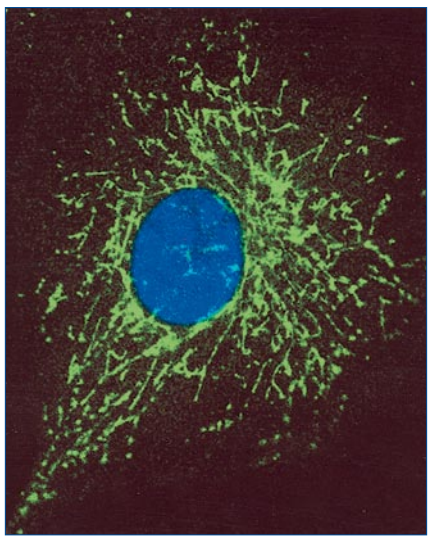
Cell Biology Basics for Discussing Culture Media

by Cheryl Scott

That water is the essential basis for all life is no revelation. Cells are the smallest discrete elements of living organisms, and they are 90% water. The remainder is half protein, 15% each carbohydrates and nucleic acids, and 10% lipids, with the last tenth representing trace compounds and elements such as vitamins and minerals. As small as their presence is relative to the water, however, all those other components are even more vital to living things. They make all the difference between life and death, health and disease, and thus the success and failure of industrial cell cultures.

Before we talk of cells growing in culture — and focus on the media necessary for their growth, production, and storage — several details of cell biology are worth reviewing. Cell culture is like farming on a very small scale. At the most basic level, it is the feeding and care of living things so that they will provide valuable products. Culture media do not serve merely as food for microbes and animal cells; the medium also represents the environment in which they live.

Animal husbandry requires an intimate knowledge of the physical needs of the animals involved. What kind of things do they like to eat? What nutrients will they need not only to thrive, but also to produce? What kind of environmental conditions make them happy and free of stress? How can we best (and most cost-effectively) provide them with the things they need? Those same



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questions are critical to the culture of microoganisms, animal cells/tissues, fungi, and plant cells. In biotechnology, the most common expression systems used to make recombinant proteins, vaccines, diagnostics, and gene therapies involve bacteria, yeast, or animal cells — in that order of increasing complexity and needs. Animal cells are the most finicky (**1**), so this special issue focuses more on their media than those used for microbial fermentation.

Cellular Structure

The first life appeared on Earth nearly four billion years ago in the form of extremophilic archaea and other prokaryotic bacteria. For a couple billion years, that was all the word *life* meant on this planet. Then eukaryotes appeared, and things got a lot more complicated. All known life forms are DNA-based, with membrane-bound cellular structures containing ribosomes and engaging in metabolic activity. But eukaryotic cells added a nucleus and numerous organelles to their cells. The DNA of prokaryotes floats unbound inside them, whereas it is held almost exclusively within the nuclei of eukaryotic cells — with mitochondrial and chloroplast DNA being the only exceptions. It is generally accepted that mitochondria in animals and chloroplasts in plants are both descended from "trapped" prokaryotes that perhaps a billion years ago entered into what has turned out to be a very successful symbiotic relationship with eukaryotes — so successful, in fact, that neither can exist without the other. All the various organelles of eukaryotic cells — mitochondria included — allow a complex intracellular division of labor, which prokaryotes do not have. Such division in turn allows for greater extracellular division of labor, which is what made truly multicellular organisms possible.

Size is another major difference between prokaryotic and eukaryotic cells. "Eukaryotic cells are, on average, 10 times the size of prokaryotic cells" (**2**) and can be as much as 1000 times greater in volume. Ova (egg cells) are among the largest animal cells. In addition, complex eukaryotic genomes are much more extensive than the DNA of prokaryotes. And the nature of cellular membranes differs radically between these two

main types of organisms. Prokaryotes have hard cell walls made of peptidoglycan, an amino-acid–sugar polymer. Animal cells are bound by very fragile membranes mostly made of proteins, lipids, and cholesterol. Some eukaryotes (such as yeasts and plants) have cell walls, but rather than peptidoglycan they are made of chitin (in fungi) or cellulose (in plants).

Prokaryotes are unicellular organisms that do not develop or differentiate into multicellular forms (3). Some bacteria grow in filaments or masses, but each cell in such a colony is identical and capable of independent existence. They may be adjacent to one another because they did not fully separate after division or because they are enclosed in a sheath or slime that the cells secrete. Typically there is no continuity or communication between them. If archaea are considered part of this domain of life (some biologists argue that they should be a separate one entirely), then it may be said that prokaryotes inhabit almost every possible environment on Earth, from the depths of the ocean to hot springs to the organs and systems of multicellular organisms.

