UNIT 3 TEACHER BACKGROUND INFORMATION

Note: Terms that are bolded are defined in glossaries for teachers and students.

WHAT IS "CELL FATE?"

A regular, non-cancerous adult stem cell is for most of its lifetime **quiescent** or quiet, suspended in **G0** and at baseline metabolic activity levels. The stem cell is activated by signals from the immediate outside environment. This puts into motion pathways (**signaling cascades**) that eventually lead to selective gene transcription and a shift in cell behavior or phenotype. When such a shift occurs, it is believed a significant "cell fate" decision has been made. Such a change could include the decision to **proliferate** and **differentiate** into a more mature cell type. For example, under certain signals, a neural stem cell may shift towards a neuronal fate instead of a **glial cell** fate.

Different stem cell types vary in the number of possible cell fate decisions, often described as their potency. Adult stem cells are more restricted in which types of mature cells they can become (they are less potent) than embryonic stem cells, which can turn into any of the body's 200+ cell types. Adult stem cells are multipotent; they may eventually become more than one mature cell type, filling the tissue's need for new cells.

If every cell is genetically identical, how are there different stem cell types? Moreover, with all the possible cell fate decisions before them, how does a stem cell choose a given path over another?

IF EVERY CELL IN AN ORGANISM HAS THE SAME DNA SEQUENCE, WHY AREN'T ALL CELLS THE SAME?

Cell phenotype depends on context-specific cues within tissues. Even though each cell in your body has the same DNA sequence, composed of **exons** and **introns**, different sets of genes are expressed in different tissues. Each tissue has a different **microenvironment** that interacts with each cell. As will later be explained in detail, the microenvironment consists of signals that, in various combinations, influence cell behavior.

Cells have the arduous task of integrating multiple extracellular signals and attempting to perform the desired response(s). A stem cell fate decision to become a certain mature cell phenotype depends on **gene expression** changes resulting in the production (**upregulation**) or inhibition (**downregulation**) of proteins. Increasing or decreasing the amount of various proteins initiates functional cellular changes that in some cases completely transform the cell type.

Suggestions

It will be helpful to review with your students how genes are expressed into proteins at the

molecular level so they can have the foundation to understand the more complex phenotype shift of a differentiating stem cell. You may wish to teach students about protein production through the example of **hematopoietic** (blood-forming) **stem cells.** These cells can receive an outside signal or set of signals, which are "passed along" by surface receptors, plasma membrane proteins, and cytoplasmic **enzymes** into the nucleus, where **transcription factors** bind to the promoter regions of genes to control their activation or deactivation. Transcription factors are very important in controlling stem cell phenotype; in many cases these factors act to keep a stem cell as a stem cell (in other words, to keep it from differentiating) by suppressing production of certain proteins and promoting others.

In the Invitation portion of this unit, the student activity helps explain to your students that outside signals, mediated by intracellular transcription factors, result in gene expression changes important for a blood stem cell to specialize into a white blood cell or a red blood cell. Exposure of blood stem cells to a different **signaling factor** or set of factors could alter the cell's fate. Your students can simulate the differentiation process from a blood progenitor cell (**common myeloid progenitor**) into either red or white blood cells, depending on the identity of the transcription factor to which each student's "cells" are exposed.

You provide them with *either* Erythropoietin *or* Colony Stimulating Factor, and the students edit their raw "partial chromosome" transcript containing two gene sequences. This models transcription and translation resulting from gene activation by one of the factors, leading to either a RED or WHITE protein (capital letters spell the amino acid sequences). This activity uses a free downloadable program called Another Plasmid Editor (ApE). It's normally used to search within and design bacterial plasmids for genetic engineering, and it has a basic interface where students can manipulate the DNA transcript. For a good review of transcription and translation, we recommend students color and work through these handouts from The Biology Corner:

http://www.biologycorner.com/worksheets/DNAcontrols.html and

http://www.biologycorner.com/bio1/notes_regulating_cell_cycle.html

TRANSCRIPTION AND RNA INTERFERENCE

During transcription, the synthesis of single-stranded, messenger RNA from a DNA template strand in the nucleus includes cutting out introns ("junk DNA") and splicing together exons that code for parts of the protein. Then, the entire coding strand of mRNA exits the nucleus and interacts with ribosomes and tRNA, which use the mRNA's codon triplets as a guide to add amino acids to the growing peptide. In addition to these roles in transcription and translation, RNA also controls the expression of genes.

