

# Nuclear Hormone Receptors, Metabolism, and Aging: What Goes Around Comes Around

Keith Pardee,\* Jeff Reinking,\* and Henry Krause

(Published 24 November 2004)

**Previous studies have linked the mysterious and inevitable process of aging to essential processes such as metabolism, maturation, and fecundity. Each of these processes is controlled to a large extent by nuclear hormone receptors (NHRs). NHRs also play important roles in the control of periodical processes, the most recently implicated being circadian rhythm. This Review stresses the mounting evidence for tight relationships between each of these NHR-regulated processes and the processes of aging.**

## Background

Nuclear hormone receptors (NHRs) are a superfamily of eukaryotic transcription factors that control a variety of systemic processes through the coordinated regulation of target gene expression (1, 2). Unlike most other transcription factors, NHR activities are controlled by small lipophilic molecules that move readily within and between cells and organs. This mechanism provides NHRs with the ability to respond directly to cues provided by distant cells, tissues, organs, or the environment; hence the name nuclear hormone receptors. Known nuclear receptor ligands range from short fatty acids to complex steroids (3, 4). They bind to the hydrophobic cores of structurally conserved ligand-binding domains located C terminal to the DNA binding domain of each NHR. The structural rearrangements that ensue can alter intramolecular interactions, subcellular localization, DNA binding, cofactor interactions, and transcriptional outputs (1). Although we have learned a lot about the effects of ligands on their cognate NHRs, more than half of NHRs are orphans, meaning that their ligands have yet to be identified.

The first NHRs to evolve, most likely related to hepatic nuclear receptor 4 (HNF4) or to peroxisome proliferator-activated receptor (PPAR) NHRs (5, 6), were probably regulators of simple lipid metabolism, regulating the expression of key metabolic genes. By binding to the precursors and/or products of these reactions, as well as to appropriate collections of target gene promoters, these prototype NHRs gained the ability to monitor lipid levels and to autoregulate accordingly. As NHRs evolved in number and ligand diversity, new roles in lipid homeostasis evolved. A summary of these roles is shown in Fig. 1. Ancillary roles related to lipid metabolism also evolved. These include roles in pattern formation, sexual diversification, inflammation, xenobiotic responses, and circadian rhythms. The interdependence between each of these biological processes and the process of aging are discussed below. Consistent with an early ap-

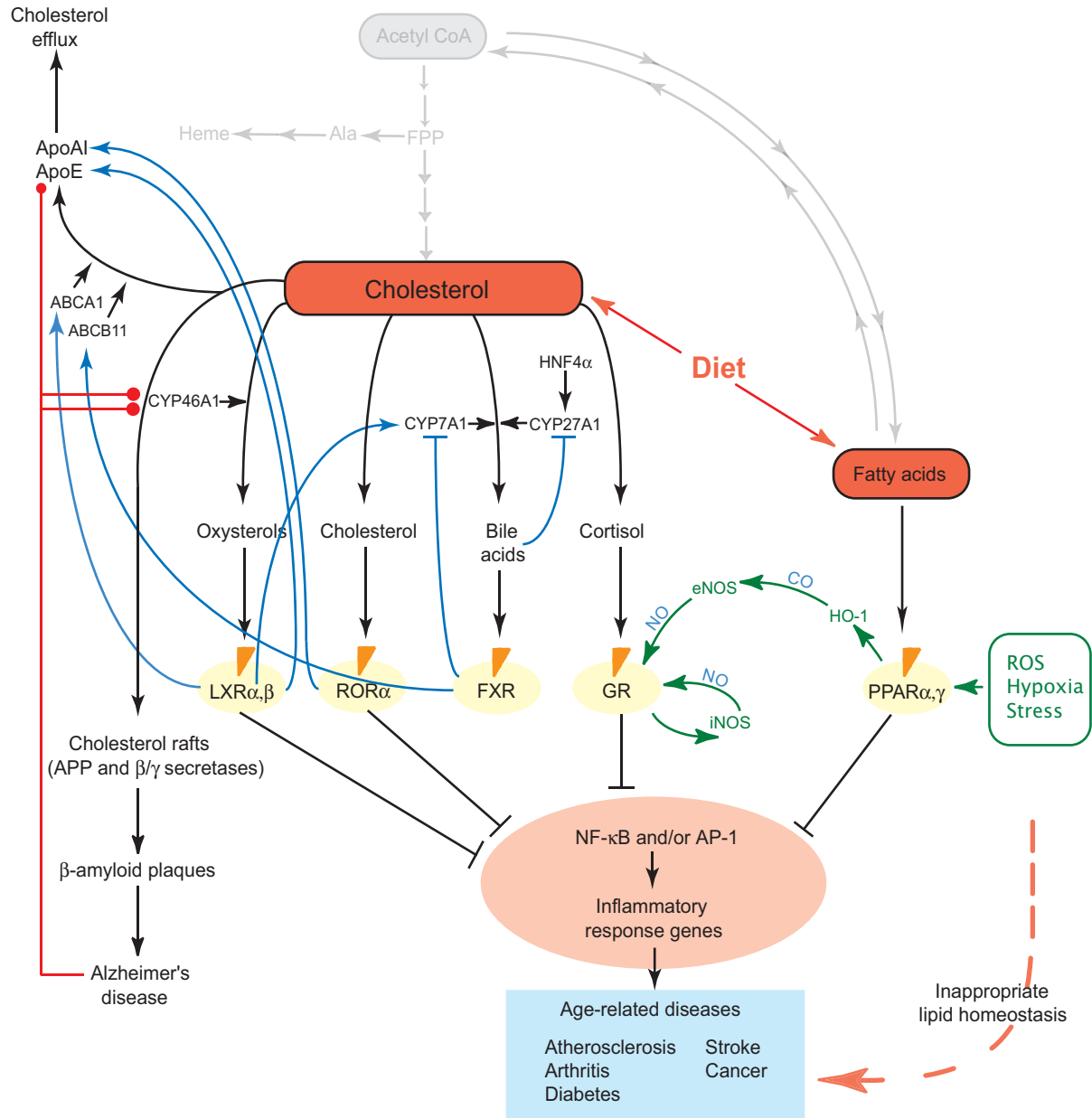
pearance in NHR evolution, the PPAR family of receptors appears to function at the hub of lipid homeostasis and most of the processes described.

## Metabolism

To understand the link between metabolism and aging, it is important to consider the hunter-gatherer environment in which our species evolved. Our ability to rapidly stockpile energy during periods of abundance and to conserve energy during times of famine has conveyed a selective advantage. However, these abilities are now ill suited to the sedentary life-styles and rich diets of modern society. The negative effects of high-calorie diets cause a variety of disorders and diseases that link metabolism and aging (see the section on Diseases of Aging below). Conversely, reduced caloric intake has been shown to increase life span and resistance to stress in most model organisms (7, 8) (see Masoro Subfield History at <http://sageke.sciencemag.org/cgi/content/full/2003/8/re2>). Specifically, restriction of caloric intake to 30 to 70% of voluntary (ad libitum) levels results in extension of life span in yeast (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbIntrvn;12>) (9), worms (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbIntrvn;13>) (10), flies (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbIntrvn;14>) (11), and mammals, including mice (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbIntrvn;9>) and rats (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbIntrvn;11>) (12).

At the top of the regulatory pathway that coordinates metabolic rate with the availability of calories in the environment is the insulin/insulin-like growth factor-1 (IGF-1)-like signaling (IIS) pathway. As with caloric restriction, down-regulation of this signaling cascade significantly increases longevity in yeast, worms, flies, and mice (13) (see Longo Perspective at <http://sageke.sciencemag.org/cgi/content/full/2004/39/pe36>, Johnson Subfield History at <http://sageke.sciencemag.org/cgi/content/full/sageke;2002/34/re4>, Warner Subfield History at <http://sageke.sciencemag.org/cgi/content/full/sageke;2003/6/re>, and "One for All" at <http://sageke.sciencemag.org/cgi/content/full/sageke;2002/49/nf15>). A growing list of studies indicates that NHR-regulated pathways are tightly interwoven with the IIS pathway, acting both upstream and downstream to coordinate responses between environmental and dietary signals and endocrine signals emanating from the central nervous system (14). Although evidence of upstream NHR actions on IIS is less extensive than that for downstream interactions, a recent influx of data suggests that many additional connections remain to be discovered. An example of an upstream interaction is the modulation of insulin secretion from pancreatic islets and  $\beta$  cells by PPAR $\alpha$  (15). Transcription of the PPAR $\alpha$  gene is also repressed by glucose (16). Another example is PPAR $\gamma$ , which, when heterodimerized with retinoid X receptor  $\alpha$  (RXR $\alpha$ ),

\*Co-first authors. The authors are in the Banting and Best Department of Medical Research, University of Toronto, Charles H. Best Institute, Toronto, Ontario, Canada. E-mail: h.krause@utoronto.ca (H.K.)

