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Understanding Diseases via Receptor Regulation

In this issue of *Chemistry & Biology*, Guy and coworkers [11] demonstrate that they can selectively recruit individual nuclear receptors by using small molecules (proteomimetics) in combination with specific agonists. This may ultimately lead to a link between the receptor's signaling pathway and its role in individual diseases.

A number of diseases, including cancer and metabolic disorders, are known to be associated with the inappropriate regulation of the nuclear hormone receptor transcription factors (NRs). [1] NRs regulate transcription based on hormone levels. The complex mechanisms involved in these signaling pathways are poorly understood. Selectively examining each nuclear receptor's signaling pathway will provide insight into their connection to specific diseases.

Uncoupling the signaling pathways of these NRs is challenging. The ability to selectively activate one NR in the presence of other NRs using small molecules has been limited by the similarity of one NR isoform over another and the challenge of replacing a protein-protein interaction with a small molecule-protein interaction. Upon discovery of lead small molecules, compound libraries targeted to individual NRs could be screened for their specificity for each NR. The discovery of unique small molecule leads for each NR would offer selective control of the signaling pathways for the individual nuclear receptor, illuminating the connection between the specific signals regulating the pathways and the disease state.

NRs bind to small molecule agonists: hormones. These hormones activate the NRs, leading the NR•agonist complex to recruit a specific steroid receptor coactivator (SRC)[1, 2] (Figure 1). This NR•agonist•SRC complex then initiates transcription. The ability to selectively bind one NR isoform to a SRC would allow the unraveling of individual signaling pathways for that NR and SRC complex. There are three known SRCs: SRC1, SRC2, and SRC3 [3–5]. They appear to play distinct but, perhaps, partially overlapping functions [4, 6]. The NRs bind to an area of a SRC protein known as the nuclear receptor-interacting domain (NID). This NID area contains multiple, conserved interfaces that have identical sequences specific for each SRC, which are known as NR boxes. The NR boxes contain sequences of the motif $L_1XXL_2L_3$, [1, 2], where L depicts a position of diversity, and X is an amino acid specific to that SRC. Thus, for example, the SRC2 NR box contains multiple, conserved interfaces within the NID that are sequence LERLL.

One series of structurally similar NRs are two isoforms of estrogen receptors: ERα and ERβ. Both ERα and ERβ bind to SRC2 to activate transcription. However, they regulate entirely different gene transcription pathways [7]. Both ERα and ERβ bind to the second box of SRC2 (SRC2-2) in the presence of a number of agonists, including estradiol, diethyl stilbesterol, or genistein.

In earlier work using a small molecule library of compounds that mimicked the second SRC2 NR box (SRC2-2), Guy and coworkers discovered a small molecule proteomimetic that selectively blocked binding of ERα in the presence of ERβ to SRC2 [8]. Thus transcription regulation by ERα was inhibited in the presence of ERβ, while transcription regulated by ERβ was unaffected. In addition, they found a proteomimetic that could preferentially inhibit the binding of ERβ to SRC2 in the presence of ERα. This new tool for selectively inhibiting individual NRs using a small molecule for regulating nuclear receptors is an excellent lead for the development of small, drug-like compounds that will ultimately illuminate the function of these individual receptors.

This issue of *Chemistry & Biology* includes an article by Guy and coworkers that demonstrates how small molecule proteomimetics can be used to selectively recruit individual NRs that are specific to the hormone
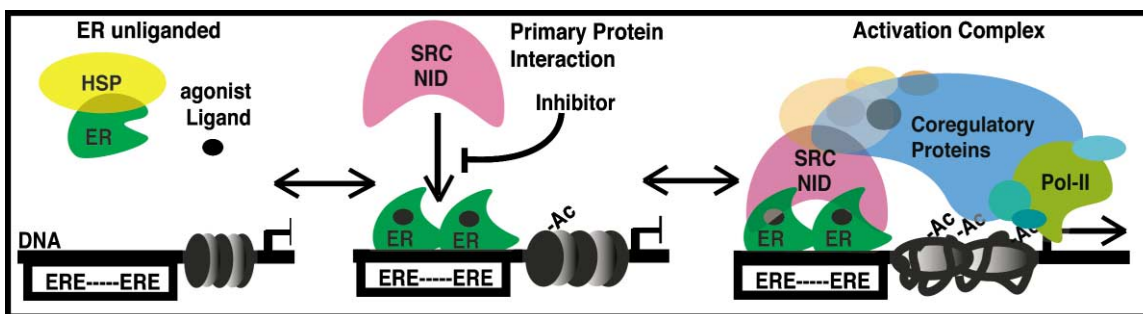
Figure 1. Ligand-Dependent Nuclear Receptor Regulatory Transcription Complex Assembly
This is Figure 1 from Geistlinger et al. [11].

agonist [11]. They show that two NR receptors, ERα and ERβ, bind to the same agonist ligand, which then exposes distinctive structural areas of that NR•agonist complex to the SRC2 NID (i.e., NR box). Using a NR box proteomimetic they had developed earlier [8], Guy and coworkers demonstrate selective recruitment of either ERα or ERβ with the same agonist hormone. These proteomimetic NR boxes were shown to selectively inhibit the binding of SRC2 to the ERα•agonist complex over the ERβ•agonist complex, preferentially forming the ERα•agonist•proteomimetic. Interestingly, Guy and coworkers found that the proteomimetic was unique to the agonist ligand used with the ER receptor. That is, three different agonists were used, estradiol, diethyl stilbesterol, or genistein, and each ERα•agonist•proteomimetic complex had unique binding selectivity compared to using the same agonist for the ERβ•agonist•proteomimetic complex.

Furthermore, in contrast to earlier work that utilized linear peptides [9, 10], Guy and coworkers found that the SRC binding pockets of the ERs have a strong selectivity for phenylalanine when utilizing a constrained scaffold. It is believed that the entropic cost of the necessary α-helix formation, which is high in linear peptides, is overcome in the constrained peptide system described by Guy. Thus, a more realistic method for screening small molecules that prevent binding of SRC to a NR•agonist complex is to utilize a constrained, induced fit, α-helix to appropriately present these hydrophobic residues. The success of these small molecule proteomimetics (Guy NR boxes) is a significant achievement, as it will allow one to observe the consequences of selectively recruiting ERβ to regulate transcription. In addition, the Guy proteomimetic approach can be generalized where one can envision screening small molecule "NR box" libraries in search of tools that will eventually uncouple the function of all NRs. This will allow the selective regulation of individual NRs and, therefore, reveal the connection between individual transcription regulation of these NRs and their associated diseases.

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How to Silence Silencing

Two recent reports [1, 2] describe the stunning crystal structures of complexes between a viral protein that suppresses RNA silencing and a 21 nucleotide small interfering (si)RNA.

RNA silencing is part of an innate defense strategy against viruses in plants [3]. Both suppressor proteins come from plant viruses belonging to the Tombusvirus