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“Very short answer” questions (10 questions, 5 points each; 50 points total)

Question 7. Leslie Orgel showed that a nonsense suppressor can be used to restore a modicum of inducibility to a particular *lacI*$^{-}$ strain of *E. coli*. What was the (correct) interpretation of this result?

Question 8. Even though the basic machinery of small-molecule-inducible gene regulation is very similar in *E. coli* and in budding yeast, the famous PaJaMo experiment would never work in yeast (or, for that matter, in any eukaryote) – i.e., if done exactly as per Arthur Pardee’s outline. Why?

Question 9. Yasuji Oshima incubated yeast cells carrying a *gal4-ts* allele in glucose at the restrictive temperature, then shifted the cells from the permissive to the restrictive temperature concomitant with the addition of galactose, and then showed that galactokinase synthesis was delayed relative to a wild-type control. What was the (correct) interpretation of this result?

Question 10. Jasper Rine constructed a special strain of budding yeast to screen for mutants defective in mating type loci silencing. The genotype of the strain was set up so as to selectively isolate mutants in the silencing process *per se*, as opposed to any other aspect of yeast physiology related to mating. The Rine screen used a “gain of signal” assay. Explain in English what that means *in the context of this experiment* (i.e., don’t just make a general statement about “gain of signal” assays).

Question 11. Michael Grunstein showed that the mating defect of a yeast strain carrying a point mutation in its histone H4 tail (lysine 16 to a glycine) can be suppressed by introducing a specific point mutation into the *SIR3* gene. What was the (correct) interpretation of that result?

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Question 12. Mendel's second law underlies the ability to "map human disease genes by linkage" because all markers linked with the gene mutations in which cause the disease will coinherit with the occurrence of the disease in human pedigrees. "Wait," – says a confused student. "But what about recombination? Won't recombination lead to the marker and the disease gene separating from each other?" In actual fact, it will – so how is this problem dealt with in mapping by linkage?

Question 13. Vernon Ingram showed that the difference between hemoglobin from individuals with sickle-cell anemia and from individuals who are unaffected by this disease is very small, perhaps as small as one amino acid. This result was very important in the development of the "central dogma of molecular biology," because it was known by then that this disease is caused by a mutation in a single gene. How could that have been known *before* the mutation itself had been identified by Ingram?

Question 14. All human cancers carry mutations in protooncogenes (for example, *Her-2/neu*, *c-myc*, or *ras*). Introduction into these cells of a wild-type copy of any of these genes has no effect on the phenotype of the cancer cell. Why?

Questions 15-16. There are three major aspects of quantitative traits that make it diffucult to identify their genetic basis. The first two are, (i) that traits vary continuously and are non-binary (i.e., there is no such thing as "tall" and "short"); (ii) the environment plays a major role in phenotype. What is the third aspect and why does it make life particularly complicated for quantitative geneticists?

(iii) ____

Why is it such a problem?

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Short essay questions

Question X (20 points)

Most sugar-metabolizing operons in *E. coli* are under some form of transcriptional control by the sugar they metabolize (just like the *lac* operon we discussed in class). Consider the hypothetical *ver* operon, which metabolizes the nonexistent sugar verbose. A forward genetic screen isolated loss-of-function mutations in the *verY* gene, which codes for the enzyme required to break down verbose. These mutant *E. coli* fail to grow on verbose as a sole source of carbon. Biochemical studies show that *verY* synthesis is positively controlled by the level of verbose in the growth medium. A screen for "constitutive" mutants in the *ver* operon yields two sets of mutants (*verA* and *verB*) with the same phenotype: *verY* is transcribed at all times, irrespective of whether verbose is present or not. While linkage analysis in the fly/human sense is impossible in *E. coli*, "interrupted mating" experiments indicate *verA* is exceedingly close to the *verY* gene, while *verB* is not. Make a prediction on the role played by the *verA* and *verB* loci in *verY* control.

verA is ____

verB is ____

Both these predictions can evaluated experimentally using a relatively simple genetic approach (note that you must use the same approach for both). Name it:

____

Draw the chromosomes of **all** the strains of bacteria you will need for these two experiments (one for *verA* and one for *verB*), and the phenotypes expected if the predictions are correct.

