Problem set 8

1. Integrins are alpha/beta heterodimeric receptors that bind to molecules in the extracellular matrix. A primary role of integrins is in adhesion, ensuring that cells attach to the matrix, and cell biologists have identified several proteins that link the intracellular tail of the beta integrin to the actin cytoskeletin. During the development of the Drosophila wing, cells on the dorsal and ventral surfaces adhere to the matrix. Mutations in the beta integrin gene *lethal(1)myospheroid* lead to recessive lethality, but hypomorphic mutations lead to wing blisters where the portions of the dorsal and ventral surfaces separate. From what you now know about integrins, propose a model for how integrins function in wing development.

You screen for mutations that lead to wing blisters using FLP/FRT induced recombination. Describe which genetic tools you will need to conduct the screen.

2. To study DNA repair mechanisms, geneticists isolated yeast mutants that were sensitive to various types of radiation; for example, mutants that were more sensitive to UV light. Ten haploid mutants were isolated.
a. How would you do the complementation tests for these mutants?

b. Based on the complementation chart below, how many genes are defined by these mutations? + indicates complementation, - failure to complement.

	1	2	3	4	5	6
1	-	-	+	-	+	+
2		-	+	-	+	+
3			-	+	-	+
4				-	+	+
5					-	+
6						-

c. The gene defined by mutation 1 was cloned. Overexpression of the wild-type gene reduces the UV sensitivity of either mutant 3 or mutant 6. Describe two models to explain the genetic interactions.

3. The *sup-7* mutation of *C. elegans* is an amber suppressor tRNA that inserts tryptophan at UAG amber codons. You isolate 20 mutations in the *unc-22* gene, which lead to a recessive phenotype causing worms to be uncoordinated in their movement. Three of the mutations are suppressed

by the *sup-7* mutation, but when you clone the gene and sequence the mutant alleles, eight mutations resulted in amber stop codons. Why do you suppose that the *sup-7* suppressor didn't suppress some of these mutations?

4. Animals homozygous for the *sup-7* mutation die at 15º C. In 1981, Bob Waterston mutagenized *sup-7*/*sup-7* hermaphodites, grew them at 25º C, and shifted the F2s to 15º C. Several strains were isolated that now could grow at 15º C. The suppressors of the *sup-7* lethality were inseparable by recombination from the *sup-7* mutation. Strains carrying *sup-7* and the tightly linked suppressor (suppressor of a suppressor) no longer suppressed amber alleles of genes that were suppressed by the original *sup-7*. Animals homozygous for the *sup-7* suppressor chromosome appear wild type. From what you know about nonsense suppressors explain these results. (Note: these suppressors were too frequently isolated to be explained by the reverting the mutant nucleotide in *sup-7* back to the wild-type nucleotide, which should be a rare event.)

5. You are working with a pure breeding stock of white-eyed flies and notice a spontaneous red-eyed male. You generate a pure breeding stock of the revertant in additional crosses. Design a crossing scheme to distinguish between two possible explanations for the mutation leading to the red-eyed male: an intragenic suppressor that restores the function of white gene and an autosomal extragenic dominant suppressor. Assume that if the suppressor is in a second gene that the recessive phenotype of the suppressor in the absence of the original *white* mutation leads to red eyes.

6. The *C. elegans lin-14* gene controls the timing of development in *C. elegans*. LIN-14 protein is high early in development and gradually decreases as development proceeds. *lin-14* is defined by both dominant and recessive mutant alleles. Animals that are homozygous for recessive alleles develop precociously (developmental events occur much earlier than normal) because LIN-14 protein levels are lowered or eliminated, similar to the levels seen later in development. Animals that contain dominant alleles are retarded in development (developmental events occur much earlier than normal) because LIN-14 levels are higher than they should be late in development. You are given three *lin-14* mutants.

