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Review

Approaches to the isolation and characterization of molecular chaperones

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Abstract

Molecular chaperones are integral components of the cellular machinery involved in ensuring correct protein folding and the continued maintenance of protein structure. An understanding of these ubiquitous molecules is key to finding cures to protein misfolding diseases such as Alzheimer's and Creutzfeldt–Jacob diseases. In addition, further understanding of chaperones will enhance our comprehension of the way the body copes with the environmental stresses that humans encounter daily. Our laboratory and our collaborators specialize in the production and characterization of chaperones from a wide variety of sources in order to gain a fuller understanding of how chaperones function in the cell. In this review, we primarily use the Hsp70/Hsp40 chaperone pair as an example to discuss recent advances in technology and reductions in cost that lend themselves to chaperone purification from both native and recombinant sources. Common assays to assess purified chaperone activity are also discussed.

Keywords: Heat shock proteins; Hsp70; Hsp40; DnaK; DnaJ; Molecular chaperones of the endoplasmic reticulum; Affinity chromatography; Codon optimization/harmonization; ATPase activity; Complementation assays

Molecular chaperones play a vital role in mediating the folding of newly synthesized proteins and the refolding of denatured proteins in the cell [1]. In addition, chaperones have also been identified in aiding the translocation of newly synthesized proteins, the rearrangement of protein oligomers, and the protection of the cell against the effects of cellular stress [2]. Originally identified as proteins up-regulated under heat stress, the major classes of molecular chaperones are defined as heat shock proteins[1] (Hsps). However, it should be noted that not all Hsps are chaperones and not all chaperones are up-regulated by heat shock.

The predominant group of heat shock proteins is the 70 kDa Hsp70s. In addition to their function in the folding of newly synthesized proteins and the refolding of denatured proteins, they have also been implicated in protein degradation, such as through the lysosomal pathway [3], the specific degradation of the heat shock transcription factor σ^{32} in *Escherichia coli* [4], and via the ubiquitination pathway in eukaryotes, for example, in the degradation of polyglutamine repeat containing proteins (reviewed in [5]). All Hsp70 functions are dependent on its chaperone property of maintaining substrate polypeptides in an extended conformation and stabilizing the exposed hydrophobic regions [6].

Expression of Hsp70s can either be inducible due to cellular stress, or constitutive. The constitutive forms of Hsp70s are termed heat shock cognate proteins (Hsc70s), and are homologous to the inducible Hsp70s

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[1] *Abbreviations used:* Hsps, heat shock proteins; ER, endoplasmic reticulum; NTA, nitrilotriacetic acid; SDS, sodium dodecyl sulfate.

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[7]. There are often significant differences in levels of enhanced expression of different inducible Hsp70s of the same organism. For example, the cytosolic Hsp70 of *Trypanosoma cruzi* has its expression enhanced approximately 4-fold after heat shock, whereas the mitochondrial Hsp70 has a lower level of enhanced expression at approximately 1.2-fold [8]. The inducibility of Hsp70 compared to Hsc70 is exemplified in HeLa cells, where there is a marked increase in the levels of Hsp70 after heat shock, as opposed to Hsc70 [9]. In addition, expression levels of different Hsp70s within an organism can vary substantially depending on the cellular organelle expressing the Hsp70, and the period of the cellular cycle [10].

Hsp40 proteins are defined by the presence of the J domain, an approximately 70 amino acid domain with similarity to the initial 73 amino acids of the *E. coli* Hsp40, DnaJ [11]. Hsp40 proteins interact with partner Hsp70 proteins through the J domain, and have been shown to facilitate the folding of nascent polypeptides through their interaction and regulation of partner Hsp70 proteins [12]. In addition, Hsp40 proteins have been implicated in protein translocation [13], protein degradation [14], clathrin uncoating [15,16], and viral infection [17]. These proteins have structural features that are conserved in *E. coli* DnaJ.