During RNA interference (RNAi), a natural process that has been adapted for use in molecular biology, small double-stranded RNAs target their matching-sequence mRNAs for

destruction by RNAi machinery. This leads to gene silencing, useful as a method for temporarily suppressing specific proteins in eukaryotic cells.

THE MICROENVIRONMENT

It is increasingly becoming known that cell interactions with the microenvironment can heavily influence their behavior. Students can think of the microenvironment as the dorm in which a stem cell lives and functions. The microenvironment has non-living materials (concrete, rebar, furniture), other cells (roommates, neighbors, and visitors), as well as signaling molecules (voices, visuals, objects in the way) that help our stem cell character navigate in the world and decide what to do. For cells, even forces (sensed as physical variations in the extracellular matrix) can influence cell fate.

A cell's microenvironment—its local interface with the outside world—feeds into its behavior. The specific microenvironment of stem cells is called the **stem cell niche**. This environment influences the development of stem cells from quiescence through stages of differentiation. Just like the ecological niche of an organism, a stem cell niche is unique to the individual or small population and guides its dynamics. Here are the four major components of the microenvironment (*soluble factors*, *cell-cell interactions*, *extra-cellular matrix proteins*, and *forces*):

Soluble factors. Received from the extracellular environment, soluble factors typically bind a cell's plasma membrane or cytoplasmic receptors. While soluble factors, such as proteins, hormones, and cytokines, vary in biochemical composition and origin, their signaling outcomes can be classified into four main cell behaviors:

Survival: Typically achieved through suppressing apoptosis.

Division: Promotes synthesis of proteins and inhibits their degradation. Also can relieve intracellular blocking of cell cycle.

Differentiation: Cell specialization occurs through changes in metabolism, gene expression, and cell shape/migration.

Death: In most cases, an intracellular proteolytic pathway is activated and the bulk of the work performed by a certain class of proteins called caspases.

Granulocyte colony-stimulating factor (G-CSF) is an example of a cytokine that activates precursors in the bone marrow to produce mature granulocytes, a type of white blood cell. A soluble factor's effect on behavior can differ by cell type. G-CSF acts as a migration signal to hematopoietic stem cells, inducing their release into the blood stream without affecting their fate. In the central nervous system, G-CSF also stimulates the differentiation of new neurons from neural precursor cells.

Cell-cell interactions. Cells can also receive extracellular signals from connections made

with their neighbors. These connections can vary in size, composition, strength, and cargo transported. Here are the major cell junction types:

Tight junctions: As the name implies, these seal cells together, forming a molecule-impermeable sheet and localizing different integral membrane proteins to either side. Epithelial tissues, like the intestines, require a strong barrier for bidirectional transport.

Anchoring junctions: The two main types are adherens and desmosome junctions. Adherens junctions are narrow bands or patches built from **cadherins** and **catenins** that provide robust mechanical attachments between cells. For example, adherens junctions help cardiac cells stay together despite constant beating of the heart. Desmosomes are small patches attaching cells that link to intermediate keratin filaments in the cytoskeleton, which provide a framework of great tensile strength.

Gap junctions: Consisting of cylindrical channels constructed from connexins, gap junctions allow passage of small molecules to and from adjacent cells. These junctions serve diverse purposes, from synchronizing heart muscle cell contraction to coordinating neural tube formation during embryogenesis. Cell adhesion molecules do more than hold cells together. They also act as receptors that transduce signals controlling contact-mediated growth suppression and differentiation.

