Nuclear hormone receptor architecture - form and dynamics: The 2009 FASEB Summer Conference on Dynamic Structure of the Nuclear Hormone Receptors

Iain J. McEwan and Ann M. Nardulli

Corresponding Author: iain.mcewan@abdn.ac.uk anardull@life.illinois.edu

Molecular and Cellular Endocrinology, School of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, Scotland, UK (IJM) and Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA (AMN)

Nuclear hormone receptors (NHRs) represent a large and diverse family of ligand-activated transcription factors involved in regulating development, metabolic homeostasis, salt balance and reproductive health. The ligands for these receptors are typically small hydrophobic molecules such as steroid hormones, thyroid hormone, vitamin D3 and fatty acid derivatives. The first NHR structural information appeared ~20 years ago with the solution and crystal structures of the DNA binding domains and was followed by the structure of the agonist and antagonist bound ligand binding domains of different NHR members. Interestingly, in addition to these defined structural features, it has become clear that NHRs also possess significant structural plasticity. Thus, the dynamic structure of the NHRs was the topic of a recent stimulating and informative FASEB Summer Research Conference held in Vermont.

Received October 20th, 2009; Accepted December 16th, 2009; Published December 31st, 2009

Cite this article: Nuclear Receptor Signaling (2009) **7**, e011

Introduction

Those in the nuclear receptor field are well acquainted with the domain organisation of the nuclear hormone receptors (NHRs) and easily recognise the globular canonical structures of the isolated DNA and ligand binding domains (Figure 1, DBD and LBD, respectively). The first DBD structures appeared in the early 1990s [Hard et al., 1990; Schwabe et al., 1993] and were followed a few years later by LBD crystal structures [Brzozowski et al., 1997; Renaud et al., 1995]. Since then, structures have been solved for the DBDs and LBDs of at least one member of nearly all NHR subclasses. Currently, there are over 300 structures deposited with the NCBI structure database that confirm the universal nature of the canonical folds of the isolated DBD and LBD (Figure 1) and the high degree of identity that is observed in the primary amino acid sequence. However, what is now emerging, and was emphasised at this meeting, is how these domains interact in the context of the full-length receptor complex and how different ligands (Figure 1: hormones, DNA response elements, and coregulatory proteins) allosterically modulate these canonical structures leading to subtle, but functionally important changes in conformation.

Allosteric regulation and biological specificity take centre stage

In the keynote address, Keith Yamamoto (UCSF) discussed his group's recent work, which elegantly emphasised the allosteric role of glucocorticoid receptor DNA binding sites (GBS) [Meijsing et al., 2009]. Whole genome analysis by ChIP-chip had previously revealed that glucocorticoid receptor binding was cell-type specific and that individual DNA binding sites were conserved across species, but also displayed considerable variation in DNA sequence. In the latest study, Yamamoto and coworkers reported on the structure-function relationships of a number of GBS with slightly different DNA sequences. Two important messages arose from these biochemical and structural studies: (1) there is not a simple relationship between the affinity of the receptor for a DNA response element and its transcriptional activation and (2) binding to different DNA sequences causes the region between the recognition helix and D-box, termed the lever arm, to adopt different conformations. Yamamoto went on to discuss how these DNA-induced conformational differences could result in the formation of distinct interaction surfaces and modulate receptor activity. The role of DNA binding and domain interactions was further explored by Fraydoon Rastinejad (University of Virginia) in his presentation of the structure of the PPARγ-RXR heterodimer bound to a DR1 response element [Chandra et al., 2008]. Thus, for the first time, information is now available on the structure of full-length receptors bound to DNA and coregulatory peptides. The key features of these structures are: (1) the degree of domain interaction between the PPARγ LBD with its own DBD, the RXR-DBD and the RXR-LBD, (2) the polarity of the complex with PPAR occupying the 5' half site, (3) the more open conformation of RXR in the complex, and (4) less surprising, but significant in light of the other interactions identified, was the confirmed disordered nature of the N-terminal domain. What both these studies emphasised is that, despite the universal nature of DBD

and LBD folding, it is important to consider the role of different DNA binding sites and to understand how the domains in the intact receptor interact when assembled on DNA.