Animals that are homozygous for loss-of-function alleles of the *lin-4* gene are retarded in development. The *lin-4* RNA is thought to bind to the *lin-14* mRNA and inhibit translation of the LIN-14 protein. What genetic epistasis result would be consistent with *lin-4* functioning as a negative regulator of *lin-14*.

7. Kin1p and Kin2p are protein kinases that act sequentially in a "kinase cascade." Kin1p places a phosphate group on Kin2p, and this increases the activity of Kin2p so that it may, in turn, place a phosphate group on its target. Two *kin1* mutants are isolated. One suppressor of each *kin1* mutant is then isolated. Both map to the *KIN2* gene. One *kin2*^{sup} mutant shows allele specificity, only suppressing the *kin1* allele that it was originally selected to suppress.

a) Propose a model to account for these observations. What is the nature of the original *kin1* mutations and how can the *kin2* mutants be allele-specific suppressors of these mutants?

b) The other *kin1* suppressor suppresses all *kin1* alleles. How do you think that this suppressor acts?

8. The supply of nitrogen regulates yeast genes affecting nitrogen catabolism (Remember the prion lectures). The Ure2 protein of *S. cerevisiae* is a negative regulator of nitrogen catabolism that inhibits the Gln3 protein, a positive transcriptional regulator of genes involved in nitrogen assimilation. For example, the Gln3 protein activates the transcription of DAL5, which encodes allantoate permease, a cell surface molecule that transports this alternate nitrogen source under poor nitrogen conditions. In the presence of a good nitrogen source like ammonia, Ure2 protein is active and inhibits Gln3 activity, whereas in a poor nitrogen source such as allantoate, Ure2 is not active and Gln3 is active. Overproduction of the Mks1 protein allows *DAL5* expression on ammonia, and lack of Mks1 protein prevents DAL5 expression on allantoate. In addition, an *mks1 ure2* double mutant (both are null mutations) expresses *DAL5* on either ammonia or allantoate.

Describe a linear genetic pathway for the function of *GLN3, MKS1, URE2* and *DAL5*, and describe which genes are active or inactive in ammonia and in allantoate.

9. The yeast *SUP35* and *SUP45* genes encode proteins that are involved in translational termination at stop codons. Mutations in these genes result in low levels of readthrough at stop codons, and were isolated as suppressor mutations. Do you think that the *sup35* and *sup45* mutations are gene specific or nonspecific? allele specific or nonspecific? Explain your reasoning.

10. Sexual development in *C. elegans* is controlled by the X:autosome ratio. In XX animals the ratio is 1.0, resulting in hermaphrodite development; in X0 animals the ratio is 0.5, resulting in male development. Amorphic or

null mutations in the genes *tra-1* and *tra-2* result in the transformation of XX animals into phenotypic males. Gain-of-function mutations in *tra-1* result in the opposite transformation: XO animals are transformed into phenotypic hermaphrodites. Double mutant combinations between *tra-2(lf)* and *tra-1(gf)* mutations have placed *tra-1* downstream of *tra-2*.

Recessive mutations in the *fem-1* gene result in the transformation of XO animals into phenotypic females. Remember that *C. elegans* hermaphrodites are females that produce sperm for a short term. The fact that *fem-1* XO animals are transformed into females and not hermaphrodites has you puzzled. You think that perhaps the mutations are hypomorphic mutations and null alleles would result in transformation of XO animals into hermaphrodites.

a. You perform a genetic test to determine whether the mutations are hypomorphic or amorphic. Describe the genetic experiments and the results that would suggest that the *fem-1* mutations are amorphic and not hypomorphic.

b. *fem-1* acts between the two *tra* genes in the sex determination pathway. Propose a genetic pathway for these three genes and define the activity states (ON/OFF) of the genes in XX and XO animals. Describe the *fem-1; tra* double mutant combinations that you would use to demonstrate this pathway, and describe the karyotypes and the phenotypes of the double mutants that support the proposed pathway.

