Review

**C. elegans** DAF-12, Nuclear Hormone Receptors and human longevity and disease at old age

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**Abstract**

In *Caenorhabditis elegans*, DAF-12 appears to be a decisive checkpoint for many life history traits including longevity. The daf-12 gene encodes a Nuclear Hormone Receptor (NHR) and is member of a superfamily that is abundantly represented throughout the animal kingdom, including humans. It is,
however, unclear which of the human receptor representatives are most similar to DAF-12, and what their role is in determining human longevity and disease at old age.

Using a sequence similarity search, we identified human NHRs similar to *C. elegans* DAF-12 and found that, based on sequence similarity, Liver X Receptor A and B are most similar to *C. elegans* DAF-12, followed by the Pregnane X Receptor, Vitamin D Receptor, Constitutive Andosteron Receptor and the Farnesoid X Receptor. Their biological functions include, amongst others, detoxification and immunomodulation. Both are processes that are involved in protecting the body from harmful environmental influences. Furthermore, the DAF-12 signalling systems seem to be functionally conserved and all six human NHRs have cholesterol derived compounds as their ligands.

We conclude that the DAF-12 signalling system seems to be evolutionary conserved and that NHRs in man are critical for body homeostasis and survival. Genomic variations in these NHRs or their target genes are prime candidates for the regulation of human lifespan and disease at old age.

**Keywords:** DAF-12; Nuclear Hormone Receptors; Longevity; Lipid metabolism; Detoxification

### 1. Introduction

The nematode worm *Caenorhabditis elegans* is a valuable experimental model for research into ageing, because its lifespan is influenced by signalling systems that are well characterised and highly conserved throughout evolution. In *C. elegans*, environmental conditions, such as food availability, temperature and population density, are monitored by sensory systems. To prevent damage and to maintain homeostasis the individual worm constantly has to adjust itself to its changing environment through alterations in essential traits, such as metabolic rate, developmental time and reproduction. Under favourable conditions, *C. elegans* develops through four larval stages and two adult stages. Under unfavourable conditions, *C. elegans* turns into an alternative highly resistant diapausal dauer larva. During this optional life stage, all major life history events (development, growth, metabolic rate and reproduction) are slowed down and although the worm is capable of movement, it is mainly inactive and non-feeding. When conditions turn favourable again, the larva resumes its normal developmental program to an adult, without loss of post-dauer lifespan, adding up to 60 days to the normal maximum lifespan of 15 days from egg to adult (Klass and Hirsh, 1976). This suggests that during the dauer state the worm does not age. Many genes in the worm’s signalling systems influence dauer formation and are therefore called *daf* genes. Mutations in these genes can prolong the lifespan of the worm by favouring dauer formation. Strikingly, some of these mutations also extend adult lifespan up to three-fold (Gems et al., 1998), indicating that these mutations also influence the rate of ageing in the adult worm.

Since the worm’s signalling systems are also present in other species, and are therefore believed to be functionally conserved, their role in longevity in other species is subject of intense research. The genetic mutations extending lifespan in the worm also extend lifespan in *D. melanogaster* and mice. For example, it was first discovered that the *C. elegans daf-2* mutant was longlived (Kenyon et al., 1993). Later, it was discovered that the *daf-2* gene shows homology to the mammalian genes encoding the Insulin Receptor (IR) and Insulin-Like Growth Factor 1 Receptor (IGF-1R) (Kimura et al., 1997), which are
conserved throughout evolution. Extended lifespan was then also demonstrated in IR mutants in *D. melanogaster* (Tatar et al., 2001) and in IR and IGF-1R mutants in mice (Bluher et al., 2003; Holzenberger et al., 2003). This exemplifies that *C. elegans* is a suitable model organism for research into ageing and that similarity searches in other organisms can yield interesting results.

