

Laboratory Worksheet, Monday, Dec. 12.

Two projects from last week which we cannot finish (I. and II.):

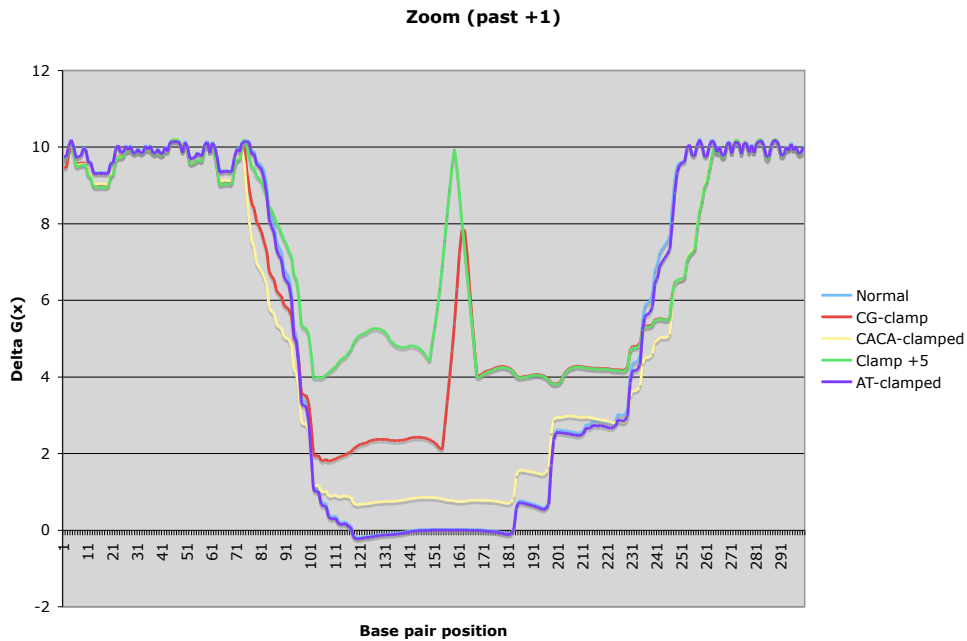
I. Aminoacyl-RNA synthetases and the RNA World. Distant Homologies, Phylogenies and the RNA World. (a) Look up the definition of a synthetase for an amino acid. (b) Look up lysine tRNA sequence for several organisms and lysyl-tRNA synthetase for the same organisms. Do a MSA for the DNA sequence, and then a phylogeny. Can one see a difference between predicted ages of these elements? What does this have to do with the “RNA World Hypothesis”?

Comment: One has to be more careful than we were last week when trying to detect a signal of the RNA world in the phylogenetic precedence of tRNAs to their corresponding aminoacyl-RNA synthetase. In general, if we look at recent species, one would expect these species to have appeared long after the transfer of many enzymatic functions from ribozymes to proteins. The paper Ribas de Pouplana et al., Genetic code origins: tRNAs older than their synthetases?, *PNAS* **95** (1998), pp. 11295-11300, carries out this analysis with the tools we are using – in fact, even cruder phylogeny tools! – but using the genes of an archaeobacterium, *Methanococcus jannaschii*. The explanation is given there as to why one uses the lysyl tRNA-synthetase as the test case (as opposed to the nineteen other possible amino acid residues). This has to do with the classification of aminoacyl tRNA-synthetases into type I and type II synthetases (approximately half and half, but not the same partition of amino acid residues as in the Miller-Urey pre-biotic/post-biotic classification below), and an apparent late (evolutionarily) shift of lysyl tRNA-synthetase from a type II to a type I synthetase. The details are in the paper, which is well worth reading, but I am afraid we will not have time to track everything down this week. The paper is in the resources section of the Ctool site for the course, under the labs section.

II. Distant Homologies, Phylogenies and the Miller-Urey Soup. Collect an example of acylsynthetase sequence for each of the 20 amino acid residues, preferably from the same organism (which will probably be simple). Align these and put them on a phylogeny. Which are the oldest and which the youngest? Look up the Miller-Urey pre-biotic soup. Compare the positions of glycine and tryptophan on the phylogeny. Is this what you would expect from the Miller-Urey and subsequent experiments?

III. The Hatfield-Benham Experiment and the Missing Calculation. This is an exercise in using WebSIDD, the online calculator of the SIDD profiles provided by the Benham laboratory at UC Davis. The URL and link are in the Ctools site. You will also find there the sequence data for the original computation. You should carry this out yourselves. In the Ctools site is also Nathan Stiennon’s calculations where there are various approximations used to try to adapt the direct calculation to a model of the mechanics of SIDD with the *IHF* protein binding at the site described by the Hatfield lab. Basically, the idea is simply to replace the binding site sequence segment by a *CG*-tract.

We will go over this calculation in the lecture. Here is Nathan's basic graphic:



IV. PHX Pipeline. This is what has to be finished today. If the tool works, one can compare “putative” versus real genes in given gene types you have already gathered, and some specific genes. For example, you can consider some genes in the *lac* operon, such as the *lac-Z* gene (β -galactosidase), *lac*-repressor or *lac-Y* (lactose permease).

Have a Good Holiday Break!

— Jeff and Dan