Molecular chaperones and proteases - their role under stress conditions and in autoimmune diseases
Current projects

- Structure and function of the heat shock – induced HtrA protease.
- The role of Hsp40 proteins in etiology of rheumatoid arthritis (RA).
- Mechanism of dissociation and degradation of protein aggregates formed in *Escherichia coli* cells during heat shock.
- Studies on heat shock response in marine bacterium *Vibrio harveyi*.
- Investigation of species-specificity of chaperone proteins *in vivo and in vitro*. 
Structure and function of the heat shock - induced HtrA protease.

HtrA protease from Escherichia coli:

A member of a novel class of proteases, that combine proteolitical and chaperone activities
**HtrA features**

- **Localization:** attached to the periplasmic side of the inner membrane.

- **Structure:**
  - serine protease: His105, Asp135, Ser210
  - oligomeric protein; monomer is composed of proteolitical domain and 2 C-terminal PDZ domains

- **N-terminal peptide** plays a role in stabilization of HtrA molecule. Reduction of the S-S bridge localized in that region leads to autocatalytic cleavage of HtrA and to disruption of the HtrA hexamer.
Functions:

- indispensable for bacterial survival at the elevated temperatures (above 42°C) and in the presence of certain oxidizing agents (Fe^{2+}).

- induced under stress conditions affecting cellular envelope (e.g., protein oxidation, changes in phospholipid composition).

- involved in degradation of misfolded periplasmic proteins, ex.: PhoA, MalS, certain hybrid proteins, recombinant proteins, mislocalized proteins – OmpF.
Latest results

• *E. coli* *htrA* mutant is more sensitive to the reducing agents than the wild type bacteria.
• HtrA prevents aggregation of the reduced insulin and lysozyme.
• However, in our hands HtrA was not able to promote reactivation of chemically denatured citrate synthase, alkaline phosphatase or lysozyme.
• CONCLUSIONS:
• HtrA acts rather as a charonin:
  1. prevents unfolded polypeptides from aggregation and
  2. presents them to proteolysis
E. coli htrA\textsuperscript{-} is more sensitive to the reducing agents than the wild type bacteria.
HtrA prevents the aggregation of reduced lysozyme

- Other nonchaperone proteins (ovalbumin, BSA) – no prevention
Homologs of HtrA

• **BACTERIAL**
  Implicated in virulence of pathogenic species

• **EUKARYOTIC**
  In humans there are at least four homologs:
  humHtrA1 – involved in oncogenesis and in general stress response
  humHtrA2 – involved in apoptosis; upregulated under heat shock conditions
  humHtrA3 and 4 – no biochemical characterization yet.
Future studies

• The influence of the reducing agents on the proteolytical activity of HtrA from *E. coli*

• The role of eukaryotic homologs in the cellular response to various stress conditions
Role of the Hsp40 proteins in etiology of rheumatoid arthritis

The multistep molecular mimicry hypothesis:

Bacterial infection leading to anti-DnaJ immune response may be one of the factors causing rheumatoid arthritis (RA), by activation of autoreactive T cells.

Could human Hsp40 protein(s) be a target for this response?
Comparison of *Escherichia coli* DnaJ and human HDJ1 (Hsp40) proteins:

- **DnaJ**
  - Molecular weight: 40.97 kDa
  - pI: 8.5

- **HDJ-1**
  - Molecular weight: 37.91 kDa
  - pI: 8.9

**Diagram:**
- **HDJ-1** contains a J domain, G/F domain, Zn-binding domain, and a low homology region.
- **DnaJ** also contains a J domain, G/F domain, and Zn-binding domain, with a low homology region.

**Homology:**
- **DnaJ** and **HDJ-1** have 45% protein sequence homology.
- The low homology region shows 12% homology.
- Overall, there is 24% homology between DnaJ and HDJ-1.
Questions:

- Is DnaJ (Hsp40) of *Escherichia coli* immunologically similar to its human homolog – HDJ-1?

- Is human HDJ1 protein involved in an autoimmune response in RA?
Anti-DnaJ monoclonal antibodies react with human HDJ-1 protein:

E. coli DnaJ and human HDJ 1 proteins are immunologically similar. The similarity is not restricted to the N terminal region conserved in evolution.
Anti-DnaJ and anti-HDJ-1 immunological response in rheumatoid arthritis patients and healthy individuals:

Patients with RA showed 3 fold increase in anti-DnaJ response and at least 2 fold increase in anti-HDJ1 response when compared to control group.
Reaction of the RA patients’ sera with HDJ-2 is 5-fold stronger than the reaction of the control group.
CONCLUSIONS:

• There is a significant immunological similarity between *E. coli* DnaJ and human HDJ-1 proteins, as judged by reactions with mono- and polyclonal anti-DnaJ antibodies. The immunological similarity is not restricted to the well conserved in evolution J-domain (45% homology), but includes also the low homology C-terminal region (12% homology).

