ROCHE Applied Science

Sales Training Manual for Cell Biology Overview

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Forward

This manual is part of the collection of technical topics for Introductory Training. This topic is an important part of the collection for the following reasons.

- *Cell Biology is an area of strategic focus*
- *To understand the needs and objectives of a cell biologist, it is very important to understand their "psyche" and challenges*
- *The manual will provide a foundation for the other cell biology manuals. Therefore, to understand the other topics, mastery of this material is essential.*

If you choose to print this manual, consider printing it double-sided, as it was designed.

Introduction

Cell biology deals with literally anything that happens in a eukaryotic organism (that is, any higher organism). Major questions studied by cell biologists include:

1. How do different parts of a cell perform their specialized functions?

2. How do genes control cell functions?

3. How do immature cells grow up (develop) into specialized adult cells ?

4. How do cells communicate with each other?

5. How do cells know when to die?

A little knowledge about cell biologists can help you understand more of your customers and their needs. It can also mean extra sales for you. However, since cell biologists are a widespread group with diverse research interests, it may seem difficult to know what they do and what to sell them.

What is Cell Biology?

Cell biology (also called cellular biology or formerly cytology, from the Greek kytos, "container") is an academic discipline that studies cells – their physiological properties, their structure, the organelles they contain, interactions with their environment, their life cycle, division and death. This is done both on a microscopic and molecular level. Cell biology research encompasses both the great diversity of single-celled organisms like bacteria and protozoa, as well as the many specialized cells in multicellular organisms like humans.

Cell biology is that branch of life science, which deals with the study of cells, their properties, structure, organelles, and interactions with environment, life cycle, division and death.

Knowing the components of cells and how cells work is fundamental to all biological sciences. Appreciating the similarities and differences between cell types is particularly important to the fields of cell and molecular biology as well as to biomedical fields such as cancer or developmental biology. These fundamental similarities and differences provide a unifying theme, sometimes allowing the

Figure 1
Cells are very complex and the field of cell biology may seem to be complex as well. Understanding more about cells and the challenges cell biologists face will improve your sales in this area and reduce the apparent complexity.

principles learned from studying one cell type to be extrapolated and generalized to other cell types. Hence, research in cell biology is closely related to genetics, biochemistry, molecular biology and developmental biology.[1]

This Learning Guide

This learning guide will help you understand cells and the people who study them. It will prepare you to understand all the other cell biology manuals, techniques, and assays. The topics in this learning guide are fundamental to cell biology and include:

- Cell and tissue culture
- Basic cell anatomy
- Cell cycle
- Mitosis and Meiosis

In addition to reading this manual, you will be directed to view some on-line videos (~20-30 minutes) and some on-line interactions. Most of the on-line interactions are optional, but you will find that they are a fast fun way of learning the material covered in this manual. They include animations of the concepts, games, puzzles, and quizzes.

A suggested approach is to do the interactions first, then skim the written material for additional information. An alternate approach is to read the manual first then use the interactions to verify your learning. Choose the approach that works best for your learning style.

After you study this learning guide, you will be able to:

- *Explain why scientists culture eukaryotic cells*
- *Describe how scientists get cells for culture and maintain them in culture (primary and secondary cell culture)*
- *Identify the major organelles in a eukaryotic cell.*
- *List the stages of a cell cycle*
- *Explain the differences between mitosis and meiosis*

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Section 1:
Cell Types, Parts, & Sizes

Learning Objectives for this Section

After you study this section, you will be able to:

- *List the differences between a prokaryotic cell and a eukaryotic cell*
- *Identify the parts of a eukaryotic animal and plant cell*
- *Describe what limits the size of a plant cell and animal cell.*

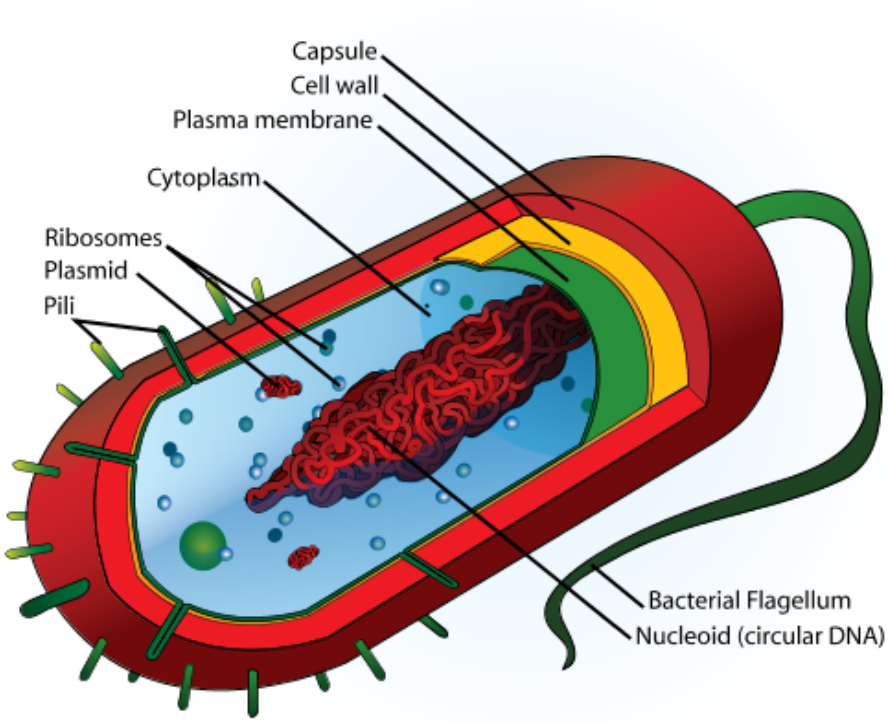
Prokayotic and Eukaryotic Cells

Prokaryotic cells

Eukaryotes have a nuclear envelope and a cell nucleus but prokaryotes do not. Prokaryotes also lack most of the intracellular organelles and structures that are seen in eukaryotic cells.

There are two kinds of prokaryotes: bacteria and archaea, but they are similar in the overall structures of their cells.

Figure 2
Diagram of a typical prokaryotic cell.



The **plasma membrane** separates the interior of the cell from its environment and serves as a filter and communications beacon.

Most prokaryotes have a **cell wall** (some exceptions are Mycoplasma (bacteria) and Thermoplasma (archaea)). This wall consists of peptidoglycan in bacteria, and acts as an additional barrier against exterior forces. It also prevents the cell from expanding and finally bursting (cytolysis) from osmotic pressure against a hypotonic environment. A cell wall is also present in some eukaryotes like plants (cellulose) and fungi, but has a different chemical composition.

A prokaryotic **chromosome is usually a circular molecule** (an exception is that of the bacterium Borrelia burgdorferi, which causes Lyme disease). Even without a real nucleus, the DNA is condensed in a nucleoid. Prokaryotes can carry extrachromosomal DNA elements called plasmids, which are usually circular. Plasmids can carry additional functions, such as antibiotic resistance.

Eukaryotic cells

Eukaryotic cells are about 10 times the size of a typical prokaryote and can be as much as 1000 times greater in volume. The major difference between prokaryotes and eukaryotes is that eukaryotic cells contain membrane-bound compartments in which specific metabolic activities take place called organelles.

Most important among these is the presence of a cell nucleus, a membrane-delineated compartment that houses the eukaryotic cell's DNA.

Cell Parts: Animal Cells

Many of your customers focus on a small part of a cell or cells. Therefore, you will better understand their work and needs when you know the parts and functions of a cell's parts.

In this section we will discuss only animal cells, which are eukaryotes. Plant and animal cells have many similarities as well as differences, which are discussed in the next section.

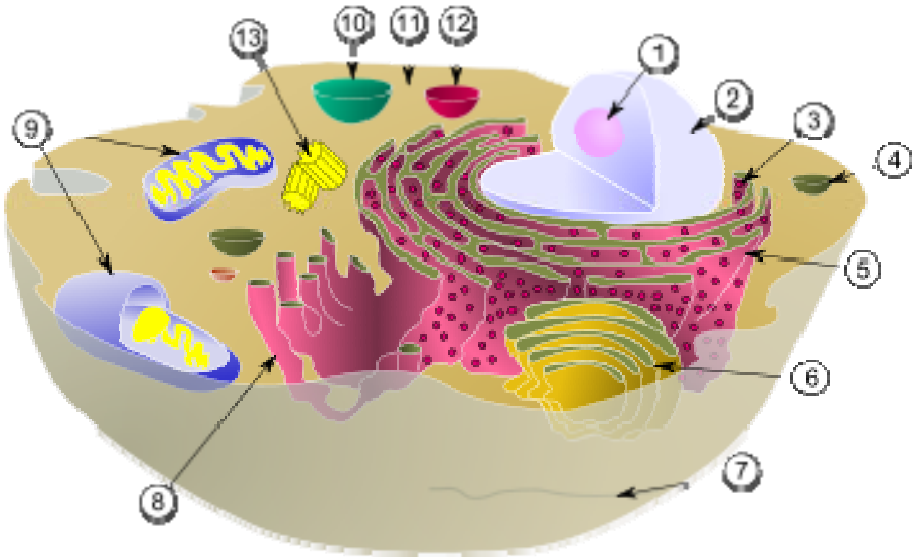
The organelles are described below. For a more interactive approach, check out the web sites in Interaction 1.

Interaction 1
Parts of a Cell
http://www.harcourtschool.com/activity/cell/cell.html (need Shockwave) Click the Info button to learn more.

http://www.cellsalive.com/cells/cell_model.htm Click on an organelle to learn more.

Try a puzzle for fun:
http://www.cellsalive.com/puzzles/index.htm

Figure 3
Organelles:
(1) nucleolus (2) nucleus
(3) ribosome (4) vesicle
(5) rough endoplasmic reticulum (ER)
(6) Golgi apparatus (7) Cytoskeleton
(8) smooth ER (9) mitochondrion
(10) vacuole (11) cytoplasm
(12) lysosome (13) centrioles



Nucleus
The nucleus contains genetic material or DNA in the form of chromatin. It is a double membrane structure, with pores on it. These pores act as a "gateway" to help the nucleoplasm to maintain continuity with the cytoplasm.

Cytoplasm (Cytosol)
The cytosol contains mainly water and numerous molecules floating in it- all except the organelles.

Mitochondria
A mitochondrion is the organelle responsible for a cell's metabolism. It synthesizes ATP through a protein called ATP synthase. Mitochondria have a double membrane; an outer membrane and a folded inner membrane. The internal membrane, called the '*cristae*AWDQS' is invaginated (folded or creased), to maximize surface area enabling it to hold more ATP synthases.

