Published in final edited form as:
Trends Endocrinol Metab. 2008 July ; 19(5): 153–160. doi:10.1016/j.tem.2008.02.005.

C. elegans Nuclear Receptors: Insights into Life Traits

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Abstract

Nuclear receptors (NRs) are a class of hormone-gated transcription factors found in metazoans that regulate global changes in gene expression when bound to their cognate ligands. Despite species diversification, NRs act similarly across taxa to play fundamental roles in detecting intrinsic and environmental signals, and subsequently in coordinating transcriptional cascades that direct reproduction, development, metabolism, and homeostasis. These endocrine receptors function *in vivo* in part as molecular switches and timers that regulate transcriptional cascades. Here we discuss in detail how several *C. elegans* NRs integrate intrinsic and extrinsic signals to regulate the dauer diapause and longevity, molting, and heterochronic circuits of development, and draw parallels to similar *in vivo* endocrine regulated processes in other animals.

Introduction

Nuclear receptors (NRs) comprise an ancient family of hormone-gated transcription factors, which regulate metazoan gene expression in response to lipophilic ligands [1]. Ligand-gated transcription provides a direct and powerful means to couple environmental and nutrient signals to coordination of metabolism, development, reproduction, and homeostasis. Given the central role of NRs in animal biology, it is perhaps not surprising that their dysfunction accounts for many major human diseases, including diabetes, obesity, cancer, and cardiovascular disease [2,3]. Because they can be pharmacologically manipulated by agonists or antagonists, NRs represent an important avenue for disease intervention.

A common molecular architecture, including a conserved N-terminal DNA binding domain (DBD) and a more variable C-terminal ligand binding domain (LBD) underlies NR signaling capabilities [1]. DNA binding is achieved by the association of two C4-Zn fingers with specific DNA response elements in the promoters of target genes. Transcriptional activation is achieved when, upon ligand binding, an NR undergoes a conformational change in which a C-terminal activation helix, AF-2, folds back onto the LBD core, thereby locking the NR in an active conformation [4–6].

NRs, having no ligand or for which no ligand(s) has been identified, are dubbed orphans [7]. Efforts to identify cognate ligands for these orphans, such as oxysterols and bile acids for the liver X receptor (LXR) [8,9] and phospholipids and sphingolipids for steroidogenic factor 1 (SF-1) [8,10,11], have helped illuminate their physiological roles and mechanism. Other NRs can be constitutively actived by virtue of hydrophobic amino acid side chains occupying the ligand binding pocket [12] or can undergo ligand-independent regulation *via* intrinsic activation domains [13]. Moreover, covalent modifications of NRs, such as phosphorylation, acetylation, sumoylation, and ubiquitylation often modulate their transcriptional activity [14]. Finally, depending on type and context, NRs can act as monomers, heterodimers, and/or

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homodimers, as well as work in heterologous transcriptional complexes, giving rise to great combinatorial diversity and mechanistic complexity [1].

An important instructive component of NR signaling arises from their association with coregulators, adapator molecules that couple the NR to activating or repressive transcriptional machinery. NR-ligand binding typically results in the recruitment of coactivators, which consequently stimulates transcription, whereas unliganded NRs often dock corepressors, which repress transcription [15]. In fact, numerous coregulator complexes have been identified, and an emerging theme is that they may serve to coordinate diverse transcription factors that work together [15]. Mechanistically, transcriptional activation potentials of ligand-bound NRs are thought to be modulated by competition between coactivators and corepressors, which allows activation-response curves to range from continuous gradients to sharp thresholds [15,16].

A comparison of the origins and functions of NRs has revealed important insights into their structural and functional diversification. Speciation, contributed in part by gene duplication events, has resulted in the amplification and subsequent divergence of NRs among metazoans. The NR superfamily is thought to have undergone two waves of expansion during metazoan evolution, giving rise to several paralogs [17]. The genome of *C. elegans* is predicted to contain a remarkable 284 NRs, whereas humans have 48, mice 49, and *Drosophila* 18 [18–21]. Roughly 15 worm NRs are homologous across taxa, whereas the remainder is thought to have evolved from an explosive expansion of the HNF4 lineage [22]. Although the reasons for this expansion are unknown, it is plausible that these novel receptors are deployed in processes such as chemical defense or immunity that require recognition of diverse molecules.