• There is a significant increase of the anti-HDJ1 and anti-HDJ2 response in the sera of RA patients.

• HDJ-1 and HDJ2 proteins could become targets for the immune response triggered by the *E. coli* DnaJ, and could be involved in the pathogenesis of rheumatoid arthritis.
Mechanism of dissociation and degradation of protein aggregates formed in Escherichia coli cells during heat shock.
IbpA, IbpB – *inclusion bodies-associated proteins*

**Small Hsps (α - Hsp):**

- C-terminal *α-crystallin* domain

- Oligomeric structure (125 kDa- 2 MDa)

- Hold non-native proteins in a folding competent state preventing their irreversible aggregation

- Deliver denatured substrates to the Hsp70 machinery for subsequent refolding
**IbpA and IbpB** bind to endogenous *E.coli* proteins aggregated by heat shock *in vivo*
Overproduction of IbpA and IbpB stabilizes aggregates of denatured proteins \textit{in vivo}
IbpA/B inhibit protein aggregation upon extreme heat shock (50°C, 4h)
IbpA/B are required for the removal of proteins aggregated during extreme heat shock.

- **50°C (15’)**
  - 37°C (20’)

- **50°C (4 h)**
  - 37°C (1 h)

**% of removed aggregates**

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<thead>
<tr>
<th></th>
<th>wt</th>
<th>ΔibpA/B</th>
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<tr>
<td><strong>50°C (15’)</strong></td>
<td>60%</td>
<td>80%</td>
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<tr>
<td><strong>37°C (20’)</strong></td>
<td>10%</td>
<td>30%</td>
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**wt** and **ΔibpA/B**
IbpA and IbpB may exhibit different affinity for unfolded proteins - most of the IbpA coaggregates with denatured proteins, whereas majority of the IbpB remains in a soluble fraction.

<table>
<thead>
<tr>
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<th>30°C</th>
<th>45°C</th>
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<tbody>
<tr>
<td>IbpB</td>
<td>![Image of IbpB at 30°C]</td>
<td>![Image of IbpB at 45°C]</td>
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Studies on heat shock response in marine bacterium *V. harveyi*

Pollution

Heat shock promoter activation

Detection

Reporter gene

*lacZ, gfp or cat*
Cloning and sequencing of the major heat shock genes
groES, groEL (2001)
Promoter mapping of the dnaK and groE operons
Species–specificity of chaperone proteins

V. harveyi groES but not groEL can complement groE mutation of E. coli.
*dnaJ* and *grpE* genes of bacterium *V. harveyi* and archaeon *M. mazei*, but not *dnaK* genes, can complement *E. coli* mutants.

<table>
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<tr>
<th>E. coli strains</th>
<th><em>V. harveyi</em> genes</th>
<th><em>M. mazei</em> genes</th>
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<tr>
<td></td>
<td>λ</td>
<td>Tr</td>
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<tr>
<td>ΔdnaK</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>ΔdnaJ</td>
<td>++</td>
<td>++</td>
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<tr>
<td>ΔgrpE</td>
<td>+++</td>
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λ - bacteriophage λ growth  
Tr- growth at elevated temperature (42°C).
DnaK, DnaJ and GrpE proteins from bacteria and archaea cooperate efficiently in protein refolding *in vitro*.

*Ec* – *Escherichia coli*

*Vh* – *Vibrio harveyi*

*Ec* – *Escherichia coli*

*Mm* – *Methanosarcina mazei*

DnaK–DnaJ– GrpE assisted luciferase refolding *In vitro*.
The *in vivo* results indicate that, in spite of high conservation in evolution, the **DnaK and GroEL chaperones are more species-specific** than the less conserved DnaJ, GrpE and GroES.
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Stable interaction of IbpA with unfolded precursor inhibits its translocation to the periplasm.

The growth of *E. coli* cells overproducing IbpA, in the medium supplemented with ampicillin, required an increased level of β-lactamase precursor.

Identification of *Vibrio harveyi* major heat shock proteins
IbpA and IbpB may exhibit different affinity for unfolded proteins – IbpA but not IbpB bound stably β-lactamase precursor \textit{in vivo}.

Protein aggregates isolated from \( \Delta ibpAB \) cells overproducing IbpA or IbpB
Further results:

1. In the presence of reducing agents not only the substrate proteins are influenced, but the enzyme, HtrA, undergoes structural changes as well (as indicated by changes in fluorescence emission spectra of the reduced HtrA and by comparison of infrared spectra of the reduced and oxidized HtrA).

2. The changes enable HtrA to bind the substrates more efficiently, possibly due to a better exposition of some hydrophobic surfaces.