Growth and Differentiation:
the Case of Hematopoiesis

As we have mentioned on several occasions this semester, there is a marked contrast between the genotypes of the cells in our body and their phenotypes. Almost all the cells in our body have an identical genotype - an identical set of genes. Yet the phenotypes of cells in our body are highly variable. The only way to reconcile these two apparently contradictory facts is to assume that the genotype of our cells contains much more information than is used by any one particular cell. In other words, different cells consult the common genomic library in different ways.

Differentiation

We imagine that during the early development of an embryo, cells in different parts of the embryo decide on which parts of their genome they will use to program their future lifestyles, their future phenotypes. This selective reading of the genome means that each distinct cell type will only consult a subset of the genes that are carried in its genome. The embryologic processes that allow cells to assume distinct phenotypes, phenotypes that distinguish them from other cells coexisting in the same embryo is termed *differentiation*.

We can imagine that as the fertilized egg divides into 2 cells, then 4 then 8 and so forth, the cells in this ever-increasing population decide on what their individual differentiated fates will be. This decision commits the embryonic cell to a distinct *differentiation pathway* that will affect not only this cell itself, but all of its lineal descendants as well. The line of descendants that leads from this early embryonic cell to its descendants is also called a *differentiation lineage*.

As an example, we might speculate that early in the development of an embryo, one cell decides that it will become the ancestor of all the blood-forming (hematopoietic) cells in the body. Another cell may commit to becoming the ancestor of all the nerve cells in the body; and yet another may become the ancestor of all the connective tissue cells. Importantly, the lineal descendants of these early embryonic cells will continue to respect the decision made by their early embryonic ancestor. Thus, the descendants of the embryonic cell that committed itself to become a blood-forming cell will, many cell generations later, continue to be specialized for the processes of blood formation.

This scheme immediately provokes two major questions, neither of which we can answer for the moment. First*,* how does a given cell in the embryo choose its fate and that of its descendants? An early, still undifferentiated embryonic cell can pick and choose among many distinct differentiation pathways. What factors and what information persuade it to choose one fate rather than another?

Second, after an early embryonic cell has decided its own fate, how is this decision remembered by its descendants? How does a particular cell 30 or 40 cell generations later know that its early ancestor was committed to a distinct differentiation pathway**?**
We might speculate that each time a cell commits itself to a particular differentiation lineage, its genome is mutated in a specific way, ensuring that this decision will be perpetuated in the DNA sequences of its genes. But in fact, this does not happen. The nucleated cells in different tissues possess identical genomes. Therefore, we conclude that differentiation decisions are transmitted from one cell generation to the next via mechanisms that do not involve changes in the structure (sequence) of the DNA.

Let's focus for a moment on the first problem. In doing so, we must assume that a complicated series of signals persuades an early embryonic cell to commit itself to take on one or another differentiated state. What kind of information could this cell use?

Positional information

A cell may use positional information when committing to a particular differentiation lineage. You can imagine that a cell knows or remembers what region of the fertilized egg it arose from. Different regions of the egg may be chemically distinct from one another and this distinctness may be passed on to the cells that descend from these different regions. Alternatively, a cell that is deciding how to differentiate may sense what part of the embryo it is located in. Such positional information may help a cell decide what it and its descendants should become. For example, a cell that finds itself in the outer layers of the embryo might decide to become the ancestor of skin cells while a cell in the middle of the embryo might decide to become ancestor to the cells that will form the gut.

How might a cell sense its own position in the embryo? We have a clue to this puzzle. Cells in an embryo concern themselves with the identities of their nearby neighbors. The identities of these neighbors govern its perceptions about its own position in the embryo.

Growth factors

If position is determined by neighboring cells, how does a cell ascertain the identities of its neighbors? A cell will communicate with its nearby neighbors by releasing and/or receiving the protein molecules that we previously termed *growth factors*, (GFs)**.** Previously, we discussed GFs in the context of decisions made by cells whether they should grow or not. GFs were depicted as *mitogens* (agents that induce mitosis), and as such convey a binary signal. When GFs are present, they induce a cell to grow; when they are absent, the cell does not grow. Now we want to add more richness to the information that they carry: maybe some GFs can also carry information that persuades a cell to differentiate in one direction or another.

Recall that GFs operate as inter-cellular messengers that convey information from one cell to another through their presence or absence. Several dozen distinct growth factors have been discovered to date*. Each is a protein molecule, usually of relatively small size (100 amino acids or less). A given cell may release several distinct growth factors and respond to a number of others. This means that the positional information received by a cell might be provided by the combinatorial mix of growth factor signals that it receives from its surrounding space, each of these factors having been released by one or more of the neighbors that surround it.