11. A deletion in the p53 gene causes a dominantly inherited form of cancer. Normal cells of an individual with cancer contain both the normal and deleted forms of the p53 gene, but the cancer cells contain only the deleted version. Explain these observations.

p53 is a tumor suppressor gene. While the cancer trait is inherited in a dominant fashion, all p53 function must be lost for cells to become cancerous. The loss of heterozygosity is caused either by mitotic recombination, loss of the normal chromosome or a spontaneous mutation in the wild-type p53 gene.

12. Utpal Banerjee's lab isolated a dominant mutation in *Sos* (Sos^{JC2}) on chromosome 2 as a suppressor of a hypomorphic *sev* allele known as sev^{E4}.

a. The investigators conducted a series of dosage experiments to determine the nature of their dominant suppressor. The data are below.

Genotype	Level of suppression
sev^{E4} / sev^{E4} ; *Sos+* / *Sos+*	0%
sev^{E4} / sev^{E4} ; Sos^{JC2} / Sos^{JC2}	36.0%

sev^{E4} / *sev*^{E4} ; *Sos*^{JC2} / *Sos+*	16.4%
sev^{E4} / *sev*^{E4} ; *Sos*^{JC2} / *Df*	4.0%

What type of mutant allele is the *Sos*JC2 allele? Be as specific as possible, and explain your reasoning.

b. Mosaic analysis of loss-of-function alleles of *Sos* isn't informative because these alleles result in a cell lethal phenotype (there are no mutant clones of *Sos* cells because the cells die). The *Sos*JC2 allele, however, is not cell lethal. Animals of the genotype *w sev*^{E4} / *w sev*^{E4} ; *Sos*^{JC2} P[*w*+] / *Sos+* were irradiated with X-rays, and mosaic ommatidia scored. Below are the results.

Cell	wild type for *w*	mutant for *w*
R1	57	12
R2	39	30
R3	42	27
R4	46	23
R5	46	23
R6	57	12
R7	69	0
R8	41	28

These are the results for 69 mosaic ommatidia scored that had R7. Each number represents the number of cells that are mutant or wild type for *white* (*w*). In the 69 ommatidia analyzed, for example, 57 of the R1 cells were wild type for *white* and 12 were mutant.

Describe a strategy that could have been used to insert the P[*w*+] transgene into chromosome 2? Be specific and use crosses if necessary.

What is the purpose of the P[*w*+] transgene?

What is (are) the genotype(s) of the *w* clones.

Do you think that *Sos* is acting cell autonomously or nonautonomously? Explain your reasoning.

13. In FLP/FRT mosaic screens for mutants with white eye clones that had expanded and overgrown at the expense of red eye tissue to generate unusually large eyes, several tumor suppressor genes were identified on chromosome 3. As we discussed in class, these genes were interesting because they normally inhibit cell division and promote apoptosis. In these same screens the investigators also identified a second class of mutants. In these mutant animals, the eyes were unusually large because there were

too many cell divisions just like the mutants that we discussed. The difference is that the red eye tissue is also overgrown in this second class of mutants. Expression of the Unpaired secreted ligand is increased in the mutant cells. Unpaired is a signal in a conserved pathway that regulates the transcription factor STAT. Loss of one copy of the STAT gene can suppress the overproliferation phenotype caused by the loss of this second class of tumor suppressor genes.

a. Describe four genetic elements/mutations present on the chromosomes (not including the induced mutations) that are used to generate and detect the mosaic eyes and define which cells are homozygous for the mutagenized chromosome in these screens.

b. In the original screen, what are the genotypes of the white cells and the red cells of the mosaic females that have the second type of tumor suppressor gene mutation?

c. Describe a model that can explain the overgrowth phenotype of this second class of tumor suppressor that incorporates the tumor suppressor, Unpaired and STAT, defining in which cells the genes act and how they might regulate one another. No molecular details are necessary.