# Functional Specificity Among Hsp70 Molecular Chaperones

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Molecular chaperones of the 70-kilodalton heat shock protein (Hsp70) class bind to partially unfolded polypeptide substrates and participate in a wide variety of cellular processes. Differences in peptide-binding specificity among Hsp70s have led to the hypothesis that peptide binding determines specific Hsp70 functions. Protein domains were identified that were required for two separate functions of a yeast Hsp70 family. The peptide-binding domain was not required for either of these specific Hsp70 functions, which suggests that peptide-binding specificity plays little or no role in determining Hsp70 functions in vivo.

Hsp70 proteins function in a diverse set of processes, including protein folding, multimer association and dissociation, translocation of proteins across membranes, and regulation of the heat shock response (*1*). All eukaryotic cells use multiple Hsp70s to carry out these functions; in the yeast *Saccharomyces cerevisiae*, 14 Hsp70s are divided into at least five functionally distinct families. Hsp70s from different families are highly conserved but cannot function interchangeably (*2*, *3*); the basis of this functional specificity is not well understood. Each Hsp70 consists of a highly conserved $NH_2$-terminal 44-kD adenosine triphosphatase (ATPase) domain, a less well conserved 18-kD peptide-binding domain, and a COOH-terminal 10-kD variable domain of unknown function (*4*–*8*). Because differences in peptide-binding specificity have been identified among Hsp70s (*9*–*11*), it has been hypothesized that peptide binding plays a central role in determining the functional specificity of each Hsp70. Here, we challenge this hypothesis by determining the source of functional differences between two families of yeast Hsp70s.

The Ssa and Ssb Hsp70 families of *S. cerevisiae* share 60% amino acid identity and reside in the cytosol (*2*) but have distinct, nonoverlapping functions in vivo (*12*). To test the role of different Hsp70 domains in determining functional specificity, we generated gene fusions that encoded chimeric proteins containing all combinations of the ATPase, peptide-binding, and variable domains of Ssa1 and Ssb1 (*13*). Chimeric junctions were placed precisely between the three Hsp70 domains (*5*–*8*, *11*).

To directly test the hypothesis that functional specificity is determined by peptide binding, we investigated whether a chimera containing the Ssa1 peptide-binding domain could rescue two phenotypes specific to the Ssb family of Hsp70s: cold sensitivity and hygromycin B sensitivity. The chimeric protein BAB (*14*), in which the peptide-binding domain of Ssb1 has been replaced by that of Ssa1, was able to rescue both Ssb-specific phenotypes (Fig. 1). Thus, the functional differences between the Ssa and Ssb protein families were not determined by the peptide-binding domain. Although the peptide-binding domain contains all of the residues that contact the peptide substrate (*8*), the variable domain was also included in most previous studies of peptide-binding function (*6*, *9*, *11*, *15*) and thus might modulate peptide binding. Because the variable domains of Ssa1 and Ssb1 are only 14% identical, they are a potential source of functional differences. However, the chimera BBA, containing the variable domain of Ssa1, was able to rescue both cold sensitivity and hygromycin B sensitivity (Fig. 1); hence, the variable domain was not required for the rescue of either Ssb-specific phenotype.

Because neither the peptide-binding nor the variable domain was required for the rescue of Ssb-specific phenotypes, we examined all of the Ssa1-Ssb1 chimeras to determine the source of Ssb-specific function (Fig. 1). Both Δ*ssb1* Δ*ssb2* phenotypes were rescued by several chimeric proteins; however, the two phenotypes were not rescued by the same chimeras. The cold-sensitive phenotype was rescued by all of the chimeras that contained the Ssb1 ATPase domain, but not by those that contained the Ssa1 ATPase domain (Fig. 1). Thus, the ATPase domain of Ssb1 was the only domain specifically required for the rescue of cold sensitivity.

The rescue of hygromycin B sensitivity was more complex. No single domain of Ssb1 was necessary for the rescue of this phenotype; BBA, BAB, and ABB all conferred resistance to hygromycin B (Fig. 1). Moreover, no single domain was sufficient; cells containing AAB, ABA, or BAA all remained sensitive to hygromycin B. Rather, the different domains of Ssb1 functioned additively, such that any two could confer resistance to hygromycin B. These domains could not function cooperatively when present in different chimeric proteins, however; when any two of the chimeras BAA, AAB, and ABA were coexpressed, no rescue of hygromycin B sensitivity was observed (*16*).

Ssb proteins appear to play a role in translation since Δ*ssb1* Δ*ssb2* mutants are sensitive to hygromycin B, and up to 70% of Ssb protein is associated with translating ribosomes (*17*). We examined five chimeras (*18*) to determine whether the rescue of hygromycin B sensitivity was correlated with polysome association. Polysomes were separated from free ribosomes by centrifugation through a sucrose gradient (Fig. 2A). Both Ssa and Ssb proteins were present in high–molecular weight complexes that were distributed throughout a sucrose gradient (Fig. 2B). These complexes were distinguished by treating extracts with ribonuclease (RNase) A, which disrupts the polysomes. Ribosome-associated proteins such as Ssb were shifted toward the top of the gradient (*17*), whereas Ssa was unaffected.

