Nuclear hormone receptors: roles of xenobiotic detoxification and sterol homeostasis in healthy aging

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Abstract

Health during aging can be improved by genetic, dietary and pharmacological interventions. Many of these increase resistance to various stressors, including xenobiotics. Up-regulation of xenobiotic detoxification genes is a transcriptomic signature shared by long-lived nematodes, flies and mice, suggesting that protection of cells from toxicity of xenobiotics may contribute to longevity. Expression of genes involved in xenobiotic detoxification is controlled by evolutionarily conserved transcriptional regulators. Three closely related subgroups of nuclear hormone receptors (NHRs) have a major role, and these include DAF-12 and NHR-8 in *C. elegans*, DHR96 in *Drosophila* and FXR, LXRs, PXR, CAR and VDR in mammals. In the invertebrates, these NHRs have been experimentally demonstrated to play a role in extension of lifespan by genetic and environmental interventions. NHRs represent critical hubs in that they regulate detoxification enzymes with broad substrate specificities, metabolizing both endo- and xenobiotics. They also modulate homeostasis of steroid hormones and other endogenous cholesterol derivatives and lipid metabolism, and these roles, as well as xenobiotic detoxification, may contribute to the effects of NHRs on lifespan and health during aging, an issue is being increasingly addressed in *C. elegans* and *Drosophila*. Disentangling the contribution of these processes to longevity will require more precise understanding of the molecular mechanisms by which each is affected, including identification of ligands and co-regulators of NHRs, patterns of tissue-specificity and mechanisms of interaction between tissues. The roles of vertebrate NHRs in determination of health during aging and lifespan have yet to be investigated.

Abbreviations

20E, 20 hydroxy ecdysone; **ABC transporter**, ATP binding cassette transporters; **AhR**, aryl hydrocarbon receptor; **CAR**, constitutive androstane receptor; **CYP**, cytochrome P450 enzyme; **DA**, dafachronic acid; **DBD**, DNA binding domain; **DR**, dietary restriction; **EcR**, ecdysone receptor; **FOXO**, forkhead Box O; **FXR**, farnesoid X receptor; **GHRKO**, growth hormone receptor knock out; **GST**, glutathione-S-transferase; **IIS**, insulin/insulin like growth factor signaling; **LBD**, ligand binding domain; **LXR**, liver X receptor; **NHR**, nuclear hormone receptor; **PXR**, pregnane X receptor; **RXR**, retinoid X receptor; **SDR**, short chain dehydrogenase; **UGT**, UDP-glucuronosyl-transferases; **USP**, Ultraspiracle; **VDR**, vitamin D receptor

Introduction

The aging process has proved to be malleable to genetic, dietary and pharmacological interventions (Kenyon, 2010, Lamming et al., 2013, Fontana et al., 2010, Fontana and Partridge, 2015). Furthermore, at least some of its mechanisms are conserved during evolution, because similar interventions, such as dietary restriction (DR), have proved capable of improving health during aging in diverse model and non-model organisms including primates (Madeo et al., 2014). However, these interventions can also have undesirable side-effects, such as impaired fecundity, immunity and wound healing (Martin et al., 2008, Lamming et al., 2013). There is therefore much interest in understanding the exact mechanisms by which different interventions improve health during aging, and in the possibility of triaging the health benefits from the side-effects.

One well established intervention to extend lifespan, in the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster,* mice and, possibly, humans, is reduction of insulin/insulin-like growth factor (Igf) signaling (IIS) (Fontana et al., 2010). The IIS pathway is conserved among metazoans, and it can modulate metabolism, development, growth, body size, fecundity and resistance to different stressors including oxidative and xenobiotic stress (Broughton and Partridge, 2009). It is therefore important to establish whether any, or all, of the many pleiotropic traits associated with reduced IIS are causal in increased lifespan and health during aging.

Binding of insulin-like peptides to the insulin/Igf receptor induces a signaling cascade leading to the phosphorylation of forkhead Box O (FOXO) transcription factors by the protein kinase AKT and sequestration of FOXO in the cytoplasm. With reduced activity of the upstream pathway, FOXO translocates to the nucleus and regulates gene expression (van der Horst and

Burgering, 2007, Salih and Brunet, 2008, Partridge and Bruning, 2008). In *C. elegans*, all phenotypes associated with reduced IIS, including xenobiotic resistance, require the gene *daf-16*, which encodes the single worm FOXO and, from this evidence, all of the pleiotropic traits could hence be relevant to the extension of lifespan (Tissenbaum and Ruvkun, 1998, Honda and Honda, 1999, Riedel et al., 2013). In contrast, in *Drosophila*, *dfoxo*, the *daf-16* orthologue, is not required for the reduction in body size and fecundity, developmental delay or the increased oxidative stress resistance from reduced IIS. Only increased resistance to xenobiotics and extension of lifespan are identified FOXO-dependent effects of reduced IIS (Slack et al., 2011). It is thus possible that the increased xenobiotic resistance of insulin mutant flies is causal in their increased lifespan, while the other traits associated with reduced IIS are irrelevant.

In accordance with their increased xenobiotic resistance, the RNA transcriptomic signature of long-lived IIS mutant worms, flies and mice is enriched for xenobiotic detoxification genes (McElwee et al., 2007). Furthermore, various genetic, pharmacological and dietary interventions that promote longevity in mice also increase expression of detoxification genes. Little mice, harboring a growth hormone releasing hormone receptor knockout, and Ames dwarf, lacking pituitary cells producing growth hormone, prolactin and thyroid stimulating hormone, have a transcriptomic signature of elevated xenobiotic detoxification genes (Amador-Noguez et al., 2004) and Little mice are resistant to hepatotoxins (Amador-Noguez et al., 2007). Other murine models of delayed aging also show increased expression of xenobiotic metabolizing enzymes (Miller et al., 2014, Steinbaugh et al., 2012). These include Snell dwarf mice, which carry mutations hindering normal pituitary development, growth hormone receptor knock out (GHRKO) mice, and mice subjected to DR, reduced access to the mother during the breastfeeding period (‘crowded litter’), or treated with

rapamycin. Furthermore, DR mice showed increased resistance to acetaminophen and other hepatotoxins, such as thioacetamide and bleomycin (Aidoo et al., 1999, Apte et al., 2003, Harper et al., 2006). Methionine-restricted mice are also less susceptible to acetaminophen (Miller et al., 2005). Taken together, these findings point to increased metabolism of endo- and xenobiotics as a potential downstream mechanism for mediating the effects of multiple interventions promoting health during aging.

Detoxification of endo- and xenobiotics is divided into three phases. In phase I, cytochrome P450 enzymes (CYPs) and short chain dehydrogenases (SDRs) bioactivate the lipophilic components, providing conjugation sites for consecutive reactions. Different classes of phase II enzymes then conjugate bulky hydrophilic groups to the molecules to increase their solubility in body fluids and facilitate their excretion. Phase II enzymes include glutathione-S-transferases (GSTs), UDP-glucuronosyl-transferases (UGTs), sulfotransferases, carboxylesterases and others. The third phase is accomplished by the ABC (ATP binding cassette) transporters, which excrete the detoxified molecules (Omiecinski et al., 2011). Notably, all steps of detoxification are highly energy demanding (Gems and McElwee, 2005).

The main transcriptional regulators of detoxifying enzymes and transporters are conserved among metazoans and include the aryl hydrocarbon receptor (AhR), the zinc-finger transcription factor Nrf-2 and members of two closely related subgroups of nuclear hormone receptors (NHRs) (Lindblom and Dodd, 2006, Brown et al., 2005, Sykiotis and Bohmann, 2008, Itoh et al., 2015, Köhle and Bock, 2009, Wang et al., 2013). NHRs are classified into groups NR1-NR6, and into subgroups according to their highly conserved domain structure (Nuclear Receptors Comittee, 1999). The NHRs relevant for xenobiotic metabolism in invertebrate models belong to the NR1J group and include DAF-12, NHR-8 and NHR-48 in *C. elegans* and DHR96 in *Drosophila.* While NHR-8 and DHR96 are demonstrated to regulate

xenobiotic detoxification, DAF-12 and NHR-48 have not yet been studied regarding this possible function (King-Jones et al., 2006, Lindblom et al., 2001). The NR1J group is closely related to the NR1I group, with mammalian pregnane X receptor (PXR), constitutive androstane receptor (CAR) and vitamin D receptor (VDR), and to the NR1H group with farnesoid X receptor (FXR) and liver X receptors (LXRs) (Fig. 1). PXR and CAR have a well-established role in regulating xenobiotic metabolism, while VDR, FXR and LXRs, like many other mammalian NHRs, induce expression of xenobiotic detoxification genes and cross-talk with xenobiotic metabolism (Haussler et al., 2013, Wang et al., 2013, Xu et al., 2005).

