Steroid Receptor Coupling Becomes Nuclear

Mario D. Galigniana[1],*
[1]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales de la Universidad de Buenos Aires/INQUIBICEN and IBYME/CONICET, Buenos Aires C1428ADN, Argentina
*Correspondence: mgali@qb.fcen.uba.ar
DOI 10.1016/j.chembiol.2012.06.001

In this issue of *Chemistry & Biology*, Grossman et al. report a study on aldosterone-dependent nuclear translocation of the mineralocorticoid receptor (MR). They analyze the dependency of MR retrotransport, DNA-binding, and transcriptional activity on Hsp90 and demonstrate that MR dimerization is a nuclear event.

Soluble protein transport is a fundamental mechanism for regulating both protein localization and protein function of a great number of factors related to signaling cascades. Therefore, it is not surprising that several pathologies leading to cell death, cell proliferation, or cancer are directly related to protein mislocalization. It is currently accepted that most soluble proteins are not confined to the cytoplasm or the nucleus in a static manner but are capable of shuttling dynamically through the nuclear pore (Heitzer et al., 2007). In this sense, steroid-receptors are excellent tools to dissect this mechanism because their localization may easily be regulated by adding or washing-out the steroid.

In the absence of ligand, some members of the steroid-receptor family such as the glucocorticoid receptor (GR) or MR reside primarily in the cytoplasm, whereas others are constitutively nuclear, for example, the estrogen receptor (Pratt et al., 2004). Regardless of their primary localization, all of them form oligomers with the chaperones Hsp90 and Hsp70, the co-chaperone p23, and proteins that possess tetratricopeptide repeat (TPR) sequences of 34 amino-acids repeated in tandem, the TPR proteins (Galigniana et al., 2010a). To date, the high molecular weight immunophilins FKBP52, FKBP51, and CyP40, and the immunophilin-like proteins PP5 and XAP2 are counted among the best characterized members of the TPR-domain family of proteins associated to factors of the nuclear receptor family (Pratt et al., 2004).

It has always been assumed that the driving force of movement for steroid-receptors is diffusion. The classical model was posited some time ago (Dahmer et al., 1984) and supported the heuristic notion that the receptor-chaperone heterocomplex is dissociated immediately after steroid binding (a process referred to as "transformation"). Therefore, transformation favors the release of the receptor from the cytoplasmic anchoring sites and permits its cytoplasmic diffusion and subsequent passage through the nuclear pore complex. Thus, the receptor reaches its nuclear sites of action. Nonetheless, the recent observation that the dynein/dynactin motor complex associates with the Hsp90-FKBP52 complex bound to GR and MR suggested that this motor protein complex could power the active retrograde movement of steroid-receptors (Echeverría et al., 2009; Galigniana et al., 2010b). If this is correct, the Hsp90-FKBP52 complex should play a significant role when it is still associated to the receptor.

While FKBP52, CyP40, and PP5 are redundant immunophilins in their ability to interact with dynein/dynactin (Pratt et al., 2004), FKBP51 is a poor interactor and is also an effective transcriptional inhibitor (Gallo et al., 2007). Therefore, it is not surprising that upon steroid binding, FKBP51 is released from the receptor complex and replaced by FKBP52, which in turn recruits dynein/dynactin (Figure 1). This immunophilin exchange assembles the molecular machinery for the efficient retrotransport of the steroid-receptor complex. Further studies demonstrated that Hsp90 is still part of the heterocomplex when MR is nuclear. Moreover, the entire Hsp90 heterocomplex cross-linked to either GR (Echeverría et al., 2009) or MR (Galigniana et al., 2010b) translocates intact through the nuclear pore of digitonin-permeabilized cells, suggesting that steroid-receptor transformation could be a nuclear event. Accordingly, members of the chaperone heterocomplex are able to interact with structures of the nuclear pore such as nucleoporins and importins (Echeverría et al., 2009).

This alternative mechanism of action differs from the classical model in that Hsp90 does not have to dissociate from the receptor to initiate its nuclear translocation. Actually, Hsp90 is required for this process (Galigniana et al., 2010a). Accordingly, it has also been suggested that GR homodimerization is nuclear (Presman et al., 2010). Nonetheless, several aspects of this model need further confirmation and some steps are still to be proven. Because the dimerization domain of the receptors is blocked by the Hsp90 complex, it is valid to wonder whether receptor dimerization is a nuclear event or whether it paves the way to favor the nuclear translocation of the receptor. In turn, DNA-binding experiments favor a model involving early dimerization prior to DNA-binding rather than consecutive binding of monomers (Segard-Maurel et al., 1996), which is also in agreement with the lower affinity of monomers for DNA (Tsai and O'Malley, 1994). One may also wonder whether this alternative mechanism is common for all members of the steroid-receptor family.