As Figure 1 illustrates, a prokaryotic cell is divided into three regions: mobility appendages called *cilia, flagella,* and *pili* (made of microtubule proteins attached to the cell surface); a cellular envelope (made up of the capsule, cell wall, and plasma membrane); and a cytoplasmic region that contains genetic information (DNA) and ribosomes along with various sorts of inclusion bodies (not organelles) mostly involved in cellular metabolism. Prokaryotic DNA comes in plasmid form, as discrete closed loops of nucleic acid, or simple chromosomes. It is translated at the ribosomes into the protein machinery of the cell.

Cytoplasm: Inside all cells is their cytoplasm (or cytosol), which is mostly water with dissolved nutrients, ribosomes, and (in prokaryotes) plasmids. The contents break down waste products and move material around through a process called *cytoplasmic streaming*. The presence of salts makes the cytoplasm a good conductor of electricity, which is important to cellular mechanics. In prokaryotes, all DNA processing takes place in there, unprotected from solutes that may be floating around nearby. When not replicating, prokaryotic chromosomes tend to coil tightly and fold up with certain proteins in a nucleoid structure that is not membrane bound.

Ribosomes are found in both prokaryotes and eukaryotes. An RNA–protein complex of one large and one small structural subunit, the ribosome is responsible for processing genetic instructions carried by messenger RNA (mRNA), translating the genetic code into amino acid sequences that make up new enzymes and other polypeptides. Protein synthesis is so vital that hundreds or even thousands of ribosomes can be present in a cell. And for most biotechnology, the protein factory is what it's all about.

Eukaryotes include fungi, animals, and plants, and most of them are multicellular organisms. Single-celled eukaryotes include yeasts, a few species of which are important in biotechnology (as listed in the "Cell Lines" box). They have tough cell walls similar to those of prokaryotes (made of chitin rather than peptidoglycan), but inside they're as complex as other eukaryotic cells.

A bilayer plasma membrane is all that serves to separate and protect most eukaryotic cells from their surrounding environment. (It is quite fragile, and most multicellular organisms use other methods of protection as well, from specialized cells to secreted materials.) A variety of special transmembrane proteins are scattered across this lipid–cholesterol barrier to act as channels and/or pumps, moving things into and out of the cell. Some eukaryotic cells have flagella or cilia for movement.

Some Cell Lines Important to Biotechnology

Prokaryotes
Bacillus subtilis

Escherichia coli

Eukaryotic Microbes
Pichia pastoris

Saccharomyces cerevisiae

Schizosaccharomyces pombe

Immortalized Animal Cell Lines
BHK-21 (baby hamster kidney fibroblasts)

CHO (Chinese hamster ovary fibroblasts)

HEK293 (human embryonic kidney epithelials)

HeLa (human cervical carcinoma epithelials)

HepG2 (human hepatoma epithelials)

HER (human embryonic retinoblasts, especially the proprietary PER.C6 line developed by Dutch company Crucell NV)

NS0 (mouse myeloma lymphoblastoid-like cells)

Sf9 (*Spodoptera frugiperda* insect ovary epithelials)

Vero (African green monkey kidney epithelials)

Figure 1A: Prokaryote cell (not to scale)

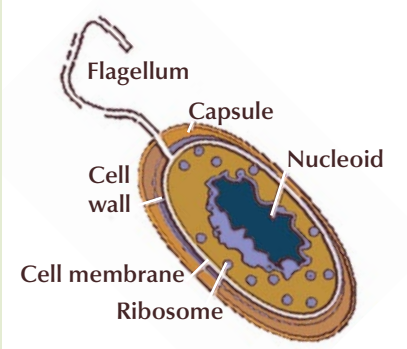
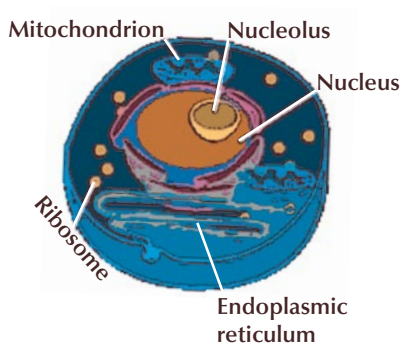


Figure 1B: Eukaryote cell (not to scale)



Whiplike flagella provide the fast, long-distance movement required by spermatazoa, for example. Short cilia act more like oars to spin or move cells around at a slower pace. Another kind of mobility — used by prokaryotic paramecia and eukaryotic white blood cells, for example — involves the temporary formation of pseudopods. The leading edge of the cell stretches out and reaches forth, then pulls the rest along after it in a wormlike motion.

Cytoskeleton: A nearly invisible protein structure called the cytoskeleton acts to organize and maintain the shape of a eukaryotic cell, anchors organelles in place, assists in the import of outside materials, and moves things around. Many types of protein filaments make up the cytoskeleton, including microtubules (like bones for holding cellular shape) and microfilaments (like muscles and tendons for changing shape and supporting various interior structures). Proteins such as myosin and kinesin act as cellular "motors" to move around parts of the cell for purposes of metabolism and overall motility.

