breathers to meet its definition of real ale.

- Barrel-ageing

Barrel-ageing (US: Barrel aging) is the process of ageing beer in wooden barrels to achieve a variety of effects in the final product. Sour beers such as lambics are fully fermented in wood, while other beers are aged in barrels which were previously used for maturing wines or spirits. In 2016 "Craft Beer and Brewing" wrote: "Barrel-aged beers are so trendy that nearly every taphouse and beer store has a section of them.

Filtering

Filtering stabilises the flavour of beer, holding it at a point acceptable to the brewer, and preventing further development from the yeast, which under poor conditions can release negative components and flavours. Filtering also removes haze, clearing the beer, and so giving it a "polished shine and brilliance". Beer with a clear appearance has been commercially desirable for brewers since the development of glass vessels for storing and drinking beer, along with the commercial success of pale lager, which - due to the lagering process in which haze and particles settle to the bottom of the tank and so the beer "drops bright" (clears) - has a natural bright appearance and shine.

There are several forms of filters, they may be in the form of sheets or "candles", or they may be a fine powder such as diatomaceous earth, also called kieselguhr. The powder is added to the beer and recirculated past screens to form a filtration bed.

Filters range from rough filters that remove much of the yeast and any solids (e.g., hops, grain particles) left in the beer, to filters tight enough to strain colour and body from the beer. Filtration ratings are divided into rough, fine, and sterile. Rough filtration leaves some cloudiness in the beer, but it is noticeably clearer than unfiltered beer. Fine filtration removes almost all cloudiness. Sterile filtration removes almost all microorganisms.

- Sheet (pad) filters

These filters use sheets that allow only particles smaller than a given size to pass through. The sheets are placed into a filtering frame, sanitized (with boiling water, for example) and then used to filter the beer. The sheets can be flushed if the filter becomes blocked. The sheets are usually disposable and are replaced between filtration sessions. Often the sheets contain powdered filtration media to aid in filtration.

Pre-made filters have two sides. One with loose holes, and the other with tight holes. Flow goes from the side with loose holes to the side with the tight holes, with the intent that large particles get stuck in the large holes while leaving enough room around the particles and filter medium for smaller particles to go through and get stuck in tighter holes.

Sheets are sold in nominal ratings, and typically 90% of particles larger than the nominal rating are caught by the sheet.

- Kieselguhr filters

Filters that use a powder medium are considerably more complicated to operate, but can filter much more beer before regeneration. Common media include diatomaceous earth and perlite.

By-products

Brewing by-products are "spent grain" and the sediment (or "dregs") from the filtration process which may be dried and resold as "brewers dried yeast" for poultry feed, or made into yeast extract which is used in brands such as Vegemite and Marmite. The process of turning the yeast sediment into edible yeast extract was discovered by German scientist Justus von Liebig.

Brewer's spent grain (also called spent grain, brewer's grain or draff) is the main by-product of the brewing process; it consists of the residue of malt and grain which remains in the lautertun after the lautering process. It consists primarily of grain husks, pericarp, and fragments of endosperm. As it mainly consists of carbohydrates and proteins, and is readily consumed by animals, spent grain is used in animal feed. Spent grains can also be used as fertilizer, whole grains in bread, as well as in the production of flour and biogas. Spent grain is also an ideal medium for growing mushrooms, such as shiitake, and already some breweries are either growing their

own mushrooms or supplying spent grain to mushroom farms. Spent grains can be used in the production of red bricks, to improve the open porosity and reduce thermal conductivity of the ceramic mass.

Brewing industry

The brewing industry is a global business, consisting of several dominant multinational companies and many thousands of smaller producers known as microbreweries or regional breweries depending on size and region. More than 133 billion liters (3.5×10^{10} U.S. gallons; 2.9×10^{10} imperial gallons) are sold per year—producing total global revenues of $294.5 billion (£147.7 billion) as of 2006.[163]SABMiller became the largest brewing company in the world when it acquired Royal Grolsch, brewer of Dutch premium beer brand Grolsch.InBev was the second-largest beer-producing company in the world and Anheuser-Busch held the third spot, but after the acquisition of Anheuser-Busch by InBev, the new Anheuser-Busch InBev company is currently the largest brewer in the world.

Brewing at home is subject to regulation and prohibition in many countries. Restrictions on homebrewing were lifted in the UK in 1963,Australia followed suit in 1972, and the US in 1978, though individual states were allowed to pass their own laws limiting production.

Cooking

Cooking,cookery, or **culinary arts** is the art, science, and craft of using heat to prepare food for consumption. Cooking

techniques and ingredients vary widely, from grilling food over an open fire to using electric stoves, to baking in various types of ovens, reflecting local conditions.

Types of cooking also depend on the skill levels and training of the cooks. Cooking is done both by people in their own dwellings and by professional cooks and chefs in restaurants and other food establishments.

Preparing food with heat or fire is an activity unique to humans. It may have started around 2 million years ago, though archaeological evidence for the same does not predate no more than 1 million years.

The expansion of agriculture, commerce, trade, and transportation between civilizations in different regions offered cooks many new ingredients. New inventions and technologies, such as the invention of pottery for holding and boiling of water, expanded cooking techniques. Some modern cooks apply advanced scientific techniques to food preparation to further enhance the flavor of the dish served.

History

Phylogenetic analysis suggests that human ancestors may have invented cooking as far back as 1.8 million to 2.3 million years ago. Re-analysis of burnt bone fragments and plant ashes from the Wonderwerk Cave in South Africa has provided evidence supporting control of fire by early humans by 1 million years ago. There is evidence that *Homo erectus* were cooking their food as early as 500,000 years ago. Evidence for the controlled use of fire by *Homo erectus* beginning some 400,000 years ago

has wide scholarly support. Archaeological evidence from 300,000 years ago, in the form of ancient hearths, earth ovens, burnt animal bones, and flint, are found across Europe and the Middle East.Anthropologists think that widespread cooking fires began about 250,000 years ago when hearths first appeared.

Recently, the earliest hearths have been reported to be at least 790,000 years old.

Communication between the Old World and the New World in the Columbian Exchange influenced the history of cooking. The movement of foods across the Atlantic from the New World, such as potatoes, tomatoes, maize, beans, bell pepper, chili pepper, vanilla, pumpkin, cassava, avocado, peanut, pecan, cashew, pineapple, blueberry, sunflower, chocolate, gourds, and squash, had a profound effect on Old World cooking. The movement of foods across the Atlantic from the Old World, such as cattle, sheep, pigs, wheat, oats, barley, rice, apples, pears, peas, chickpeas, green beans, mustard, and carrots, similarly changed New World cooking.

In the seventeenth and eighteenth centuries, food was a classic marker of identity in Europe. In the nineteenth-century "Age of Nationalism" cuisine became a defining symbol of national identity.

The Industrial Revolution brought mass-production, mass-marketing, and standardization of food. Factories processed, preserved, canned, and packaged a wide variety of foods, and processed cereals quickly became a defining feature of the American breakfast. In the 1920s, freezing methods, cafeterias, and fast food restaurants emerged.

Ingredients

Most ingredients in cooking are derived from living organisms. Vegetables, fruits, grains and nuts as well as herbs and spices come from plants, while meat, eggs, and dairy products come from animals. Mushrooms and the yeast used in baking are kinds of fungi. Cooks also use water and minerals such as salt. Cooks can also use wine or spirits.

Naturally occurring ingredients contain various amounts of molecules called *proteins*, *carbohydrates* and *fats*. They also contain water and minerals. Cooking involves a manipulation of the chemical properties of these molecules.

Carbohydrates

Carbohydrates include the common sugar, sucrose (table sugar), a disaccharide, and such simple sugars as glucose (made by enzymatic splitting of sucrose) and fructose (from fruit), and starches from sources such as cereal flour, rice, arrowroot and potato.

The interaction of heat and carbohydrate is complex. Long-chain sugars such as starch tend to break down into more digestible simpler sugars. If the sugars are heated so that all water of crystallisation is driven off, caramelization starts, with the sugar undergoing thermal decomposition with the formation of carbon, and other breakdown products producing caramel. Similarly, the heating of sugars and proteins causes the Maillard reaction, a basic flavor-enhancing technique.

An emulsion of starch with fat or water can, when gently heated, provide thickening to the dish being cooked. In European cooking, a mixture of butter and flour called a roux is used to thicken liquids to make stews or sauces. In Asian cooking, a similar effect is obtained from a mixture of rice or corn starch and water. These techniques rely on the properties of starches to create simpler mucilaginous saccharides during cooking, which causes the familiar thickening of sauces. This thickening will break down, however, under additional heat.

Fats

Types of fat include vegetable oils, animal products such as butter and lard, as well as fats from grains, including maize and flax oils. Fats are used in a number of ways in cooking and baking. To prepare stir fries, grilled cheese or pancakes, the pan or griddle is often coated with fat or oil. Fats are also used as an ingredient in baked goods such as cookies, cakes and pies. Fats can reach temperatures higher than the boiling point of water, and are often used to conduct high heat to other ingredients, such as in frying, deep frying or sautéing. Fats are used to add flavor to food (e.g., butter or bacon fat), prevent food from sticking to pans and create a desirable texture.

Proteins

Edible animal material, including muscle, offal, milk, eggs and egg whites, contains substantial amounts of protein. Almost all vegetable matter (in particular legumes and seeds) also includes proteins, although generally in smaller amounts. Mushrooms have high protein content. Any of these may be sources of essential amino acids. When proteins are heated

they become denatured (unfolded) and change texture. In many cases, this causes the structure of the material to become softer or more friable – meat becomes *cooked* and is more friable and less flexible. In some cases, proteins can form more rigid structures, such as the coagulation of albumen in egg whites. The formation of a relatively rigid but flexible matrix from egg white provides an important component in baking cakes, and also underpins many desserts based on meringue.

Water

Cooking often involves water and water-based liquids. These can be added in order to immerse the substances being cooked (this is typically done with water, stock or wine). Alternatively, the foods themselves can release water. A favorite method of adding flavor to dishes is to save the liquid for use in other recipes. Liquids are so important to cooking that the name of the cooking method used is often based on how the liquid is combined with the food, as in steaming, simmering, boiling, braising and blanching. Heating liquid in an open container results in rapidly increased evaporation, which concentrates the remaining flavor and ingredients – this is a critical component of both stewing and sauce making.

Vitamins and minerals

Vitamins and minerals are required for normal metabolism but which the body cannot manufacture itself and which must therefore come from external sources. Vitamins come from several sources including fresh fruit and vegetables (Vitamin C), carrots, liver (Vitamin A), cereal bran, bread, liver (B vitamins), fish liver oil (Vitamin D) and fresh green vegetables

(Vitamin K). Many minerals are also essential in small quantities including iron, calcium, magnesium, sodium chloride and sulfur; and in very small quantities copper, zinc and selenium. The micronutrients, minerals, and vitamins in fruit and vegetables may be destroyed or eluted by cooking. Vitamin C is especially prone to oxidation during cooking and may be completely destroyed by protracted cooking. The bioavailability of some vitamins such as thiamin, vitamin B6, niacin, folate, and carotenoids are increased with cooking by being freed from the food microstructure. Blanching or steaming vegetables is a way of minimizing vitamin and mineral loss in cooking.

Methods

There are very many methods of cooking, most of which have been known since antiquity. These include baking, roasting, frying, grilling, barbecuing, smoking, boiling, steaming and braising. A more recent innovation is microwaving. Various methods use differing levels of heat and moisture and vary in cooking time. The method chosen greatly affects the end result because some foods are more appropriate to some methods than others. Some major hot cooking techniques include:

- Roasting
- Roasting – Barbecuing – Grilling/Broiling – Rotisserie – Searing
- Baking
- Baking – Baking Blind – Flashbaking
- Boiling

- Boiling – Blanching – Braising – Coddling – Double steaming – Infusion – Poaching – Pressure cooking – Simmering – Smothering – Steaming – Steeping – Stewing – Stone boiling – Vacuum flask cooking
- Frying
- Fry – Air frying — Deep frying – Gentle frying - Hot salt frying – Hot sand frying – Pan frying – Pressure frying – Sautéing – Shallow frying – Stir frying — Vacuum frying
- Steaming
- Steaming works by boiling water continuously, causing it to vaporise into steam; the steam then carries heat to the nearby food, thus cooking the food. By many it is considered a healthy form of cooking, holding nutrients within the vegetable or meat being cooked.
- Enpapillote – The food is put into a pouch and then baked, allowing its own moisture to steam the food.
- Smoking
- Smoking is the process of flavoring, cooking, or preserving food by exposing it to smoke from burning or smoldering material, most often wood.