Despite the wealth of information on the mammalian NRs, studies in a simple model organism such as *C. elegans* offer distinct advantages. The worm's streamlined anatomy (959 cells) and completely described development provide an unparalleled view of gene regulation at the single cell level. Most importantly, genetic analysis permits a facile examination of NR mechanisms, physiology, and signal transduction in an *in vivo* setting.

In general, the *C. elegans* receptors, like their mammalian counterparts, can be classified as contributing to either reproduction and development or to nutrient cycling and metabolism [23]. Significantly, in mammals, the expression patterns, rhythmic cycling, and physiological pathways affected by NRs do not strictly correlate with their known phylogenetic relationship within the NR subfamilies [23]. Notably, even within a species, structurally-related paralogs have undergone substantial functional diversification [17]. Thus, function and mechanism of action could be more informative than structural orthology in dissecting NR biology.

Indeed, such functional studies in *C. elegans* have already led to important insights into the role of NRs in sex determination, developmental timing, molting, aging, cell fate determination, neural differentiation, and metabolism, with implications for higher animals [24]. From another perspective, NRs can act as molecular switches, timers, homeostatic devices, or gradient regulators (rheostats). These devices are utilized in a variety of biological processes. Here, we focus in detail on several *C. elegans* NRs that function as switches or timing devices in the context of dauer formation, molting, and heterochrony, while also drawing parallels to NRs of other organisms.

Dauer Formation

As transcription factors that can activate or repress, NRs have the ability to act as switches that decide between alternate fates. We discuss here how the DAF-12 NR works as a switch to regulate the choice between reproductive growth and dauer arrest.

C. elegans larval development and reproductive maturation are responsive to nutrient cues and governed by an NR-dependent switch. Development and reproduction are energetically costly processes that require proper assessment of environmental and nutritional inputs for maximal fitness. *C. elegans* develops through four larval stages (L1 to L4) to become a reproductive adult. In an environment of limited food, high temperature, or overcrowding, the animal diverts its development into an alternative third larval stage called the dauer diapause (L3d) (Figure 1). Dauer larvae are developmentally arrested, sexually immature, stress resistant, long lived, and geared for survival. Upon return to favorable conditions, these larvae exit the dauer state to resume development and reproduction [25]. Importantly, a molecular-genetic dissection of dauer formation has provided key insights into metazoan longevity, growth control, and cancer, as well as fat metabolism and diabetes. More specifically, it has revealed how environmental cues are transformed into a hormone-regulated developmental switch.

DAF-12 is perhaps the best understood *C. elegans* NR and plays a key role in the choice between the dauer diapause and reproductive development. It also influences fat metabolism, developmental timing, and adult longevity. Though most closely related to the vertebrate vitamin D (VDR) and LXR, and the *Drosophila* hormone receptor HR96 [26], DAF-12 may work analogously to mammalian estrogen receptor (ER) by coupling nutrient cues to maturation. Epistasis analysis indicates that DAF-12 works downstream of several signaling cascades including the insulin/IGF-1 signaling (IIS) and TGF-β pathways [25,27–31]. Cellular and molecular genetic studies suggest a model whereby in favorable environments, cues integrated by sensory neurons result in the graded production of TGF-β and insulin-like peptides (Figure 1), with their respective signal transduction pathways converging in endocrine tissues on biosynthetic enzymes involved in the production of the DAF-12 ligands, the dafachronic acids (DA) (see below) [27,28,32,33]. Notably, when bound to its ligand, DAF-12, along with presumptive coactivators, promotes reproductive growth (Figure 1). In unfavorable conditions, during which levels of TGF-β and insulin-like peptides decrease, DA production is thought to be repressed. Unliganded DAF-12, together with its corepressor DIN-1, a homolog of the mammalian SHARP corepressor, promotes dauer diapause and longevity [34]. Thus, DAF-12 and associated co-regulator complexes work as hormone-regulated molecular switches that specify two different life history modes, reproductive development or the dauer diapause. Importantly, hormone deficient mutants such as *daf-9* are long lived as adults and this longevity is dependent upon *daf-12*(+) [28,29]. Similarly, animals that are germline deficient are also long lived, and this too depends upon *daf-12*(+) [35]. These studies provide pioneering evidence for NR control of somatic endurance and longevity, thereby suggesting potential research avenues for the vertebrate receptors.