In *C. elegans*, DAF-12 is the decisive factor for the choice between the highly resistant dauer diapause or the developmental program (Antebi et al., 1998) and DAF-12 orchestrates the cascade of events in the dauer larva formation. DAF-12 is a member of the superfamily of Nuclear Hormone Receptors (NHRs) (Antebi et al., 2000). It is active near the end of the dauer signalling pathway (Antebi et al., 1998) and integrates information from various signalling systems (Tatar et al., 2003). NHRs are localized in the nucleus, where they act as transcription factors. When activated, they stimulate or repress the transcription of target genes. The longevity phenotype of some *daf-2* mutants is doubled by mutations in *daf-12* (Larsen et al., 1995). *daf-12* thus is a key player in the regulation of adaptations that are associated with ageing and longevity. The superfamily of the Nuclear Hormone Receptors is ubiquitously represented in phylogenetically distant organisms (Mangelsdorf et al., 1995). Although in *C. elegans* the NHR superfamily has more than 200 predicted genes (Sluder et al., 1999) and the superfamily has undergone extensive proliferation and diversification, *daf-12* belongs to the phylogenetically conserved classes of *C. elegans* NHRs. In humans, the NHR superfamily has more than 50 members (Robinson-Rechavi et al., 2001). However, it is unclear which human NHR is most similar to DAF-12 and the role of NHRs in human longevity has not been investigated.

In this review, we aim to identify human NHRs similar to *C. elegans* DAF-12, to analyse in which biological pathways they are involved and to discuss their potential role in human longevity and disease at old age. In Section 2, we briefly discuss the common and discriminating characteristics of NHRs. In Section 3, we report on the systematic search for human NHRs similar to *C. elegans*’ DAF-12 and the selection of the six most similar human NHRs. In Section 4, we review the biological pathways in which the human homologues are engaged. Special attention is paid to the presence of common gene variants in components of these human pathways. In Section 5, we envisage the potential role of the identified pathways on human longevity and disease at old age and draw conclusions.

2. Characteristics of human Nuclear Hormone Receptors

The superfamily of NHRs comprises hundreds of members throughout metazoan life. Here, some of their common and discriminative features are briefly discussed. For more detail, we refer to the reviews of Laudet (1997) and Mangelsdorf et al. (1995), and references therein.

2.1. Structural features

Nuclear Hormone Receptors are composed of four functional domains (Fig. 1): the modulator domain, the DNA-binding domain (DBD), the hinge region and the ligand
binding domain (LBD) (Giguere, 1999). The modulator domain displays the largest variability of the four domains. It specifies different splice variants of the NHR resulting in different isoforms that all contain the same LBD and DBD. Each isoform has distinct biological functions and a specific expression pattern. The Activation Function (AF1) within the modulator domain determines the biological activity of the receptor isoform, for instance with regard to tissue specificity. The DBD is the most conserved domain. It contains two zinc fingers to bind the DNA of the NHRs’ target genes. These target genes contain a Hormone Response Element (HRE) of which the organisation differs between different subfamilies of receptors (see below). The hinge region, located between the DBD and LBD, is very variable. It has been suggested that it can function as a docking site for (co-)repressor proteins. The LBD is a multifunctional region that mediates ligand binding, dimerisation, transactivation functions and interaction with chaperones, such as Heat Shock Proteins. It contains a highly conserved Activation Function 2 (AF2), which has a smaller effect on transcriptional regulation than the AF1.

2.2. Function

Most nuclear hormone receptors are constitutively localized in the nucleus. When no ligand is bound, the receptor can act as a potent repressor or activator of transcription. Ligand binding changes the transcriptional activity of the NHR by either activating or repressing it. In addition, the NHRs are highly dependent on the interaction with co-regulatory proteins, which can also either enhance or repress their activity.

2.3. Classification and nomenclature

The first classification of NHRs in subfamilies was suggested by Mangelsdorf et al. (1995) and based on DNA-binding and dimerisation properties of the NHRs. The classification distinguishes four classes: a class of steroid receptors, a class of receptors that heterodimerise with the Retinoid X Receptor, a class of homodimeric receptors and a class of monomeric receptors.

Laudet (1997) investigated the evolution of NHRs. Based on his phylogenetic analyses (with NHRs from nematodes, arthropods and vertebrates), he describes six subfamilies of NHRs. Interestingly, four of the phylogenetic classes (I–IV) that Laudet proposes based on his phylogeny coincide with the classification that Mangelsdorf et al. (1995) proposed based on dimerisation and DNA-binding properties. Since the two additional classes that Laudet describes do not contain any human NHRs, which are subject of the present study, the details of the differences between the two classifications are beyond the scope of this review.

The current nomenclature of the NHRs is based on the subfamilies of Laudet (Nuclear Receptors Committee, 1999). Each gene name starts with “NR”, followed by the Arabic number corresponding to the subfamily, then a single letter for the group and then an
Arabic number again for each individual NHR. For instance, NR1A1 belongs to subfamily 1, group A and is member number 1: Thyroid Hormone Receptor A. A representative phylogenetic tree of the classification is depicted in Fig. 2.

