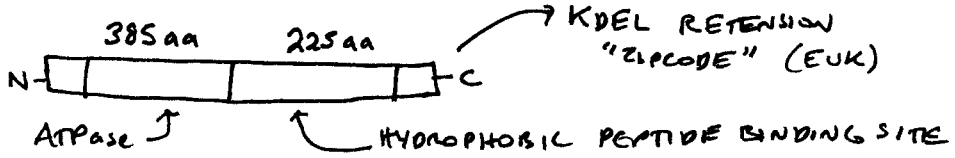
Lecture 29
4/28/04

Crypts: Proteosome- degrades proteins
Chambers: GroEl/GroES- protein folding

Chaperone proteins
Use ATP- what is the function of ATP?- still unresolved

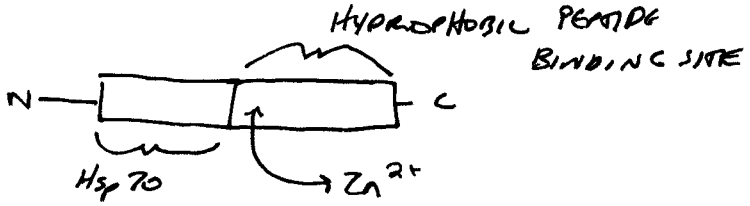
Hsp 70 (DNAK in *E.coli*) – ATPase domain (N-terminus) and peptide binding domain (C-terminus)
Hsp 40 (DNAJ)
GrpE – a nucleotide exchange factor(NEF)

PLAYERS
Hsp 70 (DNAK) –70kDa
These proteins are found in all kingdoms; Hsps are found in numerous organelles within eucaryotic cells. The N-terminus has mitochondria or ER targeting "zipcode" sequence (only in eukaryotes, in bacteria, no zipcodes, no organelle structure); in ER at the C-terminus there is also a zipcode for ER retention.
Then a 385 amino acid ATPase domain (binds NEF- GrpE)
This is linked to a 225 amino acid hydrophobic peptide binding site
The C-terminus has a "KDEL" retension "zipcode" sequence for the ER (eukaryotes)
Cartoon of Hsp 70



See page 9 of handout 4a (top left) for structures of the 2 domains of DNAK
Structural information- we have structures of the separate domains, but no intact structure.

Hsp 40 (DNAJ) –80 kDa
Cartoon of Hsp 40



Contains Hsp 70 binding site, Zn^{2+} binding site, and hydrophobic peptide binding site

Substrate is a hydrophobic patch of amino acids in a protein coming out of the ribosome exit tunnel, ~8 amino acids long

Amino acids 2,4, 6, 8 are hydrophobic amino acids- I, L, aromatics
See page 9 handout 4a, for a cartoon of interactions between hydrophobic amino acids and binding site on DNAK

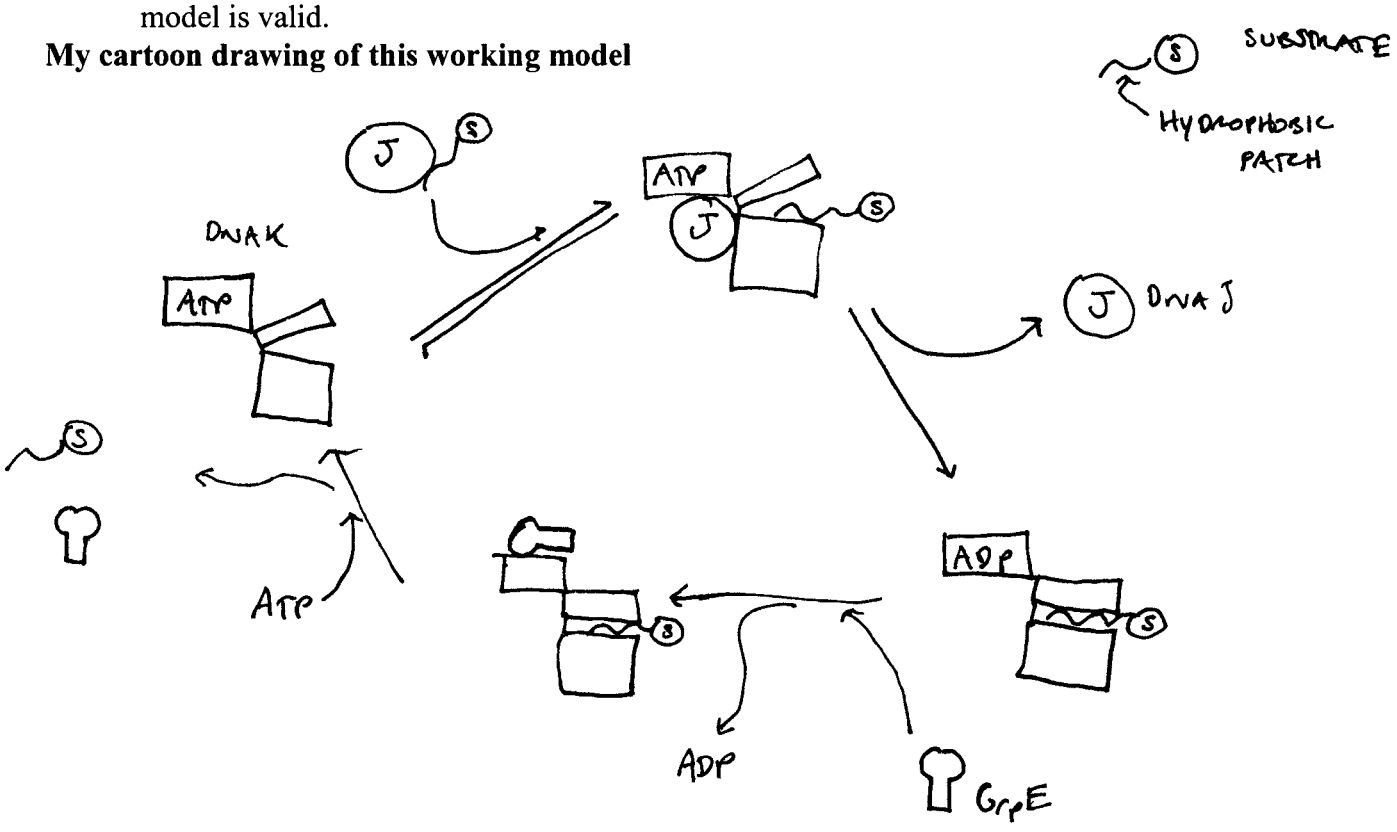
GrpE- nucleotide exchange factor, this protein catalyzes
ADP dissociation, which is followed by ATP binding. The concentration of GrpE is 1/3 that of DNAk.