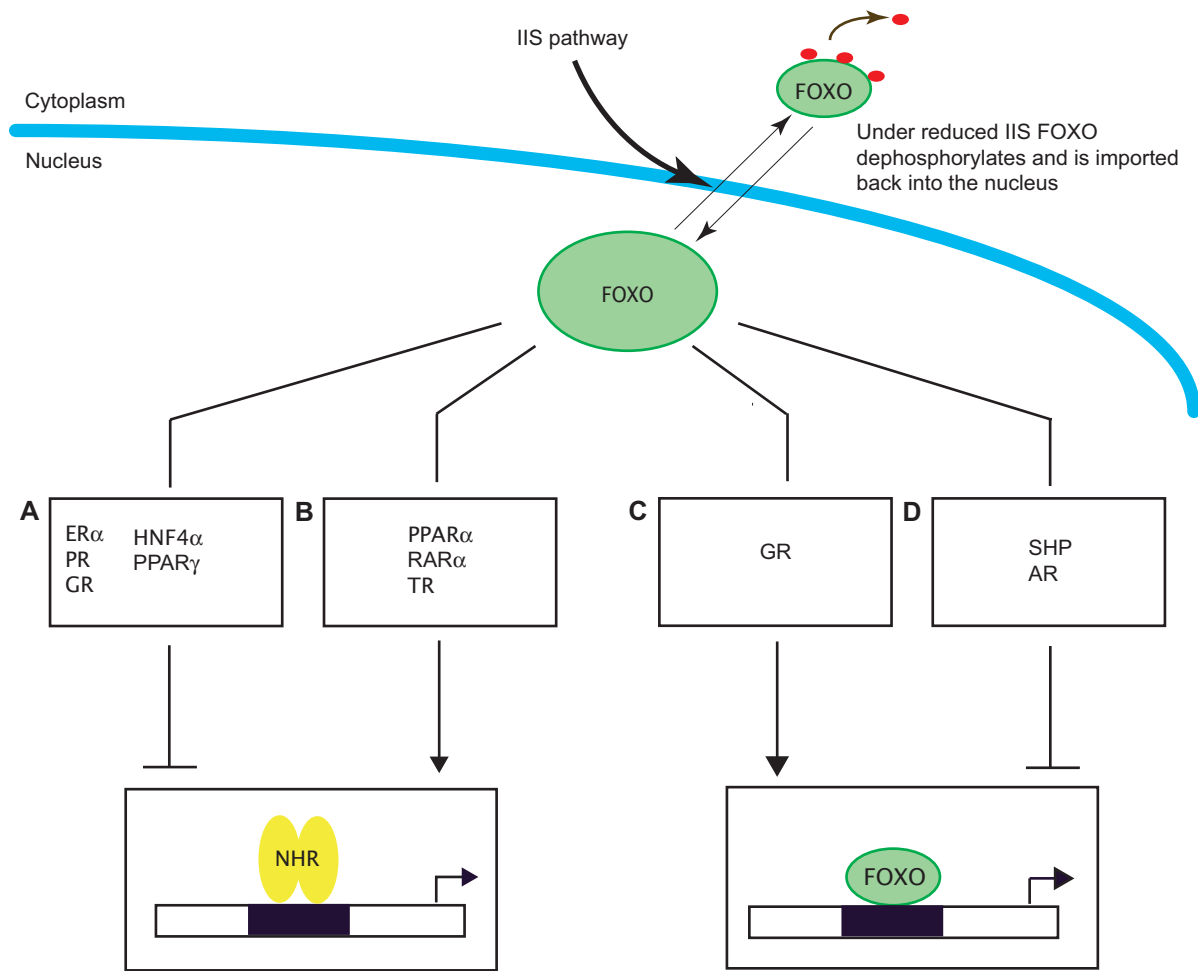


**Fig. 1.** Diet, biosynthetic pathways, and lipid clearance combine to form the complex network of lipid metabolism. NHRs are intimately involved in this regulation of lipid homeostasis and thus play a critical role in many lipid-related diseases, as well as the process of aging. Several reviews provide excellent comprehensive summaries of these interactions (31, 96, 97). Red lines and balls indicate links between AD, LXRs, and components of cholesterol metabolism.

down-regulates genes required for lipid synthesis and uptake. This is controlled indirectly through activation of *insulin-induced gene 1 (Insig-1)* expression (17). *Insig-1* acts posttranscriptionally on sterol regulatory element-binding proteins, causing them to be retained in the endoplasmic reticulum (18).

The cellular response to insulin or IGF-1 is coordinated through a number of transcription factors (19). Of these, the FOXO (<http://sageke.sciencemag.org/cgi/content/full/2004/23/pe25>) (Forkhead box, subclass O) class is most salient to the role of NHRs in aging (Fig. 2). IIS controls the amount of active FOXO protein in the nucleus, causing nuclear export under

increased insulin signaling (20). In worms, the FOXO homolog DAF-16 (<http://sageke.sciencemag.org/cgi/genedata/sageke-GdbGene;35>) is essential for the increased longevity brought about by mutations of the IIS pathway genes *daf-2* (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;38>) (which encodes an insulin/IGF-1 receptor) and *age-1* (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;2>) (which encodes a phosphatidylinositol-3-OH kinase) (21). Overexpression of DAF-16 in worms or of dFOXO in flies also extends life span (22, 23). FOXO proteins have been shown to interact with a number of NHRs (summarized in Fig. 2). In the

