Biol 213 Genetics: Wednesday, November 20, 2000
Genetics of Cancer

We've just finished three weeks of material that few of you have ever seen before. For most it was a period of feeling lost at the beginning, and for many, lost also at the end. When I find myself needing to understand an area totally new to me (which is a pretty frequent occurrence in this line of work), I'm also lost and confused. I think years of experience helps a bit to make the confusion less, but not as much as you might think. Where experience helps a LOT is in helping me realize that confusion is a temporary state. I've been confused so many times that I know if I read enough articles something somewhere will click and almost instantaneously the message will become clear. I just have to keep reading and keep wriggling, like a worm on a hook. If one paper doesn't free me from the hook, maybe the next one will. If this approach isn't helping, maybe another one is the answer. In your case, perhaps Problem 12 will be the tool that enables you to rise gloriously above the trees, but if not, then maybe it's Problem 13. So long as you go out and get it and don't wait for it to come to you, it <u>will</u> come.

<u>Reading assignment</u>: Although I've occasionally suggested useful pages to supplement certain sections, I have not built these notes around the chapter in the text (Chapter 23) concerned with cancer. It seems to me that the chapter bludgeons you with facts to the extent that the main message gets lost.

Outline

I. Clonal Nature of Cancer
II. Somatic vs. Germline Mutation
III. Lessons from Inherited Forms of Cancer (pp.643-644)
IV. *RB*: A Tumor Suppressor Gene (pp.640-643)
V. Other Genes Related to Cancer (pp.637-640)
VI. Progression of Cancer (p.642)
VII. Summary

I. Clonal nature of cancer

We've just considerable energy considering how genes suffer mutations. Before that, we spent a few weeks considering how organisms regulate the expression of their genes. The logical combination of these two subjects is the effect of mutation on gene regulation. The outcome of such mutation, in some instances, is cancer.

First of all, what is cancer? A disease, of course, but is it an infectious disease, like hepatitis, a disease caused by a virus? Alternatively, is it a genetic disease, like sickle-cell anemia, inherited by progeny from their parents? There is something right and wrong with each of these different views. Certain viruses can cause cancer (pp.633-637), though that is not the usual route. We'll see that the tumor state is certainly inherited, but in general cancer is not passed down from parent to progeny. Inherited,... not inherited... how do we resolve this paradox? The key is to understand the clonal nature of tumors. You've encountered the term "clonal" before – recall the Luria-Delbrück experiment and how mutations accumulate? Rather than define this term, let me explain it with an experiment.

Protein from 41 patients with leukemia (a cancer that results in abnormal leukocytes) were screened in the hopes of identifying genetics markers linked to the genetic defect associated with the disease. One protein studied was glucose-6-phosphate dehydrogenase (G6PD), an **X**-encoded protein that exists in the population in two common forms: **A** and **B**. Most people carry the **A** form or are heterozygous, but one patient was identified whose leukocytes carried only the **B** form. This was puzzling, because her children were type **A** (see Fig. 1A).

SQ1. What should the genotype of the mother be to produce her offspring?

To resolve this mystery, G6PD from the mother was reexamined, giving the results shown in Fig. 1B.

