

Continuous recycling: a mechanism for modulatory signal transduction

Brian C. Freeman and Keith R. Yamamoto

Modulatory signal transduction commonly requires efficient 'on demand' assembly of specific multicomponent cellular machines that convert signals to cellular actions. This article suggests that for these signaling machines to detect and respond to fluctuations in signal strength, they must be continuously disassembled in an energy-dependent process that probably involves molecular chaperones.

Cellular signaling processes can be divided into two broad types: unidirectional terminal pathways, with endpoints such as cell differentiation, and readily reversible modulatory pathways, typified by endpoints such as metabolic changes induced by food intake. A given signaling component can function in both types of pathway, and the receptors for both classes must sense signals specifically. Both types of pathway can respond rapidly and robustly to the appearance of a signal by initiating cooperative interactions that drive the assembly of multicomponent 'transducing machines' that produce decisive endpoints.

However, such multicomponent complexes present special challenges for modulatory signaling pathways, which must sense both increasing and decreasing signal intensities, and must also terminate and reinitiate signaling efficiently. Because these pathways commonly include intermittent signaling patterns of short duration, degradation or other irreversible discard routes are not proficient general strategies for termination of signaling because reinitiation would be limited by the rate of reaccumulation of the regulator. Moreover, the functional complexes commonly assemble in cellular compartments far from the plasma membrane, and therefore cannot detect fluctuations in the levels of extracellular signals. Most importantly, the transducing machines assemble spontaneously, forming specific, intrinsically stable complexes in response to signal, thus raising questions about how disassembly is accomplished in response to a decline in signal intensity.

Modulatory signaling by the intracellular receptors demonstrates rather clearly some of these special challenges. This superfamily of proteins modulates specific gene transcription in response to programmed or induced changes in circulating levels of small hydrophobic signals such as glucocorticoid, estrogen,

retinoid and thyroid hormones. For purposes of illustration, we shall focus here on transcriptional activation mechanisms conferred by intracellular receptors, particularly by the glucocorticoid receptor (GR) (Fig. 1). The unliganded apoGR resides primarily in the cytoplasm in the absence of cognate hormonal signals. Upon hormone binding, the GR-hormone complex translocates to the nucleus, where it binds to specific genomic sites termed glucocorticoid response elements (GREs) and enhances transcription of nearby genes¹⁻⁴. Upon hormone withdrawal, transcriptional activation declines promptly in conjunction with changes in nearby chromatin structure⁵; physiological studies underscore the importance of decisively terminating intracellular receptor signaling^{6,7}. Importantly, both initiation and cessation of the signaling endpoint (i.e. regulated transcription) occur with $t_{1/2} \sim 5-10$ min, much shorter than the 11-25 h half-life of the receptor itself.

Molecular chaperone complexes have been shown to play important roles in signaling mediated through intracellular receptors, including signaling mediated by steroid, retinoid and ecdysone receptors⁸⁻¹⁰. Thus, heterotypic complexes containing chaperones (e.g. Hsp90, Hsp70, p23 and large immunophilins) and co-chaperones (e.g. Hsp40, Hip and HOP) associate with apoGR and facilitate high-affinity hormone binding. Although the chaperone complex components are thought to dissociate from GR upon hormone binding, it is notable that these components also are found in the nucleus and that they function in a similar manner on nuclear aporeceptor complexes¹¹. Indeed, recent findings reveal that molecular chaperone components participate in intracellular receptor functions well after initial hormone binding¹²⁻¹⁴.

Here we summarize some of the myriad molecular interactions that define transcriptional regulatory complexes at GREs; features of these complexes lead us to suggest that ancillary factors such as molecular chaperones might be essential for promoting continuous, energy-dependent disassembly of these and other transcriptional regulatory machines. We then consider the nature and role of the putative disassembly reactions in the broader contexts of modulatory signaling and other biological processes.

