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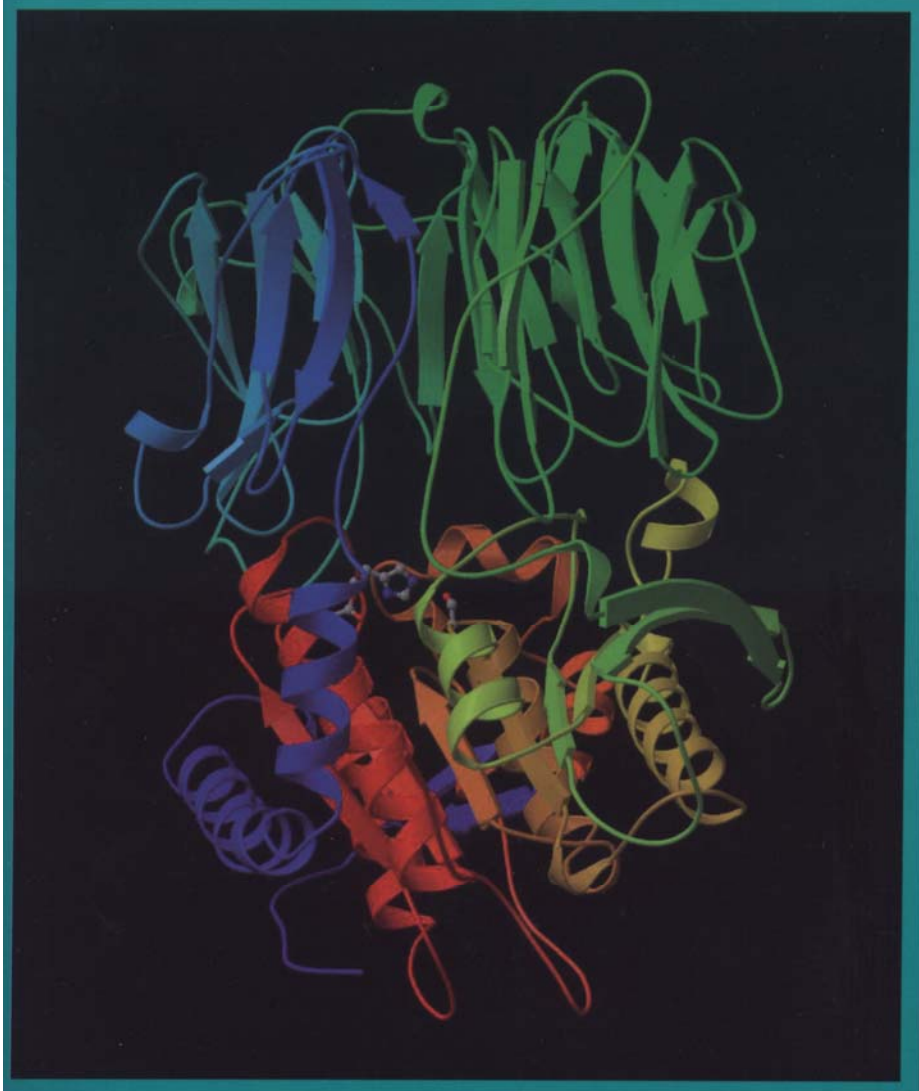

Abstracts

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F3-010P
Mutations in yeast 18S rRNA affect translational fidelity and oxidative stress

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The function of mutations *rdn1A, rdn1T* and *rdn2* in 18S rRNA of *Saccharomyces cerevisiae* has been examined. They correspond to C1054A, C1054U in helix 34 and G517A in loop 530 of helix 18 of 16S rRNA in *Escherichia coli,* respectively, in which they behave as nonsense suppressors and reduce translational fidelity. In yeast, mutation *rdn1A* caused severe loss of translational fidelity accompanied by an increase in ribosomal A-site binding, extreme sensitivity to paromomycin, and higher initial rates of protein synthesis activity, all compatible with its error-prone character. Unlike in *E. coli*, however, mutations *rdn1T* and *rdn2* caused hyperaccuracy followed by resistance to paromomycin. It is concluded that loop 530 and helix 34 of 18S rRNA participate in the accurate decoding of the genetic information. Moreover, mutational changes in conserved rRNA regions may affect the same functions in the various species but in opposite directions. Translational fidelity and oxidative stress are two of several parameters affected during a cell's life. Mutants carrying error-prone ribosomes exhibited decreased oxidative stress compared to wild type. In contrast, hyperaccurate ribosomes, such as those from *rdn2* or *rdn1T* strains seem more susceptible to oxidative modifications, i.e. lipid peroxidation and non-protein thiol oxidation. The notion is investigated that the cells consume more energy in order to achieve hyperaccuracy leading to increased production of free radicals.