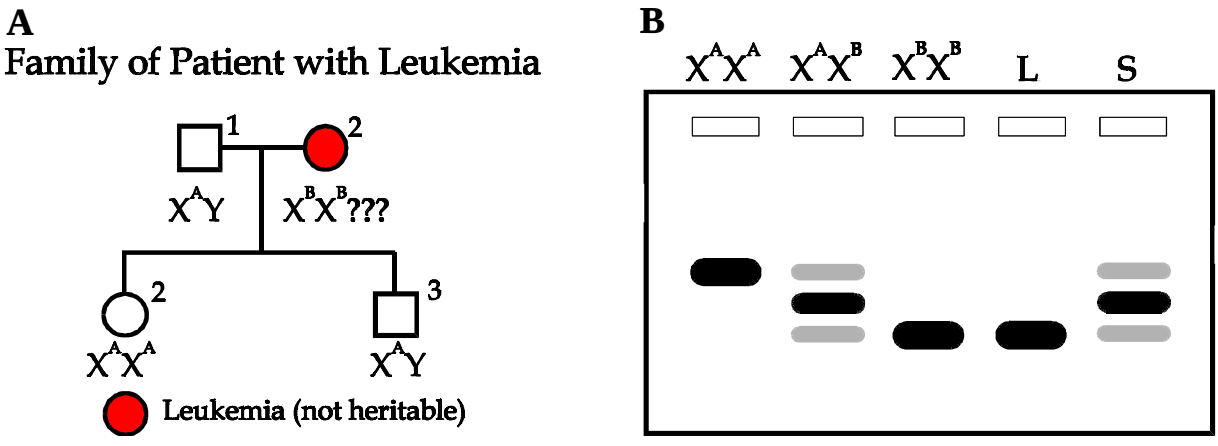

Fig. 1: Analysis of glucose-6-phosphate dehydrogenase (G6PD) protein. Gel electrophoresis of protein samples, staining for G6PD activity. Protein for the left three lanes was taken from subjects with known genotypes. **L** is the protein sample from the leukocytes of the patient. **S** is a protein sample from skin cells of the patient. (Adapted from Fialkow et al (1969) Proc Natl Acad Sci USA 163:194-196)

SQ2. What is the apparent genotype of the mother's tumor cells?

SQ3. What is the apparent genotype of the mother's normal skin cells?

SQ4. And by the way, why does the proven heterozygote have three bands?

It is evident that her cancer tissue has a different genotype than her normal tissue! It turns out that the same results could have been obtained using any X-linked marker. What does this mean?

To understand the significance of this result, I'm going to have to digress a bit to earlier in the semester when the subject, for ever so brief a time, was gender determination. Females are XX, males are XY. We know that, but we also know that nondisjunctions leading to unbalanced numbers of chromosomes are usually lethal. At the very least, aneuploidy for a chromosome, like three copies of chromosome 21, has dire effects, the mental retardation associated with Down syndrome. Why is it that either males, with one X, or females, with two, don't suffer the effects of unbalanced chromosome number?

In brief, the answer is that individuals with two X chromosomes put half of their X chromosomes to sleep and therefore end up with the same number of active X chromosomes as XY males. (Half of you will be pleased to learn that one active X chromosome is the normal human state). The inactive X chromosome is condensed into a visible structure known as a Barr body (see Fig. 7-3A, p.173 for a picture of a Barr body). The decision of which X chromosome to inactivate is made early in embryogenesis. In each cell of the multicellular embryo, a random choice is made between the two X chromosomes, and all progeny of the cell is affected by the decision.

Females are mosaics, with patches coming from different parent cells in the embryo. Fig. 7-3B is a picture of a female calico cat, in which the pattern of X-inactivation is manifest because the cat is heterozygous for an X-linked gene determining pigmentation. Each white patch arose from a parent cell that randomly chose to inactivate the X chromosome carrying the wild-type allele and retain the one carrying the allele conferring defective pigmentation. Each black patch arose from a parent cell that made the opposite decision. Such patches exist in all mammalian females, even if they are invisible, representing whatever differences there may exist between their two X chromosomes.

SQ5. Doesn't all of this sound vaguely familiar? Do you recall another case in which certain genes are inactivated early in differentiation, leading to patches of gene expression?

If you’re reminded of the red and white patches in fly eyes that came up in a problem set a few weeks ago then you’re on the right track, because heterochromatization during embryogenesis underlies both phenomena.

