Lecture #23

Lecture 23
4/9/04

EF-Tu is a paradigm for switches

See model on p. 8 of handout 3b
Initial Summary:
Initial (weak) binding k_1/k_{-1} → codon recognition k_2/k_{-2} → GTPase activation k_3 → GTP hydrolysis k_{GTP} → EF-Tu conformational change k_4 → accommodation (k_5) and peptidyl transfer (k_{pep}) OR proofreading (k_7)

Remember that conformational changes are the key
Conformational changes are coupled to a rapid irreversible chemical steps

In the first discriminatory step, (sensing cognate, near cognate, non-cogante interactions), the dissociation due to inappropriate interactions is rapid relative to the conformational change. Thus if you get dissociation, you remove the incorrectly charged tRNA without using any GTP.
In the second discrimination at the proofreading step, discrimination comes at a price, hydrolysis of GTP

MODEL (again see cartoon diagram p. 8 handout 3b)

1) EF-Tu GTP $tRNA^{aa}$ Stable ternary complex, (like a GTP binding protein, very low GTPase activity)

2) initial selection (k_1/k_{-1}, k_2/k_{-2} vs. k_3)
key: k_{-2} vs. k_3
needs to sense cognate interaction
conformational change that is slow, followed by a rapid irreversible chemical step (GTPase activity). {Note if conformational change is slow and GTP hydrolysis is fast, the rate of GTP hydrolysis will be identical to that for the conformational change, GTP hydroysis rate is masked}

select for correct interaction

	near-cognate	cognate
k_{-2}	$17 s^{-1}$	$0.2 s^{-1}$
k_{-3}	$50 s^{-1}$	$500 s^{-1}$
difference	2.5 fold	2000 fold

In the absence of GAP for cognate, the rate is only .0005 s^{-1}, 10^6 fold acceleration
In this case, if you have a non-cognate charged tRNA, you get dissociation w/out ever hydrolyzing GTP

3) Proofreading

EF-Tu GDP, delivered charged the tRNA
k_7 vs k_5 (conformational change, followed by a rapid irreversible chemical step- peptide bond formation)

	near cognate	cognate
k_5	0.1 s^{-1}	7 s^{-1}
k_7	6 s^{-1}	0.3 s^{-1}

If proofreading occurs in this step, you've sacrificed a GTP
"Book-keeping" of GTP hydrolysis is complicated, because GTP can be consumed in proofreading as well as peptide bond formation

Where did this model come from?
100s of experiments!

Model must accommodate all the kinetic data
Global analysis of all kinetic data (under the same conditions) w/ computer software give kinetic simulations which then function as your working hypothesis
The model system used by Rodnina et al give chemically and kinetically competent peptide bond formation 7 s^{-1} (this number is similar to peptide bond formation *in vivo*, rate ~10 s^{-1})

Pre-Steady State (no steady state assumptions)
1) stopped flow – continuous method
must have something colored or fluorescent
limiting is "mixing" time-> dead time ~2ms
limits observed rate constants, can't see things that happen on a faster time scale

for kobs= 500 s^{-1}
t1/2 = .69/ 500 s^{-1} = 1.3 ms
60-70% of reaction is complete w/in the dead time of the instrument!

You can initiate w/ light to measure faster rate constants

2) rapid chemical quench- discontinuous method
in general, quench with acid (Rodnina uses base) You have to be sure that the reaction stops FAST
separate products from starting material at each time point by HPLC or TLC

3 types of fluorescent probes

a. GTP[gamma-^{32}P] → ^{32}Pi + GDP
b. [^{3}H] labeled amino acids on tRNA, measure dipeptide formation
c. conformational changes
   i. proflavin labeled tRNA^{aa}
   ii. EF-Tu – you could label w/ fluorescent probe using site directed mutagenesis to place a cysteine at a desired position using the structure
   iii. GTP analogues- fluorescently labeled

Look at Rodnina paper data and look at the primary data. The rate of release of ^{32}Pi for cognate and near cognate looks very similar. However, the lag phase, prior to release of ^{32}Pi is substantially different. This is an example of how it is hard to "intuit" pre-steady state kinetics. The observed rate constant is composed of all of the steps k1, k-1, k2, k-2 etc and the model needs to account for the lag phase as well as the apparent kobs for Pi release.
In the case of dipeptide formation the cognate dipeptide is formed in substantially different amounts than the near cognate dipeptide formation (look at the differences in the axes for each experiment) proofread and dissociate, make 10x less dipeptide than w/ cognate.
These are just samples of some of the many experiments that these investigators carried out. All the information together has provided the working hypothesis we used in class.

EF-G is also a GTPase, its function is to move the mRNA from the A site to the P site and at the same time to move the tRNA charged with the growing polypeptide from the A site to the P site. The x-ray data of Noller at 5 angstroms suggests that this movement may be 25 angstroms.

EF-G looks amazingly similar to EF-Tu•GTP•tRNA^{aa} see p7 of handout3b
Example of molecular mimicry
Shape selectivity. They both fit into the same region of the ribosome

p. 10 of handout 3b
Key paper: cartoon picture of EF-G•GTP
Hydrolysis of GTP provides the energy for mRNA and tRNA to translocation

EF-G -> GTP hydrolysis -> conformational change
Energy of GTP hydrolysis somehow strains conformation, causes translocation to the P site

Understanding this movement is an active area of research

MOTORS- hydrolysis prior to movement,
Similar to myosin and actin