Introduction

Hsp70 chaperones recognize and refold misfolded proteins as part of their quality control function. They also interact with native proteins and regulate their activity and stability. In this second function Hsp70 proteins are involved in many regulatory processes like the regulation of the heat shock response in bacteria and eukaryotic cells or the initiation of DNA replication of plasmids, bacteriophages and viruses. In eukaryotic cells Hsp70s cooperate with Hsp90 chaperones in the regulation of more than 120 proteins many of which act as molecular switches. Our major research goal is to understand the role of molecular chaperones in the control of conformational changes in these switch proteins in prokaryotic and eukaryotic systems. We therefore study how Hsp70s bind native proteins, how these proteins are transferred with the help of cochaperones onto Hsp90 and how these interactions influence the conformation and activity of these so-called client proteins. In collaboration with the Bernd Bukau group, we also study the activity cycle of Hsp70 chaperones themselves and how they are regulated by cochaperones.

Conformational changes in proteins

W. Rist, F. Rodriguez, C. Graf

To analyze conformational changes in proteins we established the amide hydrogen exchange technology in combination with high-resolution mass spectrometry in the lab. Our quenched-flow setup now enables us to monitor solvent accessibility within protein structures with a time resolution of down to 100 ms. Using this technology we analyzed so far three model proteins: the *E. coli* Hsp70 chaperone DnaK and two of its native protein substrates, the *E. coli* heat shock transcription factor σ32 and the F-plasmid replicator protein RepE. For DnaK we mapped nucleotide dependent conformational changes. In σ32 we found temperature dependent conformational changes and local unfolding upon binding to DnaK (Fig. 1). In RepE we detected conformational alterations that are dependent on the dimer monomer equilibrium and on the interaction with DnaK.

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Dynamics and intramolecular control of the Hsp70 conformation
M. Vogel

Hsp70 proteins assist protein folding processes by transient association of their substrate binding domain with short hydrophobic peptide segments within their substrates. The substrate binding and release cycle is driven by the switching of Hsp70 between the low affinity, ATP bound state and the high affinity, ADP bound state. Thus, ATP binding and hydrolysis drives conformational alterations not only in the ATPase domain but also in the substrate binding domain. The molecular mechanics of this intramolecular control process was found to reside in a universally conserved proline. A cis-trans isomerization of the proline preceding peptide bond most likely constitutes the molecular switch, which controls conformational alterations in Hsp70 proteins. Currently we investigate how the switch position is transduced to the substrate binding domain.

Regulation of protein activity by the Hsp70-Hsp90 machinery
R. Nikolay, C. Graf

We started now to study the interaction of the eukaryotic Hsp70-Hsp90 machinery with their clients (e.g. p53, Hsf, steroid hormone receptors, protein kinases) with the aim to elucidate the conformational properties of the clients, the influence of Hsp70 and Hsp90 on the client conformation and the kinetics of these interactions. First, we want to elucidate the chaperone binding site using cross-linking, protease-footprinting and mass spectrometry. Second we analyze the conformational properties of the client and chaperone proteins individually and subsequently in complex with each other. Third we plan to study the kinetics of the client-chaperone complex assembly and disassembly reactions and the influence of different cochaperones on these processes.
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