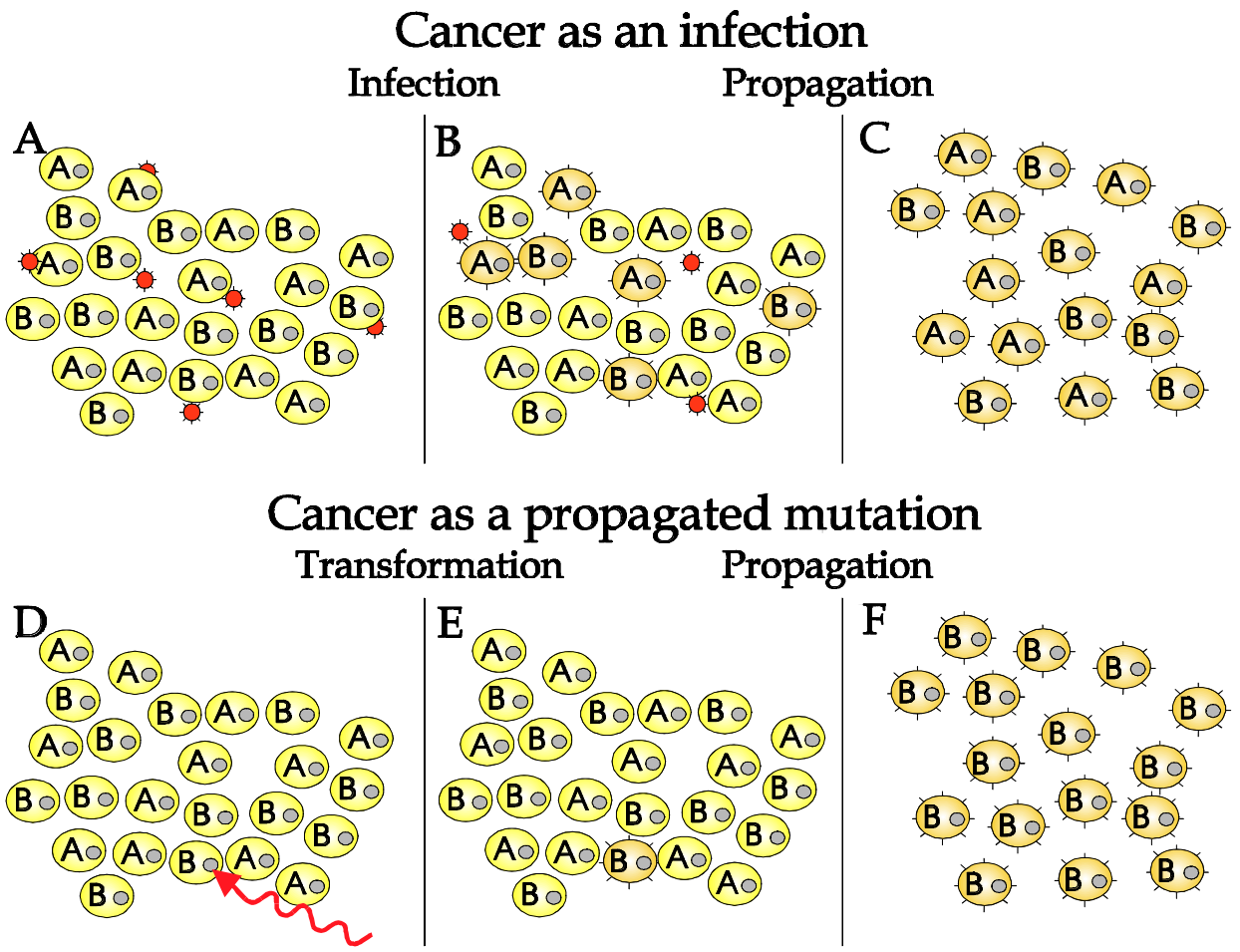

Fig. 2. Two models to explain cancer. (**A-C**) Cancer viewed as an infection. (**A**) Cancer-causing viruses (red circles) infects healthy cells of a female. Half of the cells have an active **X** chromosome carrying the *A* allele and half have an active **X** chromosome carrying the *B* allele. (**B**) Infected cells are transformed into precancer cells (gold and spiked). (**C**) Transformed cells, with a mixture of active **X** chromosomes, divide and raise havoc. (**D-F**) Cancer viewed as the result of a mutation. (**D**) A single cell suffers a mutation (red arrow). (**E**) The mutated cell is transformed into a precancer cell. Of course it contains only one type of active **X** chromosome. (**F**) The transformed cell divides and propagates itself. All its progeny carry the same active **X** chromosome.

Now we can return to our experiment. We would expect that some tissue samples from a female heterozygous for G6PD would have normal G6PD activity, if the defective allele were inactivated, while other tissue samples would have no G6PD activity, if the wild-type allele were inactivated. Suppose that cancer were determined by a viral infection (not generally true) and that a population of normal leukocytes from a heterozygote has a mixture of cells, some having G6PD type **A** and some having G6PD type **B** (true). You'd expect that the virus should infect both types of cells without regard to phenotype (Fig. 2**A-C**). The cancerous leukocytes from the patient should have shown a mixture of G6PD types.

This was not the case, so we need to find another explanation for leukemia. From the G6PD experiment and many others, it is evident that tumors arise not by infection

but by clonal growth (Fig. 2**D-F**). That is, one cell somehow becomes cancerous, and that cell becomes the progenitor of the entire tumor.

This situation is reminiscent of the T4 recombination experiment you completed a few weeks ago. You plated thousands of phage, but each individual plaque arose from a single phage that was the progeny of two different parental phage that recombined and restored *rII* to wild-type. Just as recombination within *rII* is a genetic phenomenon, so is tumor production, in that some cell, unlike thousands of its neighbors, somehow gains the genetic capacity to grow and make a huge colony, which we call a tumor. Thus at the cellular level, cancer is inherited and is demonstrably a genetic disease.