Health and safety

Security while cooking

A lot of hazards may happen while cooking such as

- Unseen slippery surfaces (such as from oil stains or water droplets)

- Cuts (1 percent of injuries in UnIted States related to knives, ended in hospital admissions).In overall 400 000 injuries from knives are recorded in the US
- Burns

To prevent those injuries there are protections such as cooking clothing, anti-slip shoes and more.

Food safety

Cooking can prevent many foodborne illnesses that would otherwise occur if the food is eaten raw. When heat is used in the preparation of food, it can kill or inactivate harmful organisms, such as bacteria and viruses, as well as various parasites such as tapeworms and *Toxoplasma gondii*. Food poisoning and other illness from uncooked or poorly prepared food may be caused by bacteria such as pathogenic strains of *Escherichia coli*, *Salmonella typhimurium* and *Campylobacter*, viruses such as noroviruses, and protozoa such as *Entamoebahistolytica*. Bacteria, viruses and parasites may be introduced through salad, meat that is uncooked or done rare, and unboiled water.

The sterilizing effect of cooking depends on temperature, cooking time, and technique used. Some food spoilage bacteria such as *Clostridium botulinum* or *Bacillus cereus* can form spores that survive boiling, which then germinate and regrow after the food has cooled. This makes it unsafe to reheat cooked food more than once.

Cooking increases the digestibility of many foods which are inedible or poisonous when raw. For example, raw cereal grains are hard to digest, while kidney beans are toxic when

raw or improperly cooked due to the presence of phytohaemagglutinin, which is inactivated by cooking for at least ten minutes at 100 °C (212 °F).

Food safety depends on the safe preparation, handling, and storage of food. Food spoilage bacteria proliferate in the "Danger zone" temperature range from 40 to 140 °F (4 to 60 °C), food therefore should not be stored in this temperature range. Washing of hands and surfaces, especially when handling different meats, and keeping raw food separate from cooked food to avoid cross-contamination, are good practices in food preparation. Foods prepared on plastic cutting boards may be less likely to harbor bacteria than wooden ones. Washing and disinfecting cutting boards, especially after use with raw meat, poultry, or seafood, reduces the risk of contamination.

Effects on nutritional content of food

Proponents of raw foodism argue that cooking food increases the risk of some of the detrimental effects on food or health. They point out that during cooking of vegetables and fruit containing vitamin C, the vitamin elutes into the cooking water and becomes degraded through oxidation. Peeling vegetables can also substantially reduce the vitamin C content, especially in the case of potatoes where most vitamin C is in the skin. However, research has shown that in the specific case of carotenoids a greater proportion is absorbed from cooked vegetables than from raw vegetables.

German research in 2003 showed significant benefits in reducing breast cancer risk when large amounts of raw

vegetable matter are included in the diet. The authors attribute some of this effect to heat-labile phytonutrients.Sulforaphane, a glucosinolate breakdown product, which may be found in vegetables such as broccoli, has been shown to be protective against prostate cancer, however, much of it is destroyed when the vegetable is boiled.

The USDA has studied retention data for 16 vitamins, 8 minerals, and alcohol for approximately 290 foods for various cooking methods.

Carcinogens

In a human epidemiological analysis by Richard Doll and Richard Peto in 1981, diet was estimated to cause a large percentage of cancers. Studies suggest that around 32% of cancer deaths may be avoidable by changes to the diet. Some of these cancers may be caused by carcinogens in food generated during the cooking process, although it is often difficult to identify the specific components in diet that serve to increase cancer risk.Many foods, such as beef steak and broccoli, contain low concentrations of both carcinogens and anticarcinogens.

Several studies published since 1990 indicate that cooking meat at high temperature creates heterocyclic amines (HCAs), which are thought to increase cancer risk in humans. Researchers at the National Cancer Institute found that human subjects who ate beef rare or medium-rare had less than one third the risk of stomach cancer than those who ate beef medium-well or well-done. While avoiding meat or eating meat raw may be the only ways to avoid HCAs in meat fully, the

National Cancer Institute states that cooking meat below 212 °F (100 °C) creates "negligible amounts" of HCAs.Also, microwaving meat before cooking may reduce HCAs by 90% by reducing the time needed for the meat to be cooked at high heat.Nitrosamines are found in some food, and may be produced by some cooking processes from proteins or from nitrites used as food preservatives; cured meat such as bacon has been found to be carcinogenic, with links to colon cancer.Ascorbate, which is added to cured meat, however, reduces nitrosamine formation.

Research has shown that grilling, barbecuing and smoking meat and fish increases levels of carcinogenic polycyclic aromatic hydrocarbons (PAH). In Europe, grilled meat and smoked fish generally only contribute a small proportion of dietary PAH intake since they are a minor component of diet – most intake comes from cereals, oils and fats. However, in the US, grilled/barbecued meat is the second highest contributor of the mean daily intake of a known PAH carcinogen benzo[a]pyrene at 21% after 'bread, cereal and grain' at 29%.

Baking, grilling or broiling food, especially starchy foods, until a toasted crust is formed generates significant concentrations of acrylamide, a known carcinogen from animal studies; its potential to cause cancer in humans at normal exposures is uncertain.

Public health authorities recommend reducing the risk by avoiding overly browning starchy foods or meats when frying, baking, toasting or roasting them.

Other health issues

Cooking dairy products may reduce a protective effect against colon cancer. Researchers at the University of Toronto suggest that ingesting uncooked or unpasteurized dairy products (see also Raw milk) may reduce the risk of colorectal cancer. Mice and rats fed uncooked sucrose, casein, and beef tallow had one-third to one-fifth the incidence of microadenomas as the mice and rats fed the same ingredients cooked. This claim, however, is contentious. According to the Food and Drug Administration of the United States, health benefits claimed by raw milk advocates do not exist. "The small quantities of antibodies in milk are not absorbed in the human intestinal tract," says Barbara Ingham, PhD, associate professor and extension food scientist at the University of Wisconsin-Madison. "There is no scientific evidence that raw milk contains an anti-arthritis factor or that it enhances resistance to other diseases."

Heating sugars with proteins or fats can produce advanced glycation end products ("glycotoxins").

Deep fried food in restaurants may contain high level of trans fat, which is known to increase levels of low-density lipoprotein that in turn may increase risk of heart diseases and other conditions. However, many fast food chains have now switched to trans-fat-free alternatives for deep-frying.

Scientific aspects

The scientific study of cooking has become known as molecular gastronomy. This is a subdiscipline of food science concerning

the physical and chemical transformations that occur during cooking. Important contributions have been made by scientists, chefs and authors such as Hervé This (chemist), Nicholas Kurti (physicist), Peter Barham (physicist), Harold McGee (author), Shirley Corriher (biochemist, author), Robert Wolke (chemist, author. It is different for the application of scientific knowledge to cooking, that is "molecular cooking"((for the technique) or "molecular cuisine" (for a culinary style), for which chefs such as Raymond Blanc, Philippe and Christian Conticini, Ferran Adria, Heston Blumenthal, Pierre Gagnaire (chef).

Chemical processes central to cooking include hydrolysis (in particular beta elimination of pectins, during the thermal treatment of plant tissues), pyrolysis, glycation reactions wrongly named Maillardreactions .

Cooking foods with heat depends on many factors — the specific heat of an object, thermal conductivity, and perhaps most significantly the difference in temperature between the two objects. Thermal diffusivity is the combination of specific heat, conductivity and density that determines how long it will take for the food to reach a certain temperature.

Home-cooking and commercial cooking

Home cooking has traditionally been a process carried out informally in a home or around a communal fire, and can be enjoyed by all members of the family, although in many cultures women bear primary responsibility. Cooking is also

often carried out outside of personal quarters, for example at restaurants, or schools. Bakeries were one of the earliest forms of cooking outside the home, and bakeries in the past often offered the cooking of pots of food provided by their customers as an additional service. In the present day, factory food preparation has become common, with many "ready-to-eat" foods being prepared and cooked in factories and home cooks using a mixture of scratch made, and factory made foods together to make a meal. The nutritional value of including more commercially prepared foods has been found to be inferior to home-made foods. Home-cooked meals tend to be healthier with fewer calories, and less saturated fat, cholesterol and sodium on a per calorie basis while providing more fiber, calcium, and iron. The ingredients are also directly sourced, so there is control over authenticity, taste, and nutritional value. The superior nutritional quality of home-cooking could therefore play a role in preventing chronic disease. Cohort studies following the elderly over 10 years show that adults who cook their own meals have significantly lower mortality, even when controlling for confounding variables.

"Home-cooking" may be associated with comfort food, and some commercially produced foods and restaurant meals are presented through advertising or packaging as having been "home-cooked", regardless of their actual origin. This trend began in the 1920s and is attributed to people in urban areas of the U.S. wanting homestyle food even though their schedules and smaller kitchens made cooking harder.

Dairy

A **dairy** is a business enterprise established for the harvesting or processing (or both) of animal milk – mostly from cows or buffaloes, but also from goats, sheep, horses, or camels – for human consumption. A dairy is typically located on a dedicated dairy farm or in a section of a multi-purpose farm (mixed farm) that is concerned with the harvesting of milk.

As an attributive, the word *dairy* refers to milk-based products, derivatives and processes, and the animals and workers involved in their production: for example dairy cattle, dairy goat. A dairy farm produces milk and a dairy factory processes it into a variety of dairy products. These establishments constitute the global dairy industry, a component of the food industry.

Terminology

Terminology differs between countries. in the United States, for example, an entire dairy farm is commonly called a "dairy". The building or farm area where milk is harvested from the cow is often called a "milking parlor" or "parlor", except in the case of smaller dairies, where cows are often put on pasture, and usually milked in "stanchion barns". The farm area where milk is stored in bulk tanks is known as the farm's "milk house". Milk is then hauled (usually by truck) to a "dairy plant", also referred to as a "dairy", where raw milk is further processed and prepared for commercial sale of dairy products. In New Zealand, farm areas for milk harvesting are also called "milking parlours", and are historically known as "milking

sheds". As in the United States, sometimes milking sheds are referred to by their type, such as "herring bone shed" or "pit parlour". Parlour design has evolved from simple barns or sheds to large rotary structures in which the workflow (throughput of cows) is very efficiently handled. In some countries, especially those with small numbers of animals being milked, the farm may perform the functions of a dairy plant, processing their own milk into salable dairy products, such as butter, cheese, or yogurt. This on-site processing is a traditional method of producing specialist milk products, common in Europe.

In the United States a *dairy* can also be a place that processes, distributes and sells dairy products, or a room, building or establishment where milk is stored and processed into milk products, such as butter or cheese. In New Zealand English the singular use of the word *dairy* almost exclusively refers to a corner shop, or superette. This usage is historical as such shops were a common place for the public to buy milk products.

History

Milk producing animals have been domesticated for thousands of years. Initially, they were part of the subsistence farming that nomads engaged in. As the community moved about the country, their animals accompanied them. Protecting and feeding the animals were a big part of the symbiotic relationship between the animals and the herders.

In the more recent past, people in agricultural societies owned dairy animals that they milked for domestic and local (village)

consumption, a typical example of a cottage industry. The animals might serve multiple purposes (for example, as a draught animal for pulling a plow as a youngster, and at the end of its useful life as meat). In this case, the animals were normally milked by hand and the herd size was quite small, so that all of the animals could be milked in less than an hour—about 10 per milker. These tasks were performed by a *dairymaid* (*dairywoman*) or *dairyman*. The word *dairy* harkens back to Middle English *dayerie*, *deyerie*, from *deye* (female servant or dairymaid) and further back to Old English *dæge* (kneader of bread).

With industrialization and urbanization, the supply of milk became a commercial industry, with specialized breeds of cattlebeing developed for dairy, as distinct from beef or draught animals. Initially, more people were employed as milkers, but it soon turned to mechanization with machines designed to do the milking.

Historically, the milking and the processing took place close together in space and time: on a dairy farm. People milked the animals by hand; on farms where only small numbers are kept, hand-milking may still be practiced. Hand-milking is accomplished by grasping the teats (often pronounced *tit* or *tits*) in the hand and expressing milk either by squeezing the fingers progressively, from the udder end to the tip, or by squeezing the teat between thumb and index finger, then moving the hand downward from udder towards the end of the teat. The action of the hand or fingers is designed to close off the milk duct at the udder (upper) end and, by the movement of the fingers, close the duct progressively to the tip to express

the trapped milk. Each half or quarter of the udder is emptied one milk-duct capacity at a time.

The *stripping* action is repeated, using both hands for speed. Both methods result in the milk that was trapped in the milk duct being squirted out the end into a bucket that is supported between the knees (or rests on the ground) of the milker, who usually sits on a low stool.

