 Name: ANSWERS

Biology 52 exam IV, May 5, 2006

You have two hours. Feel free to draw pictures to help answer the questions.

Bear in mind that some questions may cover material covered in 2006 but not this year.

1) [8 points] Suppose that you are designing a screen for mutants affecting embryonic patterning in Drosophila. You treat a population of male flies with a chemical mutagen. a) Outline what you would then do to find individual flies with mutations of interest, starting with this mutagenized population and as many wild-type flies as you want. Include in your description which flies are heterozygous or homozygous for the new mutations you are screening for.

Cross the mutagenized population to wild-type females (to transmit each new mutation), and cross the F1 progeny from these crosses to wild-type flies again (to obtain multiple male and female siblings each carrying the mutation). Mate F1 males and females from the same family (i.e. potentially carrying the same mutations) to obtain homozygous progeny (25% of progeny of matings between two carriers).

b) Some of the mutations you might find would have a maternal effect. How would you recognize individuals with maternal-effect mutations?

Look for females that produce progeny with defective development, even when crossed with a wild-type male.

2) [20 points] The embryonic patterning defects of some Drosophila mutants can be rescued by injections of cytoplasm from wild-type embryos. How would each of the following embryos develop:

a) *bicoid* mutant embryo (from a *bicoid* mutant mother):

i) untreated – no anterior end

ii) injected at the anterior end with cytoplasm from the anterior end of a wild-type embryo

would restore normal development

iii) injected in the middle with cytoplasm from the anterior end of a wild-type embryo

would have anterior structures in the middle, with anterior-posterior polarity in both directions toward the two ends

b) *toll* mutant embryo (from a *toll* mutant mother):

i) untreated – would be dorsalized

ii) injected on the dorsal side with cytoplasm from the dorsal side of a wild-type embryo

would not rescue

ii) injected on the dorsal side with cytoplasm from the ventral side of a wild-type embryo

would restore a ventral side, but on the "top" instead of the normal ventral side

c) In the injections in a and b, at what developmental stages would you need to obtain wild-type donor cytoplasm, and at what stage would you need to inject cytoplasm into the recipient mutant embryo to produce the effects you indicated?

a) early embryo

b) syncytial blastoderm stage

d) In a and b, what molecule(s) could you inject to produce the same effects on patterning?

a) *biocoid* mRNA or protein

b) Dorsal protein, or activated Pelle (component of signaling pathway that regulates Dorsal)

3) [9 points] a) Describe the phenotypes of the following Drosophila mutants. In each case indicate whether the mutation is dominant or recessive, and the biochemical function of the protein encoded by the corresponding wild-type gene.

a) *proboscipedia* – legs where mouth should be; dominant; transcription factor
b) *bithorax* – segment T3 has half a wing; recessive; transcription factor
c) *polycomb* – initial patterning would be normal, but then becomes posteriorized later; recessive; alters chromatin to repress gene expression

4) [20 points] a) On the following diagram of a wild-type Arabidopsis inflorescence apex,
i) label each of the meristems and lateral organs;
– inflorescence meristem, flower meristems
ii) Indicate which cells are dividing most actively.
– cells in the peripheral zones of these meristems

b) On the following diagram, indicate which cells express
i) the *WUSCHEL* gene, – in the center of meristems, 2-3 cell layers below the surface
ii) the *AGAMOUS* gene, – in whorls 3 and 4 of flower meristems and flowers
iii) the *LEAFY* gene. – throughout flower meristems and flowers

c) How would each of the following mutations (or combinations of mutations) affect the number and identities of flower organs formed?
i) *apetala2 apetala3* – all carpels; normal organ numbers
ii) *apetala1* – petals replaced by new flowers; overall more flower organs because of flowers-within-flowers phenotype
iii) *sepallata1 sepallata2 sepallata3* – all sepals; extra flowers in whorl 4
iv) *clavata3* – larger meristems, so more flower organs