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F3-011P
Polyamines stimulate the function of the rotary motor complex implicated in the A- to P-site translocation of tRNA substrates during protein synthesis

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Spermine, one of the naturally occurring polyamines, stimulates at 6 mM Mg2+ both the spontaneous and enzymatic translocation of AcPhe-tRNA from the A- to the P-site of poly(U)-programmed *Escherichia coli* ribosomes. To unveil the molecular basis of the spermine effect, the locations of spermine bound to rRNA were characterized by covalent binding of a photoreactive analogue of spermine, N1-azidobenzamidino (ABA)-spermine, to *E. coli* ribosomes under mild irradiation conditions. Cross-linking sites were identified by RNase H digestion and primer extension analysis at C1400 and C1411 of the 3-minor domain of 16S rRNA, at positions located in the central loop of domain V, and in helices H42-H44 and H95 of 23S rRNA. Specifically, in the central loop of domain V of 23S rRNA, ABA-spermine labels nucleosides U2584 and A2602. Nucleoside A2602 has been sug gested to propel the spiral rotation of the tRNA-3 end from the A- to P-site in concert with the tip of helix H69, while U2585, lying next to U2584, has been proposed to anchor the rotary motion by direct interaction with the aminoacyl end of the tRNA and assure the proper positioning of the P-site substrate. Interestingly, pre-translocation ribosomal complex totally labelled or partially modified in its 50S subunit by ABA-spermine exhibited higher efficiency in translocation, compared with untreated complex or complex labelled only in its 30S subunits. Our results suggest that polyamines activate the rotary machine that governs the translocation function in ribosomes, by linking in close proximity to certain nucleosides of rRNA.

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F3-012P
The FMH component of the flagellar export apparatus is a multi-zinc enzyme with phospholipase activity

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FliH and FliI are cytoplasmic components of the type III flagellar protein export apparatus. FliI is an ATPase whose enzymatic activity is necessary to drive the export process, while FliH is believed to function as a negative regulator of FliI. Our isothermal titration calorimetric experiments, however, question this regulatory role because the FliH-FliI interaction is too weak to allow effective binding in the physiological concentration range. Characterization of the metal binding ability of FliH by surface plasmon resonance spectroscopy revealed that FliH can specifically bind three zinc atoms. The binding appears to be quite strong with an overall dissociation constant of about 2×10^{-6} M, as obtained by titration calorimetric studies. Temperature induced unfolding experiments demonstrated that FliH is well folded even under zinc-free conditions suggesting that zinc is required for functional rather than structural reasons. FliH was found to exhibit structural characteristics similar to those of bacterial phospholipase C, a multi-zinc enzyme with phosphodiesterase activity. Indeed, our experiments demonstrated that FliH has a phosphatidylcholine-hydrolyzing capability. Further studies are needed to clarify how the phospholipase C activity of FliH is related to the functioning of the flagellar-specific export machinery.

F3-013P
Catenanes- simpler analogues of biomolecular rotors

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Molecular machines described in this paper are meant to be such molecular systems that make use of conformational mobility (i.e. hindered rotation around chemical bonds as well as molecular construction deformations with formation and breakage of non-valent bonds). Components of molecular machines move mainly by means of restricted diffusion. Whereas it is true to consider biological molecules-motors (various ATPases, bacterial flagellar complexes, membrane transporters) as machines, one may facilitate their studying by applying simpler models from macromolecular chemistry. As an example of molecular machines of a non-biological nature catenanes (compounds with two interlocked molecular rings) can be proposed. Thus, for example, model catenane [(2)-(cyclo-bis(paraquat-p-phenylene))-(l(2,6)-tetrathiafulvalena-16(l,5) naphtalena-3, 6, 9, 12, 15, 17, 20, 23, 26, 29-decaoxatnaconta-

phane)-catenane] changes its redox status when an electric field is applied, and rotation of the rings takes place. It occurs with fixation at certain moments of the influence. To find out characteristic properties of rings movements under various external conditions molecular dynamics (MD) simulations were carried out. Three cationic forms of the catenane were first subjected to geometrical optimization and quantum chemical calculation. Then at different temperature conditions and under varying magnitudes of external electrical field these catenane states were run in MD calculations. Summarizing of the results allowed to develop an idea about possible mechanism of the catenane's functioning.