Traditionally the cow, or cows, would stand in the field or paddock while being milked. Young stock, heifers, would have to be trained to remain still to be milked. In many countries, the cows were tethered to a post and milked.

Structure of the industry

While most countries produce their own milk products, the structure of the dairy industry varies in different parts of the world. In major milk-producing countries most milk is distributed through whole sale markets. In Ireland and Australia, for example, farmers' co-operatives own many of the large-scale processors, while in the United States many farmers and processors do business through individual contracts. In the United States, the country's 196 farmers' cooperatives sold 86% of milk in the U.S. in 2002, with five cooperatives accounting for half that. This was down from 2,300 cooperatives in the 1940s. In developing countries, the past practice of farmers marketing milk in their own neighborhoods is changing rapidly. Notable developments include considerable foreign investment in the dairy industry and a growing role for dairy cooperatives. Output of milk is

growing rapidly in such countries and presents a major source of income growth for many farmers.

As in many other branches of the food industry, dairy processing in the major dairy producing countries has become increasingly concentrated, with fewer but larger and more efficient plants operated by fewer workers. This is notably the case in the United States, Europe, Australia and New Zealand. In 2009, charges of antitrust violations have been made against major dairy industry players in the United States, which critics call **Big Milk**. Another round of price fixing charges was settled in 2016.

Government intervention in milk markets was common in the 20th century. A limited antitrust exemption was created for U.S. dairy cooperatives by the Capper–Volstead Act of 1922. In the 1930s, some U.S. states adopted price controls, and Federal Milk Marketing Orders started under the Agricultural Marketing Agreement Act of 1937 and continue in the 2000s. The Federal Milk Price Support Program began in 1949. The Northeast Dairy Compact regulated wholesale milk prices in New England from 1997 to 2001.

Plants producing liquid milk and products with short shelf life, such as yogurts, creams and soft cheeses, tend to be located on the outskirts of urban centres close to consumer markets. Plants manufacturing items with longer shelf life, such as butter, milk powders, cheese and whey powders, tend to be situated in rural areas closer to the milk supply. Most large processing plants tend to specialise in a limited range of products. Exceptionally, however, large plants producing a wide range of products are still common in Eastern Europe, a

holdover from the former centralized, supply-driven concept of the market under Communist governments.

As processing plants grow fewer and larger, they tend to acquire bigger, more automated and more efficient equipment. While this technological tendency keeps manufacturing costs lower, the need for long-distance transportation often increases the environmental impact.

Milk production is irregular, depending on cow biology. Producers must adjust the mix of milk which is sold in liquid form vs. processed foods (such as butter and cheese) depending on changing supply and demand.

Milk supply contracts

In the European Union, milk supply contracts are regulated by Article 148 of Regulation 1308/2013 - *Establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007*, which permits member states to create a requirement for the supply of milk from a farmer to a raw milk processor to be backed by a written contract, or to ensure that the first purchaser of milk to make a written offer to the farmer, although in this case the farmer may not be required to enter into a contract.

Thirteen EU member states including France and Spain have introduced laws on compulsory or mandatory written milk contracts (MWC's) between farmers and processors. The Scottish Government published an analysis of the dairy supply chain and the application of mandatory written contracts across the European Union in 2019, to evaluate the impact of

the contracts where they have been adopted. In the UK, a voluntary code of best practice on contractual relationships in the dairy sector was agreed by industry during 2012: this set out minimum standards of good practice for contracts between producers and purchasers. During 2020 the UK government has undertaken a consultation exercise to determine which contractual measures, if any, would improve the resilience of the dairy industry for the future.

The Australian government has also introduced a mandatory dairy code of conduct.

Farming

When it became necessary to milk larger cows, the cows would be brought to a shed or barn that was set up with stalls (milking stalls) where the cows could be confined their whole life while they were milked. One person could milk more cows this way, as many as 20 for a skilled worker. But having cows standing about in the yard and shed waiting to be milked is not good for the cow, as she needs as much time in the paddock grazing as is possible. It is usual to restrict the twice-daily milking to a maximum of an hour and a half each time. It makes no difference whether one milks 10 or 1000 cows, the milking time should not exceed a total of about three hours each day for any cow as they should be in stalls and laying down as long as possible to increase comfort which will in turn aid in milk production. A cow is physically milked for only about 10 minutes a day depending on her milk letdown time and the number of milkings per day.

As herd sizes increased there was more need to have efficient milking machines, sheds, milk-storage facilities (vats), bulk-milk transport and shed cleaning capabilities and the means of getting cows from paddock to shed and back.

As herd numbers increased so did the problems of animal health. In New Zealand two approaches to this problem have been used. The first was improved veterinary medicines (and the government regulation of the medicines) that the farmer could use. The other was the creation of *veterinary clubs* where groups of farmers would employ a veterinarian (vet) full-time and share those services throughout the year. It was in the vet's interest to keep the animals healthy and reduce the number of calls from farmers, rather than to ensure that the farmer needed to call for service and pay regularly.

This daily milking routine goes on for about 300 to 320 days per year that the cow stays in milk. Some small herds are milked once a day for about the last 20 days of the production cycle but this is not usual for large herds. If a cow is left unmilked just once she is likely to reduce milk-production almost immediately and the rest of the season may see her *dried off* (giving no milk) and still consuming feed. However, once-a-day milking is now being practised more widely in New Zealand for profit and lifestyle reasons. This is effective because the fall in milk yield is at least partially offset by labour and cost savings from milking once per day. This compares to some intensive farm systems in the United States that milk three or more times per day due to higher milk yields per cow and lower marginal labor costs.

Farmers who are contracted to supply liquid milk for human consumption (as opposed to milk for processing into butter, cheese, and so on—see milk) often have to manage their herd so that the contracted number of cows are in milk the year round, or the required minimum milk output is maintained. This is done by mating cows outside their natural mating time so that the period when each cow in the herd is giving maximum production is in rotation throughout the year.

Northern hemisphere farmers who keep cows in barns almost all the year usually manage their herds to give continuous production of milk so that they get paid all year round. In the southern hemisphere the cooperative dairying systems allow for two months on no productivity because their systems are designed to take advantage of maximum grass and milk production in the spring and because the milk processing plants pay bonuses in the dry (winter) season to carry the farmers through the mid-winter break from milking. It also means that cows have a rest from milk production when they are most heavily pregnant. Some year-round milk farms are penalised financially for overproduction at any time in the year by being unable to sell their overproduction at current prices.

Artificial insemination (AI) is common in all high-production herds in order to improve the genetics of the female offspring which will be raised for replacements. AI also reduces the need for keeping potentially dangerous bulls on the farm. Male calves are sold to be raised for beef or veal, or slaughtered due to lack of profitability. A cow will calve or freshen about once a year, until she is culled because of declining production, infertility or other health problems. Then the cow will be sold, most often going to slaughter.

Industrial processing

Dairy plants process the raw milk they receive from farmers so as to extend its marketable life. Two main types of processes are employed: heat treatment to ensure the safety of milk for human consumption and to lengthen its shelf-life, and dehydrating dairy products such as butter, hard cheese and milk powders so that they can be stored.

Cream and butter

Today, milk is separated by huge machines in bulk into cream and skim milk. The cream is processed to produce various consumer products, depending on its thickness, its suitability for culinary uses and consumer demand, which differs from place to place and country to country.

Some milk is dried and powdered, some is condensed (by evaporation) mixed with varying amounts of sugar and canned. Most cream from New Zealand and Australian factories is made into butter. This is done by churning the cream until the fat globules coagulate and form a monolithic mass. This butter mass is washed and, sometimes, salted to improve keeping qualities. The residual buttermilkgoes on to further processing. The butter is packaged (25 to 50 kg boxes) and chilled for storage and sale. At a later stage these packages are broken down into home-consumption sized packs.

Skimmed milk

The product left after the cream is removed is called skim, or skimmed, milk. To make a consumable liquid a portion of

cream is returned to the skim milk to make *low fat milk* (semi-skimmed) for human consumption. By varying the amount of cream returned, producers can make a variety of low-fat milks to suit their local market. Whole milk is also made by adding cream back to the skim to form a standardized product. Other products, such as calcium, vitamin D, and flavouring, are also added to appeal to consumers.

Casein

Casein is the predominant phosphoprotein found in fresh milk. It has a very wide range of uses from being a filler for human foods, such as in ice cream, to the manufacture of products such as fabric, adhesives, and plastics.

Cheese

Cheese is another product made from milk. Whole milk is reacted to form curds that can be compressed, processed and stored to form cheese. In countries where milk is legally allowed to be processed without pasteurization, a wide range of cheeses can be made using the bacteria found naturally in the milk. In most other countries, the range of cheeses is smaller and the use of artificial cheese curing is greater. Whey is also the byproduct of this process. Some people with lactose intolerance are surprisingly able to eat certain types of cheese. This is because some traditionally made hard cheeses, and soft ripened cheeses may create less reaction than the equivalent amount of milk because of the processes involved. Fermentation and higher fat content contribute to lesser amounts of lactose. Traditionally made Emmental or Cheddar might contain 10% of the lactose found in whole milk. In

addition, the aging methods of traditional cheeses (sometimes over two years) reduce their lactose content to practically nothing. Commercial cheeses, however, are often manufactured by processes that do not have the same lactose-reducing properties. Ageing of some cheeses is governed by regulations; in other cases there is no quantitative indication of degree of ageing and concomitant lactose reduction, and lactose content is not usually indicated on labels.

Whey

In earlier times, whey or **milk serum** was considered to be a waste product and it was, mostly, fed to pigs as a convenient means of disposal. Beginning about 1950, and mostly since about 1980, lactose and many other products, mainly food additives, are made from both casein and cheese whey.

Yogurt

Yogurt (or yoghurt) making is a process similar to cheese making, only the process is arrested before the curd becomes very hard.

Milk powders

Milk is also processed by various drying processes into powders. Whole milk, skim milk, buttermilk, and whey products are dried into a powder form and used for human and animal consumption. The main difference between production of powders for human or for animal consumption is in the protection of the process and the product from contamination. Some people drink milk reconstituted from powdered milk,

because milk is about 88% water and it is much cheaper to transport the dried product.

Other milk products

Kumisis produced commercially in Central Asia. Although traditionally made from mare's milk, modern industrial variants may use cow's milk. In India, which produces 22% of global milk production (as at 2018), a range of traditional milk-based productsare produced commercially.

Milking

Originally, milking and processing took place on the dairy farm itself. Later, cream was separated from the milk by machine on the farm, and transported to a factory to be made into butter. The skim milk was fed to pigs. This allowed for the high cost of transport (taking the smallest volume high-value product), primitive trucks and the poor quality of roads. Only farms close to factories could afford to take whole milk, which was essential for cheesemaking in industrial quantities, to them.

Originally milk was distributed in 'pails', a lidded bucket with a handle. These proved impractical for transport by road or rail, and so the milk churnwas introduced, based on the tall conical shape of the butter churn. Later large railway containers, such as the British Railway Milk Tank Wagonwere introduced, enabling the transport of larger quantities of milk, and over longer distances.

The development of refrigeration and better road transport, in the late 1950s, has meant that most farmers milk their cows

and only temporarily store the milk in large refrigerated bulk tanks, from where it is later transported by truck to central processing facilities.

In many European countries, particularly the United Kingdom, milk is then delivered direct to customers' homes by a milk float.

In the United States, a dairy cow produced about 5,300 pounds (2,400 kg) of milk per year in 1950, while the average Holstein cow in 2019 produces more than 23,000 pounds (10,000 kg) of milk per year.

Milking machines

Milking machines are used to harvest milk from cows when manual milking becomes inefficient or labour-intensive. One early model was patented in 1907. The milking unit is the portion of a milking machine for removing milk from an udder. It is made up of a claw, four teatcups, (Shells and rubber liners) long milk tube, long pulsation tube, and a pulsator. The claw is an assembly that connects the short pulse tubes and short milk tubes from the teatcups to the long pulse tube and long milk tube. (Cluster assembly) Claws are commonly made of stainless steel or plastic or both. Teatcups are composed of a rigid outer shell (stainless steel or plastic) that holds a soft inner liner or *inflation*. Transparent sections in the shell may allow viewing of liner collapse and milk flow. The annular space between the shell and liner is called the pulse chamber.

Milking machines work in a way that is different from hand milking or calf suckling. Continuous vacuum is applied inside the soft liner to massage milk from the teat by creating a

pressure difference across the teat canal (or opening at the end of the teat). Vacuum also helps keep the machine attached to the cow. The vacuum applied to the teat causes congestion of teat tissues (accumulation of blood and other fluids). Atmospheric air is admitted into the pulsation chamber about once per second (the pulsation rate) to allow the liner to collapse around the end of teat and relieve congestion in the teat tissue. The ratio of the time that the liner is open (milking phase) and closed (rest phase) is called the pulsation ratio.

