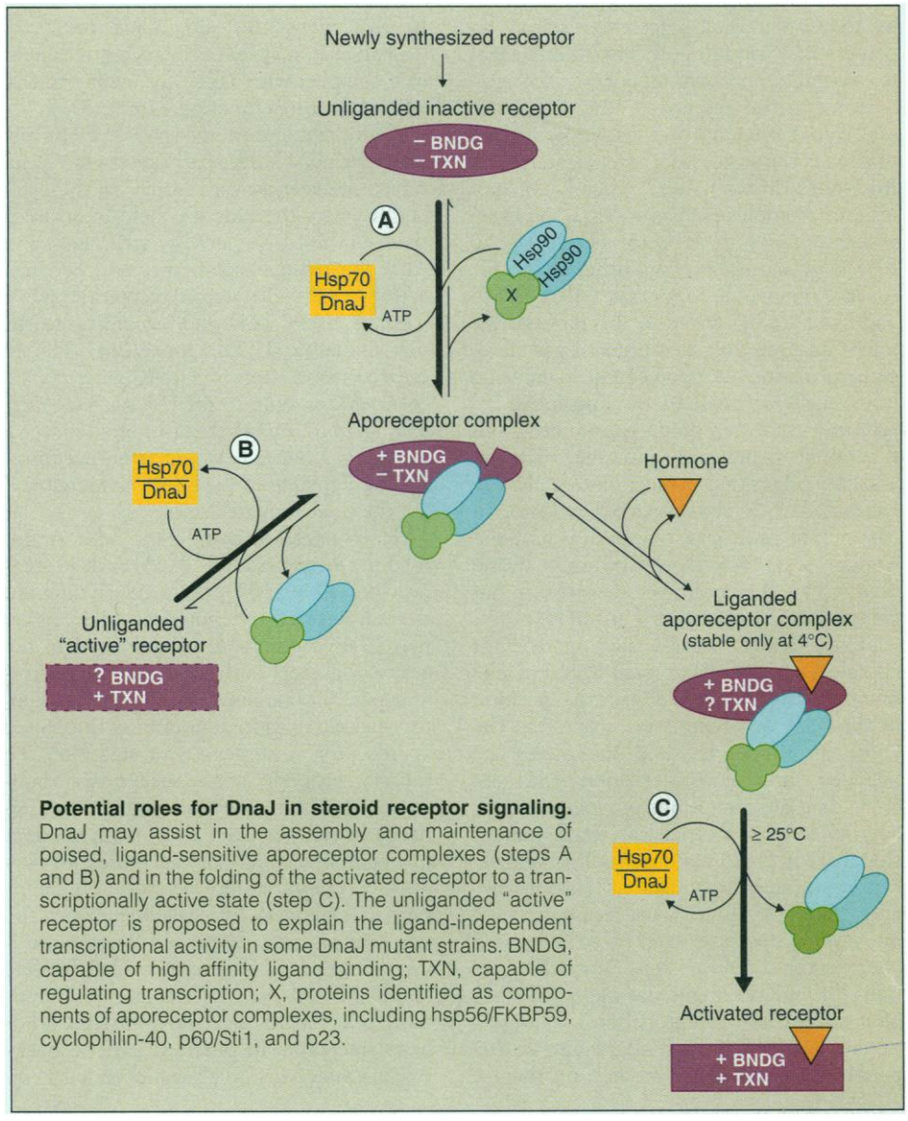
Hold 'em and Fold 'em: Chaperones and Signal Transduction

Sean P. Bohen, Anastasia Kralli, Keith R. Yamamoto

At first glance, signaling by steroid hormone receptors seems simple—these receptors are intracellular proteins that, upon activation by hormone, act as regulators of transcription. Thus, we can imagine a minimalist scheme: Hormone binds to the receptor, inducing a conformational change such that the receptor now binds DNA and modulates transcription. Alas, this fanciful view is far from accurate. There are numerous other key participants in steroid signaling, notably the molecular chaperones (*1*), proteins that assist protein folding in general. Two recent reports (*2*, *3*), one in this issue of *Science* (*2*), identify DnaJ, an old friend in chaperone circles, as a new player in steroid receptor function; the findings suggest multiple roles for DnaJ in steroid signaling. Their wider implication is that a subset of molecular chaperones may be universally required for signal transduction.