Ribosomes
Ribosomes are responsible for protein synthesis; they are composed of two subunits that to elongate an amino acid sequence.

Endoplasmic Reticulum
The Endoplasmic Reticulum (ER) acts as a transport from the nucleus and ribosomes to the Golgi apparatus. There are two types of endoplasmic reticulum:

Smooth ER
Smooth ER act as transport for various things, mainly the RNA from the nucleus to the ribosomes (RNA is a small piece of the DNA code specifically designed to tell

the ribosomes what to make). Smooth ER appears smooth in texture, hence the name.

Rough ER
Rough ER are "rough" because of the ribosomes embedded in them. The rough ER take the protein to the Golgi apparatus to be packaged into vacuoles

Golgi Complex
The Golgi Complex bonds functional groups to different biomolecules to direct them to their respective destinations. It basically "packages" the stuff into vacuoles. The Golgi Complex looks like pieces of pita bread stacked on top of each other. They are the ones that have their origin from the ER. They basically function as the delivery system of the cell.

Vacuole
Vacuoles are storage places. They store water, food or cell waste products.

Central Vacuole
The central vacuole is found only in plant cells. It is filled with water and is pressurized, like a balloon. This forces all the other organelles within the cell out toward the cell wall. This pressure is called **turgor pressure** and is what gives plants their "crisp" and firm structure.

Peroxisomes
Peroxisomes perform a variety of metabolic processes and as a by-product, produce hydrogen peroxide. Peroxisomes use peroxase enzyme to break down this hydrogen peroxide into water and oxygen.

Lysosomes
Lysosomes are vacuoles containing digestive and destructive membranes. In white blood cells, these are used to kill the bacteria or virus, while in tadpole-tail cells they kill the cell by separating the tail from the main body.

They also do much of the cellular digestion involved in apoptosis, the process of programmed cell death.

Interaction 2
Plant cell parts
http://www.harcourtschool.com/activity/cell/cell.html (need Shockwave) Click the Info button to learn more.

http://www.cellsalive.com/cells/cell_model.htm Click on an organelle to learn more. Try a puzzle for fun: http://www.cellsalive.com/puzzles/index.htm

Cell Parts: Plant Cells

Not all your customers are studying animal cells. For example, there are many opportunities in agriculture biotech. Therefore, you will better understand their work and needs when you know the parts and functions of a plant cell's parts.

In this section we will discuss plant cells, which are also eukaryotes, but have a number of important differences compared to their animal counterparts. The major ones are the chloroplasts, cell walls and vacuoles. Unlike animal cells, plant cells do not have centrioles.

The organelles are described below. For more interaction, try out the URLs in Interaction **5** and choose plant cells this time.

Chloroplasts

The chloroplasts are an organelle similar to the mitochondria in that they are self reproducing and they are the energy factories of the cell. There most of the similarities ends. Chloroplasts capture light energy from the sun and convert it into ATP and sugar. In this way the cell can support itself without food. Chloroplasts also give a cell its green color and are widely believed to have evolved from symbiotic prokaryotes that adapted to live inside eukaryotic cells. Physiologically, chloroplasts are flat discs usually 2-10 micrometer in diameter and 1 micrometer thick.

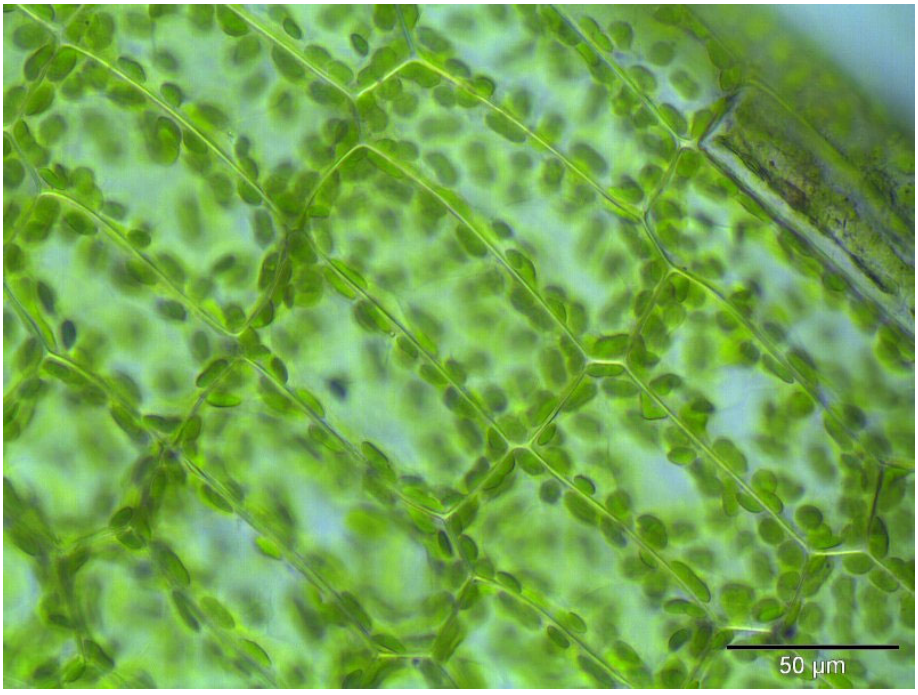


Figure 4
Light micrograph of a moss's leaf cells at 400X magnification. The green oval-like shapes are the chloroplasts.

The chloroplast has a two membrane envelope termed the *Inner & Outer* membrane respectively. Between these two layers is the *intermembrane space*.

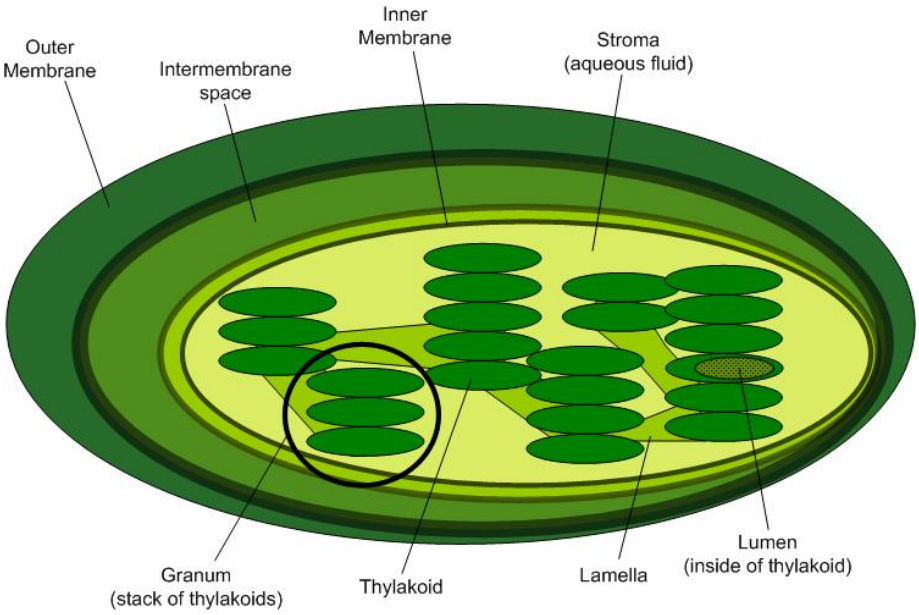


Figure 5
Illustration of a chloroplast; energy factory for a plant cell.

Vacuoles

Plants often have large structures containing water surrounded by a membrane in the center of their cells. These are vacuoles and act as a store of water and food (in seeds), a place to dump wastes and a structural support for the cell to maintain turgor. When the plant loses water the vacuoles quickly lose their water, and when plants have a lot of water the vacuoles fill up. In mature plants there is usually one large vacuole in the centre of the cell.

Cell walls

Plant cells are not flaccid like animal cells and have a rigid cell wall around them made of fibrils of cellulose embedded in a matrix of several other kinds of polymers such as pectin and lignin. The cellulose molecules are linear and provide the perfect shape for intermolecular hydrogen bonding to produce long, stiff fibrils. It is the cell wall that is primarily responsible for ensuring the cell does not burst in hypotonic surroundings.

Thylakoid

Within the stroma are stacks of thylakoids, the sub-organelles which are the site of photosynthesis. The thylakoids are arranged in stacks called grana (singular: granum). A thylakoid has a flattened disk shape. Inside it is an empty area called the thylakoid space or lumen. Photosynthesis takes place on the thylakoid membrane

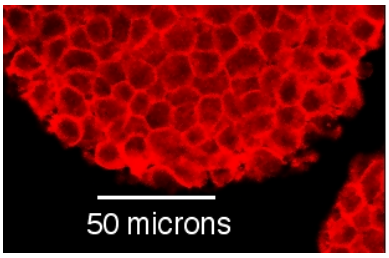


Figure 6
P19 mouse embryonal carcinoma cells. These are average size animal cells.

Organelles found in plant cells and not in animal cells:

Plastids - membrane bound organelles used in storage and food production. These are similar to entire prokaryotic cells - for example, like mitochondria they contain their own DNA and self-replicate. They include:

Chloroplasts - convert light/food into usable energy. (ATP production)

Leucoplasts - store starch, proteins and lipids.

Chromoplasts - contain pigments. (E.g. providing colors to flowers)

Cell Wall - found in prokaryotic and plant cells; provides structural support and protection.

Cell Size

Cells are so small that even a cluster of these cells from a mouse only measures 50 microns

Although it is generally the case that biological cells are too small to be seen at all without a microscope, there are exceptions as well as considerable range in the sizes of various cell types. Eukaryotic cells are typically 10 times the size of prokaryotic cells (these cell types are discussed in the next Chapter). Plant cells are on average some of the largest cells, probably because in many plant cells the inside is mostly a water filled vacuole.

So, you ask, what are the relative sizes of biological molecules and cells? See Table 1.

Table 1
Relative sizes of cells, cell parts, and other molecules

Nanometer range
0.1 nm diameter of a hydrogen atom
0.8 nm Amino Acid
2 nm Diameter of a DNA Alpha helix
4 nm Globular Protein
6 nm microfilaments
10 nm thickness cell membranes
11 nm Ribosome
25 nm Microtubule
50 nm Nuclear pore
100 nm Large Virus
150-250 nm small bacteria such as Mycoplasma
200 nm Centriole
200 nm (200 to 500 nm) Lysosomes
200 nm (200 to 500 nm) Peroxisomes

Micron (micrometer) range
1 - 10 µm the general sizes for Prokaryotes
1 µm Diameter of human nerve cell process
2 µm E.coli - a bacterium
3 µm Mitochondrion
5 µm length of chloroplast
6 µm (3 - 10 micrometers) the Nucleus
9 µm Human red blood cell
10 - 30 µm Most Eukaryotic animal cells
10 - 100 µm Most Eukaryotic plant cells
90 µm small Amoeba
100 µm Human Egg

Millimeter range
1 mm Diameter of the squid giant nerve cell
120 mm Diameter of an ostrich egg (a dinosaur egg was much larger)

Meter range
3 meters Length of a nerve cell of giraffe's neck

What limits cell sizes?