3. Human homologues of DAF-12

Sequence similarity searches are an effective tool to aid in the prediction of orthology of proteins from different species. It is unclear which human NHRs are most similar to C. elegans DAF-12. The Pregnane X Receptor and the Vitamin D Receptor have been indicated as homologues of DAF-12 (Antebi et al., 2000; Mangelsdorf et al., 1995), but other sources indicate the Liver X Receptor Alpha as the most similar human protein (http://www.wormbase.org) or the Liver X Receptor Beta as the most likely human
homologue (http://www.ensembl.org). Although Mangelsdorf et al. included C. elegans DAF-12 in their analyses, they restricted the sequence comparison to the DBD of the NHRs. Their conclusions were drawn based on a dendrogram, which is not suitable for drawing phylogenetic conclusions. More recent phylogeny searches, such as that of Laudet, also include the LBD, but did not include DAF-12. Furthermore, since the classifications of Mangelsdorf and Laudet, in 1995 and 1997, respectively, the sequencing of the human genome has been completed and new NHRs have emerged. In this section, we searched for human NHRs most similar to C. elegans DAF-12 using Basic Local Alignment Search Tool (BLAST) sequence similarity searches. Since the function of a NHR depends on both domains, we chose to use the entire amino acid sequence containing both domains for the sequence similarity.

3.1. Sequence alignment

In C. elegans, three isoforms of the DAF-12 protein are present. The longest two contain a DBD and LBD, the short transcript contains an LBD only. We used the complete amino acid sequence of the longest of the three isoforms (F11A1.3a, 753 amino acids) and performed a BLAST against the Ensembl annotated human protein database. Amino acid sequence alignment was performed with WuBLAST2.0 on Ensembl (www.ensembl.org). The following parameters, which Ensembl uses in their orthology predictions, were used: no optimisation, no filter, span 1, postsw, the BLOSUM62 matrix, gap opening penalty 9 and gap extension penalty 2. The 20 most similar human NHRs, as identified by sequence similarity search are listed in Table 1.

In bio-informatics, the criterion of bidirectional best hit is often used in orthology predictions. A bidirectional best hit means that the members of the pair are each other’s best hit in the other species. Therefore, a reverse BLAST against the Ensembl annotated C. elegans protein database was performed using each of the 20 most similar human proteins as queries. For each of the 20 proteins we noted the rank of C. elegans DAF-12 and the E-value.

3.2. Identification of most similar NHRs

The human proteins retrieved using C. elegans DAF-12, were sorted based on ascending E-value. The E-values express the probability that the score of the observed alignment(s) within the protein occurred as a result of chance. A lower E-value indicates a lower probable contribution of chance and thus a higher probability for the contribution of homology. We listed both the NCBI Reference Sequence accession number and the NHR classification number. Multiple alignments within one protein are listed as separate rows for each alignment. For each alignment length, sequence identity and score are listed. The percentage identity indicates the fraction of amino acids within the alignment that are similar for the aligned proteins. The E-value for the total protein, as reported by WuBLAST, is also listed in Table 1.

Most of the 20 human proteins show alignment with C. elegans DAF-12 on two domains: the DBD and the LBD. Two NHRs (ERA and ERB) align on the DBD only, since they do not contain an LBD. One NHR (EAR1A) aligns on three locations, with a small alignment in the hinge region besides in the DBD and LBD.
Table 1
Result of sequence similarity search of *C. elegans* DAF-12 with human Nuclear Hormone Receptors

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<th>Location</th>
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For abbreviations, see “list of abbreviations”.
Most of the 20 human proteins in Table 1 are members of the subfamily of the NHRs that heterodimerise with RXR (subfamily 1), suggesting that DAF-12 also belongs to this subfamily. The Liver X Receptor Alpha and Beta are the most similar to DAF-12, followed by the PXR, the VDR and the CAR. Both LXRα belong to group H of subfamily 1, the VDR, PXR and CAR belong to group I. Although the FXR is number 13 in the table, it belongs to the same group as both Liver X Receptors. Repeating the sequence similarity search with different BLAST parameters retrieved the same proteins, although in a different order (data not shown).

To limit our review, we selected the human members of groups H (LXRA, LXRB and FXR) and I (VDR, PXR and CAR). As can be seen in Fig. 2, these two groups together form one branch of the phylogenetic tree. In the following two sections, we will evaluate their biological function and putative role in longevity and disease in humans.