Endogenous ligands for DAF-12 were recently identified, the first for any of the 284 *C. elegans* NRs, which has been instrumental in elucidating the mechanisms underlying the dauer switch [33]. Δ^4- and Δ^7-DA are cholestenoic acid derivatives that, at nanomolar concentrations, bind and activate DAF-12, thereby promoting downstream transcription [33]. Evidence that the DAF-9/cytochrome-P450 (CYP450) enzyme acted directly upstream of DAF-12 in a cell non-autonomous fashion suggested that it was likely to help synthesize a steroidal ligand for DAF-12 [27–29,36]. Accordingly, lipid extracts from wild-type worms contained both DAs, whereas extracts from *daf-9* mutants contained neither. Moreover, exogenous Δ^4-DA could rescue all *daf-9*/CYP450 phenotypes, including those of dauer formation, gonadal maturation, and aging [32,33]. Δ^4-DA could also rescue the dauer-constitutive phenotypes of animals carrying mutations in *daf-2*/Insulin receptor, *daf-7*/TGF-β, or sterol trafficking Niemann-Pick type C1 homlogs, *ncr-1* and *ncr-2*, but not *daf-12*, supporting the predicted epistatic relationship of these genes [33].

The discovery of DA has also led to insights into the nature and regulation of the hormone biosynthetic pathway. Biochemical studies revealed that *daf-9*/CYP450 catalyzes the last step

in DA synthesis, the successive oxidation of the terminal side chain to the acid [33], in a manner similar to that of CYP27A1 (a regulator of mammalian bile acid synthesis) [37,38]. This raises the possibility that similar metabolites are found in mammals. In addition, a Rieske-like oxygenase, DAF-36, was proposed to carry out the first step, converting cholesterol to 7-dehydrocholesterol, thus outlining a pathway for DA biosynthesis [39]. Interestingly, a similar activity is seen for the *Drosophila* homolog *neverland* in ecdysteroid production [40]. The expression patterns of DAF-9 (XXX cells, epidermis, spermatheca) and DAF-36 (intestine) are non-overlapping, revealing that biosynthesis is distributed and subject to inputs from various tissues. Notably, the XXX cells are a pair of neuroendocrine cells in which several dauer signaling molecules are found. These include *sdf-9*, a protein tyrosine phosphatase homolog that may be important for insulin signaling, *ncr-1* and *ncr-2* Niemann-Pick homologs involved in sterol trafficking [41], *akt-1* kinase, and several enhancers of *akt-1* [42] Somewhat surprisingly, epidermal *daf-9* is dynamically regulated by *daf-12*, mostly in response to cholesterol availability, as well as to inputs from TGF-β and IIS [27,36]. Moreover, excess ligand is inferred to inhibit *daf-9* expression through DAF-12. Although the mechanisms underlying these various observations are currently unknown, determining the extent to which the worm homologs show similar regulatory circuitry to their mammalian counterparts will be important. For example, 25S-cholestenoic acid (akin to DA) is known to serve as a ligand for both LXR and DAF-12 [8,43], thus hinting at a potentially broader overlap. In addition, these interactions may also help us to understand how insulin, TGF-β, and steroid hormone receptor signaling converge to control reproduction in higher animals.

The identification of DA has clarified our understanding of coregulator activity, an essential component of the switching mechanism. In particular, molecular genetic data suggest that DIN-1 and DAF-12 interact to form a corepressor complex in the absence of ligand production (*i.e.*, in *daf-9* mutants). In support of this mechanism, Δ^4-DA was shown to dissociate the DAF-12/DIN-1 complex, abrogating repression in human cell culture [33,34]. Studies of DIN-1 also provide compelling *in vivo* evidence for a key biological role of the unliganded receptor and its corepressor in specifying alternate metabolic states, dauer diapause, and longevity [33,34]. The mammalian homolog SHARP works as a corepressor with several transcription factors, including unliganded retinoic acid receptor (RAR) and peroxisome proliferator activated receptor δ (PPARδ) [44,45], illustrating that this corepressor-NR interaction is evolutionarily ancient.

