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Vitamin D receptor: first full 3D observation

For the first time, a team from the Institute of Genetics and Molecular and Cellular Biology (IGBMC, Université de Strasbourg/CNRS/Inserm) has succeeded in taking a full, 3D photograph in HD[1] of a small vital, molecule, enclosed at the heart of our cells: the vitamin D receptor (VDR). Published on 18 January 2012 in the EMBO Journal, this study provides key information regarding the 3D structure of the receptor and its action mechanism at a molecular level. This data is crucial for pharmaceutical research, since the VDR is involved in several diseases, such as cancer, rickets and type 1 diabetes.

The vitamin D receptor (VDR) is part of, and plays a crucial role in, what biologists refer to as the “large family of nuclear receptors": proteins that are active in cell cores, including "steroid" receptors (sexual hormone receptors, etc.). It regulates the expression of genes involved in diverse and vital biological functions (cell growth, bone mineralisation, etc.).

Until now, researchers had only been able to study two parts of this receptor close-up: the region that interacts with DNA and the vitamin D-binding domain. These two parts were produced in a laboratory and their structure was studied individually using the crystallography technique. This method did not make it possible to visualize VDR fully since it proved to be difficult to crystallize.

To overcome this challenge, by combining the skills of several teams from across the globe for more than 15 years, the teams led by Bruno Klaholz and Dino Moras, each CNRS research directors at the IGBMC, used an innovative technique: cryo-electron microscopy (cryo-EM), which requires the latest-generation electronic “high-definition” microscope. This marvel of technology can be used to view biological objects at the molecular, or even atomic, scale. In France, the first microscope of this kind was installed at the IGBMC[2] in 2008. Prior to this research, many people thought it was impossible to study VDR using cryo-EM. Until now, the smallest molecules that had been viewed using this technique weighed more than 300 kilo Daltons[3] (kDa), or even thousands of kDa, i.e. much more than the VDR, which weighs 100 kDa and measures just 10 nm (10 x 10^{-9} m).

In concrete terms, Bruno Klaholz and his colleagues have laboratory-produced large qualities of the human VDR receptor in *Escherichia coli* bacteria (one of the most commonly-used models in biology to produce proteins). They then isolated to receptor in a physiological solution containing water and a little salt. The sample containing VDR was then frozen and immersed in liquefied ethane, which produced extremely rapid cooling (in a fraction of a second, the sample passes from 25°C to approximately -184°C). Using the microscope, approximately 20,000 photos were required of VDR particles in different directions. It is these images, aligned and combined using a software program, which finally resulted in a 3D reconstruction of VDR.

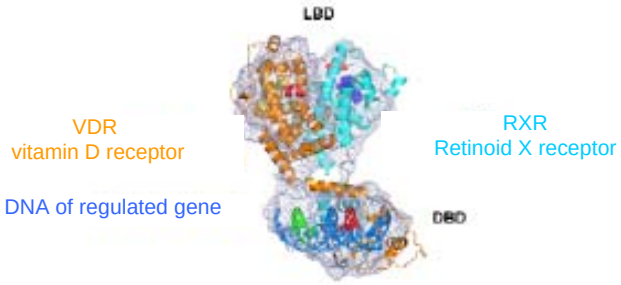
[1] 12 angstroms: 12 x10^{-10} metres (one angstrom corresponds to the average diameter of an atom).

[2] The second was inaugurated in February 2011 at the Institute of Structural Biology (CEA / CNRS / UJF) in Grenoble.

[3] One Dalton is, with relatively accurate precision, the mass of a hydrogen atom. A protein amino acid represents approximately 110 Da, an assembly of 100kDa contains approx. 900 amino acids.

This image has provided hitherto unknown information regarding how the receptor functions. It reveals that the VDR and its partner RXR (retinoid X receptor, a vitamin A derivative) form an open architecture, with the vitamin D-binding domain oriented almost perpendicularly to the DNA binding domain (see Figure below). This structure suggests cooperation between the two domains, which may work together to induce a more tight regulation of the expression of target genes.

This ground-breaking work paves the way for research into several other vital nuclear receptors, which are yet to be thoroughly investigated. In particular, biologists are now envisaging using cryo-EM to reveal the structure of steroid receptors.



View of 3D architecture of two receptors, the VDR (vitamin D receptor) and its partner RXR (retinoid X receptor, a derivative of vitamin A), after 3D reconstruction using images of individual particles. The purple mesh represents the experimental 3D map obtained through cyro-EM. The specific binding sites for DNA fragment are indicated in green and red, the ADN binding domains (BDB) and ligand binding domains (LBD) are indicated.

Bibliography

Structure of the full human RXR/VDR nuclear receptor heterodimer complex with its DR3 target DNA. Igor Orlov, Natacha Rochel, Dino Moras and Bruno Klaholz. *The EMBO Journal*. 18 January 2012 (hard copy).

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