S6-2

Substrate-binding Mechanism of Yeast Molecular Chaperone Hsp40

Soojin Lee

Department of Microbiology, Chungnam National University

The Hsp70 family members play an essential role in cellular protein metabolism by acting as polypeptide binding and release factors that interact with nonnative regions of proteins at different stages of their life cycles. Hsp40 family members regulate Hsp70s ability to bind nonnative polype ptides and thereby play an essential role in cell physiology. Type I and type II Hsp40s, such as y east Ydj1 and Sis1, form chaperone pairs with cytosolic Hsp70 Ssa1 that fold proteins with differ ent efficiencies and carry out specific cellular functions. The crystal structure of a Sis1 fragment s uggests that Type II Hsp40s utilize hydrophobic residues located in a solvent-exposed patch on ca rboxyl-terminal domain 1 to bind non-native polypeptides. We report that Lys-199, Phe-201, Ile-2 03 and Phe-251, which form a depression in carboxyl-terminal domain 1, are essential for cell via bility and required for Sis1 polypeptide binding activity. These data identify essential residues in Sis1 that function in polypeptide binding and help define the nature of the polypeptide-binding sit e in Type II Hsp40 proteins. Ydj1 and Sis1 share a high degree of sequence identity in their amino and carboxyl terminal ends, but each contains a structurally unique and centrally located protein module that is implicated in chaperone function. To test whether the chaperone modules of Ydj1 and Sis1 function in the specification of Hsp70 action, we constructed a set of chimeric Hsp40s in which the chaperone domains of Ydj1 and Sis1 were swapped to form YSY and SYS. Purified SYS and YSY exhibited protein-folding activity and substrate specificity that mimicked that of Ydj1 and Sis1, respectively. In *in vivo* studies, YSY exhibited a gain of function and, unlike Ydj1, could complement the lethal phenotype of sis1Δ and facilitate maintenance of the prion [RNQ+]. Ydj1 and Sis1 contain exchangeable chaperone modules that assist in specification of Hsp70 function.

Sis1 CTD1 Mutants Exhibit Defects in Substrate Binding

The Hsp40 family is large and structurally and functionally diverse with members classified i nto three subtypes.

Type I Hsp40s, such as yeast Ydj1, contain the J-domain, a G/F rich region, a zinc finger-like domain, and a region termed the conserved carboxyl terminal domain (CTD). Type II Hsp40s, such as human Hdj-1 and yeast Sis1, contain all of the aforementioned domains except that the zinc finger-like region has been replaced by a G/M-rich region and CTD1. Analysis of the Sis 1-(171–352) located structure revealed the existence of a hydrophobic patch of amino acids on the surface of domain 1, which was predicted to participate in Sis1 chaperone function. To test thi s model, we carried out a mutational analysis of residues present in the hydrophobic patch in C TD1 of Sis1. The results demonstrated that highly conserved residues within CTD1 (Lys-199, Ph e-201, Ile-203 and Phe-251) are required for Sis1 activity to refold the non-native polypeptides (Fig. 2) and these defects were resulted from the reduced polypeptide binding capacity of mutant Sis1 proteins (Fig. 3)

Determination of the Substrate Specificity of Ydj1 and Sis1 –

Ydj1 and Sis1 are colocalized in the yeast cytosol with the Hsp70 Ssa1-4 and Hsp70 Ssb1-2 prot eins. Genetic studies indicate that Ydj1 and Sis1 have specific functional properties that enable them to direct Hsp70 Ssa proteins to facilitate different cellular rocesses. The substrate specific ity of Ydj1 and Sis1 was investigated via screening a 7meric coliphage peptide display.

Ydj1 and Sis1 each selected phage displayed peptides that were enriched in hydrophobic am ino acids, but clear differences in the amino acid composition of the peptides selected (Fig. 4)

Domain Swapping of Sis1 and Ydj1

The overexpression of Sis1 can complement the slow growth phenotype of ydj1Δ strains, but Ydj1 cannot complement the lethal phenotype of sis1Δ strains.

Furthermore, the cellular functions of Ydj1 and Sis1 are different.

