Nuclear hormone receptors in *C. elegans**

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Table of Contents

Abstract

Nuclear receptors (NRs) are transcription factors typically regulated by lipophilic hormones, which coordinate metazoan metabolism, development and homeostasis. *C. elegans* has undergone a remarkable expansion of the family, harboring 284 of these receptors in its genome. Approximately 20 of them have been analyzed genetically, most of which correspond to conserved homologs in other metazoans. These NRs variously affect broad life history traits such as sex determination, molting, developmental timing, diapause, and life span. They also impact neural development, axon outgrowth and neuronal identity. Finally, they influence lipid and xenobiotic metabolism. The study of *C. elegans* NRs holds great promise for dissecting nuclear receptor signaling pathways *in vivo* in the context of complex endocrine networks.

1. Introduction

An early invention of metazoan signal transduction, nuclear receptors (NRs) comprise a family of transcription factors often regulated by small lipophilic molecules, such as steroids, retinoids, bile and fatty acids,

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that mediate endocrine control (Mangelsdorf et al., 1995). In addition, many so-called orphan NRs have either unidentified cognate ligands or none at all (Mangelsdorf and Evans, 1995). Although the vertebrate NRs are well-studied mechanistically, an exploration of NR function in a simple well-defined genetic organism such as *C. elegans* has illuminated their *in vivo* physiology, and their place in global regulatory networks.

2. Structure and mechanism

The key to NR signaling lies in their conserved molecular architecture (Mangelsdorf et al., 1995). The N-terminus contains a DNA binding domain (DBD) consisting of two Cys4 zinc fingers (Figure 1A). Much of the signaling intelligence resides in a C-terminal ligand binding domain (LBD), which not only sequesters ligand but also docks coactivators and corepressors—adaptor proteins that couple the NR to histone acetyltransferase and deacetylase complexes, respectively (Figure 1B). In addition, the LBD largely mediates receptor hetero- or homodimerization. Crystal structure and biochemical studies reveal that ligand binding triggers a conformational change in which a C-terminal transactivation helix, called AF-2, snaps back onto the LBD core (Bourguet et al., 1995; Renaud et al., 1995). Consequently, bound corepressors are displaced by coactivators, leading to gene expression. Moreover, NR activity is often regulated by phosphorylation, acetylation, and other covalent modifications (Fu et al., 2004).