SQ6. HIV infects helper T cells and integrates its genome at random into the human chromosome. Suppose you isolated T cells from a person with full blown aids and determined the position(s) at which HIV had integrated. Match the alternative finding to the possible conclusion(s):

Finding	**Conclusion**
a. HIV is found at several positions in the pooled DNA from the isolated T cells	**A. HIV integrated once and that infected T cell reproduced, resulting in the large number of infected cells observed today.**
b. HIV is found at only one position	**B. HIV was released from the initially infected cell and infected other T cells.**
	C. The initial infection consisted of many viruses infecting many T cells.

II. Somatic vs Germline Mutation

In most cases, however, cancer is not a genetic disease at all, at least not in the same sense as is sickle cell anemia. To explain why, I need to make the distinction between somatic and germline mutation. In some sense, you can view a chicken as the egg's way of making more eggs.

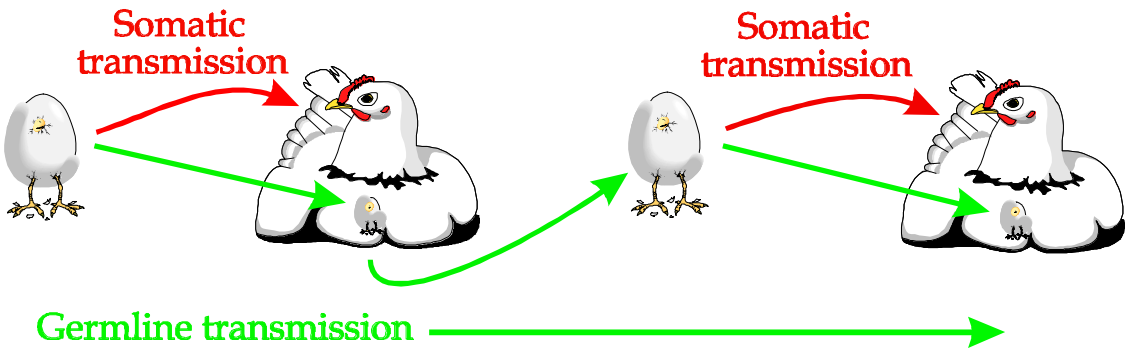

Fig. 3: Somatic vs Germline Transmission

Genetically, there is a line that runs from one egg through the gonads to the next, picking up a sperm along the way. Offshoots that go to other chicken parts besides the

gonads are genetic dead ends: they don't lead to other chickens. It may seem blasphemous or at the very least highly undignified to think of ourselves as servile appendages of our DNA, but it is a useful view from the standpoint of mutation. Mutations that occur in this line from egg to egg are passed on to succeeding generations and are called germline mutations. Those that occur in other parts of the body besides the gonads (or cells leading to the gonads) are called somatic mutations (body mutations).

SQ7. The first sanctioned attempt to treat a disease by gene therapy attacked Severe Combined Immunodeficiency (SCID), a disease caused by the absence of the enzyme adenine deaminase in T lymphocytes. A gene encoding the enzyme was introduced into bone marrow cells from which T lymphocytes are derived. Is this an example of germline or somatic gene therapy? (By the way, the boy's condition improved, but the jury is still out on the efficacy of the procedure).

The mutations that lead to cancer are generally somatic mutations. They are obviously genetic lesions -- that's what mutation means -- but since they don't occur in the germline, they are not inherited. So cancer is paradoxically a genetic disease that is usually not inherited. There are exceptions to this generality, however. People do inherit subtle predispositions to certain cancers, and there are rare but very informative cases in which cancers are very obviously inherited: a key mutation occurs in the germ line and is passed on henceforth from generation to generation.

II.C. Lessons from Inherited Forms of Cancer (pp.643-644)

Retinoblastoma, cancer of the retina, usually occurs sporadically (it is not inherited), but rarely, families are encountered with a history of the disease. Fig. 4 shows a pedigree of a family with a strong predisposition towards retinoblastoma, alongside a pedigree of the more usual situation.

The two forms of retinoblastoma differ in another way besides their heritability. In sporadic cases of the disease, usually one eye is affected, and the cancer generally appears at an advanced age. In contrast, in those with a family history of retinoblastoma, both eyes are commonly affected, and the cancer often occurs early.

Clearly, in the familial case, the phenotype appears to be inherited as a dominant trait, but just as most cancer is paradoxically a genetic disease that is not inherited, rare familial retinoblastoma is paradoxially a dominant trait that is recessive within individual cells.