Altogether, these studies reveal that steroid-like control of reproduction from biology to mechanism is evolutionarily conserved, and point in particular to the importance of bile acid-like steroids as signaling molecules, which is an emerging theme in vertebrates [46].

Molting

Timing circuits play essential roles in regulating daily, monthly, seasonal, and maturational life history events, and often rely on positive and negative feedback loops or transcriptional cascades. NRs are uniquely poised to drive such circuits because they can integrate intrinsic and environmental inputs to coordinate programs throughout the body. Moreover, ligand gating provides precise temporal control and is well suited to homeostatic feedback. Here, we discuss how the invertebrate NRs govern various timing devices, including the molt cycle and heterochronic timers.

The molt cycle, the synthesis of the new exoskeleton and shedding of the old, is a developmental clock that utilizes several coordinating NRs. During each larval stage (L1-L4), *C. elegans* undergoes a cycle of cuticle synthesis and shedding before emerging as adults. Both the nematode *C. elegans*, as well as the arthropod *Drosophila*, are considered Ecdysozoans, a clade of animals that undergo molting [47]. This process is best understood in *Drosophila*, in which

pulses of 20-hydroxyecdysone stimulate a transcriptional cascade that drives molting *via* the ecdysone receptor (EcR) and its heterodimeric partner ultraspiracle (USP) [48]. Several downstream NRs are activated, including the *Drosophila* hormone receptor DHR3, Fushi tarazu-F1 (FTZ-F1), ecdysone-inducible proteins (E75/78), DHR38, and DHR78, which are activated in strict temporal sequence to drive molting [49–51]. These NRs are largely conserved across taxa. Humans homologs include RAR-related orphan receptor (ROR), SF-1, REV-ERB, Nur-related protein 1 (Nurr1), and testicular orphan receptor 2 and 4 (TR2/TR4); and all these mammalian NRs, except SF-1, display circadian expression levels [52]. *C. elegans* has homologs to all except EcR and USP; these include NHR-23, NHR-25, SEX-1 and NHR-85, NHR-6, and NHR-41. Only a few of these *C. elegans* receptors are currently known to affect molting: NHR-23, NHR-25, NHR-41 and NHR-67/TLL (Tailless) [19]. Here, we discuss these few in more detail as well as possible ligand(s) that drive ecdysis.

NHR-23 and NHR-25 are the most extensively-characterized NRs that govern molting. Expression of *nhr-23* and *nhr-25* (also *nhr-41*) mRNA oscillates with each molt cycle, with their highest expression during intermolts (Figure 2) [19].

Reduction of *nhr-23* by RNAi knockdown at any intermolt results in defective subsequent molts, and, in addition, disrupts collagen synthesis, tail development, and placement of epidermal seam cells [53]. NHR-23 is highly similar to *Drosophila* DHR3, an NR required for stage transitions and cuticle synthesis, as well as for proper expression of EcR and FTZ-F1 [54]. NHR-23 is orthologous to vertebrate RORα, which has roles in circadian rhythms, development of Purkinje cells, bone maintenance, immunity, and cholesterol and lipid metabolism [55]. In particular, RORα works reciprocally with the REV-ERB NR as a core component of the circadian clock, with REV-ERB repressing and RORα activating *Bmal1* expression, respectively [56]. Similarly, *Drosophila* DHR3 and E75 (*nhr-85* and *sex-1* homolog) reciprocally regulate one another during the molt cycle [57,58]. However, as of yet there is no solid evidence for *sex-1* and *nhr-85* showing a similar reciprocal relationship with *nhr-23; sex-1* is involved in sex determination and *nhr-85* functions in ovulation [19,59].

