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At the Cutting Edge

Variable steroid receptor responses: Intrinsically disordered AF1 is the key

S. Stoney Simons Jr. [a,*], Raj Kumar [b,*]

[a] Steroid Hormones Section, NIDDK/LERB, National Institutes of Health, Bethesda, MD, United States
[b] Department of Basic Sciences, The Commonwealth Medical College, Scranton, PA, United States

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ABSTRACT

Steroid hormones, acting through their cognate receptor proteins, see widespread clinical applications due to their ability to alter the induction or repression of numerous genes. However, steroid usage is limited by the current inability to control off-target, or non-specific, side-effects. Recent results from three separate areas of research with glucocorticoid and other steroid receptors (cofactor-induced changes in receptor structure, the ability of ligands to alter remote regions of receptor structure, and how cofactor concentration affects both ligand potency and efficacy) indicate that a key element of receptor activity is the intrinsically disordered amino-terminal domain. These results are combined to construct a novel framework within which to logically pursue various approaches that could afford increased selectivity in steroid-based therapies.

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Steroid receptors (SRs) induce and repress gene transcription by binding to response elements in chromatin. The treatment of numerous human pathologies (e.g., inflammation, cancer, and cardiovascular disease) with SR ligands (Anbalagan et al., 2012) is complicated by the current inability to restrict SR actions to specific organ/gene targets, which is the "Holy Grail" for steroid/hormone therapies. One attractive, but limited, approach is the development of selective receptor modulators (SRMs) that regulate a subset of the normal gene repertoire (Zajchowski et al., 2000; Frasor et al., 2004; Kazmin et al., 2006; Robertson et al., 2010; Wardell et al., 2012). Here we outline a new approach to understanding steroid receptor specificity involving intrinsically disordered domains (IDs) of SRs. These IDs act as molecular rheostats to support a continuum of conformational states and interactions with multiple coregulators to generate potentially highly specialized clinical responses.

SRs regulate gene transcription via dynamic, reversible and competitive interactions with sequence-specific response elements in chromatin and subsequent reversible assembly with cofactors (McNally et al., 2000; Wang et al., 2007). Of the two activation functions in SRs, the N-terminal AF1 sequence is often more active than the AF2 sequence in the C-terminal ligand binding domain (LBD) (Hollenberg and Evans, 1988; Chen et al., 2006; Choudhry et al., 2006; Huet et al., 2009). As with the AF2 domain, coactivators such as SRC-1 and TIF2 can also increase the transcriptional activity of the AF1 domain (Onate et al., 1998; Kitagawa et al., 2002; Hill et al., 2009). Nevertheless, understanding AF1 function has languished because of their ID conformations, commonly found in many transcription factors (Dunker and Uversky, 2008; Kumar and McEwan, 2012). Interestingly, an amino-terminal fragment of a classical SR coactivator, TIF2, binds to the N-terminal domain of both glucocorticoid and progesterone receptors (Wang et al., 2007). This TIF2 fragment also increases the α-helical content of the glucocorticoid receptor ID AF1 domain, suggesting that coactivators augment the transcriptional activity of SR-agonist complexes by inducing more ordered structures beyond the LBD/AF2 region (Khan et al., 2012). Such induced folding may be general among steroid receptors as witnessed by Jun dimerization protein 2 (JDP2), which enhances the transcriptional activity of the amino-terminal domain of progesterone receptors by increasing the α-helical content and stability of the intrinsically disordered amino-terminal domain. (Hill et al., 2009). Similarly, induced folding of the N-terminal domain of mineralocorticoid receptors by trimethylamine N-oxide (TMAO) enhanced protein–protein binding with a number of coregulatory proteins, including the coactivator cAMP response element-binding protein-binding protein and the corepressors SMRT and RIP140 (Fischer et al., 2010). These coupled

* Corresponding authors. Address: Bldg. 10, Room 8N-307B, NIDDK/CEB, NIH, Bethesda, MD 20892-1772, United States. Tel.: +1 301 496 6796; fax: +1 301 402 3572 (S.S. Simons Jr.). Address: Department of Basic Sciences, The Commonwealth Medical College, 525 Pine Street, Scranton, PA 18509, United States. Tel.: +1 570 504 9675; fax: +1 570 504 9660 (R. Kumar).