In the absence of hormone, certain steroid receptors, including the glucocorticoid, androgen, and estrogen receptors, are found as "aporeceptor complexes" consisting of a receptor monomer, a dimer of the heat shock protein Hsp90, and several other proteins (*1*, *4*) (see figure). Subsequent to ligand binding, the "activated receptor" dissociates from the complex, binds to specific DNA sequences, and modulates the transcription of genes. Proper interaction of the receptors with Hsp90 is essential for efficient ligand binding and response (*5*–*7*), indicating that the aporeceptor complex represents a "poised" conformation of the receptor, ready to respond to signal. In the absence of ligand, the complex is in dynamic equilibrium with two unliganded, non-Hsp90–bound receptor forms (steps A and B in the figure). Hsp70 and other chaperones, in an adenosine triphosphate (ATP)–dependent mechanism, appear to reassemble Hsp90 onto the unliganded receptors, thereby maintaining the pool of aporeceptors that can respond to ligand (*6*). Interestingly, steroid receptor mutants lacking the "signaling domain," the region of the receptor that interacts with ligand and Hsp90, are not found in aporeceptor complexes and are constitutively active, suggesting that besides forming the signaling-competent state, the aporeceptor complex may also maintain unliganded receptors as transcriptionally inactive (*1*).

Two new genetic studies, exploiting the capacity of mammalian steroid receptors to function in yeast, now identify a role in signaling for DnaJ, the chaperone partner of Hsp70 (DnaK). Kimura *et al.* (*2*) isolated a mutant allele of the yeast *YDJ1* gene, a DnaJ family member, in a screen for mutations that are lethal to cells in combination with Hsp90 mutants. The isolation of *YDJ1* alleles in such a "synthetic-lethal" screen suggests that Hsp90 and DnaJ may function in the same cellular processes. In support of this view, the authors show that their *ydj1* mutants affect the activities of three Hsp90 targets, the estrogen and glucocorticoid steroid receptors (*1*) and the oncogene tyrosine kinase $p60^{v\text{-}src}$ (*8*), albeit with opposite effects. The glucocorticoid and estrogen receptors display a dramatic ligand-independent, constitutive activity in *ydj1* mutant strains, whereas $p60^{v\text{-}src}$ activity is significantly reduced. In a separate study, Caplan and co-workers (*3*) tested previously isolated *ydj1* mutants, reasoning that Ydj1 might contribute to steroid receptor signaling because Hsp70, the chaperone partner of DnaJ, is required for aporeceptor complex formation in vitro (*9*). Caplan *et al.* found that the activities of both steroid receptors and $p60^{v\text{-}src}$ are reduced in their *ydj1* mutant strain (*3*); in contrast to Kimura *et al.* (*2*), they observed no increase



Potential roles for DnaJ in steroid receptor signaling. DnaJ may assist in the assembly and maintenance of poised, ligand-sensitive aporeceptor complexes (steps A and B) and in the folding of the activated receptor to a transcriptionally active state (step C). The unliganded "active" receptor is proposed to explain the ligand-independent transcriptional activity in some DnaJ mutant strains. BNDG, capable of high affinity ligand binding; TXN, capable of regulating transcription; X, proteins identified as components of aporeceptor complexes, including hsp56/FKBP59, cyclophilin-40, p60/Sti1, and p23.

S. P. Bohen is in the Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892–4255, USA. A. Kralli and K. R. Yamamoto are in the Departments of Cellular and Molecular Pharmacology and Biochemistry and Biophysics, University of California, San Francisco, CA 94143–0448, USA.

in the activity of glucocorticoid or androgen receptors in the absence of ligand. Importantly, both reports show that *ydj1* mutants do not alter the constitutive transcriptional activity of the truncated receptors lacking signaling domains; thus, Ydj1 appears to be specifically involved in steroid receptor signaling.

