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Dynamic Remodeling of Transcription Complexes by Molecular Chaperones

Minireview

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The assembly and disassembly of macromolecular transcription complexes represents a key step in the regulation of gene expression. For inducible responses to hormones and stress, different combinations of molecular chaperones govern the activities of intracellular hormone receptors and heat shock transcription factor. Through their capacity to remodel the conformation of these activators, chaperones ensure tight control, dynamic range, and rapid reversible transcriptional response.

Molecular Chaperones and the Shaping of Hormone Receptors

Among the largest class of transcription factors are intracellular receptors, which signal downstream regulatory events upon binding to small molecule ligands. Of these, intracellular hormone receptors that bind to glucocorticoids, progesterone, thyroid hormone, or estrogen, for example, are assembled into intracellular heteromeric complexes that contain a consortium of accessory proteins (Figure 1) including Hsp90, Hsp70, immunophilins (FKBP50/52), cyclophilins (Cyp40), Bag1, TPR domain adaptor proteins (Hip, Hop), and p23 (Pratt and Toft, 1997). In vitro studies have unraveled a process of stepwise association of nascent receptors with accessory proteins that appears functionally analogous to an assembly line, comprised of different states of aporeceptor complexes, in which equilibrium is established by the supply of chaperones and cochaperones and the demand for the activated receptor.

The nascent aporeceptor initially binds to Hsp70 and Hsp40 and is transferred to Hsp90 by the adaptor protein Hop (Kosano et al., 1998; Chen and Smith, 1998; Hernandez et al., 2002). Release of Hop and binding to the Hsp90 cochaperone p23, immunophilins, and ATP leads to formation of a stable aporeceptor complex containing a dimer of Hsp90, immunophilins, and p23 (Davies et al., 2002; Young and Hartl, 2000). The hormone binding site of the activated receptor is buried within the hydrophobic core of the ligand binding domain; therefore, chaperones have an essential role to prevent complete folding of the aporeceptor and to stabilize the ligand-responsive state (Morishima et al., 2000). Upon hormone binding, the aporeceptor complex disassembles to release a ligand bound transcriptionally active form that binds specifically to its respective hormone response element to induce transcription within seconds. Binding of the hormone receptor to its target in vivo is transient and exhibits a half-life for the glucocorticoid receptor (GR)-DNA complex on the order of minutes (McNally et al., 2000). That the hormone receptor is in rapid exchange with its response element targets in vivo suggests yet another step of regulation at the level of receptor recycling by disassembly of the active DNA bound complex (Figure 1).

What is the process for receptor recycling in which inactivation and disassembly must occur? In addition to the Hsp90-dependent role of p23 in aporeceptor assembly, p23 has intrinsic chaperone activity, which makes it an attractive candidate for the disassembly of the active hormone receptor complex (Freeman et al., 1996, 2000). The effect of p23 on hormone-regulated transcription was examined using a combination of in vitro and in vivo assays (Freeman and Yamamoto, 2002). Thyroid receptor (TR)-dependent transcription was inhibited in vitro by p23; moreover, the inhibitory effect persisted under conditions in which transcription only from preinitiated RNA polymerase II was monitored. Using a DNA immunoprecipitation assay to assess promoter occupancy by specific factors, the level of TR and RNA polymerase II interaction with the promoter was reduced by exogenous p23. From these in vitro experiments, a role for p23 as a negative regulator of hormone receptor activity and perhaps in receptor recycling was suggested.

A critical demonstration of whether p23 has direct effects on hormone-regulated transcription was accomplished by an in vivo approach to deliver p23 and other chaperones including Hsp90 and Hsp70 directly to promoter sites engaged in hormone receptor-dependent transcription (Figure 2A). Cells were transfected with constructs expressing a chimera of the Gal4 DNA binding domain fused to p23, Hsp90, or Hsp70 together with a composite response element comprised of GR or TR binding sites adjacent to two Gal4 binding sites. Expression of Gal4-p23 had a potent 35-fold inhibitory effect on GR-dependent activity and 100-fold inhibitory effect on TR-dependent activity. By comparison, Gal4-Hsp90 had only a slight 2-fold inhibitory effect, with Gal4-Hsp70 having no effect. In previous studies, it had been shown that the coactivator GRIP1 was in competitive equilibrium with p23-mediated dissociation of TR, suggesting a possible mechanism for p23 activity (Freeman et al., 2000). To address whether GRIP1 and p23 competed for association with hormone receptor in vivo, GRIP1 was overexpressed and shown to mitigate the Gal4-p23-dependent inhibition of hormone receptor activity. These results reveal that p23 activity on receptor function, relative to other chaperones, is specific, and that changes in the local concentration of p23 have at least two effects, to compete with the association of a coactivator and as a rate-limiting component in disassembly of the hormone-receptor DNA bound complex (Figure 2B).