4. Characteristics and function of selected human NHRs

Biological characteristics of the six selected human NHRs are listed in Table 2. For each of the selected NHRs, we evaluated in which biological processes they are active. We then assessed to what extent genetic variation may influence their function. In humans common gene variants are useful to elucidate the influence of variation in the function of a gene in biological processes in vivo and phenotypes on a whole-organism level in the population at large. We used Pub Med to look for literature reporting whether in humans functional

<table>
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<th>Ligands</th>
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<th>Biological processes</th>
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<td>Oxysterols</td>
<td>Liver, macrophages</td>
<td>Cholesterol sensor</td>
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<tr>
<td>Liver X Receptor Alpha (LXRB)</td>
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<td>Ubiquitous</td>
<td>Cholesterol sensor</td>
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<td>Pregnane X Receptor (PXR)</td>
<td>Drugs (rifampicine, phenobarbital, more) Steroid hormones Dietary compounds</td>
<td>Liver and intestine</td>
<td>Detoxification and excretion of steroids and xenobiotics</td>
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<tr>
<td>Vitamin D Receptor (VDR)</td>
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<td>Ubiquitous</td>
<td>Bone metabolism and calcium homeostasis Cell differentiation and proliferation Apoptosis Genomic stability Immunomodulation Detoxification</td>
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<td>Constitutive Androstane Receptor (CAR)</td>
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<td>Bile acids</td>
<td>Terminal ileum Vascular smooth muscle</td>
<td>Bile acid synthesis Bile acid re-uptake</td>
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variants exist in the selected NHRs, and in what way these variants influence the processes the NHRs play a role in and affect phenotypes. If mice knockout models were present, we briefly reviewed their phenotype(s).

4.1. Liver X Receptor Alpha and Beta (LXRA and LXRB)

The Liver X Receptor Alpha and Beta are two different proteins transcribed from two different genes. Since they have a high sequence identity (Willy et al., 1995) and since their biological function is the same, they will be discussed together. The LXRA is expressed in liver, kidney, intestine and macrophages, whereas the LXRB is expressed ubiquitously. Both are involved in lipid metabolism and cholesterol sensing mechanisms. Their ligands are oxysterols (Janowski et al., 1999), which are oxidized derivatives of cholesterol that serve as intermediary substrates in the rate-limiting steps of steroid hormone and bile acid synthesis.

The LXRs are associated with a number of steps in cholesterol metabolism (Repa et al., 2002). Activation of LXRs leads to up-regulation of the reverse cholesterol transporter cholesterol 7-alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid synthesis. Up-regulation of CYP7A1 in the liver activates the conversion of cholesterol in bile acids (Peet et al., 1998). LXRs also up-regulates ATP Binding Cassete 1 (ABC1) (Repa et al., 2000). In peripheral cells, such as macrophages, this results in the efflux of cholesterol for transport back to the liver by high density lipoproteins. ABC1 up-regulation in the small intestine inhibits cholesterol absorption by stimulating cholesterol efflux from enterocytes back into the intestinal lumen. As a result of these combined actions, the balance between cholesterol absorption and excretion shifts in favour of its excretion.

Oxysterols accumulate in the liver when there is a surplus of cholesterol, causing tissue damage. Oxysterols also accumulate in arterial foam cells and may be generated by oxidation of lipoproteins by smooth endothelial muscle cells, endothelial cells and macrophages. Exposure of vascular cells to oxysterols leads to changes in gene expression and cellular morphology which are thought to enhance the development of atherosclerosis. Interestingly, LXR was shown to be an important endogenous inhibitor of atherosclerosis in murine models (Tangirala et al., 2002). In addition, in mice, LXR activation was found to negatively regulate macrophage inflammatory gene expression in vivo and in vitro (Joseph et al., 2003). These findings identify LXRs as lipid-dependent regulators of inflammatory gene expression that may serve to link lipid metabolism and immune functions in macrophages.

LXR null mice have an impaired cholesterol catabolism, leading to accumulation of hepatic cholesterol when fed a high cholesterol diet (Peet et al., 1998). However, the concentration of cholesterol in these diets was far higher than normal.