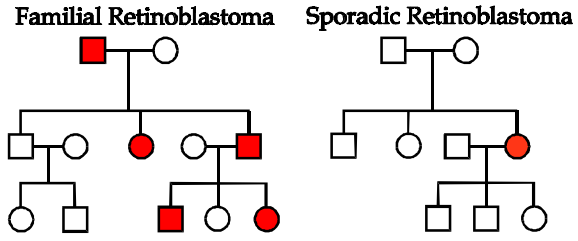

Fig. 4: Pedigrees of Inherited and Noninherited Retinoblastoma. Filled symbols represent affected individuals. (Adapted from Gelehrter et al (1997) *Principles of Medical Genetics*)

Fig. 5 illustrates how this works. Most of us are born with two good copies of *RB*, the gene that is defective in familial retinoblastoma. Almost all of our cells live out their days as they were born, with the two functional copies, but very rarely a mutation occurs over the course of our lives. A base change, a chromosomal deletion, even the loss of an entire chromosome -- all these events happen at a low frequency, but when you're talking about 100 billion cells in our body, it's pretty certain that the mutation will occur many times. Fortunately, when it does occur, the other copy of *RB* remains to keep the cell normal. It is highly unlikely that both copies of *RB* will be mutated in the same cell.

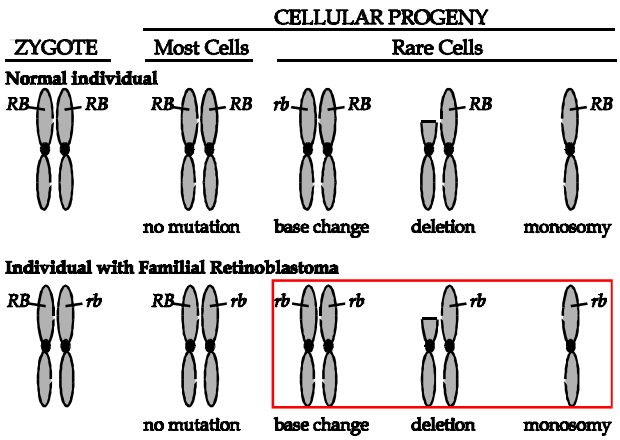

Fig. 5: Loss of heterozygosity of retinoblastoma gene. Cells possessing at least one wild-type allele (*RB*) retain retinoblastoma function. The red box identifies genotypes where retinoblastoma function is lost.

Not so with those who are born with only one good copy of *RB*. Their cells initially are perfectly normal, because they are heterozygotes at the locus, and it is the normal allele that is dominant. One good copy is enough for the moment, but in the long run, you need both. Inevitably, cells arise that have sustained a mutation of some sort in the lone *RB*. Tumors isolated from those born with a defective *RB* invariably carry a mutation in *RB*.

These events occur also with us all, but we are left with one good copy of the allele. We must lose both copies to suffer the ill effects of retinoblastoma. We can now see the paradoxical situation common with most familial cancers: at the cellular level, the trait is recessive, but at the individual level it is dominant. Over the course of a lifetime, it is almost a sure bet that a loss of heterozygosity will occur in some cells, and these are very likely to progress to tumors. A person born *RB*/*rb* is almost certain to develop retinoblastoma.

SQ8. Presuming that a loss of *RB* in retinal cells is sufficient to cause retinoblastoma, explain why sporadic cases of retinoblastoma occur generally in only one eye while those with familial retinoblastoma often have tumors in both eyes.

IV. *RB*: A Tumor Suppressor Gene (pp.640-642)

There's something strange here. One cell loses both copies of *RB*... so what? It's just one protein out of thousands, one cell out of billions. Why should the loss have such a disastrous effect? After all, each of us is born with hundreds of recessive mutations in our chromosomes. No doubt we have thousands of cells that have lost heterozygosity at these loci. Why don't we all get cancer?