The four streams of milk from the teatcups are usually combined in the claw and transported to the milkline, or the collection bucket (usually sized to the output of one cow) in a single milk hose. Milk is then transported (manually in buckets) or with a combination of airflow and mechanical pump to a central storage vat or bulk tank. Milk is refrigerated on the farm in most countries either by passing through a heat-exchanger or in the bulk tank, or both.

The photo to the right shows a bucket milking system with the stainless steel bucket visible on the far side of the cow. The two rigid stainless steel teatcup shells applied to the front two quarters of the udder are visible. The top of the flexible liner is visible at the top of the shells as are the short milk tubes and short pulsation tubes extending from the bottom of the shells to the claw. The bottom of the claw is transparent to allow observation of milk flow. When milking is completed the vacuum to the milking unit is shut off and the teatcups are removed.

Milking machines keep the milk enclosed and safe from external contamination. The interior 'milk contact' surfaces of

the machine are kept clean by a manual or automated washing procedures implemented after milking is completed. Milk contact surfaces must comply with regulations requiring food-grade materials (typically stainless steel and special plastics and rubber compounds) and are easily cleaned.

Most milking machines are powered by electricity but, in case of electrical failure, there can be an alternative means of motive power, often an internal combustion engine, for the vacuum and milk pumps.

Milking shed layouts

Bail-style sheds

This type of milking facility was the first development, after open-paddock milking, for many farmers. The building was a long, narrow, *lean-to* shed that was open along one long side. The cows were held in a yard at the open side and when they were about to be milked they were positioned in one of the bails (stalls). Usually the cows were restrained in the bail with a breech chain and a rope to restrain the outer back leg. The cow could not move about excessively and the milker could expect not to be kicked or trampled while sitting on a (three-legged) stool and milking into a bucket. When each cow was finished she backed out into the yard again. The UK bail, initially developed by Wiltshire dairy farmer Arthur Hosier, was a six standing mobile shed with steps that the cow mounted, so the herdsman didn't have to bend so low. The milking equipment was much as today, a vacuum from a pump, pulsators, a claw-piece with pipes leading to the four shells

and liners that stimulate and suck the milk from the teat. The milk went into churns, via a cooler.

As herd sizes increased a door was set into the front of each bail so that when the milking was done for any cow the milker could, after undoing the leg-rope and with a remote link, open the door and allow her to exit to the pasture. The door was closed, the next cow walked into the bail and was secured. When milking machines were introduced bails were set in pairs so that a cow was being milked in one paired bail while the other could be prepared for milking. When one was finished the machine's cups are swapped to the other cow. This is the same as for *Swingover Milking Parlours* as described below except that the cups are loaded on the udder from the side. As herd numbers increased it was easier to double-up the cup-sets and milk both cows simultaneously than to increase the number of bails. About 50 cows an hour can be milked in a shed with 8 bails by one person. Using the same teat cups for successive cows has the danger of transmitting infection, mastitis, from one cow to another. Some farmers have devised their own ways to disinfect the clusters between cows.

Herringbone milking parlours

In herringbone milking sheds, or parlours, cows enter, in single file, and line up almost perpendicular to the central aisle of the milking parlour on both sides of a central pit in which the milker works (you can visualise a fishbone with the ribs representing the cows and the spine being the milker's working area; the cows face outward). After washing the udder and teats the cups of the milking machine are applied to the cows, from the rear of their hind legs, on both sides of the

working area. Large herringbone sheds can milk up to 600 cows efficiently with two people.

Swingover milking parlours

- Swingoverparlours are the same as herringbone parlours except they have only one set of milking cups to be shared between the two rows of cows, as one side is being milked the cows on the other side are moved out and replaced with unmilked ones. The advantage of this system is that it is less costly to equip, however it operates at slightly better than half-speed and one would not normally try to milk more than about 100 cows with one person.

Rotary milking sheds

Rotary milking sheds (also known as Rotary milking parlor) consist of a turntable with about 12 to 100 individual stalls for cows around the outer edge. A "good" rotary will be operated with 24–32 (~48–50+) stalls by one (two) milkers. The turntable is turned by an electric-motor drive at a rate that one turn is the time for a cow to be milked completely. As an empty stall passes the entrance a cow steps on, facing the center, and rotates with the turntable. The next cow moves into the next vacant stall and so on. The operator, or milker, cleans the teats, attaches the cups and does any other feeding or whatever husbanding operations that are necessary. Cows are milked as the platform rotates. The milker, or an automatic device, removes the milking machine cups and the cow backs out and leaves at an exit just before the entrance. The rotary

system is capable of milking very large herds—over a thousand cows.

Automatic milking sheds

Automatic milking or 'robotic milking' sheds can be seen in Australia, New Zealand, the U.S., Canada, and many European countries. Current automatic milking sheds use the voluntary milking (VM) method. These allow the cows to voluntarily present themselves for milking at any time of the day or night, although repeat visits may be limited by the farmer through computer software. A robot arm is used to clean teats and apply milking equipment, while automated gates direct cow traffic, eliminating the need for the farmer to be present during the process. The entire process is computer controlled.

Supplementary accessories in sheds

Farmers soon realised that a milking shed was a good place to feed cows supplementary foods that overcame local dietary deficiencies or added to the cows' wellbeing and production. Each bail might have a box into which such feed is delivered as the cow arrives so that she is eating while being milked. A computer can read the eartag of each animal to ration the correct individual supplement. A close alternative is to use 'out-of-parlour-feeders', stalls that respond to a transponder around the cow's neck that is programmed to provide each cow with a supplementary feed, the quantity dependent on her production, stage in lactation, and the benefits of the main ration

The holding yard at the entrance of the shed is important as a means of keeping cows moving into the shed. Most yards have a powered gate that ensures that the cows are kept close to the shed.

Water is a vital commodity on a dairy farm: cows drink about 20 gallons (80 litres) a day, sheds need water to cool and clean them. Pumps and reservoirs are common at milking facilities. Water can be warmed by heat transfer with milk.

Temporary milk storage

Milk coming from the cow is transported to a nearby storage vessel by the airflow leaking around the cups on the cow or by a special "air inlet" (5-10 L/min free air) in the claw. From there it is pumped by a mechanical pump and cooled by a heat exchanger. The milk is then stored in a large vat, or bulk tank, which is usually refrigerated until collection for processing.

Waste disposal

In countries where cows are grazed outside year-round, there is little waste disposal to deal with. The most concentrated waste is at the milking shed, where the animal waste may be liquefied (during the water-washing process) or left in a more solid form, either to be returned to be used on farm ground as organic fertilizer.

In the associated milk processing factories, most of the waste is washing water that is treated, usually by composting, and spread on farm fields in either liquid or solid form. This is much different from half a century ago, when the main

products were butter, cheese and casein, and the rest of the milk had to be disposed of as waste (sometimes as animal feed).

In dairy-intensive areas, various methods have been proposed for disposing of large quantities of milk. Large application rates of milk onto land, or disposing in a hole, is problematic as the residue from the decomposing milk will block the soil pores and thereby reduce the water infiltration rate through the soil profile. As recovery of this effect can take time, any land-based application needs to be well managed and considered. Other waste milk disposal methods commonly employed include solidification and disposal at a solid waste landfill, disposal at a wastewater treatment plant, or discharge into a sanitary sewer.

Associated diseases

Dairy products manufactured under unsanitary or unsuitable conditions have an increased chance of containing bacteria. Proper sanitation practices help to reduce the rate of bacterial contamination, and pasteurization greatly decreases the amount of contaminated milk that reaches the consumer. Many countries have required government oversight and regulations regarding dairy production, including requirements for pasteurization.

- Leptospirosis is an infection that can be transmitted to people who work in dairy production through exposure to urine or to contaminated water or soil.
- Cowpox is a virus that today is rarely found in either cows or humans. It is a historically important

disease, as it led to the first vaccination against the now eradicated smallpox.

- Tuberculosisis able to be transmitted from cattle mainly via milk products that are unpasteurised. The disease has been eradicated from many countries by testing for the disease and culling suspected animals.
- Brucellosis is a bacterial disease transmitted to humans by dairy products and direct animal contact. Brucellosis has been eradicated from certain countries by testing for the disease and culling suspected animals.
- Listeria is a bacterial disease associated with unpasteurised milk, and can affect some cheeses made in traditional ways. Careful observance of the traditional cheesemaking methods achieves reasonable protection for the consumer.
- Crohn's disease has been linked to infection with the bacterium *M. paratuberculosis*, which has been found in pasteurized retail milk in the UK and the USA.*M. paratuberculosis* causes a similar disorder, Johne's disease, in livestock.

Animal rights

A portion of the population, including vegans and many Jains, object to dairy production as unethical, cruel to animals, and environmentally deleterious. They do not consume dairy products. They state that cattle suffer under conditions employed by the dairy industry.

Animal rights scholars consider dairy as part of the animal–industrial complex. According to Kathleen Stachowski, the animal–industrial complex "naturalizes the human as a consumer of other animals," whose enormity includes "its long reach into our lives, and how well it has done its job normalizing brutality toward the animals whose very existence is forgotten." She states that the corporate dairy industry, the government, and schools forms the animal–industrial complex troika of immense influence, which hides from the public's view the animal rights violations and cruelties happening within the dairy industry.Stachowski also states that the troika "hijacks" schoolchildren by promoting milk in the K-12 nutrition education curriculum and making them "eat the products of industrial animal production."

Bovine growth hormone

In 1937, it was found that bovine somatotropin (BST or bovine growth hormone) would increase the yield of milk. Several pharmaceutical companies developed commercial rBST products and they have been approved for use in the U.S., Mexico, Brazil, India, Russia, and at least ten others. The World Health Organization, and others have stated that dairy products and meat from BST-treated cows are safe for human consumption. However, based on negative animal welfare effects,rBST has not been allowed in Canada, Australia, New Zealand, Japan, Israel, or the European Union since 2000 – and in the U.S. has lost popularity due to consumer demands for rBST-free cows, with only about 17% of all cows in America now receiving rBST.

Chapter 5

Molecular Biology and Food Processing

Molecular biology

Molecular biology /məˈlɛkjʊlər/ is the branch of biology that concerns the molecular basis of biological activity in and between cells, including molecular synthesis, modification, mechanisms and interactions. The central dogma of molecular biology describes the process in which DNA is transcribed into RNA, which is then translated into protein.

William Astbury described molecular biology in 1961 in *Nature*, as:

...not so much a technique as an approach, an approach from the viewpoint of the so-called basic sciences with the leading idea of searching below the large-scale manifestations of classical biology for the corresponding molecular plan. It is concerned particularly with the *forms* of biological molecules and [...] is predominantly three-dimensional and structural – which does not mean, however, that it is merely a refinement of morphology. It must at the same time inquire into genesis and function.

Some clinical research and medical therapies arising from molecular biology are covered under gene therapy whereas the use of molecular biology or molecular cell biology in medicine is now referred to as molecular medicine. Molecular biology

also plays important role in understanding formations, actions, and regulations of various parts of cells which can be used to efficiently target new drugs, diagnose disease, and understand the physiology of the cell.

History

While molecular biology was established as an official branch of science in the 1930s, the term wasn't coined until 1938 by Warren Weaver. At the time, Weaver was the director of Natural Sciences for the Rockefeller Foundation and believed that biology was about to undergo significant change due to recent advancements in technology such as X-ray crystallography.

Molecular biology arose as an attempt to answer the questions regarding the mechanisms of genetic inheritance and the structure of a gene. In 1953, James Watson and Francis Crick published the double helical structure of DNA courtesy of the X-ray crystallography work done by Rosalind Franklin and Maurice Wilkins. Watson and Crick described the structure of DNA and the interactions within the molecule. This publication jump-started research into molecular biology and increased interest in the subject.

Relationship to other biological sciences

The following list describes a viewpoint on the interdisciplinary relationships between molecular biology and other related fields.

- ***Molecular biology*** is the study of the molecular underpinnings of the biological phenomena, focusing on molecular synthesis, modification, mechanisms and interactions.
- ***Biochemistry*** is the study of the chemical substances and vital processes occurring in living organisms. Biochemists focus heavily on the role, function, and structure of biomolecules such as proteins, lipids, carbohydrates and nucleic acids.
- ***Genetics*** is the study of how genetic differences affect organisms. Genetics attempts to predict how mutations, individual genes and genetic interactions can affect the expression of a phenotype

While researchers practice techniques specific to molecular biology, it is common to combine these with methods from genetics and biochemistry. Much of molecular biology is quantitative, and recently a significant amount of work has been done using computer science techniques such as bioinformatics and computational biology. Molecular genetics, the study of gene structure and function, has been among the most prominent sub-fields of molecular biology since the early 2000s. Other branches of biology are informed by molecular biology, by either directly studying the interactions of molecules in their own right such as in cell biology and developmental biology, or indirectly, where molecular techniques are used to infer historical attributes of populations or species, as in fields in evolutionary biology such as population genetics and phylogenetics. There is also a long tradition of studying biomolecules "from the ground up", or molecularly, in biophysics.