Sucrose gradients were used to examine the polysome association of the chimeras. Chimeras that failed to rescue hygromycin B sensitivity (BAA and AAB; Fig. 1) also

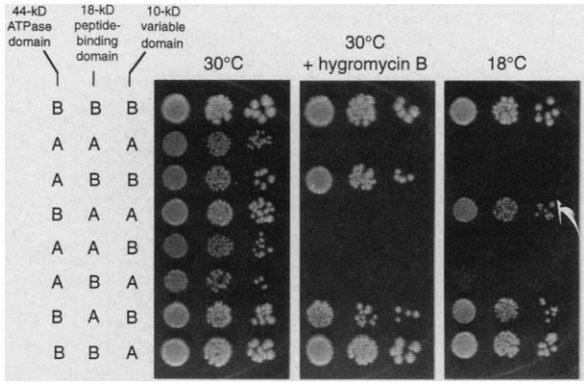


**Fig. 1.** Rescue of Δ*ssb1* Δ*ssb2* growth phenotypes by chimeric Hsp70 proteins. Δ*ssb1* Δ*ssb2* cells containing each chimera on a centromeric plasmid were grown on SC-ura media at 18°C for 8 days, or at 30°C in the presence or absence of hygromycin B (70 μg/ml) for 4 days. Immunoblotting experiments demonstrated that the wild-type proteins and all chimeras were stably expressed at similar levels at both 18° and 30°C (*16*).

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failed to associate with polysomes (Fig. 2B). In contrast, ABB and BAB, which conferred hygromycin B resistance, associated with polysomes. Thus, neither the ATPase domain nor the peptide-binding domain was required for the Ssb-specific association with translating ribosomes. Moreover, for all of the chimeras except BBA (*19*), there was a correlation between the rescue of hygromycin B sensitivity and an association with translating ribosomes, which suggested that both are the result of a single ribosomal function.