Interestingly, in worms and flies, genes that regulate xenobiotic metabolism are also implicated in healthy aging. For instance, over-expression of *skn-1*, the Nrf2 orthologue, in *C. elegans*, extends lifespan and its activity is required for the response of lifespan to DR (Tullet et al., 2008, Bishop and Guarente, 2007). Furthermore, over-expression of the Nrf2 orthologue *cnc* in *Drosophila* extends lifespan (Sykiotis and Bohmann, 2008), while *nhr-8* is required for DR to extend lifespan in *C. elegans* (Chamoli et al., 2014, Thondamal et al., 2014). Although *daf-12* has not been shown to be involved in xenobiotic metabolism, it is well established to promote healthy aging: *daf-12* is required for increased longevity induced by loss of the germline (Hsin and Kenyon, 1999, Wollam et al., 2012). Furthermore a gain of function mutant of *daf-12* is long-lived (Fisher and Lithgow, 2006). On the other hand, although *dhr96* is involved in the response to xenobiotics, it has not yet been studied regarding its involvement in healthy aging (King-Jones et al., 2006).

In addition to their possible roles in detoxification of xenobiotics, NHRs of the NR1H, NR1I and NR1J groups control homeostasis of sterol metabolites and fat metabolism. DAF-12 and NHR-8 in worms and DHR96 in flies regulate cholesterol and triacylgylceride metabolism and furthermore all three are involved in biosynthesis and/or degradation of steroid hormones

that govern developmental decisions and are involved in healthy aging (Horner et al., 2009, Bujold et al., 2009, Magner et al., 2013, Antebi, 2013b, Wang et al., 2015, King-Jones et al., 2006, Guittard et al., 2011). Mammalian LXRs have a well-established role in controlling cholesterol homeostasis, while FXR acts as the main bile acid sensor and is also involved in triacylglyceride homeostasis (Kalaany and Mangelsdorf, 2006). Next to xenobiotics, mammalian PXR and CAR bind a wide range of endogenous ligands including bile acids, bilirubin, and steroid hormones, and are involved in control of bile acid homeostasis, lipid metabolism and gluconeogenesis, as well as regulation of steroid and thyroid hormone levels (Yang and Wang, 2014, Wang et al., 2013, Moreau et al., 2008, di Masi et al., 2009). VDR is activated by bile acids and, furthermore, polyunsaturated fatty acids are ligands for VDR, albeit with low affinities (Makishima et al., 2002, Adachi et al., 2005, Haussler et al., 2013).

The increased expression of genes involved in xenobiotic metabolism, and the xenobiotic resistance seen in animal models of healthy aging, together with the dual involvement of transcription factors in regulating xenobiotic metabolism and healthy lifespan, all suggest that there may be a causal connection between the two traits. Since NHRs involved in detoxification of xenobiotics also regulate endogenous metabolites and hormones, this role could also contribute to healthy aging. To determine whether these associations are indeed causal will require unravelling the exact mechanisms by which NHRs modulate the different traits. In this review, we consider this issue, with particular emphasis on the invertebrate NHRs of the NR1J group, DAF-12, NHR-8 and DHR96, with discussion of their mammalian orthologues where relevant. Although NHR-48 also belongs to the NR1J group its functions are largely unknown, and it will hence not be further discussed. We focus on the role of these NHRs in worms and flies because their role in lifespan and health during aging has been much more extensively investigated than has that of vertebrate NHRs. We first provide

an overview over structure and function of NHRs. We then discuss whether increased xenobiotic metabolism and/or control of sterol and triacylglyceride metabolism by NHRs contributes to health during aging.

Nuclear hormone receptors

Nuclear hormone receptors comprise a large superfamily of proteins that are usually ligand-dependent transcription factors. They serve a wide variety of functions, including regulation of development, mitochondria, immunity, sex determination and reproduction, as well as lipid metabolism and detoxification of endo- and xenobiotics. They are evolutionarily conserved in metazoans, with 18 members in *Drosophila*, 48 in human, 49 in mice, and 284 in nematodes (Evans and Mangelsdorf, 2014, Fahrbach et al., 2012). Because both their DNA-binding domain (DBD) and ligand-binding domain (LBD) show high evolutionary conservation, NHRs are grouped into subfamilies and subgroups based on the phylogenetic comparison of these domains (Nuclear Receptors Comittee, 1999).

The ligands of NHRs are always lipophilic but are highly variable in size and structure (Laudet et al., 2005). Examples include glucocorticoids, steroid hormones, fatty acids, phospholipids, heme, bile acids, vitamin D and xenobiotics (Huang et al., 2010). Some NHRs are orphan receptors, meaning either that they do not have ligands or that these have not yet been identified. Furthermore, some NHRs constitutively bind their ligands in the manner of a co-factor, for instance HNF-4 and fatty acids (Evans and Mangelsdorf, 2014). Ligand specificity and affinity varies greatly between the family members. The human PXR, for example, binds various ligands with low affinities (in the micromolar range) whereas steroid receptors are

highly specific for their cognate ligands and bind these with high affinity (0.1-1 nM) (Reschly and Krasowski, 2006, Huang et al., 2010).

As illustrated in Fig. 2A, NHRs have a modular structure. The N-terminal region (A/B domain) is variable and serves in ligand-independent transactivation. The central DBD is highly conserved and has two zinc finger motifs providing DNA binding. The highly variable hinge region connects the DBD and LBD and also contains the C-terminal extensions, which bind to the DNA minor groove just C-terminal of the DBD (Huang et al., 2010, Laudet et al., 2005). The C-terminal LBD is evolutionarily conserved, but not as highly as the DBD (Bertrand et al., 2004), and contains the ligand-binding pocket. Its structure allows interaction with dimerization partners as well as co-regulators and other transcription factors.

The ligand-binding pocket is buried deeply in the molecule, in accordance with the lipophilic nature of the ligands. Upon ligand binding, helix 12 of the LBD is subjected to a conformational change, resulting in recruitment of co-regulators. Classically, in the absence of the ligand, NHRs are thought to bind to co-repressors, which are exchanged for co-activators upon ligand-binding (Fig. 2C) (Huang et al., 2010). However, regulation of the transactivating activity of NHRs is more complex. NHRs can also act only as activators or only as repressors (Fig. 2C). Furthermore, unliganded NHRs can constitutively activate transcription, with ligands acting as inverse agonists and repressing transcription, as is the case for mammalian CAR (Fig. 2C) (Evans and Mangelsdorf, 2014). Detailed models of transcriptional repression by NHRs in interaction with ligands, co-regulators and other NHRs are reviewed elsewhere (Santos et al., 2011).

Activity of NHRs is not solely regulated by presence or absence of ligands. Transactivating activity can be regulated by phosphorylation, as in the estrogen receptor (Kato et al., 1995, White et al., 1997). Furthermore, some NHRs reside in the cytoplasm bound to chaperones

in the absence of their ligands. For instance the glucocorticoid and androgen receptors are associated with a protein complex including HSP70 and HSP90, and ligand binding induces release from the complex and translocation into the nucleus (Kawata, 2001).

The spectrum of genes regulated by a given NHR, and whether it activates or represses particular genes, can differ between tissue and cell types. This specificity probably depends upon the local availability of co-activators and co-repressors, since binding of these two types of co-regulators to the surface of the NHR is mutually exclusive (Huang et al., 2010).

