1- Rb is one example of a category of antiproliferative genes in humans. Typically, when both copies of such genes are lost, cancers develop. Do you suppose that cancer could be eradicated if tumor suppressor genes such as Rb could be expressed at abnormally high levels in all human cells? What would be the effect on the human? Explain your answers.

2- Overexpression of the Myc protein is a common feature of many types of cancer cells, contributing to their excessive cell growth and proliferation. By contrast, when Myc is overexpressed in most normal cells, the result is not excessive proliferation, but cell-cycle arrest or apoptosis. How do you suppose that overexpression of Myc can have such different outcomes in nomal cells and in cancer cells?

3- About 20% of colorectal cancers have mutations in the *B-Raf* gene. B-Raf is a Serine/threonine protein kinase that functions in the Ras-Raf-Mek-Erk-MAP kinase cascade, which mediates cellular responses to growth signals. When the pathway is stimulated, Ras activate B-Raf by causing a protein kinase to add phosphates to threonine 598 and serine 601. Activated B-Raf then adds phosphates to key residues in Mek to trigger the rest of the pathway and stimulate cell growth. The mutations of *B-Raf* found in Cancer cells give rise to a constantly active form of B-Raf that does not need to be phosphorylated by Ras. In one sample of colorectal cancers, 95% of the mutant *B-Raf* genes had glutamate in place of valine at position 599. Why do you suppose that B-Raf with glutamate at position 599 is active?

4- Mouse mammary tumor virus (MMTV) is an oncogenic retrovirus that causes breast cancer in mice when it integrates into the genome. You want to know whether it carries its own oncogene or generates an oncogene upon integration. You isolate 26 different breast cancers from mice that were exposed to MMTV and determine the sites at which the retroviruses are integrated. In 18 of 26 tumors the viruses are found at a variety of sites that are all located within a 20-kb segment of the mouse genome. Upon closer examination of these 18 tumors, you find that an RNA is expressed from the region of the mouse genome near the integrated virus, but not from the corresponding region in normal mouse breast cells. Do these observations argue for MMTV carrying an oncogene or for it generating an oncogene upon integration? Explain your reasoning.

5- The *p53* gene encodes a key regulatory protein that can arrest cell growth, induce cell death, or promote cell senescence, in response to DNA damage or other types of cell stress. Its central role in governing a cell's response to stress is highlighted by the finding that it is inactivated by mutation in half of all human cancers. Somewhat surprisingly mice that lack p53 are fine in all respects-except that they develop tumors by 10 months of age. The product of a second gene, *Mdm2*, negatively regulates p53, targeting it for destruction by attaching ubiquitin to it. Your lab is investigating these genes using mouse knockouts. You can generate $Mdm2^{+/-}$ mice perfectly well, but when these mice are mated together, no viable $Mdm2^{-/-}$ offspring are born. To investigate the genetic interactions between *p53* and *Mdm2*, you generate doubly heterozygous $p53^{+/-}$ $Mdm2^{+/-}$ mice and mate them together. The genotypes of the progeny mice are shown in Table below.

A. The *p53* and *Mdm2* genes are on different chromosomes and thus assort independently during meiosis. Assuming that $p53^{+}Mdm2^{+}$, $p53^{+}Mdm2^{-}$, $p53^{-}Mdm2^{+}$, and $p53^{-}Mdm2^{-}$ haploid gametes are produced at equal frequencies by the male and female parents, calculate how frequently each of the progeny genotypes would be generated by random assortment. Which, if any, of the genotypes appear to be significantly under-represented among the progeny?

B. How would you interpret the differences in number of progeny expected and actually generated for $p53^{+/+}$ $Mdm2^{-/-}$, $p53^{+/-}$ $Mdm2^{-/-}$, and $p53^{-/-}$ $Mdm2^{-/-}$ mice?