- Prokaryotes - Limited by efficient metabolism
- Animal Cells (Eukaryotic) - Limited by Surface Area to Volume ratio
- Plant Cells (Eukaryotic) - Have large sizes due to large central vacuole which is responsible for their growth

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Section 2:
Cell Cycle, Mitosis and Meiosis

Learning Objectives for this Section

After you study this section, you will be able to:

- *List the stages of interphase, in order*
- *Briefly describe what happens during the stages of interphase*
- *List the stages of mitosis, in order*
- *Briefly describe what happens during mitosis*
- *List the stages of meiosis*
- *Briefly describe what happens in meiosis*
- *Explain how meiosis differs from mitosis*

Two Phases: Interphase & Mitotic Phase

The normal cell cycle consists of 2 major stages. The first is **interphase**, during which the cell lives and grows larger. The normal cell functions of creating proteins and organelles occur in this phase. The second is **mitotic** phase. Cells divide during the mitotic phase (mitosis). Germ cells (reproductive) are special and go through a special division phase called **meiosis** instead of mitosis.

Interaction 3
If you prefer, you may learn about cell cycles in an interactive way using one of the following links:
Cell Cycle Game:
http://nobelprize.org/educational_games/medicine/2001/cellcycle.html

The Mitotic Phase is composed of mitosis and cytokinesis.

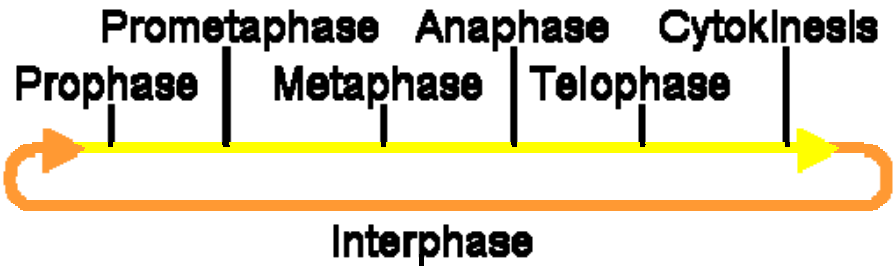

Figure 7
Schematic of interphase (orange/brown) and mitosis (yellow). The duration of mitosis in relation to the other phases has been exaggerated in this diagram.

Mitosis and meiosis are discussed in detail below starting on page 20.

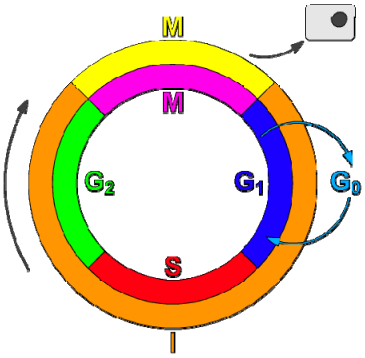

Figure 8
Schematic of the cell cycle.
I=Interphase, M=Mitosis.
The duration of mitosis in relation to the other phases has
been exaggerated in this diagram

You can test you memory of the mitosis phase by playing this game: http://www.quia.com/pp/3371.html?AP_rand=1083425077.

Interphase is composed of subphases.

1. **G_1 phase** (first gap) ↔ **G_0 phase** (resting)
2. **S phase** (synthesis)
3. **G_2 phase** (second gap).
4. **M phase** or mitosis and cytokinesis, the actual division of the cell into two daughter cells

Cell Cycle Descriptions

G_0 phase (Resting). The term "post-mitotic" is sometimes used to refer to both quiescent and senescent cells. Nonproliferative cells in multicellular eukaryotes generally enter the quiescent G0state from G1 and may remain quiescent for long periods of time, possibly indefinitely (as is often the case for neurons). This is very common for cells that are fully differentiated. Cellular senescence is a state that occurs in response to DNA damage or degradation that would make a cell's progeny nonviable; it is often a biochemical alternative to the self-destruction of such a damaged cell by apoptosis.

Interphase

G_1 phase. The first phase within interphase, from the end of the previous M phase until the beginning of DNA synthesis is called G_1 (G indicating gap or growth). During this phase the biosynthetic activities of the cell, which had been considerably slowed down during M phase, resume at a high rate. This phase is marked by synthesis of various enzymes that are required in S phase, mainly those needed for DNA replication. Duration of G_1 is highly variable, even among different cells of the same species.

S phase. The ensuing S phase starts when DNA synthesis commences; when it is complete, all of the chromosomes have been replicated, i.e., each chromosome has two (sister) chromatids. Thus, during this phase, the amount of DNA in the cell has effectively doubled. Rates of RNA transcription and protein synthesis are very low during this phase. The duration of S phase is relatively constant among cells of the same species.

G_2 phase. The cell then enters the G_2 phase, which lasts until the cell enters mitosis. Again, significant protein synthesis occurs during this phase, mainly involving the production of microtubules, which are required during the process of mitosis. Inhibition of protein synthesis during G_2 phase prevents the cell from undergoing mitosis.

The cell cycle stops at several checkpoints and can only proceed if certain conditions are met, for example, if the cell has reached a certain diameter. Some cells, such as neurons, never divide once they become locked in a G_0 phase.

Why Scientists Study the Cell Cycle

Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division. The molecular events that control the cell cycle are ordered and directional; that is, each process occurs in a sequential fashion and it is impossible to "reverse" the cycle.

Disruption of the cell cycle causes many diseases, including cancer. Tumors are typified by rapidly dividing cells, indication that there may be a problem with interphase regulation and/or the signal to begin mitosis. Therefore, customers are studying aspects of the cell cycle.

Role of Cyclins and CDKs

Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), determine a cell's progress through the cell cycle. Many of the genes encoding cyclins and CDKs are conserved among all eukaryotes, but in general more complex organisms have more elaborate cell cycle control systems that incorporate more individual components. Many of the relevant genes were first identified by studying yeast, especially Saccharomyces cerevisiae; genetic nomenclature in yeast dubs many these genes cdc (for "cell division cycle") followed by an identifying number, e.g., cdc25.

Cell cycle inhibitors

Two families of genes, the cip/kip family and the INK4a/ARF (Inhibitor of Kinase 4/Alternative Reading Frame) prevent the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.

The cip/kip family includes the genes p21, p27 and p57. They halt cell cycle in G_1 phase, by binding to, and inactivating, cyclin-CDK complexes. p21 is activated by p53 (which, in turn, is triggered by DNA damage e.g. due to radiation). p27 is activated by Transforming Growth Factor β (TGF β), a growth inhibitor.

*Note: Apoptosis (programmed cell death) is a factor in some cancers/tumors as well. Cells are dividing (proliferating) too quickly AND the cells are not dying when they are supposed to. Customers studying cell death are interested in p53, such as in the **p53 pan ELISA**.*

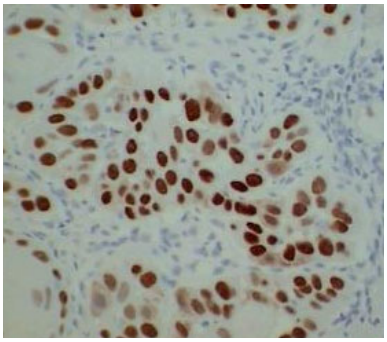

Figure 9
Diffuse and strong p53 expression (p53 positivity= 56%) [p53 IHC, Clone DO7, x400

Interaction 4
Watch one of these animations of mitosis: http://www.johnkyrk.com/mitosis.html
OR http://www.maxanim.com/genetics/Mitosis/Mitosis.htm (click ► to start)

Try this quiz to learn about mitosis or come back later to check your learning. Cell Cycle/Mitosis Quiz: http://www.purposegames.com/game/c0373f9c

Check out this mitosis crossword puzzle: http://www.cellsalive.com/puzzles/mitosis/index.html

The INK4a/ARF family includes p16INK4a, which binds to CDK4 and arrests the cell cycle in G1 phase, and p14arf which prevents p53 degradation.

Mitosis

In biology, mitosis is the process of chromosome segregation and nuclear division that follows replication of the genetic material in eukaryotic cells. This process assures that each daughter nucleus receives a complete copy of the organism's genetic material. In most eukaryotes, mitosis is accompanied with cell division or cytokinesis, but there are many exceptions, for instance among fungi.

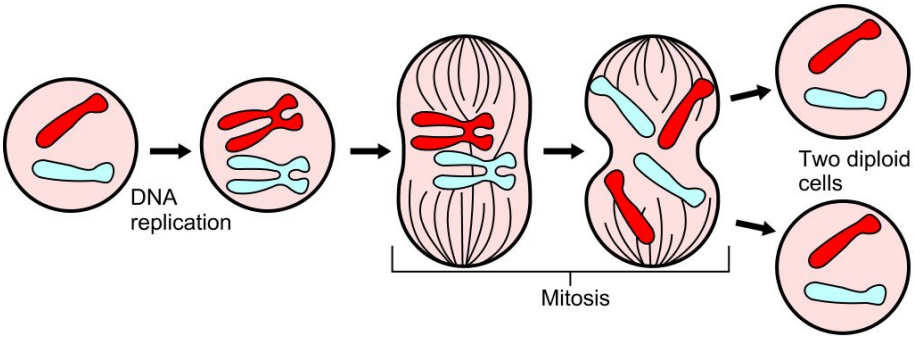

Figure 10
Illustration of mitosis. Mitosis divides genetic information during cell division.

Only the basics of mitosis will be covered in this manual. You can get more detail on mitosis at: http://en.wikipedia.org/wiki/Mitosis. There is another process called meiosis, in which the daughter nuclei receive half the chromosomes of the parent, which is involved in gamete formation and other similar processes, which makes the parent cell still active.

The steps of mitosis are:

1. Prophase
2. Prometaphase (in some text books)
3. Metaphase
4. Anaphase
5. Telophase
6. Cytokinesis

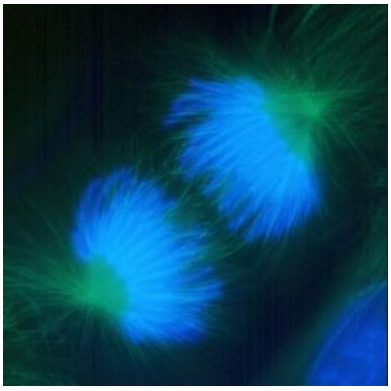

Figure 11
Lung cell undergoing mitosis.