Triggering signaling pathways

Signaling within the intracellular receptor superfamily is triggered by binding of small hydrophobic ligands such as steroids or thyroid hormone to the ligand binding domains (LBDs) of cognate receptors. There are two striking features of these interactions. First, the overall tertiary structure of the LBDs is highly conserved, despite wide variation in LBD primary sequences and ligand structures¹⁵. Second, the bound ligands are completely buried within the LBD, serving as part of the hydrophobic core of the domain¹⁶. Structural studies reveal extensive ligand-LBD contacts that account for the high potency and

Brian C. Freeman
Keith R. Yamamoto*
Dept of Cellular and
Molecular Pharmacology,
University of California,
San Francisco, 513
Parnassus, San Francisco,
CA 94143-0450, USA.
*e-mail:
yamamoto@cgl.ucsf.edu

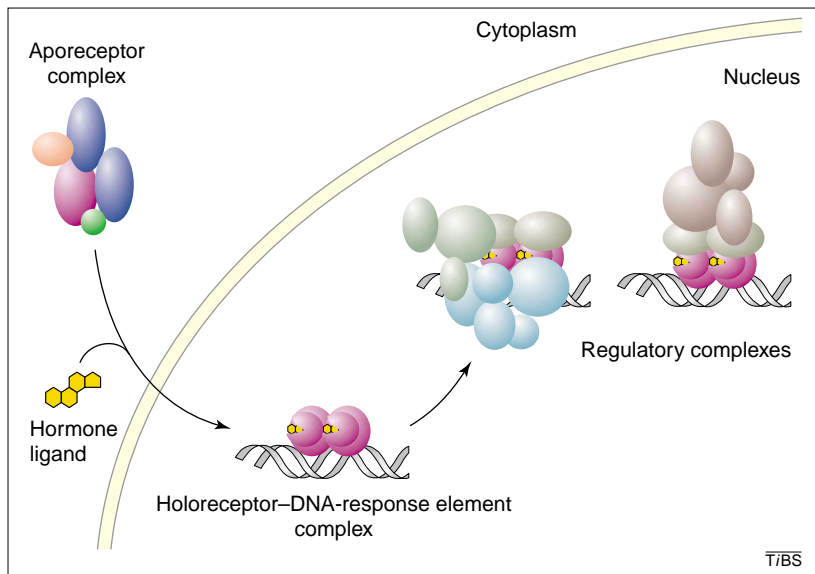


Fig. 1. Initiation of glucocorticoid receptor (GR) signaling. In the absence of ligand, apoGR (magenta) associates with molecular chaperones (cytosolic factors; dark blue, green or orange) and resides in the cytoplasm as a result of inactivation of nuclear localization and DNA-binding activity. Following ligand binding, holoGR translocates to the nucleus, associates with response elements and assembles into multiprotein regulatory complexes. These can include chromatin-remodeling and coactivator complexes (nuclear factors; tan, light blue or light green). The regulatory complexes, in turn, modulate transcription by direct or indirect interaction with the transcription initiation apparatus (not shown).

specificity of signaling by these compounds; progesterone, for example, is contacted by 18 amino-acid side chains in the progesterone receptor LBD (Ref. 17). Agonist binding triggers conformational changes in the LBD that create a novel protein-protein interface¹⁸ capable of binding to 'coactivator' proteins, which stimulate gene transcription.

The association of coactivators with the ligand-LBD complex stabilizes ligand binding¹⁹ and also serves as an early step in a cascade of further intermolecular interactions that produce site-specific transcriptional regulatory machines at cognate genomic response elements (REs) (Fig. 1). The IR-RE interactions are characterized by approximately nanomolar affinities²⁰ and are further stabilized by interactions with adjacent DNA-bound proteins²¹. The precise composition and function of the complexes vary in different cell and response element contexts²²⁻²⁴, but the final assemblies comprise many components and activities. Thus, ligand binding within the LBD core nucleates differential assembly of functional regulatory complexes containing several megadaltons of protein. This scheme seems ideal for establishing and maintaining a stable ligand-induced effect. However, these multiple layers of protein, secured by cooperative interactions and encapsulating hydrophobic ligands, bring into sharp focus a crucial issue: how might these complexes be disassembled to allow modulation or termination of intracellular receptor signaling events?

Physiological fluctuations in signaling

Metazoans maintain physiological balance, in part, through the production and recognition of hormonal ligands. For example, steroidogenesis in various endocrine organs is controlled by neuroendocrine signaling; the resultant steroid ligands circulate in the blood, cross cell membranes and are recognized by cognate receptors. Importantly, the signaling and response systems must be sensitive to fluctuations in signal strength that are either programmed, as with

diurnal cycling of cortisol levels, or induced, as with changes in cortisol levels provoked by food consumption or exercise. In humans, serum cortisol increases or decreases ~3-5 fold over 2-4 h periods in conjunction with these events (Fig. 2a)⁶. In the case of estradiol in rodents, the serum levels decline sixfold over an ~2 h period at the estrus-proestrus interface (Fig. 2b)⁷. To respond to such fluctuations requires mechanisms for efficient disassembly of intracellular receptor-ligand-triggered regulatory machines. In principle, disassembly could be driven either by degradation or by dissociation and recycling of components.

