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Hypothesis

New model for 70 kDa heat-shock proteins' potential mechanisms of function

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The 70 kDa family of heat-shock proteins (hsp 70s and hsc 70s) can facilitate protein transport to several organelles. This process is thought to involve electrostatic interactions between hsp 70s and the cellular protein targeting sequences. Analysis of the highly conserved structural and functional properties of the hsp 70 family indicated that they may cross-link cellular proteins to the actin microfilament network. Direct experimental support for this hypothesis was provided by the finding that hsp 70 is constitutively bound to actin through hydrophobic interactions. The cross-linker model may provide an explanation for the mechanism by which the cytoskeletal matrix could mediate various cellular processes.

hsp 70; Cytoskeleton; Actin, Cross-linker; Targeting sequence; Molecular chaperone

1. INTRODUCTION

The intracellular environment is extremely viscous. Its texture is more similar to a gelatinous substance than to a perfect solution. Because diffusion may be a prohibitively inefficient way for transporting macromolecules, a highly developed active transport system would be necessary as the cells evolved toward greater size and complexity.

2. CYTOSKELETAL MATRIX CROSS-LINKERS

The cytoskeletal matrix, also known as the cytoskeleton, is composed of an intricate network of filaments, motor enzymes and associated proteins. It is the basic framework for cellular organization, and it also supports the cellular transport apparatus. Although the cytoskeletal matrix has been implicated in many essential cellular processes, there is a significant gap in our current knowledge as to the manner in which the cytoskeletal matrix is physically linked to all the cellular components.

The simplest model for coupling cellular proteins to the cytoskeletal matrix is for them to bind to the matrix directly [1,2]. Such a model is dependent upon the discovery of a set of cytoskeletal matrix cross-linker molecules. These hypothetical cross-linker molecules must have at least two binding sites: one to bind the cytoskeletal matrix, and another to bind various cellular proteins.

The most probable sites on cellular proteins for interaction with cross-linkers are the targeting sequences. Cellular targeting sequences generally consist of short stretches of charged amino acids. They are necessary and often sufficient for mediating transport of proteins to a particular cellular compartment. The fact that targeting sequences can be sufficient for mediating protein transport suggests that they must interact with the transport apparatus in some way.

3. MODEL FOR hsp 70s AS CROSS-LINKERS

A number of recent discoveries have suggested that the 70 kDa family of heat-shock proteins (hsp 70s and hsc 70s) serve as cross-linker molecules between transported cellular proteins and the actin microfilaments. To establish hsp 70s as cytoskeletal cross-linkers, it is necessary to demonstrate that hsp 70s have two binding sites: one for those proteins targeted for transport, and another one for actin.

The structure of hsp 70s contains two major domains (for review see [3]). The sequences at the C-terminus are not very conserved among the different family members of the hsp 70s. This region can bind with an enormous variety of both folded and unfolded cellular proteins. It is also considered to be the substrate recognition domain that interacts with cellular proteins targeted for transport.

Hsp 70 proteins are involved in facilitating protein transport to several different organelles. These include

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the nucleus, the endoplasmic reticulum, mitochondria and lysosomes [3–11]. In those cases where the sites of protein–protein interaction have been characterized, the mechanism appears to involve direct binding of hsp 70 proteins with cellular targeting sequences [8–11].

Dice and co-workers have identified a protein that specifically recognized the lysosomal targeting sequences (KFERQ and related sequences). This protein was subsequently shown to be a member of the hsp 70 family of proteins, hsc 73 [8]. A similar mechanism was found for hsp 70s' role in facilitating protein transport to the nucleus. Yoneda and co-workers have isolated a receptor protein that specifically binds to the positively charged nuclear location signal sequences (NLSs) [10]. Based on its molecular weight, isoelectric point, cellular localization, and partial amino acid sequencing, this NLSs receptor protein has now been identified to be hsc 70 [11]. This finding is particularly relevant for the clarification of the probable in vivo functional mechanism since evidence has shown that the transport of proteins to the nucleus is independent of their conformational states. Current data therefore supports the possibility that the C-terminal substrate recognition domain of the hsp 70 family binds to the protein targeting sequences and directs protein transport to two organelles, the nucleus and the lysosomes.

The 44 kDa, N-terminal domain of hsp 70s contains the ATPase activity. Its sequence is highly conserved among all the members of the hsp 70s family [3]. The three-dimensional structure of this fragment has been determined by X-ray crystallography [12]. It revealed that this part of hsp 70 has nearly the same structure as the actin monomers that polymerize to form the actin microfilament network [13]. This evidence is also consistent with the hypothesis that the 44K ATPase portion of hsp 70s and actin may share similar binding sites and that they may bind to each other.

