Phylogenetic approach to study the origin, function and interactions between proteins of JDP/Hsp70 systems

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JDP/Hsp70 systems consisting of Hsp70 chaperone and J-domain protein (JDP) are key elements of cellular proteostasis playing critical roles both under physiological conditions and under stress or pathology. JDP/Hsp70 systems function in folding of newly synthesized proteins, polypeptide trafficking across membranes, and in preventing aggregation or promoting disaggregation of protein aggregates- thus protecting the cell against detrimental effects of stress and ageing. JDP/Hsp70 functions depend on their ability to cyclically interact with substrate protein. This substrate binding cycle is controlled by binding and hydrolysis of ATP by Hsp70. JDPs are responsible for delivering substrate proteins to Hsp70 partner or for recruiting Hsp70 into specific cellular location, thus determining the system specificity. In this thesis I used phylogenetic analyses such as reconstruction of phylogenetic trees, coevolution analyses and reconstruction of ancestral sequences to investigate relatedness, protein interactions and evolution of function of proteins constituting JDP/Hsp70 systems.

JDPs are divided into three distinct classes (A, B, C) based on their similarity to bacterial JDP DnaJ; Class B JDPs have a unique ability to prevent formation of amyloid aggregates.

In the first part of my thesis, I analysed the phylogenetic relationships among class A and B JDPs from 725 prokaryotic and eukaryotic proteomes. The obtained results support an evolutionary scenario that all eukaryotic JDPs both class A and B are directly related to bacterial DnaJ (class A). Class B JDPs has emerged independently as results of class A gene duplications in bacteria, in the cytosol of eukaryotic cell and in the endoplasmic reticulum of animals. These results demonstrate that the current classification of JDPs does not reflect their evolutionary relationships and thus should be revised.

In the second part I analyzed the evolutionary origin of mitochondrial mtHsp70 involved, together with a JDP partner (Hsc20), in the process of iron-sulfur cluster (FeS) biogenesis- a prosthetic groups required for function of many proteins. My results demonstrated that mtHsp70 descended from bacterial multifunctional Hsp70 (DnaK), which in bacteria does not function in the FeS biogenesis. I also demonstrated that bacterial Hsp70 specialized in the FeS biogenesis (HscA) is not present in eukaryotic proteomes, suggesting that HscA was lost during the evolution of mitochondria, and it was replaced by the descendant of DnaK in FeS biogenesis.

In the third part, I demonstrated using phylogenetic analyses, that the specificity of J-domain of JDP interaction with Hsp70 partner is driven by the coevolution of residues involved in this interaction. The J-domain/Hsp70 interaction is a key to the stimulation of ATP hydrolysis and thus proper functioning of JDP/Hsp70 systems. Up to now, it was not clear however whether the residues involved in this interaction are evolutionary variable and whether they coevolved- such that the changes on the J-domain's side of the binding interface are compensated by changes on the Hsp70's side and vice versa. Using two well characterized JDP/Hsp70 systems DnaJ/DnaK and Hsc20/mtHsp70 I demonstrated that residues involved in the J-domain/Hsp70 interaction are evolutionary variable and that coevolution among interacting residues determines specificity of this interaction.