NHR-25 is required for embryonic and post-embryonic functions and is homologous to *Drosophila* FTZ-F1, mammalian SF-1, and liver receptor homolog 1 (LRH-1). Embryonic loss of *nhr-25* results in failure of ventral closure and defects in epidermal elongation, causing arrest at the two-fold stage [60]. Like *nhr-23*, loss of *nhr-25* during larval stages results in severe defects in molting. In addition, *nhr-25* mutants display defects in epidermal cell fusion, abnormal seam cell morphology and number, altered vulval cell differentiation, gonadal defects and germline overproliferation [60,61]. In various developmental contexts, *nhr-25* works alongside a variety of other transcription factors, including NOB-1 and LIN-39 Hox proteins, which regulate embryogenesis and vulval cell differentiation, respectively [60], as well as with β-catenin in the establishment of distal-proximal fates in the somatic gonad precursor cells [62]. Comparably, *Drosophila*, FTZ-F1 has embryonic roles in segmentation and larval metamorphosis, and often works together with the homeodomain protein FTZ [63–66]. The mammalian SF-1 orchestrates the development of the gonad, adrenal glands, hypothalamus, and pituitary, as well as male differentiation and steroidogenesis [67,68]. Interestingly, it also works with β-catenin and Wnt signaling in a variety of contexts [67]. Lastly, LRH-1 has roles in cholesterol transport, bile acid homeostasis, and ovarian function [68].

In *Drosophila*, 20-hydroxyecdysone drives the molt process; however, the identity of such a ligand in *C. elegans* remains elusive. The ligands for the vertebrate NHR-25 homologs SF-1 and LRH surprisingly are various phospholipids and sphingolipids [8,10,11]. Given the high degree of conservation, the worm receptor, too, could have related ligands. Although nematodes lack ecdysteroids, evidence suggests that the ligand(s) driving *C. elegans* ecdysis could alternatively be a sterol, as cholesterol deprivation results in molting defects, and

molecules implicated in cholesterol disposition, such as the LDL-like receptor protein, *lrp-1*, are required for molting [69]. Additionally mutations in *let-767*, encoding a 17-β-hydroxysteroid dehydrogenase homologue, results in molting defects, suggesting a possible role in production of a steroid hormone that drives ecdysis [70].

Genome-wide RNAi screens have identified scores of genes affecting the molting process, but surprisingly, none of them obviously give clear clues to the identity of a molting hormone [53,71]. Aside from hormonal regulation, ecdysis may also be regulated by microRNAs. In particular, the *let-7* microRNA and its paralog *mir-84* regulate exit from molt cycling, and are thought to work through inhibitory feedback on *nhr-23* and *nhr-25*, though it is unclear whether this regulation is direct (Figure 2) [72].

Finally, the DAF-12 NR may have a role in integrating developmental timing programs with the molt cycle. Disrupting nicotinic acetylcholine receptors (nAChR) delays L2 cell division programs, but not the molt cycle, a process suppressed by mutations in *daf-9* and *daf-12* [73]. This suggests that nAChRs could act *via* ligand (DA)-bound DAF-12 to repress L2 cell divisions and differentiation [73]. Still, a detailed mechanism illustrating how DAF-12 could integrate the molting and heterochronic circuits awaits.

Heterochrony

During development, cells acquire temporal identity, often with distinct stage-specific programs for embryo, juvenile, and adult. Heterochrony is a change in the relative timing of such stage specific events. NRs play a critical role in developmental timing in diverse taxa. Examples include the thyroid and ecdysone receptors (TR and EcR), which mediate metamorphosis in amphibians and insects, respectively [74,75]. Additionally, androgen and estrogen receptors (AR and ER) promote reproductive maturation in mammals [76]. The vertebrate vitamin D receptor (VDR) has a role in the cycling of the hair follicles [77]. Post-developmentally, the ER and progesterone receptors (PR) orchestrate the menstrual cycle [78].

In the worm, DAF-12 promotes reproductive maturation in the context of the heterochronic pathway (Figure 2). Heterochronic loci control stage-specific programs from L1 to adult. Mutations in these genes result in temporal transformations during post-embryonic development, resulting in the misexpression of larval programs in adults (retarded) or conversely, adult programs in larva (precocious), usually in a stage- and tissue-specific manner [79,80]. Many of the heterochronic genes are highly conserved, and hence, study of this elegant circuit has led to novel insights into metazoan developmental timing, and regulation of cell proliferation, migration, and stem cell division patterns [81].