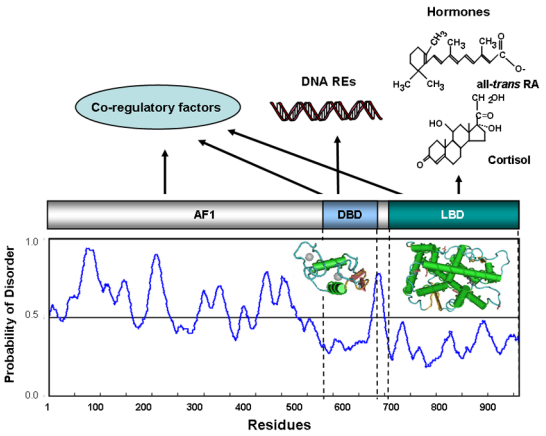

Figure 1. Structural and functional domains of nuclear hormone receptors. The basic domain organisation of a nuclear hormone receptor (NHR) is shown: LBD, ligand binding domain; DBD, DNA binding domain; and AF1 and AF2, activation functions 1 and 2 in the NTD and LBD, respectively. Above the NHR schematic are examples of allosteric modulators of NHR structure and function. These include ligands such as the vitamin A derivative retinoic acid and the steroid hormone cortisol, which bind to the LBD. NHRs interact with DNA response elements (RE), which can also induce allosteric changes in receptor structure/function. Coregulatory proteins have been described that bind to the AF2 in the LBD, AF1 in the NTD, and the DBD and may have allosteric effects on NHR conformation. Below the NHR schematic is a plot illustrating the presence of the intrinsically disordered structure within the NTD (Score above 0.5, Yang et al 2005) and the globular, canonical structures of the isolated DBD and LBD.

The structural basis for ligand binding and specificity of receptor response was considered in presentations by Geoffrey Greene (University of Chicago) and Edward Zhou (Xu Lab, Van Andel Research Institute). Interestingly, the ligand discrimination and specific activity of estrogen receptors α and β involve residues in the ligand binding pocket, as well as secondary structural elements adjacent to, and distant from, the pocket [Nettles et al., 2008]. The importance of ligand binding for differential physiological actions of the NHRs was further explored by Stoney Simons (NIDDK/NIH). Using glucocorticoid receptor signaling as an example, he demonstrated the value of considering steroid potency (EC_{50} value) in addition to the maximal response (efficacy), and considered how these parameters can be modulated by the presence of coregulatory proteins [Luo and Simons, 2009]. As with DNA binding, it was noted that the affinity of NHRs for different hormonal ligands did not relate directly to the maximal response or coregulatory protein binding.

Domain interactions and receptor dimerization were also illustrated in a talk by Robert Fletterick (UCSF), whose group recently solved the structure of the LBD of liver receptor homolog 1 (LRH-1) and an unusual member of the NHR superfamily, Dax-1, which lacks a DBD and acts as a repressor of different NHRs. Dax-1 dimers bind to LRH-1 forming a trimeric complex with the repressor helix of one Dax-1 monomer binding to the AF-2 surface of the LRH-1 LBD [Sablin et al., 2008].

Structural plasticity of the NHR N-terminal domain (NTD)

The functionally important NTD continues to pose a structural challenge. There is considerable experimental evidence that this domain is intrinsically disordered (Figure 1; [Kumar and Thompson, 2003; McEwan et al., 2007]. The low sequence complexity and underrepresentation of hydrophobic amino acids and the functional relevance of intrinsically disordered proteins or domains was considered by Chris Oldfield (Dunker Lab, Indiana University). New insights into the regulation of the stability and folding of the progesterone, glucocorticoid, androgen and mineralocorticoid receptor NTDs were discussed by Dean Edwards (Baylor College of Medicine), Raj Kumar (TCMC, Scranton), Michael Garabedian (NYU, College of Medicine) and Iain McEwan (University of Aberdeen, UK). The structural plasticity of this domain appears to be functionally important, as this domain is involved in multiple transient protein-protein interactions and contains sites for posttranslational modification (i.e., phosphorylation, sumoylation). The regulation of protein folding through coupled protein-protein interactions and posttranslational modification were key themes in the above talks ([Garza et al., 2009] and references therein). Evidence for the possible existence of folded intermediates and regions of stable structure within the NTD of the androgen and mineralocorticoid receptors was also presented [Lavery and McEwan, 2008]. Another exciting development in understanding the structure-function of the NTD is the ability to model the intrinsic disorder, leading to the hypothesis that the flexibility of this region is an evolutionary adaptation that helps to keep NHRs in a poised state, ready to respond to coregulatory proteins after binding to hormone and DNA (Vince Hilser, UTMB-Galveston) [Hilser and Thompson, 2007].