NHRs can bind to the DNA as monomers, homodimers or heterodimers (Fig. 2B). This is reflected in the arrangement of their hexameric DNA binding motifs, with monomers binding to single hexamers, homodimers binding to inverted repeats and heterodimers binding to direct repeats of the hexameric motifs (Evans and Mangelsdorf, 2014). Many NHRs, including mammalian VDR, FXR, LXRs, retinoic acid receptors (RARs), PXR and CAR, form heterodimers with retinoic X receptor (RXR). Of note, RXR is the only heterodimerization partner for all other NHRs. (Evans and Mangelsdorf, 2014). RXR heterodimers can be activated by both the RXR ligand retinoic acid and the cognate ligand of the heterodimerization partner (‘permissive heterodimer’), or only by the ligand of the partner (‘non-permissive heterodimer’) (Evans and Mangelsdorf, 2014). Permissive partners include peroxisome proliferator-activated receptors (PPARs), LXRs, FXR, PXR and CAR, while thyroid receptors (TRs), VDR and RARs are non-permissive partners. In invertebrates heterodimerization of NHRs has not been described at this level of detail. However, the *Drosophila* ecdysone receptor (EcR) heterodimerizes with USP, the RXR homolog in flies (Yao et al., 1993, Horner et al., 1995). Of note, *C. elegans* does not harbor a homolog of RXR but heterodimerization between different NHRs has been observed in worms (Li et al., 2004).

NHRs commonly have multiple isoforms that can serve different functions. Differences in the N-terminal region result in different transactivating activities, and isoforms can lack the DBD and hence sequester ligands without regulating target genes. Some isoforms act as dominant negative receptors, by binding the DNA without regulating transcription (Laudet et al., 2005). In addition, NHR signaling is autoregulated at two levels. NHRs regulate their own expression, and promotors of NHRs often hold their own binding motif (Laudet et al., 2005). NHRs can also regulate the production of their ligands by transcriptional control of enzymes necessary for their biosynthesis (Evans and Mangelsdorf, 2014).

Xenobiotic resistance: a cause for NHR dependent longevity?

The role of NHRs in longevity and healthy aging has been addressed mainly in *C. elegans* and *Drosophila*. We therefore focus on these organisms to consider the possible roles of xenobiotic, lipid and cholesterol metabolism in mediating the effects of DAF-12, NHR-8 and potentially DHR96 activity on longevity.

DAF-12, NHR-8 and DHR96 in xenobiotic resistance and longevity

The ligands of DAF-12 are dafachronic acids (DAs), which are synthesized by the CYP DAF-9 and other enzymes from a cholesterol precursor (Mahanti et al., 2014, Gerisch and Antebi, 2004, Gerisch et al., 2001, Rottiers et al., 2006, Schaedel et al., 2012, Wollam et al., 2012). While unliganded DAF-12 is associated with the co-repressor DIN-1 and represses target gene expression (Ludewig et al., 2004), binding of DAs is thought to induce expression of target genes (Antebi, 2013c). DAF-12 signaling interacts with IIS, signals from the germline and environmental conditions to modulate healthy aging and can have opposed effects on

longevity. Reduced DA/DAF-12 signaling can be achieved by mutating different components of the pathway, including the enzymes for DA biosynthesis and DAF-12 itself. The effect of *daf-9* mutation on lifespan is temperature-dependent (Tab. 1). All 4 *daf-9* alleles tested increase lifespan at 15 °C (Jia et al., 2002, Gerisch et al., 2007, Gerisch et al., 2001). However, this effect is lost or even reversed at higher temperatures up to 25 °C (Jia et al., 2002, Gerisch et al., 2007, Gerisch et al., 2001, Dumas et al., 2013, Thondamal et al., 2014). Extension of lifespan in *daf-9* mutants at 15 °C is completely dependent upon *daf-12*, indicating that unliganded DAF-12 is required for longevity at this temperature (Gerisch et al., 2001, Jia et al., 2002). Accordingly, the null allele *daf-12(rh61rh411)* alone either reduces (20 °C) or does not change (25 °C) lifespan (Fisher and Lithgow, 2006, Dumas et al., 2013) but the LBD deficient mutant *daf-12(rh273)* shows extended lifespan at 20 °C (Fisher and Lithgow, 2006). Furthermore, the co-repressor DIN-1, which is associated with DAF-12 in the absence of ligands, is required for longevity seen in *daf-9* mutants (Ludewig et al., 2004). These findings indicate that, in otherwise untreated animals, unliganded rather than liganded DAF-12 serves to extend lifespan, and DAF-12 together with DIN-1 represses genes whose functions counteract longevity.

Whether DA signaling affects xenobiotic resistance has not yet been assessed, but reduced DA signaling increases resistance to other stressors. Both long-lived *daf-9* and LBD deficient *daf-12(rh273)* mutants exhibit resistance to thermal and oxidative stress (Gerisch et al., 2007, Fisher and Lithgow, 2006). In *daf-9* mutants, resistance is abrogated when *daf-9* expression is restored or when they are treated with a DAF-12 agonist (Gerisch et al., 2007). Furthermore, increased resistance of *daf-9* mutants depends upon *daf-12* and its co-repressor *din-1*, indicating that unliganded DAF-12 acts to increase stress resistance (Gerisch et al., 2007). It has been proposed that resistance against a broad range of stressors is a

longevity-assurance mechanism (Gems and McElwee, 2005, Shore and Ruvkun, 2013), which could suggest that long-lived mutants that are resistant to thermal and oxidative stress would also be more resistant to xenobiotic stress. The hypothesis further implies that interventions that abrogate increased stress resistance should also abrogate longevity of these mutants. However, the increased stress tolerance of *daf-9* mutants is partially dependent on *daf-16* while their longevity is not abrogated by *daf-16* mutation (Gerisch et al., 2007, Gerisch et al., 2001, Jia et al., 2002), indicating that increased resistance against these two stressors can be at least partially uncoupled from longevity.

Further evidence arguing against xenobiotic detoxification as a cause for longevity in mutants with reduced DA signaling comes from comparison of genes differentially regulated between long-lived *daf12(rh273)* and short-lived *daf-12(rh61rh411)* mutants. Genes involved in phase I and II detoxification, including CYPs, GSTs and glucuronosyltransferases, are down-regulated in long-lived *daf-12(rh273)* mutants (Fisher and Lithgow, 2006) while, in contrast, xenobiotic detoxification genes are up-regulated in long-lived worms, flies and mice (McElwee et al., 2007). Xenobiotic resistance is thus unlikely to be causal for healthy aging of mutants with reduced DA/DAF-12 signaling. However, due to the correlative nature of these findings this conclusion remains speculative. Future work should assess the xenobiotic resistance of long-lived DA/DAF-12 mutants and any possible role in determination of lifespan.

NHR-8 is required for normal levels of xenobiotic resistance, because both *nhr-8(ok186)* mutants and worms subjected to nhr-8 RNAi treatment are sensitive to xenobiotics (Lindblom et al., 2001). However, *nhr-8(ok186)* mutants do not exhibit reduced resistance against phenazine indicating that NHR-8 confers resistance against a specific subset of xenobiotics (Lindblom et al., 2001). Furthermore, NHR-8 appears to have redundant functions with other transcriptional regulators in regulating xenobiotic detoxification genes,

because nhr-8 RNAi treatment does not abrogate expression of selected phase I and II enzymes (Chamoli et al., 2014, Jones et al., 2013). Interestingly, NHR-8 is necessary for the response of lifespan to DR in *C. elegans* (Chamoli et al., 2014, Thondamal et al., 2014). It remains to be tested whether xenobiotic resistance is increased by DR and contributes to longevity of DR worms. The finding that *skn-1,* the Nrf-2 ortholog in *C. elegans,* is also necessary for DR in *C. elegans* supports this idea (Bishop and Guarente, 2007).

Analogous to *nhr-8* mutants, *dhr96*^{1} loss of function mutants are sensitive to xenobiotics, including phenobarbital, DDT and permethrin (King-Jones et al., 2006, Beaver et al., 2010). Over-expression of *dhr96* in L3 larvae induces expression of xenobiotic detoxification genes independently of treatment with xenobiotics and in addition, DHR96 is required for induction of xenobiotic detoxification genes by phenobarbital treatment, including Turandot genes, acyl-CoA synthetases, CYPs, GstD7 and juvenile hormone binding proteins (JHBPs), which serve as lipid carriers in the hemolymph (King-Jones et al., 2006). However, the responses to phenobarbital of some xenobiotic detoxification genes, including Cyp6a8 and GstD2, are unaffected by loss of *dhr96* (King-Jones et al., 2006). These findings suggest that DHR96 is not solely responsible for the gene expression changes in response to phenobarbital and other transcriptional regulators, for instance the Nrf2 ortholog CNC or the aryl hydrocarbon receptor AhR, could also be involved in xenobiotic metabolism because their mammalian counterparts regulate xenobiotic detoxification genes (Köhle and Bock, 2009, Okawa et al., 2006).