Hsp40 proteins appear to be consistently produced at low levels, and over-production of full-length *E. coli* DnaJ in *E. coli* leads to a decrease in cell viability [18], implying that high levels of DnaJ are toxic to the cell. There are significant differences between the amounts of the predominant *E. coli* Hsp70 (DnaK) and DnaJ produced, with approximately 10-fold more DnaK [19], and an estimated ratio of 10:1:3 for DnaK/DnaJ/nucleotide exchange factor (GrpE) [20].

To study the functionality of the Hsp40/Hsp70 system, it is advantageous to be able to produce large amounts of pure protein for the characterization of both their individual properties and their interactions. This review focuses on various approaches and considerations for the purification and biochemical characterization of molecular chaperones from recombinant sources, especially Hsp40 and Hsp70. In addition, to illustrate the important role that molecular chaperones play in cellular processes, and how one can isolate these proteins from their native source, we discuss the approaches to the isolation and characterization of the chaperone machinery associated with the endoplasmic reticulum (ER).

Native systems: the chaperone machinery of the endoplasmic reticulum

The ER represents the cell's quality control site for many newly synthesized proteins such as secretory and/or membrane resident proteins. Correctly folded proteins are packaged into transport vesicles that deliver them to their final destination, whilst incorrectly folded proteins are retained and degraded. The ER provides an environment that allows the oxidative folding and post-translational modification of proteins and also regulates the transport of proteins ensuring that only correctly folded and assembled proteins proceed to the Golgi apparatus and the plasma membrane. A growing list of diseases, such as cystic fibrosis, result from the failure of mutant proteins to pass this quality control checkpoint. Although much is now known about how mutant or misfolded proteins are degraded, less is understood about how they are recognized and sorted out from properly folded proteins. Protein biogenesis at, and transport into, the endoplasmic reticulum involves the cytosolic and lumenal resident molecular chaperones of the Hsp70 and Hsp40 family at various stages ([21,22]; Fig. 1; Table 1) . Molecular chaperones belonging to the Hsp70 protein family play roles in post-translation protein transport into the yeast ER. The cytosolic Hsp70s and Hsp40s preserve the transport competent state of some presecretory proteins whereas the lumenal Hsp70s, Bip/Kar2 and Grp170/Lsh1, facilitate the insertion of precursor proteins into the core of the SEC-complex and cooperate with the J domain containing protein Sec63, a prominent member of the SEC-complex [23]. Moreover, both BiP/Kar2 and Sec63 are also required for co-translational translocation [24,25].

For the mammalian system, the ATP-binding proteins BiP and Grp170 were identified as the ER-resident members of the Hsp70 protein family [26,27] by using a functional approach. Proteoliposomes made from mammalian microsomal detergent extracts were depleted and/or supplemented with both Hsp70s and studied

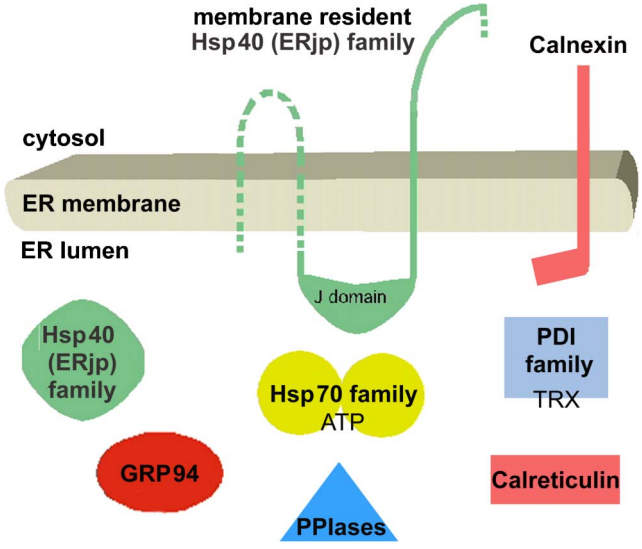

Fig. 1. Chaperones present in the endoplasmic reticulum. See Table 1 for references.