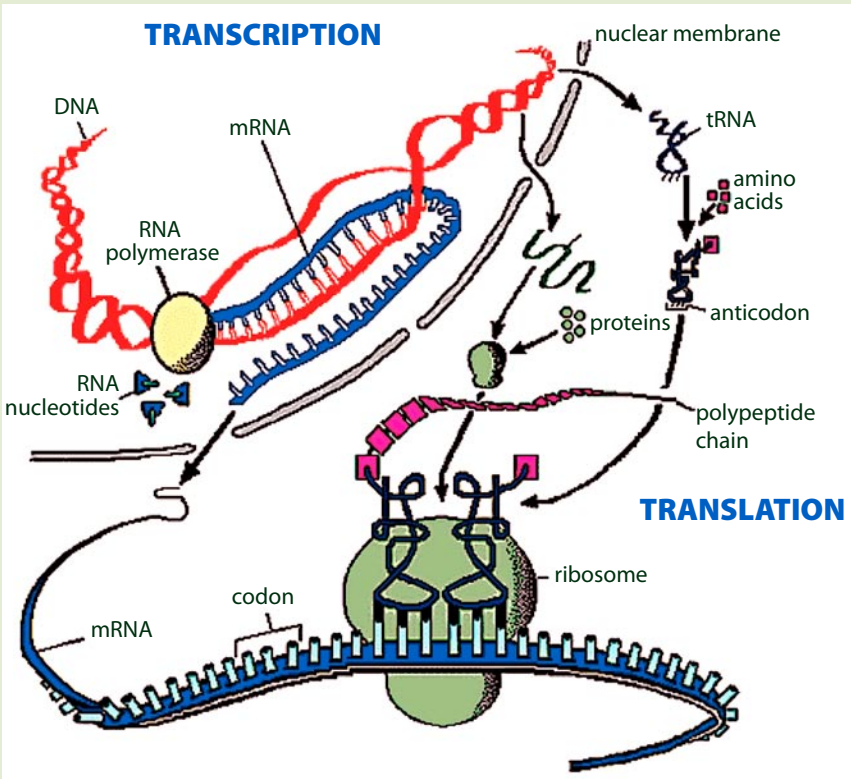
Nucleus: In eukaryotes, ribosomes are constructed in the nucleolus, a smaller organelle within the nucleus, and then transported out to the cytoplasm. The spheroid nucleus, like all organelles, is a discrete membrane-bound structure within each cell. Housing chromosomes, it is the largest, most conspicuous organelle and the site of nearly all DNA replication and RNA synthesis. A nuclear envelope (its special membrane, which is dotted with pores) isolates and protects the cellular genome from structural damage or transcription interference. Transcribed DNA blueprints leave the nucleus as messenger RNA (mRNA). Figure 2 describes the process in more detail. For most of the time, when a cell is not reproducing itself, DNA floats around inside its nucleus in an uncoiled form (chromatin) so it can be translated into RNA for needed proteins. The DNA coils up into chromosomes only during cell division.

Organelles: Eukaryotic cells have several other types of organelles that, like anatomical organs on a larger scale, are each adapted/specialized for carrying out one or more functions. Ribosomes float freely in the cytoplasm or sometimes bind to an organelle called the *endoplasmic reticulum* (ER), which provides a transport network for molecules needing certain modifications or intended for specific destinations. It is basically a mesh of interconnected membranes. A "rough ER" (called such when it is covered with ribosomes) periodically connects to the nuclear envelope to receive mRNA for translation. A smooth ER (without ribosomes) receives proteins synthesized there, passing along the secretory proteins to Golgi bodies (also called *Golgi apparati* or *Golgi complexes,* which look like flattened stacks of membrane-bound sacks) for further processing (such as glycosylation), packaging, and transport.

Vacuoles are simple organelles used as storage areas. Similarly bound by a single membrane, *vesicles* are smaller than vacuoles and involved in exporting and transporting molecules throughout the cell. Think of them as shipping containers. They carry proteins, for example, from the ER to the Golgi bodies.

Lysosomes and *peroxisomes* are somewhat spherical, single-membrane organelles containing digestive enzymes that can degrade proteins, nucleic acids, and polysaccharides. The

Figure 2: Protein synthesis is DNA transcription and translation



Transcription: One strand of a DNA double helix is used as a template by the RNA polymerase to synthesize a strand of messenger RNA (mRNA), which migrates from the nucleus out into the cytoplasm. During this process, mRNA goes through different types of maturation, including one called *splicing,* in which noncoding sequences are eliminated. Each coding mRNA sequence is a unit of three nucleotides called a *codon.*

Translation: A ribosome binds to the mRNA at the "start" codon (AUG) recognized by initiator transfer RNA (tRNA). The ribosome proceeds to "read" and translate the codon sequence into an amino acid sequence, the elongation phase of protein synthesis, in which the organelle progresses from one codon to the next along the mRNA. Complexes of amino acid linked to tRNA sequentially bind to the appropriate codons in the mRNA by forming complementary base pairs with the tRNA anticodon. Amino acids are added one by one, thus translated into polypeptidic sequences dictated by DNA and represented by mRNA. At the end, a release factor binds to the stop codon, terminating translation and releasing the completed polypeptide from the ribosome.
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Biophysics and the Laws of Energy

Energy comes in many forms: mainly heat, light, chemical energy, and electrical energy. Thermodynamics is the study of energy, and three of the four main laws of thermodynamics are relevant to biophysics. Life requires liquid water, and water is liquid only at 0–100 °C, so the fourth law (which states that all processes cease as the temperature approaches zero) does not come into play.