Receptor degradation

Conceivably, a declining level of circulating hormone might induce degradation of an intracellular receptor-triggered transcriptional regulatory complex. Such a mechanism is used in certain signaling pathways including those governed by hypoxia-inducible factors (HIFs) or nuclear factor κ B (NF- κ B). For example, NF- κ B is maintained in a quiescent state by associating with I κ B (inhibitor of NF- κ B), and NF- κ B signaling is triggered upon degradation of I κ B (Ref. 25). For hypoxia signaling, an HIF subunit fails to accumulate under normoxia because it is immediately degraded, whereas it is stabilized and functional under hypoxic conditions²⁶. However, receptor turnover seems unlikely as a general mechanism for modulation of intracellular receptor-mediated effects. First, it fails to explain how the hormone-receptor complex, embedded in a several megadalton machine and bound to genomic DNA within the cell nucleus, can sense and respond to changes in hormone levels in the bloodstream. Second, both aporeceptors and holoreceptors are relatively stable, with half-lives longer than the time course of serum ligand fluctuations and the responses to those fluctuations. Moreover, the effects of hormones on intracellular receptor stability are typically in the wrong direction to account for the observed modulations^{27,28}.

For example, the estrogen receptor (ER) is destabilized in the presence of estradiol: its half-life declines from ~4 h to ~3 h, and steady-state levels drop by 5-10-fold with chronic hormone treatment^{29,30}. Similarly, glucocorticoids reduce GR half-life from ~25 h to ~11 h, with an accompanying approximately threefold reduction in steady-state levels³¹. Inhibition of proteasome function leads to a decrease in ER transcriptional activation activity, yet receptor protein levels do not decrease^{27,32}; in the case of GR, neither activity nor protein levels are affected by proteasome inhibitors²⁷. In addition, it has been observed for GR that addition of ligand can lead to an increase in receptor protein levels²⁸. These findings indicate that the mechanisms of hormone-induced destabilization of steroid receptors are unknown, and that the alterations cannot account for declines in signaling upon hormone withdrawal.

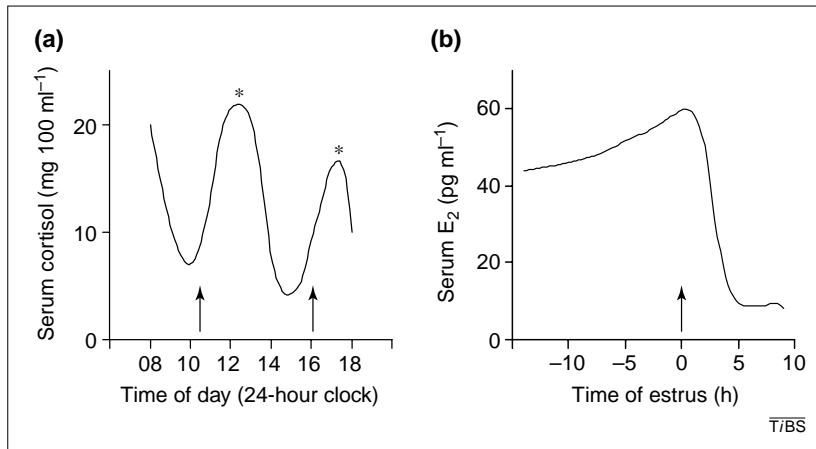


Fig. 2. Serum hormone levels fluctuate. (a) Serum cortisol levels fluctuate in humans diurnally with sleep and wake cycles, and are also modulated by food consumption (*) and stress or exercise (arrows). (b) In rodents, serum estradiol reaches high levels preceding ovulation (arrow), then declines sharply following ovulation. The graphs are composites adapted from the work of many labs (for specific examples see Refs 6,7).

Therefore, current evidence fails to support a receptor degradation model for intracellular receptor responsiveness to hormonal fluctuations. Thus, although there might be cases in which proteolysis is used for such regulation, we suggest that hormonal effects on receptor turnover probably serve to buffer responses to chronic hormone exposure, rather than to sensitize signaling pathways to fluctuations in signal strength.