GENOTYPE	PROGENY MICE (NUMBER)	PROGENY MICE (EXPECTED)
$p53^{+/+}$ $Mdm2^{+/+}$	3	
$p53^{+/+}$ $Mdm2^{+/-}$	5	
$p53^{+/+}$ $Mdm2^{-/-}$	0	
$p53^{+/-}$ $Mdm2^{+/+}$	7	
$p53^{+/-}$ $Mdm2^{+/-}$	11	
$p53^{+/-}$ $Mdm2^{-/-}$	0	
$p53^{-/-}$ $Mdm2^{+/+}$	1	
$p53^{-/-}$ $Mdm2^{+/-}$	7	
$p53^{-/-}$ $Mdm2^{-/-}$	2	

Table 1 Genotypes of progeny mice from cross between doubly heterozygous p53+/- Mdm2+/- mice

6- Remarkably, the *Ink4A-Arf* locus encodes two different tumor suppressor proteins, Ink4A and Arf, which share a common exon but are translated in different reading frames (figure 1A). Mutations in human that were initially thought to affect Ink4A were shown to affect a novel protein encoded in a different reading frame; hence, the name of the gene: *Arf*, for alternative reading frame. One of the principal functions of Arf is to inhibit Mdm2, which in turn inhibits p53. The relationship among Arf, Mdm2, and p53 is commonly represented as shown in Figure 1B. The sort of 'double-negative' implied in this relationship can be confusing: Arf is an inhibitor of an inhibitor of p53.

A. Would you expect *Arf*-knockout mice to be more prone, or less prone, to getting tumors than a wild-type mouse? Explain your reasoning.
B. Do you suppose that a $p53^{+/-}$ $Mdm2^{-/-}$ mouse, which will die in early embryogenesis, would be rescued by knockout of the *Arf* gene? That is, would you expect a $p53^{+/-}$ $Mdm2^{-/-}$ $Arf^{-/-}$ mouse to be viable or dead? Explain your reasoning.
C. The *Myc* oncogene, in addition to stimulating cell-proliferation pathways, also activates Arf, thereby indirectly influencing the activity of p53. How would you account for the observation that mice expressing the *Myc* oncogene get tumors more quickly in $Arf^{-/+}$ mice than in $Arf^{+/+}$ mice (Figure 1C)?

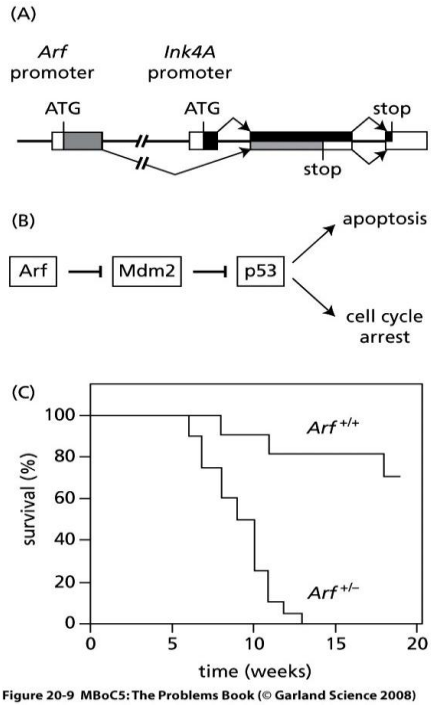

Figure 20-9 MBoC5: The Problems Book (© Garland Science 2008)

Figure 1 Gene structure and function of Arf. (A) Gene structures of *Arf* and *Ink4A*. The ATG in the initial exon of each gene indicates the start site of translation, which corresponds to AUG in the RNA transcript. The reading frame for Ink4A is shown in *black*, and the one for Arf is shown in *gray*. Note that the amino acid sequences of Ink4A and Arf are completely different. (B) Functional relationship between Arf, Mdm2, and p53. The *on-side* T symbol indicates inhibition; for example, Arf inhibits Mdm2. (C) Survival of mice expressing the *Myc* oncogene on a genetic background that is either $Arf^{+/+}$ or $Arf^{+/-}$. Mice that have survived for a given length of time are expressed as a percentage of the initial population. All the dead mice died of cancer.