Prophase. The first step of cell division is prophase, the nucleus dissolves and the chromosomes (called chromatids in this phase) begin migration to the midline of the cell. The two round objects above the nucleus are the centrosomes (see Figure **12**). Note the condensed chromatin.

Prometaphase. Some biology textbooks insert a phase called "prometaphase" at this point. In this phase, the nuclear membrane dissolves in some eukaryotes, reforming later once mitosis is complete. This is called open mitosis, found in most multicellular forms.

Metaphase. The second step, known as metaphase, occurs when all the chromosomes are aligned in pairs along the midline of the cell. The midline is called the "metaphase plate." See Figure **13**.

Anaphase. As the cell enters anaphase, the chromatids, which form the chromosomes, will separate and drift toward opposite poles of the cell. See Figure **14**.

Telophase. Telophase is the opposite of prophase. A nuclear membrane reforms around each of the daughter cells; nucleoli reappear. The spindles and asters (in animals) disappear. The chromatids start to elongate and become less condensed, changing their form to the long and thin chromatin. See Figure **15**.

Cytokinesis. Cytokinesis refers to the physical division of one eukaryotic cell. Except for some special cases, the amount of cytoplasm in each daughter cell is the same. In animal cells, the cell membrane forms a cleavage furrow and pinches apart like a balloon. In plant cells, a cell plate forms, which becomes the new cell wall separating the daughters.

The whole procedure is very similar among most eukaryotes, with only minor variations. As prokaryotes lack a nucleus and only have a single chromosome with no centromere, they cannot be properly said to undergo mitosis.

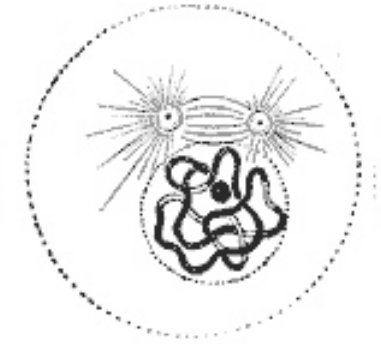

Figure 12
Prophase:

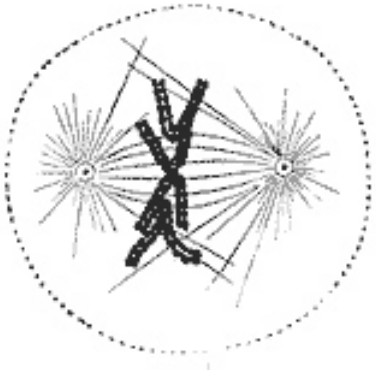

Figure 13
Metaphase:

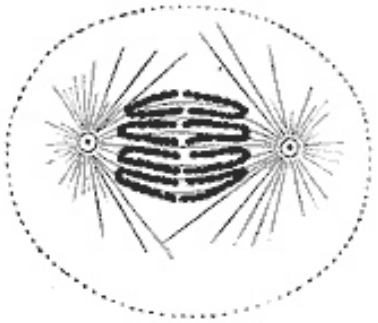

Figure 14
Early anaphase

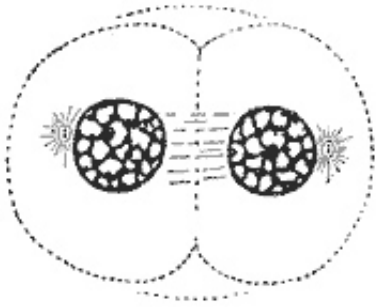

Figure 15
Telophase

Meiosis

Meiosis (pronounced my-oh-sis) is cell division where the number of chromosomes per cell is cut in half. In animals, meiosis always results in the formation of gametes, while in other organisms it can give rise to spores. Only the basics of meiosis will be covered in this manual. For more detail, see: http://en.wikipedia.org/wiki/Meiosis.

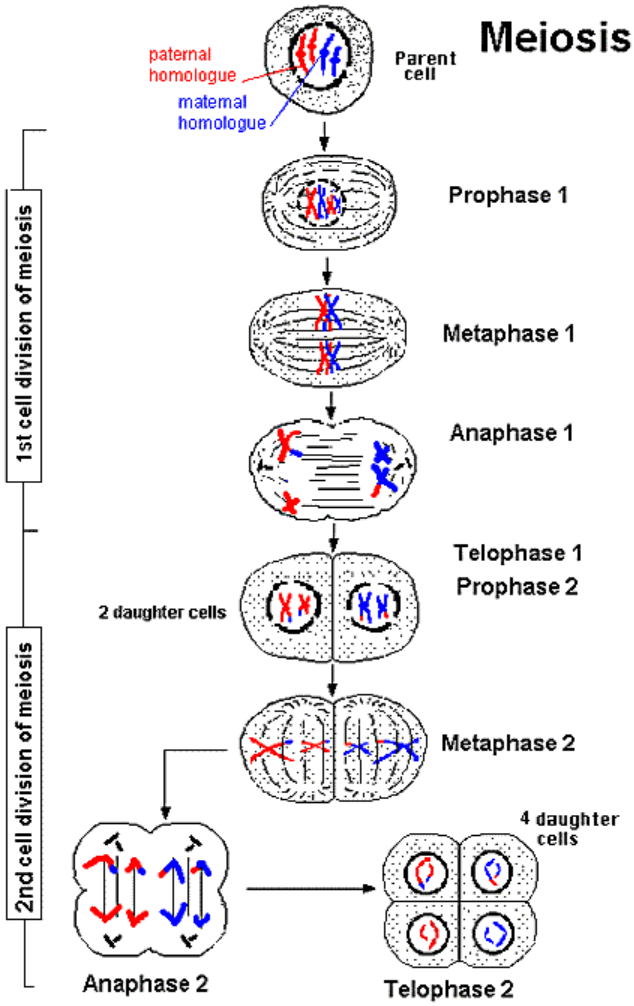

Figure 16
Illustration of the meiotic process.

During meiosis, the genome of a diploid germ cell, which is composed of long segments of DNA packaged into chromosomes, undergoes DNA replication followed by *two* rounds of division, resulting in four haploid cells. Each of these cells contain one complete set of chromosomes, or half of the genetic content of the original cell.

Interaction 5
Watch one of these animations of meiosis: http://www.johnkyrk.com/meiosis.html OR
http://www.cellsalive.com/meiosis.htm
(click ► to start)

Try this quiz to learn what the stages of meiosis really look like..
http://www.purposegames.com/game/real-meiosis-quiz HINT: study the illustration in Figure **16** before you start.

In all plants, and in many protists (formerly called protozoa), meiosis results in the formation of haploid cells that can divide vegetatively without undergoing fertilization, referred to as spores. In these groups, gametes are produced by mitosis.

Meiosis uses many of the same biochemical mechanisms employed during mitosis to accomplish the redistribution of chromosomes. There are several features unique to meiosis, most importantly the pairing and genetic recombination between homologous chromosomes.

For cells undergoing meiosis, interphase is followed by meiosis I and then meiosis II.

Meiosis I consists of separating the pairs of homologous chromosome, each made up of two sister chromatids, into two cells. One entire haploid content of chromosomes is contained in each of the resulting daughter cells; the first meiotic division therefore reduces the ploidy of the original cell by a factor of 2.

Meiosis II consists of decoupling each chromosome's sister strands (chromatids), and segregating the individual chromatids into haploid daughter cells. The two cells resulting from meiosis I divide during meiosis II, creating 4 haploid daughter cells.

The stages of meiosis are:

Meiosis I

1. Prophase I
2. Metaphase I
3. Anaphase I
4. Telophase I

Meiosis II

1. Prophase II
2. Metaphase II
3. Anaphase II
4. Telophase II

Prophase I. Homologous chromosomes pair (or synapse) and crossing over (or recombination) occurs - a step unique to meiosis. The paired and replicated chromosomes are called bivalents or tetrads, which have two chromosomes and four chromatids, with one chromosome coming from each parent. At this stage, non-sister chromatids may cross-over at points called chiasmata. Prophase I has 6 stages:

- Leptotene
- Zygotene
- Pachytene
- Diplotene
- Diakinesis
- Synchronous processes

In human fetal oogenesis (egg production) all developing oocytes develop to the diplotene stage and stop before birth. This suspended state is referred to as the dictyotene stage and remains so until puberty. In males, only spermatogonia (intermediate step in sperm production) exist until meiosis begins at puberty.

Metaphase I. Homologous pairs move together along the metaphase plate.

Anaphase I. Kinetochore microtubules shorten, pulling chromosomes toward opposing poles, forming two haploid sets. Each chromosome still contains a pair of sister chromatids.

Telophase I. The last meiotic division effectively ends when the chromosomes arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids.

Cytokinesis, the pinching of the cell membrane in animal cells or the formation of the cell wall in plant cells, occurs, completing the creation of two daughter cells. Sister chromatids remain attached during telophase I.

Cells may enter a period of rest known as interkinesis or **interphase II**. No DNA replication occurs during this stage.

Meiosis II

Meiosis II is the second part of the meiotic process. Much of the process is similar to mitosis. The end result is production of four haploid cells.

Prophase II takes an inversely proportional time compared to telophase I. In this prophase we see the disappearance of the nucleoli and the nuclear envelope again as well as the shortening and thickening of the chromatids. Centrioles move to the polar regions and arrange spindle fibers for the second meiotic division.

Metaphase II. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate.

Anaphase II. The sister chromatids by convention are now called sister chromosomes as they move toward opposing poles.

Telophase II. Similar to telophase I, it is marked by uncoiling and lengthening of the chromosomes and the disappearance of the spindle. Nuclear envelopes reform and cleavage or cell wall formation eventually produces a total of four daughter cells, each with a haploid set of chromosomes.

Meiosis is now complete.

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Section 3: Cell Culture: Basic Concepts & Getting the Cells

Learning Objectives for this Section

After you study this section, you will be able to:

- *Explain the difference between adherent and suspension cell cultures*
- *Compare similarities and differences between cell culture and tissue culture*
- *Describe how primary cell culture differs from cell lines*
- *List the three general steps for starting a new culture of cells*
- *Explain, in general, why cell biologists tend to be resistant to change*
- *Name at least three dissociating enzymes*

Why Scientists Culture Cells

To tap into the cell biology market, you need to understand a little about cell culture. Why? Because cell culture is the one thing most cell biologists have in common.

If they have a choice, cell biologists seldom study whole animals (intact, eukaryotic organisms). Studying one type of cell inside a whole animal is a lot like searching for a needle in a haystack (except that the haystack keeps changing its structure and occasionally walks away from you). One cell type interacts with and influences another. Biological rhythms (time of day, time since feeding, and so forth) affect each cell type differently. The same type of cell may function differently in different parts of the animal.