Normal cells grown in a petri dish will divide until they completely cover the surface, when they experience **contact inhibition** and stop dividing. But cancer cells lose this contact inhibition, suggested by the observation that they continue growing on top of each other after they cover the whole petri dish. Cancer cells do not respond normally to growth-regulatory signals induced by cell-cell interactions.

Carcinomas are cancerous epithelial growths in which the cells have lost their desmosomes, leading to metastasis. Also, in several developmental systems, cell-cell contact is important in determining cell fate through interactions of specific cell surface receptors on neighboring cells. Another function of cell adhesion molecules, in combination with cytoskeletal proteins, is maintenance of cell shape. Cell shape can be experimentally altered by coating different thicknesses of polyHEMA (a hydrogel polymer used for growing cells) onto plastic petri dishes. It has been shown that cells plated on a thick layer of polyHEMA are spherical and have reduced DNA synthesis (they do not enter S-phase) as compared to cells of the same type grown on a thinner layer, which become flatter and are able to enter S-phase and divide. For more information about and examples of each of these junctions, visit http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/J/Junctions.html.

Extracellular matrix proteins. These proteins make up the extracellular scaffold within which cells adhere and grow. Extracellular matrices are made of collagen, fibronectin,

laminin, and other proteins produced by connective tissue cells. They all have unique structures that contribute to both the biochemical and mechanical signaling in a given tissue.

Collagen is a triple helix and occurs in many places throughout the body. 29 types of collagen have been identified. Over 90% of the collagen in the body belongs to types I, II, III, and IV.

1. Collagen I: Skin, tendon, vascular, ligature, organs, bone (main component of bone)
2. Collagen II: Cartilage (main component of cartilage)
3. Collagen III: Reticulate (main component of reticular fibers), commonly found alongside type I.
4. Collagen IV: Basement membranes
5. Collagen V: Cells surfaces, hair and placenta

Fibronectin is a **dimer**, present as a soluble protein in the blood (a liquid matrix for blood cells) and as an insoluble component of the extracellular matrices in our bodies. Fibronectin plays a role in cell adhesion, growth, migration and differentiation, and is important in embryonic development and wound healing. In embryonic development, fibronectin guides migration and cell attachment. Without it, mesodermal, neural tissue, and vascular tissue do not develop normally.

In wound healing, plasma fibronectin and fibrin are deposited and form a clot. **Fibroblasts** bind to it and replace the bound plasma fibronectin with new extracellular fibronectin in amounts matching the surrounding tissue. The breaking down of fibronectin thus promotes contraction of the surrounding tissue extracellular matrix and healing of the wound. Abnormal expression of fibronectin is implicated in lung cancer and tumor cell morphology.

Laminin is featured (along with collagen IV) in the basement membrane of tissues. Its cross-like structure allows the short arms of laminin molecules to link into sheets and the long-arms to attach to cells. Cellular **integrins** mediate this attachment and signaling. Laminins can control, directly or indirectly, cellular activities such as adhesion, migration, differentiation, polarity, proliferation, apoptosis, and gene expression. It is important in branching morphogenesis of the lung, kidney, breast, and salivary gland, where cells clump together and protrude to form glands and ducts.

Laminin is expressed in the central and peripheral nervous systems, notably by Schwann cells, which insulate PNS neurons with myelin (oligodendrocytes myelinate neurons in the CNS.) It heavily influences Schwann cell proliferation, differentiation, and survival during PNS development. Mutations in laminin causing decreased nerve insulation and other effects are associated with the symptoms of Muscular Dystrophy.

Forces. The last section described collagen, fibronectin, and laminin as proteins that can assemble into extracellular matrices in the body and signal to cells. These proteins, plus adherent cells, form a matrix that varies in stiffness, the degree to which a material can maintain its original form under force, relative to its density and organization. Because of this, tissues differ in stiffness; for example, brain is soft and very elastic, skin has intermediate stiffness, muscle is stiffer, and bone is very stiff and inelastic.

