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To Bind or Not To Bind: The Taxonomic Scope of Nuclear Receptor Mediated Endocrine Disruption in Invertebrate Phyla

L. Filipe C. Castro*[,†,‡] and Miguel M. Santos*[,†,‡]

[†]CIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

[‡]FCUP, Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

The origin and diversification of metazoan endocrine systems represent a fundamental research issue in biology. Critical components of these systems are the nuclear receptors (NRs). NRs form an important superfamily of ligand-dependent and independent transcription factors that regulate numerous biological processes in vertebrates, including morphogenesis, differentiation, metabolism and reproduction. Given their central role in mammalian physiology, an in-depth understanding of their molecular modes of action has been built over the last decades. NRs are activated or antagonized by a diverse array of small hydrophobic ligands through direct interactions with key amino acids within the ligand pocket. Examples include hormones such as estrogens, androgens, and thyroid hormones, but also other compounds such as fatty acids and retinoids. Ligand binding typically occurs at rather low concentrations in the nanomolar or micromolar range, which makes them prime targets of endocrine disrupting chemicals (EDCs). In fact, some of the best characterized examples of endocrine disruption involve the modulation of NRs signaling. Classical examples include the impact of estrogenic chemicals mediated through Estrogen Receptors (ERs) on teleost fishes, which was associated with feminization, behavior and reproductive disruption and population decline.[1] More recently, the mammalian NR-mediated endocrine disruption has known an additional disquieting chapter. Organotins, well-known for their role in imposex development in gastropod mollusks, have been associated with the increased incidence of obesity, through the direct binding to the NRs retinoid X receptor (RXR) and peroxisome proliferator-activated receptor gamma (PPARγ).[2]

The fact that most research on NR-mediated disruption has focused on vertebrate models significantly hinders our understanding on the wider biological and phylogenetic impact of EDCs and emerging pollutants. This is particularly relevant as invertebrates represent more than 95% of the known animal species, and have key roles in ecosystem functions. With the exception of the imposex phenomenon in prosobranch gastropods, that has indisputably been associated with tributyltin binding to RXR,[3] very few studies have linked NRs and endocrine perturbation in invertebrate phyla. This is most likely related with the lack of knowledge of invertebrate endocrinology and genomic NR collection. In contrast to vertebrates, the NR gene family diversity outside chordates was assumed to be comparatively smaller for a considerable time. However, the full genome sequences from a few invertebrate species suggest that this is a problem of biased taxonomic sampling.[4] Invertebrate model organisms such as the ecdysozoans *Drosophila melanogaster*, *Caenorhabditis elegans* and *Daphnia magna*, are highly derived and have suffered extensive gene loss. Therefore, these lineages are not fully representative of NR gene repertoire diversity in invertebrate phyla.[4] In invertebrates, RXR is perhaps the best characterized example of NR functions. RXR orthologues have been reported or predicted in virtually all Bilateria lineages. Available data points to a mostly conserved binding profile to either natural ligands and/or xenobiotics (e.g., tributyltin) across phyla that have diverged over 600 million years ago. These initial findings suggested that the presence of a NR orthologue could establish a conserved ligand/xenobiotic binding affinity for the receptor, and hence could be a good indicator of an apparently phylogenetic *stasis* in response toward an EDC insult. Importantly, recent findings involving other NR gene families such as ER, TR, and RAR suggest otherwise. For example, whereas the mollusk and amphioxus ER do not bind estrogen (or xenoestrogens in the case of mollusks), the annelid orthologue does and therefore is probably targeted by ECs *in vivo*. This hypothesis has recently been confirmed by life-cycle annelid exposure to ECs, where similar impacts to those reported in fish have been observed. Similarly, the cephalochordate TR binds a different ligand than the vertebrate orthologues, while the binding affinities of the same receptor