Techniques of molecular biology

Molecular cloning

One of the most basic techniques of molecular biology to study protein function is molecular cloning. In this technique, DNA coding for a protein of interest is cloned using polymerase chain reaction (PCR), and/or restriction enzymes into a plasmid (expression vector). A vector has 3 distinctive features: an origin of replication, a multiple cloning site (MCS), and a selective marker usually antibiotic resistance. Located upstream of the multiple cloning site are the promoter regions and the transcription start site which regulate the expression of cloned gene. This plasmid can be inserted into either bacterial or animal cells. Introducing DNA into bacterial cells can be done by transformation via uptake of naked DNA, conjugation via cell-cell contact or by transduction via viral vector. Introducing DNA into eukaryotic cells, such as animal cells, by physical or chemical means is calledtransfection. Several different transfection techniques are available, such as calcium phosphate transfection, electroporation, microinjection and liposome transfection. The plasmid may be integrated into the genome, resulting in a stable transfection, or may remain independent of the genome, called transient transfection.

DNA coding for a protein of interest is now inside a cell, and the proteincan now be expressed. A variety of systems, such as inducible promoters and specific cell-signaling factors, are available to help express the protein of interest at high levels. Large quantities of a protein can then be extracted from the bacterial or eukaryotic cell. The protein can be tested for

enzymatic activity under a variety of situations, the protein may be crystallized so its tertiary structure can be studied, or, in the pharmaceutical industry, the activity of new drugs against the protein can be studied.

Polymerase chain reaction

Polymerase chain reaction (PCR) is an extremely versatile technique for copying DNA. In brief, PCR allows a specific DNA sequence to be copied or modified in predetermined ways. The reaction is extremely powerful and under perfect conditions could amplify one DNA molecule to become 1.07 billion molecules in less than two hours. The PCR technique can be used to introduce restriction enzyme sites to ends of DNA molecules, or to mutate particular bases of DNA, the latter is a method referred to as site-directed mutagenesis. PCR can also be used to determine whether a particular DNA fragment is found in a cDNA library. PCR has many variations, like reverse transcription PCR (RT-PCR) for amplification of RNA, and, more recently, quantitative PCR which allow for quantitative measurement of DNA or RNA molecules.

Gel electrophoresis

Gel electrophoresis is one of the principal tools of molecular biology. The basic principle is that DNA, RNA, and proteins can all be separated by means of an electric field and size. In agarose gel electrophoresis,

DNA and RNA can be separated on the basis of size by running the DNA through an electrically charged agarose gel. Proteins can be separated on the basis of size by using an SDS-PAGE

gel, or on the basis of size and their electric charge by using what is known as a 2D gel electrophoresis.

Macromolecule blotting and probing

The terms *northern*, *western* and *eastern* blotting are derived from what initially was a molecular biology joke that played on the term *Southern blotting*, after the technique described by Edwin Southern for the hybridisation of blotted DNA. Patricia Thomas, developer of the RNA blot which then became known as the *northern blot*, actually didn't use the term.

Southern blotting

Named after its inventor, biologist Edwin Southern, the Southern blot is a method for probing for the presence of a specific DNA sequence within a DNA sample. DNA samples before or after restriction enzyme (restriction endonuclease) digestion are separated by gel electrophoresis and then transferred to a membrane by blotting via capillary action.

The membrane is then exposed to a labeled DNA probe that has a complement base sequence to the sequence on the DNA of interest.

Southern blotting is less commonly used in laboratory science due to the capacity of other techniques, such as PCR, to detect specific DNA sequences from DNA samples. These blots are still used for some applications, however, such as measuring transgene copy number in transgenic mice or in the engineering of gene knockoutembryonic stem cell lines.

Northern blotting

The northern blotis used to study the presence of specific RNA molecules as relative comparison among a set of different samples of RNA. It is essentially a combination of denaturing RNA gel electrophoresis, and a blot. In this process RNA is separated based on size and is then transferred to a membrane that is then probed with a labeled complement of a sequence of interest. The results may be visualized through a variety of ways depending on the label used; however, most result in the revelation of bands representing the sizes of the RNA detected in sample. The intensity of these bands is related to the amount of the target RNA in the samples analyzed. The procedure is commonly used to study when and how much gene expression is occurring by measuring how much of that RNA is present in different samples, assuming that no post-transcriptional regulation occurs and that the levels of mRNA reflect proportional levels of the corresponding protein being produced. It is one of the most basic tools for determining at what time, and under what conditions, certain genes are expressed in living tissues.

Western blotting

In western blotting, proteins are first separated by size, in a thin gel sandwiched between two glass plates in a technique known as SDS-PAGE. The proteins in the gel are then transferred to a polyvinylidene fluoride (PVDF), nitrocellulose, nylon, or other support membrane. This membrane can then be probed with solutions of antibodies. Antibodies that specifically bind to the protein of interest can then be visualized by a variety of techniques, including colored

products, chemiluminescence, or autoradiography. Often, the antibodies are labeled with enzymes. When a chemiluminescentsubstrate is exposed to the enzyme it allows detection. Using western blotting techniques allows not only detection but also quantitative analysis. Analogous methods to western blotting can be used to directly stain specific proteins in live cells or tissue sections.

Eastern blotting

The eastern blotting technique is used to detect post-translational modification of proteins. Proteins blotted on to the PVDF or nitrocellulose membrane are probed for modifications using specific substrates.

Microarrays

A DNA microarray is a collection of spots attached to a solid support such as a microscope slide where each spot contains one or more single-stranded DNA oligonucleotide fragments. Arrays make it possible to put down large quantities of very small (100 micrometre diameter) spots on a single slide. Each spot has a DNA fragment molecule that is complementary to a single DNA sequence. A variation of this technique allows the gene expression of an organism at a particular stage in development to be qualified (expression profiling). In this technique the RNA in a tissue is isolated and converted to labeled complementary DNA (cDNA). This cDNA is then hybridized to the fragments on the array and visualization of the hybridization can be done. Since multiple arrays can be made with exactly the same position of fragments, they are particularly useful for comparing the gene expression of two

different tissues, such as a healthy and cancerous tissue. Also, one can measure what genes are expressed and how that expression changes with time or with other factors. There are many different ways to fabricate microarrays; the most common are silicon chips, microscope slides with spots of ~100 micrometre diameter, custom arrays, and arrays with larger spots on porous membranes (macroarrays). There can be anywhere from 100 spots to more than 10,000 on a given array. Arrays can also be made with molecules other than DNA.

Allele-specific oligonucleotide

Allele-specific oligonucleotide (ASO) is a technique that allows detection of single base mutations without the need for PCR or gel electrophoresis. Short (20–25 nucleotides in length), labeled probes are exposed to the non-fragmented target DNA, hybridization occurs with high specificity due to the short length of the probes and even a single base change will hinder hybridization. The target DNA is then washed and the labeled probes that didn't hybridize are removed. The target DNA is then analyzed for the presence of the probe via radioactivity or fluorescence. In this experiment, as in most molecular biology techniques, a control must be used to ensure successful experimentation.

In molecular biology, procedures and technologies are continually being developed and older technologies abandoned. For example, before the advent of DNA gel electrophoresis (agarose or polyacrylamide), the size of DNA molecules was typically determined by rate sedimentation in sucrose gradients, a slow and labor-intensive technique requiring expensive instrumentation; prior to sucrose gradients,

viscometry was used. Aside from their historical interest, it is often worth knowing about older technology, as it is occasionally useful to solve another new problem for which the newer technique is inappropriate.

Food processing

Food processing is the transformation of agricultural products into food, or of one form of food into other forms. Food processing includes many forms of processing foods, from grinding grain to make raw flour to home cooking to complex industrial methods used to make convenience foods. Some food processing methods play important roles in reducing food waste and improving food preservation, thus reducing the total environmental impact of agriculture and improving food security.

Primary food processing is necessary to make most foods edible, and secondary food processing turns the ingredients into familiar foods, such as bread. Tertiary food processing has been criticized for promoting overnutrition and obesity, containing too much sugar and salt, too little fiber, and otherwise being unhealthful in respect to dietary needs of humans and farm animals.

Process

Primary food processing

Primary food processing turns agricultural products, such as raw wheat kernels or livestock, into something that can

eventually be eaten. This category includes ingredients that are produced by ancient processes such as drying, threshing, winnowing and milling grain, shelling nuts, and butchering animals for meat. It also includes deboning and cutting meat, freezing and smoking fish and meat, extracting and filtering oils, canning food, preserving food through food irradiation, and candling eggs, as well as homogenizing and pasteurizing milk.

Contamination and spoilage problems in primary food processing can lead to significant public health threats, as the resulting foods are used so widely. However, many forms of processing contribute to improved food safety and longer shelf life before the food spoils. Commercial food processing uses control systems such as hazard analysis and critical control points (HACCP) and failure mode and effects analysis (FMEA) to reduce the risk of harm.

Secondary food processing

Secondary food processing is the everyday process of creating food from ingredients that are ready to use. Baking bread, regardless of whether it is made at home, in a small bakery, or in a large factory, is an example of secondary food processing.Fermenting fish and making wine, beer, and other alcoholic products are traditional forms of secondary food processing.Sausages are a common form of secondary processed meat, formed by comminution (grinding) of meat that has already undergone primary processing. Most of the secondary food processing methods known to human kind are commonly described as cooking methods.

Tertiary food processing

Tertiary food processing is the commercial production of what is commonly called processed food. These are ready-to-eat or heat-and-serve foods, such as TV dinners and re-heated airline meals.

History

- Food processing dates back to the prehistoric ages when crude processing incorporated fermenting, sun drying, preserving with salt, and various types of cooking (such as roasting, smoking, steaming, and oven baking), Such basic food processing involved chemical enzymatic changes to the basic structure of food in its natural form, as well served to build a barrier against surface microbial activity that caused rapid decay. Salt-preservation was especially common for foods that constituted warrior and sailors' diets until the introduction of canning methods. Evidence for the existence of these methods can be found in the writings of the ancient Greek, Chaldean, Egyptian and Roman civilizations as well as archaeological evidence from Europe, North and South America and Asia. These tried and tested processing techniques remained essentially the same until the advent of the industrial revolution. Examples of ready-meals also date back to before the preindustrial revolution, and include dishes such as Cornish pasty and Haggis. Both

during ancient times and today in modern society these are considered processed foods.

- Modern food processing technology developed in the 19th and 20th centuries was developed in a large part to serve military needs. In 1809Nicolas Appert invented a hermetic bottling technique that would preserve food for French troops which ultimately contributed to the development of tinning, and subsequently canning by Peter Durand in 1810. Although initially expensive and somewhat hazardous due to the lead used in cans, canned goods would later become a staple around the world. Pasteurization, discovered by Louis Pasteur in 1864, improved the quality and safety of preserved foods and introduced the wine, beer, and milk preservation.

In the 20th century, World War II, the space race and the rising consumer society in developed countries contributed to the growth of food processing with such advances as spray drying, evaporation, juice concentrates, freeze drying and the introduction of artificial sweeteners, colouring agents, and such preservatives as sodium benzoate. In the late 20th century, products such as dried instant soups, reconstituted fruits and juices, and self cooking meals such as MRE food ration were developed. By the 20th century, automatic appliances like microwave oven, blender, and rotimatic paved way for convenience cooking.

In western Europe and North America, the second half of the 20th century witnessed a rise in the pursuit of convenience. Food processing companies marketed their products especially

towards middle-class working wives and mothers. Frozen foods (often credited to Clarence Birdseye) found their success in sales of juice concentrates and "TV dinners". Processors utilised the perceived value of time to appeal to the postwar population, and this same appeal contributes to the success of convenience foods today.

Benefits and drawbacks

Benefits

Benefits of food processing include toxin removal, preservation, easing marketing and distribution tasks, and increasing food consistency. In addition, it increases yearly availability of many foods, enables transportation of delicate perishable foods across long distances and makes many kinds of foods safe to eat by de-activating spoilage and pathogenic micro-organisms. Modern supermarkets would not exist without modern food processing techniques, and long voyages would not be possible.

Processed foods are usually less susceptible to early spoilage than fresh foods and are better suited for long-distance transportation from the source to the consumer. When they were first introduced, some processed foods helped to alleviate food shortages and improved the overall nutrition of populations as it made many new foods available to the masses.