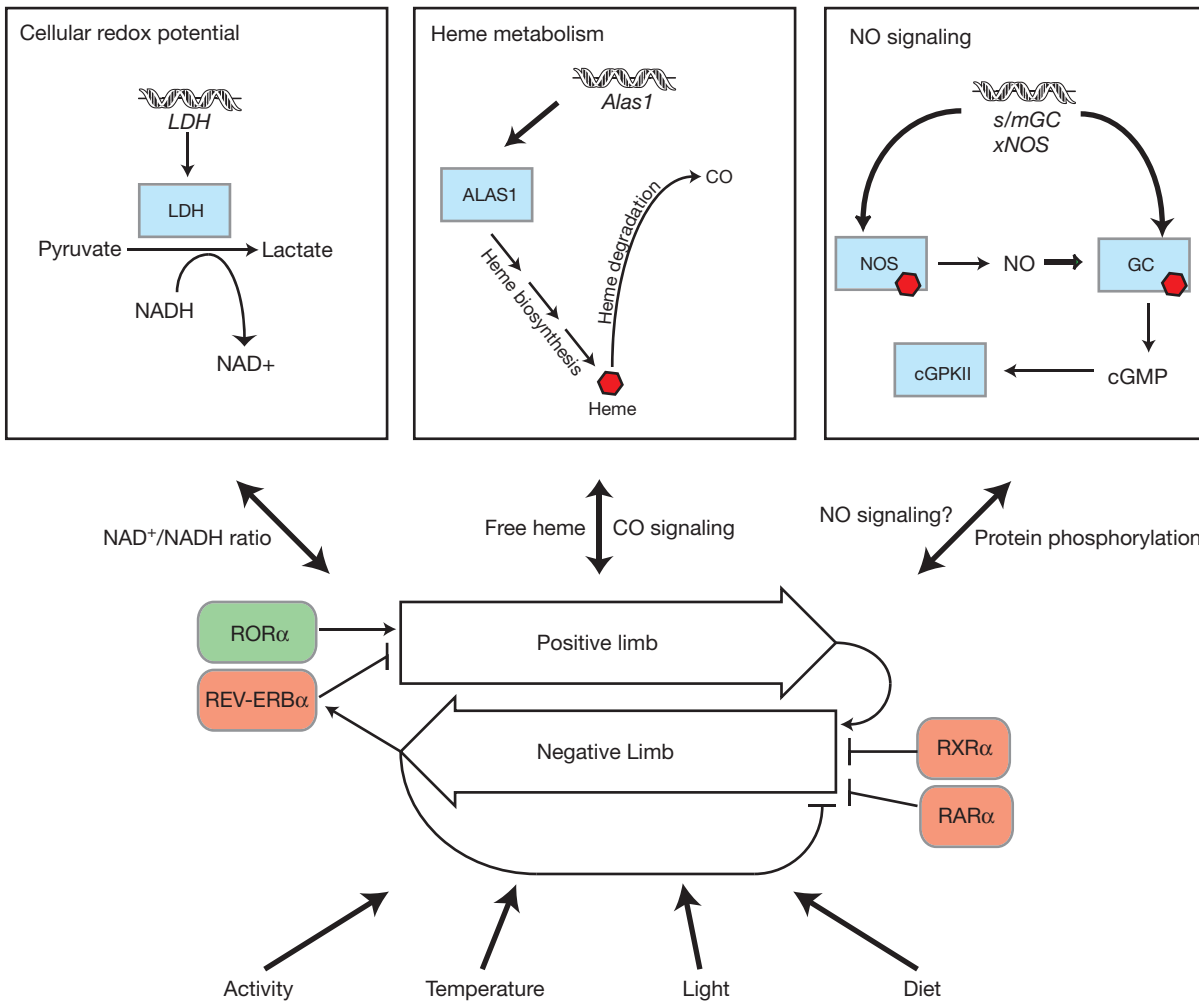


**Fig. 2.** Interactions between FOXO proteins and NHRs regulate genes critical to metabolism and aging. IIS causes FOXO nuclear export via phosphorylation (red dots). **(A)** Ectopic expression of Foxo1 in MCF-7 breast cancer cells represses ER $\alpha$ , progesterone receptor (PR), and GR-mediated transcription (26); FOXO1 interacts with the DNA binding domain of HNF4 $\alpha$ , preventing DNA binding (25). PPAR $\gamma$  and FOXO1 are reciprocally antagonistic (27). **(B)** Ectopic expression of FOXO1 up-regulates PPAR $\alpha$ -mediated transcription of lipoprotein lipase in skeletal muscle cells (106); ectopic expression of FOXO1 in MCF-7 breast cancer cells stimulates transcription mediated by RAR $\alpha$  and TR (26). **(C)** In hepatoma cells, both FOXO1 and GR interact to activate the expression of a glucose-6-phosphate transporter (107). **(D)** SHP (small heterodimer partner) displaces FOXO1's coactivator CBP; AR inhibits FOXO1-mediated transcription by blocking DNA binding (28).

cases of estrogen receptor  $\alpha$  (ER $\alpha$ ), retinoic acid receptor (RAR), and thyroid receptor (TR), FOXO acts as an essential cofactor in transcriptional activation (24–26). Conversely, both ER $\alpha$  and its ligand have been shown to act as both repressors of transactivation brought about by FOXO1 and of cell cycle arrest brought about by FOXO3a (24). FOXO1 has also been shown to interact with PPAR $\gamma$ , with the two proteins acting as reciprocal antagonists. This interplay is augmented by the antidiabetic PPAR $\gamma$  ligand rosiglitazone (27). Similar types of NHR/FOXO interactions appear to exist with the NHRs HNF4 and androgen receptor (AR) (25, 28).

Among the NHRs, PPARs appear to play a particularly important role in IIS and dietary restriction. Of the three ( $\alpha$ ,  $\delta$ , and  $\gamma$ ), PPAR $\alpha$  and  $\gamma$  are the best understood. For example, a recent microarray study showed that PPAR target genes are among the first and most prevalent genes to be up-regulated in the liver by re-

duced-calorie diets (29). As stated above, PPAR $\alpha$  regulates fatty acid catabolism, and in doing so, coordinates lipid metabolism and glucose homeostasis with feeding and fasting cycles. In contrast, PPAR $\gamma$  is a fundamental regulator of adipogenesis, controlling the expression of genes involved in lipid storage (see Melloul Perspective at <http://sageke.sciencemag.org/cgi/content/full/2004/9/pe9>, “Counterattack” at <http://sageke.sciencemag.org/cgi/content/full/2004/23/nf57>, and “Wasting Away: Stress Response Keeps Aging Cells from Fattening Up” at <http://sageke.sciencemag.org/cgi/content/abstract/2002/8/nw25>). More recently, studies with PPAR $\delta$  have shown that it is a potent regulator of fatty acid catabolism and energy homeostasis (30, 31). The expression of an activated form of PPAR $\delta$  in skeletal muscle yielded transgenic mice with a considerably greater ability to metabolize lipids, run long distances, and live longer than the wild type (32, 33) (see “Going the Extra Mile” at <http://sageke.sciencemag.org/cgi/>



**Fig. 3.** The role of nuclear receptors in circadian oscillators. Input, output, and reciprocal signaling pathways are presented as a schematic. Nuclear receptor proteins acting as transcriptional activators and repressors are highlighted in green and red, respectively. Other key proteins are shown in blue. NAD<sup>+</sup>, nicotinamide adenine dinucleotide; cGMP, cyclic guanosine monophosphate; LDH, lactate dehydrogenase; ALAS1, 5-aminolevulinic acid synthase 1; GC, guanylyl cyclase; cGPKII, cGMP-dependent protein kinase II.

content/full/2004/34/nf79). As might be expected, the opposite phenotype is displayed by *PPARδ* null mice (34).

### Cellular Oxidation and Stress

From a cellular perspective, aging is a process of progressive cellular damage caused by the products of metabolism. Cells with high metabolic turnover are particularly subject to hypoxia, the buildup of reactive oxygen species (<http://sageke.sciencemag.org/cgi/content/full/sageke;2001/1/oa5>) (ROS), stress, and inflammation. Cells combat these insults with a typical response pathway that is known to involve NHR inputs at several key points (Fig. 1). Two gas molecules, nitric oxide (NO) and carbon monoxide (CO), also play major roles in the sensing and response to symptoms of cellular stress (35–38). The following sections will show that the functions of NHRs and the production and function of these gases may be intertwined at many different levels.

NO is produced by nitric oxide synthases (eNOS, iNOS, and nNOS) and CO is produced by heme oxygenases (HO-1, HO-2,

and HO-3). Both types of enzymes are widely distributed and come in constitutive and inducible forms. The interplay and effects of these molecules are complex, depending on cell type and the extent and nature of the damage. Hence, there are likely to be many nuances and exceptions to the generalized interactions shown in Fig. 1.

The antioxidant and anti-inflammatory mechanisms of NO and CO actions include (i) increased expression and activation of enzymes that neutralize and remove ROS {for example, manganese superoxide dismutase [MnSOD, also known as SOD2 (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;146>)], cytochrome P450s, and sulfotransferases}, (ii) reduction of the concentrations of certain inflammatory agents [such as cytokines and prostaglandins (<http://sageke.sciencemag.org/cgi/content/full/2002/29/re3#SEC6>)], and (iii) smooth muscle relaxation (35, 39) (see “NO-aspirin, No Atherosclerosis” at <http://sageke.sciencemag.org/cgi/content/abstract/2002/36/nw126> and “Shear Benefits” at <http://sageke.sciencemag.org/cgi/content/full/>

2003/3/nw13). NO and CO also protect tissues affected by ROS, inflammation, and hypoxia by suspending cell growth and division (40–42) and by preventing apoptosis (36, 43) (but see also “Cell Death, Start to Finish” at <http://sageke.sciencemag.org/cgi/content/full/2004/6/nf17>).

Nuclear receptors play key roles in each of these responses. For example, PPAR $\alpha$ ,  $\delta$ , and  $\gamma$ , which play central roles in lipid metabolism, also play major roles in ROS and inflammation responses. Increased PPAR $\alpha$  activity, for example, leads to up-regulation of certain enzymes, including some of those listed above, that break down and remove lipid-derived ROS {for example, MnSOD and catalase [CAT (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;15>) (44)]}. PPAR $\gamma$ , on the other hand, is activated by prostaglandins produced at sites of inflammation, allowing it to up-regulate *HO-1* gene expression (45).

The glucocorticoid receptor (GR) is also a key mediator of CO/NO effects and anti-inflammation (37, 46). GR expression is up-regulated by NO and in turn induces expression of the *iNOS* gene (37, 47), resulting in further production of NO and the creation of a positive feedback loop (Fig. 1). Another important role of GR is to block activities of the transcription factors nuclear factor kappa B (NF- $\kappa$ B) and AP-1. The latter are well-established activators of inflammatory response genes. PPAR $\alpha$ , PPAR $\gamma$ , ER $\alpha$ , and the liver X receptor [LXR (<http://sageke.sciencemag.org/cgi/content/abstract/2002/34/nw121>)] are also able to block inflammation by directly binding and neutralizing NF- $\kappa$ B and/or AP-1 activities (48, 49).