5) [6 points] A great diversity of flower forms are found in nature and in greenhouses. Suggest how regulation of flower development in each of the following flower forms may differ from regulation of Arabidopsis flower development. Assume in each case that the same regulatory genes are present in the indicated species as in Arabidopsis.
a) azaleas with two whorls of petals instead of one.
– ectopic B and E function in first whorl (PI, AP3, SEPx)

b) dogwood trees with white or pink bracts (a bract is a leaf-like organ found immediately beneath the flower in some plants.)
– ectopic expression of A, B, and E functions in bracts (AP2, PI, AP3, SEPx)

c) Acacia trees in which flowers are produced by the shoot apical meristems, which then grow leaves again in the following season
– the vegetative SAM converts to a flower meristem upon flower induction, and then converts back to a vegetative meristem after the flower has formed

6) [12 points] a) The following diagrams show an Arabidopsis gynoecium and a close-up diagram of an ovule within the gynoecium. On the diagrams, indicate how a pollen grain fertilizes the ovule. Include the site of pollination, the route by which sperm is delivered, and where fertilization takes place. Indicate the ploidy of each of the cells in the ovule both before and after fertilization.

Pollen germinates on the stigma, the pollen tube grows down the transmitting tract and up the funiculus to the micropylar end of an ovule, where it bursts and releases two sperm cells. One haploid (1N) sperm fertilizes the haploid egg to produce a diploid (2N) embryo. One sperm fertilizes the diploid central cell to produce a triploid (3N) endosperm.

b) Maize plants that are homozygous for an *ea1* mutation are female sterile, although the female gametopyhyte has normal anatomy, and they also have have normal pollen production and function. On the following diagram of a maize female gametophyte, indicate i) which cells express the *EA1* gene and ii) where the EA1 protein is present.

The synergids express EA1, and the protein is present outside these cells near the microphyle.

iii) What goes wrong during fertilization to cause the *ea1* mutant plant to be female sterile?

The pollen tubes can't find the egg sac so don't fertilize.

iv) Do you expect that a heterozygous *EA1/ea1* plant would be sterile or fertile?

Male fertile (100%); 50% female sterile.

7) [12 points] a) Sketch an antibody protein. Indicate the antigen binding region, and which part of the antibody the i) V and ii) Cα heavy chain gene segments encode.

(See slide in lectures.)

b) What are the functions of the part of the protein encoded by these segments?

i) V – antigen-binding, near tip of antibody arms

ii) Cα – "lower" part of heavy chain near base (stem of "Y"), recruits immune defenses such as phagocytosis by macrophages

c) Describe two types of genetic changes that occur in antibody genes prior to a secondary immune response? In which cells do these changes occur?

somatic hypoermutation, class switching

occur in memory cells

8) [13 points] A scientist makes a vaccine using an attenuated virus that can grow slowly in human cells but does not normally cause disease. The vaccine is injected into an individual who mounts a cellular immune response. a) Describe how lymphocyte cells mount the immune reaction against the antigen. Include in your answer i) which genes are rearranged, ii) which cells are induced to proliferate, iii) the molecular recognition events that provide specificity of response, and iv) how the body actually gets rid of the infection. v) Indicate which events occur before and which occur after immunization.

Before immunization, T-Cell receptor genes are rearranged to produce naive T cells. Upon immunization, those T cells having receptors that recognize a petide piece of the antigen (in the context of an MHC molecule) are stimulated to proliferate. These may be cytotoxic T cells (MHCI) or helper T cells (MHCII). Cytotoxic T cells recognize infected host cells through their TCR-MHCI+peptide interaction, and induce apoptosis in the infected cells (cell suicide). (Helper T cells might also contribute, to the extent that they may stimulate cytotoxic T cells or B cells that produce antibodies against the virus. However, the question asked about cellular immune response.)

b) How might the immune system of someone with AIDS respond to this vaccination?

To the extent that helper T cells are required to stimulate cytotoxic T cells, then the person with AIDS might mount a poor immune response and therefore suffer disease even from the attenuated virus.