Increased xenobiotic resistance of long-lived IIS mutants – mediated by NHRs?

In flies, long-lived IIS mutants are resistant to xenobiotics and furthermore, xenobiotic detoxification genes are up-regulated in long-lived mutants with reduced IIS in worms, flies

and mice (McElwee et al., 2007, Gronke et al., 2010, Slack et al., 2011). DHR96 is likely to act down-stream of reduced IIS in flies because it is a direct target gene of FOXO and xenobiotic resistance is a FOXO-dependent trait of IIS mutants (Alic et al., 2011, Slack et al., 2011, King-Jones et al., 2006). These findings suggest that DHR96 might contribute to increased xenobiotic resistance and longevity of IIS mutants.

In *C. elegans*, long-lived *daf-2* mutants share transcriptomic signatures of increased detoxification genes with fly IIS mutants (McElwee et al., 2007, McElwee et al., 2004). Whether long-lived daf-2 mutants are actually resistant to xenobiotics has not been assessed but further evidence suggests that xenobiotic detoxification and longevity are coupled in worms (Shore et al., 2012): many mutations that disrupt cytoprotective mechanisms including resistance to different xenobiotics also abrogate longevity of *daf-2* mutants (Shore et al., 2012). Necessity of *nhr-8* or *daf-12* for *daf-2*-dependent longevity would provide an indirect hint for the putative involvement of these NHRs in the regulation of xenobiotic detoxification down-stream of IIS. *nhr-8* is not necessary for longevity from reduced IIS indicating that it does not contribute to xenobiotic resistance and longevity from reduced IIS (Thondamal et al., 2014). In contrast, DA/DAF-12 signaling interacts with IIS in a context- and temperature-dependent manner to modulate lifespan (Tab. 1). Longevity induced by both weak *daf-2(e1368)* and strong *daf-2(e1370)* alleles is abrogated by *daf-9* mutation at 15 °C, but at higher temperatures *daf-9* mutation shortens lifespan of *daf-2(e1368)* and further extends lifespan of *daf-2(e1370)* (Gerisch et al., 2001, Dumas et al., 2013). Correspondingly, Δ^4-DA supplementation further extends *daf-2(e1368)* lifespan but has no effect on *daf-2(e1370)* longevity (Gerisch et al., 2007). Furthermore, different *daf-12* mutations affecting different DAF-12 isoforms influence longevity of *daf-2* mutants in opposing directions (Gems et al., 1998, Larsen et al., 1995, McCulloch and Gems, 2007, Dumas et al., 2013). These

complex phenotypes suggest intense cross-talk between DA/DAF-12 and IIS pathways. However, since combination of different *daf-2* mutations with mutants impaired in DA/DAF-12 signaling have different effects on longevity, assessing their xenobiotic resistance in parallel to their lifespan phenotypes could prove useful to test whether xenobiotic resistance and longevity are coupled in these long-lived mutants.

Xenobiotic resistance as a cause for longevity – open questions

The experimental evidence is not yet sufficient to determine whether xenobiotic resistance *per se* improves health during aging. In *C. elegans,* a causal connection between xenobiotic detoxification and longevity could be implied by the common role of *skn-1*, reduced activity of which interferes with both xenobiotic resistance and longevity (Shore et al., 2012, Tullet et al., 2008). However, it remains to be assessed whether xenobiotic resistance is increased by interventions that extend lifespan in *C. elegans*. Many DR-like interventions increase xenobiotic resistance in rodents. Therefore it would be interesting to investigate whether DR worms are resistant to xenobiotics as well and, if so, to assess the role of *skn-1* and *nhr-8* as candidates for mediating the effect, since both regulate expression of xenobiotic detoxification genes and are necessary for longevity of DR worms (Bishop and Guarente, 2007, Chamoli et al., 2014, Thondamal et al., 2014, Lindblom et al., 2001).

In flies and rodents, a correlation between increased xenobiotic resistance and longevity has been observed. However, it is unclear whether increased resistance is causal for healthy aging. In flies, longevity and increased xenobiotic resistance are phenotypes resulting from reduced IIS in a FOXO-dependent manner (Slack et al., 2011). Because DHR96 is a target gene of FOXO (Alic et al., 2011), DHR96 is a promising candidate to mediate the beneficial effect of

reduced IIS on xenobiotic resistance and longevity. To confirm this hypothesis it needs to be tested whether interventions that increase the activity of DHR96 extend lifespan. Furthermore, if IIS mutants are long-lived because of their increased xenobiotic resistance, then longevity of these mutants should be abrogated in a DHR96 null background. However, since DHR96 is probably not the only transcriptional regulator of xenobiotic metabolism in flies, other relevant regulators of xenobiotic detoxification genes, namely CNC and AhR, should also be examined for a possible role downstream of interventions that improve health during aging.

In rodents, the causal connection between increased xenobiotic resistance and longevity also requires testing. However, this will not be a trivial undertaking, because ~~several~~ many transcriptional regulators, including PXR, CAR, VDR, FXR, Nrf2, and AhR, regulate detoxification enzymes, and it is likely that they can compensate for each other (Wang et al., 2013, Haussler et al., 2013, Itoh et al., 2015, Köhle and Bock, 2009). One possibility would be to test whether longevity of Little mice is abrogated in a FXR null background. These mice have elevated levels of xenobiotic detoxification genes, which is lost in an FXR null background, while xenobiotic gene expression is unaffected in Little mice in a PXR/CAR double mutant background (Amador-Noguez et al., 2007). If longevity of Little mice is also abrogated in a FXR null background then this would suggest that increased xenobiotic resistance could be causal for longevity. However, FXR has a well-established role in regulation of bile acid levels by regulating detoxification enzymes, and this function of FXR could also contribute to longevity of Little mice.

Xenobiotic resistance of long-lived models – a bystander effect?

Deregulated expression of xenobiotic detoxification genes in long-lived mutants could also point to changes in levels of steroid hormones or other lipophilic metabolites. In mammals, homeostasis of lipophilic signaling molecules and metabolites such as steroid hormones, bile acids or bilirubin, is controlled by the enzyme classes that act in xenobiotic detoxification (Gibson and Skett, 1986, di Masi et al., 2009). This implies that, for example, steroid hormone homeostasis and xenobiotic detoxification are intimately connected. It remains to be studied whether these processes are interrelated also in the long-lived invertebrate models, although it seems very likely given the structural similarities of, for example, DAs with bile acids (Mahanti et al., 2014). Therefore, the unexpected down-regulation of detoxification genes in long-lived LBD deficient *daf-12(rh273)* mutants in *C. elegans* might reflect changes in DA homeostasis. Bile acids are degraded by CYPs (di Masi et al., 2009) and the same is likely to apply to degradation of DAs. Furthermore, the production of DAF-12 ligands is achieved by CYPs and short chain dehydrogenases and other enzymes that are yet to be identified (Mahanti et al., 2014). Given the broad substrate specificities of CYPs (Gibson and Skett, 1986), some of the enzymes that have, as of yet, been described only as detoxification enzymes might also be involved in DA biosynthesis. In further support of this idea, both DAF-12 and DHR96 regulate CYPs involved in steroid hormone homeostasis. DAF-12 regulates expression of DAF-9, required for biosynthesis of DAs (Gerisch and Antebi, 2004, Mak and Ruvkun, 2004) and DHR96 regulates CYP18a1, which is involved in catabolism of 20 hydroxy ecdysone (20E), a major steroid hormone in *Drosophila* (King-Jones et al., 2006, Guittard et al., 2011). In summary, differential expression of xenobiotic detoxification genes in long-lived animal models suggests that, as well as the potential to reduce toxic endo- and xenobiotic molecules, levels of steroid hormones and their metabolites are changed. This implies that

physiological functions that are under control of these hormones may be causal for healthy aging of these animals. Accordingly, steroid hormones like DAs in worms and 20 E in flies modulate lifespan (Gerisch et al., 2007, Simon et al., 2003, Tricoire et al., 2009). The roles of steroid signaling in healthy aging are reviewed in detail elsewhere (Toivonen and Partridge, 2009, Galikova et al., 2011). Comprehensive analyses of the substrate specificities of CYPs, SDRs and other enzymes involved in detoxification and steroid hormone biosynthesis could prove very useful to disentangle cause from effect.