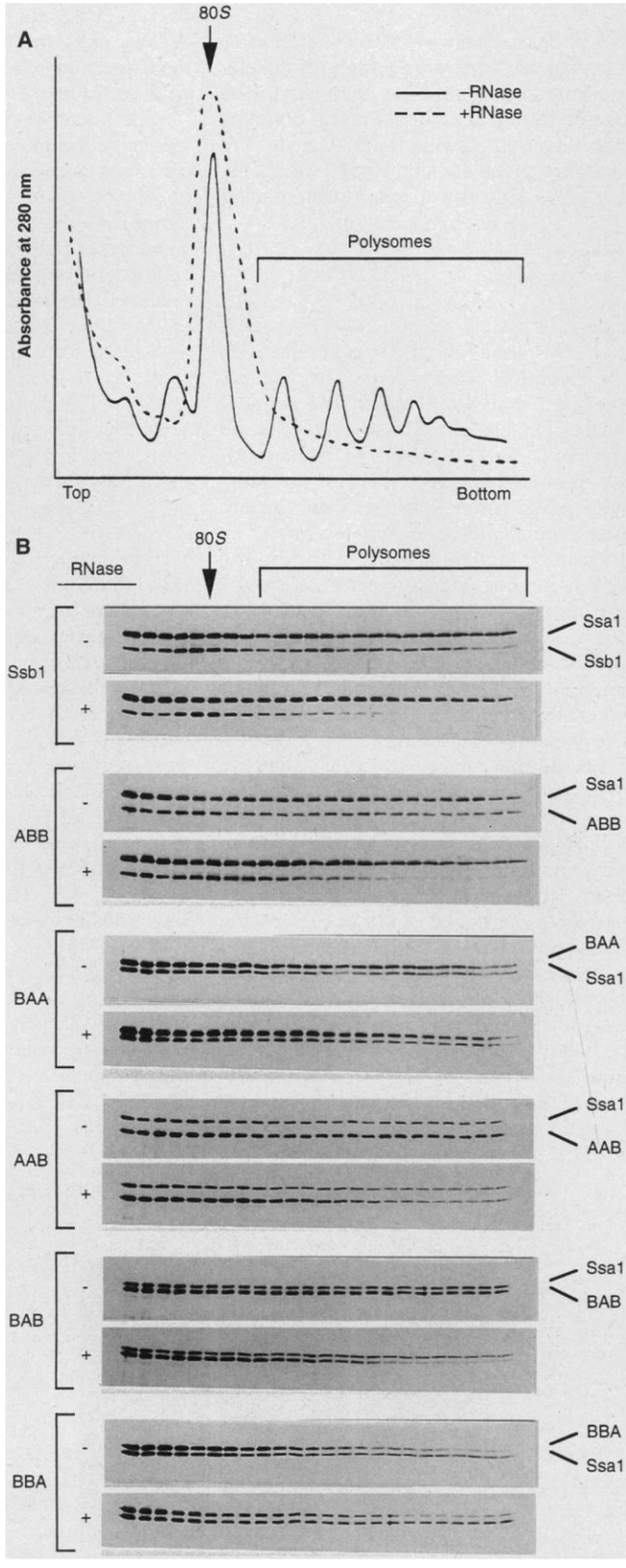


**Fig. 2.** Association of chimeric Hsp70 proteins with polysomes. (**A**) Separation of free ribosomes (80*S* peak) and polysome complexes on a sucrose gradient (*25*). RNase A treatment disrupts polysome complexes, causing a shift into the 80*S* peak. (**B**) Immunoblots of polysome profile fractions. Each panel shows fractions taken from across a sucrose gradient, with the positions of the 80*S* peak and the polysomes indicated. Immunoreactive bands are identified in the top panel of each pair. Compare each upper panel (−RNase A) with its partner below (+RNase A). Proteins associated with polysomes are shifted toward the 80*S* peak upon RNase A treatment.

In contrast, the cold-sensitive and hygromycin B–sensitive phenotypes of a Δ*ssb1* Δ*ssb2* strain were separable (Fig. 1). The chimera BAA rescued only the cold-sensitive phenotype of Δ*ssb1* Δ*ssb2* cells, whereas ABB rescued only hygromycin B sensitivity. This separation of phenotypes suggested that cold sensitivity and hygromycin B sensitivity represent two distinct cellular functions of the Ssb proteins. The chimera BAB rescued both of these phenotypes; thus, the peptide-binding domain of Ssb1 was dispensable for two different Ssb-specific functions. In addition, the ability of BAA to rescue cold sensitivity in the absence of detectable polysome association suggested that Ssb proteins performed these two functions at different sites. This suggestion was supported by cotransformation experiments. When both BAA and ABB were present in the same cell, they did not interfere with one another but functioned additively, rescuing both cold sensitivity and hygromycin B sensitivity (*16*, *20*).

Together, these results indicate that functional specificity among the Hsp70 class of molecular chaperones does not depend on peptide-binding specificity. We favor a model in which functional specificity is determined by physical interactions between one or more Hsp70 domains and other components of the cellular machinery (*21*). Once established, these interactions may be stabilized by binding to substrate polypeptides; however, we view peptide binding as an activity of the Hsp70s, similar to their ATPase activity, that is ferried to particular functions by the specific interactions of other parts of the protein. Such interactions may serve to target Hsp70 to a site of function, such as the ribosome, or to direct association with cohort proteins such as DnaJ homologs, which function cooperatively with Hsp70s (*22*, *23*).

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12. Ssa proteins function in protein translocation into organelles and regulation of the heat shock response (*2*). Ssb1 and Ssb2 are 99% identical and are associated with translating ribosomes (*17*); *Δssb1 Δssb2* cells are sensitive to cold and to certain translation-inhibiting drugs such as hygromycin B. Reduced numbers of polysomes, previously reported in *Δssb1 Δssb2* cells (*17*), are a general characteristic of slowly growing cells (*24*). Ssa and Ssb proteins are each unable to rescue the phenotypes of the other, even when Ssa1 is expressed from the *SSB1* promoter, or vice versa (*2*).
13. Chimeras were generated using Nde I and Xho I sites created by polymerase chain reaction (PCR) at codons 368 and 540 in *SSA1* and 374 and 547 in *SSB1*, respectively. The sites are in regions of Ssa1-Ssb1 identity and did not alter the amino acid sequence or function of either protein. The Nde I site occurs in the final α helix of the 44-kD ATPase domain (*5*). The Xho I site occurs in the long α helix shared by the 18-kD peptide-binding domain and the 10-kD variable domain, at the point of flexibility between them (*8*).
14. Chimeras were named with three-letter acronyms indicating the protein domains they contain. For example, BAB contains the 44-kD ATPase domain of Ssb1, the 18-kD peptide-binding domain of Ssa1, and the 10-kD variable domain of Ssb1.
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17. R. J. Nelson, T. Ziegelhoffer, C. Nicolet, M. Werner-Washburne, E. Craig, *Cell* **71**, 97 (1992).
18. The chimera ABA could not be tested because it comigrates with Ssa1 protein.
19. The chimera BBA rescued hygromycin B sensitivity but did not detectably associate with polysomes in this assay. It is possible that BBA associates only weakly with polysomes, allowing rescue of the hygromycin B phenotype, but that the complex does not survive the conditions of our analysis.
20. It is unlikely that a single site of function is shared, because both phenotypes were rescued even when ABB was single copy and BAA was overexpressed from a high-copy plasmid.
21. Domain interactions vary for different functions. Growth at low temperature requires primarily the ATPase domain of Ssb, whereas ribosome association depends on interaction at several sites, with each domain contributing to overall binding. These interactions are likely additive, because any one can be lost or weakened by replacement with Ssa sequences without losing Ssb-specific function.
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23. The DnaJ homolog Sis1 appears to be a likely partner for Ssb, because it is associated with polysomes. However, Sis1 stimulates the ATPase activity of Ssa1, but not that of Ssb1, in vitro (T. Ziegelhoffer and E. Craig, unpublished data).
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25. Polysomes were prepared and fractionated as described (*17*) from the *Δssb1 Δssb2* strain JN208, except that gene fusions were present on centromeric plasmids and cells were grown in complete synthetic media minus uracil (SC − ura media) to an optical density of 1.0 at 600 nm. Gradient fractions were immunoblotted with a mixture of two antibodies to the COOH-termini of Ssa1 and Ssb1.
26. We thank R. Aramayo, J. Dahlberg, C. Fox, P. Kiley, and M. Krebs for helpful discussions. Supported by grants from NIH (E.A.C. and C.P.) and the Jane Coffin Childs Fund (P.J.).

