Period 13 (Apr. 30)

***E. coli* DNA microarrays and databases; PubMed**

Glass slide DNA microarrays are used to monitor gene expression in an organism in a global manner. Microarrays for *E. coli* carry all 4400 genes of the organism (amplified from the genome by PCR). To compare mRNA levels in one strain of *E. coli* grown under two conditions or in two strains grown under the same condition, you isolate RNA from each and convert it to cDNA using reverse transcriptase. During (or after) cDNA synthesis you label each cDNA with a nucleotide analogue that fluoresces. One common analogue, "Cy3," fluoresces green, whereas another, "Cy5," fluoresces red. You then mix the cDNAs from the two cultures and hybridize them to the glass slide. If the two cultures contained equal amounts of mRNA for a given gene, the "spot" of that gene on the glass slide will be yellow after hybridization (equal amounts of red and green fluorescent label will have bound to it). Otherwise the spot will be red or green, depending on which culture had more mRNA for that gene. Adriane Jones, who took PMB112L two years ago and now works in my lab, will demonstrate the use of *E. coli* glass slide DNA microarrays to monitor gene expression in this organism globally. We will discuss DNA microarray experiments in Section before you see the demonstration.

Daniel Zimmer, a postdoctoral fellow in my lab, wrote a computer program to rearrange the spots on glass slides in the order in which they occur on the genome of *E. coli*. He calls the resulting images "genome images." (WHY DO YOU THINK SUCH IMAGES WOULD BE USEFUL TO BIOLOGISTS TRYING TO INTERPRET ONE OR MORE MICROARRAY EXPERIMENTS?) You will examine a genome image comparing mRNA levels in a wild-type strain of *E. coli* before and after induction of transcription of the lactose operon with IPTG. (WHAT CAN YOU LEARN FROM THIS EXPERIMENT THAT YOU DID NOT LEARN BY DETERMINING THE DIFFERENTIAL RATE OF SYNTHESIS OF β-GALACTOSIDASE IN THE LAB?) You will also examine a genome image comparing mRNA levels in a wild-type strain of *E. coli* grown with taurine as sulfur source to those with sulfate as sulfur source. Like lactose, taurine (2-aminoethane sulfonate) is found in the human intestine.

Dan Zimmer also constructed a database called the *E. coli* Entry Point, which assists biologists in analyzing DNA microarray data. Using the Entry Point, the Ecocyc database, and PubMed you will: 1) determine the structure of lactose and its degradation products (which you already know); 2) determine the pathway for lactose degradation (which you already know); 3) obtain references on the lactose degradative enzymes/pathway. This preliminary exercise will allow you to learn to use the databases. Then you will examine the second genome image to determine which operons are induced on taurine as the sulfur source. Using the same tools mentioned above, half the class will: 1) determine the structure of taurine; 2) determine what the *tau* genes are; 3) determine the degradative pathway for taurine; 4) obtain pertinent references on the taurine operon and its regulation in response to the sulfur source. Using PubMed this half of the class will determine what is known about the regulation of the taurine operon. The second half of the class will do the same things for the *ssu* operon. HOW DOES A SULFONATE LIKE TAURINE DIFFER FROM A SULFATE ESTER?