ONE-MINUTE WRITE 2/1/00

Question: An amber suppressor mutation is needed for some useful bacterial genetic tricks. How could you select for a suppressor mutation in *Salmonella enteritidis*?

Answer: Begin with a strain with amber mutations in two different genes and select for the simultaneous repair of both mutant phenotypes. There are many ways you could do this, but the example discussed in class is shown below: Begin with a *S. enteritidis* strain that has two amber mutations: one mutation inactivates a gene required for resistance to the antibiotic tetracycline, and the other mutation results in auxotrophy for tryptophan. This strain will have the following properties.

Bacterial strain	**Growth conditions**				
	Rich medium − Tetracycline	Rich medium + Tetracycline	Minimal medium + Tryptophan	Minimal medium − Tryptophan	Minimal medium − Tryptophan + Tetracycline
S. enteritidis tet (Am) *trp* (Am)	+	−	+	−	−

By plating this strain on minimal medium − tryptophan + tetracycline, you can select for repair of both mutations. Reversion of two separate mutations is very rare – for example, if the reversion frequency of the *tet* (Am) mutation is 10^{-6} and the reversion of the *trp* (Am) mutation is 10^{-7}, then the probablity of the two events occuring at the same time is the multiple of these numbers or $10^{-6} \times 10^{-7} = 10^{-13}$, a frequency too rare to readily detect. Therefore, simultaneous repair of both mutations is much more likely to occur by another mutation that can suppress both amber mutations (i.e. an amber suppressor mutant) designated "*sup*". The phenotype of such a mutant will be:

Bacterial strain	**Growth conditions**				
	Rich medium − Tetracycline	Rich medium + Tetracycline	Minimal medium + Tryptophan	Minimal medium − Tryptophan	Minimal medium − Tryptophan + Tetracycline
S. enteritidis tet (Am) *trp* (Am) *sup*	+	+	+	+	+