"Zeroth Law": Called such because there is some contention as to whether it should be considered a law, the Zeroth Law of Thermodynamics states that when two systems are placed in contact with one another, there will be an exchange of energy/matter between them unless/until they are in thermodynamic equilibrium. Conversely, two systems are said to be in thermodynamic equilibrium if they both remain the same while in contact.

First Law: The Law of Conservation states that energy can be converted from one form to another, but it cannot be created or destroyed. The total amount of energy/matter in the Universe remains constant.

Second Law: The Law of Entropy states that in all if no energy enters a system, its potential energy at any given point will always be less than that of the initial state. All processes involve a positive heat flow from a colder body to a hotter one. "A watchspring-driven watch will run until the potential energy in the spring is converted, and not again until energy is reapplied to the spring to rewind it. Once the potential energy locked in carbohydrates is converted into kinetic energy (energy in use or motion), the organism will get no more until energy is input again" (1). Some energy dissipates as heat. "Entropy is a measure of disorder: Cells are not disordered and so have low entropy. The flow of energy maintains order and life. Entropy wins when organisms cease to take in energy and die" (1).

Thermokinetics: Potential energy is that which has not yet been used; kinetic energy is that in use (or motion). "A tank of gasoline has a certain potential energy that is converted into kinetic energy by the engine. Batteries, when new or recharged, have a certain potential. Chemicals may also be considered from a potential energy or kinetic energy standpoint. One pound of sugar has a certain potential energy. If that pound of sugar is burned, then the energy is released all at once as heat" (1). Living things would actually burn up if all the energy they took in were released at once through straightforward oxidation. So instead, their cells release it a little bit at a time. "Cells convert potential energy, usually in the form of carbon covalent bonds or ATP molecules, into kinetic energy for cell division, growth, biosynthesis, and active transport, among other things" (1).

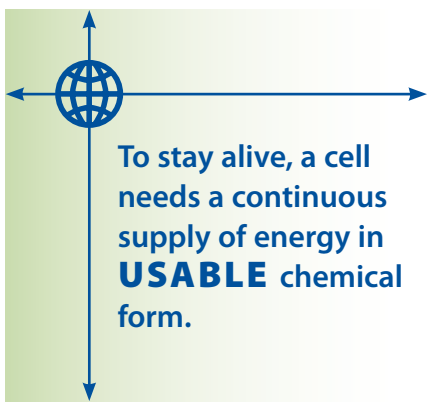
Reference

1 Farabee MJ. *Online Biology Book.* Estrella Mountain Community College: Avondale, AZ, 2002; www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookTOC.html

enzymes work best at the low pH conditions inside their respective organelles, reducing the risk of digesting the contents and structure of their own cell should they somehow escape — illustrating the value of intracellular compartmentalization. No cell could house such destructive enzymes if they were not contained.

Made by Golgi bodies, lysosomes digest invading bacteria, help recycle used membrane components, and degrade worn-out organelles. They sometimes serve to patch plasma membranes, sealing holes if a cell is damaged. Peroxisomes rid the cell of toxic substances, such as hydrogen peroxide and other metabolites. Resembling lysosomes, they are self replicating.

Mitochondria are self-replicating organelles that occur in various numbers, shapes, and sizes in the cytoplasm of all eukaryotic cells. They each contain a plasmid genome that is separate and distinct from the nuclear genome of the associated cell. Although the mitochondrial genome represents a tiny fraction of the eukaryote's overall genome, it codes for some very important proteins. Mitochondria have two functionally distinct membrane systems separated by a space: an outer membrane surrounding the whole mitochondrion and an inner membrane with folds or reticulations called *cristae* that project inward to increase its surface area. The cristae are where the mitochondria's work is done, and their number and shape differ according to the tissue and organism in which a cell is found. *Chloroplasts* are similar to mitochondria but found only in plants, where they use light energy from the sun to make ATP (photosynthesis) in the phototrophic equivalent of cellular metabolism described below.