Most scientists who study eukaryotic cells will culture the cells before they study them. When cell biologists isolate a particular type of cell from the animal and culture it **in vitro** (that is, outside the animal) they can simplify their experiments by:

- Starting with a group of identical cells, an easy task in cell culture, an impossible task inside a whole animal
- Creating an environment favorable to their experiments, by feeding the cells a nutrient solution that influences the cell's growth and properties
- Delivering their experimental stimulus (chemicals, DNA, or whatever) directly to the cells, instead of shooting the stimulus into the whole animal and hoping it reaches the right cells

What You Should Know About Cell Culture

In this section of the manual, you'll learn the basics of cell culture and how cells are acquired. The next section will describe general maintenance of cells. These two sections are key to understanding the needs and attitudes of cell culturists (that is, those who grow eukaryotic cells in vitro and use those cells as experimental tools).

The Cell Biologist's Psyche

At first, cell biologists may seem to be inexplicably quirky, superstitious, or down-right paranoid. These are not uncommon characteristics for scientists, but cell biologist tend be excel at them. To effectively serve your cell biology customers, you will need to understand a little about their psyche.

A good way to understand another person's attitudes and actions is to "take a walk in their shoes." If you have a customer who seems interested in giving you a peek into how they do their cell culture, take the offer! You will also get to take a virtual peek into cell and tissue culture using some on-line videos.

Cell Acquisition and Counting Videos

The purpose of the video web site[1] you will visit is to share video protocols amongst the scientific community. We are using the videos to illustrate the complex, difficult, and detailed processes your customers use to acquire and maintain their cells cultures.

Focus on the number of steps and skill it takes to perform the procedure. Also look for the routine aseptic technique skills involved. Think about how you would value and treat the resulting cells if *you* were the technician in the video. Finally, do not be concerned with understanding all the terms and science of their work.

Several of the videos include protocols if you want to look at the written version when you are done. Simply scroll down a little on the screen.

More detail is available on each video in the Appendix on page 48. You will need to watch 3 videos but you are welcome to watch more.

Choose one of the following:

- Counting and Determining the Viability of Cultured Cells: http://www.jove.com/index/details.stp?ID=752 time: 5:46
- Counting Human Neural Stem Cells http://www.jove.com/index/details.stp?ID=262 time: 6:37

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[1] If you have trouple viewing the videos, call 317-521-2051 or use a different computer. Note: videos take a minute or two to load.

Watch this video:

- Trypsinizing and Subculturing Mammalian Cells: http://www.jove.com/index/details.stp?ID=755 time: 5:59

Choose one of the following:

- Mouse Adrenal Chromaffin Cell Isolation: http://www.jove.com/index/details.stp?ID=129 time: 18:30. Note: shows removal of adrenal from mouse. Also, this video does not have sound.
- Method for Culture of Early Chick Embryos ex vivo (New Culture): http://www.jove.com/index/details.stp?ID=903 time: 8:45
- Primary Dissociated Midbrain Dopamine Cell Cultures from Rodent Neonates: http://www.jove.com/index/details.stp?ID=820 time: 14:13

*NOTE: All cell culturists use an **inverted microscope** (Figure 18) to look at the cells in their culture flasks. If you notice an inverted microscope in a laboratory you visit, chances are that lab is doing cell culture.*

Adherent vs. Suspension Cell Culture

Some Cells Float; Some Don't

Cells may be classified according to where they stay in the culture flask (that is, the glass or plastic vessel in which they are grown):

Adherent Cell Cultures (Non-Floaters)

Most cultured cells (such as muscle or skin cells) are anchorage-dependent (Figure 17, top). That means the cells don't like to float in the nutrient solution. Instead, they attach to a solid surface called a substrate (for example, the bottom of the culture flask) and let the media wash over them. Cultures of anchorage-dependent cells are called adherent cultures (because they adhere or stick to the flask as they grow) or monolayer cultures (because the cells grow until they have formed a single layer that covers all the available space on the bottom of the culture flask).

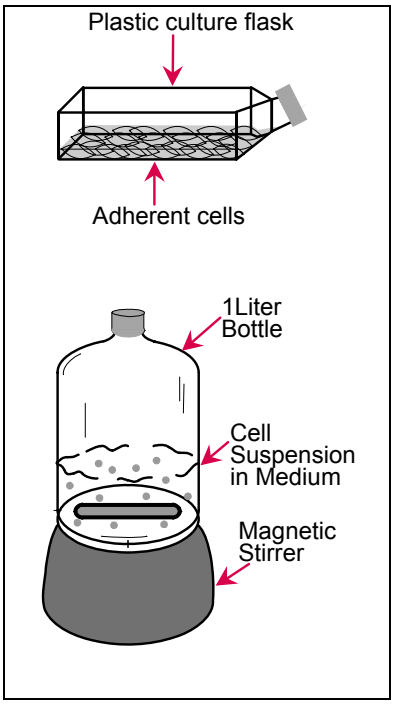

Figure 17
Adherent cell cultures (top) stick to the bottom of the culture flask. Suspension cell cultures (bottom) float in the nutrient solution. Note that suspension cultures may also be in ordinary culture flasks like the one at the top of the figure.

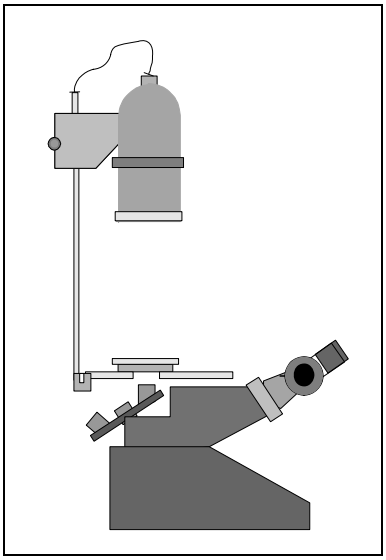

Figure 18
An inverted microscope has the light source above the viewing mechanism under the stage where the sample sits. This allows the cell culturists to view cells on the bottom of a cell culture flask when the flask is place on the stage.

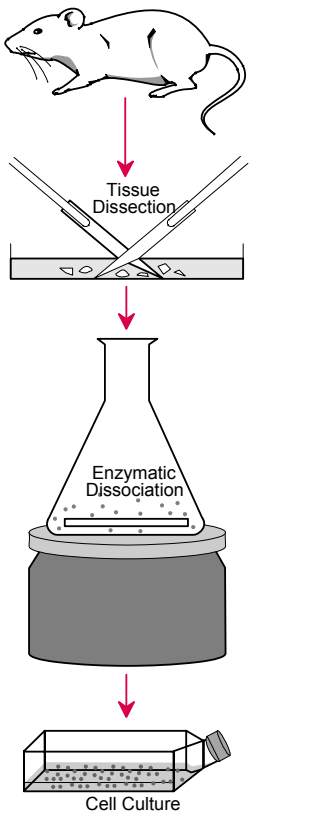

Figure 19
In primary cell culture, tissues are removed from an animal, dissociated, and placed into a culture flask along with a nutrient solution.

*NOTE: Roche Applied Science sells fibronectin and **collagen** for coating the insides of culture vessels. These preparations make the culture vessels better substrates for adherent cell cultures.*

Most cells derived from solid tissues are adherent. Another type of adherent culture is *organotypic culture* which involves growing cells in a three-dimensional environment as opposed to two-dimensional culture dishes. This 3D culture system is biochemically and physiologically more similar to *in vivo* tissue, but is technically challenging to maintain because of many factors (e.g. diffusion).

Suspension Cell Cultures (Floaters)

A few cultured cells do like to float in the nutrient solution (Figure 17, bottom). For instance, blood cells (such as the important immune system cells called lymphocytes) do not have to be attached to anything when they grow in vitro. Instead, they form suspension cultures (that is, they grow while suspended in the growth media).

Adherent and suspension cultures are dissociated cells, which means that they are no longer in their original conformation, i.e. unstuck. When it is important to understand how cells who touch each other interact, a customer may use tissue culture instead.

Tissue Culture

The term "tissue culture" can be used to refer to the culturing of tissue pieces, for example explant culture or whole organs, AKA organ culture.

A customer may have thin slices of an organ or tissue she is growing or even small chunks. Regardless, tissue cultures do not live as long as cell cultures in general, due to the challenge of getting nutrients and oxygen to the cells.

The term tissue culture and cell culture are commonly used interchangeably. Therefore, get specifics from your customer on exactly what she is growing.

Primary Cell Culture: Getting The Cells

All cell cultures are derived from whole animals or plants (eukaryotic organisms). The process of getting individual cells from whole animals (Figure 19) is called primary cell culture.

To get adherent cell cultures, cell culturists:

1. Remove a slice of tissue (that is, a collection of functionally related cells) from one spot in the whole animal

2. Break apart (dissociate) the extracellular matrix (network that glues cells together into a tissue) to release the individual cells

3. Transfer the individual cells to a glass or plastic vessel (culture flask) and cover them with a nutrient solution (media) that contains chemicals the cells need for growth

As you saw in the videos, the above process steps are very simplified. The reality is much tougher!

NOTE: Originally, scientists did not break tissues apart. They placed the isolated tissue (called the primary explant) in the culture flask and let individual cells find their way out of the tissue. This was called tissue culture. Tissue culture eventually became the broad term for culturing part of an organism, whether a single cell or a whole organ, in vitro, that is, outside the organism.

To get suspension cell cultures, cell culturists:

1. Take a rich source of these cells (such as bone marrow) from the animal

2. Gently disperse the clumps of cells (since they're not held together by an extracellular matrix as in tissue)

3. Transfer the individual cells to a vessel (culture flask or dish) and cover them with a nutrient solution (media)

Dissociating Enzymes for Primary Cell Culture

Dissociating enzymes break up (dissociate) the extracellular matrix in a eukaryotic tissue (Figure 20). Some of these enzymes are proteases (enzymes that chew up the many proteins in the extracellular matrix). Others chew up non-protein parts of the matrix.

No one dissociating enzyme is best for primary cell culture, since different tissues respond differently to each enzyme. Among the Roche Applied Science enzymes frequently used for primary cell culture are:

- **Collagenase**
  Collagenase is a protease that chews up collagen, a major protein in the extracellular matrix.
- **Dispase**
  Dispase is a relatively gentle protease that will dissociate cells without damaging their membranes (outer coatings). Sometimes, dispase is used in combination with collagenase, so you will see collagenase/dispase listed as a single preparation in the Roche Applied Science catalog.
- **Trypsin**
  Trypsin is an aggressive protease that will readily release cells from tissue, but will also destroy the cell membranes if it remains in contact with the cells for very long.