HO, NOS, and other key enzymes in these cytoprotective pathways, including SOD, catalase, cytochrome P450s, cytochrome c oxidase, and NAPH-oxidase, could all potentially be regulated directly by NO or CO. This is because all of these proteins contain heme (protoporphyrin IX + Fe) moieties within their active centers, which provides them with the potential to bind gases such as O<sub>2</sub>, CO<sub>2</sub>, NO, and CO. Other potential roles for heme, NO, and CO will be revisited below.

Many toxic by-products of lipid metabolism are also damaging to the cell. The NHRs pregnane X receptor (PXR) and constitutive androstane receptor up-regulate the expression of genes encoding certain enzymes, including glutathione S-transferases and sulfur transferases, that facilitate the clearance of bile acids, estrogens, and bilirubin (heme) (50). These receptors are also charged with the job of recognizing and detoxifying other ingested toxins. In mammals, the majority of these xenobiotic NHRs reside in the liver. In organisms such as worms and insects, which literally live within their food, the presence of more widely dispersed xenobiotic receptors is necessitated. The nematode is an extreme example, with approximately 270 evolutionarily divergent NHRs, many of which are believed to function as xenobiotic sensors (51). Dealing with these toxins, and the stresses that they impose, is a critical aspect of slowing the aging process.

### Sexual Maturation and Diapause

A fundamental aspect of the aging process is the tradeoff between reproductive and somatic function (see Mobbs Perspective at <http://sageke.sciencemag.org/cgi/content/full/2004/35/pe33> and Tatar Perspective at <http://sageke.sciencemag.org/cgi/content/full/sageke;2002/3/pe2>). In this sense, aging can be defined as the process that begins after sexual maturation. Sex hormones that are recognized by nuclear receptors, such as estrogen, progesterone, and androgen (testosterone), play essential

roles in both sexual maturation and resulting senescence. After sexual maturity, the concentrations of these hormones are programmed to decline. One way to counteract this decline in hormone levels, and potentially in life span, is to supplement the diminished hormone titers. This process, referred to as hormone replacement therapy (HRT) has been explored as a viable mechanism not only to alleviate the acute symptoms of menopause and andropause (such as hot flashes and depression) but also to treat chronic aging diseases such as osteoporosis, heart disease, and neurodegeneration (see “More Than a Hot Flash” at <http://sageke.sciencemag.org/cgi/content/full/sageke;2002/10/ns3>). At first considered very promising, recent results have brought into question the safety and effectiveness of HRT in humans (52–56) (see “Weathering the HRT Storm” at <http://sageke.sciencemag.org/cgi/content/full/2003/38/nf18>). Some of the variability in results may be due to (i) the nature of the hormones used, (ii) how and when they are applied, and (iii) the genotypes of the test participants [reviewed by (57)]. Future research in this area will also have to address the intrinsic complexity of NHR interactions.

A second approach to combating the decline that follows sexual maturity is to prevent or postpone it. An elegant substantiation for this tactic is found in the reproductive diapause of insects. Diapause is a state of developmental arrest often used by insects to time their life cycles to the appropriate season or surroundings. This process occurs in adults and results in the arrest of oogenesis, vitellogenesis, accessory gland activity, and mating behavior. Several threads of evidence suggest that reproductive diapause is induced by down-regulation of the endocrine juvenile hormone (JH). Surgical removal of the source of JH in monarchs and grasshoppers induces diapause and also doubles adult longevity [reviewed in (58)]. In *Drosophila*, diapause also reduces mortality rates. JH is widely believed to be a ligand for an orphan NHR waiting to be adopted.

In *Caenorhabditis elegans*, a form of diapause, known as dauer, is an alternate to the larval pathway that leads to reproductive adults (see “Dauer Power” at <http://sageke.sciencemag.org/cgi/content/abstract/2002/31/nw110>). Nematodes enter dauer as a result of environmental stress and are long-lived as compared to reproductively growing animals. As conditions improve, diapausing animals are able to resume development and become reproductive adults with normal life spans. Recent evidence suggests that dauer diapause is controlled by an unidentified lipophilic hormone, synthesized by the cytochrome P450 hydroxylase DAF-9 (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;443>) and sensed by the NHR DAF-12 (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;34>) (59) (see “Hard Times Teach Life-Extending Lessons” at <http://sageke.sciencemag.org/cgi/content/full/2001/11/nf5> and “Homing In on a Hormone” at <http://sageke.sciencemag.org/cgi/content/full/2004/41/nf92>).

The only NHR with a known ligand in invertebrates is the ecdysone receptor (EcR), which is involved in many aspects of insect development and maturation. A recent study focusing on EcR demonstrated that the endocrine hormone 20-hydroxyecdysone can have a measurable impact on life span (60) (see Tatar Perspective at <http://sageke.sciencemag.org/cgi/content/full/2003/9/pe5>). By creating EcR mutants less capable of binding the hormone than the wild type or by simply down-regulating hormone biosynthesis, the longevity of the flies could be extended by up to 40%. This extension also brought with it an increased tolerance of stress, but not the usual loss in fecundity

brought about by down-regulation of the entire insulin response pathway. The latter suggests that it may be possible to uncouple the link between sexual maturation/activity and senescence.

A phenomenon similar to diapause is observed when *Drosophila* embryos are subjected to severe hypoxia. Larvae reversibly enter a state of suspended animation, curtailing cell division, protein synthesis, and membrane permeability. The signaling molecule that appears to mediate this response is NO (42).

### Circadian Rhythm

Gaseous signaling also plays a key role in entraining circadian rhythms. Circadian oscillators are complex molecular loops that are entrained by environmental stimuli (zeitgebers), most importantly light, but also temperature, food intake, and activity. The conventional model establishes the suprachiasmatic nucleus (SCN) of the brain as the site of the central or master pacemaker, with optic lobe input as its major driving force. "Light" and "dark" inputs are thought to be converted to signals of NO and CO, respectively, through activation of nNOS or HO-2 within nonoverlapping cells of the SCN (61, 62). NO and CO, in turn, are believed to signal the central clock proteins of the SCN: Clk and Bmal1 in the positive limb and mPer2 and mCry2 in the negative limb (Fig. 3). With the exception of mCry2, each of these proteins contains a PAS domain. Like NHR ligand-binding domains, PAS domains can act as small molecule-activated triggers [reviewed in (63)]. For the PAS domain proteins mPer2 and the peripheral clock component NPAS-2, the small molecule ligand is heme (64, 65), which gives them the ability to bind and respond to diatomic gases. In the case of NPAS-2, physiological levels of CO block its ability to bind DNA (64). This suggests the possibility that PAS domain proteins of the central clock may also respond directly to NO or CO.

So how does circadian rhythm relate to NHRs and aging? Although NHRs do not appear to be part of the central clock "core," several have recently been shown to play important ancillary roles. For example, retinoid acid-related orphan receptor  $\alpha$  (ROR $\alpha$ ) is a positive transcriptional regulator of *Bmal1* expression (66). The orphan receptor Rev-erb $\alpha$  blocks this activity (67) by competing for the same DNA binding sites and acting as a repressor (68). Rev-erb $\alpha$  and ROR $\alpha$ , in turn, are regulated by the negative limb of the central loop. In the peripheral oscillators of vascular cells, retinoid nuclear receptors RXR $\alpha$  and RAR $\alpha$  negatively regulate positive limb gene expression in a ligand-dependent manner (69).

Based on their roles in metabolism, and the cyclical nature of feeding, many more NHRs are likely to exhibit circadian transcription profiles, and conversely, play important roles in the entrainment of core and peripheral clocks. In fact, it has been known for some time that the serum concentrations of many hormones oscillate with a 24-hour cycle (70–74). One example is cortisone, a glucocorticoid produced in the adrenal gland that binds to GR. As described above, GR mediates important immunosuppressive and anti-inflammatory responses (75). Other NHRs that have been shown to oscillate in expression or activity in various tissues include PPAR $\alpha$ , mineralocorticoid receptor (MR), TR, and estrogen-related receptor alpha (ERR $\alpha$ ) (76–80). It will be interesting to see what the roles of these receptors are, how many more NHRs exhibit circadian rhythmicity, and how their functions interconnect.

Melatonin, a hormone produced by the pineal gland, is known to play a major role in sleep cycles and the restorative nature

thereof. Melatonin operates reciprocally with the circadian clock mechanism and has been shown to have direct and indirect roles to counter ROS [reviewed in (81)]. Heme metabolism, which also plays a role in ROS detoxification, is also reciprocally regulated by circadian rhythms (65). Other relevant processes, such as immune system activity, also vary with the circadian clock. Thus, circadian rhythms help to couple appropriate metabolic processes with active parts of the cycle and necessary recuperative functions with the resting parts of the cycle.