Apart from endo- and xenobiotic metabolism members of the NR1J group control lipid metabolism and reproduction. These functions, which we shall consider in the next section, are likely to mediate healthy aging and, furthermore, their effects on longevity might be interrelated.

NHRs, lipid metabolism, germline and aging

Deregulated fat and cholesterol homeostasis has a major impact on health during aging resulting in type II diabetes and cardiovascular disease (Barzilai et al., 2012). In addition, lipid metabolism and reproduction mutually influence each other and both affect the aging process (Hansen et al., 2013). However, the connection of the three processes is as of yet unresolved. Interestingly, DAF-12, NHR-8 and DHR96 all either cross-talk with signals from the germline to modulate healthy aging or control reproduction in response to nutrient availability.

DA/DAF-12 signaling is required for longevity of worms lacking a germline, a phenomenon referred to as germline longevity or gonadal longevity (Tab. 1) (Hsin and Kenyon, 1999, Gerisch et al., 2001, Yamawaki et al., 2010). The primordial germline of *C. elegans* comprises

four stem cells, with two somatic and two germline stem cells. When the germline stem cells are ablated by laser microsurgery, worms are sterile and live 60 % longer than intact animals (Hsin and Kenyon, 1999). However, if the gonadal stem cells are ablated as well, animals are not long-lived (Hsin and Kenyon, 1999, Arantes-Oliveira et al., 2002). Importantly, modulation of adult lifespan by the germline is conserved in flies and mice (Flatt et al., 2008, Cargill et al., 2003, Mason et al., 2009). Much effort has been made to understand how signals from the germline affect aging reviewed in detail by (Antebi, 2013a). One candidate mechanism by which germline signals may modulate healthy aging is through regulation of fat metabolism. This idea is supported by the finding that the triacylglycerol lipases *lips-17* and *lipl-4* and the fatty acyl-CoA reductase *fard-1* are necessary for germline longevity, and that overexpression of *lipl-4* is sufficient to extend lifespan in *C. elegans* (Wang et al., 2008, McCormick et al., 2012). Interestingly, *fard-1* is a target gene of DAF-12, suggesting that control of lipid metabolism is a mechanism by which DAF-12 modulates healthy aging. In further support of this idea, DA/DAF-12 signaling induces mobilization of fat stores and fatty acid oxidation (Wang et al., 2015).

NHR-8 also regulates lipid metabolism. RNAi against *nhr-8* increases fat content and, furthermore, NHR-8 controls cholesterol homeostasis in worms that are cholesterol auxotroph (Ashrafi et al., 2003, Magner et al., 2013). By regulation of apolipoproteins, NHR-8 regulates distribution of cholesterol throughout the body and, specifically, its transport into eggs (Magner et al., 2013). Evidently, this function has implications for the control of reproduction in the adult worm, because NHR-8 is necessary for decreased proliferation of germline stem cells under nutrient deprivation (Thondamal et al., 2014). This opens up the possibility that NHR-8 mediates the beneficial effects of DR by the reduction of germ cell signals that shorten lifespan. In addition, by controlling cholesterol uptake, NHR-8 also

regulates availability of precursors for biosynthesis of steroid hormones such as DAs, reflected in the developmental phenotypes of *nhr-8* mutants, which resemble those of DA-deficient animals (Magner et al., 2013). This suggests that NHR-8 signaling might also interfere with effects of DA/DAF-12 signaling on health during aging.

DHR96 performs very similar functions in flies to those of NHR-8 in worms. Flies are also cholesterol auxotroph, and DHR96 adjusts cholesterol metabolism to varying levels of cholesterol in the food (Horner et al., 2009, Bujold et al., 2009). As implied by the parallel regulation of DA levels by NHR-8 in worms, DHR96 may also control availability of precursors for steroid hormones that modulate healthy aging. Furthermore, DHR96 also regulates reproduction in response to nutrient availability. In response to starvation, germline stem cells, as well as follicular stem cells, which give rise to the somatic gonad in flies, cease to proliferate until nutrients are available again (LaFever and Drummond-Barbosa, 2005). DHR96 is necessary for proliferation of follicular stem cells when flies are re-fed after a starvation period (Hartman et al., 2013). The response to re-feeding is further dependent on the cholesterol content of the food, indicating that the cholesterol-sensing function of DHR96 is responsible for the control of the response. Although it has not yet been addressed whether DHR96 also controls germline stem cell proliferation, by inference from the role of NHR-8 in adjusting germline stem cell proliferation to cholesterol availability, DHR96 could play a role in germline longevity in flies.

Finally, DHR96 regulates many genes involved in fat metabolism including *magro*, a gastric triacylglycerol lipase and cholesterol esterase (Sieber and Thummel, 2009). In addition to its function in cholesterol homeostasis, *magro* facilitates uptake of lipids from the food by liberating fatty acids from triacylglycerides (Horner et al., 2009, Bujold et al., 2009, Sieber and Thummel, 2009, Sieber and Thummel, 2012). Since *magro* is an ortholog of *lipl-4* in *C.*

elegans, it would be interesting to investigate whether over-expression of *magro* also improves health during aging in flies.

Molecular functions of NR1J group members

While invertebrate animal models have proven very useful to gain insights into the biological mechanisms of the aging process and have helped to identify members of the NR1J group as important mediators of interventions that promote health during aging, we lack comprehensive knowledge of their molecular functions. To better understand the proximal mechanisms by which these NHRs might confer longevity, it will be necessary to decipher how they function molecularly to control the correlated phenotypes, i.e. xenobiotic resistance, steroid/lipid homeostasis and lifespan.

In *C. elegans* DAF-12 has three isoforms, with DAF-12A1 and DAF-12A3 being very similar and DAF-12B lacking the DBD (Antebi et al., 2000). Interestingly, the *daf-12(m20)* mutation, that affects only the DAF-12A isoforms, has different effects on longevity from those of the *daf-12(rh61rh411)* null mutation, which deletes all isoforms. Strikingly, these differences are apparent in two different contexts. On one hand, at higher temperatures longevity of IIS mutants is further increased by the *daf-12(m20)* mutation while the *daf-12(rh61rh411)* null allele does not change their lifespan (Tab. 1) (Gems et al., 1998, Larsen et al., 1995, McCulloch and Gems, 2007, Dumas et al., 2013). On the other hand, the different *daf-12* mutations have different effects on germline longevity (Tab. 1). The original experiments showing that germline longevity is dependent on *daf-12*, with deletion of the somatic gonad having no effect, were done with the *daf-12(m20)* allele, which leaves the DAF-12B isoform unaffected (Hsin and Kenyon, 1999, Antebi et al., 2000). However, in *daf-12(rh61rh411)* null

mutants, ablation of germline stem cells and somatic gonad increases lifespan slightly (Yamawaki et al., 2010). These findings imply that different DAF-12 isoforms exhibit important differences in their impact on longevity. DAF-12B lacks the DBD, but the functional relevance of this isoform is unknown. It is thought to sequester DAs without affecting transcription of DAF-12 targets and/or to heterodimerize with other NHRs and to modulate their transactivating activity (Fig. 3) (Antebi et al., 2000, Gissendanner et al., 2004). If DAF-12B acts solely as a ligand scavenger, this finding would imply that DAs have targets other than DAF-12. Since the fully functional DAF-12 protein is not present in the *daf-12(m20)* mutants, scavenging of DAs can only be effective if there is another receptor that can respond to their absence or presence. If DAF-12B acts as a heterodimerization partner for other NHRs, then their interaction has considerable relevance for the modulation of healthy aging. In either case the functional differences of DAF-12 isoforms and their impact on healthy aging could provide detailed molecular evidence on the possible roles of both xenobiotic and lipid metabolism in healthy aging from DAF-12.