While in vitro reconstitution experiments and the targeted expression of chimeric chaperones support a role for chaperone suppression of hormone receptor-regu-

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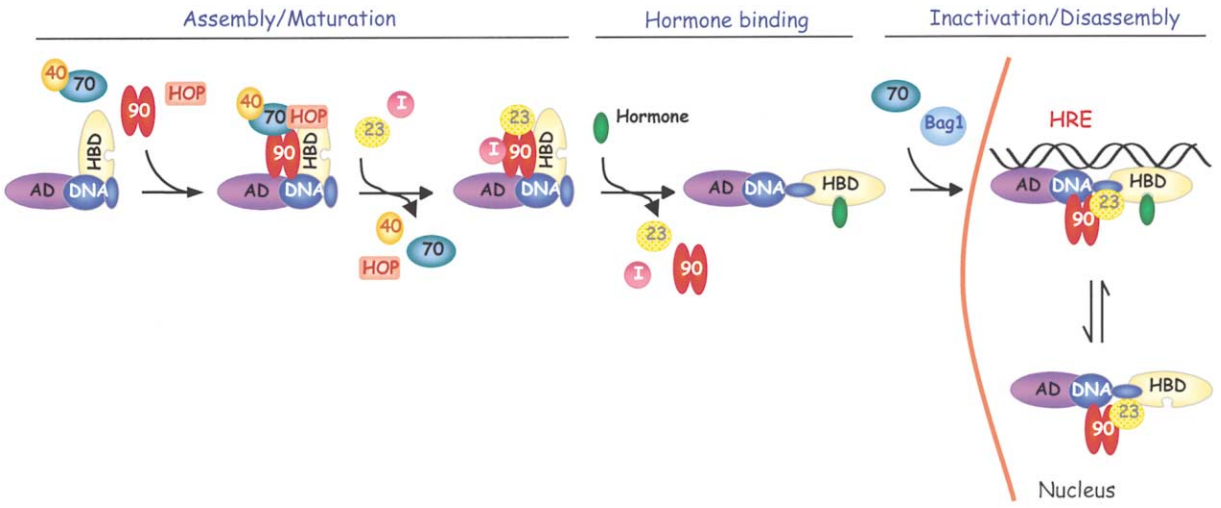

Figure 1. Assembly and Disassembly of Intracellular Hormone Receptors

A stepwise process is described in which the hormone receptor is assembled into the aporeceptor state by sequential interactions with different molecular chaperones (modified from Nollen and Morimoto, 2002). Upon ligand binding, the hormone receptor binds to the response element. The association of the DNA bound form of the hormone receptor with p23 and Hsp90 leads to the disassembly of the hormone receptor complex and release from DNA. Abbreviations: AD, activation domain; DNA, DNA binding domain; HBD, hormone binding domain.

lated gene expression, a question remains whether these chaperones are bound in vivo to transcription complexes of hormone-responsive genes. Chromatin immunoprecipitation assays were employed to identify proteins bound to the GREs of two liver-specific hormone-inducible genes, tyrosine aminotransferease and tryptophan oxygenase. Upon hormone treatment, GR activity was induced and shown to be associated in vivo with the GREs for both genes; and consistent with their previous experiments, both p23 and Hsp90 but not Hsp70 were recruited to the functional GR complex associated with the respective promoters. The combination of in vivo studies demonstrating that p23 and Hsp90 are associated with transcription factor complexes bound to hormone-response elements, transient transfection experiments in which p23 delivered to the activated hormone receptor causes repression, and in vitro studies indicating a reduction in hormone receptor DNA binding following p23 addition offers snapshots from which a new view (Figure 2B) on the role of chaperones in receptor recycling and disassembly can be proposed.