E-mail addresses: stoneys@helix.nih.gov (S.S. Simons Jr.), rkumar@tcmedc.org (R. Kumar).

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binding and folding processes may be modifiable by drugs. Furthermore, the ID AF1 may also induce local unfolding within adjacent structured SR sequences and facilitate allosteric communication between these domains (Motlagh and Hilser, 2012). Finally, the size of AF1 domains in SRs is often quite different. It may be significant that the length of the N-terminal domain correlates with AF1 "strength" (Kumar and McEwan, 2012).

SRMs have the clinically useful but enigmatic property of evoking anywhere from full agonist to full antagonist activity in a gene/tissue-dependent manner. Thus SRMs can display between 100% and 0% efficacy. This variability is thought to result from allosteric and functional synergy between AF1 and AF2 (Hollenberg and Evans, 1988; Frasor et al., 2004), although similar changes in the activity of glucocorticoid complexes can occur in the absence of the N-terminal domain (Cho et al., 2005). Because crystal structures of only LBD/AF2 are available (Brzozowski et al., 1997), the current design of SRMs is primarily based on their modulation of coregulatory protein motif (e.g., LxxLL) interactions with AF2 to further perturb the binding of cofactors (Brzozowski et al., 1997; Johnson and O'Malley, 2012). However, this and related strategies often fail to inactivate AF1 (Shang and Brown, 2002; Shiau et al., 2002; Simons, 2010), leading to unwanted side-effects during endocrine-based therapies. For example, simply changing cofactor concentrations can influence the amount of agonist activity, or efficacy, of SRMs (Simons, 2003, 2010). The above ability of cofactors to alter AF1 conformation plus the capacity of inter-domain coupling to modify the stabilities of SR microstates (Motlagh and Hilser, 2012) suggest that small molecules could tune SRM activities. An example of this approach is EP1-001, which binds the androgen receptor's ID AF1 and inhibits AF1-coactivator interaction. EP1-001 also prevents transactivation of androgen receptor AF1 on target genes without attenuating transcriptional activities of related SRs (Andersen et al., 2010). Importantly, EP1-001 blocks androgen-induced proliferation and causes cytoreduction of castration-recurrent prostate cancer in xenografts dependent on androgen receptor for growth and survival without causing toxicity in other tissues (Andersen et al., 2010), thus revealing the potential of targets outside of the LBD pocket to be tissue-specific modulators of AF1 activity.

Numerous studies over the past decade with various steroid receptors have demonstrated that changing cofactor concentrations can produce a sliding scale of values not just for the total amount of gene expression and/or SRM activity (Heemers et al., 2009; Johnson and O'Malley, 2012) but also for steroid potency, which is defined by the position of the dose–response curve (or EC_{50}) (Simons, 2003; Ong et al., 2010; Simons, 2010; Zhang et al., 2013). As exemplified by Drosophila development in response to ecdysone, differences in steroid potency can be an extremely effective method for generating selective gene expression (Karim and Thummel, 1992). It is now known that cofactors can interact with both N- and C-terminal regions of SRs (Bevan et al., 1999; Benecke et al., 2000; Kumar and Thompson, 2003; Tian et al., 2006; Kressler et al., 2007; Wang et al., 2007) in ways that influence the conformation of at least the AF1 ID domain (Hill et al., 2009; Khan et al., 2012) and probably the distribution of microstates for SR as a whole (Motlagh and Hilser, 2012). Furthermore, considerations of both A_{max} (or maximal efficacy) and EC_{50} yield previously inaccessible information about the kinetic mode and site of action (Ong et al., 2010; Dougherty et al., 2012; Zhang et al., 2013), which will be invaluable in fine-tuning SR activities (Simons, 2010).

