Central Dogma

DNA → RNA → Protein sequence → Protein fold

Proline cis-trans isomerisation
Disulfide bond formation
Glycosylation
Proteolysis
FOLDING
MOLECULAR CHAPERONES: Definition
A large group of unrelated protein families whose role is to stabilize unfolded proteins, unfold them for translocation across membranes or for degradation, and/or to assist in their correct folding and assembly.

Properties
• Molecular chaperones interact with unfolded or partially folded protein subunits, e.g. nascent chains emerging from the ribosome, or extended chains being translocated across subcellular membranes.
• They stabilize non-native conformation and facilitate correct folding of protein subunits.
• They do not interact with native proteins, nor do they form part of the final folded structures.
• Some chaperones are non-specific, and interact with a wide variety of polypeptide chains, but others are restricted to specific targets.
• They often couple ATP binding/hydrolysis to the folding process.
• Essential for viability, their expression is often increased by cellular stress.

Main role: They prevent inappropriate association or aggregation of exposed hydrophobic surfaces and direct their substrates into productive folding, transport or degradation pathways.

Examples of molecular chaperones

Heat shock proteins: hsp104, 90, 70, 60 and small hsps, including homologues of lens α-crystallin.

Catalysts of folding: Protein disulfide isomerase, Peptidyl prolyl cis-trans isomerase

Nucleoplasmin: nucleosome assembly

Prosequences: subtilisin, α-lytic protease (intramolecular chaperones)
Unfolded or misfolded proteins
- Aggregation-prone
- Protease-sensitive
- Bind chaperones
- Non functional

Globular soluble protein
- Stable and soluble
- Protease resistant
- No chaperone binding
- Functional

Unfolded protein chains are prone to proteolysis
**FAMILIES OF MOLECULAR CHAPERONES**

Small heat shock proteins (hsp25) [holders]
- protect against cellular stress
- prevent aggregation in the lens (cataract)

Hsp60 system (cpn60, GroEL) *ATPase* [(un)folders]
- protein folding

Hsp70 system (DnaK, BiP) *ATPase* [(un)folders]
- stabilization of extended chains
- membrane translocation
- regulation of the heat shock response

Hsp90 [holder]
- binding and stabilization/regulation of steroid receptors, protein kinases
- Buffer for genetic variation?

Hsp100 (Clp) *ATPase* [unfolder]
- thermotolerance, proteolysis, resolubilization of aggregates

Calnexin, calreticulin
- glycoprotein maturation in the ER
- quality control

**Folding catalysts**: PDI, PPI [folders]

**Prosequences**: alpha-lytic protease, subtilisin (intramolecular chaperones) [folders]
Molecular chaperones

Small heat shock proteins
- Sponges for denatured protein
- Chaperonins
  - Hsp60/Hsp10
  - GroEL/GroES
  - Nascent chain folding/refolding
- ER chaperones
  - Disulfide, prolyl isomerases; oligosaccharide processing

Hsp70
- Folding/unfolding
- Translocation/disaggregation

Hsp90
- Stabilize steroid receptors/kinases;
  - Buffer genetic variation?

Hsp100
- Clp ATPases
- AAA+
- Unfolding/disaggregation

Small heat shock proteins
- A dimer of β-sandwich folds forms the conserved building block of small hsp5

Crystal structure of sHsp from *Methanococcus jannaschi* (Kim et al, 1998)
Small Hsp function

- Superfamily includes the eye lens protein $\alpha$-crystallin
- Protective role in suppressing protein aggregation *in vivo* and *in vitro*
- Bind and stabilise denatured protein under conditions of cellular stress, ageing and degenerative disease
- Do not appear to have unfolding and refolding activity
- High capacity for protein binding: up to 2 denatured proteins per subunit

Formation of substrate complexes

Heat to 44°C with substrate protein
**Immunoglobulin fold chaperones**

**HSP70 FAMILY**

<table>
<thead>
<tr>
<th>Location</th>
<th>Chaperone</th>
<th>Roles</th>
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<tbody>
<tr>
<td>Prokaryotic cytosol</td>
<td>DnaK</td>
<td>Stabilizes newly synthesised polypeptides and preserves folding competence; reactivates heat-denatured proteins; controls heat-shock response</td>
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<tr>
<td></td>
<td>cofactors DnaJ, GrpE</td>
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<tr>
<td>Eukaryotic cytosol</td>
<td>SSA1, SSB1(yeast)</td>
<td>Protein transport across organelle membranes; binds nascent polypeptides; dissociates clathrin from coated vesicles; promotes lysosomal degradation of cytosolic proteins</td>
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<td></td>
<td>Hsc/hsp70, hsp40 (mammalian)</td>
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<tr>
<td>ER</td>
<td>KAR2, BiP/Grp78</td>
<td>Protein translocation into ER</td>
</tr>
<tr>
<td>Mitochondria/ Chloroplasts</td>
<td>SSC1 ctHsp70</td>
<td>Protein translocation into mitochondria; Insertion of light-harvesting complex into thylakoid membrane</td>
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</tbody>
</table>
Substrate-binding domain ATPase domain

Asymmetric complex of Hsp90 dimer, cofactor and substrate protein

Hsp90-cdc37-cdk4 (kinase) Vaughan et al, 2006
AAA Proteins

• A large and diverse family of ATPases associated with unfolding, unwinding, assembly and disassembly of protein and nucleic acid complexes (ATPases Associated with various cellular Activities)

• Includes the Hsp100/Clp family of chaperones/proteases, components in DNA replication, recombination and restriction, the NSF protein in vesicle fusion, dynein motor proteins and many others

• Hsp100 proteins can totally unfold their substrate proteins, in order to deliver them to associated proteases, or they can dissolve large aggregates, in cooperation with the Hsp70 system
Hsp100 chaperone-protease complexes

Architecture of the Proteasome

Sousa et al, Cell (2000)
CHAPERONINS

Group I
- GroEL/Hsp60 - 14 x 60 kD subunits
- GroES/Hsp10 - 7 x 10 kD subunits
- found in eubacteria, mitochondria, chloroplasts
- very abundant and non-specific, will interact with most non-native proteins

Group II
- 16 or 18 x 55 kD subunits
- TF55 & thermosome in archaea
- TCP-1 in eukaryotic cytosol, >8 related gene products
- TCP-1 not very abundant, folds actin, tubulin, transducin, WD-40 domain proteins

Chaperonins assist folding without imparting steric information

Subunit structures:

Oligomeric structures:
**E. coli GroEL**

**14-mer**

- **apical domain**
- **equatorial domain**
- **intermediate domain**
- **hinge 1**
- **hinge 2**
- **ATP contact 1**
- **ATP contact 2**
- **14-mer**
- **hydrophobic binding sites**

**Allosteric states in GroEL**

1. **ATP binding**
   - 7 ATP
   - 7 ATP

2. **Substrate protein binding**
   - T
   - T
The chaperonin functional cycle

GroEL ATPase cycle
Conformational changes induced by ATP binding to GroEL

GroEL-GroES-ADP
Crystal structure

GroES

ADP
Rotation and separation of hydrophobic binding sites on GroEL could unfold a substrate protein bound to several sites (Shtilerman et al, 1999).

Group I
GroEL subunit in GroES-bound conformation

Group II
Thermosome subunit in closed conformation
Molecular Chaperones: References

Reviews

Research papers & additional references on lecture notes web site: people.cryst.bbk.ac.uk/~ubcg16z/hsplec.html