4.2. Pregnane X Receptor (PXR)

The Pregnane X Receptor is involved in detoxification and removal of xenobiotic compounds as well as endogenous hormones. It is a broad specificity, low affinity sensing receptor that triggers metabolising enzymes. It is expressed in liver, small intestine and colon (Bertilsson et al., 1998; Blumberg et al., 1998). Ligands are mainly steroids, such as glucocorticoids, but also include drugs like rifampicine and phenobarbital (Lehmann et al.,
Target genes include cytochrome P450 enzymes (CYPs), of which CYP3A1 and CYP3A4 are the major forms, and multiple drug resistance gene 1 (MDR1) (reviewed in Wang and LeCluyse, 2003). Because of its role in these biological pathways, research on the PXR has focused on adverse drug effects and drug–drug interactions.

The PXR is also associated with inflammatory disease. For instance, expression of the PXR is negatively regulated by IL-6 (Pascussi et al., 2000b), a pro-inflammatory cytokine. Inflammatory bowel disease is associated with dysregulation of PXR target genes (Langmann et al., 2004).

Four studies report on natural variants in the PXR in humans (Hustert et al., 2001; Koyano et al., 2002; Uno et al., 2003; Zhang et al., 2001). PXR genes of a total of several hundreds of individuals from different races were sequenced, revealing a small number of infrequent mutations but no common variants. A six base pair deletion in the promoter region influences PXR transcription in vitro (Uno et al., 2003). However, no associations were found with a specific phenotype.

PXR null mice are viable and fertile in the normal laboratory environment (Xie et al., 2000). Although there is no loss of basal expression of CYP3A, expression is no longer induced by specific ligands, such as pregnenolone, which is a key intermediate in steroid hormone synthesis.

4.3. Vitamin D Receptor (VDR)

The Vitamin D Receptor is expressed in almost all tissues of the human body (Minghetti and Norman, 1988). It is activated by the Vitamin D hormone 1,25(OH)2D3, a secosteroid hormone that is synthesised in the skin from 7-dehydrocholesterol under the influence of sunlight. Target genes of the VDR are active in different processes discussed below (Minghetti and Norman, 1988).

The biological functions of the VDR are diverse (reviewed in Lin and White, 2004), including roles in bone metabolism, cellular proliferation and differentiation, chemoprevention, neuroprotection and immunomodulation. Knowledge on the role of Vitamin D in bone metabolism and homeostasis comes from the observation that rickets is the result of poor dietary intake and low exposure to sunlight. Low levels of circulating Vitamin D cause a decrease in calcium absorption from the intestine and re-absorption from the kidney, thereby decreasing circulating calcium levels. This leads to a decreased calcium content of bone, that becomes deformed and more vulnerable to fractures. The role of the VDR in bone mineral density has been confirmed by the observation that VDR knockout mice develop osteomalacia, a disease that can be reversed by calcium supplementation (Yoshizawa et al., 1997). In humans, polymorphisms in the VDR have been associated with decreased bone mineral density and increased fracture risk. For instance, in a meta-analysis the Cdx-2 polymorphism was associated with hip fracture risk (Fang et al., 2003).

The active hormone has been suggested to have anticancer properties (Hutchinson et al., 2000) in several different ways. Active Vitamin D was shown to inhibit cell proliferation while it stimulates cellular differentiation and apoptosis in a number of tissues expressing VDR. When the level of apoptosis is limited cells that have accumulated damage can evolve into cancerous cells. Furthermore, the VDR participates in maintaining genomic stability. The target genes are involved in DNA repair mechanisms in response to reactive
oxygen species (ROS). This role for the VDR is especially important in the skin, where ultraviolet radiation causes ROS. In clinical studies, both serum Vitamin D levels and VDR polymorphisms have been associated with susceptibility to, and outcome of malignancies, such as breast cancer, prostate cancer, colon cancer and malignant melanoma. On the other hand, metastatic growth of lung cancer cells is extremely reduced in VDR knockout mice (Nakagawa et al., 2004).

The VDR is also implicated in immunomodulation. For instance, knockout mice fail to develop experimental asthma (Wittke et al., 2004), suggesting a role for the receptor in inflammation at least in the lung. Immunological factors are of great importance in late life survival (van den Biggelaar et al., 2004). There is also a role for VDR in detoxification processes. For instance, dehydroepiandrosterone-sulfotransferase (SULT2A1), which is a target gene of the VDR, mediates the inactivation and excretion of steroid hormones, amongst others (Echchgadda et al., 2004). Other functions of the VDR have yet to be explained. For instance, VDR knockout mice also showed gonadal deformities, implying a role for the receptor in gonadal development (Yoshizawa et al., 1997).