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F3-014P

The flagellum-specific type III secretion signal is confined to a 22-amino-acid sequence in the disordered N-terminal region of flagellin

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Bacteria swim by rotating their flagellar filaments, each of which has a helical shape and works as a propeller. External flagellar proteins, lying beyond the cytoplasmic membrane, are synthesized in the cell and exported by the flagellum-specific export apparatus to the site of assembly through the central channel of the filament. The flagellum-specific export system is a specialized, signal-peptide independent type III export machinery. Earlier studies suggested that the N-terminal region of flagellar proteins is essential for recognition for export, however, comparison of their amino acid sequences failed to identify a common recognition signal at the primary structure level. In this work we aimed at identifying the secretion signal in *Salmonella* flagellin. Two approaches were followed: fragments of flagellin from the N-terminal part of the molecule were expressed and their secretion efficiency was characterized. Also, various segments of the N-terminal region were fused to the 74-amino-acid long CCP2 module of human complement Clr protein and secretion of these fusion constructs was investigated by Western blot assays of the culture media. We found that residues 26-47 of *Salmonella* flagellin are sufficient and essential for secretion. This region lies within the disordered N-terminal part of flagellin. Amino acid sequence alignment of flagellins from various sources revealed that the region containing the export signal is highly conserved. Our experiments demonstrate that the flagellum-specific export system can be utilized to secret over-expressed recombinant proteins into the culture media by generating fusions to the secretion signal.

G1-Molecular Chaperones

G1-001

Stress and misfolded proteins: modulators of neurodegenerative diseases and longevity

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Misfolded proteins, aggregates, and inclusion bodies are hallmarks of the cytopathology of neurodegenerative disorders including Huntington's disease, Amyotropic lateral sclerosis, Parkinson's disease, Prion diseases, and Alzheimer's disease. The appearance of proteins with altered folded states is regulated by the protein folding quality control machinery and age-dependent. We have identified an unexpected molecular link between metabolic state, accumulation of damaged proteins, the heat-shock response and chaperones, and longevity. Mutations (age-1, daf-2) in the insulin-like signaling (ILS) pathway in *C. elegans* leading to longevity results in the suppression of polyglutamine toxicity and aggregate formation. Because overexpression of HSF-1, a known regulator of chaperone networks and quality control, was also shown to suppress polyglutamine aggregation, we examined whether HSF-1-regulated lifespan. Downregulation of hsf-1 by RNAi in neurons and muscle cells suppressed longevity, which reveals a new molecular link between longevity and stress resistance. To identify other modifiers of protein quality control, we screened transgenic polyglutamine-expressing strains using genome-wide RNAi to identify genes that regulate polyglutamine aggregation. Nearly 200 genes were identified defining a "protein quality control proteome" corresponding to five principal classes of polyglutamine regulators: genes involved in RNA metabolism, protein synthesis, protein folding, protein degradation, and those involved in protein trafficking. We propose that each of these classes represents a molecular machine that collectively comprises the protein homeostatic buffer that responds to the expression of damaged proteins to prevent their misfolding and aggregation.

G1-002

Chaperone machines of the cytosol

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The ensemble of molecular chaperones constitutes the cellular system that assists folding and assembly of newly synthesized proteins, translocation of unfolded proteins across membranes, as well as refolding and degradation of misfolded and aggregated proteins. In the *Escherichia coli* cytosol, the ribosome-associated trigger factor assists the first steps in the co-translational folding of nascent polypeptide chains. The major Hsp70 chaperone, DnaK, uses the energy of ATP and the assistance by the DnaJ and GrpE co-chaperones, to prevent aggregation and support refolding of damaged proteins. DnaK furthermore cooperates with the AAA + ATPase, ClpB, to solubilize and refold aggregated proteins. The remarkable remodeling activity of ClpB is essential for cell survival under severe heat stress. This seminar will describe our current knowledge of the working mechanism of these chaperone machines.