Cells can sense the stiffness of their surroundings by anchoring and pulling using myosin-based contraction and adhesion molecules (like integrins and cadherins). This pulling also generates small contractile forces that can change the organization of the extracellular matrix. Cells can respond to the stiffness they feel by reorganizing their cytoskeletons and initiating other cellular processes. In these ways, there is a dynamic, reciprocal relationship between the cells and the extracellular matrix with regards to forces felt and applied. Put differently, when the matrix imposes forces on cells, cells feel these forces. They then respond by changing their own stiffness—sometimes even locally reorganizing the matrix to change its stiffness, which can signal a different response from the cells!

Research on how stem cells sense and respond to forces in the microenvironment has implications for tissue engineering. When creating a scaffold for cell transplantation, knowing exactly how the organization of the engineered matrix will influence cell behavior, and vice versa, could mean the difference between a successful transplant and rejection. A scaffold usually refers to a 3-dimensional gel (similar to Jell-O) consisting of matrix proteins like collagen or synthetic polymers. A scaffold can also refer to an organ that has been treated with chemicals to remove all cells, with just the extracellular matrix left in the shape of the organ.

A researcher embeds cells inside the scaffold and bathes it in growth media and factors that promote survival. To a stem cell, being in "3D" is a drastically different experience than growing in "2D" on top of a petri dish; the stiffness of the surface, among other things, influences cell shape, growth, and responses to signaling molecules. Because of this, cell biologists should carefully consider how the surface on/in which their cells are grown plays into the phenomenon they are studying.

Varying substrate stiffness can even influence stem cell fate. Mesenchymal stem cells—multipotent cells found in the bone marrow that naturally differentiate into bone, cartilage, fat, tendon, muscle, and marrow stroma—can be isolated and propagated in culture. A study from the University of Pennsylvania (Discher et al., 2005) showed that mesenchymal stem cells commit to different lineages depending on the stiffness of the substrate. When grown on a collagen gel that mimics the stiffness of brain tissue, the mesenchymal stem cells differentiate into neurons! Grown on a 10-fold stiffer gel, reminiscent of muscle tissue, the stem cells turn into muscle cell precursors. Finally, when grown on a very stiff gel, the

stem cells differentiate into bone cells. In addition to these observations, the researchers pinpointed the mechanism of the stem cell response. Using a drug called blebbistatin, they inhibited nonmuscle myosin II—involved in sensing matrix elasticity—in the stem cells. Without the action of nonmuscle myosin II, the cells could not differentiate according to the stiffness.

CURRENT RESEARCH ON THE MICROENVIRONMENT

Research on how cell behavior and the microenvironment are related is progressing rapidly. We can maintain one cell type for long periods of time (proliferation), differentiate stem cells into mature progeny, and induce cells to perform other cell behaviors in a dish because we have identified and applied the necessary and sufficient microenvironmental factors that direct cells in these ways. By identifying powerful factors characteristic of the abnormal microenvironment—one that allows cancer to grow, for example—we will learn much more about diseases and potentially how to stop them.

Cell culture is an example of "outside-in" phenotype control because we are using factors outside of the cell to induce gene expression changes or to control cells to stay the same. How do we identify factors outside the cell that influence cell behavior? There are many possible combinations of microenvironmental factors to which cells can respond, so we would need a way to test a large number of possibilities. A tool called the Microenvironment Array (MEArray) helps researchers test thousands of individual microenvironments on stem cells to observe how they respond.

In the Application portion of this unit, students will learn about the work of Mark LaBarge and Mina Bissell, who narrowed down the components of the breast microenvironment required for mammary gland progenitor cells to differentiate into myoepithelial cells or luminal epithelial cells.

On the other hand, "inside-out" phenotype control manipulates the genomic sequence using recombinant DNA technology, causing a phenotype shift or change in cell behavior. This enables us to perform dramatic manipulations of cell fate—to the point of being able to reprogram skin cells into stem cells, and one fully differentiated cell into another. Being able to turn one cell type into another is dependent on the introduced genes' expression inside the cell, guided by the correct signals from the new, simulated, outside microenvironment.

You may want to teach students about different types of arrays as examples of technologies used to see what is going on inside cells and how they change behavior.