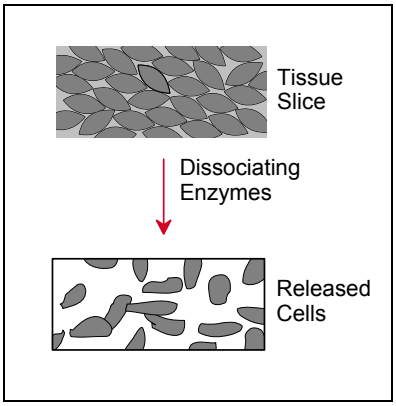

Figure 20
Dissociating enzymes chew up the extracellular matrix that holds cells together in a tissue. The cells that are released can be placed in a cell culture.

- **Papain**
  Works with collagenase, esterase, trypsin to dissociate tissue Note: the addition of cystein is essential for enzyme activity.
- **Pronase**
  Mixture of proteinases isolated from the extracellular fluid of *Streptomyces griseus*. Activity extends to both denatured and native proteins leading to complete or nearly complete digestion into individual amino acids
- **Protease mixtures**, especially crude collagenases
  Often a mixture of proteases is more effective at digesting a tissue than any one protease. Roche Applied Science offers several combinations of proteases that are best for dissociating certain types of tissue.

In fact, the most commonly used dissociating enzymes in our product line are actually impure preparations of collagenase. These "crude collagenases" (collagenases A, B, D, H, P, Liberase) are custom protease blends that contain mostly collagenase along with varying amounts of other proteases. Each of these custom blends is designed to release cells effectively from a particular tissue. For example, Liberase works well for pancreatic islet cells, the cells that produce the hormone insulin.

- **Hyaluronidase**
  Hyaluronidase, is an enzyme that chews up a large carbohydrate molecule commonly found in connective tissue (tissue that holds together major organs such as lungs and heart). Roche Applied Science no longer sells this enzyme; direct customers to Sigma-Aldrich.
- **DNase I**
  Since some cells are inevitably destroyed as tissue is broken up, some DNA is released from the ruptured cells. Since DNA can stick to the outside of cells and make them clump together, a cell culturist may add DNase I (deoxyribonuclease I, a nonspecific endonuclease) to get rid of the released DNA.

Secondary Cell Culture: Moving The Cells

Healthy cells proliferate (reproduce or increase in number) in primary cell cultures. Eventually, they grow to fill the available area or volume. This can generate several issues:

- Nutrient depletion in the growth media
- Accumulation of apoptotic/necrotic (dead) cells.
- Cell-to-cell contact can stimulate cell cycle arrest, causing cells to stop dividing known as contact inhibition or **senescence**.
- Cell-to-cell contact can stimulate cellular differentiation.

If nothing is done to the primary cell culture, the cells will stop growing. When cells stop growing, the culture will die or deteriorate.

In order to keep the cells healthy, cell culturists must periodically give them more room to grow (Figure 21). To do this, cell culturists establish a secondary cell culture by diluting the cells from the primary culture with fresh media and transferring them to a new culture flask. Once diluted, the cells have room to roam and grow again. Each of these periodic dilutions is called a **passage** and the new culture is said to be a subculture of the original.

Keeping cells alive is covered in more detail in the next section starting on page 37.

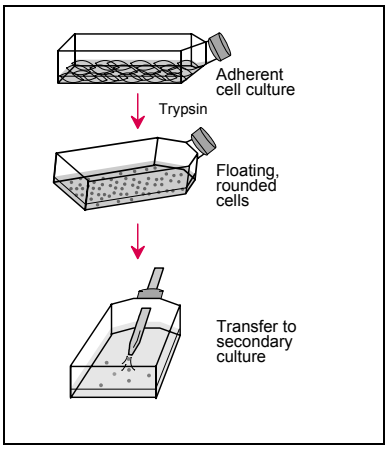

Figure 21
Preparing a secondary cell culture.

Cell Lines

Cell culturists know that passaging has another benefit besides keeping the cells healthy. In a primary cell culture, the fastest growing cells tend to take over most of the space. Therefore, the secondary cell culture is a fairly homogeneous population of growing cells. This homogeneous, growing cell population is called a cell line. Cell lines behave much more predictably and respond more uniformly to experimental manipulation than a mixed population of cells.

Note: Cell lines that originate with humans have been somewhat controversial in bioethics, as they may outlive their parent organism and later be used in the discovery of lucrative medical treatments. In the pioneering decision in this area, the Supreme Court of California held in Moore v. Regents of the University of California that human patients have no property rights in cell lines derived from organs removed with their consent. [2]

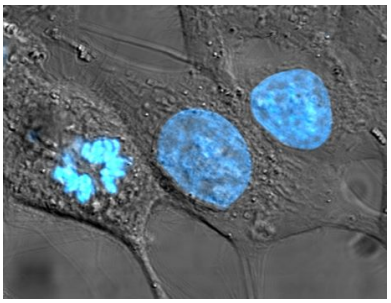

Figure 22
One of the earliest human cell lines, descended from Henrietta Lacks, who died of the cancer that those cells originated from, the cultured HeLa cells shown here have been stained with Hoechst turning their nuclei blue.

Transformation and continuous cell lines

There is a second way that a homogeneous population of cells can arise in culture. This process is called transformation.

Normal (primary) cell lines survive only a limited time in culture. For unknown reasons, they will, after several weeks in culture, grow old and die. Even passaging can't prevent this normal aging process.

However, a certain portion of the aging cells may not die. Instead, they change (mutate) in such a way that they can continue to grow after all the other cells die. Cells that mutate so they can live in culture for a very long time are said to have **transformed**. Homogeneous populations of transformed cells are called continuous cell lines or established cell lines.

Note: The term "transform" has a different meaning for prokaryotic cells, e.g. E. coli. *For prokaryotes, transform means that foreign DNA has been inserted into the cells. In eukaryotes, the process of inserting foreign nucleic acids into a cell is called "transfection." Transfection is covered in detail in another manual. If your customer uses the term transform, just make certain which type of cells he is using to avoid confusion.*

Note two things about continuous cell lines.

- Each continuous cell line is a homogeneous population of cells. After the normal cells die, only a homogeneous population of transformed cells are present in the culture.
- Continuous cell lines do not need to be transferred to secondary cell cultures frequently. Unlike normal cell lines, continuous cell lines will live even if they are not passaged very often.

Common Cell Lines

The list below contains some common cell lines. To look up your customer's cell line, try http://www.biotech.ist.unige.it/cldb/cname-1c.html.

Human cell lines

DU145 (Prostate cancer)
Lncap (Prostate cancer)
MCF-7 (breast cancer)
MDA-MB-438 (breast cancer)
PC3 (Prostate cancer)
T47D ([breast cancer])
THP-1 (acute myeloid leukemia)
U87 (glioblastoma)
SHSY5Y Human neuroblastoma cells, cloned from a myeloma
Saos-2 cells (bone cancer)

Primate cell lines
Vero (African green monkey Chlorocebus kidney epithelial cell line initiated 1962)
Rat tumor cell lines
GH3 (pituitary tumor)
PC12 (pheochromocytoma)
Mouse cell lines
MC3T3 (embryonic calvarial)
Plant cell lines
Tobacco BY-2 cells (kept as cell suspension culture, they are model system of plant cell)
Other species cell lines
zebrafish ZF4 and AB9 cells.
Madin-Darby Canine Kidney (MDCK) epithelial cell line
Xenopus A6 kidney epithelial cells.

Enzymes For Secondary Cell Culture

If the cells are growing in suspension, subculturing only requires removing a portion of the cell suspension and transferring it to a new flask containing fresh media.

However, cells in an adherent cell culture must first be released from the walls of the culture vessel (Figure 21) before they are transferred to a new flask, as seen in the video: Trypsinizing and Subculturing Mammalian Cells: http://www.jove.com/index/details.stp?ID=755. Two enzymes, **trypsin** and **dispase** are frequently used to release cells for subculturing.

Cells in culture are much more sensitive to enzymatic destruction than are cells in a tissue. So proteases (especially trypsin) are used carefully during subculturing. Small amounts of the enzyme are added to the culture flask for very short periods of time (a few minutes, or even seconds). Other chemicals (such as EDTA) may be added to the flask to speed up the release.

As soon as the cells break free from the flask wall, they are transferred to a new flask containing fresh media. Sometimes, an inhibitor (such as trypsin inhibitor) is included in the new media to stop the protease activity from chewing up the cells.

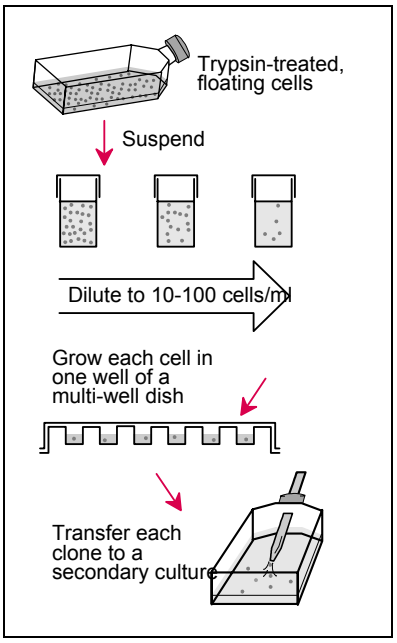

Figure 23
Isolating individual clones in cell culture.

Cloning: Obtaining Purebred Cells

Even relatively homogeneous cell lines are not predictable enough for some cell biology experiments. Only absolutely pure cell lines will do.

To get pure cell lines, cell culturists clone the cells in the cell line (Figure 23), that is, isolate single cells from the cell culture. Each of these single cells is placed in a culture of its own. All the offspring arising from that single cell should respond identically to experimental stimuli.

Hybridomas

Hybridomas are a special type of continuous cell line as well as a special cell clone. They are cells that have been engineered to produce a desired antibody in large amounts. Customers with hybridomas need special reagents such as “Hybridoma

Fusion and Cloning Supplement" and "IsoStrip Mouse Monoclonal Antibody Isotyping Kit."

NOTE: Roche Applied Science sells two preparations, HAT and HT, that can be used during cloning of the cells that make monoclonal antibodies.

For more information on hybridomas and monoclonal antibodies, see the "Sales Training Manual for Antibodies, Monoclonal Antibodies"

Plant Cell Culture

Plant cell cultures are typically grown as **cell suspension** cultures in liquid medium or as **callus cultures** on solid medium.

