Lecture 26
4/16/04

No class Monday(patriots day) OR Wednesday(4/21)

New X-ray structure of the translocon:
Nature (2004) 427, 36-44

Targeting of proteins to membranes and for secretion
p.2 Handout 4a
Cartoon overview of SRP pathway
Equipment required for inserting proteins into membranes

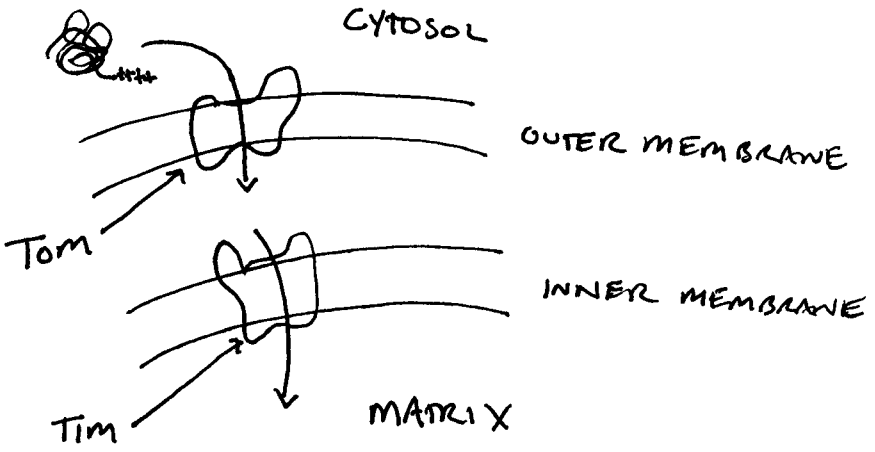
Both SRP and SRP-R contain GTPase domains

Somehow (we don't completely understand the whole process yet) SRP interacts with signal sequence (hydrophobic) at N-terminous of growing polypeptide as it exits the ribosome, a pause in polypeptide formation occurs because the SRP can interact with the A site of the ribosome. The pause allows time for the SRP with its cargo to interact with SRP-R in the membrane, and transports protein to translocon (ER membrane)

How are multiple transmembrane domains inserted? We don't know yet.

Translocon x-ray structure does not agree with other methods (FRET, cryoEM)
We need multiple methods to really understand function

How to get proteins into the mitochondria?
p.4 handout 4a
Must cross inner and outer membrane to get into mitochondrial matrix
+++++, a positively charged signal sequence at the N-terminus (first end to exit ribosome), targets the protein for transport into the mitochondria
TOM (transporter outer membrane) complex of proteins- equipment to get protein across outer membrane
TIM (transporter inner membrane) complex of proteins- equipment to get protein across inner membrane
Cartoon Diagram:



Delta Psi, a change in membrane potential is required for transport across the inner mitochondrial membrane.

p.4 handout 4a
To go from cytosol into matrix you have to unfold and then refold the protein
Why do this?

3 possibilities for how folding and refolding could be accomplished

a. spontaneous global unfolding
(not as far-fetched as it sounds, the energy difference between folded/unfolded states is very small)
b. catalyzed unfolding – membrane potential
c. catalyzed unfolding – chaperone protein (uses ATP)

Misfolding:
We know that misfolding occurs
Can we refold misfolded proteins? Yes
If we cannot correct the misfolding, can we remove the misfolded protein? Yes, via the proteosome
If we can't correct or destroy the misfolded protein, what are the consequences?
-disease, (ex. Mad cow disease, alzheimer's, huntington's disease, parkinson's)
See p. 5 handout 4a
Misfolded proteins can form amyloid plaques and other aggregates

Protein Folding *in vitro*

Energy difference between folded/unfolded state is very small (~5-15 kcal/mol)
A typical H-bond is ~5 kcal/mol
People would love to be able to predict protein folded structure from primary sequence, but very difficult problem
There are huge numbers of interactions in both the folded and unfolded states, and the difference between the overall energies is very small- makes prediction of protein folding very difficult

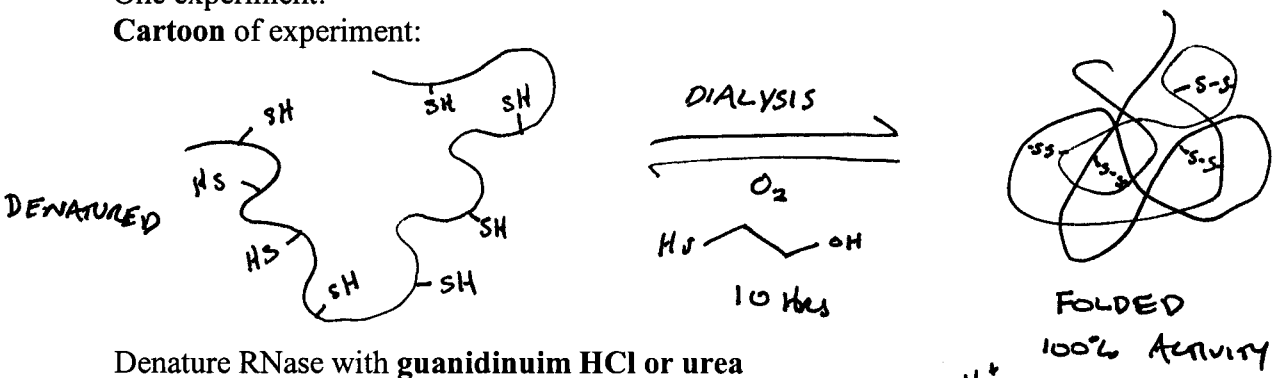
The Levinthol Paradox
Proteins cannot undergo unbiased conformational space searches to fold

If a protein were to sample all the different conformational space available it would take billions of years for proteins to fold!
Need a biased search.

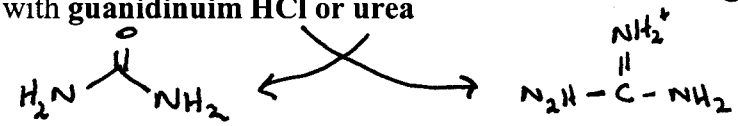
Anfinsen's Hypothesis: The primary sequence is sufficient for protein folding
Did experiments on RNase (ribonuclease) – cleaves RNA
124 amino acid protein
RNase was chosen because it was available in gram quantities, also the protein folding can be easily measured by catalytic activity (very sensitive)

RNase has 8 cysteines-and the active form of the protein contains four disulfides. How do you form the correct disulfide bonds?

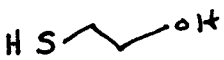
One experiment:
Cartoon of experiment:



Denature RNase with **guanidinuim HCl or urea**



Then dialyze away the denaturant in the presence of **beta-mercapethanol** (beta-Me)
After 10 h the unfolded protein is refolded as evidenced by the recovery of 100% of its catalytic activity



Dialysis without (beta-Me) and in the presence of oxygen gives only 1% activity

Why only 1% activity w/out beta-Me? The chance of randomly forming the correct disulfide bonds on the first go is very small
1 in 7 chance of forming first bond correctly, for the next bond you have a 1 in 5 chance, etc

1/7x1/5x1/3= 1/105 chance forming all four disulfide bonds with no corrections
Therefore, only 1% of the protein is folded correctly and is active

Beta-Me
Allows disulfide bonds to form, break and reform
Protein can sample different disulfide bond combinations, eventually get 100% folded and active protein

Cartoon of Beta-Me allowing disulfides to break and reform