> *Examples of GFs are platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), tumor growth factor- (TGF-) and a series of growth factors that are specialized to affect hematopoietic cells and are called interleukins.*
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> *At least 14 distinct interleukins have been found, each having its own specific amino acid sequence. Each of these various growth factors/interleukins is released by one cell type and impinges upon a second cell type which senses its presence and responds in one or another way.*

Replenishing the ranks

We can understand some of the features of embryonic decision-making by looking at the process of blood formation that occurs in the adult bone marrow and spleen where most of the red and white cells of our blood are formed. The picture that we have painted until now suggests that cells decide early during embryonic development about differentiation fates; their differentiated descendants then form the tissues in the later embryo, in the newborn, and ultimately in the adult. Why now are we suddenly talking about differentiation occurring in an adult, long after embryogenesis?

In fact, all throughout embryonic development and through our entire lives, we continue to carry within our bodies large numbers of undifferentiated cells that retain the potential to commit themselves to specific differentiation pathways. You might think of these as primitive, undifferentiated embryonic cells that remain throughout life scattered about the body. Blood forming cells are particularly attractive to study experimentally because they are so plentiful; as many as 10^{10} blood cell progeny are formed in an hour. For this reason, undifferentiated blood forming cell precursors are a highly useful experimental model for certain early embryonic processes.

The reason for the continued presence of primitive, undifferentiated cells in our body is simple and straightforward. The cells in almost all of our organs are constantly wearing out**. Some cells have lifetimes of days, yet others live weeks, months or years. When these cells die, they must be replaced by an equivalent number of new cells. These replacements are usually newly differentiated cells that are recruited to fill the ranks of their recently fallen comrades.

> ***Red blood cells in humans have lifetimes of about 120 days. Blood platelets have lifetimes of several days. Skin cells have a lifetime of several weeks. As red blood cells, platelets, and skin cells die, newly differentiated cells of the same type are produced. An exception to this picture is in the brain. Unlike virtually all other organs, when nerve cells (neurons) in the adult brain die, they are not replaced, resulting in a slow steady loss of a hundred thousand or more neurons a day and inexorable shrinkage of brain power!*

One way to replace a missing, recently departed skin or blood cell might be to induce a surviving, differentiated skin cell to grow and divide into two. But this rarely happens for a very important reason: most cell types that become highly differentiated give up the option of ever dividing again. Thus, in many organs in the body, the highly differentiated, specialized cells are *post-mitotic*.   They may be viable and healthy, but they have lost the ability to divide (go through mitosis) again. If their ranks are going to be replenished by new recruits, these new recruits must arise through the growth and division of those cells within the organ that retain the ability to grow and divide. Only cells, called *stem cells*, have remained undifferentiated and retain the option to divide.

Stem cells: mothers and daughters

The primitive, undifferentiated stem cells are scattered about in various of the body.   When a stem cell divides, its daughters have two possible fates. One or both daughter cells may assume the undifferentiated phenotype of their mother, or alternatively, one or both daughters may decide to take on a more differentiated phenotype. In fact, it is usually the case that at least one daughter cell retains the phenotype of the mother cell.

The logic of this binary decision-making is clear. In general, an organ or tissue wants to maintain rather constant numbers (*pools*) of stem cells and differentiated cells. The one daughter that retains the phenotype of the mother stem cell becomes itself a new stem cell, ensuring that the total number of stem cells does not change. The other daughter cell, by choosing to

differentiate, replenishes the pool of differentiated cells whose presence is essential for the functioning of a tissue.

Why, in fact, does a tissue wish to maintain a constant, minimum number of stem cells? Again the logic is simple: stem cells constitute a safety back-up for the tissue. Normal tissue can lose some of its differentiated cells by normal attrition (i.e. wear and tear) or by damage or injury. When this occurs the tissue can call upon its stem cell population to divide and create progeny that will rapidly repopulate the temporarily depleted ranks of the differentiated cells within the tissue. If both daughter cells of an undifferentiated stem cell chose to differentiate, then the tissue or organ would lose a cell from its stem cell compartment. If this occurred repeatedly, the stem cell compartment would be depleted and the tissue would lose its ability to repair and replenish itself.

Multiple differentiation pathways

More often than not, the cells that form a given tissue or organ are representatives of several distinct differentiated cell types. These distinct cell types coexist with one another and collaborate to create the functional organ. Importantly, in many such organs, these various distinctive cell types may all descend from a common stem cell precursor. There are exceptions to this. In the liver most of the cells hepatocytes or muscle cells, and the contractile tissue is composed of cells identical to one another.