Another important step to understand the role of NR1J group members in healthy aging will be to identify their cognate ligands and the enzymes involved in their biosynthesis and degradation. NHR-8 is an orphan, however, given its modular structure and its close homology to DAF-12, it is highly probable that it has a ligand. Extrapolation from the DAF-12 ligands is unlikely to be informative because worm NHRs show low sequence identity within the LBD (< 30 %) (Gissendanner et al., 2004). In an attempt to identify NHR-8 ligands, the xenobiotics chloroquine and colchicine, as well as several sterol derivatives known to be bound by mammalian LXR, were tested in a ligand-sensor screen, but they did not transactivate NHR-8 (Magner et al., 2013). Several attempts have been made to identify DHR96 ligands. A promising candidate is cholesterol or a closely related derivative, because

cholesterol co-purifies with the DHR96 LBD (Horner et al., 2009) However, neither supplementation of cholesterol nor reduction of cholesterol availability by different means changed DHR96 activity (Horner et al., 2009). To identify and/or confirm the ligands of NHR-8 and DHR96 it will be preferable to use *in vivo* rather than *in vitro* assays, because their transactivating activity is dependent on heterodimerization partners and co-regulators which may not be present in cell culture systems.

Apart from the known interaction of DAF-12 with the co-repressor DIN-1, it is unclear which dimerization partners and/or co-regulators are required for DAF-12, NHR-8 and DHR96 to exert their functions, and whether availability of these proteins differs at different life history stages and in different tissues. DAF-12 contains a homodimerization domain in its LBD (Antebi et al., 2000) and its response elements commonly contain inverted or direct repeats, indicating that it binds to the DNA as a dimer (Shostak et al., 2004). However, formation of homo- or heterodimers by DAF-12 has not yet been reported. One interesting candidate for a DHR96 dimerization partner is Ultraspiracle (USP), the ortholog of RXR in flies. USP forms the receptor for 20E together with ecdysone receptor (EcR), which has been implicated in modulating healthy aging (Yao et al., 1993, Horner et al., 1995, Maletta et al., 2014, Simon et al., 2003, Tricoire et al., 2009). Interestingly, DHR96 binds the hsp27 ecdysone response element, suggesting a possible heterodimerization between DHR96 and USP (Fisk and Thummel, 1995). Notably, in mammals RXR is the only heterodimerization partner for all other NHRs and USP may have a similar function in flies. However, it is also possible that DHR96 competes with EcR/USP for binding sites and shares target genes with the 20E receptor. Given that DHR96 also regulates catabolism of 20E by regulating expression of CYP18a1 (King-Jones et al., 2006, Guittard et al., 2011), EcR and DHR96 signaling might cross-talk in modulating healthy aging, possibly by sharing the heterodimerization partner USP.

Studying the interaction of DHR96 and USP could thus be revealing of molecular mechanisms.

Conclusion and outlook

Increased xenobiotic resistance is a commonly observed phenotype in many long-lived worms, flies and rodents. Members of the NR1J group are important modulators of health during aging. These NHRs and their close mammalian homologs control xenobiotic detoxification but also sterol and triglyceride metabolism. Evidence is not yet sufficient to assess xenobiotic resistance as a cause for healthy aging, because most of the data are correlative in nature, and they are also largely confined to *C. elegans* and *Drosophila*. Furthermore, due to the overlapping functions of xenobiotic detoxification genes with sterol metabolism, xenobiotic resistance is tightly linked with the biosynthesis and degradation of steroid hormones and bile acids. It is therefore possible that the increased expression of xenobiotic detoxification genes in long-lived models reflects changes in levels of bile acids or steroid hormones. In invertebrates, steroid hormones modulate health during aging and in mice bile acids elicit expression profiles of detoxification genes similar to the ones found in long-lived mice. Finally, it is likely that NHRs of the NR1J group contribute to health during aging by controlling lipid metabolism because they regulate lipid-modifying enzymes that are necessary and/or sufficient to extend lifespan in *C. elegans*. NHRs of the NR1J group and their mammalian homologs may therefore contribute to health during aging by balancing sterol and lipid metabolism and xenobiotic resistance (Fig. 4). On one hand, by regulating enzymes of phase I, II and, III of endo-and xenobiotic detoxification NHRs can decrease the load of toxic endo- and xenobiotic molecules and promote somatic maintenance. On the

other hand, they provide for homoeostasis of steroid hormones, bile acids and other sterol metabolites and can maintain a healthy lipid profile.

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Table 1: DA/DAF-12 signaling affects longevity in a context dependent manner.

DA/DAF-12 signaling mutant/ intervention	temp. (C°)	genetic background/intervention							Reference
		wt	*daf-2 (class 1)*		*daf-2 (class 2)*	daf-2 RNAi	*daf-16*	germ cell ablat.	
			e1368	*m41*	*e1370*				
daf-9 (e1406)	**15**	**+**							Jia et al., 2002
daf-9 (e1406)	**15**	**+**							Gerisch et al., 2001
daf-9 (dh8)	**15**	**+**							Gerisch et al., 2001
daf-9 (dh6)	**15**	**+**							Gerisch et al., 2007
daf-9 (e1406)	**15**	**+**							Gerisch et al., 2007
daf-9(rh50)	**15**		**-**		**-**				Gerisch et al., 2001
daf-9 (e1406)	**15**						**=**		Jia et al., 2002
daf-9(rh50)	**15**						**=**		Gerisch et al., 2001
daf-9 (e1406)	**20**	**=**							Jia et al., 2002
daf-9(rh50)	**20**					**-**			Thondamal et al., 2014
daf-9(rh50)	**22.5**	**-**	**-**		**+**				Gerisch et al., 2001
daf-9 (e1406)	**25**	**=**							Jia et al., 2002
daf-9 (k182)	**25**	**-**	**-**						Dumas et al., 2013
daf-9 (k182)	**25**					**--**			Dumas et al., 2013
***daf-9(rh50)* + Δ7-DA**	**20**	**=**							Thondamal et al., 2014
Δ4-DA	**22.5**		**+**		**=**				Gerisch et al., 2007
daf-12 (m20)*	**15**			**-**	**=**				Gems et al., 1998
daf-12 (rh61rh411)**	**20**				**-/=**				Dumas et al., 2013
daf-12 (rh61rh411)**	**20**	**-**							Fisher and Lithgow, 2006
daf-12 (rh273)***	**20**	**+**							Fisher and Lithgow, 2006
daf-12 (m20)*	**22.5**			**-/=**	**=/+**				Gems et al., 1998
daf-12 (m20)*	**22.5**	**-/=**		**-**	**+**				McCulloch et al., 2007
daf-12 (rh61rh411)**	**25**	**=**	**=**		**=**				Dumas et al., 2013
daf-12 (rh61rh411)**	**25**					**--**			Dumas et al., 2013
daf-12 (m20)*	**25.5**				**+**				Larsen et al., 1995
daf-9(rh50)	**20**							**--**	Gerisch et al., 2001
daf-9 (dh6)	**20**							**--**	Gerisch et al., 2001
daf-12 (m20)*	**20**							**--**	Hsin and Kenyon, 1999
daf-12 (rh61rh411)**	**20**							**=**	Yamawaki et al., 2010

*non null, only DAF-12A is affected, DAF-12B is transcribed normally; ** null; *** LBD deficient; **+** DA/DAF-12 mutant/intervention increased longevity compared to background; **=** no change compared to background;