Studies using a monoclonal antibody against hsp 70 have revealed that the hsp 70 proteins and the actin microfilaments [14,15]. This suggests that hsp 70s is an actin-associated protein. The most convincing set of evidence for the association of hsp 70s with actin was found by Margulis and Welsh [16,17]. They have discovered that hsp 70 binds to actin directly. The data indicated that the tight association between actin and hsp 70s is constitutive, and that it is due to hydrophobic types of interactions. These results are consistent with earlier observation by Morimoto and co-workers lab that a protein with same molecular weight as actin is the major hsp 70-binding protein throughout the cell cycle and after heat shock [18].

Other researchers studying actin-binding proteins are reporting that hsp 70 binds to actin. Condeelis and co-workers have isolated a 70 kDa actin-binding protein called aginactin [19]. Recently, at the thirty-second annual meeting of the American Society for Cell Biology (ASCB), it was reported that cloning and sequencing of aginactin cDNA showed that aginactin is hsp 70 [20]. Thus, data from different labs using independent experimental approaches have led to the same conclusion that hsp 70 binds to actin. The nature of these interactions are distinct from hsp 70s' association with other cellular proteins in that different binding sites appeared to be involved.

These results form a significant body of experimental evidence to support proposing a new model for the function of hsp 70s. This new cross-linker model proposes that hsp 70 binds to cellular proteins through its C-terminal substrate recognition domain. The binding sites on the proteins include the protein targeting sequences, but other binding sites on the protein are certainly possible. In the eukaryotic cytosol, hsp 70 is proposed to bind with actin through its highly conserved N-terminal domain. The nature of interaction appears to be hydrophobic, and it is regulated by hydrolysis of ATP (Fig. 1).

4. APPLICATION OF THE CROSS-LINKER MODEL

Hsp 70 family binds to a variety of proteins, and it effects the folding, assembly, disassembly and transport of these cellular proteins and structures [3,21,22]. It is currently thought that hsp 70s functioned as molecular chaperones [23–26]. However, their roles in these diverse and seemingly unrelated processes can all potentially be more precisely defined by the cross-linker model.

Beckmann et al. have determined that hsp 70 binds to nascent polypeptide chains as they emerged from the ribosomes [27]. Coupling the nascent polypeptides to the cytoskeletal matrix can physically separate the immature polypeptides and prevent protein agglutination. This will restrict the thermal motion of the molecules and also provide a consistent starting point for the processes of protein folding and structural assembly to

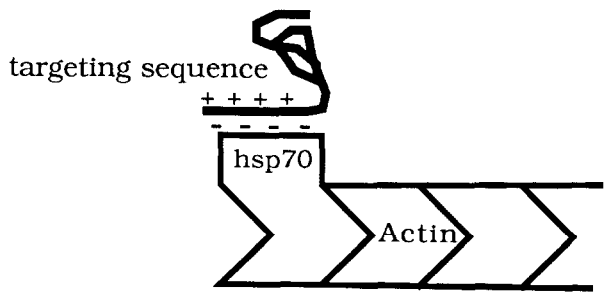

Fig. 1. Proposed mechanism for hsp 70s' cross-linker function. The N-terminal ATPase fragment of the hsp 70s is proposed to bind the actin microfilaments through hydrophobic interactions. The hsp 70s' C-terminal substrate recognition domain is proposed to bind cellular proteins through electrostatic interactions The potential binding sites include protein targeting sequences.

begin. Thus, the cross-linkers may facilitate folding without changing the principle of self assembly of proteins [28].

Physical attachment to the cytoskeletal matrix can couple various cellular components to the mechanical forces generated by the motor enzymes. This is particularly relevant in the processes of disassembly where it often requires de-stabilization of existing intra- and inter-molecular bonds. The mechanical energy generated by the cytoskeletal matrix based motor enzymes could be directly applied onto these bonds if some or all of the components involved were coupled to the matrix through the cross-linkers.

A significant number of heat-shock proteins are associated with the cytoskeletal matrix [28–31]. Thus, the functions of heat-shock proteins may be directly linked to the functions of the cytoskeletal matrix. One of the most dramatic effects after heat-shock is the near instantaneous collapse of the cytoskeletal matrix [32,33]. A major role of heat-shock proteins could be to reform the cytoskeletal matrix after it has been damaged.

A very important feature of the cross-linker model is that it provides an interpretation for the extraordinary conservation of the hsp 70s. The set of specific demands on the structure of the cross-linkers could maintain a great deal of selective pressure on their sequences. Cross-linkers must be able to form at least two high affinity bonds, and the strength of bonding must be strong enough to withstand the stress generated during transport of bulky components. Furthermore, the overall folding of the cross-linkers must be stable enough so that it will not partially denature when the stress is applied onto its structure. Such stringent functional constraints would have caused both the overall folding of the protein and its surface binding domains to remain conserved throughout evolution.

5. PERSPECTIVES

The cytoskeletal matrix and the heat-shock proteins are both areas of great interest in biology. Their interactions therefore should open up many new exciting avenues for future research.

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