Induced or continuous disassembly

The other general mechanistic class for responding to changes in intracellular receptor signal levels is the induced or continuous disassembly of intracellular receptor-containing transcriptional regulatory complexes. An 'induced' disassembly model suffers from the same problem as the induced degradation scheme because the large genome-bound complex cannot monitor changes in serum hormone levels. In the 'continuous' dissociation model, both the regulatory machines and the receptor-hormone complex itself are dynamic, rapidly dissociating into components that are competent for reassembly in the presence of sufficient levels of intracellular hormone. According to this scheme, termination of intracellular receptor signaling would not require a separate mechanism, but rather would be an intrinsic consequence of the dynamic receptor-hormone interaction.

Recent findings are consistent with a continuous disassembly hypothesis. Using GR-green fluorescent protein fusions to visualize the dynamics of holoGR-GRE complexes *in vivo*³³, it was discovered that the half-life of these complexes is in the order of seconds. Thus, despite the long duration ($t_{1/2} > 100$ min) of GR-GRE complexes *in vitro*³⁴, *in vivo*, these complexes (adorned with additional molecular interactions that should in principle stabilize them further) nevertheless are released from the DNA at rates more than three orders of magnitude higher than those observed *in vitro*. Regardless of the mechanism for this highly dynamic behavior *in vivo*, it clearly provides a sensitive way for intracellular receptors to monitor and respond to rapid fluctuations in serum hormone levels. Presumably, the hormone is also released from the dissociated receptor, and the

newly generated unliganded receptor is converted to a form competent for hormone binding and for all subsequent holoreceptor functions; that is, the receptor is continuously recycled.

In early studies, it was thought that receptors such as GR might need to return to the cytoplasm to interact again with molecular chaperone complexes and to regain hormone-binding competence^{35,36}. However, it is now apparent that molecular chaperones are present in both the cytoplasm and the nucleus, and that aporeceptor-molecular chaperone complexes can form within the nucleus following ligand withdrawal¹¹. What, then, are the requirements and challenges of continuous recycling, and how might molecular chaperones participate?

Role of molecular chaperones in continuous recycling

At least three of the intermolecular interactions involved in forming functional transcriptional regulatory complexes – hormone with LBD, holoreceptor with response element and holoreceptor with coactivator – are specific and relatively long-lived *in vitro*, raising the possibility that additional factors might be essential for the rapid reversal of those contacts *in vivo*. Consideration of the steroid-receptor interaction alone underscores the point: a small hydrophobic molecule embedded in a hydrophobic binding pocket in which it is contacted by ~20 amino-acid side chains would have little probability of spontaneously breaking these contacts and re-entering an aqueous environment. The detailed composition, structures and stabilities of the full transcriptional regulatory machines are not known, but many more interactions are clearly implicated, elevating further the complexity of disassembling these machines.

Molecular chaperones appear to be strong candidates as factors that might effect continuous dissociation and recycling because: (1) they interact physically and functionally with intracellular receptor LBDs; (2) they harbor ATPase activities that could drive disassembly of hydrophobic interactions; and (3) they can promote dissociation of intracellular receptors from coactivators and response elements. Moreover, there is precedent for chaperone participation in modulatory signaling pathways; for example, the oxygen-dependent proteasomal degradation of the HIF α subunits is facilitated by the Von Hippel-Lindau (pVHL)-elongin complex³⁷, which is assembled by the chaperonin TRiC (Ref. 38).

Freeman *et al.*¹⁴ demonstrated that the purified p23 molecular chaperone can disrupt thyroid hormone receptor (TR)-DNA complexes *in vitro* and that p23 binds preferentially to the holoTR-response element ternary complex. In addition, p23 appears to compete with a coactivator for association with the TR LBD. These opposing interactions have opposite effects on the stability of the receptor-DNA interaction. Moreover, coactivator binding increases the apparent affinity