Another type of array, called a cDNA array, compares gene expression profiles of different cell types or disease states. High-throughput cDNA arrays give us a huge amount of information about what's happening inside cells as they change phenotype and are used

widely in biology. This technique is described clearly in an interactive animation you can do with your students, found at:

http://www.bio.davidson.edu/Courses/genomics/chip/chip.html

FOCUS ON CANCER

In cancer, the genomic sequence is mutated, causing problems with the produced proteins. This results in cells with a cancerous phenotype, which can be considered an "inside-out" phenotypic shift. Some examples of cancer phenotypes and causes (carcinogens such as viruses, toxins, and UV damage) are explained in the Supplementary PowerPoint presentation (http://www.cirm.ca.gov/curriculum_unit-3) for students. While reading this section and teaching about the causes of cancer, try to brainstorm answers to these questions:

- Is a genetic mutation necessary for cancer?
- Could cancer occur only by manipulating the microenvironment?
- If you took a piece of normal tissue and inserted it inside a tumor, what would happen?
- If you infect a chicken embryo with a cancer-causing virus, and the chicken grew up cancer-free, would you assume the chicken's cells were noncancerous?
- How might we test ways to see if certain microenvironments can stop cancer from growing?

What causes cancer?

Cancer is an abnormal cell phenotype characterized by uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes **metastasis** (spread to other locations in the body via lymph or blood). For a cell to turn malignant—highly cancerous—multiple mutations in important regulatory genes must accumulate. Generally speaking, mutations in two basic classes of genes— **tumor suppressor genes** and **proto-oncogenes**—are what lead to cancer:

Tumor Suppressor Genes. These are vital for stopping a cell or group of cells from spontaneously dividing without obtaining the correct signals for initiation or continuation of the cell cycle ("growth"). Tumor suppressor genes are normal genes that slow down cell division, repair DNA mistakes, and tell cells when to die (a process known as **apoptosis** or programmed cell death). So, a mutated tumor suppressor gene leads to cancer because these cells make an abnormal protein that doesn't correctly act to suppress "cancerous attributes" like rapid cell growth, survival, and metastasis. Many different tumor suppressor genes have been identified, including p53, BRCA1, BRCA2, APC, and RB1 (ACS). See:

http://www.cancer.org/cancer/cancercauses/geneticsandcancer/genesandcancer/genes-and-cancer-oncogenes-tumor-suppressor-genes

An example pathway of a specific tumor suppressor is provided below for discussion with advanced or AP students.

Proto-oncogenes. Mutations in proto-oncogenes, which normally code for proteins regulating cell growth, **mitosis**, and differentiation, can lead to their abnormal or overactive function—and lead to cancer. Once a proto-oncogene has been mutated, it is called an **oncogene**, the expression of which contributes to a cancerous phenotype. Oncogenes cause some sort of interference with or acceleration of the cell cycle. They tend to be caused by radiation, toxic compounds, or some biological stress, which degrades the gene or mutates it, thus changing its normal function.

Transcription and translation of an oncogene can have a devastating effect on other cellular functions either by affecting the amount of protein produced or the movement of nucleic DNA (transposons), or by causing another **point mutation** in another proto-oncogene. Other causes of oncogene activation include chromosomes that have broken and rejoined incorrectly, and **translocation** of gene fragments from one chromosome to another. If a translocated oncogene ends up near an active promoter (or other transcriptional control element), its activity may increase, making it an oncogene.

Specific examples of Tumor suppressor genes: Cell cycle regulators

Cyclin dependent kinases and inhibitors are cell cycle regulator proteins that can be the targets of cancer-causing mutations. For example, **p53** controls key elements in the cell cycle. The expression of p53 is activated by damaged DNA somewhere else in the genome. If the cell were to continue dividing, a DNA replication error, possibly a serious one, would be made and passed along to the daughter cells. P53 acts to halt the cell cycle so the broken DNA can be successfully repaired before division.

