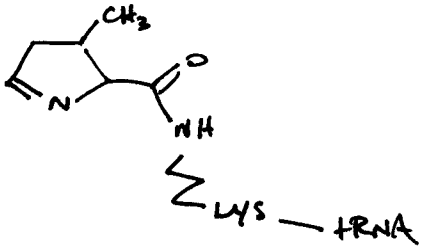
Lecture 20
3/19/04

References:
GTPases: Wittinghofer, Science (2001) **294**, 1299-1304
Ras-Effector Inter. Curr.Opin. Struct. Biol. (2003) **13**, 122-129

There are 61 tRNAs (64 possible)
And at least 31 tRNA synthatases (RS). The number varies between prokaryotes and eucaryotes
p. 2 of handout 3b
Class I and Class II RS
Three proteins, p38, p43, p18 co-purify with a multi-RS complex in eucaryotic systems. Those that co-purify are indicated by bold lines in the figure.
Amino acids can be modified after they are attached to a tRNA, these are also indicated in the figure: one of these is serine that can be changed to a selenocysteine and a second is methionine that can be formylated (cofactor is N-10 formyl tetrafolate)

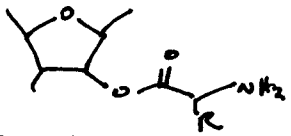
A 22nd amino acid was recently found in archae and is pyrrolysine

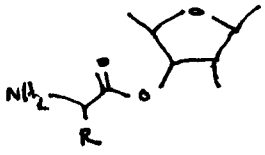


Drawing of 22nd amino acid
The tRNA is actually charged with this unusual amino acid- it is not simply a post-attachment modification.

Class I
alpha monomers
protein binds in minor groove of tRNA
Predominantly generates 2'
acylated product

Class II
dimers
protein binds in the major groove
Predominantly generates 3'
acylated product





Drawing of acylated products

We will focus on Class I-RS specifically: an RS where editing has been studied

The two classes have different defined active site motifs

Our focus will be isoleucine tRNA synthetase (class I). You can look at Rasmol scripts on the 5.08 website

Nomenclature:
RS^{Ileu} = uncharged
Ileu RS^{Ileu} = charged with isoleucine
Val RS^{Ileu} = charged incorrectly with valine

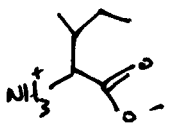
Looking at structure:
Has a beta-alpha-beta structure. This is typical of NT folds called Rossman folds. These are unique folds to Class I

Drawing of beta-alpha-beta motif

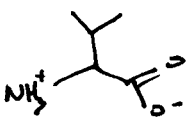
-Active site motif: HIGH (64-67) colored magenta on script
KMSKS (595-98) colored yellow
(can find using Blast/ClustalW)

-Inhibitor structure: pseudominic acid is a bisubstrate analog of ATP and amino acid bound. The binding of this compound will help you locate the active site (This inhibitor is used as a therapeutic)

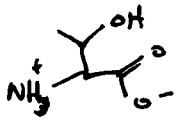
Editing Model:
Know from *in vivo* studies that errors, that is the incorrect amino acid, is incorporated in 1 of every 10^3 to 10^4 amino acids incorporated
Isoleucine looks a lot like valine (one methylene different) and like threonine (sterically)



Isoleucine



Valine



Threonine

How much is that methylene group worth in terms of interaction energy
-it's a weak interaction, the interaction has been estimated to be worth about 2.8 kcal/mol
-remember that 1.38 kcal/mol -> factor of ten
-so, the most you can have is a factor of 100 in discrimination between valine and isoleucine
-but the mistake rate is much lower! So, this is evidence for some sort of editing mechanism

Specificity: use kcat/Km as key parameter for measuring specificity

$v = V_{max}[S]/([S] + K_m) = k_{cat}[E][S]/([S] + K_m)$

At low [S] conditions, conditions under which the enzyme can discriminate between two alternative substrates, v approaches $(k_{cat}/K_m)[E][S]$, where k_{cat}/K_m is a second order rate constant for binding of enzyme to substrate
Compare v_{Ileu} to v_{val} :