Processing can also reduce the incidence of food-borne disease. Fresh materials, such as fresh produce and raw meats, are more likely to harbourpathogenic micro-organisms (e.g. Salmonella) capable of causing serious illnesses.

The extremely varied modern diet is only truly possible on a wide scale because of food processing. Transportation of more exotic foods, as well as the elimination of much hard labor gives the modern eater easy access to a wide variety of food unimaginable to their ancestors.

The act of processing can often improve the taste of food significantly.

Mass production of food is much cheaper overall than individual production of meals from raw ingredients. Therefore, a large profit potential exists for the manufacturers and suppliers of processed food products. Individuals may see a benefit in convenience, but rarely see any direct financial cost benefit in using processed food as compared to home preparation.

Processed food freed people from the large amount of time involved in preparing and cooking "natural" unprocessed foods. The increase in free time allows people much more choice in life style than previously allowed. In many families the adults are working away from home and therefore there is little time for the preparation of food based on fresh ingredients. The food industry offers products that fulfill many different needs: e.g. fully prepared ready meals that can be heated up in the microwave oven within a few minutes.

Modern food processing also improves the quality of life for people with allergies, diabetics, and other people who cannot consume some common food elements. Food processing can also add extra nutrients such as vitamins.

Drawbacks

Processing of food can decrease its nutritional density. The amount of nutrients lost depends on the food and processing method. For example, heat destroys vitamin C. Therefore, canned fruits possess less vitamin C than their fresh alternatives. The USDA conducted a study of nutrient retention in 2004, creating a table of foods, levels of preparation, and nutrition. New research highlighting the importance to human health of a rich microbial environment in the intestine indicates that abundant food processing (not fermentation of foods) endangers that environment. Using some food additives represents another safety concern. The health risks of any given additive vary greatly from person to person; for example using sugar as an additive endangers diabetics. In the European Union, only European Food Safety Authority (EFSA) approved food additives (e.g., sweeteners, preservatives, stabilizers) are permitted at specified levels for use in food products. Approved additives receive an E number (E for Europe), simplifying communication about food additives included in the ingredients' list for all the different languages spoken in the EU. As effects of chemical additives are learned, changes to laws and regulatory practices are made to make such processed foods more safe. Food processing is typically a mechanical process that utilizes extrusion, large mixing, grinding, chopping and emulsifying equipment in the production process. These processes introduce a number of contamination risks. Such contaminants are left over material from a previous operation, animal or human bodily fluids, microorganisms, nonmetallic and metallic fragments. Further processing of these contaminants will result in downstream equipment failure and the risk of ingestion by the consumer.

Example: A mixing bowl or grinder is used over time, metal parts in contact with food will tend to fail and fracture. This type of failure will introduce into the product stream small to large metal contaminants. Further processing of these metal fragments will result in downstream equipment failure and the risk of ingestion by the consumer. Food manufacturers utilize industrial metal detectors to detect and reject automatically any metal fragment. Large food processors will utilize many metal detectors within the processing stream to reduce both damage to processing machinery as well as risk to consumer health. Food processing does have some benefits, such as making food last longer and making products more convenient. However, heavily processed foods also have drawbacks. Whole foods and those that are only minimally processed, like frozen vegetables without any sauce, tend to be more healthy. An unhealthy diet high in fat, added sugar and salt, such as one containing much highly processed food, can increase the risk for cancer, type 2 diabetes and heart disease, according to the World Health Organization.

Added sodium

One of the main sources for sodium in the diet is processed foods. Sodium, mostly in the form of sodium chloride, i.e. salt, is added to prevent spoilage, add flavor and improve the texture of these foods. Some processed food may contain over 2% salt. Americans consume an average of 3436 milligrams of sodium per day, which is higher than the recommended limit of 2300 milligrams per day for healthy people, and more than twice the limit of 1500 milligrams per day for those at increased risk for heart disease.

Added sugars

While it is not necessary to limit the sugars found naturally in whole, unprocessed foods like fresh fruit, eating too much added sugar found in many processed foods increases the risk of heart disease, obesity, cavities and Type 2 diabetes. The American Heart Association recommends women limit added sugars to no more than 420 kilojoules (100 kilocalories), or 25 grams, and men limit added sugars to no more than 650 kJ (155 kcal), or about 38.75 grams, per day. Currently, Americans consume an average of 1,490 kJ (355 kcal) from added sugars each day.

Nutrient losses

Processing foods often involves nutrient losses, which can make it harder to meet the body's needs if these nutrients aren't added back through fortification or enrichment. For example, using high heat during processing can cause vitamin C losses. Another example is refined grains, which have less fiber, vitamins and minerals than whole grains. Eating refined grains, such as those found in many processed foods, instead of whole grains may increase the risk for high cholesterol, diabetes and obesity, according to a study published in "The American Journal of Clinical Nutrition" in December 2007.

Trans fats

Foods that have undergone processing, including some commercial baked goods, desserts, margarine, frozen pizza,

microwave popcorn and coffee creamers, sometimes contain trans fats. This is the most unhealthy type of fat, and may increase risk for high cholesterol, heart disease and stroke. The 2010 Dietary Guidelines for Americans recommends keeping trans fat intake as low as possible.

Other potential disadvantages

Processed foods may actually take less energy to digest than whole foods, according to a study published in "Food & Nutrition Research" in 2010, meaning more of their food energy content is retained within the body. Processed foods also tend to be more allergenic than whole foods, according to a June 2004 "Current Opinion in Allergy and Clinical Immunology" article. Although the preservatives and other food additives used in many processed foods are generally recognized as safe, a few may cause problems for some individuals, including sulfites, artificial sweeteners, artificial colors and flavors, sodium nitrate, BHA and BHT, olestra, caffeine and monosodium glutamate.

Performance parameters for food processing

When designing processes for the food industry the following performance parameters may be taken into account:

Hygiene, e.g. measured by number of micro-organisms per mL of finished product

Energy efficiency measured e.g. by “ton of steam per ton of sugar produced”

Minimization of waste, measured e.g. by “percentage of peeling loss during the peeling of potatoes”

Labour used, measured e.g. by “number of working hours per ton of finished product”

Minimization of cleaning stops measured e.g. by “number of hours between cleaning stops”

Chapter 6

Protein Biosynthesis

Protein biosynthesis (or **protein synthesis**) is a core biological process, occurring inside cells, balancing the loss of cellular proteins (via degradation or export) through the production of new proteins. Proteins perform a number of critical functions as enzymes, structural proteins or hormones. Protein synthesis is a very similar process for both prokaryotes and eukaryotes but there are some distinct differences.

Protein synthesis can be divided broadly into two phases - transcription and translation. During transcription, a section of DNA encoding a protein, known as a gene, is converted into a template molecule called messenger RNA (mRNA). This conversion is carried out by enzymes, known as RNA polymerases, in the nucleus of the cell. In eukaryotes, this mRNA is initially produced in a premature form (pre-mRNA) which undergoes post-transcriptional modifications to produce mature mRNA. The mature mRNA is exported from the cell nucleus via nuclear pores to the cytoplasm of the cell for translation to occur. During translation, the mRNA is read by ribosomes which use the nucleotide sequence of the mRNA to determine the sequence of amino acids. The ribosomes catalyze the formation of covalentpeptide bonds between the encoded amino acids to form a polypeptide chain.

Following translation the polypeptide chain must fold to form a functional protein; for example, to function as an enzyme the polypeptide chain must fold correctly to produce a functional active site. In order to adopt a functional three-dimensional

(3D) shape, the polypeptide chain must first form a series of smaller underlying structures called secondary structures. The polypeptide chain in these secondary structures then folds to produce the overall 3D tertiary structure. Once correctly folded, the protein can undergo further maturation through different post-translational modifications. Post-translational modifications can alter the protein's ability to function, where it is located within the cell (e.g. cytoplasm or nucleus) and the protein's ability to interact with other proteins.

Protein biosynthesis has a key role in disease as changes and errors in this process, through underlying DNA mutations or protein misfolding, are often the underlying causes of a disease. DNA mutations change the subsequent mRNA sequence, which then alters the mRNA encoded amino acid sequence. Mutations can cause the polypeptide chain to be shorter by generating a stop sequence which causes early termination of translation. Alternatively, a mutation in the mRNA sequence changes the specific amino acid encoded at that position in the polypeptide chain. This amino acid change can impact the protein's ability to function or to fold correctly. Misfolded proteins are often implicated in disease as improperly folded proteins have a tendency to stick together to form dense protein clumps. These clumps are linked to a range of diseases, often neurological, including Alzheimer's disease and Parkinson's disease.

Transcription

- Transcription occurs in the nucleus using DNA as a template to produce mRNA. In eukaryotes, this mRNA molecule is known as pre-mRNA as it

undergoes post-transcriptional modifications in the nucleus to produce a mature mRNA molecule. However, in prokaryotes post-transcriptional modifications are not required so the mature mRNA molecule is immediately produced by transcription.

Initially, an enzyme known as a helicase acts on the molecule of DNA. DNA has an antiparallel, double helix structure composed of two, complementary polynucleotide strands, held together by hydrogen bonds between the base pairs. The helicase disrupts the hydrogen bonds causing a region of DNA - corresponding to a gene - to unwind, separating the two DNA strands and exposing a series of bases. Despite DNA being a double stranded molecule, only one of the strands acts as a template for pre-mRNA synthesis - this strand is known as the template strand. The other DNA strand (which is complementary to the template strand) is known as the coding strand.

Both DNA and RNA have intrinsic directionality, meaning there are two distinct ends of the molecule. This property of directionality is due to the asymmetrical underlying nucleotide subunits, with a phosphate group on one side of the pentose sugar and a base on the other. The five carbons in the pentose sugar are numbered from 1' (where ' means prime) to 5'. Therefore, the phosphodiester bonds connecting the nucleotides are formed by joining the hydroxyl group of on the 3' carbon of one nucleotide to the phosphate group on the 5' carbon of another nucleotide. Hence, the coding strand of DNA runs in a 5' to 3' direction and the complementary, template DNA strand runs in the opposite direction from 3' to 5'.

The enzyme RNA polymerase binds to the exposed template strand and reads from the gene in the 3' to 5' direction. Simultaneously, the RNA polymerase synthesizes a single strand of pre-mRNA in the 5'-to-3' direction by catalysing the formation of phosphodiester bonds between activated nucleotides (free in the nucleus) that are capable of complementary base pairing with the template strand. Behind the moving RNA polymerase the two strands of DNA rejoin, so only 12 base pairs of DNA are exposed at one time. RNA polymerase builds the pre-mRNA molecule at a rate of 20 nucleotides per second enabling the production of thousands of pre-mRNA molecules from the same gene in an hour. Despite the fast rate of synthesis, the RNA polymerase enzyme contains its own proofreading mechanism. The proofreading mechanisms allows the RNA polymerase to remove incorrect nucleotides (which are not complementary to the template strand of DNA) from the growing pre-mRNA molecule through an excision reaction. When RNA polymerases reaches a specific DNA sequence which terminates transcription, RNA polymerase detaches and pre-mRNA synthesis is complete.

The pre-mRNA molecule synthesized is complementary to the template DNA strand and shares the same nucleotide sequence as the coding DNA strand. However, there is one crucial difference in the nucleotide composition of DNA and mRNA molecules. DNA is composed of the bases - guanine, cytosine, adenine and thymine (G, C, A and T) - RNA is also composed of four bases - guanine, cytosine, adenine and uracil. In RNA molecules, the DNA base thymine is replaced by uracil which is able to base pair with adenine. Therefore, in the pre-mRNA molecule, all complementary bases which would be thymine in the coding DNA strand are replaced by uracil.

Post-transcriptional modifications

Once transcription is complete, the pre-mRNA molecule undergoes post-transcriptional modifications to produce a mature mRNA molecule.

There are 3 key steps within post-transcriptional modifications:

- Addition of a 5' cap to the 5' end of the pre-mRNA molecule
- Addition of a 3' poly(A) tail is added to the 3' end pre-mRNA molecule
- Removal of introns via RNA splicing

The 5' cap is added to the 5' end of the pre-mRNA molecule and is composed of a guanine nucleotide modified through methylation. The purpose of the 5' cap is to prevent break down of mature mRNA molecules before translation, the cap also aids binding of the ribosome to the mRNA to start translation and enables mRNA to be differentiated from other RNAs in the cell. In contrast, the 3' Poly(A) tail is added to the 3' end of the mRNA molecule and is composed of 100-200 adenine bases. These distinct mRNA modifications enable the cell to detect that the full mRNA message is intact if both the 5' cap and 3' tail are present.