Consequently, we can imagine at least one dimension of greater complexity beyond that described above: when stem cells differentiate they may choose several distinct differentiation pathways. What external stimuli might influence a stem cell to choose one differentiation pathway rather than another? For the moment, we can speculate that certain growth factors encountered by the stem cell may induce them to choose one or another pathway of differentiation. Evidence for this will follow later.

In blood cells[§] we have a dramatic example a common stem cell becoming the ancestor of a multitude of distinct cell types including a variety of white blood cells as well as the red cells (erythrocytes). As listed below, there are as many as 9 different blood cell types in the body. Some of these, such as T lymphocytes, are further divided into sub-classes that play distinct roles in the immune system.

The full complexity of the hematopoietic system can be understood in terms of the multiple functions of blood cells. They serve several basic functions: to transport gases (erythrocytes), to prevent bleeding by forming blood clots (platelets), and to form an immune defense system that protects us against a variety of foreign infectious agents and irritants (all the rest).

[§]*We normally think of a tissue or organ as being a solid mass such as the kidney or the liver or the brain. Blood is also a tissue, but the constituent cells that form blood happen to be single, circulating cells rather than cells that form a solid, physical mass. The dispersed nature of the blood cells throughout the body in no way detracts from the fact that the cells in the hematopoietic system constitute a single interacting organ system.*

It is also important to note that not all of the blood cells are in fact free found throughout our circulatory system. Some of cells spend extended periods of time in solid organ host tissues. Many of the precursor, undifferentiated cells live in the bone marrow and spleen. The thymus (at the base of our throat) and the lymph nodes (scattered throughout the body) are homes for many lymphocyte populations.

Inventory of the cell types in blood: (per liter of blood)

Red blood cells (erythrocytes): transport CO_2 and O_2. (5×10^{12} cells)

White blood cells

Granulocytes

Neutrophils, engulf and destroy bacteria. (5×10^9 cells)

Eosinophils, destroy larger parasites, modulate inflammatory response. (2×10^8 cells)

Basophils, release histamine and serotoninin, modulate certain inflammatory responses. (4×10^7 cells)

Monocytes, develop into tissue macrophages that phagocytose invading microbes and senescent cells. (4×10^8 cells)

Lymphocytes

B cells, make antibodies. (2×10^9 cells)

T cells, kill virus infected cells, and regulate activities of other leukocytes. (1×10^9 cells)

Natural killer cells, kill virus-infected cells and cancer cells. (1×10^8 cells)

Platelets are cell fragments that derive from megakaryocyte precursors. They initiate blood clotting. (3×10^{11} "cells")

A multi-talented cell

The average person creates 3-10 billion red cells, platelets, and neutrophils per hour! This represents an enormous biosynthetic effort. How is all this arranged and regulated? As we will see, blood cell formation represents a highly controlled system that regulates both mitogenic activity and differentiation decisions.

There is compelling evidence that the wide array of blood cells derives from a single common precursor. This cell is termed a *pluripotent* stem cell to denote the fact that it has the potential to differentiate into a multitude of distinct cell types. Importantly, the pluripotent stem cell does not itself differentiate directly into all of the end-stage, highly differentiate progeny in a single step. Rather, we see a multi-step hierarchy involving a number of intermediate cell types. The fact that there are cells at various stages of differentiation means that we can no longer speak simply in terms of undifferentiated and differentiated cells. Instead, there are degrees of differentiation.

A highly branched family tree

The pluripotent stem cell can replenish itself or it can spawn two distinct types of daughter cells, one in the *lymphoid* lineage, the other in the *myeloid* (marrow) lineage. Each of these daughter cells has itself just begun the differentiation pathway. Although committed to either the lymphoid or the myeloid lineage, each of these daughters retains a stem cell phenotype in the sense that it retains the option of replenishing itself. Thus, we learn that the daughter of a stem cell may itself be another kind of stem cell, but one that has already limited its options.

The lymphoid lineage is initially associated with the thymus where two types of lymphocytes, B-lymphocytes and T-lymphocytes may develop. Each of these takes on specific functions in the immune system. The end stage T and B lymphocytes are not created in one step from their lymphoid stem cell precursor. Instead, there are intermediate, less-differentiated cell types are formed along the path toward the end stage cells. Note that lymphocytes, unlike almost all other highly differentiated cell types, retain the option of dividing.

The myeloid stem cell lineage is much more complex. There are least five different sub-lineages. Among these is the *erythrocyte* (red cell) lineage. An additional three lineages - *eosinophils, basophils, and neutrophils/macrophages* are devoted to various aspects of recognizing and gobbling up invading infectious agents or cells that have been infected by such agents and need to be destroyed. Another lineage leads the *megakaryocytes* that form the small, anucleate platelets that participate in forming clots. Cytoplasmic fragments are budded off the plasma membranes of megakaryocytes to yield the small, anucleate platelets that participate in forming clots.

