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Review

Nuclear receptors in nematode development: Natural experiments made by a phylum☆,☆☆

Marta Kostrouchova [a,*], Zdenek Kostrouch [b]

[a] Laboratory of Molecular Biology and Genetics, Charles University in Prague, Prague, Czech Republic
[b] Laboratory of Molecular Pathology, Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

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ABSTRACT

The development of complex multicellular organisms is dependent on regulatory decisions that are necessary for the establishment of specific differentiation and metabolic cellular states. Nuclear receptors (NRs) form a large family of transcription factors that play critical roles in the regulation of development and metabolism of Metazoa. Based on their DNA binding and ligand binding domains, NRs are divided into eight NR subfamilies from which representatives of six subfamilies are present in both deuterostomes and protostomes indicating their early evolutionary origin. In some nematode species, especially in *Caenorhabditis*, the family of NRs expanded to a large number of genes strikingly exceeding the number of NR genes in vertebrates or insects. Nematode NRs, including the multiplied *Caenorhabditis* genes, show clear relation to vertebrate and insect homologues belonging to six of the eight main NR subfamilies. This review summarizes advances in research of nematode NRs and their developmental functions. Nematode NRs can reveal evolutionarily conserved mechanisms that regulate specific developmental and metabolic processes as well as new regulatory adaptations. They represent the results of a large number of natural experiments with structural and functional potential of NRs for the evolution of the phylum. The conserved and divergent character of nematode NRs adds a new dimension to our understanding of the general biology of regulation by NRs. This article is part of a Special Issue entitled: Nuclear receptors in animal development.

1. Introduction

1.1. Overall characteristics of NRs in nematodes

Nuclear receptors (NRs) are very characteristic transcription factors found only in metazoan species. NRs are critically important for the regulation of metabolism, development and reproduction. Their molecular structure includes two highly conserved domains. The DNA binding domain (DBD) which interacts with response elements in promoters of regulated genes and the ligand binding domain (LBD), which in the case of proteins with known ligands binds and is regulated by small-molecule ligands. The DBD consists of two protein loops (called zinc fingers), each contain a zinc ion coordinating tetrahedrically four cysteine residues. The number of amino acid residues forming the molecular signature is also conserved across the animal phyla and has in an absolute majority of cases the formula Cys-*X*2-Cys-X13-Cys-*X*2-Cys-X15-Cys-X5-Cys-X9-Cys-*X*2-Cys [1–4]. There are exceptions to this rule. Genome sequencing projects and focused studies aimed at cloning NRs from different species identified homologues of classical NRs with slightly variable length of the sequence separating the two zinc fingers. The sequence separating the two zinc fingers contains in most NRs 15 amino acid residues. In some NRs, such as in the vertebrate thyroid hormone receptors, it contains 17 amino acid residues. Less frequently there is a variable number of amino acids in sequences forming the