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# Chaotic Dynamics in an Insect Population

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A nonlinear demographic model was used to predict the population dynamics of the flour beetle *Tribolium* under laboratory conditions and to establish the experimental protocol that would reveal chaotic behavior. With the adult mortality rate experimentally set high, the dynamics of animal abundance changed from equilibrium to quasiperiodic cycles to chaos as adult-stage recruitment rates were experimentally manipulated. These transitions in dynamics corresponded to those predicted by the mathematical model. Phase-space graphs of the data together with the deterministic model attractors provide convincing evidence of transitions to chaos.

The mathematical theory of nonlinear dynamics has led population biology into a new phase of experimental and theoretical research (*1*–*6*). Explanations of observed fluctuations of population numbers now include dynamical regimes with a variety of asymptotic behaviors: stable equilibria, in which population numbers remain constant; periodic cycles, in which population numbers oscillate among a finite number of values; quasiperiodic cycles, which are characterized by aperiodic fluctuations that are constrained to a stable attractor called an invariant loop; and chaos, where population numbers change erratically and the pattern of variation is sensitive to small differences in initial conditions. There is, however, a need for new experiments to confirm these hypothetical possibilities (*7*, *8*). A key theoretical prediction, which is subject to experimental challenge, is that specific sequences of transitions among qualitatively different dynamical regimes occur in response to changing biological parameters (*9*, *10*). Here, we address a route to chaos predicted by a mathematical model of insect populations (*11*) in which the onset of chaos is preceded by transitions from stable equilibrium to quasiperiodic and periodic cycles.

We modeled the relation of larval, pupal, and adult *Tribolium* numbers at time $t + 1$ to the numbers at time $t$ by means of a system of three stochastic difference equations:

$$L_{t+1} = bA_t \exp(-c_{el}L_t - c_{ea}A_t + E_{1t}) \quad (1)$$

$$P_{t+1} = L_t(1 - \mu_l) \exp(E_{2t}) \quad (2)$$

$$A_{t+1} = [P_t \exp(-c_{pa}A_t) + A_t(1 - \mu_a)] \exp(E_{3t}) \quad (3)$$

In this model (*12*, *13*), $L_t$ is the number of feeding larvae (referred to as the L-stage) at time $t$; $P_t$ is the number of large larvae, nonfeeding larvae, pupae, and callow adults (collectively the P-stage) at time $t$; and $A_t$ is the number of sexually mature adults (A-stage animals) at time $t$. The unit of time is 2 weeks; this is the approximate average amount of time spent in the L-stage under our experimental conditions, as well as the approximate average duration of the P-stage. The quantity $b > 0$ is the number of larval recruits per adult per unit of time in the absence of cannibalism. The fractions $\mu_l$ and $\mu_a$ are the larval and adult rates of mortality in one time unit. The exponential nonlinearities account for the cannibalism of eggs by both larvae and adults and the cannibalism of pupae by adults. The fractions $\exp(-c_{el}L_t)$ and $\exp(-c_{ea}A_t)$ are the probabilities that an egg is not eaten in the presence of $L_t$ larvae and $A_t$ adults in one time unit. The fraction $\exp(-c_{pa}A_t)$ is the survival probability of a pupa in the presence of $A_t$ adults in one time unit. The terms $E_{1t}$, $E_{2t}$, and $E_{3t}$ are random noise variables assumed to have a joint multivariate normal distribution with a mean vector of zeros and a variance-covariance matrix denoted by $\Sigma$. The noise variables represent unpredictable departures of the observa-

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