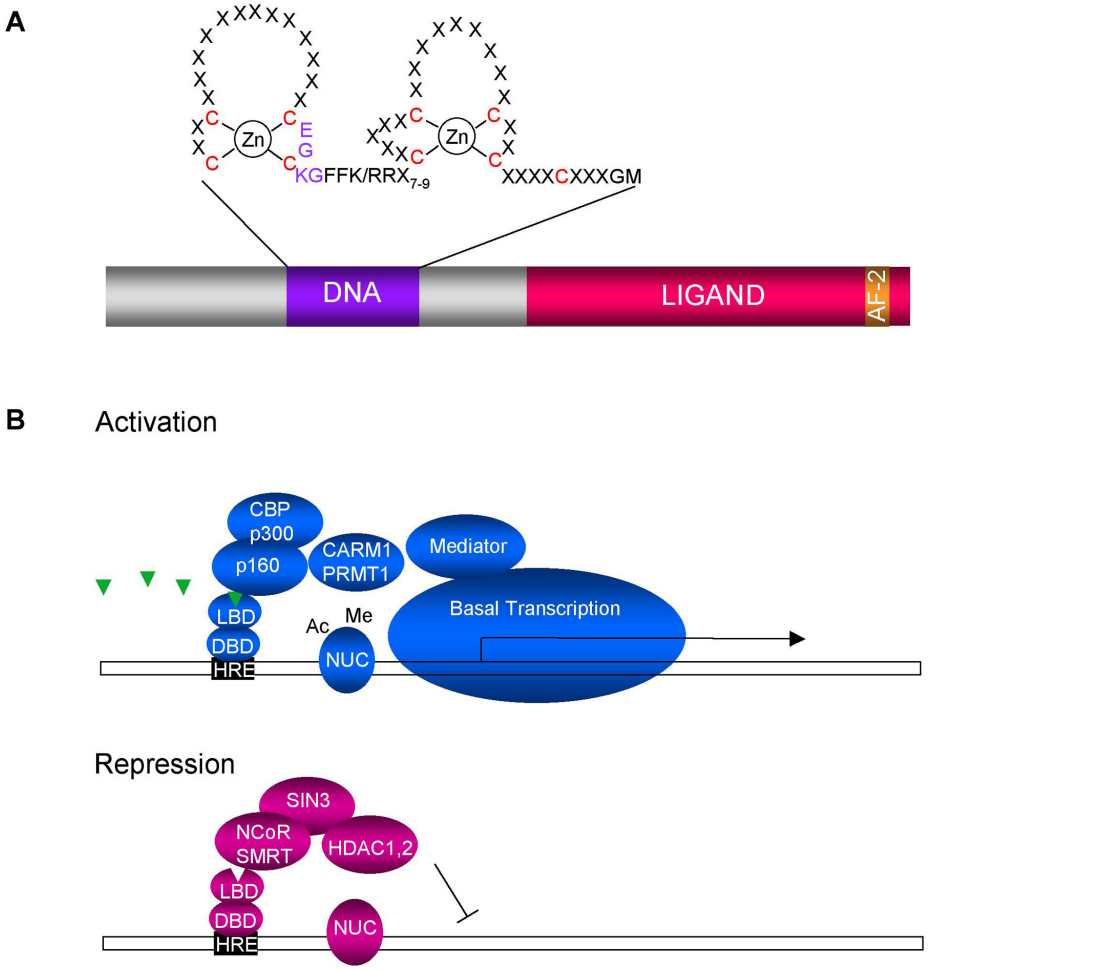

Figure 1. (A) Schematic diagram of an exemplary nuclear hormone receptor. DNA binding domain (DNA) comprised of two Cys4 Zn fingers. Nine conserved cysteines are highlighted in red. A typical P-box, (CEGCKG) which confers DNA binding specificity, is shown. The ligand binding domain (ligand) contains multiple surfaces that recruit coactivators or corepressors. The AF-2 transactivation helix closes the ligand binding pocket during hormonal activation. (B) General model for transcriptional activation and repression based on vertebrate receptors. In the presence of hormone (triangle), NRs bind to their hormone response elements (HRE), and assemble coactivator complexes that acetylate (ac, p160, p300) or methylate (me, CARM1, PRMT1) nucleosomes (NUC). Mediator components contact NRs and facilitate the recruitment of the basal transcription machinery, turning on gene expression. Some nuclear receptors repress in the absence of hormone, by assembling corepressor complexes, such as SMRT and NCoR, which recruit histone deacetylases (HDACs) that inhibit transcription. The composition of activation and repression complexes depends on the NR as well as the promoter context. In addition, many NRs work as homo- or heterodimers.

3. Comparative phylogeny

Evidently, NRs have undergone an explosive expansion and divergence in the worm. *C. elegans* has an astounding 284 receptors, compared to 48 for humans and 21 for flies. (For comparative phylogenetic trees see Maglich et al., 2001; Robinson-Rechavi et al., 2005; Sluder and Maina, 2001; Sluder et al., 1999). Fifteen NRs have clear homologs in other species (Table 1), and include relatives of the mammalian HNF4, Vitamin D receptor, COUP-TF, SF1, ROR, PNR, GCNF, TLX as well as the *Drosophila* DHR3, DHR38, E75, E78, DHR78, and DHR96. The other 279 receptors arose from an ancestral HNF4 (Robinson-Rechavi et al., 2005). Why do worms have so many receptors? One thought is that gene duplication has been deployed instead of receptor combinatorics. Consistent with this, *C. elegans* lacks an apparent RXR/USP, a heterodimeric partner with numerous NRs (Sluder and Maina, 2001). However, yeast two-hybrid screens identify several interacting NRs (Li et al., 2004), suggesting *C. elegans* may have evolved a combinatorial system different from RXR.