In *Drosophila*, members of the Rev-erb and ROR protein families (E75 and dHR3, respectively) interact molecularly in much the same way as their mammalian counterparts (82). Furthermore, transcriptional expression of the B isoform of E75 appears to oscillate with a regular circadian rhythm (83). What makes this correlation particularly intriguing is the recent finding that the E75 ligand-binding domain, like the PAS domain of NPAS2, binds heme (84). For E75, this provides it with the ability to bind NO or CO interchangeably with equally high affinities. This ability could provide E75/Rev-erb proteins with a major role in decoding NO and CO signals (in the SCN for example) or in monitoring concentrations of systemic heme.

An ability of NHRs to monitor concentrations of heme, NO, and CO would also provide them with obvious potential roles in regulating cellular metabolism, stress, and aging. A further indication of the potential roles that Rev-erb and ROR family proteins might play in aging is that E75 and dHR3 function both upstream and downstream of ecdysone signaling (85, 86). Appropriately timed decreases in E75, dHR3, or EcR expression extend the duration of larval development (85, 87, 88). As described earlier, reduction of ecdysone concentrations or EcR activity increases life span (60). It will be interesting to see if the same is true for E75 and dHR3.

### Diseases of Aging

Diseases associated with aging, such as diabetes, Alzheimer's disease (<http://sageke.sciencemag.org/cgi/content/full/2001/1/dn2>) (AD), Parkinson's disease (<http://sageke.sciencemag.org/cgi/content/full/2001/7/dn4>) (PD), cardiac disease, and cancer, are in many cases related at some level to inappropriate or dysfunctional lipid homeostasis. Use of the PPAR $\alpha$ - and PPAR $\gamma$ -specific agonists fibrates and thiazolidinediones, respectively, have implicated these NHRs in each of these diseases (30, 89–91). These pleiotropic effects are consistent with the central roles that PPARs play in lipid and IIS homeostasis (32, 33).

In cardiovascular disease, GR, PPARs, and LXRs act as inhibitors of proinflammatory genes related to atherosclerosis (see Lee *Science* article at <http://sageke.sciencemag.org/cgi/content/abstract/2003/37/or16>). If unchecked, inflammation of the arterial walls attracts macrophages. When serum cholesterol concentrations are high, these macrophages accumulate high concentrations of lipids, turning into foam cells and eventually producing artery-clogging plaques (92). Consistent with this relationship, atherosclerotic lesions that occur in male mice deficient in the low-density lipoprotein receptor are dramatically reduced by PPAR $\gamma$  agonists (93). PPARs and LXRs may also play a role in atherosclerosis via their roles in lipid efflux, lipid metabolism, and associated decreases in obesity (32, 34, 92) (see "From Cheeseburgers to Chest Pains" at <http://sageke.sciencemag.org/cgi/content/full/2003/4/nw18>).

ROR $\alpha$  is another receptor that regulates serum cholesterol concentrations as well as suppressing the expression of athero-

genic genes regulated by NF- $\kappa$ B (94). Accordingly, *ROR $\alpha$*  mutant mice also show increased susceptibility to atherosclerosis (95). The ability of the *ROR $\alpha$*  ligand-binding domain to bind cholesterol is consistent with its role in sensing and regulating plasma cholesterol concentrations (96). The role that these receptors play in controlling inflammation in cardiovascular disease is similar to their role in rheumatoid arthritis (<http://sageke.sciencemag.org/cgi/content/full/2002/50/oa1#SEC7>), another disease of aging.

As first indicated by studies with the cholesterol-lowering statin drugs, cholesterol levels also play an important role in AD. Although the nature of this relationship is currently unclear, one hypothesis is that enzymes responsible for the synthesis of  $\beta$ -amyloid peptides, which are found in cholesterol-rich membrane "rafts," are augmented by the increased concentrations of cellular cholesterol (97). It is through their roles in cholesterol clearance that LXRs are thought to affect AD (98). Cholesterol efflux is mediated, in part, through LXR-dependent activation of the ApoE carrier protein (99). An allele of the *apoE* gene (<http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;7>) (*epsilon-4*) is currently the best molecular marker for AD (100) (see Raber Review at <http://sageke.sciencemag.org/cgi/content/full/2004/11/re2> and "Detangling Alzheimer's Disease" at <http://sageke.sciencemag.org/cgi/content/full/2003/43/oa2>). Polymorphisms in the gene encoding cholesterol-24-hydroxylase (CYP46), an enzyme that converts cholesterol to an oxysterol form, have also been correlated with AD (101). Bringing these interactions full circle, LXRs have been shown to bind and be activated by oxysterols (102) (Fig. 1).

As described earlier, a number of studies indicate that estrogen therapy and ER $\alpha$  protect against bone loss, cognitive decline, and the risk of AD, PD, and death from stroke in postmenopausal women (103). However, the benefits and risks of estrogen replacement are still being weighed.

Several other NHRs have been implicated in the onset of PD. Le *et al.* (104) found that 10 of 107 individuals with familial PD carry mutations in the NHR Nurr-1 (also known as NR4A2). RXR, the heterodimeric partner of Nurr1, and related RARs also play a potential role in PD, because they are involved in the regulation of brain plasticity and regeneration through binding of their retinoid ligands (105).

## Conclusion

As stated at the beginning of this Review, it has been suggested that the first ligand-regulated NHRs evolved based on their ability to monitor and regulate lipid homeostasis. The hormonal nature of new ligands enabled the establishment of new homeostatic processes based on, or related to, lipid metabolism. As discussed here, these include development, growth, sexual maturation, and circadian rhythm, all of which are tightly linked to aging. The current state of the literature places PPARs at the center of these homeostatic loops. Because of their wide-ranging ligand sensitivities, these NHRs will likely prove to be important therapeutic targets for many age-related diseases. The incorporation of heme, NO, and CO as key signaling molecules in most of the processes discussed, and as NHR ligands, provides further connectivity between the pieces of this complex puzzle. To complete the puzzle, ligands for the remaining orphan receptors will need to be discovered. Nearly half of the mammalian NHRs are still orphans, as are much higher percentages in other model organisms (17 out of 18 in flies, 278

out of 278 in worms). With discoveries of new ligands will come new tools to probe the function of cognate NHRs. Such studies will go a long way toward bringing the picture of aging into sharper focus.