A series of discrete molecular events for intracellular receptor regulation, through the involvement of chaperones, becomes a continuum of assembly and disassembly events in which p23 and to a lesser extent Hsp90 serves more than one master; not only an essential role in the assembly of the ATP-dependent, hormone-responsive aporeceptor complex, but also in the inactivation and disassembly of the hormone receptor (Figure 1). The effects of p23 and Hsp90, however, are not limited to hormone receptors, as the targeting of these chaperones to NF-κB or AP1 sites also has inhibitory effects (Freeman and Yamamoto, 2002). Whether this reflects common mechanisms for inactivation and disassembly of a broadening class of inducible transcription factors remains for future studies. What we can conclude for now is that both p23 and Hsp90 are associated in vivo with transcription factor complexes and that the inhibitory effects of p23 on a cotransfected reporter are dramatic and suggest distinct functional specificities of chaperones.

Linking Stress-Induced Pathways by Chaperone Networks

Hormone-activated intracellular receptors share many features in common with the regulation of the heat shock response. Both are inducible responses that are regulated at the level of transcriptional control, involve families of transcription factors that cycle between inert and activated states by adopting multiple conformations, and exhibit kinetics of activation that are rapid and reversible. Additionally, chaperone networks are employed in both systems to maintain intracellular receptors and heat shock factor (HSF1) in an inert but responsive state, and again, to repress and disassemble the activated state.

At the heart of the heat shock response is the regulation of HSF1. Under "normal" conditions of cell growth, the majority of HSF1 exists in a repressed state associated transiently with molecular chaperones Hsp90, Hsp70, and Hsp40 and distributed in the cytoplasm or nucleus of human cells. In response to environmental and physiological stress signals, this equilibrium is shifted to a DNA bound trimeric state that subsequently becomes hyperphosphorylated and acquires transcriptional activity within minutes (Morimoto, 1998). The high level of heat shock gene transcription, induced by heat shock stress, occurs rapidly, transiently, and is autoregulated by molecular chaperones that feedback through HSF1 to attenuate transcription. The kinetics of activation, the duration, and magnitude of the transcriptional response are precisely titrated rather than an absolute "all out" response. Consequently, the heat shock response via regulation of HSF1 utilizes chaperone networks to maintain HSF1 as a "stress" sensor and to inactivate and disassemble the trimer.