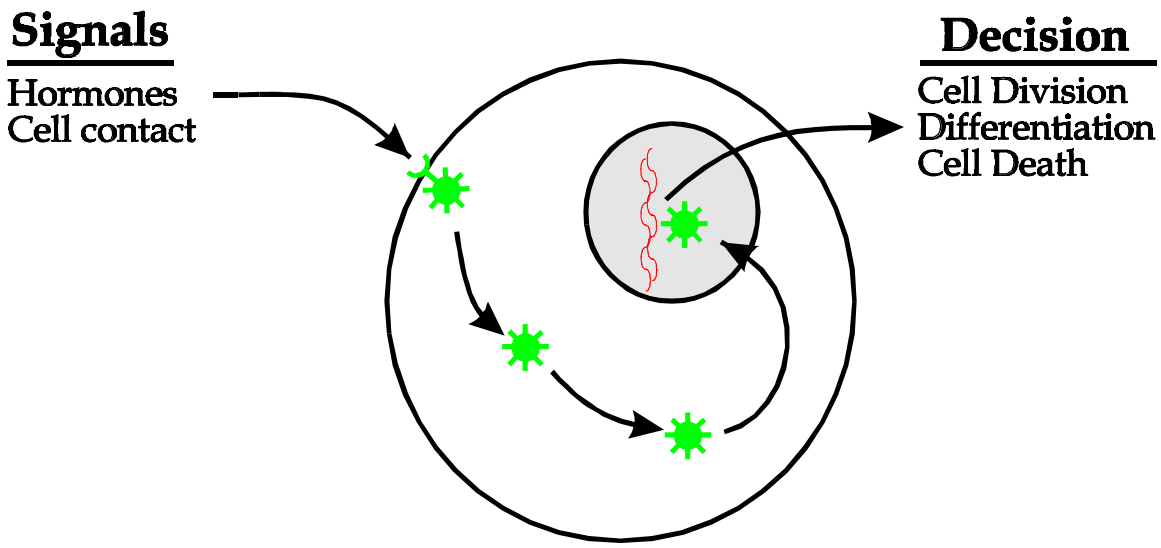

Fig. 6. Signal transduction. External signal molecule binds to receptor protein on the surface of the cell. The interaction causes the receptor to activate an internal enzyme that acts on different protein (typically by adding phosphate groups to them), activating them in turn. The final step in the cascade is the interaction of an activated transcription factor with genes whose expression is important for the cell's ultimate response to the external signal.

There is something special about the function of *Rb* and other genes whose loss in even a single cell can lead to cancer. This is not the place to talk about the cell biology of cancer -- Val Kish offers an entire course on that. But let me summarize a few points that will make the genetics of cancer more understandable.

From information gained from cloned genes and other experiments, many of the functions are known of genes whose mutations lead to cancer. They all generally deal with the critical decisions of a cell, the most important of which are shown in Fig. 6. The most important decisions a cell makes are when to grow, when to differentiate, and when to die. You may be surprised at the last one, but death, too, is programmed at the cellular level. These decisions are influenced by inputs from the environment: hormones from afar, signals from neighboring cells, and the internal state of the cell.

External signals are sensed by receptors on the cell membrane, and the message is passed to protein within the cell (Fig. 6), usually by phosphorylating them (putting phosphate groups on them). The modification of the protein is an internal signal that alters the activity of the proteins. Interestingly, it is often the case that the modified proteins gain the ability to modify other proteins, creating a cascade effect.

Eventually, the series of protein modification steps reaches protein that are transcriptional regulators. RB protein falls within this class. Normally, RB protein binds to a transcriptional factor E2F preventing it from binding to its enhancer on DNA and turning on the synthesis of genes critical to the progress of the cell cycle. If RB is not there, owing to mutation, then unauthorized cell division can proceed.

SQ9. Suppose two cells in the retina suffer mutation at the same moment. Cell #1 loses the ability to make an enzyme required for eye pigmentation. Cell #2

loses the ability to make RB protein. If cell division of mature retinal cells is normally repressed by RB but without RB cells can divide once every day, what will be the ratio of progeny of Cell #1:Cell #2 after a month?

Now we can see how mutation in a single cell in our body can have such disastrous results. Whether it is two mutations to RB in a normal person or one additional mutation in *Rb* in a person who has already inherited the first mutation, the result is a cell that has relaxed control over cell division. While the unaffected surrounding cells are appropriately frozen in the cell cycle, the *Rb* double mutant is able to divide. Just as the single recombinant T4 phage produce a plaque while the surrounding phage are ineffective, so does the single tumorigenic cell grow into a tumor.

V. Other Genes Related to Cancer (pp.637-640)

RB is by no means the only critical protein involved in the decision to divide. p53 is another tumor suppressor protein that controls cell division and when mutated loses the ability to prevent cell division. One of its functions is to detect DNA damage and, if there is any, to stop cell division. This makes sense, as we saw last week, because DNA synthesis makes mutations permanent. If the cell cycle is temporarily halted by p53, then there is time for DNA repair to take place. But if the damage is too great, then p53 directs the cell to commit suicide, a better fate than risking mutation that might lead to cancer. In most tumors, p53 itself has been mutated, thereby removing a formidable defence against the production of defective cells.