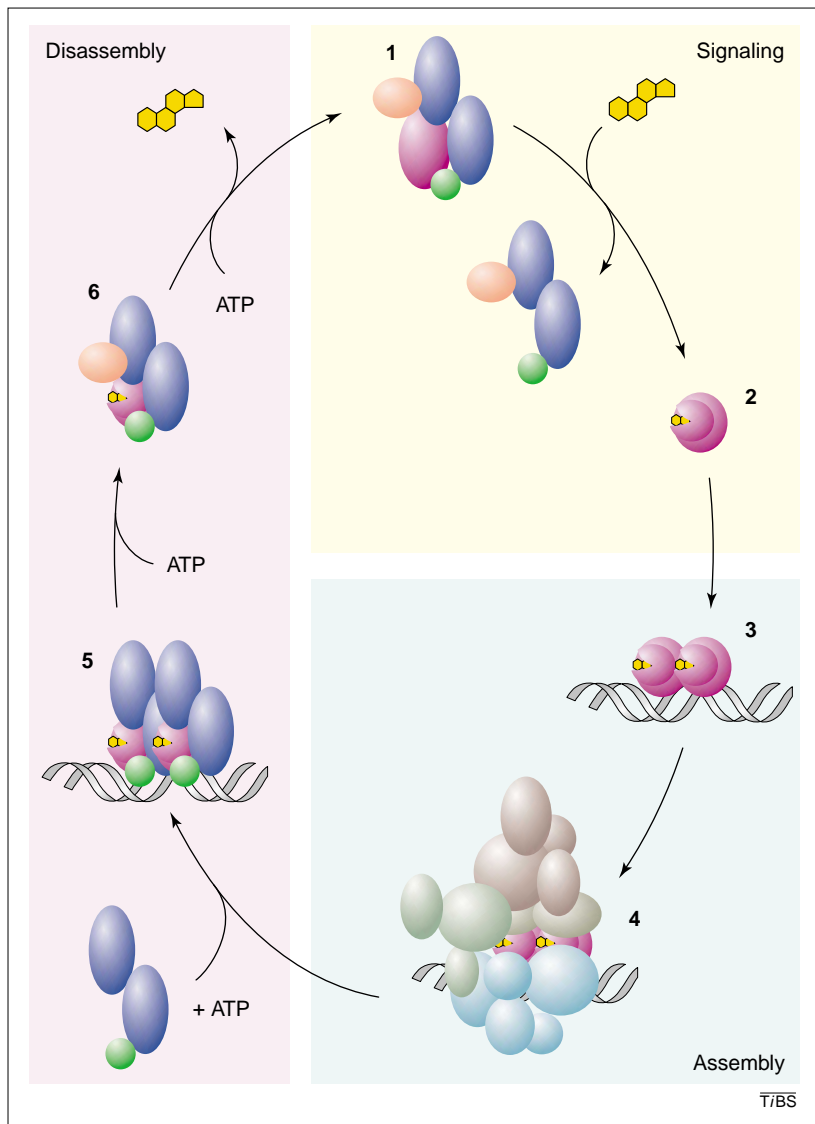


Fig. 3. Continuous recycling of components in regulatory complexes containing intracellular receptors. In the absence of ligand, apoGR (magenta) associates with molecular chaperone complexes (dark blue, green or orange) (1). Following ligand binding, the GR-hormone complexes (2) associate with GREs (3), and these ternary complexes recruit additional regulatory factors in a context-specific manner to form regulatory complexes (4). We propose that the holoreceptor within regulatory complexes is also a substrate for molecular chaperones (dark blue, green), which consume energy to drive an overall disassembly process that involves three events: release of the nonreceptor factors (4 → 5), release of the receptor from DNA (5 → 6) and release of hormone from the receptor (6 → 1); the actual order of the disassembly process, and the steps requiring energy expenditure, are unknown. This three-phase recycling process, comprising signaling, assembly and disassembly phases, sensitizes the receptor to periodic fluctuations in hormone levels. Abbreviations: GR, glucocorticoid receptor; GRE, glucocorticoid response element.

of hormone binding by 20–60-fold¹⁹, so p23 might act directly or indirectly to facilitate hormone release. Whether p23 plays a role in dissociation of other components in the regulatory machinery has not been examined.

Hsp90, a member of the molecular chaperone complex that interacts directly with intracellular receptor LBDs, also might have a role in receptor recycling. Thus, inhibitors of Hsp90 abrogate release of GR from chromatin following hormone withdrawal¹³, and elevated levels of nuclear Hsp90 lead to reduced GR activity after prolonged hormone treatment¹². It has not yet been determined whether Hsp90 acts

directly or through association with p23. Additionally, purified Hsp90 can dissociate ER-ERE (estrogen receptor response element) complexes *in vitro*³⁹.

Together with dissociation of the holoreceptor from the RE, the intracellular receptor-hormone complex must be disassembled and the aporeceptor re-established to reset the signaling 'trigger'. In our model, intracellular receptor-mediated signaling is a cyclical process comprising three distinct phases (Fig. 3). The first two phases, signaling and assembly, involve binding of the cognate ligand by receptor which, in turn, drives cooperative assembly of regulatory complexes. The third phase, disassembly, disrupts the regulatory complexes including the intracellular receptor-hormone association. Given the spontaneous and cooperative formation of the complexes, disassembly would require expenditure of energy. We suggest that disassembly might be driven by the ATPase activities associated with molecular chaperone complexes.