Table 1. Some functionally described *C. elegans* NRs and their homologs

C. elegans	**Function**	***D. melanogaster***	**Function**	***M. musculus/ H. sapiens***	**Function**
SEX-1	Sex determination	E78	Molting	revERB	Circadian clock, transcriptional repression
NHR-85	Egg laying, molting?	E75	Molting		
NHR-23	Molting, epidermal differentiation, dauer formation	DHR3	Embryonic development, molting, metamorphosis	ROR alpha	Cerebellar differentiation
				beta	Circadian clock
				gamma	Thymopoesis
NHR-25	Ventral closure, epidermal differentiation, molting, dauer formation	FTZ-F1 alpha	Segmentation	SF1	Steroidogenesis
		FTZ-F1beta	Molting, metamorphosis	LHR	Cholesterol homeostasis, bile acid metabolism
NHR-41	Dauer formation	DHR78	Molting, metamorphosis	TR2/TR4	Unknown
NHR-6	Ovulation	DHR38	Molting	Nurr1	Dopaminergic differentiation
				NGFI-B/nur 77	Apoptosis, immediate early response
NHR-67	Molting, growth, vulval formation	Tailless	A/P patterning, neurogenesis	TLX	Forebrain development, neural stem cell maintenance
NHR-91	No obvious function	DHR4	Unknown	GCNF	Germ cell differentiation, embryogenesis
DAF-12	Dauer formation, lipid metabolism, stage specification, life span	DHR96	Unknown	VitD	Bone differentiation, bile acid metabolism
				CAR, PXR	Xenobiotic and bile metabolism
NHR-8	Xenobiotic metabolism				
NHR-48	Unknown				

C. elegans	**Function**	***D. melanoga-ster***	**Function**	***M. musculus/ H. sapiens***	**Function**
NHR-49	Fatty acid metabolism	dmHNF4	Unknown	HNF4	Glucose homeostasis, liver metabolism
UNC-55	Neural differentiation	Seven-up	Photoreceptor fate	COUP TF1 COUP TF2	Neural development
FAX-1	Neural differentiation	dmFAX-1	Unknown	PNR	Photoreceptor fate
ODR-7	Olfaction	None		None	

4. Physiology

Only about twenty *C. elegans* NRs have described visible phenotypes. Below we highlight a handful.

5. Sex determination

Acting early in development, SEX-1 regulates *C. elegans* sex determination and dosage compensation by downregulating a sex determining gene called *xol-1*(Carmi et al., 1998). Encoded on the X chromosome, *sex-1*´s dose determines the level of *xol-1* repression. When SEX-1 is high, XOL-1 is low, and animals develop as hermaphrodites. Conversely, when SEX-1 is low, XOL-1 is high, and animals become males. SEX-1 is most homologous to E78A, a *Drosophila* ecdysone-induced NR of unknown function (Stone and Thummel, 1993), and to vertebrate rev-Erb, an orphan NR that behaves as a transcriptional repressor in circadian oscillators (Preitner et al., 2002). The SEX-1 ligand binding domain is quite diverged, and it is unknown whether its activity is hormone regulated. Interestingly, *C. elegans* sex is also influenced by diet; paternal X disjunction is increased by unknown metabolites from log phase *E. coli* (Prahlad et al., 2003). Conceivably, such metabolites might somehow impinge on SEX-1.