This modified pre-mRNA molecule then undergoes the process of RNA splicing. Genes are composed of a series of introns and exons, introns are nucleotide sequences which do not encode a protein while, exons are nucleotide sequences that directly encode a protein. Introns and exons are present in both the underlying DNA sequence and the pre-mRNA molecule,

therefore, in order to produce a mature mRNA molecule encoding a protein, splicing must occur. During splicing, the intervening introns are removed from the pre-mRNA molecule by a multi-protein complex known as a spliceosome (composed of over 150 proteins and RNA). This mature mRNA molecule is then exported into the cytoplasm through nuclear pores in the envelope of the nucleus.

Translation

During translation, ribosomes synthesize polypeptide chains from mRNA template molecules. In eukaryotes, translation occurs in the cytoplasm of the cell, where the ribosomes are located either free floating or attached to the endoplasmic reticulum. In prokaryotes, which lack a nucleus, the processes of both transcription and translation occur in the cytoplasm.

Ribosomes are complex molecular machines, made of a mixture of protein and ribosomal RNA, arranged into two subunits (a large and a small subunit), which surround the mRNA molecule. The ribosome reads the mRNA molecule in a 5'-3' direction and uses it as a template to determine the order of amino acids in the polypeptide chain. In order to translate the mRNA molecule, the ribosome uses small molecules, known as transfer RNAs (tRNA), to deliver the correct amino acids to the ribosome. Each tRNA is composed of 70-80 nucleotides and adopts a characteristic cloverleaf structure due to the formation of hydrogen bonds between the nucleotides within the molecule. There are around 60 different types of tRNAs, each tRNA binds to a specific sequence of three nucleotides (known as a codon) within the mRNA molecule and delivers a specific amino acid.

The ribosome initially attaches to the mRNA at the start codon (AUG) and begins to translate the molecule. The mRNA nucleotide sequence is read in triplets - three adjacent nucleotides in the mRNA molecule correspond to a single codon. Each tRNA has an exposed sequence of three nucleotides, known as the anticodon, which are complementary in sequence to a specific codon that may be present in mRNA. For example, the first codon encountered is the start codon composed of the nucleotides AUG. The correct tRNA with the anticodon (complementary 3 nucleotide sequence UAC) binds to the mRNA using the ribosome. This tRNA delivers the correct amino acid corresponding to the mRNA codon, in the case of the start codon, this is the amino acid methionine. The next codon (adjacent to the start codon) is then bound by the correct tRNA with complementary anticodon, delivering the next amino acid to ribosome. The ribosome then uses its peptidyl transferase enzymatic activity to catalyze the formation of the covalent peptide bond between the two adjacent amino acids.

The ribosome then moves along the mRNA molecule to the third codon. The ribosome then releases the first tRNA molecule, as only two tRNA molecules can be brought together by a single ribosome at one time. The next complementary tRNA with the correct anticodon complementary to the third codon is selected, delivering the next amino acid to the ribosome which is covalently joined to the growing polypeptide chain. This process continues with the ribosome moving along the mRNA molecule adding up to 15 amino acids per second to the polypeptide chain. Behind the first ribosome, up to 50 additional ribosomes can bind to the mRNA molecule forming a polysome, this enables simultaneous synthesis of multiple

identical polypeptide chains. Termination of the growing polypeptide chain occurs when the ribosome encounters a stop codon (UAA, UAG, or UGA) in the mRNA molecule. When this occurs, no tRNA can recognise it and a release factor induces the release of the complete polypeptide chain from the ribosome. Dr. HarGobind Khorana, a scientist originating from India, decoded the RNA sequences for about 20 amino acids. He was awarded the Nobel Prize in 1968, along with two other scientists, for his work.

Protein folding

Once synthesis of the polypeptide chain is complete, the polypeptide chain folds to adopt a specific structure which enables the protein to carry out its functions. The basic form of protein structureis known as the primary structure, which is simply the polypeptide chain i.e. a sequence of covalently bonded amino acids. The primary structure of a protein is encoded by a gene. Therefore, any changes to the sequence of the gene can alter the primary structure of the protein and all subsequent levels of protein structure, ultimately changing the overall structure and function.

The primary structure of a protein (the polypeptide chain) can then fold or coil to form the secondary structure of the protein. The most common types of secondary structure are known as an alpha helix or beta sheet, these are small structures produced by hydrogen bonds forming within the polypeptide chain. This secondary structure then folds to produce the tertiary structure of the protein. The tertiary structure is the proteins overall 3D structure which is made of different secondary structures folding together. In the tertiary

structure, key protein features e.g. the active site, are folded and formed enabling the protein to function. Finally, some proteins may adopt a complex quaternary structure. Most proteins are made of a single polypeptide chain, however, some proteins are composed of multiple polypeptide chains (known as subunits) which fold and interact to form the quaternary structure. Hence, the overall protein is a multi-subunit complex composed of multiple folded, polypeptide chain subunits e.g. haemoglobin.

Post-translational modifications

When protein folding into the mature, functional 3D state is complete, it is not necessarily the end of the protein maturation pathway. A folded protein can still undergo further processing through post-translational modifications. There are over 200 known types of post-translational modification, these modifications can alter protein activity, the ability of the protein to interact with other proteins and where the protein is found within the cell e.g. in the cell nucleus or cytoplasm. Through post-translational modifications, the diversity of proteins encoded by the genome is expanded by 2 to 3 orders of magnitude.

There are four key classes of post-translational modification:

- Cleavage
- Addition of chemical groups
- Addition of complex molecules
- Formation of intramolecular bonds

Cleavage

Cleavage of proteins is an irreversible post-translational modification carried out by enzymes known as proteases. These proteases are often highly specific and cause hydrolysis of a limited number of peptide bonds within the target protein. The resulting shortened protein has an altered polypeptide chain with different amino acids at the start and end of the chain. This post-translational modification often alters the proteins function, the protein can be inactivated or activated by the cleavage and can display new biological activities.

Addition of chemical groups

Following translation, small chemical groups can be added onto amino acids within the mature protein structure. Examples of processes which add chemical groups to the target protein include methylation, acetylation and phosphorylation.

Methylation is the reversible addition of a methyl group onto an amino acid catalyzed by methyltransferase enzymes. Methylation occurs on at least 9 of the 20 common amino acids, however, it mainly occurs on the amino acids lysine and arginine. One example of a protein which is commonly methylated is a histone. Histones are proteins found in the nucleus of the cell. DNA is tightly wrapped round histones and held in place by other proteins and interactions between negative charges in the DNA and positive charges on the histone. A highly specific pattern of amino acid methylation on the histone proteins is used to determine which regions of DNA are tightly wound and unable to be transcribed and which regions are loosely wound and able to be transcribed.

Histone-based regulation of DNA transcription is also modified by acetylation. Acetylation is the reversible covalent addition of an acetyl group onto a lysine amino acid by the enzyme acetyltransferase. The acetyl group is removed from a donor molecule known as acetyl coenzyme A and transferred onto the target protein.Histones undergo acetylation on their lysine residues by enzymes known as histone acetyltransferase. The effect of acetylation is to weaken the charge interactions between the histone and DNA, thereby making more genes in the DNA accessible for transcription.

The final, prevalent post-translational chemical group modification is phosphorylation. Phosphorylation is the reversible, covalent addition of a phosphate group to specific amino acids (serine, threonine and tyrosine) within the protein. The phosphate group is removed from the donor molecule ATP by a protein kinase and transferred onto the hydroxyl group of the target amino acid, this produces adenosine diphosphate as a biproduct. This process can be reversed and the phosphate group removed by the enzyme protein phosphatase. Phosphorylation can create a binding site on the phosphorylated protein which enables it to interact with other proteins and generate large, multi-protein complexes. Alternatively, phosphorylation can change the level of protein activity by altering the ability of the protein to bind its substrate.

Addition of complex molecules

Post-translational modifications can incorporate more complex, large molecules into the folded protein structure. One common example of this is glycosylation, the addition of a

polysaccharide molecule, which is widely considered to be most common post-translational modification.

In glycosylation, a polysaccharide molecule (known as a glycan) is covalently added to the target protein by glycosyltransferases enzymes and modified by glycosidases in the endoplasmic reticulum and Golgi apparatus. Glycosylation can have a critical role in determining the final, folded 3D structure of the target protein. In some cases glycosylation is necessary for correct folding. N-linked glycosylation promotes protein folding by increasing solubility and mediates the protein binding to protein chaperones. Chaperones are proteins responsible for folding and maintaining the structure of other proteins.

There are broadly two types of glycosylation, N-linked glycosylation and O-linked glycosylation. N-linked glycosylation starts in the endoplasmic reticulum with the addition of a precursor glycan. The precursor glycan is modified in the Golgi apparatus to produce complex glycan bound covalently to the nitrogen in an asparagine amino acid. In contrast, O-linked glycosylation is the sequential covalent addition of individual sugars onto the oxygen in the amino acids serine and threonine within the mature protein structure.

Formation of covalent bonds

Many proteins produced within the cell are secreted outside the cell to function as extracellular proteins. Extracellular proteins are exposed to a wide variety of conditions. In order to stabilize the 3D protein structure, covalent bonds are formed

either within the protein or between the different polypeptide chains in the quaternary structure. The most prevalent type is a disulfide bond (also known as a disulfide bridge). A disulfide bond is formed between two cysteine amino acids using their side chain chemical groups containing a Sulphur atom, these chemical groups are known as thiol functional groups. Disulfide bonds act to stabilize the pre-existing structure of the protein. Disulfide bonds are formed in an oxidation reaction between two thiol groups and therefore, need an oxidizing environment to react. As a result, disulfide bonds are typically formed in the oxidizing environment of the endoplasmic reticulum catalyzed by enzymes called protein disulfide isomerases. Disulfide bonds are rarely formed in the cytoplasm as it is a reducing environment.

Role of protein synthesis in disease

Many diseases are caused by mutations in genes, due to the direct connection between the DNA nucleotide sequence and the amino acid sequence of the encoded protein. Changes to the primary structure of the protein can result in the protein mis-folding or malfunctioning. Mutations within a single gene have been identified as a cause of multiple diseases, including sickle cell disease, known as single gene disorders.

Sickle cell disease

Sickle cell disease is a group of diseases caused by a mutation in a subunit of hemoglobin, a protein found in red blood cells responsible for transporting oxygen. The most dangerous of the sickle cell diseases is known as sickle cell anemia. Sickle cell

anemia is the most common homozygous recessive single gene disorder, meaning the sufferer must carry a mutation in both copies of the affected gene (one inherited from each parent) to suffer from the disease.

Hemoglobin has a complex quaternary structure and is composed of four polypeptide subunits - two A subunits and two B subunits. Patients suffering from sickle cell anemia have a missense or substitution mutation in the gene encoding the hemoglobin B subunit polypeptide chain. A missense mutation means the nucleotide mutation alters the overall codon triplet such that a different amino acid is paired with the new codon. In the case of sickle cell anemia, the most common missense mutation is a single nucleotide mutation from thymine to adenine in the hemoglobin B subunit gene. This changes codon 6 from encoding the amino acid glutamic acid to encoding valine.

This change in the primary structure of the hemoglobin B subunit polypeptide chain alters the functionality of the hemoglobin multi-subunit complex in low oxygen conditions. When red blood cells unload oxygen into the tissues of the body, the mutated haemoglobin protein starts to stick together to form a semi-solid structure within the red blood cell.

This distorts the shape of the red blood cell, resulting in the characteristic "sickle" shape, and reduces cell flexibility. This rigid, distorted red blood cell can accumulate in blood vessels creating a blockage. The blockage prevents blood flow to tissues and can lead to tissue death which causes great pain to the individual.

Peritrich nuclear code

The **peritrich nuclear code** (translation table 30) is a genetic code used by the nuclear genome of the peritrichciliates*Vorticella* and *Opisthonecta*.

The code (30)

- AAs = FFLLSSSSYYEECC*WLLLAPPPPHHQQRRRRIIIMTTTTNNKKSSRRVVVVAAAADDEEGGGG
- Starts = ----------*--------------------M----------
- Base1 = TTTTTTTTTTTTTTTTCCCCCCCCCCCCCCCCAAAAAAAAAAAAAAAAGGGGGGGGGGGGGGGG
- Base2 = TTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGG
- Base3 = TCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAG

Bases: adenine (A), cytosine (C), guanine (G) and thymine (T) or uracil (U).

Amino acids: Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N), Aspartic acid (Asp, D), Cysteine (Cys, C), Glutamic acid (Glu, E), Glutamine (Gln, Q), Glycine (Gly, G), Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S), Threonine (Thr, T), Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V).