Notably, high-affinity hormone binding by newly synthesized apoGR requires that the receptor associates with Hsp90 (Refs 40,41), an interaction that is facilitated by Hsp70 (Ref. 42); parallel studies with various intracellular receptors suggest that co-chaperones such as Hsp40 and HOP also contribute to the establishment of ligand binding activity⁸. Thus, according to our model, molecular chaperone components would 'bridge' the cycle by functioning in both the disassembly and signaling phases (Fig. 3).

Perspectives

In this article we have focused on intracellular receptor action to illustrate a crucial feature of modulatory signaling pathways – the requirement to sense and respond efficiently to fluctuations in the level of the signaling ligand – and the mechanistic implications of this requirement. Ligands for intracellular receptors trigger assembly of multifactor regulatory complexes that include both receptor and ligand, and the specificity and affinity of the intermolecular interactions enable strong selectivity in the structure and activity of the assembled machines. By contrast, the specificity and affinity vital to the assembly of these machines, together with their intranuclear location far from the circulating ligand, run counter to the efficient complex disassembly and receptor recycling needed for modulation and termination of signaling. We propose that responsiveness to signal fluctuations could be achieved by an energy-requiring process of continuous recycling, perhaps conferred by molecular chaperone complexes.

Many biological processes employ precision assemblies of multicomponent machines to carry out complex reactions at particular points in a multistep process, and then require efficient alteration of these machines for subsequent steps in the process. In this sense, the requirements of the processes for disassembly and perhaps for recycling of components

are similar to those of modulatory signaling. We illustrate this point with a few brief examples:

- The mechanics of translation termination and reinitiation⁴³ appear to parallel our proposed recycling pathway for modulatory signaling complexes; both involve ancillary factors and require an energy source. As in our intracellular receptor recycling model, ribosomal subunits are disassembled and re-assembled using a common factor, dissociation factor/initiation factor-3 (DF/IF-3). Thus, DF/IF-3 serves as a bridging component that promotes recycling of the ribosomal subunits. Before the transition to recycling, the 70S ribosome is disassembled in an energy-dependent process that involves the auxiliary factors ribosome recycling factor (RRF) and elongation factor-G (EF-G).
- As a second example, consider mRNA nuclear export. Newly synthesized mRNAs associate with at least 30 proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) in the nucleus^{44,45}. Some of the associated factors are exported to the cytoplasm with the mRNA and shuttle back to the nucleus; however, most remain nuclear^{44,46}. Thus, a specific subset of nuclear proteins and hnRNPs dissociates from the hnRNP-mRNA-protein complex before export, a process that presumably produces the export-competent species and is driven, at least in part, by the DEAD-box ATPase Dbp5 (Ref. 47). (DEAD-box protein family members contain the Asp-Glu-Ala-Asp motif and unwind RNA in an ATP-dependent manner.) Upon export and cytoplasmic localization, the shuttling factors are thought to dissociate and return to the nucleus,

competent for another round of export. We suggest that both steps of this dynamic reconfiguration of the mRNA export and shuttling machineries require energy input, perhaps provided in some cases by molecular chaperones.

- A third case involves the spliceosome. This is a highly dynamic apparatus that is rapidly assembled, rearranged and disassembled during the process of pre-mRNA splicing⁴⁸. Precise removal of intronic sequences minimally involves a six-step process that uses distinct snRNP complexes. Transitions between each stage are dependent upon both ancillary factors and energy expenditure; a final disassembly step involving ATP hydrolysis and protein factors Prp22 and Prp43 regenerates the various snRNP components for additional rounds of splicing. Certain factors, such as Prp22, Prp24 and Prp43, might serve recycling or bridging roles, in some cases energy-dependent, that facilitate both termination and reinitiation of splicing. Additionally, the spliceosome component U5-116kD, which is related to EF-2, might provide energy for recycling of certain snRNP complexes⁴⁹.

In general then, we anticipate that multicomponent machineries that mediate a broad range of complicated biological processes might employ energy-consuming ancillary factors to drive expeditious transitions between functional complexes in stepwise pathways or cycles. In many of these cases, it seems likely that the energy-requiring steps serve also to help keep the complexes discrete and distinct, their stable states separated by an energy barrier.

Acknowledgements

We appreciate helpful comments on the manuscript by D. Agard, R. Derynck, M. Diamond, C. Guthrie, H. Luecke, R. Nissen, M. Van Gilst and J. Weissman. Our apologies to the many colleagues whose original manuscripts could not be cited owing to space constraints. B.C.F. was supported by a fellowship from the American Heart Association. Research support was from the National Science Foundation and National Institutes of Health.