An important probe for stabilizing or inducing structure in the NTD of the NHRs is the natural osmolyte trimethylamine N-oxide (TMAO). TMAO belongs to a family of small organic molecules including sacrosine and sucrose that are used by organisms from bacteria to humans to maintain cell volume and protein structure/function under stress conditions. The mechanism of action and thermodynamic basis for the action of osmolytes on protein folding was discussed by Wayne Bolen (UTMB-Galveston). Compounds such as TMAO are found in elasmobranchs like sharks, as well as the kidney medulla and, due to their solvophobic properties, act by increasing free energy of the denatured state, making it less favourable than the native state [Auton et al., 2008]. This drives protein folding through the formation of hydrogen bonds within the peptide backbone, without affecting the amino acid side chains or protein function. TMAO increases both α-helical secondary structure and folding of the glucocorticoid and androgen receptor NTDs and enhances target protein

binding of this domain [Kumar and Thompson, 2003; McEwan et al., 2007].

Intrinsic disorder is not restricted to the NHRs, but appears to be a common feature of eukaryotic transcriptional machinery including transcription factors, coactivators (for example CREB-binding protein and p160 proteins) and corepressors (for example NCoR and SMRT). Jane Dyson (Scripps Research Institute) illustrated the value of NMR to study intrinsic disorder and discussed recent work on the structural basis for IκB-NFκB interactions [Cervantes et al., 2009]. Strikingly, the corepressors NCoR and SMRT are almost entirely predicted to be intrinsically disordered. The structural basis for the assembly of different multi-protein repressor complexes by discrete domains within these proteins was presented by John Schwabe (University of Leicester, UK) [Codina et al., 2005].

You are never alone in a complex

NHRs are intimately involved in the transient assembly and disassembly of protein complexes at the promoter and/or enhancer elements of hormone-regulated genes. Given the active role DNA binding is likely to play in NHR action, Ann Nardulli and coworkers (University of Illinois) used the DNA-bound estrogen receptor to isolate receptor-interacting protein complexes. By then identifying the individual components of the complexes, they were able to define novel protein networks and cellular processes regulated by the receptor [Schultz-Norton et al., 2008]. The assembly of complexes at estrogen-regulated genes was also discussed by Mike Stallcup (University of Southern California). His presentation focused on NHR interacting proteins that coordinate the recruitment of complexes with different enzymatic activities, for example Flightless-I protein and the SWI/SNF chromatin remodeling complex [Jeong et al., 2009]. The regulation of enzymatic activity of complexes containing the androgen receptor and lysine demethylation enzymes formed at androgen-regulated genes was the theme for Roland Schüle (University of Freiburg, Germany) [Wissmann et al., 2007]. This work revealed the exciting possibility that the substrate specificities for the different enzymatic activities in receptor recruited complexes can be differentially regulated. David Lonard (O'Malley Lab, Baylor College of Medicine) focused on the p160 family of coactivators and discussed recent studies in which the roles of these proteins are being defined in normal physiology and in disease. Taken together, the findings from these groups and others are beginning to explain why so many coactivators and enzymatically-active complexes interact with NHRs and reveal the possibility of additional regulation and cross talk during development and adult life.

A glimpse of protein folding and NHR dynamics inside cells

In an exciting series of presentations, the participants learned of novel approaches to studying protein folding in cells (Lila Gierasch, University of Massachusetts), the potential to rescue a folding-defective mutation of the glucocorticoid receptor *in situ* (Brad Thompson, UTMB-Galveston), and the role of HMG chaperone proteins in the binding of DNA by NHRs and other transcription factors (Mair Churchill, University of Colorado; Jean Thomas, University of Cambridge). Gierasch illustrated how protein engineering and novel chemical probes could be used to follow protein folding/unfolding in the macromolecular-crowded environment of the cell. The biarsenical-based fluorophore 4',5'-bis (1,3,2-dithioarsolan-2-yl)fluorescein (FlAsH) binds to the amino acid sequence Cys-Cys-X-X-Cys-Cys (where X is any amino acid), and, when incorporated into a protein of interest, it was found that the fluorescence (quantum yield) was a powerful read out of folding/unfolding of the protein. Gierasch and co-workers have gone on to create 'split-tetra cys motifs' with the idea that the dye binds only to the folded polypeptide [Krishnan and Gierasch, 2008]. The availability of such chemical probes, together with recent advancements in NMR technology [Burz and Shekhtman, 2009], opens up real possibilities for investigating folding/unfolding of NHRs in the context of the cellular environment and relating these findings to the wealth of *in vitro* data that already exists. Sam John (Hager Lab, NIH) presented recent findings on the interaction of the glucocorticoid receptor with chromatin using both a single cell model containing an engineered MMTV array and genome-wide analysis by ChIP-seq and DNase I hypersensitivity. Using these approaches, it was possible to distinguish, in different cell types, inducible and constitutive DNase hypersensitive binding sites for the receptor and to identify a requirement for different chromatin remodeling complexes at different sites throughout the genome [John et al., 2008].

Pathophysiology and NHRs

NHRs have long been important clinical targets and several speakers highlighted the translational potential of their research. Marianne Sadar (BC Cancer Agency) described the isolation of several natural compounds from a marine organism, which were able to modulate androgen receptor signaling in cell culture and tumour growth in a mouse model. Kim Kemper (University of Illinois) elegantly demonstrated how biochemical and cell biology studies on posttranslational modification (acteylation) of NHRs could lead to dysregulation of receptor function in metabolic disease. Ongoing and future work from these groups will further explore the mechanistic basis of these effects and define how these discoveries could benefit prostate cancer patients and patients presenting with the wide spectrum of metabolic syndrome disorders.

Ron Margolis (NIDDK, NIH) closed the meeting with a broad discussion of the Nuclear Receptor Signaling Atlas (NURSA). NURSA (http://www.nursa.org) is an interdisciplinary project that provides a valuable resource of methods and experimental data, together with short 'biographies' of individual NHRs, tutorials for students

and access to the online journal *Nuclear Receptor Signaling*.

Future studies

To fully understand the mechanism of action and the regulation of NHRs in normal physiology and disease states, it will be necessary to consider the cellular environment and the allosteric effects of different ligands (hormones, DNA and binding partners). Thus, outstanding questions concern the *in vivo* folding of the receptor protein and the dynamic nature of NHR structure. The development and application of new techniques, together with cross-disciplinary collaborations, will assist researchers in the NHR field in answering these questions. The FASEB Summer Conference on *Dynamic Structure of the Nuclear Hormone Receptors* is possibly unique in the NHR arena in that it has a strong focus on the structural dynamics of the receptor proteins that underpin their action in health and disease. Another unique aspect of the meeting is that it encourages investigators with different scientific backgrounds to actively participate and so leads to wider-reaching discussions and, in some cases, active, new collaborations. The 2009 meeting was considered a great success by those who participated.

Acknowledgements

We would like to thank each of the invited speakers and attendees for their active participation and for helping to make the conference informative and enjoyable. We would also like to acknowledge FASEB, The National Institute of Diabetes and Digestive and Kidney Diseases (Award Number R13DK083798), The Endocrine Society, The Society for Endocrinology (UK), and Oxford Journals-Nucleic Acid Research for their financial support of this meeting.

References

Auton, M., Bolen, D. W. and Rosgen, J. (2008) Structural thermodynamics of protein preferential solvation: osmolyte solvation of proteins, aminoacids, and peptides *Proteins* **73**, 802-13.

Brzozowski, A. M., Pike, A. C., Dauter, Z., Hubbard, R. E., Bonn, T., Engstrom, O., Ohman, L., Greene, G. L., Gustafsson, J. A. and Carlquist, M. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor *Nature* **389**, 753-8.

Burz, D. S. and Shekhtman, A. (2009) Structural biology: Inside the living cell *Nature* **458**, 37-8.

Cervantes, C. F., Markwick, P. R., Sue, S. C., McCammon, J. A., Dyson, H. J. and Komives, E. A. (2009) Functional dynamics of the folded ankyrin repeats of I κ B α revealed by nuclear magnetic resonance *Biochemistry* **48**, 8023-31.

Chandra, V., Huang, P., Hamuro, Y., Raghuram, S., Wang, Y., Burris, T. P. and Rastinejad, F. (2008) Structure of the intact PPAR-γ-RXR-α nuclear receptor complex on DNA *Nature* **456**, 350-356.

Codina, A., Love, J. D., Li, Y., Lazar, M. A., Neuhaus, D. and Schwabe, J. W. (2005) Structural insights into the interaction and activation of histone deacetylase 3 by nuclear receptor corepressors *Proc Natl Acad Sci U S A* **102**, 6009-14.

Garza, A. M. S., Khan, S. H. and Kumar, R. (2009) Site-specific phosphorylation induces functionally active conformation in the intrinsically disordered N-terminal activation function (AF1) domain of the glucocorticoid receptor *Mol. Cell. Biol.* **30**, 220-230.

Hard, T., Kellenbach, E., Boelens, R., Maler, B. A., Dahlman, K., Freedman, L. P., Carlstedt-Duke, J., Yamamoto, K. R., Gustafsson, J. A. and Kaptein, R. (1990) Solution structure of the glucocorticoid receptor DNA-binding domain *Science* **249**, 157-60.

Hilser, V. J. and Thompson, E. B. (2007) Intrinsic disorder as a mechanism to optimize allosteric coupling in proteins *Proc Natl Acad Sci U S A* **104**, 8311-5.

Jeong, K. W., Lee, Y. H. and Stallcup, M. R. (2009) Recruitment of the SWI/SNF chromatin remodeling complex to steroid hormone-regulated promoters by nuclear receptor coactivator flightless-I *J Biol Chem* **284**, 29298-309.

John, S., Sabo, P. J., Johnson, T. A., Sung, M. H., Biddie, S. C., Lightman, S. L., Voss, T. C., Davis, S. R., Meltzer, P. S., Stamatoyannopoulos, J. A. and Hager, G. L. (2008) Interaction of the glucocorticoid receptor with the chromatin landscape *Mol Cell* **29**, 611-24.

Krishnan, B. and Gierasch, L. M. (2008) Cross-strand split tetra-Cys motifs as structure sensors in a β-sheet protein *Chem Biol* **15**, 1104-15.

Kumar, R. and Thompson, E. B. (2003) Transactivation functions of the N-terminal domains of nuclear hormone receptors: protein folding and coactivator interactions *Mol Endocrinol* **17**, 1-10.

Lavery, D. N. and McEwan, I. J. (2008) Structural characterization of the native NH2-terminal transactivation domain of the human androgen receptor: a collapsed disordered conformation underlies structural plasticity and protein-induced folding *Biochemistry* **47**, 3360-9.

Luo, M. and Simons, S. S., Jr. (2009) Modulation of glucocorticoid receptor induction properties by cofactors in peripheral blood mononuclear cells *Hum Immunol* **70**, 785-9.

McEwan, I. J., Lavery, D., Fischer, K. and Watt, K. (2007) Natural disordered sequences in the amino terminal domain of nuclear receptors: lessons from the androgen and glucocorticoid receptors *Nucl Recept Signal* **5**, e001.

Meijsing, S. H., Pufall, M. A., So, A. Y., Bates, D. L., Chen, L. and Yamamoto, K. R. (2009) DNA binding site sequence directs glucocorticoid receptor structure and activity *Science* **324**, 407-10.

Nettles, K. W., Bruning, J. B., Gil, G., Nowak, J., Sharma, S. K., Hahm, J. B., Kulp, K., Hochberg, R. B., Zhou, H., Katzenellenbogen, J. A., Katzenellenbogen, B. S., Kim, Y., Joachmiak, A. and Greene, G. L. (2008) NFkappaB selectivity of estrogen receptor ligands revealed by comparative crystallographic analyses *Nat Chem Biol* **4**, 241-7.

Renaud, J. P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Gronemeyer, H. and Moras, D. (1995) Crystal structure of the RAR-γ ligand-binding domain bound to all-trans retinoic acid *Nature* **378**, 681-9.

Sablin, E. P., Woods, A., Krylova, I. N., Hwang, P., Ingraham, H. A. and Fletterick, R. J. (2008) The structure of corepressor Dax-1 bound to its target nuclear receptor LRH-1 *Proc Natl Acad Sci U S A* **105**, 18390-5.

Schultz-Norton, J. R., Ziegler, Y. S., Likhite, V. S., Yates, J. R. and Nardulli, A. M. (2008) Isolation of novel coregulatory protein networks associated with DNA-bound estrogen receptor α *BMC Mol Biol* **9**, 97-108.

Schwabe, J. W., Chapman, L., Finch, J. T. and Rhodes, D. (1993) The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements *Cell* **75**, 567-78.

Wissmann, M., Yin, N., Muller, J. M., Greschik, H., Fodor, B. D., Jenuwein, T., Vogler, C., Schneider, R., Gunther, T., Buettner, R., Metzger, E. and Schule, R. (2007) Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression *Nat Cell Biol* **9**, 347-53.