Cell Metabolism

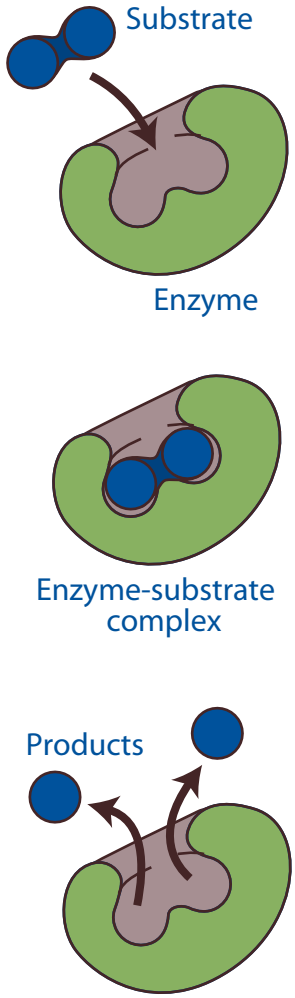
To stay alive, an organism or cell needs a continous supply of energy in usable chemical form. The "Biophysics" box explains the relevant thermodynamics in more detail. *Catabolic* processes break larger organic molecules into their smaller constituents, usually accompanied by the release of energy. *Anabolic* processes assemble small precursors into larger organic molecules, requiring energy input. *Metabolism* is a catch-all word to describe the energy-related chemical processes occurring within an organism or cell. In our discussion of culture media, we're most interested in cellular respiration, as discussed below.

Energy for living things is stored chemically in the form of a nucleotide called adenosine 5´-triphosphate (ATP). Hydrolysis of ATP yields free energy, and intact ATP molecules participate actively in most processes that involve conversion or expenditure of energy. Phototrophic organisms (green plants, algae, and some prokaryotes) derive their energy from light through photosynthesis of ATP. Chemotrophic organisms (animals, fungi, and most prokaryotes) get their energy through food chemistry. For both, the movement of molecules into and out of cells is a complicated and vital business.

Membrane Transport: Membranes are key to life. They keep the essential molecules and machinery of cells together, and they protect those components from damaging conditions and molecules in the environment. Water has a tendency to move from areas of high concentration to areas of low concentration because of a difference in potential energy between where it is and where it's going. The process is called diffusion (or osmosis when it's across a membrane), and because of the "Zeroth Law" of thermodynamics (in the "Biophysics" box), it is common to most gases and liquids. Without membranes, the contents of a cell would mix with its exterior until equilibrium was reached, and thus life could not exist. What cells need is a controlled influx of nutrients and a way to get rid of waste.

Very small, electrically neutral molecules such as water, carbon dioxide (CO_2), and molecular oxygen (O_2) can easily diffuse across cell membranes. Solutes in water decrease its tendency to diffuse, so all the substances mixed together inside a cell help keep the water inside. This depends, of course, on the environment outside: Cells placed in highly concentrated salt-water will lose their free water through osmosis outward; those placed in absolutely pure water will take more and more of it on until they burst. CO_2 is the main waste product of most cellular respiratory processes — especially those important to bioindustrial cell culture — so it is usually present in higher concentration within chemotrophic cells than without. Thus it will diffuse outward naturally. Oxygen, on the other hand, is present at higher concentrations outside the cell, so it will diffuse into the cell, where it is just as important as water is to survival.

Figure 4: Enzyme activity



SOURCE: NATIONAL INSTITUTES OF HEALTH, NATIONAL HUMAN GENOME RESEARCH INSTITUTE, DIVISION OF INTRAMURAL RESEARCH

Those are all examples of passive transport across the membrane. Facilitated diffusion is another. Because the lipids in cell membranes are hydrophobic (4), water-soluble molecules and ions cannot easily diffuse through. Transport proteins embedded through the membrane have structural binding sites that recognize specific molecules or ions. Most transmembrane proteins in higher lifeforms are glycosylated polypeptides: amino-acid chains folded into complex structures that have some hydrophobic and some hydrophilic molecular domains, with sugars attached by Golgi body modification. After binding its target ion/molecule, a transport protein changes shape and moves it across the membrane, where it is released. Then the protein returns to its original configuration, awaiting the next arrival. Facilitated diffusion does not involve a change in energy, so it is not considered an "active" transport.

Active transport is mediated transport of biochemicals and other substances across membranes by processes that require chemical energy. In this case, molecules move against an electrochemical gradient in defiance of the laws of thermodynamics described in the "Biophysics" box. Energy is burned either to alter the affinity of a protein's binding site or alter the rate at which the protein changes conformation. In *primary active transport,* ATP is enzymatically coupled with a given substance to move it across a membrane. *Secondary transport* (either countertransport or cotransport, described below) is diffusion of one molecular type across a membrane to drive the transport of another. The sodium-potassium pump is a form of primary transport, and ion channels are a type of secondary transport. Both are common to all cells.

In *counter-transport,* two types of solute are pumped in opposite directions across a membrane. The flow of one from high to low concentration provides entropic energy to drive movement of the other from low to high. For example, a sodium-calcium exchanger allows three sodium ions into the cell for every calcium ion it moves out. *Cotransport* uses the natural flow of one solute from high to low concentration to piggy-back another molecule with it from low to high. The glucose symporter, for instance, cotransports two sodiums for every molecule of glucose it imports into a cell.

