

Solutions Systems Microbiology 1.084J/20.106J PROBLEM SET #3

Problem 3.1

a. Describe the stringent response, and at what level and what components of cellular activity it regulates. The stringent response is triggered in response to two modified nucleotides called *alarmones*—*guanosine tetraphosphate* (ppGpp) and *guanosine pentaphosphate* (pppGpp). These nucleotides accumulate rapidly during a shift-down from amino acid excess to starvation and are synthesized by an STP-dependent protein, RelA that is associated with the ribosomal 50S subunit. These alarmones have global control effects that inhibit rRNA and tRNA synthesis, and thus adjust the cell's biosynthesis in response to amino acid limitation.

b. Most biosynthetic operons need only be under negative control for effective regulation, while most catabolic operons need to be under both negative and positive control. Why might this be?

Biosynthetic operons normally require only negative control for effective regulation because the cell only needs to know when there is "enough" of that biosynthetic product for growth. Catabolic operons, on the other hand, have to be regulated both positively and negatively, since these operons provide the ATP for biosynthesis, and also the carbon intermediates for biosynthesis.

c. Why are promoters from *E. coli* under positive control not close matches to the promoter consensus sequence for *E. coli*?

If a promoter closely matches the DNA consensus sequence one would expect RNA polymerase to bind to that promoter and induce transcription in the absence of other factors. Therefore, promoters under positive control would be expected to be quite different.

d. The attenuation control of some of the pyrimidine biosynthetic pathway genes in *E. coli* actually involves coupled transcription and translation. Can you propose a mechanism whereby the cell could somehow make use of translation to help it measure the level of pyrimidine nucleotides?

If there were a shortage of pyrimidine nucleotides, one would expect transcription to go more slowly. Such a system might include a termination loop that forms unless the ribosome is closely following the RNA polymerase. When polymerase is functioning at maximal rates, it moves out ahead of the ribosome, allowing the termination loop to form. When transcription slows because of pyrimidine shortage, the ribosome follows RNA polymerase closely enough so that the loop does not form.

e. How could quorum sensing be considered a regulatory mechanism for conserving cell resources? Quorum sensing systems can be considered a regulatory mechanism for conserving cell resources is that the genes under its control are all induced at the same times in response to cell density, and conditions that enhance productive use of those resources. For example, pathogenesis in many *Pseudomonas aeruginosa* requires high cell densities for invasion and colonization, and also for production of biofilms that increase pathogenicity and protect the cells from antibiotics.

Problem 3.2

a. How does homologous recombination differ from site-specific recombination?

Homologous recombination refers to recombination involving extensive homology between two regions of DNA. In site-specific recombination, a site on the DNA, usually a few base pairs in length, is recognized by a specific protein and results in recombination between two DNA molecules that often do not share homology.

b. What does an F⁺ cell need to do before it can transfer chromosomal genes?

Before a donor cell can transfer chromosomal genes it must initiate DNA replication and continue DNA synthesis during transfer.

c. What do you think are the most useful transposons for isolating a variety of bacterial mutants? Why are such transposons so useful for this purpose?

Transposons containing antibiotic resistance genes are useful for obtaining a variety of mutants since the introduction of the transposons into a population of cells allows the use of antibiotic selection to identify viable cells containing the transposon while counter selecting against those cells not stably integrating the transposon.

d. Describe why the discovery and use of transformation and the use of the F plasmid were important milestones in the history of genetics. What insights did they contribute?

Transformation experiments lead to proof that DNA was the genetic material. F plasmids allow for chromosomal mapping.

e. Describe three different ways that foreign DNA can enter the cell. How can homologous recombination favor the integration of more than a single or a few genes into the chromosome?

Conjugation--Direct cell-to-cell transfer of plasmid DNA through pili or channels between bacteria.

Transduction--Transfer of DNA mediated by independently replicating bacterial viruses (bacteriophages).

Bacteriophages have variations in specificity, but typically DNA transferred via transduction is among closely related bacteria.

Transformation--Uptake of naked DNA from the environment either naturally (via transport proteins) or artificially (electroporation, or other methods of making holes in cell walls).

Homologous recombination requires sequence homology, thus with prokaryotes this is likely to be triggered by multiple genes.

Problem 3.3

- a. Explain why in generalized transduction one always refers to a transducing particle but in specialized transduction one refers to a transducing virus or phage.

Generalized transduction refers to the “accidental”, random incorporation of host DNA into the virus particle. Thus, in a given population of phage particles, only a few will contain host DNA. For this reason, the particles are called transducing particles. Specialized transduction involves a mutant phage that has lost some of its original DNA and has picked up a specific fragment of host genetic material. In a suspension of a specialized transducing phage, essentially all of the virus particles will contain the transduced gene.

- b. What are the essential characteristics of a cloning vector? What characteristics of plasmids make them especially useful as vectors for molecular cloning? What characteristic(s) of the F plasmid makes it less useful for use *in vitro*?

The essential characteristics of a cloning vector are 1) small size making DNA isolation and manipulation easier, 2) independent origin of replication, 3) multiple copy number allowing for amplification of cloned DNA, and 4) selectable markers for identification of the cells containing the cloning vector or recombinant clone.

Characteristics that make plasmids especially useful for molecular cloning (including those just stated) include 1) a polylinker, or multiple cloning site, 2) color selection for immediate identification of recombinant plasmids, and 3) promoters for expression of the cloned gene. (e.g. M13 phage promoters for generation of single-stranded DNA, etc). The F plasmid is much too large to be useful as a cloning vector and does not contain any selectable markers.

- c. What are the general properties of insertion elements? Class II transposons?

Insertion elements (or sequences) have no genes other than those required for them to move to a new location. They are ~1000bp in length and become integrated at specific sites on the genome (both plasmids and chromosomal DNA, and a few bacteriophages). Homologous recombination often occurs between IS elements causing the integration of new DNA. Transposons are larger than IS elements and contain other genes such as antibiotic resistance. Conjugative transposons are able to move between bacterial species via conjugation, not solely around in one genome.

Problem 3.4

Five Hfr strains A through E are derived from a single F⁺ strain of E. coli. The following table shows the time of entry (in minutes) of the first 5 genetic markers observed in interrupted mating experiments. Please answer a-c, below, based on the data.

STRAIN A		STRAIN B		STRAIN C		STRAIN D		STRAIN E	
mal ⁺	1	ade ⁺	3	pro ⁺	3	pro ⁺	10	his ⁺	7
str ^s	11	his ⁺	28	met ⁺	29	gal ⁺	16	gal ⁺	17
ser ⁺	16	gal ⁺	32	xyl ⁺	32	his ⁺	26	pro ⁺	23
ade ⁺	36	pro ⁺	44	mal ⁺	37	ade ⁺	41	met ⁺	49
his ⁺	51	met ⁺	70	str ^s	47	ser ⁺	61	xyl ⁺	52

- a) Draw a map of the F⁺ plasmid, showing the positions of all the genes relative to the origin, and their distance apart in minutes.

See figure.

- b) Show the insertion point and orientation of each Hfr strain.

See figure.

- c) In using each of these strains, which gene would you choose to use as a marker, to get the greatest number of “exconjugants” (eg. recipient cells that have successfully received an F plasmid)?

In each strain one should use the last gene to be expressed to ensure that the full plasmid is present. Thus Strain A = xyl, Strain B = ser, Strain C = gal, Strain D = met, Strain E = ade