Not all tumor suppressors are transcription factors. Families carrying a mutant allele of *MSH2* are prone to a type of colon cancer that initiates when the wild-type copy of *MSH2* is lost. The gene encodes a protein required for DNA mismatch repair (in fact, the protein is very similar to MutS, whose function we've already seen in Friday's notes). The loss of mismatch repair that results from a full defect in *MSH2* presumably leads to the further mutation of genes required for control of cell division.

SQ10. Why do you think that a defect in a gene encoding a DNA repair enzyme leads specifically to predisposition to cancer of the colon rather than, say, prostate cancer? Consider where in the body mutagens are likely to be found.

The signal transduction proteins upstream of RB are also often found mutated in tumors. Many of these are encoded by what you may read about in your text: proto-oncogenes. Proto-oncogenes are genes which, when mutated, gain an activity that lead to tumors. Certain mutations of these genes make faulty signal transduction protein that are always on, always telling the cell that it is time to divide. Ras protein is an example of a proto-oncogene. It normally serves to regulate the phosphorylation of the proteins of the signal transduction cascade that allow the cell to react to its environment. Table 23-7 is a list of some of other oncogenes and their functions.

SQ11. What are some differences between tumor suppressors and proto-oncogenes?

SQ12. A protein monitors the genome for DNA damage. When damage is detected, it blocks cell division until the damage is repaired. Would you expect this protein to be a tumor suppressor or a proto-oncogene?

Myc protein is a transcriptional factor and a proto-oncogene of clinical importance. The gene encoding Myc normally resides on the tip of chromosome 8. For some reason, this region is particularly prone to translocate to the tip of chromosome 14, just to the spot where the gene encoding an immunoglobulin lies. Maybe the two regions are similar in their DNA sequence. The translocation places *myc* under the control of the enhancers normally controlling the expression of immunoglobulin (Fig. 7). This translocation has no effect in most cells, since immunoglobulins are expressed only in lymphocytes. But, if the translocation occurs in a lymphocyte, watch out! No doubt the lymphocyte's ability to make immunoglobulins is compromised, but that doesn't matter, there are thousands of other lymphocytes to pick up the slack. The problem is that *myc* will be highly expressed in these cells, bad news, because Myc turns on genes that lead to aberrant growth. The result is a type of cancer known as Burkitt's lymphoma.

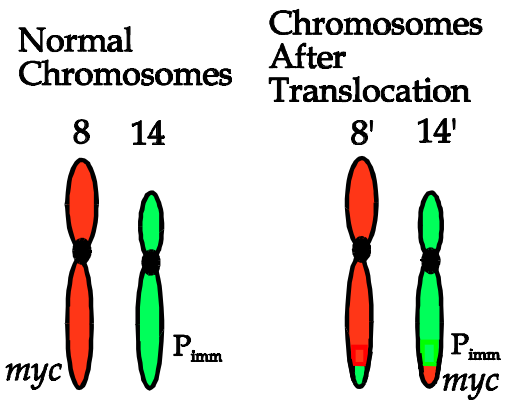

Fig. 7: Translocation leading to Birkitt's lymphoma. Reciprocal translocation between chromosomes 8 and 14 may place *myc* under the control of the enhancer region normally regulating the transcription of the heavy chain immunoglobulin locus (*imm*).

SQ13. Why does the chromosome 8/14 translocation shown above lead to lymphomas and not other types of cancer?

VI. The Progression of Cancer (p.642)

You might get the idea that if you get a mutation in a key gene, your day is ruined. Actually, for such an important process as control over cell growth and cell death, the body maintains a number of redundant protective mechanisms. It therefore requires several hits to damage a cell sufficiently that it loses control over growth. That's why cancer is a disease that typically develops over decades. It takes that long for a cell to accumulate the level of damage to render it out of control.

A useful lesson is learned from another rare inherited cancer. Colon cancer is one of the most common cancers in the industrialized world. A small number of cases are inherited. One form of inherited colon cancer is called familial adenomal polyposis coli -- coli for colon, familial for inherited, polyposis for the peculiar blobs that appear on the colons of affected individuals at an early age, and adenomal for their shape, I think.