Comparing Microbes and Animal Cells: Cellular respiration is the process by which the chemical bonds of energy-rich molecules such as glucose are exploited for energy. In cellular respiration (Figure 3), some of that energy is trapped in the form of ATP.

CO_2 and water are the waste products. In a multicellular organism, complex sugars in food (carbohydrates, for example) are broken down by digestive systems into the simple sugar glucose, which enters cells through membrane proteins called glucose transporters. Oxidation (burning) of organic material releases energy most often in the form of heat, a main reason animals are warm compared with plants.

Glycolysis, the breakdown of glucose to pyruvic acid (pyruvate), does not require oxygen and is performed by all living things. One molecule of glucose yields two molecules of pyruvate and energy stored as ATP (Figure 4). Glycolysis takes place in the cytoplasm of a cell, and it is a more complicated series of chemical reactions than Figure 4 suggests. Each reaction produces some hydrogen ions that are then used to make ATP energy packets. Only four ATP molecules can be made from one molecule of glucose by this method.

For prokaryotes, glycolysis is the only method used for converting energy. It is anaerobic metabolism, requiring no oxygen. In fact, oxygen was toxic to early life-forms — and still is to many archaea today. Oxygen can be toxic to any form of life in some forms: ozone (O_3), hydrogen peroxide, hydroxyl radicals, superoxide, and free oxygen at high concentrations. And O_2 is thermodynamically unstable, so we owe thanks for its abundance in Earth's atmosphere to green plants, which release it as a waste product. Without continual replenishment in the atmosphere, all O_2 on Earth would end up in unusable (some toxic) molecules through the processes of cellular metabolism discussed here. Eukaryotes improved on the concept of metabolism by oxidizing the pyruvic acid produced by glycolysis in aerobic cellular respiration. They do this through the efforts of their mitochondria, which are probably related to some of the early prokaryotes who were working on the concept a couple billion years ago.

Fermentation is a less efficient, anaerobic method of cellular respiration, and it probably came first. Some prokaryotes improved on glycolysis by using fermentation to extract more energy from food, but the chemical reactions involved produce no ATP for storing energy. They do, however, make one of the main compounds needed for glycolysis, thus feeding backward into the cycle of respiration. Some bacteria and archaea can ferment sugar using a variety of substances: nitrogen and sulfur compounds (nitrates and nitrites; sulfates, sulfites, sulfur dioxide), carbon dioxide, and metal compounds (based on iron, manganese, cobalt, and even uranium). Those various types of fermentation produce different compounds such as ethanol, lactic acid, and hydrogen.

The yeasts we are familiar with in baking, brewing, and biotechnology are masters of fermentation. Unlike bacteria, however, eukaryotes have a choice: In anaerobic conditions, they can get energy through fermentation; in aerobic conditions, they can do something else for even better results. For example, when your muscles don't get enough oxygen, they use fermentation to break pyruvate into lactic acid, CO_2, and water. The lactic acid is what makes you sore. And "walking off the pain" actually works: When the localized conditions in your muscles are made more favorable (aerobic) by increased circulation, they can use oxygen for more efficient metabolism.

Figure 3: Cellular respiration

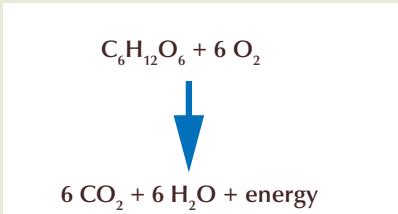
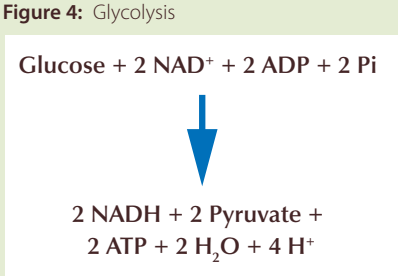


Figure 4: Glycolysis



Aerobic respiration squeezes the most usable energy out of food possible. Pyruvate enters the mitochondria for a complicated process called the Krebs cycle (Figure 5), which involves citric acid and oxygen, among other things, to ultimately yield 24–28 ATP molecules for every glucose molecule entering the system. So in eukaryotic cells, mitochondria play a critical role in turning food into energy.