Abbreviations: AR (NR3C4), androgen receptor; CAR (NR1I3), constitutive androstane receptor; COUP-TF (NR2F), chicken ovalbumin upstream promoter transcription factor; DAF-12, (abnormal) dauer formation (NR)-12; DAX-1 (NR0B1), dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1; DBD, DNA binding domain; (D)HR-3 (HR46, NR1F4), (*Drosophila*) hormone receptor-3, hormone receptor-like in 46; DPR-1, dauer pheromone responsive (NR)-1; E75, -78, ecdysone-induced protein 75, -78; EAR2 (NR2F6), V-erbA-related protein 2; EcR (NR1H1), ecdysone receptor; ER (NR3A1), estrogen receptor; ERR (NR3B4), estrogen related receptor; FAX-1, (defective) fasciculations of axons phenotype (NR)-1; FXR (NR1H4), farnesoid X receptor; GCNF, germ cell nuclear factor; GFP, green fluorescence protein; GR (NR3C1), glucocorticoid receptor; HNF4 (NR2A1), hepatocyte nuclear factor-4; LBD, ligand binding domain; LRH1 (NR5A2), liver receptor homologue-1; LXR (NR1H), liver X receptor; MR (NR3C2), mineralocorticoid receptor; NGFIB (NUR77, NR4A1), nerve growth factor IB; NHR(s), nuclear hormone receptor(s); NR(s), nuclear receptor(s); NOR1 (NR4A3), neuron-derived orphan receptor-1; NURR1 (NR4A2), nuclear receptor related-1; ODR-7, odorant response abnormal (NR)-7; PNR (NR2E3), photoreceptor cell-specific nuclear receptor; PPAR (NR1C), peroxisome proliferator-activated receptor; PR (NR3C3), progesterone receptor; PXR (NR1I2), pregnane X receptor; RAR (NR1B), retinoic acid receptor; RNAi, RNA interference; ROS, reactive oxygen species; ROR (NR1F), retinoid-related orphan receptor; RXR (NR2B), retinoid X receptor; SEX-1, signal element on X-1; SF-1 (NR5A1), steroidogenic factor-1; SHP (NR0B2), small heterodimer partner (NR); SVP (NR2F3), seven-up (NR); TLL (NR2E2), tailless (NR); TLX (NR2E1), vertebrate homologue of the *Drosophilla* tailless gene (NR); TR2, −4 (NR2C1, −2), testicular receptor 2, −4; TR (THR, NR1A), thyroid hormone receptor; UNC, uncoordinated (NR); USP (NR2B4), ultraspiracle protein; VDR (NR1I1), vitamin D receptor

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* Corresponding author.
E-mail address: marta.kostrouchova@lf1.cuni.cz (M. Kostrouchova).

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loops between the coordinating cysteines. Exceptionally, NRs with two DBD domains are found in some species, e.g. in *Platyhelminthes Schistosoma mansoni* [5], *Schmidtea mediterranea*, *Dugesia japonica*, in the mollusk *Lottia gigantea*, and in the arthropod *Daphnia pulex* [6]. Another exception is represented by TLX, whose DBD differs from other NRs and forms a longer signature with additional cysteines [7]. TLX emerged very likely early in the evolution of metazoans since this NR is found in Cnidaria [8] and the unusual DBD is conserved in many metazoan species whose genomes have been sequenced. The LBD of NRs is also very characteristic at the structural level. It consists of 11 to 12 helices that form a highly conserved secondary structure despite the quite high variability in the primary sequence of various NRs [9]. Based on the sequence similarities of both DBD and LBD, it was possible to detect homologous NRs in several species of vertebrates, insects and nematodes and comparison of NRs from more or less distant species allowed subclassification of NRs into eight main subfamilies [10].

It is surprising that this division, which was based mostly on vertebrate and insect NRs [10] is very consistent with the evolutionary scheme that can be built from the large number of NRs now identified in sequenced genomes of distant taxa [8,11–13]. This most likely reflects the inherent properties for their regulatory and evolutionary potential.

Comparison of nematode, vertebrate and insect NRs indicates that the six main subfamilies of NRs were already evolved in animal species existing before the split of deuterostomes and protostomes [14,15] (Fig. 1). The number of NRs, however, differs in-between species not only in the total number of NR genes in the genomes but also in the number of NRs within certain NR subfamilies, especially in NR1 through NR4. Contrary to that, subfamilies NR5 and NR6 contain in sequenced genomes only one representative of each. Species as distant as those of Cnidaria and vertebrates show examples of multiplication and subspeciation of NRs within certain NR subfamilies. In certain phyla, the multiplication of NRs can be attributed to whole genome duplications (WGD), such as in vertebrates which experienced WGD twice [16]. Radiation and multiplication of NRs within subfamilies, however, cannot be simply explained by WGD and the duplication of NRs may be considered a primordial feature of their regulatory and evolutionary potential. This is strongly supported by the multiplication of NR2A members in *Caenorhabditis* [17,18]. The number of NRs varies between Rhabditida species reaching 289 in *C. elegans*, 232 in *C. briggsae* and 256 in *C. remanei* [19].