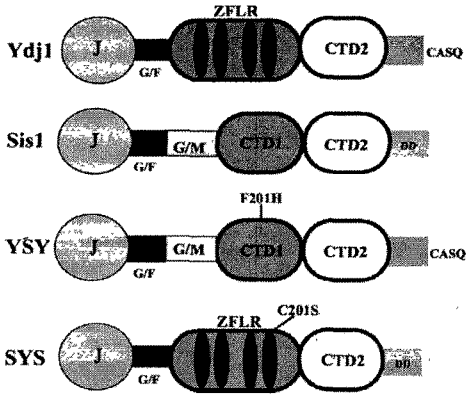
Ydj1 and its human homolog Hdj2 function on the cytoplasmic face of the endoplasmic retic ulum to promote membrane protein fIding and protect cells from stress. Whereas Sis1 is found in association with translating ribosomes where it facilitates the assembly of translation initiation. I n addition, Sis1, but not Ydj1, is required of the assembly of the prion [RNQ+] into insoluble fibr ils. The reason why type I and type II Hsp40s exhibit differences in chaperone activity is unknow n. Insight into the answer to this question comes from biochemical and structural studies, which s uggest that type I and II Hsp40s have evolved to contain structurally distinct polypeptide binding domains. It is possible that the structural differences exhibited by the chaperone modules of Ydj1 and Sis1 helps confer their ability to specify Hsp70 Ssa1 function in the yeast cytosol. To test thi s model we constructed a set of chimeric Hsp40s in which the chaperone domains of Ydj1 and S is1 were swapped to form YSY and SYS.

The data presented in Figure 5 demonstrate that the selectivity of Ydj1 and Sis1 in binding coliphage-displayed peptides can be switched by swapping the chaperone modules located in the middle of these Hsp40s. Then we characterized the ability of YSY and SYS to cooperate with H sp70 Ssa1 to fold proteins, bind substrates, support cell viability, and promote the propagation of the prion [RNQ+]. We found that YSY, but not Ydj1, could support the Growth of a sis1Δ strain and Sis1 and YSY, but not SYS maintained [RNQ+] (Fig. 6).

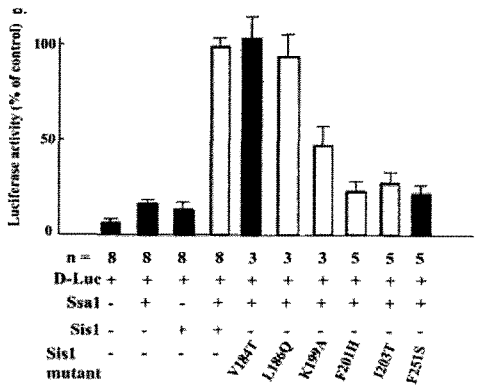
The experimental results demonstrated that Ydj1 and Sis1 contain exchangeable chaperone mo dules that control their protein folding activity and *in vivo* functions. These collective data demonst rate that type I and type II Hsp40s are not equivalent as chaperones, and we propose that this functional difference plays a role in the specification of Hsp70s cellular action.

References

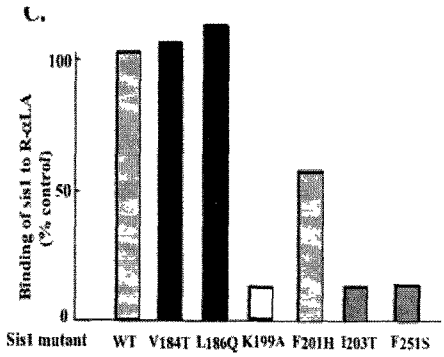
1. Caplan, A.J., Cyr, D.M., and Douglas, M.G. (1992). Cell *71*, 1143–1155.
2. Lee, S., Fan, C.Y., Younger, J.M., Ren, H., and Cyr, D.M. (2002). J. Biol. Chem. *277*, 21 675-21682.
3. Sha, B., Lee, S., and Cyr, D.M. (2000). Structure Fold Des. *8*,799–807.
4. Fan CY, Lee S, Cyr DM. (2004) Mol Biol Cell, 15, 761-773.
5. Sondheimer N, Lopez N, Craig EA, Lindquist S. (2001) EMBO J 20: 2435–2442.
6. Fan CY, Lee S, Cyr DM. (2003) Cell Stress & Chaperones 8, 309-316.