These polyps are abnormal but benign growths that arise when a cell loses its second copy of a gene, called *APC* (for *A*denomal *P*olyposis *C*oli). The first copy was

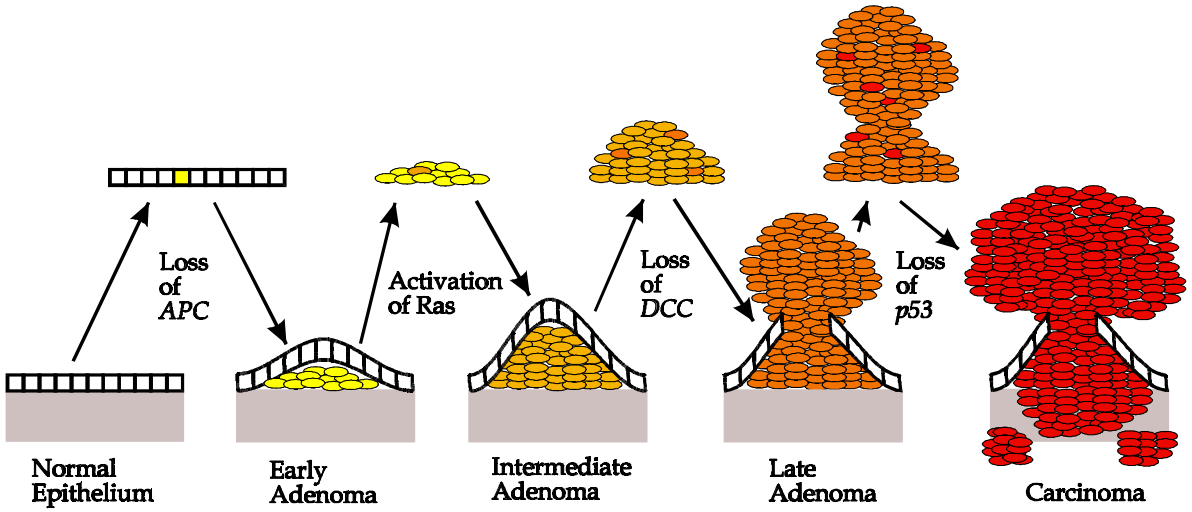

Fig. 8: Multiple stages of cancer.

defective from the start in individuals with familial polyposis coli. Each polyp represents clonal growth from a single cell that has partially lost the ability to control its cell division. I must stress that these cells are not tumors; however, they stand a much better chance of suffering additional chance to becoming tumors. Fig. 8 shows why.

A second mutation in *APC* leads to a partial loss of control of cell division, resulting in small polyps. Amongst those cells, one arises that has experienced a mutation in Ras, giving rise to larger but still benign polyps. The successive loss of other tumor suppressor genes, *DCC* and *p53*, in cells within the clonal growth eventually leads to a cell that has lost the regulatory mechanism that normally confines growth within tissue boundaries. There are many other required mutations not shown here.

It is clear from this why removal of the colon is called for in people with familial polyposis, even though no cancer has yet appeared. The large number of polyps, each with a large number of partly defective cells, increases the likelihood that one cell will suffer a mutation that brings it to the dangerous phase of carcinogenesis.

If you had been asked what is the chance of a cell sustaining perhaps the dozen different mutations required for malignancy, where any one mutation is a rare event, you would have calculated a probability so small that cancer could not possibly have arisen during the history of humankind. But it does. The reason is that mutations increase the growth of the affected cell, and the increased number of affected cells vastly increases the probability that the next mutation will be observed. (The same reasoning may be applied to arguments against evolution that multiply small probabilities of multiple events).

SQ14. Why is it that colds come and go in a day or two but tumors sometimes take decades to develop?

SQ15. You are a molecular coroner of the 21st century. Your job is to sequence the genomes of the dead to determine their causes of death. You've seen a lot gene

sequences in your time and have gotten a feel for which somatic mutations are common and which are not. You've found that mutations in p53 gene stand out far above the crowd, way more common than mutations in genes encoding glucose-6-phosphate dehydrogenase (G6PD) and other run-of-the mill genes. Why?

A. Klutz Hypothesis: p53 is just error prone. It is more susceptible to mutation than other genes.

B. Celebrity Gene Hypothesis: Mutations in p53 occur no more frequently than mutations in other genes, but when they occur you hear about them... if you're a sequencer of dead people's DNA.

C. Microphone Hypothesis: Point the microphone in most directions and you get noise. Point it at the speaker and you blow out your ears. Maybe mutations in p53 are like that.

VII. Summary

- **Cancer is caused by somatic cell mutations**
  - Tumors are monoclonal
  - Tumors are generally not inherited
- **Rare, inherited tumors provide important clues**
  - Familial cancers act as a dominant trait
  - The defect is recessive at the cellular level
  - Heterozygotes are much more prone to full loss
- **Genes involved in tumorigenesis**
  - Many are related to control over cell division
  - Tumor suppressers: loss leads to cancer
    - Examples: RB and p53
  - Proto-oncogenes: activation leads to cancer
    - Examples: Ras and Myc