- DA/DAF-12 mutant/intervention decreased longevity compared to background

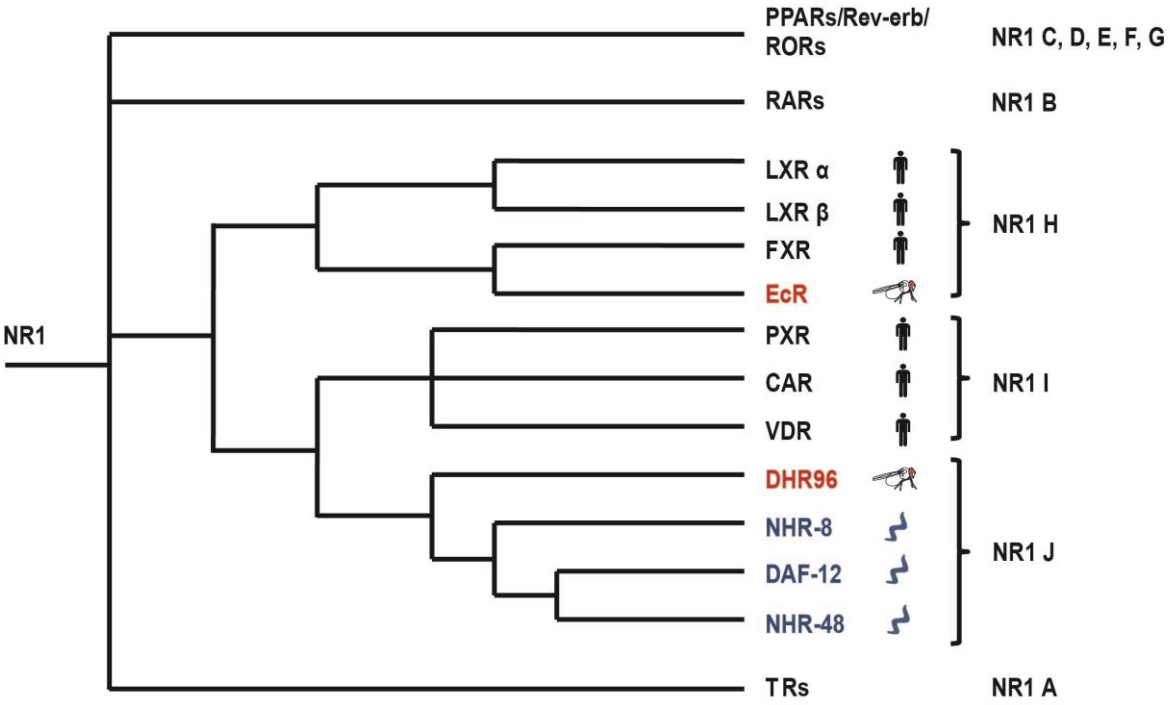

Fig. 1. Phylogenetic relationships between NHRs implicated in xenobiotic metabolism in worms, flies and mammals. The large superfamily of NHRs is classified into 6 classes of which only NR1 is depicted here. NHRs involved in xenobiotic metabolism in invertebrates belong to subgroup NR1J and include DHR96, NHR-8, DAF-12 and NHR-48. Subgroup NR1J is closely related to subgroup NR1I, which harbors the mammalian xenobiotic receptors PXR, CAR and VDR. In close proximity, mammalian bile acid and cholesterol sensors, FXR and LXRs, respectively, are located in subgroup NR1H. Distances between nodes are not to scale and branches of other subgroups have been merged for simplicity. Adapted from (Maglich et al., 2001) and (Bertrand et al., 2004).

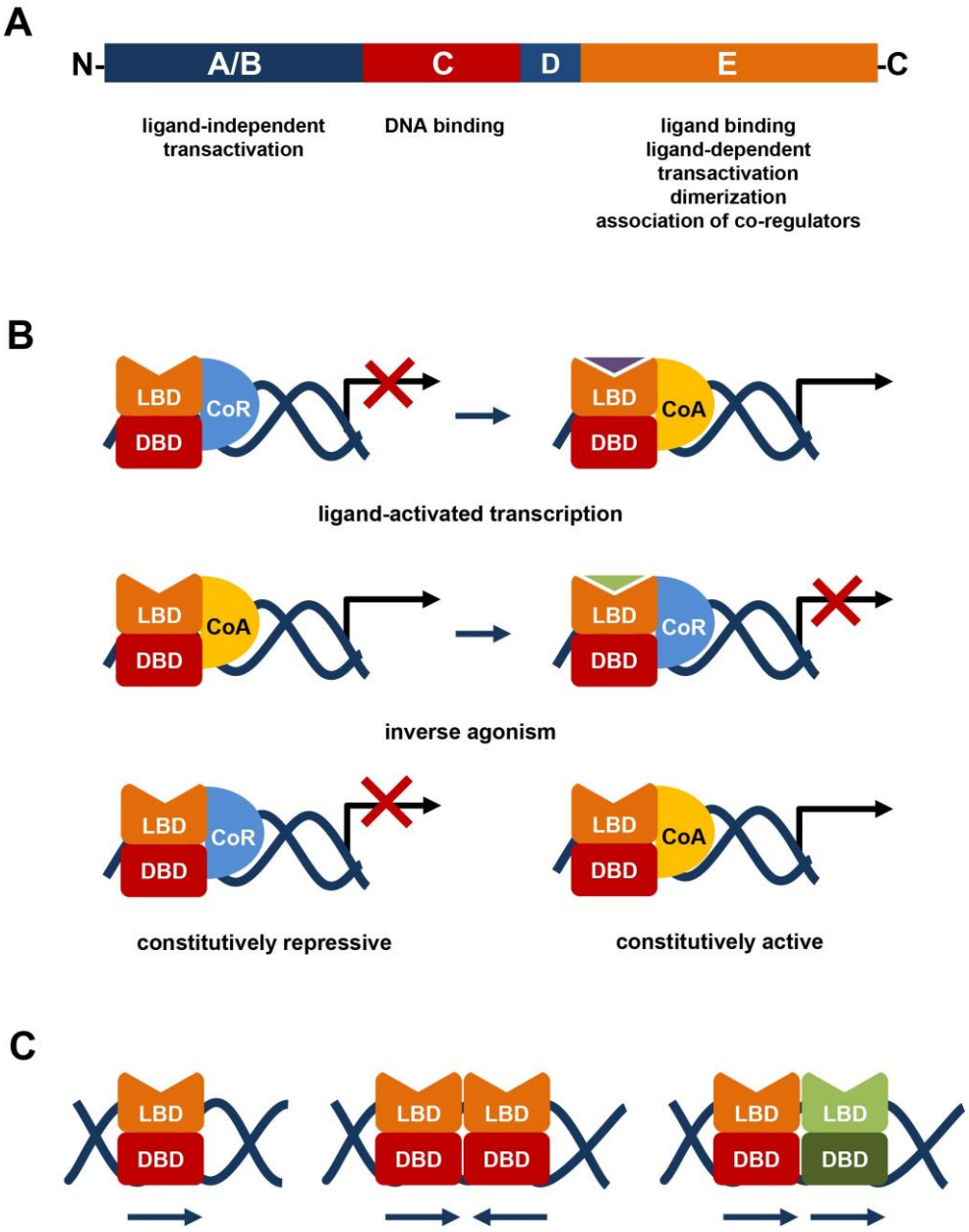

Fig. 2. Structure and molecular functions of NHRs. A) The N-terminal A/B domain is variable and serves in ligand-independent transactivation. The central C domain is highly conserved and harbors two zinc finger motifs through which the NHR binds to the DNA. The highly variable hinge region or D domain connects DBD and LBD and also facilitates DNA binding through the C terminal extensions. The E domain comprises the LBD, which serves in ligand-dependent transactivation. The LBD furthermore contains motifs necessary for dimerization and association of co-regulators, which are modulated by ligand binding. B) NHRs can bind the DNA as monomers, homo- or heterodimers. This is reflected in the arrangement of the hexameric half sites of their binding motif. Monomers bind to single half sites, while homodimers and heterodimers bind to inverted and direct repeats, respectively. C) Modes of transcriptional regulation through NHRs and their ligands. Top: Classicly, in the absence of the ligand, NHRs are associated with co-repressors and silence target gene expression. Upon binding of a lipophilic ligand, NHRs form homo- or heterodimers and associate with co-activators, resulting in expression of target genes. Middle: Some NHRs are constitutively active and ligand binding induces repression of transcription (inverse agonism). Bottom: Independent of ligand-binding, NHRs can constitutively repress (bottom left), or constitutively activate (bottom right) transcription.