Polysome

A **polyribosome** (or **polysome** or **ergosome**) is a group of ribosomes bound to an mRNA molecule like “beads” on a “thread”. It consists of a complex of an mRNA molecule and two or more ribosomes that act to translatemRNA instructions into polypeptides. Originally coined "ergosomes" in 1963, they were further characterized by Jonathan Warner, Paul M. Knopf, and Alex Rich.

Polysomesare formed during the elongation phase when ribosomes and elongation factors synthesize the encoded polypeptide. Multiple ribosomes move along the coding region of mRNA, creating a polysome. The ability of multiple ribosomes to function on an mRNA molecule explains the limited abundance of mRNA in the cell. Polyribosome structure differs between prokaryotic polysomes, eukaryotic polysomes, and membrane bound polysomes.Polysome activity can be used to measure the level of gene expression through a technique called polysomal profiling.

Structure

Electron microscopy technologies such as staining, metal shadowing, and ultra-thin cell sections were the original methods to determine polysome structure. The development of cryo-electron microscopy techniques has allowed for increased resolution of the image, leading to a more precise method to determine structure. Different structural configurations of polyribosomes could reflect a variety in translation of mRNAs. An investigation of the ratio of polyribosomal shape elucidated

that a high number of circular and zigzag polysomeswere found after several rounds of translation. A longer period of translation caused the formation of densely packed 3-D helical polysomes. Different cells produce different structures of polysomes.

Prokaryotic

Bacterial polysomeshave been found to form double-row structures. In this conformation, the ribosomes are contacting each other through smaller subunits. These double row structures generally have a “sinusoidal” (zigzag) or 3-D helical path. In the “sinusoidal” path, there are two types of contact between the small subunits- “top-to-top” or “top-to-bottom”. In the 3-D helical path, only “top-to-top” contact is observed.

Polysomes are present in archaea, but not much is known about the structure.

Eukaryotic

In cells

in situ (in cell) studies have shown that eukaryotic polysomes exhibit linear configurations. Densely packed 3-D helices and planar double-row polysomeswere found with variable packing including “top-to-top” contacts similar to prokaryotic polysomes. Eukaryotic 3-D polyribosomes are similar to prokaryotic 3-D polyribosomes in that they are “densely packed left-handed helices with four ribosomes per turn”. This dense packing can determine their function as regulators of

translation, with 3-D polyribosomes being found in sarcoma cells using fluorescence microscopy.

Cell free

Atomic force microscopy used in *in vitro* studies have shown that circular eukaryotic polysomes can be formed by free polyadenylated mRNA in the presence of initiation factor eIF4E bound to the 5' cap and PABP bound to the 3'-poly(A) tail. However, this interaction between cap and poly(A)-tail mediated by a protein complex is not a unique way of circularizing polysomal mRNA. It has been found that topologically circular polyribosomes can be successfully formed in the translational system with mRNA with no cap and no poly(A) tail as well as a capped mRNA without a 3'-poly(A) tail.

Membrane-bound

Polyribosomes bound to membranes are restricted by a 2 dimensional space given by the membrane surface. The restriction of inter-ribosomal contacts causes a round-shape configuration that arranges ribosomes along the mRNA so that the entry and exit sites form a smooth pathway. Each ribosome is turned relative to the previous one, resembling a planar spiral.

Profiling

Polysomal Profiling is a technique that uses cycloheximide to arrest translation and a sucrose gradient to separate the resulting cell extract by centrifugation. Ribosome-associated mRNAs migrate faster than free mRNAs and polysome

associated mRNAs migrate faster than ribosome associated mRNAs. Several peaks corresponding to mRNA are revealed by the measurement of total protein across the gradient. The corresponding mRNA is associated with increasing numbers of ribosomes as polysomes. The presence of mRNA across the gradient reveals the translation of the mRNA. Polysomal profiling is optimally applied to cultured cells and tissues to track the translational status of an identified mRNA as well as measure ribosome density. This technique has been used to compare the translational status of mRNAs in different cell types.

For example, polysomal profiling was used in a study to investigate the effect of vesicular stomatitis virus (VSV) in mammalian cells. The data from polysomal profiling showed that host mRNAs are outcompeted by viral mRNAs for polysomes, therefore decreasing the translation of host mRNA and increasing the translation of viral mRNA.

Post-translational modification

Post-translational modification (**PTM**) refers to the covalent and generally enzymatic modification of proteins following protein biosynthesis. Proteins are synthesized by ribosomestranslatingmRNA into polypeptide chains, which may then undergo PTM to form the mature protein product. PTMs are important components in cell signaling, as for example when prohormones are converted to hormones.

Post-translational modifications can occur on the amino acidside chains or at the protein's C- or N- termini. They can extend the chemical repertoire of the 20 standard amino acids

by modifying an existing functional group or introducing a new one such as phosphate. Phosphorylation is a very common mechanism for regulating the activity of enzymes and is the most common post-translational modification. Many eukaryotic and prokaryotic proteins also have carbohydrate molecules attached to them in a process called glycosylation, which can promote protein folding and improve stability as well as serving regulatory functions. Attachment of lipid molecules, known as lipidation, often targets a protein or part of a protein attached to the cell membrane.

Other forms of post-translational modification consist of cleaving peptide bonds, as in processing a propeptide to a mature form or removing the initiator methionine residue. The formation of disulfide bonds from cysteine residues may also be referred to as a post-translational modification. For instance, the peptide hormoneinsulin is cut twice after disulfide bonds are formed, and a propeptide is removed from the middle of the chain; the resulting protein consists of two polypeptide chains connected by disulfide bonds.

Some types of post-translational modification are consequences of oxidative stress. Carbonylation is one example that targets the modified protein for degradation and can result in the formation of protein aggregates. Specific amino acid modifications can be used as biomarkers indicating oxidative damage.

Sites that often undergo post-translational modification are those that have a functional group that can serve as a nucleophile in the reaction: the hydroxyl groups of serine, threonine, and tyrosine; the amine forms of lysine, arginine,

and histidine; the thiolateanion of cysteine; the carboxylates of aspartate and glutamate; and the N- and C-termini. In addition, although the amide of asparagine is a weak nucleophile, it can serve as an attachment point for glycans. Rarer modifications can occur at oxidized methionines and at some methylenes in side chains.

Post-translational modification of proteins can be experimentally detected by a variety of techniques, including mass spectrometry, Eastern blotting, and Western blotting. Additional methods are provided in the external links sections.

PTMs involving addition of functional groups

Addition by an enzyme *in vivo*

Hydrophobic groups for membrane localization

- myristoylation (a type of acylation), attachment of myristate, a C_{14} saturated acid
- palmitoylation (a type of acylation), attachment of palmitate, a C_{16} saturated acid
- isoprenylation or prenylation, the addition of an isoprenoid group (e.g. farnesol and geranylgeraniol)
- farnesylation
- geranylgeranylation
- glypiation, glycosylphosphatidylinositol (GPI) anchor formation via an amide bond to C-terminal tail

Cofactors for enhanced enzymatic activity

- lipoylation (a type of acylation), attachment of a lipoate (C_8) functional group
- flavin moiety (FMN or FAD) may be covalently attached
- heme C attachment via thioether bonds with cysteines
- phosphopantetheinylation, the addition of a 4'-phosphopantetheinyl moiety from coenzyme A, as in fatty acid, polyketide, non-ribosomal peptide and leucine biosynthesis
- retinylideneSchiff base formation

Modifications of translation factors

- diphthamide formation (on a histidine found in eEF2)
- ethanolamine phosphoglycerol attachment (on glutamate found in eEF1α)
- hypusine formation (on conserved lysine of eIF5A (eukaryotic) and aIF5A (archaeal))
- beta-Lysine addition on a conserved lysine of the elongation factor P (EFP) in most bacteria. EFP is a homolog to eIF5A (eukaryotic) and aIF5A (archaeal) (see above).

Smaller chemical groups

- acylation, e.g. *O*-acylation (esters), *N*-acylation (amides), *S*-acylation (thioesters)
- acetylation, the addition of an acetyl group, either at the N-terminus of the protein or at lysine

residues.*See also histone acetylation*. The reverse is called deacetylation.

- formylation
- alkylation, the addition of an alkyl group, e.g. methyl, ethyl
- methylation the addition of a methyl group, usually at lysine or arginine residues. The reverse is calleddemethylation.
- amidation at C-terminus. Formed by oxidative dissociation of a C-terminal Gly residue.
- amide bond formation
- amino acid addition
- arginylation, a tRNA-mediation addition
- polyglutamylation, covalent linkage of glutamic acid residues to the N-terminus of tubulin and some other proteins. (See tubulin polyglutamylase)
- polyglycylation, covalent linkage of one to more than 40 glycine residues to the tubulin C-terminal tail
- butyrylation
- gamma-carboxylation dependent on Vitamin K
- glycosylation, the addition of a glycosyl group to either arginine, asparagine, cysteine, hydroxylysine, serine, threonine, tyrosine, or tryptophan resulting in a glycoprotein. Distinct from glycation, which is regarded as a nonenzymatic attachment of sugars.
- *O*-GlcNAc, addition of *N*-acetylglucosamine to serine or threonine residues in a β-glycosidic linkage
- polysialylation, addition of polysialic acid, PSA, to NCAM
- malonylation
- hydroxylation: addition of an oxygen atom to the side-chain of a Pro or Lys residue

- iodination: addition of an iodine atom to the aromatic ring of a tyrosine residue (e.g. in thyroglobulin)
- nucleotide addition such as ADP-ribosylation
- phosphate ester (*O*-linked) or phosphoramidate (*N*-linked) formation
- phosphorylation, the addition of a phosphate group, usually to serine, threonine, and tyrosine (*O*-linked), or histidine (*N*-linked)
- adenylylation, the addition of an adenylyl moiety, usually to tyrosine (*O*-linked), or histidine and lysine (*N*-linked)
- uridylylation, the addition of an uridylyl-group (i.e. uridine monophosphate, UMP), usually to tyrosine
- propionylation
- pyroglutamate formation
- *S*-glutathionylation
- *S*-nitrosylation
- *S*-sulfenylation (*aka**S*-sulphenylation), reversible covalent addition of one oxygen atom to the thiol group of a cysteine residue
- *S*-sulfinylation, normally irreversible covalent addition of two oxygen atoms to the thiol group of a cysteine residue
- *S*-sulfonylation, normally irreversible covalent addition of three oxygen atoms to the thiol group of a cysteine residue, resulting in the formation of a cysteic acid residue
- succinylation addition of a succinyl group to lysine
- sulfation, the addition of a sulfate group to a tyrosine.

Non-enzymatic additions *in vivo*

- glycation, the addition of a sugar molecule to a protein without the controlling action of an enzyme.
- carbamylation the addition of Isocyanic acid to a protein's N-terminus or the side-chain of Lys.
- carbonylation the addition of carbon monoxide to other organic/inorganic compounds.
- spontaneous isopeptide bond formation, as found in many surface proteins of Gram-positive bacteria.

Non-enzymatic additions *in vitro*

- biotinylation: covalent attachment of a biotin moiety using a biotinylation reagent, typically for the purpose of labeling a protein.
- carbamylation: the addition of Isocyanic acid to a protein's N-terminus or the side-chain of Lys or Cys residues, typically resulting from exposure to urea solutions.
- oxidation: addition of one or more Oxygen atoms to a susceptible side-chain, principally of Met, Trp, His or Cys residues. Formation of disulfide bonds between Cys residues.
- pegylation: covalent attachment of polyethylene glycol (PEG) using a pegylation reagent, typically to the N-terminus or the side-chains of Lys residues. Pegylationis used to improve the efficacy of protein pharmaceuticals.

Other proteins or peptides

- ISGylation, the covalent linkage to the ISG15 protein (Interferon-Stimulated Gene 15)
- SUMOylation, the covalent linkage to the SUMO protein (Small Ubiquitin-related MOdifier)
- ubiquitination, the covalent linkage to the protein ubiquitin.
- neddylation, the covalent linkage to Nedd
- pupylation, the covalent linkage to the prokaryotic ubiquitin-like protein

Chemical modification of amino acids

- citrullination, or **deimination**, the conversion of arginine to citrulline
- deamidation, the conversion of glutamine to glutamic acid or asparagine to aspartic acid
- eliminylation, the conversion to an alkene by beta-elimination of phosphothreonine and phosphoserine, or dehydration of threonine and serine

Structural changes

- disulfide bridges, the covalent linkage of two cysteine amino acids
- proteolytic cleavage, cleavage of a protein at a peptide bond

- isoaspartate formation, via the cyclisation of asparagine or aspartic acid amino-acid residues
- racemization
- of serine by protein-serine epimerase
- of alanine in dermorphin, a frog opioid peptide
- of methionine in deltorphin, also a frog opioid peptide
- protein splicing, self-catalytic removal of inteins analogous to mRNA processing