THE MODULATION OF RETINOIC ACID SIGNALLING PATHWAYS BY ENVIRONMENTAL POLLUTANTS IN MARINE METAZOANS

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Abstract
Nuclear receptors (NRs) form a superfamily of ligand-dependent transcription factors in metazoans, where they regulate many biological functions (e.g. reproduction, development and homeostasis). NRs can be modified by anthropogenic contaminants, which can cause cancer and endocrine disruption in humans and wildlife. Marine organisms disturbed by endocrine disrupting chemicals, can have reduced testicular development and fertility, masculinization of female specimens (imposex), etc. In this context, we evaluated the genetic characterization of the retinoid X receptor (RXR) in varied metazoan species (from invertebrates to vertebrates), and we modulated RXR 3D structures to predict the binding affinity of environmental pollutants, obtaining insight into the resilience of RXRs in marine organisms to the effects of endocrine disrupters.
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The study of biological systems in atomic detail is a multidisciplinary field combining knowledge from sciences such as Mathematics, Physics, Chemistry, Biochemistry, Biology and Informatics. It as been previously used with great success to understand enzymatic mechanisms, to predict the binding free energy of two molecules, and in the design of new drugs based on structural information. Although this approach may have the availability to predict if a pollutant may activate a hormone receptors or interfere with the enzymatic activities of an organisms, it has not been a common approach in risk assessment studies of aquatic contaminants, despite it potential great utility in Ecotoxicology. Moreover, integrative efforts using state-of-the-art methodologies at the gene-level (comparative genomics) and protein-level (comparative proteomics) may be the ultimate bridge between structural biology and molecular evolution [1]. The evolutionary study of the RXR protein across a wide range of metazoans (from invertebrates to vertebrates) is important to reveal how mutations have accumulated in this functional constrained protein over millions of years of evolution. The understanding of such genetic basis can provide fundamental biological insight about protein evolution and ecological fitness [2, 3].

In this study, we have retrieved RXR sequences from 57 metazoan organisms (invertebrates and vertebrates) and we have performed a detailed search for selection signatures. First, we have used single-site analyses for detecting selection at the gene level [1].
However, these analyses may be biased against even moderately conservative proteins because the primary criterion involves a comparison of nonsynonymous and synonymous substitution rates, not allowing for the possibility that adaptation may come in the form of very few amino acid changes [1].
Second, we have additionally used powerful statistical methods at the protein-level considering the nature of the amino acid change ("conservative" or "radical" depending on the magnitude of the physicochemical difference between amino-acids, and the physical location of amino-acid sites in the three-dimensional (3D) protein structure [1].
Finally, third, we have determined the 3D structure of several RXRs, using homology-modeling [4], a reliable technique to computationally infer an unknown protein 3D-structure (>50% amino-acid identity) based on the experimentally determined 3D-structure of a related protein [e.g. Human and Biomphalaria (mollusc) RXR crystal structures].

We identified several aminoacids that seem to be essential to RXR activation by organotins and methoprene acid, and therefore, will allow the future identification of potential environmental pollutants RXR agonists based on their conformational characteristics. Typically, RXR ligands contain a carboxylate group, which is important in their ability to be buried stably in the predominantly hydrophobic pocket. This functional group is involved in an ionic interaction with the strictly conserved basic residue of R316 of helix H5 and forms a hydrogen bond with the backbone carbonyl amide group of the β-turn residue A327. Although tributyltin (TBT), a widespread marine contaminant, bind RXR with high specificity and induce RXR activation (similarly to the natural ligand 9-cis retinoic acid), it lacks a carboxylate group. Hence, the protein-ligand interaction of TBT is very different from those seen with other known RXR agonists.

In order to further demonstrate the utility of bioinformatics in foreseeing the capability of environmental pollutants to bind hormone receptors and enzymes, the RXR of a Cnidarian (Actinia equina, the most common species of sea anemone along coastal areas in Europe and the Mediterranean) have been partially cloned (based on degenerate primers, PCR on cDNA, cloning with pGemTeasy system from Promega, and sequencing), and the 3 D structure of Actinia equina was developed based on homology modelling with the Human and Biomphalaria (mollusc) RXR crystal structure, which will be of great utility to subsequently evaluate the capability of TBT, triphenyltin (TPT), methoprene acid, and 9-cis retinoic acid to activate the cnidarian RXR.

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