Bone marrow transplants

How can we prove that the pluripotent stem cell is more than a figment of some biologist's fertile imagination? The procedure of bone marrow transplantation makes this possible. In such a procedure, a mouse is irradiated with X-rays. These X-rays are delivered at a dose such that all of the bone marrow stem cells are wiped out. These cells turn out to be more sensitive to X-ray killing than other stem cells throughout the body. Consequently, it is possible to find a dose of X-rays that kills bone marrow cells but leaves the remaining tissues of the mouse intact.

Such irradiation is usually lethal because most of the blood cell types turn over relatively rapidly and need to be replaced continually. For this reason, the death of precursor stem cells places the mouse in a precarious position. It will quickly run out of platelets to form clots and lymphocytes to fight infections. The longer-lived red blood cells are more gradually depleted.

These mice can be rescued by injection of a small number of marrow cells prepared from a normal, untreated donor mouse. After injection, these donor cells migrate into the depleted bone marrow of the irradiated mouse. Physiological signals indicate a need for many types of differentiated cells and begin to divide frenetically. Over several weeks, the donor stem cell progeny expand exponentially and replenish the marrow and the various compartments of end stage differentiated cells. Once the various differentiated cell types in the blood have been replenished, the stem cells revert to their normal, lower level of activity. Of course, the same processes can be envisioned in human beings subjected to bone marrow transplantation.

The elusive pluripotent stem cell

The fact that a small number of donor bone marrow cells can rescue a lethally irradiated mouse (or human) suggests but does not prove that a single precursor stem cell has pluripotency, i.e., the ability to spawn multiple differentiated cell lineages.

Pluripotency can be proven creating a population of normal stem cells, such that each cell is marked with a distinctive tag. Such tagging involves attaching a unique genetic sequence to each of the thousands of cells in the stem cell where no two tags are the same.

How can cells be tagged in such a fashion? One method uses a light dose of X-rays. This light dose will not kill the donor stem cells but instead will create a small amount of genetic damage in each cell. Included in this damage will be random breaks and rearrangements of chromosomes that can be seen under the light microscope. As a consequence, each of the donor cells in such a lightly irradiated population will have a distinctive, unique chromosomal abnormality that serves to distinguish it from its sister cells in the population.

A small number of these randomly tagged donor stem cells can then be injected into a lethally irradiated animal. These cells will enter into the marrow of the irradiated animal and begin to repopulate it, thereby rescuing the animal from death. Subsequently, the various blood cells types of the now-rescued animal can be examined for chromosomal abnormalities. If different blood cell types in the rescued animal (e.g. eosinophils, macrophages, lymphocytes) all carry the same unique chromosomal abnormality (distinctive tag), then all the blood cells descended from the same common ancestral pluripotent stem cell (a cell that also carried this unique abnormality, having acquired it following the light X-ray treatment). Hence, we can conclude that this ancestor was capable of spawning a diverse array of descendant cells that populated the entire bone marrow. Indeed, this is just what is observed after doing such an experiment.

Homeostasis - a balancing act

The pool sizes of each distinct type of blood cell must be kept under careful control. If there are too many of a given type, then a *leukemia* (a blood cancer) will occur. If there are too few, than the animal or person will die from insufficient gas transport (*anemia*) or from an overwhelming infection because of the absence of immune system cells.

The process by which the number of cells (or other vital physiologic components) is kept under careful, highly balanced control is termed *homeostasis*. As you may imagine, many biological homeostatic systems depend on carefully balanced negative feedback loops (Purves, pp. 699, 721-723). When the end product of a metabolic pathway accumulates to a high concentration, it acts to damp down the earlier steps in the pathway. Conversely, when the end product is present at low levels, the earlier steps in the pathway are induced. If designed properly, such a control loop can ensure constant levels of the end product. Hundreds of such negative feedback loops have been described in as many biological systems.

Homeostasis of erythropoiesis - keeping the right shade of red

It is obvious from what was discussed above that the levels of all the various blood cell types must be kept under very careful homeostatic control. We will focus now on only one of these control loops - that involving the production of erythrocytes. The process of producing red blood cells, as opposed to other of the cell types in the blood, is termed *erythropoiesis*.

What biosensors can the bone marrow use to determine whether it has too many or too few erythrocytes? In principle, the bone marrow might monitor the viscosity of the blood. Since the red cells constitute the great bulk of the cells circulating in the blood, too many red cells might create a hyper-viscous blood. But in fact, biological systems don't monitor viscosity very well.

