

A NUCLEAR RECEPTOR COMPLEX REVEALED

Regulation of many aspects of metabolism is managed by small molecules that circulate in the blood, pass through the plasma membrane, and bind to nuclear receptors that can affect DNA transcription. These nuclear receptors are responsible for appropriate uptake, storage, and breakdown of lipids and carbohydrates in our cells. For patients with severe metabolic disorders such as diabetes mellitus, regulation of metabolism is crucial. One group of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs), has been identified as important for lipid metabolism; their activation by a class of drugs known as thiazolidinediones can improve glucose uptake to relieve symptoms of diabetes in patients. Peroxisome proliferator-activated receptors work as part of a complex with retinoid X receptors (RXR) to bind specific DNA sequences and activate transcription of metabolic genes. Recent work completed by researchers using the SER-CAT 22-ID beamline at the APS determined the structure of one of these transcription complexes for a nuclear receptor called PPAR- γ . This structure increases our understanding of how nuclear receptors regulate genes involved in human disease and could lead to drugs that are more effective with fewer side effects.

Drugs that activate PPAR- γ , such as rosiglitazone, have shown promise as treatment for metabolic disorders such as type 2 diabetes, hyperinsulinemia, and hyperglycemia. In addition, due to other beneficial effects, rosiglitazone is being considered for treatment of inflammatory diseases, atherosclerosis, hypertension, and Alzheimer's disease. However, these drugs have been reported to have side effects including increased risk of heart attack. Drug design efforts and previous structural studies have focused on the structure of the PPAR- γ ligand binding domain (LDB) responsible for interaction with the thiazolidinediones. Elucidation of the structure of the entire DNA binding complex of PPAR- γ provides additional information that may allow science to design drugs with increased efficacy and fewer side effects.

First, the researchers, from the University of Virginia Health System, the ExSAR Corporation, and the Louisiana State University Health System, crystallized the complex, which included PPAR- γ ; RXR- α , the ligand for RXR- α called 9-*cis*-retanoic acid; rosiglitazone, the DNA sequence that is recognized by the two protein receptors; and two co-activator peptides. In order to determine whether different ligands would change the configuration of the complex, two other complexes were crystallized that contained a partial activator or a suicide inhibitor for PPAR- γ instead of rosiglitazone. The *"Nuclear"* continued on page 80

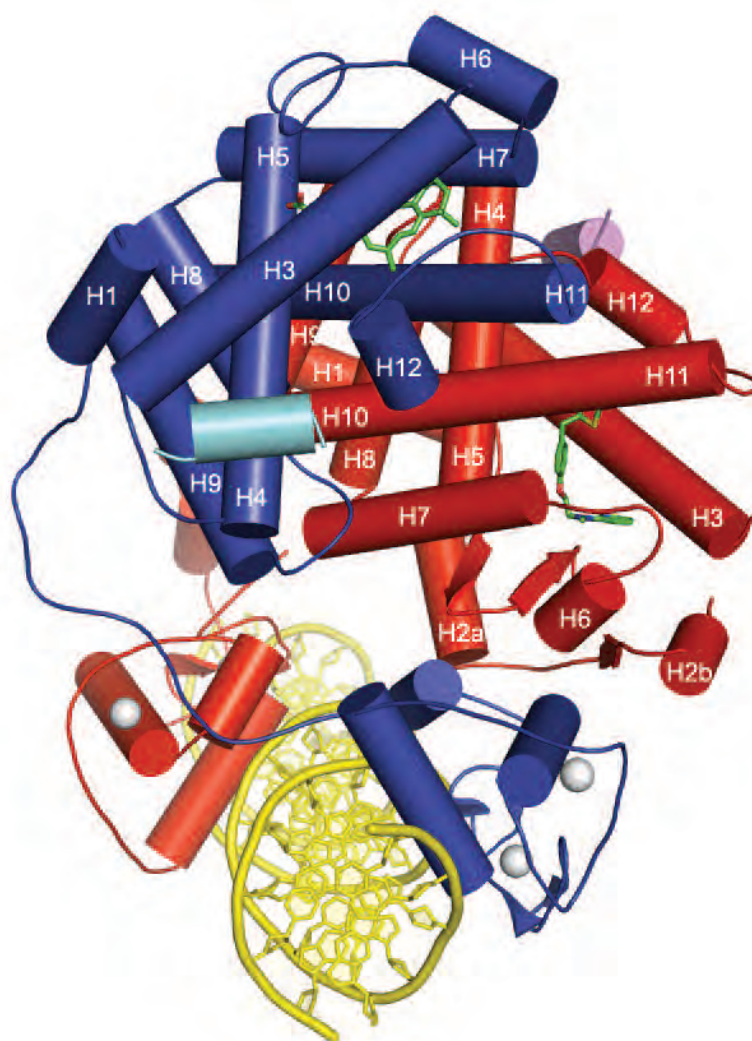


Fig. 1. Structure of the PPAR- γ nuclear complex. PPAR- γ is shown in red, RXR- α is shown in blue, DNA response element is yellow, receptor ligands rosiglitazone and 9-*cis*-retanoic acid are shown in green, Zn(II) ions are white and coactivator peptides are in light blue and purple.