$v_{Ileu} / v_{val} = (k_{cat}/K_m)_{Ileu} [E][S] / (k_{cat}/K_m)_{Val} [E][S]$

[E] cancels out, because both amino acids are competing for the same E

In bacteria [Val] = 5x[Ileu]
(concentration of valine is five times higher that isoleucine)

also we know the ratio of kcat/Km for ileu/val is 180 (measured experimentally)

$v_{Ileu} / v_{val} = 180*(1/5) = 30$ Thus the ability to discriminate between ileu and val is way below the 1 in 10^3 to 10^4 that is observed under in vivo conditions

Paul Berg was the first to propose that there exists an editing domain within some RSs. This led to the "double sieving, which came out of the Fersht lab in Cambridge

There are two selections, both based on steric constraints.
The 1st discrimination occurs at active site and the enzyme discriminates between ileu and anything larger. Thus valine which is smaller than ileu can be incorporated.
The 2nd discrimination occurs at editing site, 34 angstroms away, and it is also based on size. In this case the editing site precludes the larger ileu from binding and allows the valine to bind where it can be hydrolyzed.

In active site, coarse sieve selects sterically against bigger structures
Isoleucine is bigger than valine, but this will help select against larger amino acids

Fine sieve: also selects sterically against bigger amino acids
Only the smaller amino acids get hydrolyzed
V,T get hydrolyzed

This model is used in many RNA synthatases, but not all
RS^{Ile}, RS^{Leu}, RS^{Val} (all class I)
Class II has a different type of editing domain based on chemistry rather than sterics.
One enzyme uses a metal ion where serine and threonine can coordinate, but no amino acids without hydroxyl groups can bind

Model: from RS^{Ile}

$E^*Val\text{-}AMP + tRNA^{Ileu} \rightleftharpoons E^*ValAMP^*\ tRNA^{Ileu}$ (active site)

$E^*Val\ tRNA^{Ileu}$

Movement to editing site

$Val + tRNA^{Ileu}$

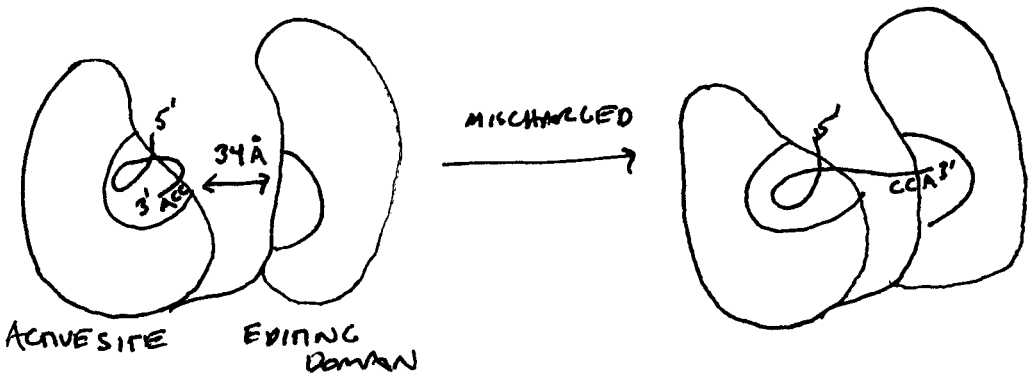
Sacrificed ATP in the name of getting the right amino acid on the tRNA
Working hypothesis: will be clearer if you pull up the scripts and look at the structure

See page1 of handout 3b - cartoon from Steitz's paper

Synthetic mode Editing Mode

Polymerase domain and editing (exonuclease) domain. The same strategy for DNA polymerase and synthetases

Editing domain: 34 angstroms removed from active site



Cartoon Drawing of Editing
-the CCA end is bent back into the active site
(look for this in the scripts)
-once it's charged, the base pairing is changed so that is unbends and reaches over to editing domain

How do you know that there are two distinct sites?

Set of experiments done by Schimmel: Science (1997) **276**, 1250
Biochemistry (2000) **31**, 8180-8188

-If you look at the gene sequence, see an insert called CP1 in all these RS's