Lecture 21
4/5/04

Problem sets are due April 12th

References
G-proteins
Science (2001) 294, 1299-1304
Curr Opin Struct Biol (2003) 13, 122-4
Acta Biochimica Polonira (2001) 48, 829-50

Remember that amino acids are first activated by adenylation and then loaded onto tRNAs.
There are two editing possibilities: It could occur before the amino acid is covalently loaded on the tRNA or after it is loaded.
Look at lecture 20 notes for details

The 5.08 website has rasmol scripts that you should look at to understand what we know about the editing process with ileuRS.

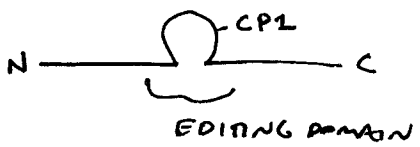
Page 1 of handout 3b also has a cartoon picture of the editing mechanism and the parallels to the editing mechanism proposed for DNA polymerase I and its exonuclease domain that cuts out an incorrectly inserted nucleotide.
The charged 3' end of the tRNA (CCA) is looped back into the active site of the synthetase domain. The editing domain is 34 angstroms away from the active site. If the wrong amino acid is incorporated onto the tRNA, the 3' CCA end of the tRNA straightens out and moves into the editing domain, where the mischarged amino acid can be hydrolyzed.

Testing the editing domain model

Are there two separate sites? Or is the active site the same as the editing site?
(As it turns out, there are two separate sites)

Experiments to test these models
Make mutants of the putative editing domain active site

Cp1



WTT[T]PWX_3[N]

Alanine scanning methods were carried out in which each amino acid in the putative editing site was changed to an alanine by site directed mutagenesis Each mutant was then examined biochemically.

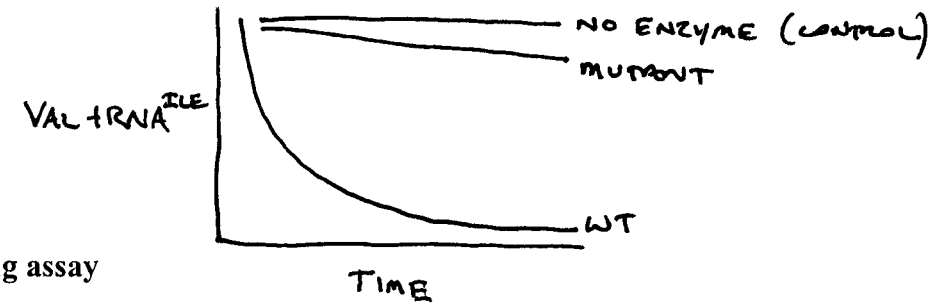
Alanine scanning is a common method to try to establish which groups are important in an active site or binding site for function.

T242A and N250A were interesting mutants based on biochemical assays

We need separate assays for charging (active site) and hydrolysis (editing site)

Editing assay

Compare wt to mutant
Give RS^{Ileu} a $tRNA^{Ileu}$ that is mischarged with labeled Val (Val $tRNA^{Ileu}$)
Monitor the release of Valine as a function of time. To do this Val is radiolabeled.
For the wt, the amount of Val $tRNA^{Ileu}$ will decrease exponentially with time
For the mutant, there will be only a slight decrease, corresponding to a small amount of contaminating wt RS activity. The wt-RS activity cannot be removed from E. coli as this protein is essential.



Graph of editing assay

Active site assay- two possible assays
There are 2 half-reactions, you can assay both

1st half reaction:

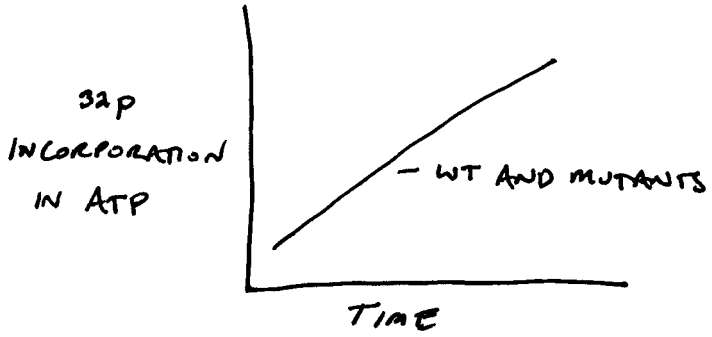
$$E + Val + ATP \rightleftharpoons E\ Val\text{-}CO\text{-}OAMP + PPi$$

Do assay in presence of inorganic ^{32}PPi and no tRNA
Then, monitor the incorporation of radiolabeled pyrophosphate into ATP through the reverse reaction. This reaction is reversible and requires that PPi dissociates and ^{32}PPi binds. Revesal of the reaction gives ^{32}P into the ATP. The rate of this incorporation is the actual assay. Recall that for this RS, this step is fast relative to the second half reactio.

2nd half reaction:

Use labeled Ile ^{14}C
Monitor attachment to tRNA through ^{14}C incorporation into the large tRNA

Results: The mutants have no effect on either half reaction- same as wt results



Graphs of active site activity assays
Conclusion: strongly suggests a separate editing domain from the active site
Editing domains can discriminate based on steric factors and other mechanisms (ex.chelation (S,T) to a metal)

You don't always need an editing domain if the amino acid is sufficiently unique for it to bind specifically

IV. **G-PROTEINS** (Ef-Tu/ EF-G)
-Paradigms
-switch or motor??

Overview: GTPase- superfamily of proteins (domain 20-25 kDa) that are involved in many functions
Examples:
Ras – intracellular signaling, cell differentiation
Rho/Rac – cytoskeleton rearrangements
Rab – directional vesicle transport
Ran – nuclear import and export
Alpha subunit of heterotrimeric G-proteins- involved in diverse things including glycogen breakdown, taste, smell, etc….)

GTP-> GDP + Pi (break high energy phosphate bond)

When is GTP hydrolyzed relative to the work?

GTP hydrolysis (chemical energy) to do work
OR
GTP binding energy to do conformational change-> work-> made irreversible by coupling to chemistry of GTP hydrolysis
(This second option is the true for EF-Tu)

Science (2001) 294, 1299-1304
Gives the structure of the generic GTPase domain -> Ploop, 2 switches (by convention, switch I is usually colored green, and switch II is blue)

The conserved GTPase domain may be pasted into many different protein machines

Overview:

"Active form" - GTPase domain binds GTP very tightly ($K_D \sim 10^{-11}$ M for EF-Tu) but catalysis is very slow. For EF-tu, the GTPase activity is only 0.003 min^{-1}. The effector (in the case of EF-tu, the effector is tRNA) is also tightly bound. Different G proteins can interact with a wide variety of effectors, and the effectors always interact with the 2 switches of the GTPase.

GAP= GTPase activating protein (partner) (actually RNA in the case of EF-Tu)
When the GTP-GTPase-Effector complex interacts with GAP, the rate of hydrolysis is rapidly increased (accelerates hydrolysis of GTP 10^3 to 10^5 fold for EF-Tu).

Hydrolysis of GTP-> GDP creates a new state-> conformational change, that allows the dissociation of the effector

Finally, Guanine (Or Nucleotide) Exchange Factor (GEF, NEF) facilitates GDP dissociation so that GTP (mM concentration in the cell) can rebind, and the process can begin again. The GEF for the EF-TU is EF-Ts. GDP is tightly bound to GTPase in the absence of a GEF.

Cartoon Diagram: