ORPHAN NUCLEAR RECEPTORS EXTEND THE VARIETY OF MECHANISMS FOR THE LIGAND-DEPENDENT REGULATION OF TRANSCRIPTION

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Nuclear receptors bind to DNA and activate in general the transcription of sets of genes in response to the binding of cognate ligands, usually small lipophilic molecules such as steroids, vitamins, and fatty acid derivatives. About 50 nuclear receptor genes have been identified in the human genome, but most of them have no known ligand and are therefore called orphans; their biological functions are also poorly understood.

Among the orphan nuclear receptors, some appear to be constitutively active. We have solved the structure of the ligand-binding domain (LBD) of two such receptors: RORβ and ERR3. The human RORβ LBD was found in the transcriptionally active conformation thanks to the combined action of stearate, a fortuitous ligand, and a coactivator peptide. Mutagenesis based on the structure showed that RORβ transcriptional activity is in fact ligand-dependent, and thus RORβ constitutive activity is only apparent. The human ERR3 LBD in complex with a coactivator peptide was also found in the transcriptionally active conformation, but this time in the absence of any ligand, suggesting that ERR3 is indeed constitutively active.

References:
1) Stehlin C., Wurtz J.M., Steinmetz A., Greiner E., Schuele R., Moras D., and Renaud J.P. (2001) X-ray structure of the orphan nuclear receptor RORβ ligand-binding domain in the active conformation. EMBO J. 20: 5822-5831.
2) Greschik H., Wurtz J.M., Sanglier S., Bourguet W., van Dorsselaer A., Moras D., and Renaud J.P. (2002) Structural and functional evidence for ligand-independent transcriptional activation by the estrogen-related receptor 3. Mol. Cell 9: 303-313.

Keywords: NUCLEAR RECEPTORS, ORPHANS, LIGAND BINDING DOMAIN

THE ANTAGONISTIC CRYSTAL STRUCTURE OF THE GLUCOCORTICOID RECEPTOR, LIGAND-BINDING DOMAIN

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The glucocorticoid receptor (GR) belongs to a large class hormone induced transcription factors. Its primary natural ligand in human is cortisol. This hormone and a large number of synthetic steroids such as dexamethasone act as agonists. GR plays a major role in several important cell processes with a broad therapeutic window. It is known that compounds that bind to the glucocorticoid receptor are potentially useful in the treatment a wide range of disease states. Here we describe the crystal structure of human GR-LBD in complex with the antagonist RU-486 at 2.8 Å resolution. The structure was solved by molecular replacement in two different crystal forms with the corresponding Helix 12 (human estrogen receptor nomenclature) intact and one with Helix 12 removed by proteolysis. This is the first antagonist structure from this class of nuclear hormone receptors. The GR structure consists of three layers of α helices arranged in an anti parallel sandwich fashion. This is a conserved fold of all nuclear receptors solved to date. The main difference is the new position Helix12. The fact that GR has been a target for structure based drug design for many years but so far failed to produce crystals for structure determination, questions have been raised (by non-crystallographers?) that GR would have a unique three dimensional structure with completely novel architecture. We show now that this is not the case. Comparing the structures with and without Helix 12 gives insights on the flexibility of the molecule.

Keywords: NUCLEAR RECEPTORS, CRYSTAL STRUCTURE, ANTAGONIST

FRONT-LINE DRUG RECOGNITION IN HUMANS BY THE NUCLEAR RECEPTOR PXR

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The human nuclear receptor PXR detects a wide variety of drugs, xenobiotics, and toxins, and controls the transcription of genes central to drug metabolism. We determined crystal structures of the ligand binding domain of PXR both alone and in complexes with human drugs.

The high-affinity ligand SR12813 binds within the smooth, hydrophobic PXR ligand binding cavity in three distinct orientations – a direct observation of binding promiscuity. The active agent of St. John's wort, hyperforin, binds in a single orientation but causes the pocket to expand to accommodate this larger drug. The hyperforin structure also provides a molecular explanation for the dangerous drug-drug interactions mediated by St. John's wort, an unregulated herbal therapy widely used to treat depression. Two surface electrostatic interactions gate a potential ligand entry-exit site and tune the basal transcriptional activity of the receptor. The targeted mutation of four residues within the ligand binding pocket effectively "humanizes" mouse PXR's response to ligands. Taken together, these structural and biochemical results reveal that PXR uses a conformable ligand binding pocket and the ability to allow drugs to bind in multiple orientations to protect the body from potentially harmful compounds. The downside of PXR activation, however, is the potentially life-threatening drug-drug interactions mediated by the efficient activation of PXR by St. John's wort.

Keywords: NUCLEAR RECEPTORS, DRUG DRUG INTERACTIONS, ST JOHNS WORT

CAN RADIATION DAMAGE IN CRYOCOOLED PROTEIN CRYSTALS BE MITIGATED?

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Over the last ten years, the development and widespread use of macromolecular cryocrystallographic techniques has allowed second and third generation synchrotron wiggler and undulator produced X-ray beams to be utilized effectively for structural studies, since radiation damage at 100 K is greatly reduced compared to that observed at room temperature.

However, significant radiation damage to crystals is still observed even at 100K, resulting in more than one crystal being required to obtain a complete dataset, the failure of MAD experiments, and the possibility of erroneous biological conclusions on mechanisms being drawn because the damage is structurally specific. Intense synchrotron beams are commonly attenuated, thus not utilizing their full potential.

In order to mitigate the damage, we need an understanding of both the processes involved and the factors affecting the rate of damage to protein crystals held at cryogenic temperatures. Relevant parameters include externally controlled variables such as the incident beam conditions (e.g. wavelength, dose, dose rate, flux) and the cooling regime used in the experiment (e.g. nitrogen or helium as cryogen, temperature, flow rate) and also the physical and chemical environment of the crystal within the fiber loop (e.g. cryoprotectant agent and concentration, solvent content of the crystals).
Our current understanding will be summarized and some new experimental results on the potential for using radical scavengers will be presented.

Keywords: CRYOCRYSTALLOGRAPHY, RADIATION DAMAGE, RADICAL SCAVENGERS