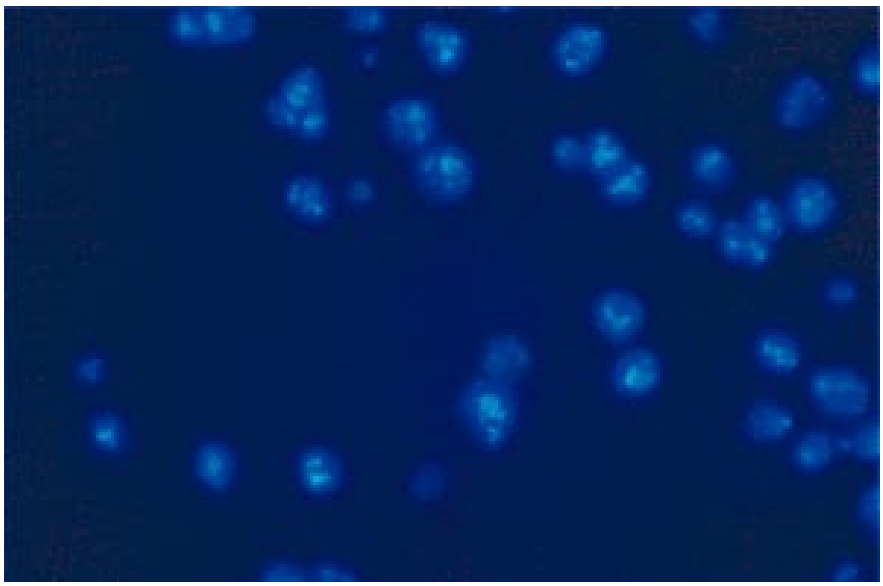
Life and Death

Culturing cells is done in six steps: formulation of media, sterilization of equipment and media, creation of an active culture, growth of the organisms, induction/extraction of the product, and disposal of effluents (**5**). The choice of a batch, continuous, or fed-batch culture process usually depends on the type of product being made (**6**) and the type of organism being used. Figure 6 shows growth kinetics for the three types of culture. Regardless of the methods involved, growth and production are the goal. So there are several facts of life and death that must be considered in process development.

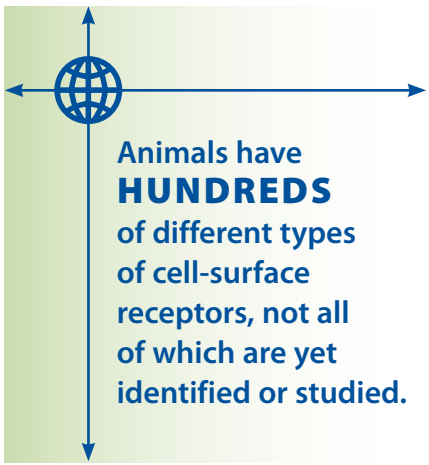
Receptors and Growth: Cells, especially those of multicellular organisms, do not (cannot) exist in a vacuum. They need to somehow communicate with their environment so they can adapt to changing conditions.

> For example, if an *E. coli* bacterium detects a high concentration of lactose in the surrounding environment it begins synthesizing proteins to take in and metabolize the lactose. But if the *E. coli* also detects a high concentration of glucose in the environment it instead begins synthesizing proteins to take in and metabolize the glucose. It also needs to pump in nutrients and release toxic products of its metabolism. How does the *E. coli* know? It depends on its membrane proteins to gather information about the environment in various ways. (**2**)

Each cell in a multicellular organism communicates with dozens to hundreds of other types of cells for



Chromatin-stained hybridoma cells REPRINTED FROM FRANEK F. "PEPTIDES MODULATE GROWTH AND PRODUCTIVITY OF MAMMALIAN CELL CULTURES AND SUPPRESS APOPTOSIS." *BIOPROCESS INTERNATIONAL* 2(6) JUNE 2004: 48–52.



the good of the whole body. They need to know when to grow or go dormant or die, when to secrete proteins or other molecules needed elsewhere, and what other cells to join with for building complex tissues and organs. But their cell membranes act as a barrier to outside influence, as described above. So in addition to embedded transport proteins that move molecules in and out, the membrane has various receptor proteins for communication. Some transporters serve a dual function, but most receptors are separate entities, often adsorbed onto the surface of the cell rather than embedded into its membrane.

Animals have hundreds of different types of cell-surface receptors, not all of them yet identified or studied. For discussion of cell culture (and media in particular) two transmembrane receptors are of the most interest: growth factor receptors and G protein receptors. Guanine nucleotide binding proteins (G-proteins) provide a signaling mechanism that, like a toggle switch, allows or inhibits certain biochemical reactions inside the cell. The "Biomolecules" box provides more detail. Growth factor proteins induce cells to grow and divide (mitosis), so they are sometimes called mitogens. Each type of growth receptor binds only to a specific factor, which allows different parts of a body to grow at different rates.

> The body uses seven membrane-spanning serpentine receptors for an astounding variety of biological signalling functions. Receptors on the cells lining our tongue convey taste. Hundreds of distinct receptor species in the cells of our olfactory bulbs in our nose convey information about the presence of odors (odorant ligands). A carotenoid molecule related to vitamin A is bound in the ligand position of rhodopsin in the rods and cones of our eyes where it serves to pick up photons, alter its conformation, and cause the receptor to which it is bound to release signals into the rod/cone cytoplasm that result in our perception of light. These serpentine receptors are of very ancient lineage. Baker's yeast cells communicate their sexual identity to each other by release of polypeptide mating factors. The cell surface receptors that recognize these mating factors are once again seven membrane-spanning serpentine receptors! (**2**)

Binding of a signal protein to its receptor is only the beginning of a cascade of chemical reactions known as signal transduction. Growth factor receptors, for example, have enzymatic domains at the end opposite their binding site. Capture of a growth factor molecule induces enzymatic activity inside the cell. And that in turn sets off a chain reaction of protein activation and alteration within the cell, ultimately leading to its growth/division. The ligand itself need not be physically transported into the cell for that to occur. And in many cases, signal amplification occurs: A single bound ligand may cause the enzymatic activation of dozens of proteins inside the cell. Each of those in turn can start its own signaling cascade.