The emerging picture from studies with several SRs, therefore, is that different surfaces within the SRs' AF1 may be created and used to manipulate gene expression (Kumar and McEwan, 2012). Cell/tissue-specific effects of SRs are tightly regulated through specific kinase(s)/phosphatase(s) and site-specific phosphorylation-induced conformational changes in AF1 that have been correlated with its interaction with specific coactivators and subsequent gene expression (Garza et al., 2010). Studies of N/C-terminal interactions illustrate the importance of both AF1 and AF2 for target gene expression and provide a starting point for evaluating mechanisms for this selectivity, which are likely to involve specific protein–protein interactions and post-translational modifications. ID AF1s may act as molecular rheostats to support a continuum of conformational states and transitions capable of mediating highly specific interactions with multiple coregulators. Most known SR phosphorylation sites and tissue-specific SR splice variants are located within the N-terminal domain, which can modulate AF1 conformation, giving rise to multivalent interactions and thereby regulating diverse cellular processes.

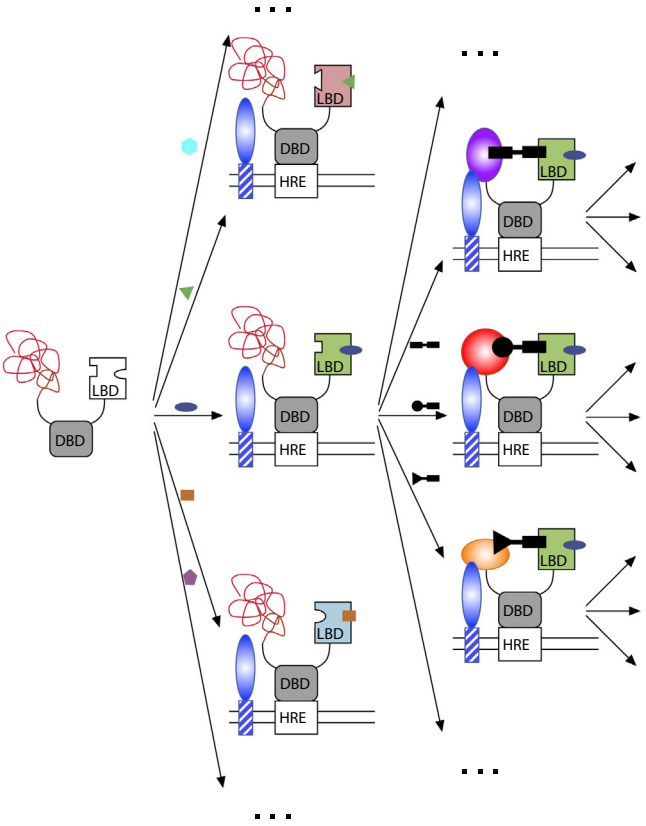

Fig. 1. Model for control of cell- and gene-selective transactivation with agonists and SRMs of SRs. Ligand-free SR without chaperone proteins is represented as a three-domain protein consisting of LBD, DBD, and unstructured (red, unlabeled) N-terminal ID/AF1 domains. Ligand (colored geometric shapes) binding modifies LBD conformation and the complex binds to its hormone response element (HRE) on chromatin to form a dimer (only one monomer is illustrated). Gene-selective variations can arise both from near-by or distant interacting, DNA-associated transcription factors (one proximal factor is illustrated; dark blue striped box and shaded oval) (Metivier et al., 2006; Datson et al., 2011; Altintas et al., 2012) and from HRE-induced conformational changes (not shown) (Meijsing et al., 2009; Kim et al., 2013). Differences in cellular concentrations of cofactors (solid shapes), which then bind either simultaneously (as shown) or separately (not illustrated) to the ID/AF1 and LBD to induce varied ID/AF1 structures (different shaped colored ellipses), could yield cell-selective responses. Additional possibilities at this, and the previous step, are indicated by arrows pointing to undesignated structures (. . .) and subsequent steps. The resultant receptor/steroid/cofactor complexes can then uniquely interact with other transcriptional machinery components to afford varied transcriptional responses. Not indicated are perturbations due to further influences such as protein alterations (e.g., phosphorylation, acetylation, and sumoylation), epigenetic modifications, and other factors/molecules interacting with the receptor and/or illustrated cofactors. SR-mediated gene repression would involve a parallel set of possibilities starting, in most cases, with tethered SR complexes.