Name __________________________

Gene 5102-05
Final Examination
December 13, 2005

For each item, your tasks are highlighted in **bold face**. Please ask for clarification if you do not understand a question!

*Possible answers are given in italics*

I. Basis for principles (35 points)
   A. Principle:
      In the replication-cell division cycles of mammalian cells, origins of replication acquire a protein complex, called licensing factor, prior to initiation of DNA synthesis, but not after. Presence of this factor on the DNA allows the origin to which it is bound to function in initiating replication.

   Assignment (9 points):
   Describe the evidence suggesting presence of the factor bound to DNA surrounding origins before S phase, but not during S phase.

   *DNase I footprints of origins cover more residues when chromatin from G1 cells is tested than when chromatin is from S phase cells. Footprinting measures the protection of the DNA from digestion by bound proteins.*

   B. Principle:
      Repressed chromatin is structurally, functionally and morphologically distinct from ‘poised’ or ‘active’ chromatin.

   Assignment (8 points):
   List four differences between the two kinds of chromatin, being sure to explain which characteristic is associated with which state (for example, condensed chromatin is associated with repressed chromatin).

   *Condensed chromatin has supernucleosomes, deacetylated histones, CpG methylated DNA (in some organisms), inactivity in transcription. Active chromatin is active in transcription, can be wound in nucleosomes, but lacks*
defined higher order structure, is undermethylated and has acetylated histones.

C. Principle: Many kinds of mobile DNA exist and modify genomes in various ways.

The following is a list of movable or removable genetic information mentioned in this course. There follows a list of descriptions.

Movable or removable genetic information:

0. Copia LTR retrotransposon
1. P element
2. Lambda bacteriophage DNA
3. Immunoglobulin inter V-J region DNA
4. 2 micron plasmid invertible segment
5. I-factor non-LTR retrotransposon
6. Tn916 conjugative transposon
7. Agrobacterium T-DNA
8. LINE-1 non-LTR retrotransposon
9. Salmonella flagellar antigen regulation invertible region
10. Tn3 transposon
11. DIRS-1 YR retrotransposon

Assignment (9 points):

For each description, choose one item from the first list that is appropriate for the description. Note that some descriptions have more than one correct choice from the top list. You need choose only one.

A host-encoded recombination protein complex recognizes specific DNA sequences, different at left and right ends and excises the intervening sequences ___3___

An element-encoded recombination protein complex recognizes specific sequences and cuts the element ends to reconnect them to the host strands that were associated with the opposite ends ___4___

An element-encoded recombination protein complex recognizes a specific unique sequence on circular extrachromosomal DNA and a specific unique sequence on chromosomal DNA, catalyzing insertion of the extrachromosomal DNA into the chromosome ___2___
Element-encoded proteins recognize a specific unique sequence on chromosomal DNA of one cell and transfer the element to another cell, where insertion into the chromosome occurs ___6__

Proteins not encoded by the movable element recognize element sequences in one cell and transfer them to another cell, where insertion into the chromosome occurs ___7__

An element-encoded recombination protein recognizes specific sequences at the ends of the element and excises the element and inserts the excised DNA into sites in the same genome ___1__

No target site duplication occurs on insertion of this element ___10__.

Double-stranded cDNA made by an element-encoded protein is inserted into host DNA by another element-encoded protein ___0__.

Nicked host DNA serves as primer for copying element RNA into DNA that then becomes part of the host DNA ___8__

D. Hypothesis:

Mitochondria arose by engulfment of bacteria related to Agrobacterium and Rhizobium followed by extensive loss of genes from the engulfed genome.

**Assignment (9 points):**

What observations support the above hypothesis?

*Sensitivity of ribosomes of bacteria and mitochondria, but not those of eukaryotic cytoplasms, to same antibiotics. Phylogenetic analysis of amino acid sequences of common enzymes. RNA polymerase similarities.*

II. Interpretation (35 points total)

A. Facts and observations:

- First observation.
  - A DNA segment of 240 bp, containing part of the P element transposase gene’s ORF2, the ORF2-ORF3 intron, and a small part of ORF3 was fused to a lacZ reporter gene.
  - The fused structure was placed under control of a hsp70 promoter.
  - The construct led to expression of beta-galactosidase in germline cells but not in somatic cells.

- Second observation. Mutations in ORF2, just upstream of the splice donor site, activated splicing in somatic cells.

- Third observation.
o Cell-free extracts of Drosophila somatic cells do not splice the ORF2-3 intron, but do splice other introns.

o Adding increasing amounts of RNA corresponding just to ORF2 to these extracts gradually activated this splicing in the extracts.

Assignment (8 points):

Provide a hypothesis regarding the mechanism accounting for the tissue specificity of P element transcript splicing.

A site in the RNA sequence corresponding to ORF2 has a specific affinity for a protein factor that interferes with intron ORF2-3 splicing. Its mutation prevents binding of the interfering factor. Supplying extra copies of ORF2 RNA competes with the intact transcript for binding to the factor. With enough extra copies, the inhibition is relieved.

B. Facts and observations:

- Tc1 is a 1.6 kbp transposable element of the nematode Caenorhabditis elegans.
- Molecular methods can recover nematodes whose genomes contain Tc1 insertions in a gene of interest without selecting for a mutant phenotype.
- Most such insertion mutants appear phenotypically normal when homozygous, even though homozygous null mutations (such as deletions) in the same gene have severe phenotypes.
- Northern blots probed with gene specific probes show, in addition to a band that is 1.6 knt larger than the wild type transcript, several other bands, including one that has the same apparent size as the wild type transcript.

Assignment (9 points):

Provide a mechanistic hypothesis explaining the absence of a phenotypic effect of these Tc1 insertions.

