The mammalian mitochondrial Hsp70 chaperone system, new GrpE-like members and novel organellar substrates

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Summary

The DnaK (Hsp70), DnaJ and GrpE heat shock proteins of *Escherichia coli* work synergistically in a diverse number of vital cellular processes including the folding of nascent polypeptides, assembly and disassembly of multimeric proteins, refolding of malformed proteins, degradation of unstable and non-native polypeptides, regulation of the stress response and the mediation of protein translocation across membranes. Various biochemical and genetic studies have identified homologues of the DnaK, DnaJ, GrpE triad within all cells and the major compartments thereof that participate in similar functions. Thus the concept of a universally conserved Hsp70 chaperone system (‘machine’ or ‘team’) has arisen and the *E. coli* triad is considered the prototype. In this study DnaK-affinity purification was employed to identify a mammalian mitochondrial GrpE homologue (mt-GrpE#1) for the first time. Isolation of a cDNA sequence encoding rat mt-GrpE#1 and deduction of its polypeptide sequence, permitted the generation of a consensus sequence for GrpE members from several biological kingdoms that revealed only six invariant residues at the amino acid level. Utilising this consensus sequence a second mammalian mt-GrpE homologue (mt-GrpE#2) was identified and shown to exhibit ~47% positional identity to mt-GrpE#1 at the amino acid level. Following synthesis in *E. coli*, the functional integrity of mt-GrpE#1 and #2 was verified by their ability to stably interact with and stimulate the ATPase activity of mammalian mitochondrial Hsp70 (mt-Hsp70). A constitutive expression of both mitochondrial GrpE transcripts was observed in 22 distinct mouse tissues but the presence of putative destabilisation elements in the 3'-untranslated region of the mt-GrpE#2 transcript, which are not present in the mt-GrpE#1 transcript, may confer a different expression pattern of the encoded proteins. Evidence is also provided for the existence of a distinct cytosolic GrpE-like protein within mammalian cells.

In conjunction with these studies, several organelar polypeptides (from mitochondria, peroxisomes and the endoplasmic reticulum) were observed to be selectively retained on an immobilised Hsp70 member, the consequence of which is speculated to be of fundamental importance in maintaining proper organelar biogenesis and may constitute a new level of metabolic regulation. The cDNA sequence of one of the retained polypeptides was determined and subsequently characterised as a putative peroxisomal isoform of 2,4-dienoyl CoA reductase. Should this be the case, it is concluded that distinct isoforms of this protein exist in mammalian mitochondria and peroxisomes rather than the dual targeting of the known mitochondrial reductase to both organelles, this has been a subject of debate for several years.