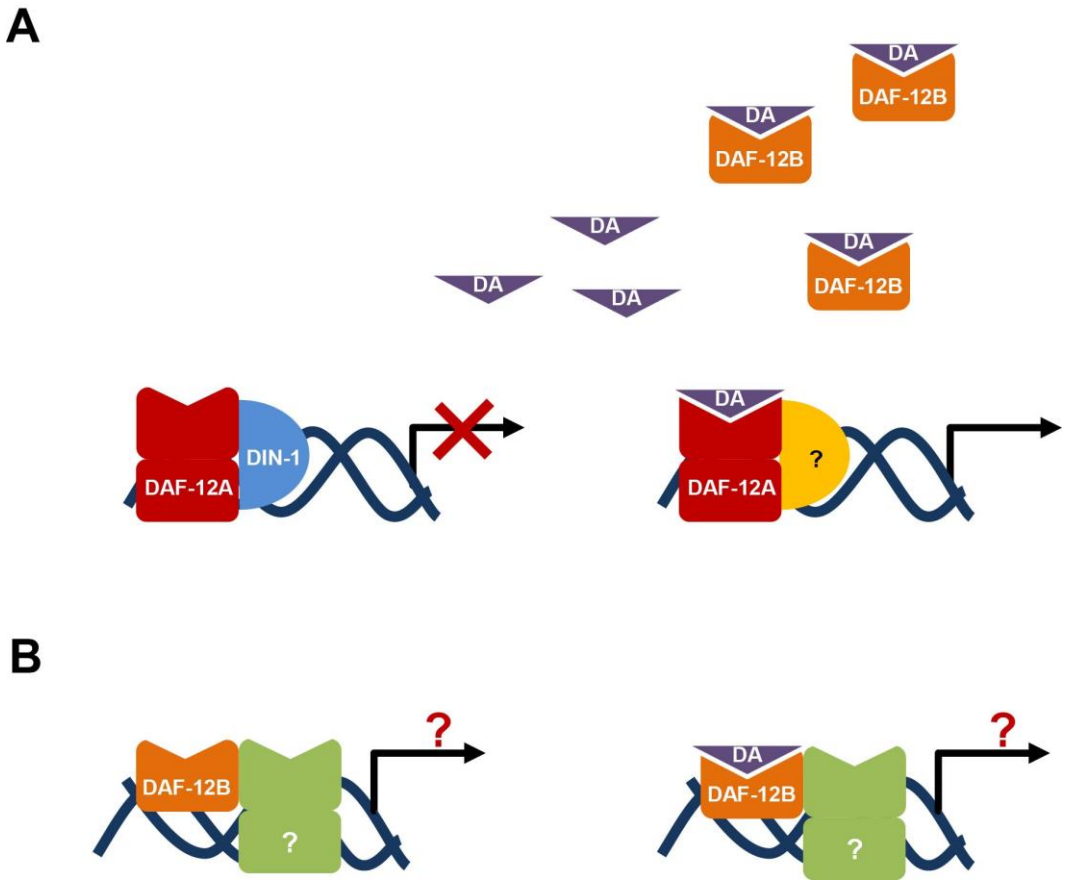

Fig. 3. Different molecular functions of DAF-12 isoforms. DAF-12 A isoforms comprise both LBD and DBD while DAF-12B lacks the DBD. In the absence of DAs, DAF-12A is bound to the co-repressor DIN-1 and represses transcription of target genes while transcription is activated when DA is bound. DAF-12B might either sequester DAs and thereby repress transcription from DAF-12A (A) or heterodimerize with another unknown NHR and regulate its transactivating activity dependent or independent of DA availability (B).

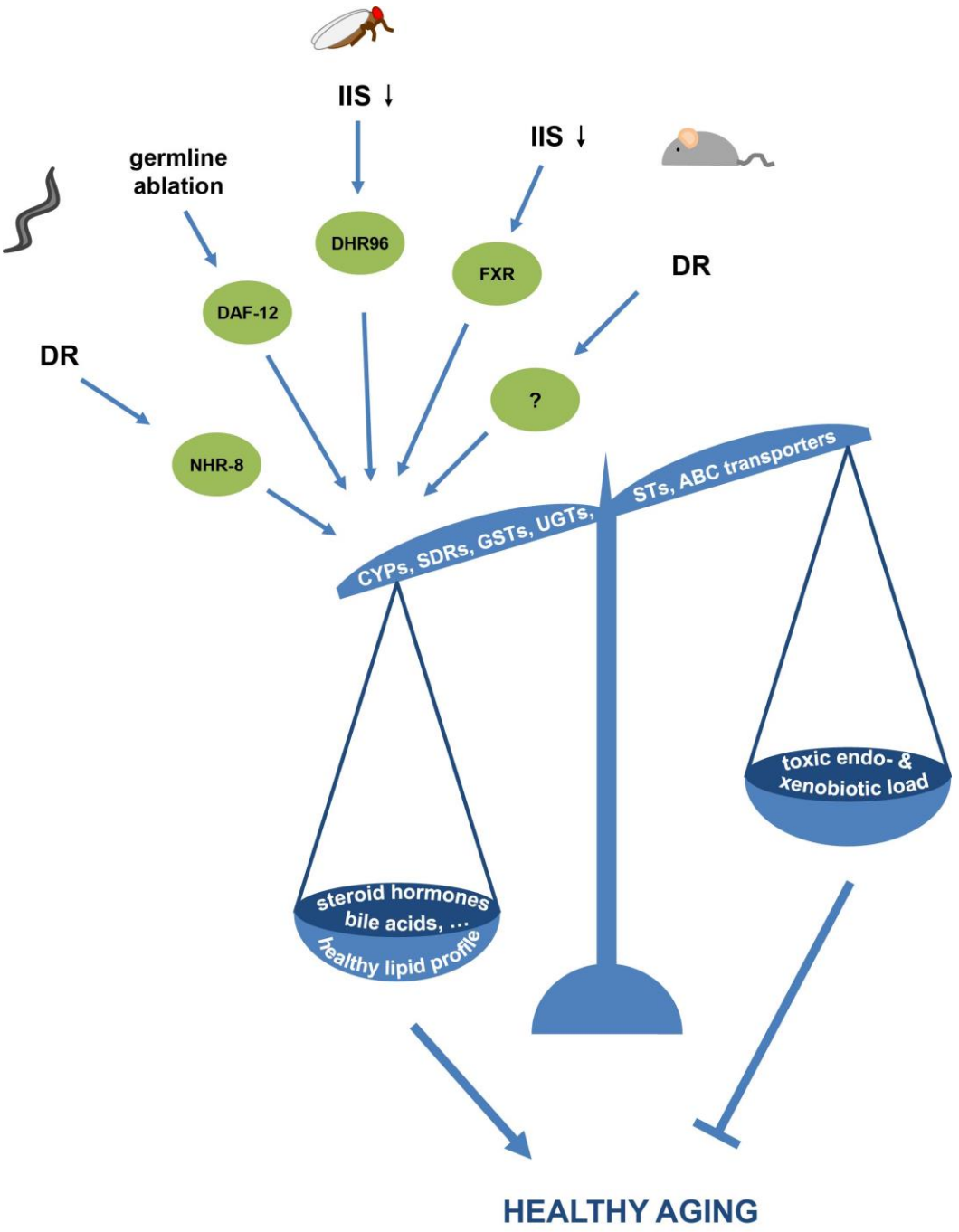

Fig. 4. Model how NHRs could balance sterol and xenobiotic metabolism to modulate healthy aging. NHR-8, DAF-12, DHR96, and FXR regulate enzymes involved in phase I, II, and III of endo- and xenobiotic detoxification. Due to their broad substrate specificities these enzymes are also involved in biosynthesis and degradation of sterol metabolites like steroid hormones or bile acids whose homeostasis is critical for healthy aging, possibly by maintaining healthy lipid profiles. Different interventions that extend lifespan in C. elegans, Drosophila and rodents either act through the NHRs depicted and/or increase expression of xenobiotic detoxification genes and xenobiotic resistance (for details see text). Noteably, these interventions also act through other downstream mediators to modulate healthy aging which are not depicted here for clarity. CYPs, cytochrome P 450 enzymes; DR, dietary restriction; GSTs, glutathione-S-transferases; IIS, insulin/insulin-like growth factor signaling; SDRs, short chain dehydrogenases; STs, sulfotransferases; UGTs, UDP-glucuronosyltransferases.