## References

1. R. V. Weatherman, R. J. Fletterick, T. S. Scanlan, Nuclear-receptor ligands and ligand-binding domains. *Annu. Rev. Biochem.* **68**, 559–581 (1999).
2. T. M. Willson, J. T. Moore, Genomics versus orphan nuclear receptors—a half-time report. *Mol. Endocrinol.* **16**, 1135–1144 (2002).
3. A. A. Bogan, F. E. Cohen, T. S. Scanlan, Natural ligands of nuclear receptors have conserved volumes. *Nat. Struct. Biol.* **5**, 679–681 (1998).
4. G. A. Francis, E. Fayard, F. Picard, J. Auwerx, Nuclear receptors and the control of metabolism. *Annu. Rev. Physiol.* **65**, 261–311 (2003).
5. P. Barnett, H. F. Tabak, E. H. Hetta, Nuclear receptors arose from pre-existing protein modules during evolution. *Trends Biochem. Sci.* **25**, 227–228 (2000).
6. H. Escriva, S. Bertrand, V. Laudet, The evolution of the nuclear receptor superfamily. *Essays Biochem.* **40**, 11–26 (2004).
7. V. D. Longo, C. E. Finch, Evolutionary medicine: From dwarf model systems to healthy centenarians? *Science* **299**, 1342–1346 (2003).
8. L. Partridge, D. Gems, Mechanisms of ageing: Public or private? *Nat. Rev. Genet.* **3**, 165–175 (2002).
9. J. C. Jiang, E. Jaruga, M. V. Repnevskaya, S. M. Jazwinski, An intervention resembling caloric restriction prolongs life span and retards aging in yeast. *FASEB J.* **14**, 2135–2137 (2000).
10. B. P. Braeckman, K. Houthoofd, J. R. Vanfleteren, Insulin-like signaling, metabolism, stress resistance and aging in *Caenorhabditis elegans*. *Mech. Ageing Dev.* **122**, 673–693 (2001).
11. T. Chapman, L. Partridge, Female fitness in *Drosophila melanogaster*: An interaction between the effect of nutrition and of encounter rate with males. *Proc. R. Soc. London Ser. B* **263**, 755–759 (1996).
12. E. J. Masoro, Caloric restriction and aging: An update. *Exp. Gerontol.* **35**, 299–305 (2000).
13. M. Barbieri, M. Bonafe, C. Franceschi, G. Paolisso, Insulin/igf-1-signaling pathway: An evolutionarily conserved mechanism of longevity from yeast to humans. *Am. J. Physiol. Endocrinol. Metab.* **285**, E1064–E1071 (2003).
14. M. Tatar, A. Bartke, A. Antebi, The endocrine regulation of aging by insulin-like signals. *Science* **299**, 1346–1351 (2003).
15. M. C. Sugden, M. J. Holness, Potential role of peroxisome proliferator-activated receptor- $\alpha$  in the modulation of glucose-stimulated insulin secretion. *Diabetes* **53** (Suppl. 1), S71–S81 (2004).
16. R. Roduit, J. Morin, F. Masse, L. Segall, E. Roche, C. B. Newgard, F. Assi-macopoulos-Jeannet, M. Prentki, Glucose down-regulates the expression of the peroxisome proliferator-activated receptor- $\alpha$  gene in the pancreatic beta cell. *J. Biol. Chem.* **275**, 35799–35806 (2000).
17. H. R. Kast-Woelbern, S. L. Dana, R. M. Cesario, L. Sun, L. Y. de Grandpre, M. E. Brooks, D. L. Osburn, A. Reifel-Miller, K. Klausung, M. D. Leibowitz, Rosiglitazone induction of *insig-1* in white adipose tissue reveals a novel interplay of peroxisome proliferator-activated receptor  $\gamma$  and sterol regulatory element-binding protein in the regulation of adipogenesis. *J. Biol. Chem.* **279**, 23908–23915 (2004).
18. T. Yang, P. J. Espenshade, M. E. Wright, D. Yabe, Y. Gong, R. Aebbersold, J. L. Goldstein, M. S. Brown, Crucial step in cholesterol homeostasis: Sterols promote binding of scap to *insig-1*, a membrane protein that facilitates retention of srebps in er. *Cell* **110**, 489–500 (2002).
19. R. M. O'Brien, R. S. Streeper, J. E. Ayala, B. T. Stadelmaier, L. A. Hornbuckle, Insulin-regulated gene expression. *Biochem. Soc. Trans.* **29**, 552–558 (2001).
20. K. U. Birkenkamp, P. J. Coffey, Regulation of cell survival and proliferation by the foxo (forkhead box, class o) subfamily of forkhead transcription factors. *Biochem. Soc. Trans.* **31**, 292–297 (2003).
21. C. Kenyon, J. Chang, E. Gensch, A. Rudner, R. Tabtiang, A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464 (1993).
22. M. E. Giannakou, M. Goss, M. A. Junger, E. Hafen, S. J. Leivers, L. Partridge, Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* **305**, 361 (2004).
23. S. T. Henderson, T. E. Johnson, Daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr. Biol.* **11**, 1975–1980 (2001).
24. E. R. Schuur, A. V. Loktev, M. Sharma, Z. Sun, R. A. Roth, R. J. Weigel, Ligand-dependent interaction of estrogen receptor- $\alpha$  with members of the forkhead transcription factor family. *J. Biol. Chem.* **276**, 33554–33560 (2001).
25. K. Hirota, H. Daitoku, H. Matsuzaki, N. Araya, K. Yamagata, S. Asada, T. Sugaya, A. Fukamizu, Hepatocyte nuclear factor-4 is a novel downstream target of insulin via *fkhr* as a signal-regulated transcriptional inhibitor. *J. Biol. Chem.* **278**, 13056–13060 (2003).