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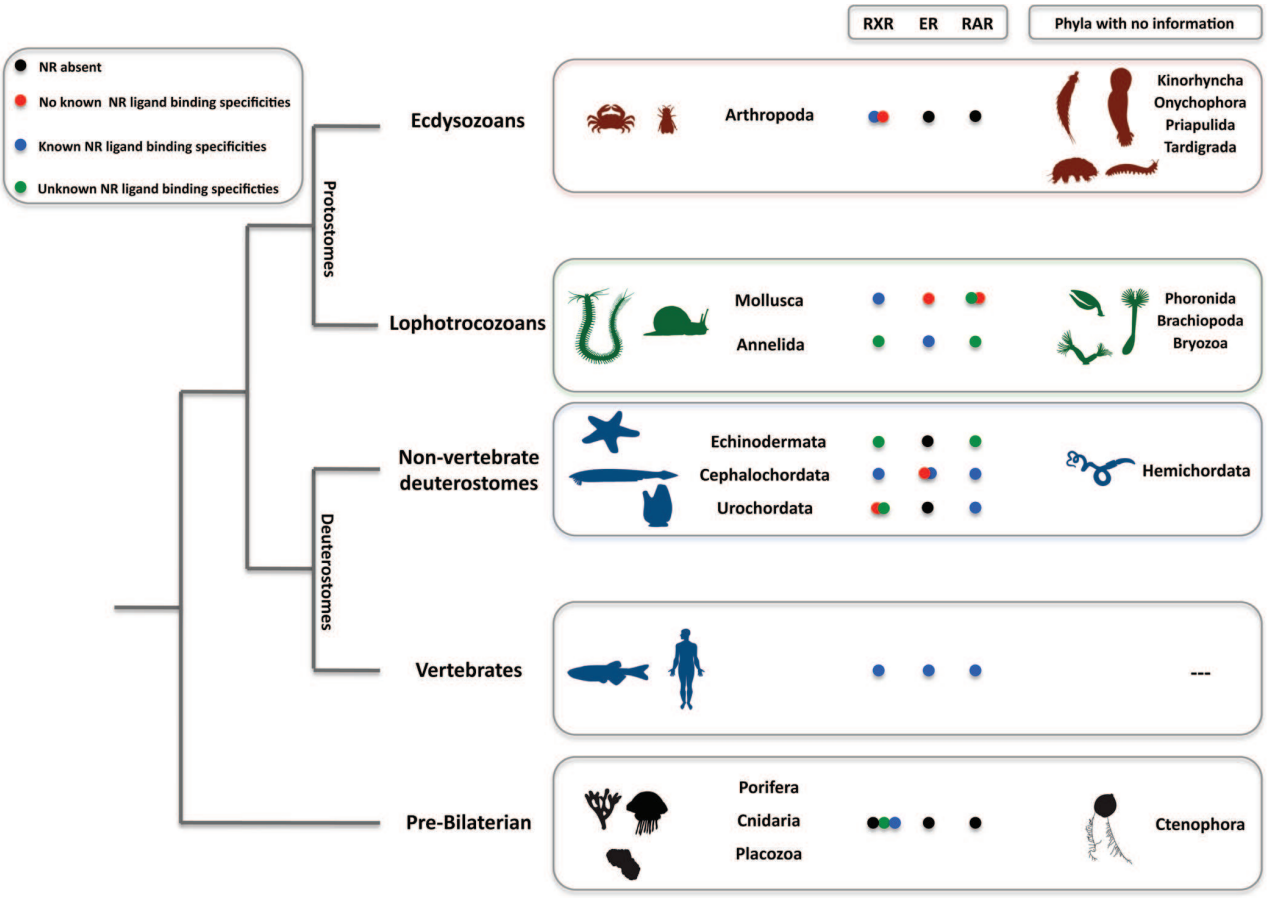

Figure 1. Phylogenetic relationships between major extant Metazoa lineages. Examples of NR repertoire (RXR, ER and RAR) and known ligand binding affinities toward endogenous compounds and/or EDCs are identified.

toward natural ligands and EDCs in mollusks and annelids is presently unknown. Finally, the gastropod RAR does not bind polar retinoids as their vertebrate counterparts although it remains to be investigated whether it is a target of organic pollutants. These findings have a fundamental ecological implication since they suggest that the ligand/NR complex has evolved multiple alternative molecular specificities throughout Bilateria evolution.[5] In addition, many other NR gene families though present in invertebrate phyla (e.g., PPAR and ROR) remain largely uncharacterized. Consequently a fundamental question emerges: what is the animal taxonomic scope of NR-mediated endocrine disruption? We believe that the answer to this question is very much linked to the phylogenetic distribution and molecular specificity of NR binding profile in Bilateria (Figure 1). Thus, we think that diversity is a key word, which has often been overlooked while addressing the toxicological risk and the mode of action of organic pollutants. In the wise words of Sumpter and Johnson:[1] "*one animal's poison may not be another's*". In our opinion a comprehensive analysis and functional characterization of the NR repertoire in the majority of invertebrate lineages is missing, hampering proper research regarding the ecological scale of NR-mediated disruption. Hence, the characterization of NRs from different phyla is urgently needed (Figure 1). We consider that *in vitro* bioassays with these characterized NRs should be a first step to address the risk and the mode of action of priority and emerging organic pollutants, while at the same time allowing a cost-effective high-through put testing method. This approach integrates well with the recently EPA and OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors that is currently skewed toward mammalian models due to the lack of *in vitro* assays with invertebrate NRs. In our opinion, this should represent an imperative step in identifying hazard and addressing potential mechanisms of disruption.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: filipe.castro@ciimar.up.pt.

*E-mail: santos@ciimar.up.pt.

Notes

The authors declare no competing financial interest.

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