What role is DnaJ playing in the function of Hsp90 target proteins like steroid receptors and $p60^{v\text{-}src}$? Kimura *et al.* find that a portion of the glucocorticoid receptor pool is physically associated with wild-type Ydj1 (*2*), suggesting a direct action. In other arenas, proteins of the DnaJ family, like Ydj1, act in concert with the Hsp70 (DnaK) family of molecular chaperones to assist in protein folding (*10*). DnaJ stimulates the ATPase activity of Hsp70 and likely contributes to its substrate specificity. In vitro, both Hsp70 and DnaJ, in combination with the TRiC (GroEL) chaperone, are needed for proper folding of nascent polypeptides (*11*).

Taken together, the results of Kimura *et al.* and Caplan *et al.* suggest that steroid receptors may require both Hsp70 and DnaJ for assembly and maintenance of the aporeceptor in the absence of ligand (steps A and B in the figure) and for proper folding of the activated receptor after ligand binding (step C in the figure). Assembly of aporeceptor complexes in vitro is an ATP-dependent process that requires Hsp90, Hsp70 (*9*), p23 (*12*), and perhaps other factors (*1, 4*). It is reasonable to speculate that Hsp70 and DnaJ act in concert in this process. We might imagine that mutations in *ydj1* abrogate formation of aporeceptor complexes from newly synthesized or unliganded receptors, or that they produce misfolded, nonfunctional complexes. However, Kimura and co-workers find as much or more Hsp90 associated with glucocorticoid receptor in the mutant as in wild-type strains (*2*), and Caplan and co-workers find that ligand binding by the androgen receptor is not compromised by mutations in *ydj1* (*3*).

These results suggest that aporeceptor complexes form and can bind ligand in *ydj1* mutant strains, but leave open the question of the relative amounts of receptor in the aporeceptor complex and the other unliganded states. The observation of Kimura *et al.* that *ydj1* mutations provoke receptor activity in the absence of ligand implies that Ydj1 helps to maintain unliganded receptors in an inactive state. Thus, if Ydj1 and Hsp70 facilitated the reassembly of unliganded receptor back to the poised aporeceptor complex (steps A and B), certain *ydj1* mutants might allow accumulation of an unliganded receptor that is transcriptionally active. Even a small increase in this pool of receptors might account for the activity seen by Kimura *et al.* Alternatively, the *ydj1* mutants could allow the poised aporeceptor complex itself to assume a transcriptionally active conformation. Quantitation of relative levels of free receptor and aporeceptor complex in wild-type versus *ydj1* mutant cells would help to distinguish between these possibilities.

Do chaperones participate in the ligand-dependent dissociation of aporeceptor complexes? Formation of the "activated receptor" (step C in the figure) requires bound ligand, moderate temperatures (25° to 37°C), and is accelerated by the presence of ATP (*1, 13*). Ligand binding per se may not increase the rate of dissociation of Hsp90 but may simply preclude the reverse reaction, reassembly of the aporeceptor complex (*6*). In this context, the findings of Caplan *et al.* suggest that DnaJ, and by inference Hsp70, are needed to fold the receptor into its transcriptionally active conformation (step C in the figure), analogous to the role of DnaJ and DnaK in the activation of phage P1 repA protein dimers. In this view, the *ydj1* allele tested by Caplan *et al.* may leave the receptor trapped in a complex with Hsp90 or unable to fold efficiently into a functional form (*3*).

Can the maturation of $p60^{v\text{-}src}$ be fit into this scheme? Newly synthesized $p60^{v\text{-}src}$ interacts transiently with Hsp90 in the cytoplasm where the kinase is held in an inactive state until it is myristylated, associates with the plasma membrane, and becomes active (*8*). As with steroid receptors, $p60^{v\text{-}src}$ requires Hsp90 (*14*) and, according to the new studies (*2, 3*), Ydj1 for activity. The increased association of Hsp90 with $p60^{v\text{-}src}$ in a *ydj1* mutant strain (*3*) suggests that, analogous to the last step in steroid receptor signaling, DnaJ may be important for dissociating Hsp90 from the kinase or for achieving the final activated state.