Working Model
See page 9 handout 4a for the working model- cycle of DnaK, DnaJ, GrpE

Think about concentrations inside the cell
30-35 micro M peptide exit tunnel
50 microM DnaK
5 micro M DnaJ
15 microM GrpE

1) DnaK with "lid" in open conformation, ATP bound low ATPase activity ($3x10^{-4}$ s^{-1})
2) Substrate and DnaJ bind OR DnaJ interacts with substrate and delivers it to DnaK,
Now substrate is bound to DnaK (loosely),
Binding of DnaJ accelerates the ATPase activity by 10^4
3) ATP is hydrolyzed to ADP and DnaJ dissociates, the substrate is now tightly bound (very stable) in absence of NEF (GrpE) this complex can be stable for 20 s to minutes, sufficient to translate a protein of 300 amino acid – This is an example of a "holdase" model. The DNAk holds onto the folding protein for sufficiently long time to allow to fold.
4) GrpE interacts with DnaK substrate complex and causes release of ADP, then ATP can rebind and substrate is released. If there is a lot of GrpE, then the time that the peptide is bound to DNAk would be very short and this would negate the holdase model. The question would then be raised is whether a holdase model or potentially an unfoldase model is valid.

My cartoon drawing of this working model



Controversy: The model does not really take into account what we know about concentrations inside the cell
Unresolved issue: how does GrpE affect the lifetime of the DnaK/substrate/ADP complex? Think about concentrations, kinetics, and Kd's (affected by ionic strength) inside the cell vs. *in vitro*

CHAMBERS GroEl/GroES

Machine- 800kDa with two-7 membered rings (stacked) (GroEL with 57 KDa subunits) and a lid (GroES). GroES is also a 7 membered ring, but the subunits are only 10 KDa.

To get into the chamber, protein must be unfolded

Model: there exists a chamber, the machine coordinates unfolded peptide binding and ATP hydrolysis
GroEL/GroES is an allosterically regulated machine
Both positive and negative cooperativity
Governed by the ATP state of binding

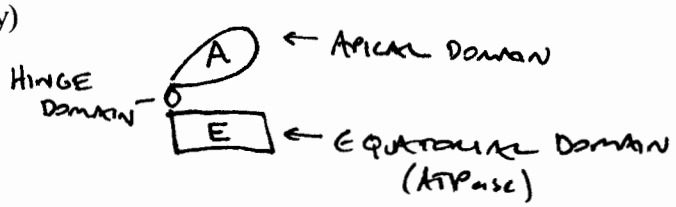
See page 10 handout 4a for a picture of GroEL structure

PLAYERS

In bacteria there is GroEL and Gro ES, in some eukaryotes these is a single protein where the GroES lid is attached to the GroEL chamber

GroEL- (57.3 kDa – 1 subunit of 7 that form the ring)
x-ray structures, cryo EM

Cartoon of one subunit
Contains apical domain, hinge domain, equatorial domain (ATP binding region, controls +/- cooperativity)



The A and E domains can move relative to each other using the hinge
The two 7 membered rings are stacked
Cross-section showing just 2 of the 7 subunits in each ring and GroES
Conformational change with ATP binding
See page 10 handout4a for more pictures of the GroEl/GroES system