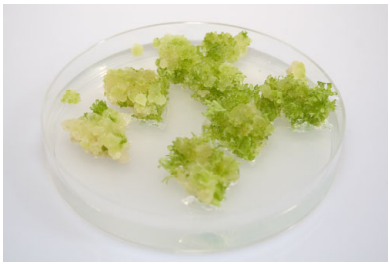

Figure 24
Callus *Nicotiana tabacum*

Callus Cultures

In biological research and biotechnology, a **callus of cells** is a mass of undifferentiated cells. In plant biology, callus cells are those cells that cover a plant wound. [3]

Cell Suspensions

Tobacco BY-2 cells are nongreen, fast growing plant cells which can multiply their numbers up to 100-fold within one week in adequate culture medium and good culture conditions. This cultivar of tobacco is kept as a cell suspension culture. This model plant system is comparable to HeLa cells for human research. Because the organism is relatively simple and predictable it makes the study of biological processes easier, and can be an intermediate step towards understanding more complex organisms. They are used by plant physiologists and molecular biologists as a model organism because of their exceptionally high homogeneity and high growth rate, featuring still general behavior of plant cell.

The diversity of cell types within any part of a naturally grown plant (in vivo) makes it very difficult to investigate and understand some general biochemical phenomena of living plant cells. The transport of a solute in or out of the cell, for example, is difficult to study because the specialized cells in a multicellular organism behave differently. The influence of a neighboring cell's behavior in the suspension is not as important as it would be in an intact plant. This model plant system is especially useful for studies of cell division, cytoskeletons, plant hormone signaling, intracellular trafficking, and organelle differentiation.[4]

Section 4:
Cell Culture: Keeping the Cells Alive

Learning Objectives for this Section

After you study this section, you will be able to:

- *Explain why cell culturists sometimes clone cells*
- *List the components that are found in almost all cell growth media*

Basic Needs of Cells

Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO2 for mammalian cells) in a cell incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed.

Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the **growth medium**. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients.[5] The topic below describes media in more detail.

Nurturing the Cells: Media and Supplements

Cells in culture are finicky. Different cell lines require different nutrients to survive the rigors of growing in culture. So, cell culturists use many different nutrient formulations (called growth media) to keep the cells healthy.

Basal media, serum, and antibiotics

All growth media have certain components:

- **Amino acids**
  Amino acids are the building blocks for proteins. Most cells cannot synthesize all the different amino acids. Media supply at least the amino acids the cell can't synthesize (essential amino acids).

- **Vitamins**
  Vitamins help enzymes to perform the many essential processes that keep the cell going.

- **Inorganic salts**
  Common chemicals such as sodium chloride (NaCl) and sodium bicarbonate ($NaHCO_3$) help the cell maintain the proper osmotic pressure (force necessary to keep all the salts from flowing out of the cell membrane) and pH (relative acidity of the solution) while growing.
  Other salts such as copper sulfate ($CuSO_4$) and zinc sulfate ($ZnSO_4$) provide trace minerals that, like vitamins, help enzymes work.

- **Food (energy source)**
  Cells need food, just like animals do, to fuel the many chemical reactions essential to life. The most common food for cells is a simple sugar like glucose.

The combination of amino acids, vitamins, inorganic salts, and food is often called basal media.

NOTE: Some of your customers may ask you for one of the many formulations of basal media (such as RPMI 1640 or DMEM) that are commercially available. However, Roche Applied Science doesn't sell any basal media in the U.S.

Most basal media are supplemented with:

- **Fetal bovine (calf) serum**
  Serum, the liquid part of animal blood, is a rich nutrient soup that can feed many cell lines. Serum from several animals (horses, sheep, etc.) are sometimes used in cell culture. However, fetal bovine serum (FBS), the liquid part of calf blood, is by far the most common type of serum used in cell culture.

  NOTE: FBS is the most common supplement to basal media. However, Roche Applied Science doesn't sell FBS or serum-containing media in the U.S.

- **Serum substitutes**
  FBS contains many components that are not well characterized. Therefore, a cell biology experiment may yield unexpected results due to an unknown component of the serum. In an effort to replace FBS with a better defined media component, a cell culturist may use mixtures such as Roche Applied Science's Nutridoma® media supplement. These serum substitutes provide some of the essential nutrients the cell normally gets from FBS. If cell lines can tolerate it, a cell culturist completely replaces FBS with a serum substitute, thus creating serum-free media. Frequently, though, the cell culturist must add just a little FBS to supply things that the serum substitute can't. Such semi-defined media (containing both serum and a serum substitute) support more cell lines than serum-free media can.

- **Antibiotics**
  Antibiotics (such as penicillin or streptomycin) kill bacteria or other microorganisms. Adding antibiotics to media reduces the risk of bacteria contaminating the cell cultures and devouring the nutrients intended for the cell.

NOTE: In addition to bacteria, other microorganisms can contaminate cell culture. The most difficult to detect and eliminate are ***mycoplasma.*** *A sensitive kit for detecting mycoplasma was released in early 1995. It is called the* Mycoplasma PCR ELISA. *Also, an exclusive Roche Applied Science product,* BM-Cycline*, is a combination of two antibiotics that can eliminate mycoplasma from cell cultures.*

Plant Cell Culture

A callus cell culture is usually sustained on gel media, much in the same manner as bacteria are grown. Sufficient media consists of agar and the usual mix of macronutrients and micronutrients for the given cell type. For plant cells, enrichment with nitrogen, phosphorus, and potassium is especially important. Water is provided as a constituent part of the gel media. Ex. sandalwood callus[6]

The culturing of undifferentiated plant cells and calli requires the proper balance of the plant growth hormones **auxin** and **cytokinin**.

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Section 5: Growth Factors and Cytokines

Learning Objectives for this Section

After you study this section, you will be able to:

- *Explain how growth factors are used in cell culture*
- *Explain what a cytokine is.*
- *Describe why researchers use cytokines in their work.*
- *Name the types of behavior that can be induced with cytokines*
- *List similarities and differences between growth factors and cytokines*
- *Recognize the names of other cytokines and growth factors that Roche Applied Science sells.*

Growth Factors

A cell culturist wants the cells to grow as much as possible. Only living, growing cells are useful in most cell biology experiments. However, the media described above may not be enough to ensure that some of the cell lines grow. These cells require specific "signal molecules" (growth factors) to tell them when to grow (Figure 25).

*NOTE: Many growth factors are also called **mitogens**, that is, chemicals that stimulate cell division. However, not all mitogens are growth factors, so the terms shouldn't be used interchangeably.*

Cell culturists could rely on FBS to supply the growth factors. Serum is a rich source of growth factors and that's one of the reasons serum helps cells grow in culture. However, it's risky to rely on serum to provide the correct growth factor or the right amount of growth factor.

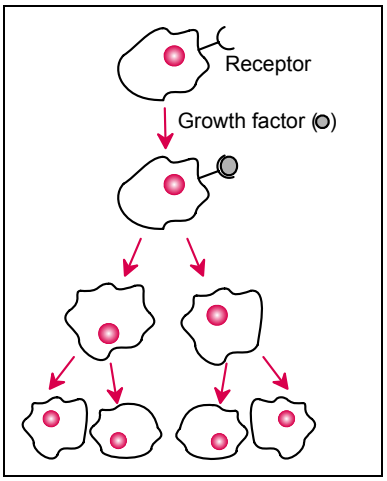

Figure 25
A growth factor binds a receptor molecule on the surface of certain cells. The binding of the growth factor to the receptor causes the cell to divide (reproduce)

To be certain each of the cell lines gets the right "grow signal" at the right time, a cell culturist may add purified growth factors to the media. Each growth factor:

- **Is a polypeptide (small protein).**
- **Is recognized by only a particular type of cell.**
  Each growth factor is bound by a different receptor molecule. Cells have different receptor molecules in their membranes. Only the cells that have the right receptor molecule will be influenced by a particular growth factor.
- **Doesn't actually enter the cell.**
  The growth factor exerts its effect by merely binding to the receptor. Once a receptor in a cell's membrane binds a growth factor, a number of things begin to happen inside the cell that lead to growth.
- **Causes the target cell to begin making DNA and preparing to divide (reproduce).**

Some growth factors target a particular type of cell, as indicated by the name of the factor. For instance, nerve growth factor (NGF) stimulates neurons in culture.

Some growth factors (for instance, epidermal growth factor or EGF) stimulate a lot of different kinds of cell (including cells of the epidermis or outer layer of skin). Usually, all the cells stimulated by such a growth factor have a similar function or originate from one type of tissue.

Cytokines

When the body mounts an immune response against a foreign invader, there is a need to coordinate the different functions of the cellular immune system. This coordination is possible because the cells in the immune system can communicate with each other via chemical messengers. Cytokines (or lymphokines) are these chemical messengers.

Sometimes, cell culturists want more from the cell than simple growth. Just as a milk cow is no good unless it produces milk, certain cells are useless for experiments unless they produce certain chemicals or have activated certain functions.

For instance, certain immune system cells called T cells have a resting mode and an attack mode. Only when they are in attack mode (that is, activated) can they fulfill their role of fighting off a foreign invader. For cell culturists to study the attack functions of the T cells, the cells have to be activated.

NOTE: For more information on the function of the immune system, read the "Sales Training Manual for an Overview of Immunology,"

To activate T cells in culture, cell culturists can add such "command" chemicals as interleukin 1 (IL-1) and interleukin 2 (IL-2) to the T cell culture.

IL-1 and IL-2 are cytokines (literally, cell movers). Cytokines (Figure 26) are natural chemical messengers that eukaryotic organisms produce to command various cells to do the right thing at the right time.

NOTE: A subset of cytokines (including IL-1 and IL-2) are only produced by immune system cells, especially the white blood cells called lymphocytes. Thus, IL-1, IL-2, and related cytokines are usually called ***lymphokines.***

Sometimes, cytokines are considered growth factors. Like growth factors, cytokines:

- Are polypeptides or proteins
- Affect only certain kinds of cells
- Work by binding to specific receptor molecules on the membranes of target cells
- Often induce cells to grow

In fact, one of the principal reasons cell culturists buy IL-2 (also called T cell growth factor) is to get activated T cells to survive and reproduce in culture. Without IL-2, activated T cells wouldn't survive in vitro.