To date, some of the answers to these questions were indirect extrapolations that deserved a more convincing experimental demonstration. In this issue of *Chemistry & Biology*, Grossman et al. (2012) have addressed this biological conundrum and have elegantly confirmed those previous studies. In addition, the study also proves the accuracy of the alternative model. The authors report that during aldosterone-induced nuclear translocation, MR is still bound to Hsp90, and by using extended bioluminescence resonance energy transfer and fluorescence resonance energy transfer, it is univocally demonstrated that MR

homodimerization is a nuclear process. After optimizing the proper amounts of transfected enhanced yellow-green fluorescent protein MR, it is shown that aldosterone, but not spironolactone, causes a significant nuclear MR-MR homodimerization, such that the MR remains cytoplasmic with the antimineralocorticoid. On the other hand, cell treatment with the Hsp90-disrupting agent geldanamycin shows that MR homodimerization takes place even in the absence of ligand when Hsp90 is dissociated from the receptor in the cytoplasm. However, Hsp90 inhibition prevents the nuclear translocation of the MR and it partially inhibits DNA-binding of MR. Interestingly, this inhibition is less efficient for GR. This dissimilarity could contribute to the mechanism by which MR differs from GR in those cells where both receptors recognize equal hormone-response elements. Importantly, only homodimers formed in the nucleus regulate gene expression, whereas those formed in the cytoplasm do not possess the ability to translocate to the nucleus and consequently influence transactivation.

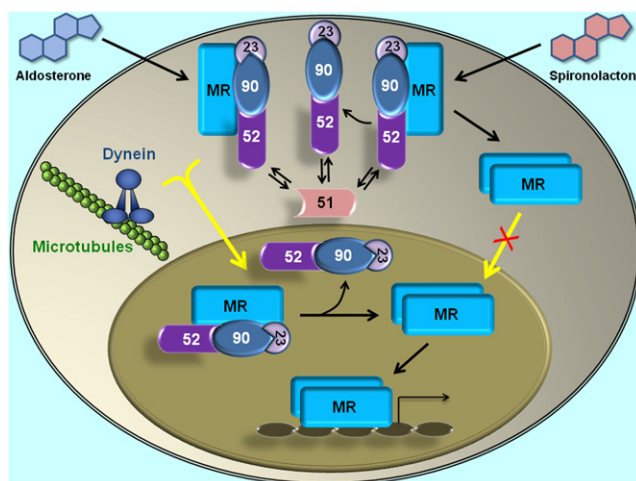These findings by Grossman et al. (2012) clarify mechanistic aspects of the MR signaling, although some aspects remain unknown. In this regard, the overall elucidation of the nuclear-cytoplasmic mechanism of steroid-receptor shuttling prompts the subsequent search for new potential partners of the receptor as well as for active drugs that can influence receptor relocalization. This will contribute to development of new strategies for preventing the pathophysiological effects of steroid hormones.

Figure 1. Model of MR Activation
In the absence of ligand, MR exists in the cytoplasm associated to the Hsp90-based heterocomplex. Upon aldosterone binding, the immunophilin FKBP51 is exchanged by FKBP52, an immunophilin able to bind dynein/dynactin. Thus, the MR heterocomplex is transported toward the nucleus via the motor protein complex. Then, MR translocates to the nucleoplasm still associated to the Hsp90 heterocomplex, and MR transformation occurs in the nuclear compartment followed by MR homodimerization. The receptor is finally targeted to the promoter binding-sites to trigger the proper biological response. When the antimineralocorticoid spironolactone binds, MR transformation and MR homodimerization can also take place in the cytoplasm, but the steroid-receptor complex translocation to the nucleus is impaired. It should be emphasized that FKBP51 is a very weak dynein-interacting protein and is also a strong transcriptional inhibitor of MR. Yellow arrows represent active transport of the MR complex.

REFERENCES

Dahmer, M.K., Housley, P.R., and Pratt, W.B. (1984). Annu. Rev. Physiol. 46, 67–81.

Echeverría, P.C., Mazaira, G., Erlejman, A., Gomez-Sanchez, C., Piwien Pilipuk, G., and Galigniana, M.D. (2009). Mol. Cell. Biol. 29, 4788–4797.

Galigniana, M.D., Echeverría, P.C., Erlejman, A.G., and Piwien-Pilipuk, G. (2010a). Nucleus 1, 299–308.

Galigniana, M.D., Erlejman, A.G., Monte, M., Gomez-Sanchez, C., and Piwien-Pilipuk, G. (2010b). Mol. Cell. Biol. 30, 1285–1298.

Gallo, L.I., Ghini, A.A., Piwien Pilipuk, G., and Galigniana, M.D. (2007). Biochemistry 46, 14044–14057.

Grossman, C., Ruhs, S., Langenbruch, L., Mildenberger, S., Strätz, N., Schumann, K., and Gekle, M. (2012). Chem. Biol. 19, this issue, 742–751.

Heitzer, M.D., Wolf, I.M., Sanchez, E.R., Witchel, S.F., and DeFranco, D.B. (2007). Rev. Endocr. Metab. Disord. 8, 321–330.

Pratt, W.B., Galigniana, M.D., Harrell, J.M., and DeFranco, D.B. (2004). Cell. Signal. 16, 857–872.

Presman, D.M., Alvarez, L.D., Levi, V., Eduardo, S., Digman, M.A., Martí, M.A., Veleiro, A.S., Burton, G., and Pecci, A. (2010). PLoS ONE 5, e13279.

Segard-Maurel, I., Rajkowski, K., Jibard, N., Schweizer-Groyer, G., Baulieu, E.E., and Cadepond, F. (1996). Biochemistry 35, 1634–1642.

Tsai, M.J., and O'Malley, B.W. (1994). Annu. Rev. Biochem. 63, 451–486.