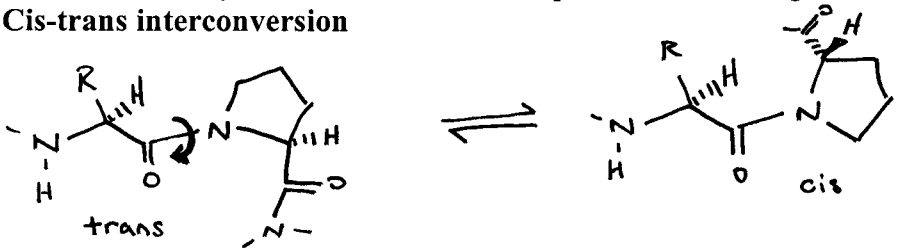
Lecture 28
4/26/04

Protein Folding *in vivo*

1. Peptidoproylisomerase- interconverts cis/trans proline configuration.
Proline is the only amino acid that is not capable of H-bonding, often found in turns
Cis-trans interconversion



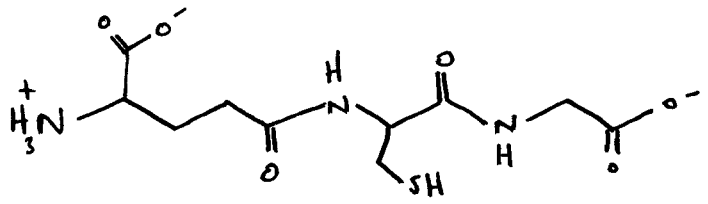
You must destroy the planarity of the carbonyl to allow rotation around the OC-N bond of the amide
Proline isomerization is often the rate-limiting step for folding in vitro (we don't know about *in vivo*)
It does not involve covalent catalysis in the enzymes that have been examined thus far

The immuno-suppresants cyclophilin and FK506BP have proylisomerase activity; TF, trigger factor, a chaperone protein also has this activity

2. Disulfide bond formation
Key step in maturation of extra-cellular membrane proteins and secreted proteins
The intracellular reducing environment (cytosol) prevents disulfide bond formation
Disulfide bond formation occurs in the lumen of the E.R. Requires SRP and SRP-R (eukaryotes) to take proteins there The counterpart to the lumen of the ER in gram negative bacteria, is the periplasm

Equipment: oxidases and isomerases
Very similar in pro and eukaryotes

Why the lumen vs. the cytosol in eukaryotes?
Redox buffer- glutathione (GSH – reduced; GSSG –oxidized) a tripeptide gamma-ECG
Glutathione



The ratio of reduced to oxidized glutathione has been determined experimentally

	GSH :GSSG
Cytosol	30-100:1
Lumen	1-3:1

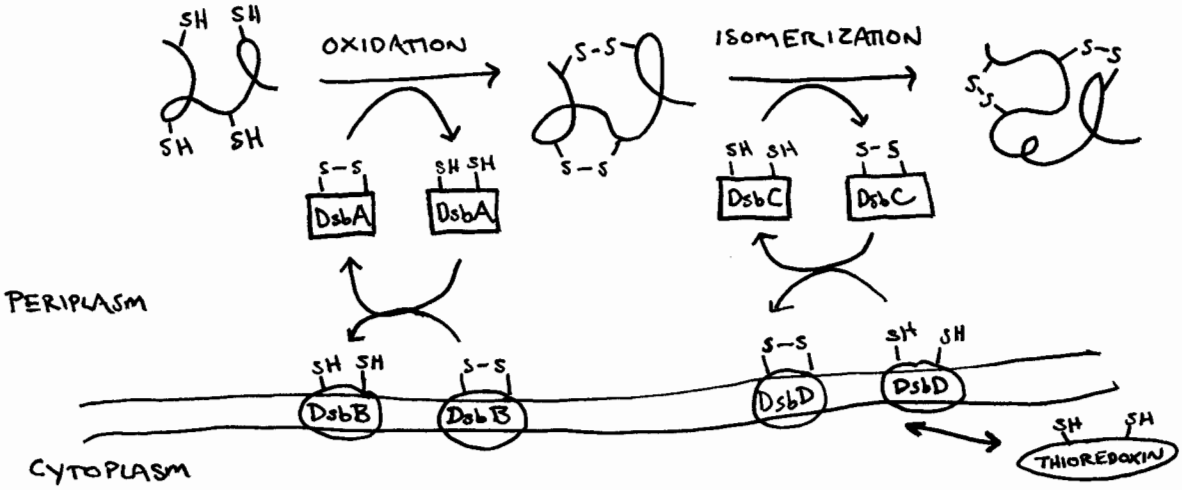
The lumen is much more oxidizing!

See page 7 of handout 4a for a scale of reduction potentials

Remember: more positive reduction potential, the easier the molecule is reduced

These potentials are measured in the test tube, may be significantly modulated inside the cell

DsbB, DsbA, DsbC, DsbD are all involved in disulfide bond formation in *E. coli* in the periplasm
See page 7 of handout 4a for a **cartoon model**



DsbA (soluble protein) involved in oxidation of disulfides in protein, DsbB is an intergral membrane protein that recyles DsbA back to oxidized state
Then a mechanism may be needed to reorganize and form correct disulfides
DsbC (reduced, soluble) catalyzes this, and DsbD (membrane associated) uses redox cysteines to take DsbC back to reduced state.
There is also communication between the periplasm and cytosol via thioredoxin
Note that these mechanisms involve soluble proteins interacting with membrane bound proteins

In eukaryotes PDI= protein disulfide isomerase

Chaperone proteins- facilitate correct folding by preventing side reactions (use ATP)

-still no paradigm for how these proteins use energy of ATP to accomplish their tasks

-Chaperones do not provide specific information about how protein folds- might call them "holdases"
this is controversial- might also be "unfoldases"

Two functions of chaperones:

1. protein folding from the ribosome
-is it co-translational? Or does the protein exit the ribosome completely unfolded and then fold?
2. what happens under stress? (UV, heat, cold, oxidative)
–can get aggregation
-do chaperone proteins have unfoldase activity?

See page 8 of handout 4a for a list of players
Players- two sets of chaperone proteins in bacteria and eukaryotes
1)Hsp70 system (uses ATP) with Hsp40 and NEF (nucleotide exchange factor) =GrpE
Called DnaK, DnaJ, and GrpE in bacteria
2) Chambers
GroEL/GroES system
Chamber (GroEL) with a lid-(GroES) proteins fold in the chamber (uses ATP)
Kinetics and timing are again important

Cartoon overview- see page 9 of handout 4a
The exit tunnel of the ribosome is 100 angstroms long and it can fit 30 amino acids in an extended conformaton and 65 amino acids in a helical conformation. The exit channel is too narrow to accommodate any other secondary structure. The protein can't fold inside the tunnel
The sizes of proteins in prokaryotes are much smaller than in eukaryotes-affects folding. In E. coli only 13% of the proteins are > 55 KDa, while in yeast 38% are > 55 KDa.

In prokaryotes (model A in handout)
Small proteins can fold spontaneously (65-80% of proteins)

Or DnaK and DnaJ interact- "holdase" model- may hold on to a hydrophobic patch, allow protein to fold in that reduced conformational space (10-20% of proteins)

Or GroEL/GroES may be needed (~10-15% of proteins)

In archea and eukaryotes (models B and C in the handout)
Very similar equipment
But more complex and less well studied

Next time: we will talk specifically about the 2 systems and their players