The Tc1 insertion sequence contains splicing signals that when used result in the production of a mature RNA that is only slightly different from the wild type RNA.

C. Facts and observations:

- Comparisons of substitutions between sequences of mitochondrial genes of different Drosophila species show that transitions are about equal to transversions in frequency.
• In similar comparisons between strains of *D. yakuba*, transitions considerably outnumber transversions.

**Assignment (9 points):**

Provide a hypothesis explaining the difference between the transition/transversion ratios in the two situations.

*In the intrastrain comparison, only limited evolutionary time has passed and the ratio of transitions to transversions thus reflects the rate at which they occur. In the interstrain comparison, a greater evolutionary distance separates the sequences. The positions that can undergo transitions have likely undergone transitions many times. The transversions have caught up, so to speak, to this number.*

D. Facts and observations:

Protein-coding genes in the mitochondrial genome of *D. melanogaster* are interspersed with tRNA genes with few extragenic nucleotides between genes.

**Assignment (9 points):**

Provide a hypothesis about how such an arrangement was selected for in evolution of these genomes.

*Two features have combined to make efficient use of mitochondrial genome information. Pre-tRNA molecules need to be trimmed to the write size. The pre-tRNA processing enzymes result not only in the release of tRNA, but also in the release of mRNAs. These mRNAs, often ending in U nucleotides are the target for polyadenylation, resulting in the production of UAA termination codons.*

III. Experimentation (30 points)

A. Gene identification

• You have isolated several *D. melanogaster* mutants that have indistinguishable phenotypes.

• The mutations map to the same region of the *D. melanogaster* chromosome 2.

• In the DNA sequence of this region, you identify a candidate gene likely to have been the site of the mutations.

**Assignment (7.5 points):**

Describe two separate approaches that can provide convincing support that mutations in the candidate gene are indeed responsible for the mutant phenotypes. List the experimental steps required to carry out the approaches.
Determine the nucleotide sequence of the candidate gene in each of the mutants. If the correct gene was identified, then differences between the mutant and the wild type sequence should be identifiable.

Place the gene under control of a constitutive promoter and introduce it into the mutant lines using a P element vector and a wings clipped helper. Phenotype should be restored.

B. Genetic engineering

- Linear DNA molecules introduced into host cells integrate at random in host chromosomes.
- The plasmid depicted below, when linearized at the unique restriction site B, stably transforms a small proportion of target cells. Different clones of these cells have widely differing expression levels of the green fluorescent protein (GFP).

\[ \text{Plasmid Diagram} \]

(E = enhancer, P = promoter, GFP = gene for GFP, HygR = gene for hygromycin resistance, A B C D = unique restriction sites in multiple cloning site, ori = origin of replication in E. coli, ampR = ampicillin resistance gene)

Assignment (7.5 points):

Describe how you would modify the plasmid DNA to reduce the variation in expression levels on transformation.

Introduce a boundary element at site A and another at site C or D. Make the molecule linear by restriction at site B. Inject the linear DNA intro embryos.

C. Applying methods

Three methods (among others) for relatively precise location of certain nucleotides on DNA sequences use, for detection, separation of polynucleotide chains on nucleotide sequencing gels (gels that allow one nucleotide length differences to be detected). These methods are:
Primer extension
S1 nuclease protection
DNase I footprinting.

Assignment:
For each each of the three purposes below, choose the most appropriate method. Briefly describe the method (using labeled diagrams may save time). Sketch what the result will look like. Describe how the result you sketch should be interpreted (7.5 points). Each method should be chosen just once.

1. Identification of the site of cleavage of a DNA sequence by a transposase.

   Primer extension. Design a primer complementary to a sequence near the 5' end of the DNA being studied. Make it tagged (radioactivity or fluorescence). Anneal it to denatured DNA. Add a DNA polymerase to elongate the primer til it comes to the end of the template. Measure the length of the product by polyacrylamid gel electrophoresis with markers.

2. Location of splice junctions.

   S1 nuclease protection. Produce a DNA fragment (for example, a restriction fragment) that spans the suspected splice junction site. Produce it with label on one end of a single strand, that complementary to the RNA. Anneal the labeled strand to the RNA and digest with S1 nuclease. It will clip where the strand does not match the RNA (where the intron is). Measure the length of the product by polyacrylamide gel electrophoresis.

3. Identification of the nucleotides to which a repressor protein binds.

   DNAsel footprinting. Prepare DNA containing the supposed binding site so that the DNA is labeled on one end only. Divide it into two parts. To one add the repressor under binding conditions. To the other add some non-binding protein. Lightly digest both with the enzyme DNAsel. Extract and denature the DNA. Size the labeled fragment.

D. Protein function

   Imagine a protein, PSI, (and its gene) identified as interacting with sequences (F1) whose mutation allows splicing of the intron between ORFs 2 and 3 of the P element transposase gene. The hypothesis is that PSI binding to F1 prevents splicing in somatic cells.

   You have available:
   • Cultures of somatic cells of Drosophila
   • A PSI cDNA clone with two identical tandem inserts, except that one insert is inverted with respect to the other. The clone is in a plasmid vector that
has promoters for T7 and SP6 RNA polymerases flanking the insertion point.

• The RNA polymerases.
• A good assay for production of active P transposase,
• Common tissue culture and molecular biological tools

Assignment (7.5 points):
• How would you use the tools to test the hypothesis?

I would introduce the clone into the somatic cells in culture. Because of the structure of the insert, a transcript shoud form a double-stranded RNA hairpin that should induce RNA silencing (interference).

• What result would you expect if the hypothesis is correct?

I expect that ORF2-3 intron splicing would be activated in the tissue culture cells

• What result would you expect if the hypothesis is wrong?

The intron should survive splicing and be part of the mature RNA making a P element repressor protein.