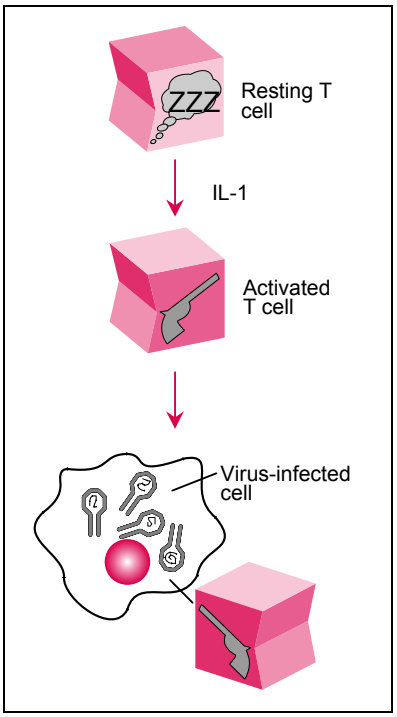

Figure 26
Cytokines order cells to do the right thing at the right time. For instance, IL-1 commands T cells to become activated and start to destroy foreign invaders.

However, in addition to commanding cells to grow, cytokines can induce a wide variety of new behavior in target cells, including:

- **Activation**
  (See T cell example above.)
- **Differentiation**
  Cytokines can help a cell decide what it wants to be when it grows up. For instance, granulocyte-macrophage colony stimulating factor (GM-CSF) helps bone marrow cells differentiate into a more mature form and become granulocytes or macrophages (two specific types of blood cells).
- **Production of receptor molecules**
  In some cases, the presence of a cytokine can cause a cell to make the receptor molecule that recognizes the cytokine. So, to study receptors and their interaction with their signal molecules in vitro, cell culturists may add cytokines to a cell culture.
- **Death**
  Remarkably, some cytokines such as tumor necrosis factor can kill certain types of cells, especially transformed cancer cells.

A list of the cytokines RAS sells is on the facing page.

Table 2: Cytokines Sold by Roche Applied Science*

Cytokine Name	Full Name and Effect on Target Cells
EGF	**Epidermal Growth Factor**. Stimulates the proliferation and differentiation of a wide variety of cells of ectodermal and mesodermal origin.
GM-CSF	**Granulocyte-Macrophage Colony Stimulating Factor**. Controls the differentiation, activation, and proliferation of blood progenitor cells from bone marrow into granulocytes or macrophages. Also enhances the survival of mature neutrophils, eosinophils and macrophages.
IFN-γ	**Interferon-gamma**. Anti viral and anti tumor activity. Activator of certain parts of the immune system.
IL-1α	**Interleukin-1 alpha**. Activates B-cells and T-cells. Induction of IL-6 and IL-2. Other effects too numerous to mention here.
IL-2	**Interleukin-2**. Activates B and T-cells and NK cells.
IL-6	**Interleukin-6.** Stimulates the maturation of B-cells.
NGF	**Nerve Growth Factor**. Stimulates nerve tissues.
TNF-α	**Tumor Necrosis Factor-Alpha**. Inhibits or lyses many tumor cells.
TGF-β1	**Transforming Growth Factor-Beta 1**. Stimulates growth of many mammalian cells.

*List current as of February 2009. Check web site for current availability.

Table 3: Other Common Cytokines Not Currently Sold by Roche Applied Science**

Cytokine Name	Full Name and Effect on Target Cells
G-CSF	**Granulocyte Colony-Stimulating Factor**. Controls the differentiation, activation and proliferation of blood progenitor cells from bone marrow into granulocytes.
IGF I and IGF II	**Insulin-like Growth Factors**. Stimulates the division of many types of cells in culture.
IL-1β	**Interleukin-1 Beta**. Activates B-cells and T-cells. Induction of IL-6 and IL-2. Other effects too numerous to mention here.
IL-3	**Interleukin-3**. Growth and differentiation of precursor cells.
IL-4	**Interleukin-4**. Proliferation and differentiation of many types of immune cells.
IL-7 and IL-11	**Interleukin-7 and Interleukin-11**. May be used as a growth and maturation factor for early cells of B-cell and T-cell lineage.
TGF-α	**Transforming Growth Factor-Alpha**. Stimulates growth of many mammalian cells.

**Most were previously sold by Roche Applied Science. Therefore, your customers may read papers where these cytokines were purchased from Roche Applied Science or Boehringer Mannheim.

Section 6: Appendix

Contents of This Section

This section includes:

- *Table of Interactions*
- *Tables of Videos*

Table of Interactions

The table below lists all the interactions and games available in this document.

Topic	URL	Additional Information
1-Cell Parts	http://www.harcourtschool.com/activity/cell/cell.html	Click the Info button to learn more. Choose animal cell. (need Shockwave)
	http://www.cellsalive.com/cells/cell_model.htm	Choose animal cell. Click on an organelle to learn more.
	http://www.cellsalive.com/puzzles/index.htm	Try a puzzle for fun:
2-Plant cell parts	http://www.harcourtschool.com/activity/cell/cell.html	Click the Info button to learn more. Choose plant cell. (need Shockwave)
	http://www.cellsalive.com/cells/cell_model.htm	Choose plant cell. Click on an organelle to learn more.
	http://www.cellsalive.com/puzzles/index.htm	Try a puzzle for fun:
3-Cell Cycle Game	http://nobelprize.org/educational_games/medicine/2001/cellcycle.html	Learn about cell cycles in an interactive way using one of the following links: Cell Cycle Game:
4-Mitosis	http://www.johnkyrk.com/mitosis.html	Animation of mitosis
	http://www.maxanim.com/genetics/Mitosis/Mitosis.htm	(click ► to start)
	http://www.purposegames.com/game/c0373f9c	Try this quiz to learn about mitosis or come back later to check your learning. Cell Cycle/Mitosis Quiz:
	http://www.quia.com/pp/3371.html?AP_rand=1083425077.	You can test you memory of the mitosis phase by playing this game
	http://www.cellsalive.com/puzzles/mitosis/index.html	Mitosis crossword puzzle:
5-Meiosis	http://www.johnkyrk.com/meiosis.html	Animation of meiosis
	http://www.cellsalive.com/meiosis.htm	(click ► to start)
	http://www.purposegames.com/game/real-meiosis-quiz	Try this quiz to learn what the stages of meiosis really look like. HINT: study the illustration in Figure 16 before you start.

Tables of Videos

Below are details about the videos referenced in this document.

Counting and Determining the Viability of Cultured Cells

URL	http://www.jove.com/index/details.stp?ID=752
Total Time	5:46
Segments & time stamps	0:00 Title 0:58 Preparing the hemacytometer 1:31 Preparing cell suspension 2:09 Loading hemacytometer 2:42 Counting and calculating number of cells 4:24 Determination of cell viability 5:25 Conclusion
Protocol?	No (see Current Protocols of Cell biology)
Citation	Ricardo R, Phelan K (2008). Counting and Determining the Viability of Cultured Cells JoVE. 16. http://www.jove.com/index/details.stp?id=752, doi: 10.3791/752

Counting Human Neural Stem Cells

URL	http://www.jove.com/index/details.stp?ID=262
Total Time	6:37
Segments & time stamps	0:00 Title 0:12 Passaging Human Embryonic Neural Stem Cells 0:30 Preparing the Cell Suspension for Counting 3:54 Counting Cells in the Hemocytometer
Protocol?	Yes w/ illustrations
Citation	Marchenko S, Flanagan L (2007). Counting Human Neural Stem Cells JoVE. 7. http://www.jove.com/index/details.stp?id=262, doi: 10.3791/262
Notes	Narrator not very animated but good support material below video.

Trypsinizing and Subculturing Mammalian Cells:

URL	http://www.jove.com/index/details.stp?ID=755
Total Time	5:59
Segments & time stamps	0:00 Title 0:37 Introduction 0:56 Trypsinizig and Subculturing Mammalian cells from a Monolayer 4:00 Passaging Cells in Suspension Culture 5:36 Conclusion
Protocol?	No
Citation	Ricardo R, Phelan K (2008). Trypsinizing and Subculturing Mammalian Cells JoVE. 16. http://www.jove.com/index/details.stp?id=755, doi: 10.3791/755

Mouse Adrenal Chromaffin Cell Isolation:

URL	http://www.jove.com/index/details.stp?ID=129
Total Time	18:30
Segments & time stamps	0:45 Tools and reagents 1:09 Dissection procedure 5:04 Isolation of medullae 9:03 Enzymatic digestion of tissue 16:18 Plating cells
Protocol?	Yes
Citation	Kolski-Andreaco A, Cai H, Currle DS, Chandy KG, Chow RH. (2007). Mouse Adrenal Chromaffin Cell Isolation JoVE. 2. http://www.jove.com/index/details.stp?id=129, doi: 10.3791/129
Notes	No sound. Removal of adrenal glands may seem a bit graphic to some viewers.

Method for Culture of Early Chick Embryos ex vivo (New Culture)

URL	http://www.jove.com/index/details.stp?ID=903
Total Time	8:45
Segments & time stamps	0:00 Title 0:37 Introduction 1:09 Explanting the Embryo in Saline 1:58 Centering the Embryo on a Ring 3:09 Setting Up the Culture 4:07 Transferring the Culture to the Incubator 5:02 Fixing the Embryo 7:35 Representative Results/Outcome 8:17 Conclusion
Protocol?	Yes, w/ photos of results
Citation	Psychoyos D, Finnell R (2008). Method for Culture of Early Chick Embryos ex vivo (New Culture) JoVE. 20. http://www.jove.com/index/details.stp?id=903, doi: 10.3791/903

Primary Dissociated Midbrain Dopamine Cell Cultures from Rodent Neonates

URL	http://www.jove.com/index/details.stp?ID=820
Total Time	14:13
Segments & time stamps	0:00 Title 0:14 Introduction 1:00 Preparing Tools and Reagents 5:03 Dissecting Midbrain of P0-P2 Rodent 9:10 Triturating Brain Segments 10:49 Plating Dissociated Neurons 12:53 Primary Dissociated Midbrain Dopamine Cultures 13:28 Conclusion
Protocol?	Yes
Citation	Pothos EN, Frank LE, Caldera-Siu AD (2008). Primary Dissociated Midbrain Dopamine Cell Cultures from Rodent Neonates JoVE. 21. http://www.jove.com/index/details.stp?id=820, doi: 10.3791/820

[1] Excerpt is retrieved from "http://en.wikipedia.org/wiki/Cell_biology"

[2] Moore v. Regents of University of California (1990) 51 C3d 120, [S006987, Cal Sup Ct, July, 9, 1990]

[3] Excerpt is retrieved from "http://en.wikipedia.org/wiki/Callus_(cell_biology)"

[4] Excerpt is retrieved from "http://en.wikipedia.org/wiki/Tobacco_BY-2_cells"

[5] Excerpt is retrieved from http://en.wikipedia.org/wiki/Cell_culture#Maintaining_cells_in_culture

[6] Excerpt is retrieved from http://en.wikipedia.org/wiki/Callus_(cell_biology)

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