Lecture #27

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4/23/04

Exam 3: Avg. 62, grades from 8-87

From Exam question #2

Many mechanisms by which enzymes catalyze reactions
Yarus inhibitor= transition state analog for peptide bond formation
Mimics tetrahedral intermediate in peptide bond formation
Select for RNA that binds tightly to Yarus, it can catalyze peptide bond formation!
Supports transition state stabilization theory of enzyme catalysis

#3

Correct answer ultimately depends on kinetics and concentrations

a) fMet – intitation- goes into the P site (requires energy)
b) proofreading: non-cognate discriminates w/out GTP hydrolysis'
near-cognate uses a GTP when proofread out

Use 2 GTP, 1 for Ef-Tu, and 1 fro Ef-G

For more info on exam 3 see the exam answers and the T&D9

PROTEIN folding and degradation

The Small Difference in the free energy of the folded and unfolded states makes it very difficult to predict structure from amino acid sequence.

Remember the Levinthol paradox and Anfinsen's hypothesis from last lecture

Anfinsen's – (*in vitro*) RNase has 4 disulfide bonds- if a thiol isomerase is present, it can spontaneously fold

See page 5 of handout 4a for *in vitro* protein folding models

1) Framework: local secondary stucture elements form first (independently), the the secondary structural elements diffuse and collide to fold into tertiary structure

2) Hydrophobic collapse model: protein collapses rapidly around hydrophobic sidechains, this restricts the conformational space, potentiates folding (molten globule intermediates)- this is a popular model, strong evidence for some proteins

3) Nucleation Model- neighboring residues adajacent in sequence space form some element of the native secondary structure, this structure acts as a nucleus for cooperative folding (no intermediates)

4) Jigsaw model- each molecule folds by a distinct pathway (need single molecule methods to test this theory)

See page 6 of handout 4a for a description of different <u>methods</u> used to study protein folding
Pay attention to timescales- each method has a different timescale associated with it
Different proteins fold at different rates, small proteins can fold in ns-ms, RNase with all the disulfide bonds took hours
Fluorescence, Circular Dichroism, IR, NMR, and Mass-spec(hydrogen/deuterium exchange) are all common methods used for studying protein folding in vitro

Conclusion (from all studies, using a wide range of different methods)- There is no specific pathway, but a multidimensional landscape- called a folding funnel
Some pathways are more populated than other(this is protein dependent- different proteins may take different pathways)
(See diagram of this model on page 6 of handout 4a)

<u>Protein folding</u> – *<u>in vitro</u>* vs. *<u>in vivo</u>*
In the last five years we've learned that many proteins are actually unfolded in the cell!
Think about **timescales**: *In vitro* : nsec to hrs –protein dependent
In vivo : rate constant for peptide bond formation in *E.coli* is ~ 10-20 s-1
With a 300 amino acid protein on the ribosome, the half-life for folding as it comes out of the exit tunnel is ~30s (folding limited by rate of peptide bond formation)

What are the conditions under which protein folding occurs??
In vitro: most studies of *in vitro* folding are done in the absence of other proteins in dilute conditions- avoid aggregation (very different from conditions inside the cell)
Usually study small proteins
In vivo : 340 mg/mL of macromolecules in bacteria
Concentration of ribosome is 30-35 microM
Concentration and kinetics are important!!!

In vitro: no assistance in folding (except maybe beta-mercaptoethanol)
In vivo: more complex
Multi-domain proteins, sub-unit interactions
A large repertoire of proteins are required

<u>Folding Process:</u> *<u>in vivo</u>* (overview)
a) Proylisomerase (inter-converts the cis/trans forms of proline)
b) Disulfide bond formation: requires an oxidase to form disulfides
isomerase to rearrange incorrect disulfides
c) protein chaperones

1. Hsp70/Hsp40 (Heat shock protein- elevated when bacteria is under stress) "Holdases" or "unfoldases" (Use ATP)
2. Clp B/Hsp104- "deaggregases"
3. Chambers- GroEL/GroES- big chambers