A much better parameter to monitor is *oxygenation* - the extent to which the organs throughout the body are receiving an adequate oxygen supply. Oxygenation is a direct reflection of red cell levels since the red cells are responsible for transporting oxygen from the lungs to the peripheral tissues. If the oxygen levels in the tissues are too low, this is a clear sign that more red blood cells are required to transport oxygen from the lungs.

Since low oxygen levels affect all peripheral organs, the extent of oxygenation could, in principle, be gauged at a number of organ sites. In fact, through a quirk of mammalian evolution, the kidneys have been assigned the role of monitoring oxygenation of the blood. They have this task in addition to their main job of purifying blood by excreting urine. When our kidneys sense low oxygen levels in the blood, they respond by sending a message to the bone marrow to increase red cell production.

Erythropoietin (EPO) and very high mountains

The message sent by the kidneys when low blood oxygen is sensed is the release of a protein called *erythropoietinI (EPO)*. This growth factor released by kidney cells passes through the circulation from the kidneys into the marrow where it specifically stimulates the red cell precursors. Other branches of the hematopoietic system are not affected by EPO.

This signaling system has interesting consequences for us. The altitude sickness experienced after rapid movementI to high altitudes is due to the fact that our normal red cell levels are not concentrated enough to scavenge sufficient oxygen from mountain air with low oxygen tension. Sensing inadequate oxygenation, our kidneys produce large amounts of EPO, which in turn mobilizes the erythrocyte stem cell precursors residing in the marrow. They respond by churning out astronomical numbers of red cells in several days' time. Soon we feel much better. Our blood is now thick with red cells that compensate for the lower oxygen levels of the air pulled into the lungs. As a result, we are able to oxygenate peripheral tissues effectively once again.

Failing kidneys

Humans with failing kidneys are unable to filter and detoxify their blood effectively. Much of this defect can be cured by kidney dialysis, which eliminates many of the wastes from the blood of such patients. But the dialysis does not deal with the fact that these patients rapidly become anemic (inadequate levels of red cells). Their anemia is due to the absence of EPO secretion from their malfunctioning (or absent) kidneys.

One way of curing this has been by giving these patients blood transfusions. But these are expensive, cumbersome, and with the identification of AIDS and HIV infections, potentially dangerous. The alternative is to give these patients exogenous EPO. One biotechnology company earned more than 10^9 dollars (US) last year by producing EPO through the recombinant DNA techniques. This recombinant EPO can be injected into the kidney patients, compensating for their own inadequate EPO production.

Cross-country skiers

EPO, like other growth factors, acts on a specific receptor, in this case a receptor displayed by a specific stem cell in the bone marrow. In response to binding EPO, this receptor induces the proliferation of the stem cell and the differentiation of daughter cells into erythrocytes. In certain Scandinavian families, there is a mutation in the gene encoding the EPO receptor. This mutation hyperactivates the receptor, much like the mutations that create hyperactive GF receptors identified as oncogene proteins. The hyperactivation in this case is mild and as a consequence, the stem cells of these patients churn out a bit too many red blood cells. This has been used to explain the fact that individuals from these families have done extremely well in Olympic cross-country skiing competitions, since they have hyper-efficient transport systems for oxygenating their peripheral tissues!

Bone marrow transplants and cancer therapy

The existence of bone marrow stem cells makes other things possible as well. Often during the treatment of cancer, chemotherapeutic drugs or X-rays are used to kill off the cancer cells. Unfortunately, these treatments also kill other cells in the body that are rapidly dividing. Included among these unintended victims are the cells of the bone marrow, the cells lining the gut, and the skin cells. The sensitivity of the skin cells explains the temporary loss of hair in many patients receiving anti-cancer therapy.

These various rapidly dividing organ systems can usually regenerate, given enough time. In the case of gut and the skin, this may lead to temporary discomfort, but the restoration usually occurs quite nicely. In the case of bone marrow, the temporary depression of various blood cell levels can be fatal because of anemia and reduced immune defenses. Thus, most chemotherapeutics are given in relatively low, sub-optimal doses because the use of higher doses may kill the patient by destroying too much bone marrow.

Bone marrow transplantation offers a possible solution to this problem. Bone marrow stem cells can be removed from a patient prior to chemotherapy and stored. Anti-cancer, chemotherapeutic drugs can then be administered at very high (and much more effective) doses. The anti-cancer drugs do their job quickly and are cleared from the body within hours after injection. Thereafter, the healthy bone marrow stem cells that have been stored away are injected into the treated cancer patient and rescue hematopoietic function in the marrow.