Accumulated data from genome sequencing projects and functional studies indicate that some NRs are highly conserved and are likely to keep orthologous functions in different phyla. In the majority of other NRs, it is possible to detect a structural and functional relationship to a particular NR subfamily but not to a particular receptor within this subfamily.

Of the 289 NRs (denominated as *nhr —*, *daf-12*, *fax-1*, *sex-1*, *unc-55*, *odr-7*, *dpr-1*) found in the *C. elegans* genome WormBase WS241 [20] only 15 to 20 are considered conserved [21–23] based on higher sequence similarity and functional studies (Fig. 2).

The other 269 NRs show variable relation to HNF4 and are more related to other NRs found only in nematodes than to vertebrate or insect homologues [18,22,24]. Their sequence conservation and their proven expression indicates that the majority of them are functional genes but their exact function or relation to particular NRs from other phyla based on mutual comparison of sequence may be difficult or misleading (as pointed out by Laudet and others [25]).

In *C. elegans*, the conserved NRs have been intensively studied. NRs have been shown to have critical roles in the regulation of nematode development and metabolism (reviewed below). Functional data not only revealed new facts concerning particular nematode developmental pathways but also shed light on the relation of nematode NRs to their closest vertebrate and insect homologues. They further support the evolutionary links between particular members of NR subfamilies in distant phyla. Several NRs that are classified as non-conserved (but still related to NR2A–HNF4) were shown to have critical functions in nematodes. Despite the diversity of the primary sequence an absolute majority of these seemingly non-conserved NRs is still highly conserved and consistently new studies are revealing important developmental and metabolic roles for these multiplied nematode specific NRs. Detailed knowledge about their functions is likely to contribute to the general

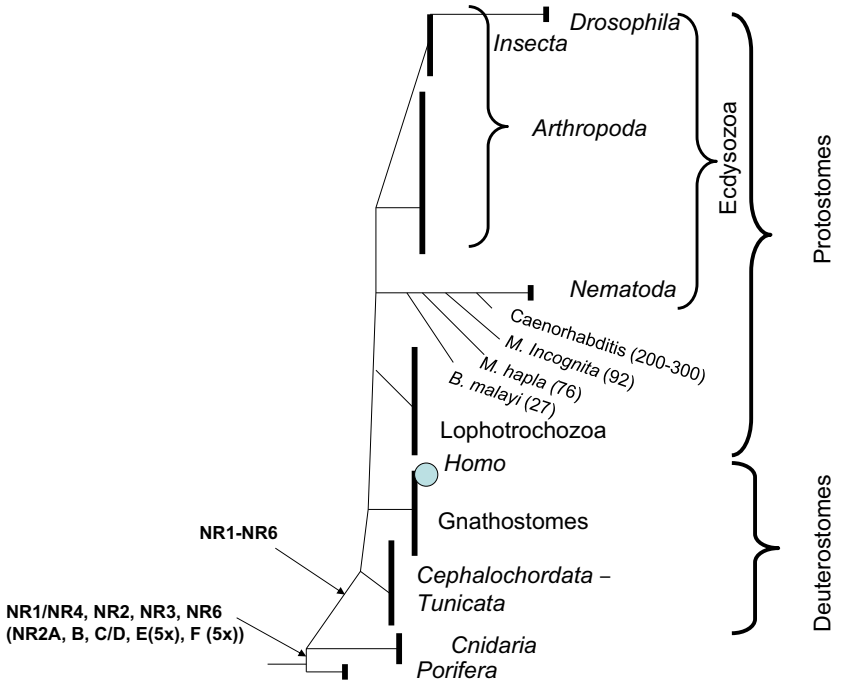

Fig. 1. Schematic representation of nuclear receptors found in sequenced genomes in relation to deuterostome and protostome evolution. The phylogenetic tree is derived from [227]. The occurrence of representative NR subfamilies indicates the early origin of founders of NRs (based on [8,10,14]). Numbers in brackets indicate the number of NRs in selected species.