6. The molt cycle

Nematodes are postulated to belong to the Ecdysozoa, a proposed broad clade of animals that molt (Aguinaldo et al., 1997). In *Drosophila* and other insects, pulses of the hormone 20-hydroxyecdysone stimulate the ecdysone receptor, which initiates transcriptional cascades that drive molting and metamorphosis (Riddiford et al., 2003). Downstream, several NR transciption factors, including DHR3, FTZ-F1, E75/E78, DHR38, DHR78 and others are turned on in a stereotypical sequence (Ashburner, 1974; Riddiford et al., 2000; Sullivan and Thummel, 2003). Remarkably, *C. elegans* lacks ecdysone, the ecdysone receptor, and its heterodimeric partner USP. Little is known about how the molt cycle is driven. Conceivably, another sterol hormone does the job, since cholesterol deprivation, as well as disruption of genes implicated in sterol transport result in molting, growth or fecundity defects (Matyash et al., 2004; Merris et al., 2003; Shibata et al., 2003; Shim et al., 2002; Yochem et al., 1999). In addition, *C. elegans* harbors five orthologs of the ecdysone inducible NRs mentioned above. Several are expressed periodically, attuned to the molt cycle, and some mediate ecdysis although others do not (Asahina et al., 2000; Gissendanner et al., 2004; Gissendanner and Sluder, 2000; Kostrouchova et al., 2001).

C. elegans NHR-23 is a homolog of *Drosophila* DHR3, which mediates the pre-pupal to pupal transition in fly (Lam et al., 1999). Similarly, NHR-23 functions in ecdysis; RNAi knockdown results in molting defects at all four molts. Other phenotypes indicate aberrant epidermal differentiation, including disrupted collagen synthesis, epidermal seam cell displacement and blunted male tail development (Kostrouchova et al., 1998; Kostrouchova et al., 2001). Accordingly, NHR-23 is expressed in the epidermis. The vertebrate homologs, RORα,β,γ, function in various processes including Purkinje cell generation, circadian rhythms, and thymopoesis (Jetten et al., 2001). It may be significant that orthologs in all three species are part of biological clocks—the molt cycle and the circadian oscillator.

C. elegans NHR-25 belongs to a highly conserved receptor subtype that includes *Drosophila* FTZ-F1 and the human SF1 and LRH. *Drosophila* FTZ-F1 functions in embryonic segmentation and larval metamorphosis (Broadus et al., 1999; Guichet et al., 1997; Lavorgna et al., 1993; Ueda et al., 1990; Yu et al., 1997). Similarly, *nhr-25* mutants arrest at the two-fold stage of embryogenesis, prior to elongation, with defects in ventral closure of the epidermis (Chen et al., 2004; Silhankova et al., 2005). Larvae also have defects in molting, epidermal and vulval cell

fusion, and cell elongation. Adults exhibit aberrant somatic gonadal development, tumorous germlines, and are sterile (Asahina et al., 2000; Gissendanner et al., 2004; Gissendanner and Sluder, 2000; Hwang and Sternberg, 2004). Consistent with its phenotype, NHR-25 is expressed in epidermis and somatic gonad (Asahina et al., 2000; Gissendanner and Sluder, 2000). Interestingly, mammalian SF1 also controls differentiation of the gonad, as well as the adrenal gland, pituitary and hypothalamus, where it initiates transcription of key genes involved in steroidogenesis (Parker et al., 2002). LRH regulates bile acid and cholesterol metabolism (Fayard et al., 2004).

The rudimentary sketches of an ecdysis signaling pathway are beginning to take shape. Interestingly, *let-767* encodes a 17-betahydroxysteroid reductase, which when mutated gives rise to molting defects and embryonic lethality reminiscent of NHR-25 (Kuervers et al., 2003). Conceivably, this protein works upstream in the production of a molting hormone. Downstream of both NHR-25 and NHR-23, *acn-1* encodes a metalloprotease implicated in molting (Brooks et al., 2003).Work from *Drosophila* suggests that DHR3 inhibits expression of FTZ-F1 in ecdysone regulatory cascades (Kageyama et al., 1997; Lam et al., 1999; White et al., 1997), but no such regulation has been seen in the worm (Kostrouchova et al., 2001). Fly FTZ-F1 has also been shown to form a heterodimeric complex with the FTZ homoedomain transcription factor to regulate embryonic patterning (Guichet et al., 1997; Yu et al., 1997). Similarly, NHR-25 may form a functional complex with two Hox genes, NOB-1 and LIN-39, to influence embryonic and larval cell fates (Chen et al., 2004).