26. H. H. Zhao, R. E. Herrera, E. Coronado-Heinsohn, M. C. Yang, J. H. Ludes-Meyers, K. J. Seybold-Tilson, Z. Nawaz, D. Yee, F. G. Barr, S. G. Diab, *et al.*, Forkhead homologue in rhabdomyosarcoma functions as a bifunctional nuclear receptor-interacting protein with both coactivator and corepressor functions. *J. Biol. Chem.* **276**, 27907–27912 (2001).
27. P. Dowell, T. C. Otto, S. Adi, M. D. Lane, Convergence of peroxisome proliferator-activated receptor gamma and foxo1 signaling pathways. *J. Biol. Chem.* **278**, 45485–45491 (2003).
28. P. Li, H. Lee, S. Guo, T. G. Unterman, G. Jenster, W. Bai, Akt-independent protection of prostate cancer cells from apoptosis mediated through complex formation between the androgen receptor and fkrh. *Mol. Cell. Biol.* **23**, 104–118 (2003).
29. M. Bauer, A. C. Hamm, M. Bonaus, A. Jacob, J. Jaekel, H. Schorle, M. J. Pankratz, J. D. Katzenberger, Starvation response in mouse liver shows strong correlation with life-span-prolonging processes. *Physiol. Genomics* **17**, 230–244 (2004).
30. R. M. Evans, G. D. Barish, Y. X. Wang, Ppars and the complex journey to obesity. *Nat. Med.* **10**, 355–361 (2004).
31. A. Chawla, J. J. Repa, R. M. Evans, D. J. Mangelsdorf, Nuclear receptors and lipid physiology: Opening the X-files. *Science* **294**, 1866–1870 (2001).
32. Y. X. Wang, C. L. Zhang, R. T. Yu, H. K. Cho, M. C. Nelson, C. R. Bayuga-Ocampo, J. Ham, H. Kang, R. M. Evans, Regulation of muscle fiber type and running endurance by ppardelta. *PLoS Biol.* **2**, E294 (2004).
33. F. S. Celi, A. R. Shuldiner, The role of peroxisome proliferator-activated receptor gamma in diabetes and obesity. *Curr. Diabetes Rep.* **2**, 179–185 (2002).
34. Y. X. Wang, C. H. Lee, S. Tiep, R. T. Yu, J. Ham, H. Kang, R. M. Evans, Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell* **113**, 159–170 (2003).
35. B. Demple, Signal transduction by nitric oxide in cellular stress responses. *Mol. Cell. Biochem.* **234–235**, 11–18 (2002).
36. R. Zamora, Y. Vodovotz, K. S. Aulak, P. K. Kim, J. M. Kane 3rd, L. Alarcon, D. J. Stuehr, T. R. Billiar, A DNA microarray study of nitric oxide-induced genes in mouse hepatocytes: Implications for hepatic heme oxygenase-1 expression in ischemia/reperfusion. *Nitric Oxide* **7**, 165–186 (2002).
37. J. Y. Ji, S. L. Diamond, Exogenous nitric oxide activates the endothelial glucocorticoid receptor. *Biochem. Biophys. Res. Commun.* **318**, 192–197 (2004).
38. S. W. Ryter, D. Morse, A. M. Choi, Carbon monoxide: To boldly go where NO has gone before. *Sci. STKE* **2004**(230), re6 (2004).
39. S. W. Ryter, L. E. Otterbein, D. Morse, A. M. Choi, Heme oxygenase/carbon monoxide signaling pathways: Regulation and functional significance. *Mol. Cell. Biochem.* **234–235**, 249–263 (2002).
40. G. Enikolopov, J. Banerji, B. Kuzin, Nitric oxide and *Drosophila* development. *Cell Death Differ.* **6**, 956–963 (1999).
41. P. J. DiGregorio, J. A. Ubersax, P. H. O'Farrell, Hypoxia and nitric oxide induce a rapid, reversible cell cycle arrest of the *Drosophila* syncytial divisions. *J. Biol. Chem.* **276**, 1930–1937 (2001).
42. R. O. Teodoro, P. H. O'Farrell, Nitric oxide-induced suspended animation promotes survival during hypoxia. *EMBO J.* **22**, 580–587 (2003).
43. J. Li, T. R. Billiar, The anti-apoptotic actions of nitric oxide in hepatocytes. *Cell Death Differ.* **6**, 952–955 (1999).
44. M. Takahashi, N. Tsuboyama-Kasaoka, T. Nakatani, M. Ishii, S. Tsutsumi, H. Aburatani, O. Ezaki, Fish oil feeding alters liver gene expressions to defend against pparalpha activation and ros production. *Am. J. Physiol. Gastrointest. Liver Physiol.* **282**, G338–G348 (2002).
45. J. D. Liu, S. H. Tsai, S. Y. Lin, Y. S. Ho, L. F. Hung, S. Pan, F. M. Ho, C. M. Lin, Y. C. Liang, Thiol antioxidant and thiol-reducing agents attenuate 15-deoxy-delta 12,14-prostaglandin j2-induced heme oxygenase-1 expression. *Life Sci.* **74**, 2451–2463 (2004).
46. P. J. Barnes, Anti-inflammatory actions of glucocorticoids: Molecular mechanisms. *Clin. Sci. (London)* **94**, 557–572 (1998).
47. M. W. Radomski, R. M. Palmer, S. Moncada, An l-arginine/nitric oxide pathway present in human platelets regulates aggregation. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5193–5197 (1990).
48. P. Delerive, K. De Bosscher, S. Besnard, W. Vanden Berghe, J. M. Peters, F. J. Gonzalez, J. C. Fruchart, A. Tedgui, G. Haegeman, B. Staels, Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors nf-kappab and ap-1. *J. Biol. Chem.* **274**, 32048–32054 (1999).
49. X. Xu, M. Otsuki, H. Saito, S. Sumitani, H. Yamamoto, N. Asanuma, H. Kouhara, S. Kasayama, Pparalpha and gr differentially down-regulate the expression of nuclear factor-kappab-responsive genes in vascular endothelial cells. *Endocrinology* **142**, 3332–3339 (2001).
50. W. Xie, M. F. Yeuh, A. Radominska-Pandya, S. P. Saini, Y. Negishi, B. S. Bottruff, G. Y. Cabrera, R. H. Tukey, R. M. Evans, Control of steroid, heme, and carcinogen metabolism by nuclear pregnane x receptor and constitutive androstane receptor. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 4150–4155 (2003).
51. M. Van Gilst, C. R. Gissendanner, A. E. Sluder, Diversity and function of orphan nuclear receptors in nematodes. *Crit. Rev. Eukaryot. Gene Expr.* **12**, 65–88 (2002).
52. J. E. Rossouw, G. L. Anderson, R. L. Prentice, A. Z. LaCroix, C. Kooperberg, M. L. Stefanick, R. D. Jackson, S. A. Beresford, B. V. Howard, K. C. Johnson *et al.*, Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. *J. Am. Med. Assoc.* **288**, 321–333 (2002).
53. J. Hays, J. K. Ockene, R. L. Brunner, J. M. Kotchen, J. E. Manson, R. E. Paterson, A. K. Aragaki, S. A. Shumaker, R. G. Brzyski, A. Z. LaCroix, *et al.*, Effects of estrogen plus progestin on health-related quality of life. *N. Engl. J. Med.* **348**, 1839–1854 (2003).
54. G. L. Anderson, M. Limacher, A. R. Assaf, T. Bassford, S. A. Beresford, H. Black, D. Bonds, R. Brunner, R. Brzyski, B. Caan *et al.*, Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: The women's health initiative randomized controlled trial. *J. Am. Med. Assoc.* **291**, 1701–1712 (2004).
55. M. A. Espeland, S. R. Rapp, S. A. Shumaker, R. Brunner, J. E. Manson, B. B. Sherwin, J. Hsia, K. L. Margolis, P. E. Hogan, R. Wallace *et al.*, Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's health initiative memory study. *J. Am. Med. Assoc.* **291**, 2959–2968 (2004).
56. S. A. Shumaker, C. Legault, L. Kuller, S. R. Rapp, L. Thal, D. S. Lane, H. Filit, M. L. Stefanick, S. L. Hendrix, C. E. Lewis *et al.*, Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's health initiative memory study. *J. Am. Med. Assoc.* **291**, 2947–2958 (2004).
57. N. J. MacLusky, Estrogen and Alzheimer's disease: The apolipoprotein connection. *Endocrinology* **145**, 3062–3064 (2004).
58. M. Tatar, C. Yin, Slow aging during insect reproductive diapause: Why butterflies, grasshoppers and flies are like worms. *Exp. Gerontol.* **36**, 723–738 (2001).
59. B. Gerisch, A. Antebi, Hormonal signals produced by daf-9/cytochrome p450 regulate *C. elegans* dauer diapause in response to environmental cues. *Development* **131**, 1765–1776 (2004).
60. A. F. Simon, C. Shih, A. Mack, S. Benzer, Steroid control of longevity in *Drosophila melanogaster*. *Science* **299**, 1407–1410 (2003).
61. J. M. Ding, D. Chen, E. T. Weber, L. E. Faiman, M. A. Rea, M. U. Gillette, Resetting the biological clock: Mediation of nocturnal circadian shifts by glutamate and NO. *Science* **266**, 1713–1717 (1994).
62. L. R. Artinian, J. M. Ding, M. U. Gillette, Carbon monoxide and nitric oxide: Interacting messengers in muscarinic signaling to the brain's circadian clock. *Exp. Neurol.* **171**, 293–300 (2001).
63. M. A. Gilles-Gonzalez, G. Gonzalez, Signal transduction by heme-containing pas-domain proteins. *J. Appl. Physiol.* **96**, 774–783 (2004).
64. E. M. Dioum, J. Rutter, J. R. Tuckerman, G. Gonzalez, M. A. Gilles-Gonzalez, S. L. McKnight, NPAS2: A gas-responsive transcription factor. *Science* **298**, 2385–2387 (2002).
65. K. Kaasik, C. C. Lee, Reciprocal regulation of haem biosynthesis and the circadian clock in mammals. *Nature* **430**, 467–471 (2004).
66. Y. Nakajima, M. Ikeda, T. Kimura, S. Honma, Y. Ohmiya, K. Honma, Bidirectional role of orphan nuclear receptor roralpha in clock gene transcriptions demonstrated by a novel reporter assay system. *FEBS Lett.* **565**, 122–126 (2004).
67. N. Preitner, F. Damiola, L. Lopez-Molina, J. Zakany, D. Duboule, U. Albrecht, U. Schibler, The orphan nuclear receptor rev-erbalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* **110**, 251–260 (2002).
68. T. K. Sato, S. Panda, L. J. Miraglia, T. M. Reyes, R. D. Rudic, P. McNamara, K. A. Naik, G. A. FitzGerald, S. A. Kay, J. B. Hogenesch, A functional genomics strategy reveals rora as a component of the mammalian circadian clock. *Neuron* **43**, 527–537 (2004).
69. P. McNamara, S. P. Seo, R. D. Rudic, A. Sehgal, D. Chakravarti, G. A. FitzGerald, Regulation of clock and mop4 by nuclear hormone receptors in the vasculature: A humoral mechanism to reset a peripheral clock. *Cell* **105**, 877–889 (2001).
70. K. Krajnak, M. L. Kashon, K. L. Rosewell, P. M. Wise, Aging alters the rhythmic expression of vasoactive intestinal polypeptide mRNA but not arginine vasopressin mRNA in the suprachiasmatic nuclei of female rats. *J. Neurosci.* **18**, 4767–4774 (1998).
71. C. Ankarberg, E. Norjavaara, Diurnal rhythm of testosterone secretion before and throughout puberty in healthy girls: Correlation with 17beta-estradiol and dehydroepiandrosterone sulfate. *J. Clin. Endocrinol. Metab.* **84**, 975–984 (1999).
72. R. Mitamura, K. Yano, N. Suzuki, Y. Ito, Y. Makita, A. Okuno, Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol secretion before the onset of female puberty in short children. *J. Clin. Endocrinol. Metab.* **85**, 1074–1080 (2000).
73. G. Morineau, A. Boudi, A. Barka, M. Gourmelen, F. Degeilh, N. Hardy, A. al-Halnak, H. Soliman, J. P. Gosling, R. Julien, *et al.*, Radioimmunoassay of cortisone in serum, urine, and saliva to assess the status of the cortisol-cortisone shuttle. *Clin. Chem.* **43**, 1397–1407 (1997).