In support of multiple roles for chaperones in autoreg-

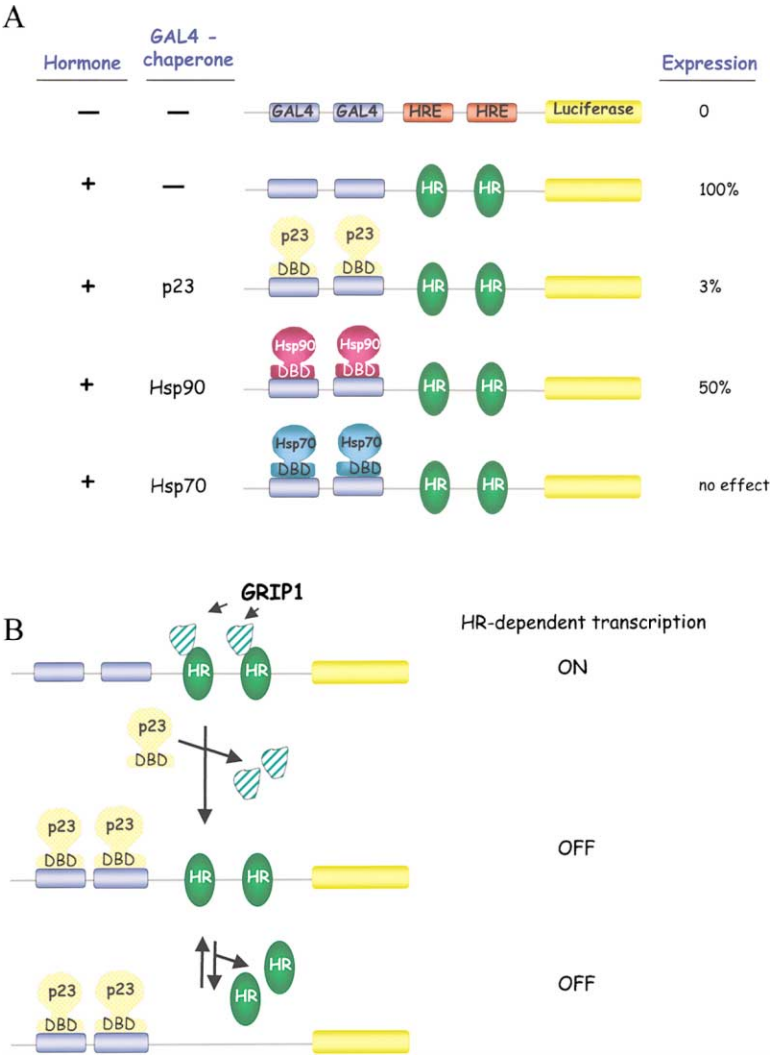

Figure 2. Role of p23 in the Disassembly of Transcription Complexes

(A) The "repression-in-*cis*" experiments require coexpression of chimeric Gal4-chaperone proteins together with a luciferase reporter regulated by a composite response element comprised of two hormone response elements adjacent to two Gal4 binding sites. Upon hormone stimulation, luciferase activity is induced. Coexpression of Gal4-p23, Gal4-Hsp90, or Gal4-Hsp70 differentially influences hormone receptor activity.

(B) p23 inactivates hormone receptors by competition with the coactivator GRIP1 and initiates the disassembly of the bound receptor from DNA. Binding of the ligand bound HR to DNA induces transcription; association with p23 competes with the coactivator GRIP1 (and perhaps release) and continued disassembly of HR from the DNA template.

ulation of HSF1 are observations from genetic, biochemical, and cell physiological studies on the transcriptional control of the Hsp70, Hsp40, and Hsp90 genes (Morimoto, 1998). For example, in the yeast *S. cerevisiae*, multiple Hsp70 genes are expressed; when the cytosolic Hsp70 gene, Ssa1, was overexpressed, the inducible expression from two other Hsp70 genes was dampened, and likewise expression of the Hsp70 gene, Ssa3, was induced in an HSF-dependent manner in cells deficient for two different Hsp70 genes. Similar roles for DnaJ proteins (Sis1, Ddj-1, and Hsp40) and Hsp90 in the negative regulation of the heat shock response, dependent upon HSF1 activity, have also been proposed (Guo et al., 2001). Biochemical studies on HSF1 have described interactions with Hsp70 and Hsp40 as the heat shock response attenuates (Abravaya et al., 1992). Moreover, conditional overexpression of either chaperone to the levels achieved during heat shock inhibited heat shock-induced transcription with little effect on the hyperphosphorylation or DNA binding of HSF1 (Shi et al., 1998). These observations collectively reveal that the heat shock transcriptional response activity is influenced by the levels of specific chaperones; that HSF1 DNA binding activity is negatively regulated by high levels of chaperones; and that the magnitude and duration of the heat shock response is autoregulated by direct binding of chaperones to the activated state of HSF1.

A Strategy for Chaperones in Dynamic Remodeling of Stress Signaling Events

An increasing number of cell signaling and biosynthetic pathways have been shown to involve molecular chaperones (Arbeitman and Hogness, 2000; Donze et al., 2001). Perhaps transcription factors such as hormone receptors and heat shock factor and other signaling molecules including kinases and caspases have complex folding requirements that may have arisen from exon shuffling and other evolutionary pressures, for which chaperones have become essential to ensure proper folding. Or did this arise because of the unique properties of chaperones and cochaperones to generate stable folded intermediates that have become prime targets in the design of regulatory complexes? From a systems perspective, the linkage of signaling pathways that feedback through a shared pool of chaperones posits the presence of hierarchical genetic networks through which changes in the cellular environment can

be transduced (Nollen and Morimoto, 2002). In support of this speculation, aberrant expression of individual chaperones or cochaperones influences proliferation and development by preventing or enhancing cell growth and cell death. Decreasing the levels of Hsp90, whether by mutation or by the Hsp90 inhibitor geldanamycin, uncovers a Pandora's box of developmental abnormalities in *Drosophila* and *Arabidopsis* (Queitsch et al., 2002). Likewise, heat shock and elevated levels of Hsp70 have growth inhibitory effects on signaling pathways (Song et al., 2001). As chaperones are highly conserved, this codependency between protein conformation, protein function, and the transcriptional control of intracellular signaling pathways provides the framework for adaptation and survival in a changing environment.

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