Among these highly conserved genes are the first discovered microRNAs, *lin-4* [82,83] and *let-7* [84], 22-nucleotide RNAs that inhibit expression by base pairing with the 3′ UTR of their target mRNAs. Interestingly, transitions in stage programs are often triggered by up-regulation of distinct sets of microRNAs, which down-regulate their targets. For example, *lin-4* down-regulates the nuclear factor *lin-14* and the cold shock domain protein *lin-28* during L1/L2 transitions [83,85]. The microRNAs *mir-84*, *48*, and *241* down-regulate *hbl-1*/*hunchback* during L2/L3 transitions [86] and *let-7* microRNA down-regulates *lin-41*/RBCC ring finger protein and *hbl-1*, as well as *daf-12* during L4 to adult transitions [86–88] (Figure 2). Components involved in the processing and assembly of microRNA complexes also have heterochronic phenotypes [89–91]

NR DAF-12 primarily promotes L2/L3 transitions in gonadal and extragonadal tissues, as *daf-12* mutants repeat L2 programs during the L3 stage [92]. In general, *daf-12* LBD mutants have more severe heterochronic phenotypes than DBD mutants [26], possibly because they

lock the receptor in a repressive state and interfere with other heterochronic activities [34]. One potential target of *daf-12* regulation is *lin-28*, which is genetically epistatic to *daf-12* [92] and whose protein accumulates at late stages in *daf-12* LBD mutants [93,94]. However, *lin-28* mRNA levels remain unchanged during development, suggesting that regulation must be post-transcriptional and likely indirect. Presumably, other loci involved in the L2/L3 transition could be *daf-12* targets.

Remarkably, homologs of several circadian clock genes, including *lin-42*/*period*, *tim-1*/*timeless*, and *kin-20*/*double-time*, also function in the *C. elegans* heterochronic circuit, but lack aspects of the feedback regulation and diurnal fluctuations found in classical circadian circuits [95,96]. Instead, *lin-42* mRNA levels fluctuate with the molt cycle (Figure 2) and functions to prevent early expression of adult fates. While RNAi knockdown of *tim-1* and *kin-1* resembles the precocious cell fusion patterns seen in *lin-42* mutants [96], it is unclear whether they directly regulate *lin-42*. Interestingly, *lin-42* and *daf-12* act antagonistically with *lin-42* mutants producing a precocious dorsal turn of the gonad at the L2 molt, whereas *daf-12* mutant animals fail to make this turn appropriately at the L3 molt [92,97,98]. Epistasis analysis further suggests that *daf-12* functions in parallel or downstream of *lin-42*. Speculatively, this interaction may mimic the relationship of PERIOD and the NRs REV-ERB and RORα in the circadian clock.

DAF-12 acts at the nexus of the dauer and heterochronic pathways, telling of its importance in regulating developmental programs. By integrating inputs from the dauer pathways, DAF-12 conveys information about stress, nutrients, and the environment into the heterochronic circuit, thereby either advancing or arresting development through a hormone-dependent mechanism (Figure 1). In a similar manner, the ER may govern puberty in response to environmental input [76]. Conceivably, global transitions such as mammalian puberty or tissue-specific clocks such as the hair follicle cycle may be embedded in the rich circuitry of similar heterochronic timers, which drive forward regulatory hierarchies and specify stage appropriate events.

Summary

The basic biology of metazoans dictates tight regulatory control of reproduction, development, metabolism, and homeostasis. NRs are well-poised to govern these processes because they can couple environmental and physiologic cues to coordinate organism-wide events. Although diversified through evolution, NRs across taxa regulate similar fundamental biological processes. Here, we have discussed in detail how a few of the best understood NRs from *C. elegans* function as switches and timers to control diapause, molting, and developmental timing. A comparison of these conserved circuits and mechanisms promises to illuminate *in vivo* transcriptional networks that underlie analogous processes in other animals.

A key challenge for the future will be to further dissect the molecular basis of how NRs act as switching and timing devices, as well as homeostatic controllers and rheostats in various biological contexts. To this end, it will important to identify and understand all the major components that control the regulatory circuitry. A systems approach, which takes into account not only the dimensions of ligand, receptor, and target gene, but also ratios of coactivator and corepressor complexes, tissue-specific licensing factors, and signaling modulators, will be needed to fully describe such circuits. In this light, *in vivo* quantitative readouts in a genetically tractable organism such as *C. elegans* should prove invaluable. A second critical challenge will be to understand the transcriptional cascades and target genes that underlie the physiology controlled by NRs. Such studies should reveal important insights into conserved NR mechanisms across taxa.

Acknowledgments

The authors wish to acknowledge grant support from the NURSA, NIA, NIH, and AFAR for A.A. and NIA for D.B.M.