NHR-23 and NHR-25 as well as NHR-41, a DHR78 homolog (Fisk and Thummel, 1998), affect the dauer molt and morphogenesis (Gissendanner et al., 2004). Two other NRs implicated in *Drosophila* ecdysone cascades have no obvious molting phenotypes. RNAi depletion of NHR-6, the ortholog of DHR38/NGF-IB, instead results in ovulation defects, while knockdown of NHR-85 (E75) causes an egg laying phenotype, suggesting responsibilities in reproductive biology (Gissendanner et al., 2004). Alternately, egg laying defects could result from localized obstruction of cuticle deposition or shedding from the vulva.

Surprisingly, NHR-67 plays an unexpected role in the molt cycle and vulval morphogenesis (Gissendanner et al., 2004). RNAi knockdown results in animals that have difficulty shedding the L3 cuticle and a protruding vulva phenotype, but the cellular basis of these defects remains to be determined. By contrast, the *Drosophila* homolog TLL has no known role in molting or metamorphosis. Instead it influences anterior/posterior patterning including that of the embryonic CNS (Strecker et al., 1988). The mouse homolog, TLX, functions in the forebrain where it is involved in the generation and differentiation of neurons destined for superficial cortical layers, (Land and Monaghan, 2003; Roy et al., 2002), as well as maintenance of adult neural stem cells (Shi et al., 2004).

7. Dauer formation

From the perspective of signal transduction, DAF-12 is perhaps the best characterized NR in *C. elegans*. The outline of an entire hormonal signaling pathway—from signaling inputs, genes involved in hormone transport and metabolism, transcriptional complexes, binding sites, and gene targets—is emerging (Figure 2).

DAF-12 couples environmental cues to life history alternatives, acting at the nexus of pathways governing metabolism, dauer diapause, heterochronic stage selectors, and life span (Antebi et al., 1998; Gerisch et al., 2001; Hsin and Kenyon, 1999; Jia et al., 2002; Larsen et al., 1995; Riddle et al., 1981). For dauer formation, DAF-12 integrates signals from Insulin/IGF-I, TGF-beta and cGMP pathways to mediate either reproductive development or arrest at the dauer diapause. DAF-12 relatives include Vitamin D, Pregnane-X, Liver-X and Androstane receptors (Antebi et al., 2000; Snow and Larsen, 2000), all of which can respond to hormones ultimately derived from cholesterol. Although a DAF-12 hormone has not yet been identified, clear evidence argues for its existence. Notably, DAF-9 encodes a cytochrome P450 related to mixed function oxygenases involved in steroid hormone and xenobiotic metabolism (Gerisch et al., 2001; Jia et al., 2002). *daf-9* mutants phenotypically resemble *daf-12* LBD mutants: they form dauer larvae constitutively, have heterochronic delays in gonadal outgrowth, and are long lived. Moreover, phenotypes are *daf-12(+)* dependent. Expressed from a handful of endocrine tissues, DAF-9 works cell non-autonomously to control programs throughout the body (Gerisch and Antebi, 2004; Mak and Ruvkun, 2004).