74. T. J. Jechura, J. M. Walsh, T. M. Lee, Testicular hormones modulate circadian rhythms of the diurnal rodent, octodon degus. *Horm. Behav.* **38**, 243–249 (2000).
75. M. R. Yudt, J. A. Cidlowski, The glucocorticoid receptor: Coding a diversity of proteins and responses through a single gene. *Mol. Endocrinol.* **16**, 1719–1726 (2002).
76. T. Lemberger, B. Desvergne, W. Wahli, Peroxisome proliferator-activated receptors: A nuclear receptor signaling pathway in lipid physiology. *Annu. Rev. Cell Dev. Biol.* **12**, 335–363 (1996).
77. B. Horard, B. Rayet, G. Triqueneaux, V. Laudet, F. Delaunay, J. M. Vanacker, Expression of the orphan nuclear receptor erralpha is under circadian regulation in estrogen-responsive tissues. *J. Mol. Endocrinol.* **33**, 87–97 (2004).
78. P. Kitchener, F. Di Blasi, E. Borrelli, P. V. Piazza, Differences between brain structures in nuclear translocation and DNA binding of the glucocorticoid receptor during stress and the circadian cycle. *Eur. J. Neurosci.* **19**, 1837–1846 (2004).
79. B. Zandieh Doulabi, M. Platvoet-Ter Schiphorst, A. Kalsbeek, E. Fliers, O. Bakker, W. M. Wiersinga, Diurnal variation in rat liver thyroid hormone receptor (tr)-alpha messenger ribonucleic acid (mrna) is dependent on the biological clock in the suprachiasmatic nucleus, whereas diurnal variation of tr beta 1 mrna is modified by food intake. *Endocrinology* **145**, 1284–1289 (2004).
80. V. J. Poirrel, M. Masson-Pevet, P. Pevet, F. Gauer, Mt1 melatonin receptor mrna expression exhibits a circadian variation in the rat suprachiasmatic nuclei. *Brain Res.* **946**, 64–71 (2002).
81. R. J. Reiter, D.-X. Tan, Melatonin: Reducing intracellular hostilities. *Endocrinologist* **14**, 222–228 (2004).
82. K. P. White, P. Hurban, T. Watanabe, D. S. Hogness, Coordination of *Drosophila* metamorphosis by two ecdysone-induced nuclear receptors. *Science* **276**, 114–117 (1997).
83. G. E. Duffield, DNA microarray analyses of circadian timing: The genomic basis of biological time. *J. Neuroendocrinol.* **15**, 991–1002 (2003).
84. J. Reinking, M. Lam, K. Pardee, S. Liu, P. Yang, S. Williams, A. Yudin, A. Edwards, H. M. Krause, The nuclear receptor E75 binds heme and is regulated by nitric oxide, in preparation.
85. M. Bialecki, A. Shilton, C. Fichtenberg, W. A. Segraves, C. S. Thummel, Loss of the ecdysteroid-inducible e75a orphan nuclear receptor uncouples molting from metamorphosis in *Drosophila*. *Dev. Cell* **3**, 209–220 (2002).
86. A. A. Sullivan, C. S. Thummel, Temporal profiles of nuclear receptor gene expression reveal coordinate transcriptional responses during *Drosophila* development. *Mol. Endocrinol.* **17**, 2125–2137 (2003).
87. G. T. Lam, C. Jiang, C. S. Thummel, Coordination of larval and prepupal gene expression by the dhr3 orphan receptor during *Drosophila* metamorphosis. *Development* **124**, 1757–1769 (1997).
88. T. Li, M. Bender, A conditional rescue system reveals essential functions for the ecdysone receptor (ecr) gene during molting and metamorphosis in *Drosophila*. *Development* **127**, 2897–2905 (2000).
89. E. L. Schiffrin, Peroxisome proliferator-activated receptors and cardiovascular remodeling. *Am. J. Physiol. Heart Circ. Physiol.* **16** September 2004 [e-pub ahead of print].
90. J. Bassaganya-Riera, K. Reynolds, S. Martino-Catt, Y. Cui, L. Hennighausen, F. Gonzalez, J. Rohrer, A. U. Benninghoff, R. Hontecillas, Activation of ppar gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. *Gastroenterology* **127**, 777–791 (2004).
91. U. Kintscher, C. J. Lyon, R. E. Law, Angiotensin II, PPAR-gamma and atherosclerosis. *Front. Biosci.* **9**, 359–369 (2004).
92. A. F. Valledor, M. Ricote, Nuclear receptor signaling in macrophages. *Biochem. Pharmacol.* **67**, 201–212 (2004).
93. A. C. Li, K. K. Brown, M. J. Silvestre, T. M. Willson, W. Palinski, C. K. Glass, Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in ldl receptor-deficient mice. *J. Clin. Invest.* **106**, 523–531 (2000).
94. A. F. Valledor, M. Ricote, Nuclear receptor signaling in macrophages. *Biochem. Pharmacol.* **67**, 201–212 (2004).
95. A. C. Li, K. K. Brown, M. J. Silvestre, T. M. Willson, W. Palinski, C. K. Glass, Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in ldl receptor-deficient mice. *J. Clin. Invest.* **106**, 523–531 (2000).
96. F. Delerive, D. Monte, G. Dubois, F. Trottein, J. Fruchart-Najib, J. Mariani, J. C. Fruchart, B. Staels, The orphan nuclear receptor ror alpha is a negative regulator of the inflammatory response. *EMBO Rep.* **2**, 42–48 (2001).
97. A. Mamontova, S. Seguret-Mace, B. Esposito, C. Chaniala, M. Bouly, N. Delhaye-Bouchaud, G. Luc, B. Staels, N. Duverger, J. Mariani *et al.*, Severe atherosclerosis and hypoalphalipoproteinemia in the staggerer mouse, a mutant of the nuclear receptor roralpha. *Circulation* **98**, 2738–2743 (1998).
98. F. Boukhtouche, J. Mariani, A. Tedgui, The “cholesterol” protective pathway in the vascular system. *Arterioscler. Thromb. Vasc. Biol.* **24**, 637–643 (2004).
99. N. V. Patel, B. M. Forman, Linking lipids, Alzheimer’s and LXRs. *NURSA* **2**, ID 3.03082004.1 (<http://www.nursa.org/template.cfm?threadId=393>) (2004).
100. K. D. Whitney, M. A. Watson, J. L. Collins, W. G. Benson, T. M. Stone, M. J. Numerick, T. K. Tippin, J. G. Wilson, D. A. Winegar, S. A. Kliewer, Regulation of cholesterol homeostasis by the liver x receptors in the central nervous system. *Mol. Endocrinol.* **16**, 1378–1385 (2002).
101. B. A. Laffitte, J. J. Repa, S. B. Joseph, D. C. Wilpitz, H. R. Kast, D. J. Mangelsdorf, P. Tontonoz, Lxrs control lipid-inducible expression of the apolipoprotein e gene in macrophages and adipocytes. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 507–512 (2001).
102. E. H. Corder, A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses, J. L. Haines, M. A. Pericak-Vance, Gene dose of apolipoprotein e type 4 allele and the risk of Alzheimer’s disease in late onset families. *Science* **261**, 921–923 (1993).
103. A. Papassotiropoulos, J. R. Streffer, M. Tsolaki, S. Schmid, D. Thal, F. Nicosia, V. Iakovidou, A. Maddalena, D. Lutjohann, E. Ghebremedhin *et al.*, Increased brain beta-amyloid load, phosphorylated tau, and risk of Alzheimer disease associated with an intronic cyp46 polymorphism. *Arch. Neurol.* **60**, 29–35 (2003).
104. J. M. Lehmann, S. A. Kliewer, L. B. Moore, T. A. Smith-Oliver, B. B. Oliver, J. L. Su, S. S. Sundseth, D. A. Winegar, D. E. Blanchard, T. A. Spencer *et al.*, Activation of the nuclear receptor lxr by oxysterols defines a new hormone response pathway. *J. Biol. Chem.* **272**, 3137–3140 (1997).
105. P. S. Green, J. W. Simpkins, Neuroprotective effects of estrogens: Potential mechanisms of action. *Int. J. Dev. Neurosci.* **18**, 347–358 (2000).
106. W. D. Le, P. Xu, J. Jankovic, H. Jiang, S. H. Appel, R. G. Smith, D. K. Vassilatis, Mutations in nr4a2 associated with familial Parkinson disease. *Nat. Genet.* **33**, 85–89 (2003).
107. J. Mey, P. McCaffery, Retinoic acid signaling in the nervous system of adult vertebrates. *Neuroscientist* **10**, 409–421 (2004).
108. Y. Kamei, J. Mizukami, S. Miura, M. Suzuki, N. Takahashi, T. Kawada, T. Taniguchi, O. Ezaki, A forkhead transcription factor fkhr up-regulates lipoprotein lipase expression in skeletal muscle. *FEBS Lett.* **536**, 232–236 (2003).
109. A. Kallwellis-Opara, X. Zaho, U. Zimmermann, T. G. Unterman, R. Walther, D. Schmoll, Characterization of cis-elements mediating the stimulation of glucose-6-phosphate transporter promoter activity by glucocorticoids. *Gene* **320**, 59–66 (2003).
110. We thank T. Willson, L. Partridge, C. Thummel, and A. Edwards for taking the time to read and comment on the manuscript.