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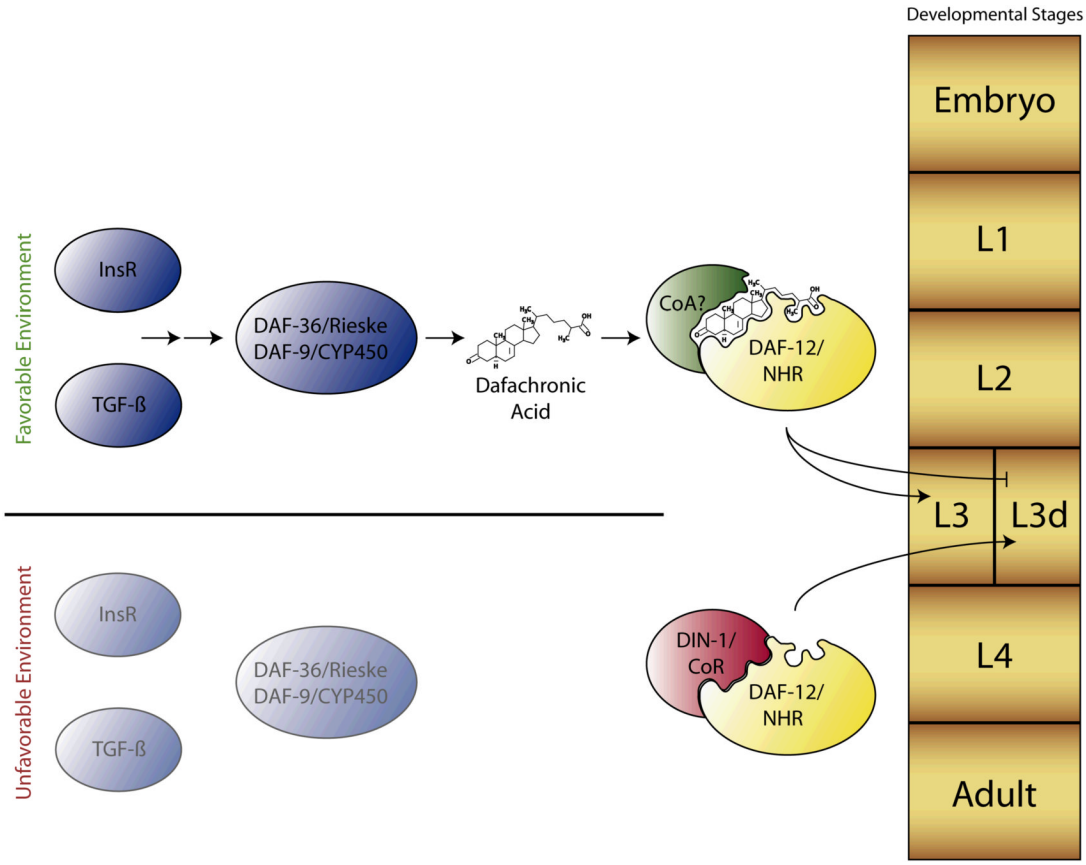

Figure 1.

The choice between two life history fates, reproductive (L3) or dauer (L3d), results from graded environmental cues impacting production of a ligand for DAF-12, a key nuclear receptor that controls the switch between these two fates. Depending upon its ligand-bound state, DAF-12 governs this switch through the DIN-1 corepressor (CoR) and unidentified coactivator(s) (CoA). In favorable conditions, signals from the environment are translated *via* the Insulin/IGF-1 (InsR) and TGF-β pathways to promote transformation of cholesterol by the DAF-36/Rieske-like oxygenase and DAF-9/CYP450 (and others) to produce dafachronic acid, the ligand for DAF-12. DAF-12 drives transcription of genes involved in reproductive development and fat metabolism. In unfavorable environments (*e.g.*, low food, overcrowding, other stresses), low Insulin/IGF-1 and TGF-β signaling translates to little or no dafachronic acid production, resulting in unliganded DAF-12 binding to its corepressor DIN-1, and promoting the dauer specific lineage characterized by stress resistance, increased fat storage, and longevity.