Once *p53* is produced, it acts as a transcription factor, activating **p21.** This gene stops the cell's growth by creating an inhibitory protein (*p21*) that binds to **cyclin-dependent kinases** A & B. Cyclin-dependent kinases normally bind to cyclin, setting off signaling pathways enabling cell division to begin.

Cyclin-dependent kinase inhibitors such as *p21* bind to and inactivate the cyclin-dependent kinase/cyclin complex, halting cell division before the S-phase. *p53* then activates DNA repair genes to fix the damaged DNA. If repair is successful, the cell will continue past the G1 checkpoint into S-phase and divide. If repair is impossible, *p53* activates a suicide gene which prompts **lysosomes** to hydrolyze (dissolve) the cell apoptosis. If *p53* doesn't function correctly, these regulatory cycles cannot occur and the cell can become cancerous. In fact, in 50% of cancers *p53* is missing or mutated.

Cancer stem cell hypothesis

A tumor is a collection of cell types; some are terminally differentiated, some can proliferate and differentiate to some degree, and a significant number may be able to form all tumor cell types—called **cancer stem cells.** These cells give rise to additional tumors when transplanted to another animal, and those tumors contain all cell types in the original tumor. The cancer stem cell hypothesis argues that mutations to stem cells activate them into cancer stem cells, which cause tumors and retain the capacity to differentiate into new tumors. When different cell types in a mouse tumor were dissociated and then sorted into populations of similar types, only one small population was able to give rise to new tumors when injected into different immune-deficient mice. This implies there could be a population of "adult stem cells gone wrong, which have gained the ability to proliferate and differentiate into multiple tumor cell types and are responsible for initiation of the cancer.

The Microenvironment and Cancer

The four aspects of the microenvironment—soluble factors, extracellular matrix proteins, cell-cell interactions, and forces—are implicated in cancerous responses of cells. As discussed earlier, cancer arises from DNA mutations in a cell that cause proliferation, differentiation, and metastasis (migration and spreading).

Could cancer arise from a normal stem cell in the wrong microenvironment? When embryonic stem cells (pluripotent, and incredibly similar in their characteristics to immortalized cancer cell lines) are injected into immune-system–deficient mice (**SCID mice**) so that the immune system cannot fight the stem cells, these mice form tumors that contain cell types from all three germ layers, as a pluripotent cell would be expected to contain. Here, the embryonic stem cells suddenly were moved to a wildly different microenvironment than embryonic stem cells require to remain unchanged. This barrage of abnormal differentiation cues to the pluripotent stem cells caused them to turn into many different cell types.

Like the above example, adult stem cells, or other cells exposed to abnormal cues, might be expected to turn cancerous, in combination with or even without cancer-causing mutations to their genomes. Mutations in surrounding cells, or something else irregular about the niche, might push a cell toward a cancer phenotype.

It would be therapeutically useful to know if a normal microenvironment could cause inhibition of a cancer cell. Early studies with **embryonal carcinoma cells**, which are undifferentiated cancer cells that form different cell types when introduced into adult mice, showed that if injected into a **blastocyst**, the cells that were derived from the cancer cells no longer caused cancer in the mice that developed from the injected blastocyst. This suggested that the microenvironment prevented or reversed the cancerous properties, then caused them to develop normally in the presence of cues from the normal blastocyst

environment.

Another study, supporting the idea that cancer cells require additional signals besides genetic abnormalities to become cancerous, used a cancer-causing virus that required a certain microenvironment for tumor growth. The Rous Sarcoma Virus (RSV) caused **sarcomas** (cancerous growth of the connective tissue) in mature chickens at the site of injection. Scientists observed that when the virus was injected into chick embryos, it did not cause cancer even though the chick cells derived from those embryos had the ability to gain cancerous properties or "transform" when cultured **in vitro**. Surprisingly, a later study showed that creating a lesion or wound would cause the surrounding cells to turn cancerous and cause tumors. More specifically, it was shown that a protein called TGF-beta, involved in wound healing, was sufficient to induce tumor formation.
These findings indicate that factors involved in wound healing could promote cell transformation, and that this particular microenvironment provided important signals for the cancer growth.