<Fig. 1>



<Fig. 2>

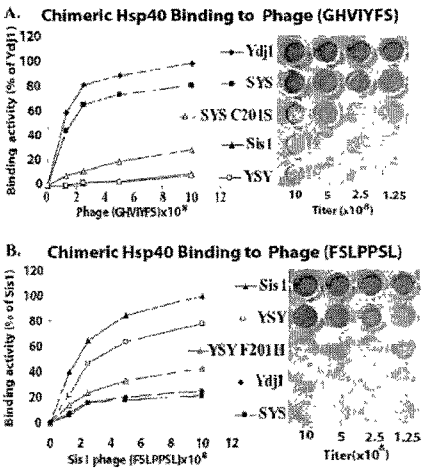


<Fig. 3>

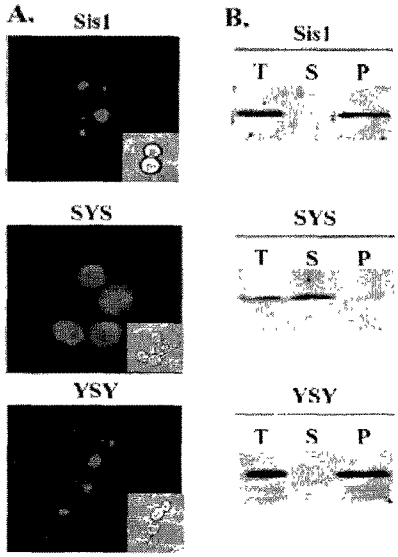
Ydj1 Peptides	#selected
G-H-I-I-Y-L-S	3
S-H-T-I-Y-L-S	5
M-H-V-I-Y-L-S	2
N-H-E-I-Y-L-S	1
S-H-T-I-W-T-D	1
S-H-K-I-H-L-S	1
A-H-T-I-Y-L-S	1
W-H-T-I-F-F-T	1
G-H-V-I-Y-F-S	1
T-H-T-I-Y-L-S	1
W-T-L-S-W-S-Q	3
W-T-F-S-F-S-A	3
W-T-L-R-W-T-D	2
W-T-L-H-F-S-N	2
W-T-I-H-L-S-T	1
W-T-I-N-F-S-D	1
K-L-P-G-W-S-G	1
I-P-T-L-P-S-S	1
Y-S-T-M-W-A-L	1
L-P-L-T-P-L-P	1
M-S-K-A-M-S-P	1

Sis1 Peptides	#selected
K-L-W-K-I-P-T	1
K-L-W-I-I-P-K	1
K-I-Y-V-L-P-R	1
S-V-N-K-P-P-T	1
G-S-P-Q-A-P-H	1
L-P-F-T-T-P-F	1
K-L-W-V-I-P-Q	1
L-S-S-T-W-P-N	1
Y-L-H-P-T-P-T	1
T-S-T-S-T-P-S	1
S-I-L-P-Y-P-Y	1
M-P-A-Y-K-N-Y	1
I-P-S-R-T-A-T	1
Q-P-T-V-R-H-L	1
V-P-P-F-L-A-T	1
Y-F-N-L-A-T-H	1
W-P-T-L-Q-T-A	1
W-T-P-Q-Q-W-A	1
T-Q-P-P-R-L-H	1
Q-H-P-F-L-S-W	1
S-L-S-P-A-M-V	1
F-S-L-P-P-S-P	1
L-M-V-P-D-V-P	1
H-R-A-P-W-P-P	1
N-N-Y-P-R-L-S	1
M-L-T-A-P-R-A	1
Q-S-I-H-L-V-L	1
S-A-T-S-S-Y-T	1
K-V-W-I-V-S-T	1
E-L-H-L-S-A-T	1
K-V-W-N-L-S-A	1
S-I-H-V-T-H-R	1
Y-A-N-W-A-T-H	1
N-Q-D-S-A-K-T	1

<Fig. 4>



<Fig. 5>



<Fig. 6>