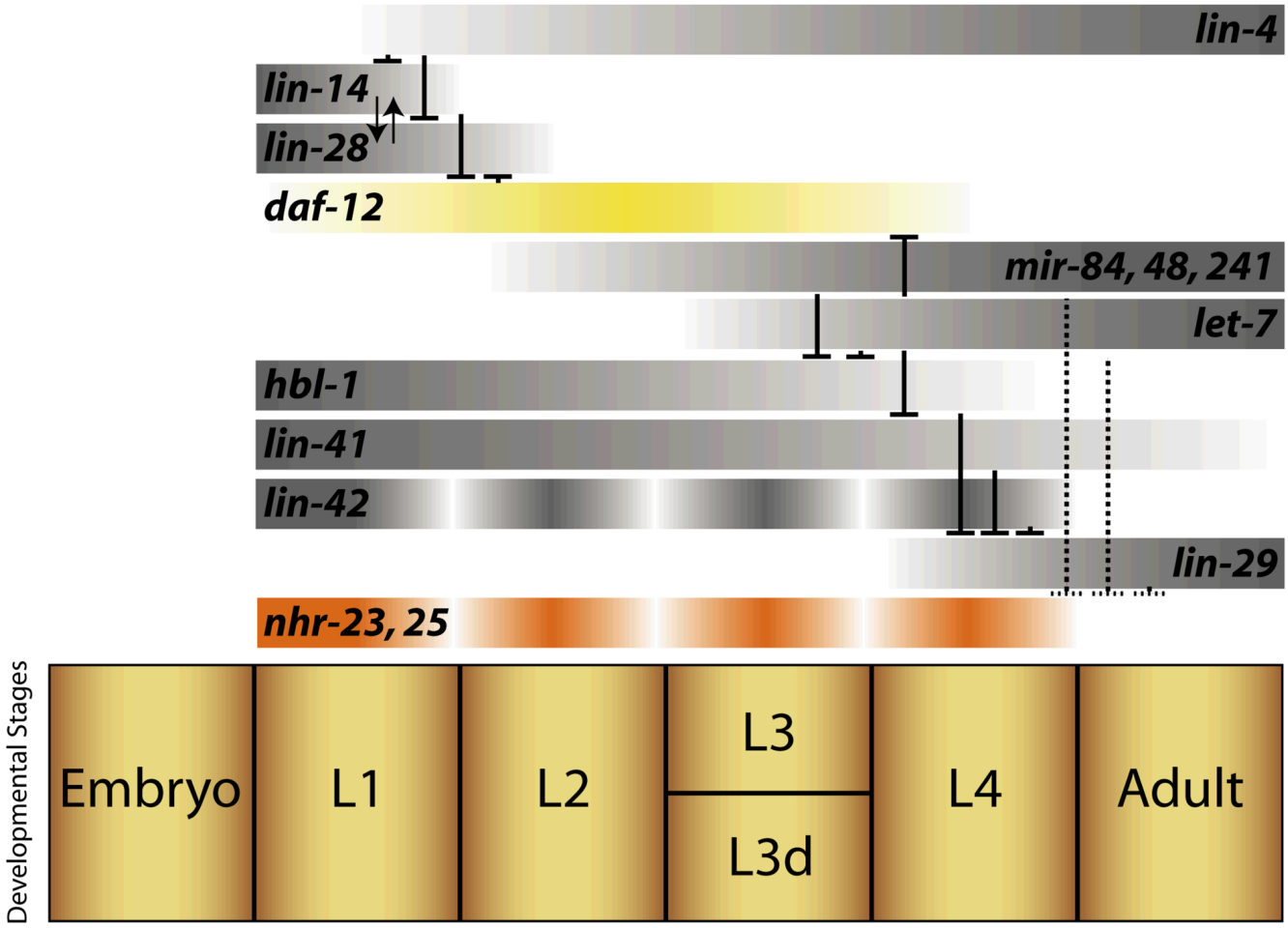

Figure 2.

Heterochronic genes control stage-specific programs of development and regulate expression of the DAF-12, NHR-23, and NHR-25 NRs. Graded expression levels of several selected heterochronic genes are shown, with higher expression levels indicated by increasing darkness. Together, these genes form an elegant network of gene regulation (repression is indicated by lines with blocked ends) that helps drive development. Dashed lines represent interactions in which it is unclear whether regulation is direct.