Preliminary evidence supports the hypothesis that the DAF-12 hormone could be a sterol derivative. Interestingly, cholesterol deprivation produces defects similar to *daf-9* and *daf-12* LBD mutants (Gerisch et al., 2001, Jia et al., 2002). Moreover, the *C. elegans* Niemann-Pick C1 homologs, NCR-1 and NCR-2, act at the same point as DAF-9 in the dauer signaling pathways (Li et al., 2004; Sym et al., 2000); such proteins are implicated in intracellular cholesterol trafficking in mammals (Ribeiro et al., 2001). Finally, crude lipid fractions can rescue dauer formation induced by cholesterol starvation (Matyash et al., 2004), as well as the Daf-c phenotypes of *daf-9* mutants (Gill et al., 2004; A. Antebi, B. Gerisch, unpublished). Together, these data constitute the first functional evidence

for any kind of lipophilic hormone in the worm. Further work should ultimately reveal the molecular identity.

More recently, the coregulator DIN-1 has been shown to bind DAF-12 to specify diapause and long life in the absence of hormone (Ludewig et al., 2004), indicating that the unliganded complex is central to organismal biology. DIN-1 is homologous to human SHARP, a corepressor for nuclear receptors and other transcription factors (Oswald et al., 2002; Shi et al., 2001; Shi et al., 2002). A unifying model is that a DAF-12/coregulator complex works as a hormone regulated switch specifying fast life history in the presence of ligand and slow life history in its absence (Figure 2).

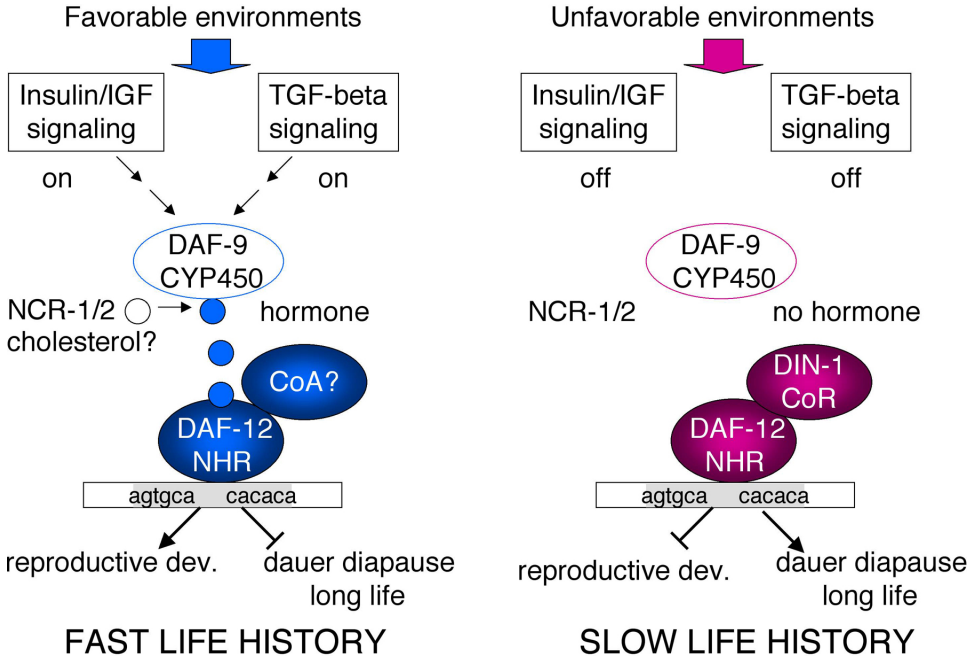

Figure 2. Life history regulation by DAF-12 . In favorable environments, Insulin/IGF-I and TGF-beta peptide signal converge on the nuclear receptor branch of the dauer pathways. Niemann-Pick C1 homologs NCR-1/2 deliver cholesterol to DAF-9/cytochrome P450 and perhaps other biosynthetic enzymes, triggering synthesis of a hormone, presumably a sterol. In the presence of hormone, DAF-12/NHR assembles postulated coactivator complexes, and directs expression of genes involved in reproductive development, developmental advance, fat metabolism, and accelerated aging (fast life history traits). In unfavorable environments, hormonal pathways are suppressed. Unliganded DAF-12 together with DIN-1/coregulator specify programs of dauer diapause, delayed development, fat storage, and retarded aging (slow life history traits). Insulin/IGF-I signaling also independently regulates longevity and aspects of diapause.