References

- Baxter, J.D. and Tomkins, G.M. (1970) The relationship between glucocorticoid binding and tyrosine aminotransferase induction in hepatoma tissue culture cells. *Proc. Natl. Acad. Sci. U. S. A.* 65, 709–715
- Wira, C.R. and Munck, A. (1974) Glucocorticoid-receptor complexes in rat thymus cells. 'Cytoplasmic'-nuclear transformations. *J. Biol. Chem.* 249, 5328–5336
- Ringold, G.M. (1979) Glucocorticoid regulation of mouse mammary tumor virus gene expression. *Biochem. Biophys. Acta* 560, 487–508
- Ucker, D.S. and Yamamoto, K.R. (1984) Early events in the stimulation of mammary tumor virus RNA synthesis by glucocorticoids. Novel assays of transcription rates. *J. Biol. Chem.* 259, 7416–7420
- Zaret, K.S. and Yamamoto, K.R. (1984) Reversible and persistent changes in chromatin structure accompany activation of a glucocorticoid-dependent enhancer element. *Cell* 38, 29–38
- Brandenberger, G. and Follenius, M. (1975) Influence of timing and intensity of muscular exercise on temporal patterns of plasma cortisol levels. *J. Clin. Endocrinol. Metab.* 40, 845–849
- Banks, P.K. *et al.* (1991) Regulation of ovarian steroid biosynthesis by estrogen during prooestrus in the rat. *Endocrinology* 129, 1295–1304
- Pratt, W.B. and Toft, D.O. (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr. Rev.* 18, 306–360
- Holley, S.J. and Yamamoto, K.R. (1995) A role for Hsp90 in retinoid receptor signal transduction. *Mol. Biol. Cell* 6, 1833–1842
- Arbeiterman, M.N. and Hogness, D.S. (2000) Molecular chaperones activate the *Drosophila* ecdysone receptor, an RXR heterodimer. *Cell* 101, 67–77
- Hache, R.J. *et al.* (1999) Nucleocytoplasmic trafficking of steroid-free glucocorticoid receptor. *J. Biol. Chem.* 274, 1432–1439
- Kang, K.I. *et al.* (1999) The molecular chaperone Hsp90 can negatively regulate the activity of a glucocorticosteroid-dependent promoter. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1439–1444
- Liu, J. and DeFranco, D.B. (1999) Chromatin recycling of glucocorticoid receptors: implications for multiple roles of heat shock protein 90. *Mol. Endocrinol.* 13, 355–365
- Freeman, B.C. *et al.* (2000) The p23 molecular chaperones act at a late step in intracellular receptor action to differentially affect ligand efficacies. *Genes Dev.* 14, 422–434
- Weatherman, R.V. *et al.* (1999) Nuclear-receptor ligands and ligand-binding domains. *Annu. Rev. Biochem.* 68, 559–581
- Wagner, R.L. *et al.* (1995) A structural role for hormone in the thyroid hormone receptor. *Nature* 378, 690–697
- Williams, S.P. and Sigler, P.B. (1998) Atomic structure of progesterone complexed with its receptor. *Nature* 393, 392–396
- Darimont, B.D. *et al.* (1998) Structure and specificity of nuclear receptor-coactivator interactions. *Genes Dev.* 12, 3343–3356
- Gee, A.C. *et al.* (1999) Coactivator peptides have a differential stabilizing effect on the binding of estrogens and antiestrogens with the estrogen receptor. *Mol. Endocrinol.* 13, 1912–1923
- Perlmann, T. *et al.* (1990) Quantitative analysis of the glucocorticoid receptor-DNA interaction at the mouse mammary tumor virus glucocorticoid response element. *J. Biol. Chem.* 265, 17222–17229
- Boonyaratankornkit, V. *et al.* (1998) High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding *in vitro* and transcriptional activity in mammalian cells. *Mol. Cell. Biol.* 18, 4471–4487
- Yamamoto, K.R. (1985) Steroid receptor regulated transcription of specific genes and gene networks. *Annu. Rev. Genet.* 19, 209–252
- Tsai, M.J. and O'Malley, B.W. (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.* 63, 451–486
- Moras, D. and Gronemeyer, H. (1998) The nuclear receptor ligand-binding domain: structure and function. *Curr. Opin. Cell Biol.* 10, 384–391