Research on polymorphisms in the VDR is abundant and includes a large number of polymorphisms and their effect on a large number of phenotypes, such as bone mineral density and cancer risk. Uitterlinden et al. (2004) reviewed the genetics and biology of common VDR polymorphisms. The associations found with the polymorphisms also indicate roles for the VDR that have to be established. For instance, a polymorphism in the VDR gene has been associated with the onset of diabetes (Motohashi et al., 2003).

4.4. Constitutive Androstane Receptor (CAR)

The Constitutive Androstane Receptor is closely related to PXR and has a comparable but distinct function in xenobiotic detoxification (Maglich et al., 2002). There are indications that the PXR and CAR arose from a common ancestor (Handschin et al., 2004). Like the PXR, CAR is expressed mainly in the liver and intestine. Ligands include androstanol, which inhibits constitutive activity. CAR is induced by the binding of phenobarbital-like substances which activates the transcription of CYP genes. Dexamethasone also enhances expression of CYPs through CAR (Pascussi et al., 2000a), illustrating the overlapping functions of CAR and PXR.

Together with the PXR CAR is involved in the transcription of UDP-glucuronosyltransferase gene UGT1A1 (reviewed in Wang and LeCluyse, 2003). CAR is responsible for the conjugation of bilirubin (a breakdown product of haemoglobin) which makes the compound suitable for excretion and thus accelerates its clearance. High levels of unconjugated bilirubin are toxic. Furthermore, CAR was identified as a key regulator of acetaminophen metabolism and hepatotoxicity (Zhang et al., 2002).

However, apart from xenobiotic metabolism CAR exerts other functions (Goodwin and Moore, 2004). CAR participates in the molecular mechanisms contributing to homeostatic resistance to weight loss, as CAR influences thyroid metabolism during caloric restriction (Maglich et al., 2004). Furthermore, CAR appears to be a primary glucocorticoid receptor-response gene (Pascussi et al., 2003a).

The loss of CAR expression in knockout mice did not result in any overt phenotype in the normal laboratory environment (Wei et al., 2000). However, loss of CAR function
altered sensitivity to toxins (such as cocaine), increasing or decreasing it depending on the compound (Wei et al., 2000).

4.5. Farnesoid X Receptor (FXR)

The Farnesoid X Receptor functions in bile acid metabolism and transport as well as cholesterol and lipid homeostasis (Kuipers et al., 2004). Bile acids are the product of cholesterol degradation, involving a large number of enzymatic steps. The FXR regulates

Fig. 3. Schematic representation of the biochemical processes that involve the selected NHRs, emphasizing the central role for cholesterol.
the transcription of several of these enzymes. For instance, it represses CYP7A1, the rate-limiting step in the conversion of cholesterol into bile acids, thereby influencing both cholesterol and bile acid homeostasis. This repression was shown to be dominant over the CYP7A1 induction by the LXR (Lu et al., 2000).

FXR is expressed in the terminal ileum where it regulates the re-uptake of bile acids from the intestine, as a part of the so-called entero-hepatic circulation of bile acids. Bile acids lower triglyceride levels via a pathway involving FXR (Watanabe et al., 2004). Recently, FXR has been shown to induce very low density lipoprotein expression (Sirvent et al., 2004a) and to repress hepatic lipase gene expression (Sirvent et al., 2004b), emphasizing the role for FXR in cholesterol metabolism. The FXR is also expressed in vascular smooth muscle. In an in vitro study (Bishop-Bailey et al., 2004), stimulation of the FXR by its ligands resulted in apoptosis of the smooth muscle cells. This hints at an important role for the FXR in atherogenic processes.

FXR null mice develop normally and are outwardly identical to wild-type littermates. However, they have elevated serum bile acid, cholesterol and triglycerides, increased hepatic cholesterol and triglycerides and a proatherogenic serum lipoprotein profile (Sinal et al., 2000).

4.6. Summary

The NHRs are engaged in various biological pathways. A simplified scheme of the biochemical mechanisms that involve the six selected NHRs is shown in Fig. 3 and highlights the central role for cholesterol. For both LXR, FXR and CAR, we were unable to retrieve any article on common gene variants. For some of the genes mouse models are available, but usually without a clear phenotype in relation to ageing and longevity. However, potential effects may become visible when tested in environments more mimicking the natural environment of the mouse (Zwaan, 2003).