At least two disparate response elements and several target genes have been identified for DAF-12 (Ao et al., 2004; Shostak et al., 2004). One element (AGTGCA; Shostak et al., 2004) resembles the half sites of VitDR (Freedman et al., 1994) and DHR96 relatives (Fisk and Thummel, 1995). The other (CACACA) is often found juxtaposed to PHA-4/forkhead binding sites in pharyngeal expressed genes, employed in pharyngeal remodeling (Ao et al., 2004). In addition, several key genes in the heterochronic circuit (e.g. *lin-28*; Antebi et al., 1998; Seggerson et al., 2002) *let-7* (Johnson et al., 2003) and dauer pathways (*daf-9*; Gerisch and Antebi, 2004; Mak and Ruvkun, 2004) are regulated by *daf-12*, but it is unknown if regulation is direct or indirect. Finally, NHR-23, NHR-25 and NHR-41 also influence dauer morphogenesis and molting (Gissendanner et al., 2004). Understanding their roles in the dauer transcriptional hierarchies will be interesting to pursue.

8. Xenobiotic response

Xenobiotic defense is key to *C. elegans* survival given its soil ecology and exposure to plant, fungal and bacterial toxins. Homologous to the xenobiotic receptors CAR and PXR, NHR-8 is proposed to manage xenobiotic stress. Mutants are more sensitive to colchicine and chloroquine (Lindblom et al., 2001). Moreover, NHR-8 is expressed in the worm intestine, the equivalent to the mammalian liver. Xenobiotics often themselves behave as ligands for xenobiotic receptors but whether colchicine, chloroquine or any other compounds stimulate NHR-8 is unknown. Nor is it known whether NHR-8 induces phase 1 and 2 detoxifying enzymes. Several cytochrome P450 enzymes are induced by xenobiotics, but the regulation of this response remains unexplored (Menzel et al., 2001). More recently, NHR-8 has been shown to also influence lipid metabolism since Nile red deposition is altered in RNAi knockdown experiments (Ashrafi et al., 2003).

9. Metabolic control

Vertebrate receptors, such as PPAR, FXR, and LXR, are lipid sensors that regulate fatty acid, bile and cholesterol metabolism. Though orthologs of these receptors are absent in *C. elegans*, genome-wide screens identified several NRs that increase (NHR-8, NHR-49, C56E10.4, F16B4.9, H12C20.3) or decrease (DAF-12, NHR-25, Y69A2A_7278, C33G8.9, KO8A2.b) lipid deposition when knocked down by RNAi (Ashrafi et al., 2003). Further studies on NHR-49 reveal that it upregulates genes for fatty acid beta oxidation (*acs-2*, *ech-1*), desaturation (*fat-5*, *fat-7*), and transport (Van Gilst et al., 2004) as well as genes for synthesis of monomethyl branched chain fatty acids (Kniazeva et al., 2004). Mutants accumulate saturated fatty acids and are short lived. Overall, NHR-49 is thought to coordinate fat consumption and the balance of fatty acid saturation. Despite homology to HNF4, NHR-49 may have assumed many of the responsibilities of PPARα, based on its regulatory spectrum (Van Gilst et al., 2004). Other NRs perhaps more similar to HNF4, such as NHR-64 and NHR-69, have no overt phenotype (Gissendanner et al., 2004), but their regulatory spectra have yet to be examined. Interestingly, NHR-49 physically interacts with numerous other NRs by yeast two-hybrid (Li et al., 2004), suggesting it may work as a common heterodimeric partner.