- 25 Baldwin, A.S. (1996) The NF- κ B and I κ B proteins: new discoveries and insights. *Annu. Rev. Immunol.* 14, 649–683
- 26 Wenger, R.H. (2000) Mammalian oxygen sensing, signaling and gene regulation. *J. Exp. Biol.* 203, 1253–1263
- 27 Lonard, D.M. *et al.* (2000) The 26S proteasome is required for estrogen receptor- α and coactivator turnover and for efficient estrogen receptor- α transactivation. *Mol. Cell* 5, 939–948
- 28 Denton, R.R. *et al.* (1993) Differential autoregulation of glucocorticoid receptor expression in human T-cell and B-cell lines. *Endocrinology* 133, 248–256
- 29 Gorski, J. *et al.* (1974) The regulation of uterine concentration of estrogen binding protein. *Adv. Biosci.* 7, 5–10
- 30 Nardulli, A.M. and Katzenellenbogen, B.S. (1986) Dynamics of estrogen receptor turnover in uterine cells *in vitro* and *in uteri in vivo*. *Endocrinology* 119, 2038–2046
- 31 Dong, Y. *et al.* (1988) Regulation of glucocorticoid receptor expression: evidence for transcriptional and posttranslational mechanisms. *Mol. Endocrinol.* 2, 1256–1264
- 32 Lange, C.A. *et al.* (2000) Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1032–1037
- 33 McNally, J.G. *et al.* (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science* 287, 1262–1265
- 34 Perlmann T. *et al.* (1990) Quantitative analysis of the glucocorticoid receptor–DNA interaction at the mouse mammary tumor virus glucocorticoid response element. *J. Biol. Chem.* 265, 17222–17229
- 35 Munck, A. *et al.* (1972) Glucocorticoid–receptor complexes and the earliest steps in the action of glucocorticoids on thymus cells. *J. Steroid Biochem.* 3, 567–578
- 36 Kassib, J.A. and Gorski, J. (1981) Estrogen receptor replenishment. Evidence for receptor recycling. *J. Biol. Chem.* 256, 7378–7382
- 37 Maxwell, P.H. *et al.* (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399, 271–275
- 38 Feldman, D.E. *et al.* (1999) Formation of the VHL–elongin BC tumor suppressor complex is mediated by the chaperonin TRiC. *Mol. Cell* 4, 1051–1061
- 39 Sabbah, M. *et al.* (1996) The 90 kDa heat-shock protein (hsp90) modulates the binding of the oestrogen receptor to its cognate DNA. *Biochem. J.* 314, 205–213
- 40 Wheeler, R.H. *et al.* (1981) Glucocorticoid receptor activation and inactivation in cultured human lymphocytes. *J. Biol. Chem.* 256, 434–441
- 41 Mendel, D.B. *et al.* (1986) Glucocorticoid receptors lacking hormone-binding activity are bound in nuclei of ATP-depleted cells. *Nature* 324, 478–480
- 42 Morishima, Y. *et al.* (2000) Stepwise assembly of a glucocorticoid receptor.hsp90 heterocomplex resolves two sequential ATP-dependent events involving first hsp70 and then hsp90 in opening of the steroid binding pocket. *J. Biol. Chem.* 275, 18054–18060
- 43 Kisselev, L.L. and Buckingham, R.H. (2000) Translational termination comes of age. *Trends Biochem. Sci.* 25, 561–566
- 44 Krecic, A.M. and Swanson, M.S. (1999) hnRNP complexes: composition, structure, and function. *Curr. Opin. Cell Biol.* 11, 363–371
- 45 Nakielnny, S. and Dreyfuss, G. (1999) Transport of proteins and RNAs in and out of the nucleus. *Cell* 99, 677–690
- 46 Pinol-Roma, S. and Dreyfuss, G. (1991) Transcription-dependent and transcription-independent nuclear transport of hnRNP proteins. *Science* 253, 312–314
- 47 Snay-Hodge, C.A. *et al.* (1998) Dbp5p/Rat8p is a yeast nuclear pore-associated DEAD-box protein essential for RNA export. *EMBO J.* 17, 2663–2676
- 48 Staley, J.P. and Guthrie, C. (1998) Mechanical devices of the spliceosome: motors, clocks, springs, and things. *Cell* 92, 315–326
- 49 Fabrizio, P. *et al.* (1997) An evolutionarily conserved U5 snRNP-specific protein is a GTP-binding factor closely related to the ribosomal translocase EF-2. *EMBO J.* 16, 4092–4106