5. Discussion and conclusions

The human NHRs that were selected based on their structural similarity to C. elegans DAF-12 orchestrate a large number of processes that are essential for maintaining body integrity. They all play a role in detoxification and immunomodulation. Furthermore, cholesterol is a key component in these biochemical pathways.

5.1. Detoxification and immunomodulation

All six selected NHRs are involved in detoxification and immunomodulation. Detoxification decreases the harmful effects of a wide range of endogenous and exogenous compounds, such as bilirubin, cholesterol and its metabolites, steroid hormones, drugs and xenobiotics. Regulation of detoxifying genes has recently been implicated in longevity in C. elegans (McElwee et al., 2004). The transcriptional profile of long-lived daf-2 mutants in C. elegans showed up-regulation of detoxification enzymes, such as cytochrome P450, short-chain dehydrogenase/reductase, UDP-glucuronosyltransferase and glucagon S-
Some of these genes were recently identified as target genes of DAF-12 (Shostak et al., 2004). Strikingly, human NHRs have similar target genes. In humans, decreased detoxification by the PXR in the intestine has been associated with inflammatory bowel disease (Langmann et al., 2004). Other studies report on the association of NHRs with immune function and inflammation (Joseph et al., 2003; Lin and White, 2004). The immune system is one of the major lines of defence against harmful environmental influences. At old age, a reduced inflammatory response is associated with increased morality risk (van den Biggelaar et al., 2004). Taken together, NHRs are involved in biological processes that involve protection against harmful environmental influences. These processes have been associated with longevity in either model organisms, humans or both. However, no systematic research has been performed into the specific role of NHRs or their target genes in these processes and their association with human longevity and disease at old age.

5.2. Cholesterol

Cholesterol is a common component in the biochemical pathways in which the selected NHRs are involved (Fig. 3). Cholesterol is a structural component of cell membranes and a precursor in hormones synthesis. Cholesterol metabolism includes production, uptake, transport to target tissues and the excretion of toxic (by)products of cholesterol and its derivatives. Because of its hydrophobic nature, cholesterol is transported in lipoprotein particles consisting of cholesterol, triglycerides and apolipoproteins.

In C. elegans, cholesterol is a substrate in DAF-12 signalling. DAF-9, a member of the cytochrome P450 superfamily, is involved in the conversion of cholesterol into the potential ligand for DAF-12, which is believed to be a steroid (Jia et al., 2002). Conversely, DAF-12 negatively regulates DAF-9 expression in a feedback loop (Gerisch and Antebi, 2004). Recently, DIN-1 was discovered as an important cofactor for DAF-12 (Ludewig et al., 2004). It interacts with DAF-12 to form a transcriptionally active complex. In parallel, the selected human NHRs all have cholesterol derivatives as ligands and Cytochrome P450s are involved in the production of these ligands. The LXR and FXR are involved in the metabolism, transport and excretion of cholesterol. The PXR, VDR and CAR have cholesterol derived hormones as ligands. Furthermore, the expression of some major cytochromes is regulated by a network of nuclear receptors, including the PXR, CAR and VDR (Pascussi et al., 2003b). The C. elegans cofactor DIN-1 also has distinct homologues in humans. These homologues act as regulators of transcription by interaction with NHRs (Ariyoshi and Schwabe, 2003; Shi et al., 2001). Taken together, these data suggest that both the sequence and the function of the components of DAF-12 signalling are conserved throughout evolution, hinting at a critical role of such components in the processes that they regulate. Besides putative homology, there are more arguments that hint at essential functions of the NHR signalling systems. The fact that there are two LXR duplicates with highly similar function and that PXR and CAR have strongly overlapping function, indicates functional redundancy. Secondly, although research in this area is far from complete, gene variants or polymorphism do not appear to be common.