10. Neural development

Because the *C. elegans* nervous system is described down to the synaptic level (White et al., 1986), there is unparalleled opportunity to dissect nematode neurobiology. Several identified NRs affect neural development. UNC-55, an ortholog of the orphan receptor COUP-TF/Seven-up specifies the synaptic wiring of VD motorneurons during the L1stage (Walthall and Plunkett, 1995; Zhou and Walthall, 1998). In mutants, the post-embryonicVD motorneurons differentiate like their embryonic DD counterparts. Expressed in the VD neurons, UNC-55 autonomously prevents expression of the DD fate. Similarly, in the *Drosophila* eye, Seven-up quells the R7 fate in neighboring photoreceptor cells (Mlodzik et al., 1990). In mice, COUP-TFI affects neural crest ganglionic precursor cells, axon guidance, and early neocortical regionalization (Qiu et al., 1997; Zhou et al., 1999; Zhou et al., 2001). FAX-1 is a homolog of mammalian PNR, a nuclear receptor associated with hyperproliferation of blue cone cells and retinal degeneration (Gerber et al., 2000; Haider et al., 2000). FAX-1 is also related to Tailless/Tlx. Expressed in 20 neurons, FAX-1 alters late aspects of neural fate (Much et al., 2000). In *fax-1* mutants, AVK interneurons fail to extend along the ventral cord and into the nerve ring, and fail to express specific neuronal markers. Expression of FAX-1 in AVK suggests a cell autonomous role. However, it also affects the outgrowth of neurons in which it is not expressed (Wightman et al., 1997), suggesting a non-autonomous role in guidepost cells. Recently, FAX-1 has been shown to work in a complementary or combinatorial fashion with the UNC-42/paired-homeodomain protein in specifying aspects of interneuron cell fate (Wightman et al., 2005).

ODR-7 controls the fate of specific olfactory neurons (Sengupta et al., 1996; Sengupta et al., 1994). In mutants, AWA neurons express markers of AWC, suggesting that ODR-7 specifies late aspects of AWA fate, while repressing the AWC fate. Interestingly, ODR-7 lacks an obvious LBD, and the DBD is displaced to the C-terminus. How much of the NR machinery is coopted by this divergent receptor is unclear. *odr-7* reveals a surprising level of complexity, with specific residues differently influencing autoregulation, activation, or repression of downstream target genes (Colosimo et al., 2003). Several other divergent NRs are neuronally expressed but their functions are unknown (Miyabayashi et al., 1999).

11. Future directions

Undoubtedly the future of NRs lies in the full exploitation of genomic tools available, such as RNAi, two-hybrid, and transcriptional profiling, as well as classical approaches of suppressor and enhancer genetics and transgenesis, to explore regulatory networks. Elucidation of NR signaling may be realized by investigating candidates with similar or contrary phenotypes. In particular, this approach could be used to identify inputs from signal transduction pathways, potential hormone metabolic genes, coactivators and corepressors, and perhaps unknown factors that impinge on receptor activity. By perturbing NR function itself, we may gain further insight into the physiological output by scrutinizing detailed patterns of target gene regulation or metabolic spectra. Furthermore, these analyses may also reveal transcriptional hierarchies, combinatorial control and connectivity with other signaling pathways .

Several specific challenges lie ahead. The dissection of the molting pathways, identification of hormones, the elucidation of lipid metabolic networks, the coordination of neural development, and a detailed exploration of the xenobiotic response come immediately to mind. In addition, little is known about the role of *C. elegans* NRs in ion

balance, stress response, immunity, and numerous other processes where vertebrate receptors have a proven function. With the vast number of *C. elegans* receptors largely unexplored, many with no obvious phenotype, the challenge will be to discern their physiological responsibility. The other task will be to relate the roles of both ancestral and diverged receptors to functional vertebrate counterparts. Finally, solidifying genetic inferences with biochemistry, e.g. dissecting transcriptional complexes and identifying hormones, will prove crucial to spanning molecular mechanism to physiology.

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