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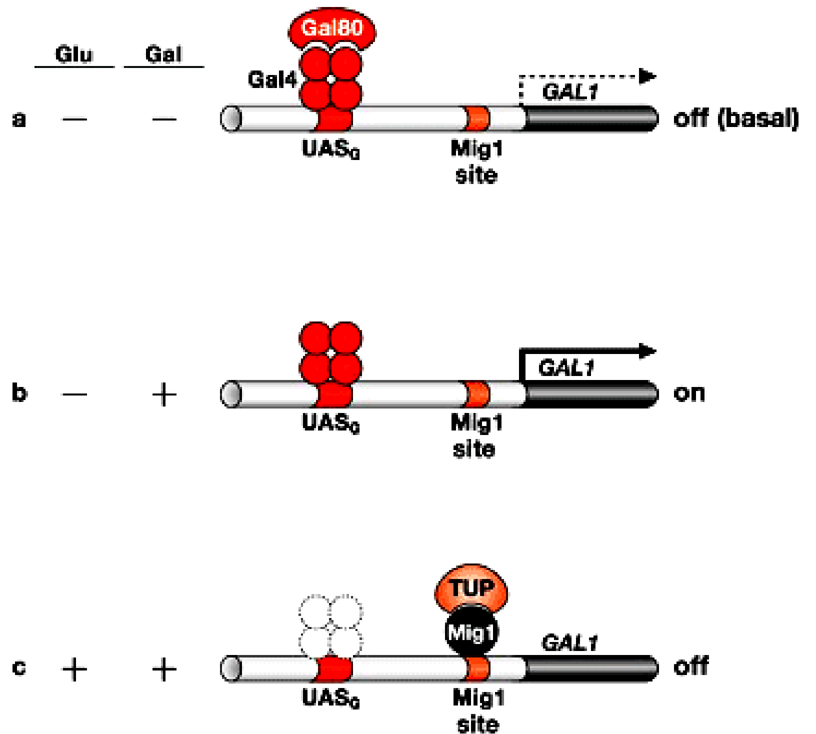
Question X (20 points)

We discussed extensively the interplay between Gal4p, Gal80p, and Mig1p in regulating the response of galactose-metabolizing genes in *S. cerevisiae* to glucose and galactose (see schematic on the right). Pick any **one** of these three proteins, and describe the genetic approach that was used in identifying the gene coding for it as being involved in galactose metabolism, and in determining what the function of its protein product is. Be sure to state explicitly, what the genotype of the starting strain was, what experiment was done, what the phenotype of the resulting strain was, and how this phenotype was interpreted to assign function to the gene being studied. For full credit, you *must* describe *all* the relevant evidence from that particular experiment that helped elucidated the function of the gene product.



Genes and Signals, © 2002 by Cold Spring Harbor Laboratory Press, Chapter 2, Figure 2.