In humans, the role of cholesterol itself in disease at old age is well established. High levels of cholesterol cause cardiovascular disease at middle age. At old age, high levels of
total cholesterol are associated with decreased mortality from cardiovascular disease and infectious disease (Weverling-Rijnsburger et al., 1997). The genetic determinants of cholesterol homeostasis are currently being uncovered, as is the role of cholesterol metabolism in longevity. Strikingly, a number of the discovered genes are under regulation of NHRs: CETP and APOE are target genes of the LXR, whereas MTP is a target gene of the FXR. A linkage analysis of longlived subjects identified a linkage region on chromosome 4 (Puca et al., 2001). Further association analysis of the region identified variations in the gene encoding Microsomal Transfer Protein (MTP) to associate with longevity (Geesaman et al., 2003). MTP is a rate-limiting protein in the production of lipoproteins (Jamil et al., 1998). In a group of longlived subjects a single nucleotide polymorphism (SNP) in the gene encoding the Cholesterol Ester Transfer Protein (CETP) was enriched in a group of longlived subjects compared to young controls (Barzilai et al., 2003). CETP plays a role in reverse cholesterol transport. The SNP was associated with alterations in HDL and LDL particle size, and it was therefore concluded that CETP affects longevity by influencing cholesterol metabolism. Another example is the gene encoding apolipoprotein E (APOE). A genetic variant causing variation in the structure of the ApoE protein affects its biological activity, resulting in alteration in circulating levels of cholesterol. The variant “ε2” allele has been associated with decreased cardiovascular mortality and the variant “ε4” allele with early cognitive decline, although the results have been inconsistent. Although the serum levels of ApoE are highly heritable (Beekman et al., 2002), the APOE ε2/ε3/ε4 genotype only explains 15% of this genetic component suggesting that variation in other genes regulating ApoE transcription, such as the LXR, largely contribute to these serum levels. Taken together, these data show that cholesterol metabolism is a key process in human longevity and disease at old age, and that NHRs play a crucial role in cholesterol metabolism. Similarly, in a sequence similarity analysis across species, the vitellogenin gene family has been identified as a conserved pathway regulating energy storage and lipid metabolism in species from nematodes to humans (Brandt et al., 2005). All this fits the suggestion that the mechanisms that regulate human longevity are conserved throughout evolution.

5.3. Evolutionary background

The disposable soma theory on ageing states that each individual has to allocate limited energy resources to either maintenance of the soma or reproduction (Kirkwood, 1977). This trade-off between reproduction and longevity was shown to exist also in humans (Westendorp and Kirkwood, 1998). Therefore, it is likely that human longevity necessitates more than average investments in somatic maintenance. Toxic compounds or radiation can generate excess ROS in the body. ROS cause damage to organelles and the important macromolecules, such as lipids and DNA, ultimately leading to cell death. High levels of oxidative stress have been associated with accelerated ageing (Finkel and Holbrook, 2000). Genetic factors influencing the maintenance processes may influence the rate of damage accumulation in the cell and thus the rate of ageing. Loss-of-function mutations lead to inefficient adaptation and accumulation of damage, causing accelerated ageing and disease. Gain-of-function mutations lead to more efficient adaptation and decreased accumulation of damage, and may thus slow the rate of ageing and therefore promote
longevity. For example, mice lacking the LXRA have impaired cholesterol and bile acid metabolism when fed a high cholesterol diet, leading to cholesterol accumulation in the liver and eventually hepatic failure (Peet et al., 1998). Conversely, a synthetic LXR ligand inhibits the development of atherosclerosis in mice (Joseph et al., 2002). Mice knockout models of the selected NHRs show no overt phenotype under standard laboratory conditions. However, when challenged with changing environmental conditions the null mice are less able to cope, resulting in disease and early mortality. This implies an essential role for NHRs in maintaining of homeostasis under changing environmental conditions, in line with the function of *C. elegans* DAF-12. In *C. elegans*, DAF-12 is downstream of signalling systems that translate environmental cues, as well as germline signals (Tatar et al., 2003). Interestingly, the longlived phenotype exhibited by germline ablated worms is dependent on DAF-12 and DAF-9 (Hsin and Kenyon, 1999). This positions DAF-12 at the crossing point of maintenance of the soma and germline signalling. In humans, the PXR, CAR and VDR are involved in homeostasis of steroid hormones and detoxification of xenobiotic compounds, implicating a role for these NHRs in both germline signalling and maintenance. This observation identifies these NHRs as candidates for longevity regulation.

5.4. Conclusions

Our results indicate that cholesterol is a key player in the regulation of NHR activity. We have limited our review to the two groups of human NHRs that are most similar to DAF-12, but we do not exclude the possibility that other groups are equally interesting. It remains possible that during evolution specific NHRs have changed into forms different from DAF-12 without loosing their function in longevity associated processes. Also, cross-talk between different NHRs may play a role in orchestrating the effector pathways. We conclude that the approach we used is powerful to detect signalling pathways involved in essential processes, yielding a new list of candidate genes. The approach can be extended to more, if not all NHRs. We think it likely that human longevity and disease at old age depend on NHR function.

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