Mitosis and Reproduction: In multicellular organisms, cell division (cytokinesis) is induced by growth factors; in microorganisms, it usually comes about in response to an abundance of food. Mitosis is the process of chromosome segregation and nuclear division that replicates genetic material in eukaryotic cells, assuring that each daughter nucleus receives a complete copy of the organism's genome.

Lacking a cellular nucleus, prokaryotes do not undergo mitosis; instead they reproduce through binary fission, which is simple division into two equal (or near equal) parts. First the cellular genome is duplicated (replicated), then each circular strand of DNA attaches itself to the plasma membrane, which grows inward at the middle and splits the cell into two daughter cells. Bacterial DNA mutates more readily than eukaryotic DNA, not only because it is not protected by a cell nucleus, but also because bacterial reproduction is so rapid. That's what makes bacteria capable of adapting to such a variety of environments.

A typical eukaryotic cell cycle is divided into a series of stages: cell growth, the duplication of genetic material, further growth, and nuclear division through mitosis. Mitosis itself is divided into several stages: prophase, prometaphase, metaphase, anaphase, and telophase (Figure 7). With animal cells in culture, such proliferation can be induced by growth factors. Adding food will usually do the trick for microbes. Growth can also be temporarily suspended in either type of cell — usually by a reduction of food.

Apoptosis and Death: Most microorganisms will proliferate as long as conditions allow — that is, as long as there is enough food and water, and temperatures and pressures are favorable. Animal cells, such as those used in biotechnology cell culture, naturally divide only a limited number of times before they die off (a process called *senescence*). The ends of their DNA strands are "capped" with *telomeres*: repeat nucleotides that, like the tight plastic sheaths on the ends of shoelaces, act to protect the real genetic information from "fraying." With each mitotic division those telomeres shorten, and when they're gone the cell can no longer divide. This is one of the bases of the aging process, and it is the main method by which most animal cells "count" divisions (**7**). Exceptions to the rule include stem cells and germ cells.

Continuous cell lines are desirable for industrial processes. Biotechnology manufacturing involves genetically transformed cell lines, many of which are engineered to produce telomerase enzyme in addition to the protein of interest. Telomerase replicates and replenishes the terminal DNA sequences of telomeres, thus extending the life of those cells by making it possible for them to continue dividing without senescence. Tumor cell lines (hybridomas) thus transformed can go on forever. Sometimes, as in the development of malignant tumors, such transformations are spontaneous.

> A number of the properties of continuous cell lines, such as reduced serum requirement, reduced density limitation on growth, growth in semisolid media, aneuploidy, and more — are associated with malignant transformations. . . . Similar morphological and behavior changes can also be observed in cells that have undergone virally or chemically induced transformation. (**8**)

An important thing to remember about continuous cell lines is that they tend to be genetically unstable. "They should only be used continuously for approximately three months before stock replacement. . . . Validated and authenticated frozen stocks of all cell lines should be maintained to protect against cell line instability and to give insurance against contamination, incubator failure, or other accidental loss" (**9**). Industrial cell lines are tested often for their genetic stability.

Apoptosis is different from senescence in that it occurs in response to changing conditions around or within a cell. Disease states are often the trigger. When things are going irreparably wrong inside the cell (as in a contaminating viral infection, for example), it may commit suicide for the protection of its neighbors. Numerous caspases (proteolytic enzymes) are produced, which efficiently break down the protein building-blocks of the cell until it can no longer function. Certain peptide inhibitors of those caspase enzymes can be added to cell cultures for preventing apoptosis, but they will not correct the conditions that led to the process in the first place.

Most of the detailed work described in Chapters Three, Four, and Five of this supplement addresses the issues of life and death raised here. Determination of media (as discussed in Chapter Two) and culture conditions is never easy or straightforward. What worked for someone else's culture of the same cell line may not work for yours. Once a medium has been selected through empirical testing, it must be supplemented (Chapter Three) and optimized (Chapter Four) for the specific culture type (batch, fed-batch, or continuous as well as cell species) being fed and the specific product being made. Apoptosis, senescence, tumorigenicity, and contamination are just as important to consider as water, nutrients, physical conditions, and waste removal. And choices made regarding media are inextricably tied to all of those. So a discussion of cell culture media is by necessity a discussion of cell culture.

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