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structures were solved at 3.1-Å to 3.2-Å resolution by single anomalous dispersion phasing for the rosiglitazone structure and molecular replacement for the other ligands. All structures were similar in the relative arrangement of the receptors and their domain interactions with each other, and with the DNA sequence. The center of the complex is the PPAR- γ LBD, which has contacts with many other domains in the structure (Fig. 1). In contrast, the LBD of the RXR- α has no additional contacts with other parts of the complex except for the PPAR- γ LBD.

Both RXR- α and PPAR- γ interact with DNA through C-terminal DNA binding domains (DBD) that have α -helical structures aligned with the DNA major groove at the conserved sequences for binding. The C-terminal extension of RXR- α forms a DNA-dependent contact with the PPAR- γ DBD, but has no other secondary structure. This may be due to the fact that RXR- α is known to interact with other nuclear receptors and requires

flexibility in this region to accommodate these alternate interactions.

In addition to the interaction between the DBDs of the two proteins, the structure of the PPAR- γ complex revealed two other DNA-dependent interactions between RXR- α and PPAR- γ . First, the two proteins interact at an interface between the two LBDs, and second, the PPAR- γ LBD interacts with the C-terminal DNA-binding domain of RXR- α . This previously unknown DNA-dependent interaction helps to explain why intact receptors have a higher affinity for DNA than the isolated DNA binding domain alone. In fact, the researchers show here that mutations that disrupt this binding interaction reduce the affinity of the receptor for DNA and also disrupt transcriptional activation by the complex.

The insights provided by this increased understanding of the PPAR- γ nuclear receptor complex will be useful to researchers and clinicians hoping to expand the therapeutic value of nuclear receptor activation in human disease.

— *Sandy Field*

See: Vikas Chandra¹, Pengxiang Huang¹, Yoshitomo Hamuro², Srilatha Raghuram¹, Yongjun Wang³, Thomas P. Burris³, and Fraydoon Rastinejad^{1*}, “Structure of the intact PPAR- γ -RXR- α nuclear receptor complex on DNA,” *Nature* **456**, 350 (20 November 2008). DOI:10.1038/nature07413

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two other known synthetic agonists, GW4064 and fexaramine.

Once the researchers had identified MFA-1 as being a potent agonist of FXR, they next sought to glean some insights into how MFA-1 interacts with FXR by determining the crystal structure of the FXR in complex with MFA-1. Notably, the structure of human FXR in complex with MFA-1 was similar to previously reported structures of rat FXR in complex with bile. FXR was also similar in structure to other members of the same receptor family, which includes the estrogen and progesterone receptors.

X-ray crystallography carried out at the IMCA-CAT beamline 17-ID showed that the FXR consisted of 12 coils or helices and that MFA-1, when bound, was buried deep within the FXR structure. The researchers also found that the steroid ring system of MFA-1 was bound in an orientation that was flipped by 180 degrees compared with the nat-

ural bile acid ligands. They suggest that the presence of a negative charge on MFA-1 may play a role in dictating the orientation of the ligand in the binding pocket. These findings help to dispel previous suggestions that FXR uses shape discrimination to preferentially bind bile acids over conventional steroids. In fact, FXR may use elements other than the binding orientation and steroid rings to select ligands. The researchers also speculated that MFA-1, like other FXR agonists, might activate the FXR by helping to stabilize the structure of the receptor.

Future efforts are likely to focus on developing additional molecules that can bind to the FXR and that may be useful in preventing or treating the metabolic syndrome. — *Emma Hitt*

See: Stephen M. Soisson*, Gopalakrishnan Parthasarathy, Alan D. Adams, Soumya Sahoo, Ayesha Sitlani, Carl Sparrow, Jisong Cui, and Joseph W. Becker, “Identification of a

potent synthetic FXR agonist with an unexpected mode of binding and activation,” *Proc. Nat. Acad. Sci. USA* **105**(14), 5337 (April 8, 2008). DOI: 10.1073/pnas.0710981105

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