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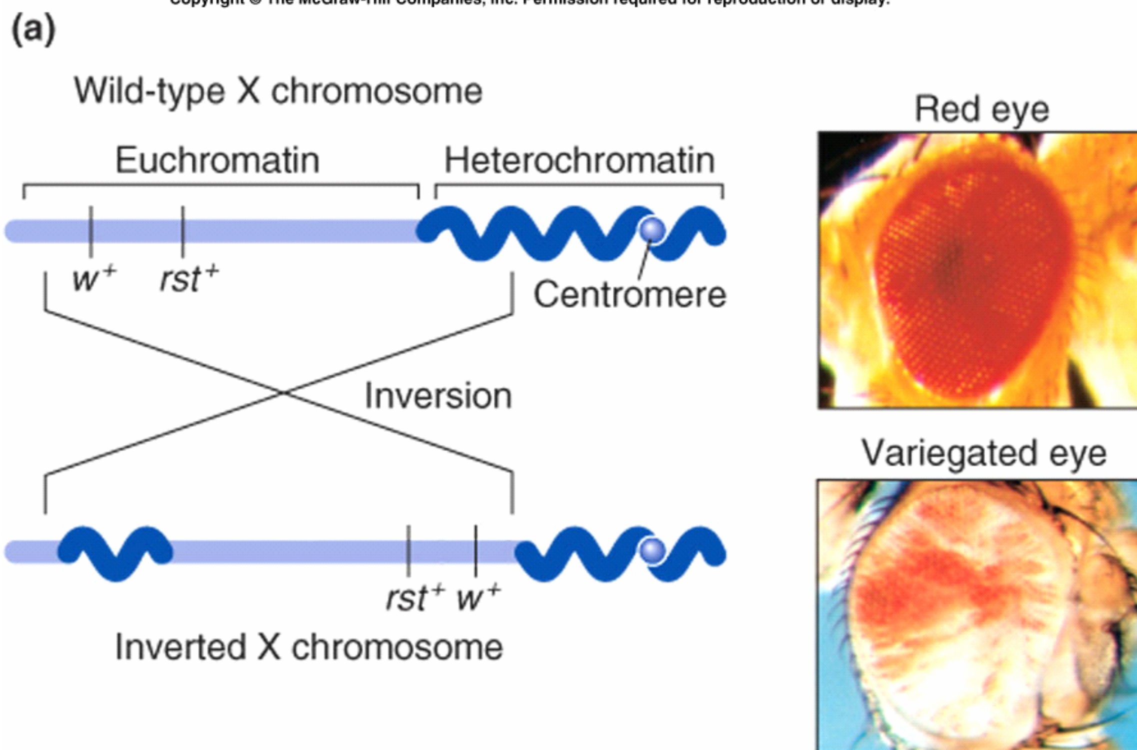
Question X (15 points)

In class, we discussed over several lectures the remarkable parallels between seemingly disparate processes such as dosage compensation in human females, mating type loci regulation in budding yeast, pistle number in *Arabidopsis*, and regulation of tumor suppressor genes in metastatic prostate cancer. What general phenomenon links all these processes?

As you know, position effect variegation (PEV; see below) of the *white* gene discovered by Hermann Muller also falls into this category. One difference between PEV and all the events described in the first paragraph is that PEV does not occur naturally (it only happens in laboratory strains of *Drosophila* and other model organisms). Nonetheless, study of PEV was fundamental for some major recent advances in genome biology.



In what *specific* way is PEV **analogous** to regulation of the mating type loci?

In what *specific* way is PEV **homologous** to regulation of tumor suppressor genes in prostate cancer by E(z), the human homolog of the fruit fly *Enhancer of zeste* gene?

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Data analysis and experimental design questions

Question X (30 points).

Leland Hartwell received a Nobel prize for his studies on cell cycle control in budding yeast, *S. cerevisiae*. His forward genetic screen identified a large number of *cdc* genes in the yeast genome, products of which are required for the cell to correctly execute the G1–S–G2–M sequence. Importantly, many yeast *cdc* genes turned out to have homologs in genomes of higher eukaryotes, and this illuminated the conservation of fundamental cell cycle control mechanisms in all eukarya. It is interetesting to note, though, that the yeast genome does not contain genes homologous to the human tumor suppressor genes p53 and pRB. We know this because the yeast genome has been sequenced in its entirety. Even if yeast had both p53 and pRB, Dr. Hartwell would not have been able to isolate mutants in those two genes in his famous *cdc* screen. Why? (10 points)

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Let's pretend that yeast has a p53 gene, and that the function of this (actually nonexistent) "yeast p53" gene is **exactly analogous** to that in human cells. Describe a yeast forward genetic screen for genes, products of which function downstream of "yeast p53" (the same way that human p21 does). Please use a "numbered list" format for your answer. Your screen starts with haploid wild-type yeast (10 points)

1. ____

2. ____

3. ____

If you do this screen properly, then the majority of mutations that you isolate will be in the "yeast p53" gene itself. In their screen for O_C mutants in the *lac* operon in *E. coli,* F. Jacob and J. Monod used an elegant genetic trick to prevent isolation of mutations in the *lacI* gene. What was that trick? (5 points)

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Why wouldn't that trick work in your p53 screen? Note that we are continuing to assume precise conservation of function between human and "yeast" p53 (5 points)

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