How might the different *ydj1* strains used in the two studies (*2, 3*) result in similarly defective $p60^{v\text{-}src}$ but produce different steroid receptor phenotypes? The answer may lie in a common role for DnaJ in the activation step both of steroid receptors and $p60^{v\text{-}src}$, combined with steroid receptor–specific roles in maintaining the poised aporeceptor complex conformation. To function properly, steroid receptors, *src* family oncogene tyrosine kinases, and other signaling machines must exist in more than one conformational state. In principle, each distinct conformation defines a different potential step for Ydj1 involvement in the signaling pathway. In turn, this complicates interpretation of the results: On the one hand, different *ydj1* alleles may preferentially affect particular steps; on the other hand, receptors in different genetic backgrounds may vary in their dependence on Ydj1 at different steps. Notably, overexpression of SIS1, another yeast DnaJ protein, can partially complement *ydj1* mutants (*10*), so depending on the strain background and the *ydj1* allele, distinct steps might be preferentially affected. Thus, the opposing effects of *ydj1* mutants on glucocorticoid receptor function observed by Kimura *et al.* and Caplan *et al.* may simply represent the differential effects of their respective mutants on different steps in the pathway.

Hsp90, DnaJ, and Hsp70 molecular chaperones probably contribute to the function of a broad range of signaling proteins; genetic analyses imply a role for Hsp90 in signaling by the *sevenless* and *torso* receptor tyrosine kinases, the *wee1* cell cycle tyrosine kinase, and the basic helix-loop-helix dioxin receptor (*15–17*). Why would certain signaling proteins depend on chaperones for proper function? These chaperones may establish and maintain in such signaling proteins a poised conformation that is optimally sensitive to the signal. Any particular conformation of a signaling protein may be intrinsically unstable, producing a mixed population that is at once unresponsive, active in the absence of signal, or responsive to signal. Clearly, such molecular indecisiveness is incompatible with precise signaling control. The "signaling chaperones" could shepherd their target into a conformation that is inactive but highly responsive—triggered only by the signal into the active conformation.

References

1. S. P. Bohen and K. R. Yamamoto, in *The Biology of Heat Shock Proteins and Molecular Chaperones*, R. I. Morimoto, A. Tissieres, C. Georgopoulos, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994), pp. 313–334.
2. Y. Kimura, I. Yahara, S. Lindquist, *Science* **268**, 1362 (1995).
3. A. J. Caplan, E. Langley, E. M. Wilson, J. Vidal, *J. Biol. Chem.* **270**, 5251 (1995).
4. D. F. Smith and D. O. Toft, *Mol. Endocrinol.* **7**, 4 (1993).
5. E. H. Bresnick, F. C. Dalman, E. R. Sanchez, W. B. Pratt, *J. Biol. Chem.* **264**, 4992 (1989).
6. D. F. Smith, *Mol. Endocrinol.* **7**, 1418 (1993).
7. S. P. Bohen and K. R. Yamamoto, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 11424 (1993).
8. J. S. Brugge, *Curr. Top. Microbiol. Immunol.* **123**, 1 (1986).
9. K. A. Hutchison, K. D. Dittmar, M. J. Czar, W. B. Pratt, *J. Biol. Chem.* **269**, 5043 (1994).
10. D. M. Cyr, T. Langer, M. G. Douglas, *Trends Biochem. Sci.* **19**, 176 (1994).
11. J. Frydman, E. Nimmesgern, K. Ohtsuka, F. U. Hartl, *Nature* **370**, 111 (1994).
12. J. L. Johnson and D. O. Toft, *J. Biol. Chem.* **269**, 24989 (1994).
13. D. F. Smith, B. A. Stensgard, W. J. Welch, D. O. Toft, *ibid.* **267**, 1350 (1992).
14. Y. Xu and S. Lindquist, *Proc. Natl. Acad. Sci. U. S. A.* **90**, 7074 (1993).
15. T. Cutforth and G. M. Rubin, *Cell* **77**, 1027 (1994).
16. R. Aligue, H. Akhavan-Niak, P. Russell, *EMBO J.* **13**, 6099 (1994).