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Steroid hormone regulation of *C. elegans* and *Drosophila* aging and life history

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In the last two decades it has become clear that hormones and gene mutations in endocrine signaling pathways can exert major effects on lifespan and related life history traits in worms, flies, mice, and other organisms. While most of this research has focused on insulin/insulin-like growth factor-1 signaling, a peptide hormone pathway, recent work has shown that also lipophilic hormones play an important role in modulating lifespan and other life history traits. Here we review how steroid hormones, a particular group of lipophilic hormones, affect life history traits in the nematode worm (*Caenorhabditis elegans*) and the fruit fly (*Drosophila melanogaster*), with a particular focus on longevity. Interestingly, a comparison suggests that parallel endocrine principles might be at work in worms and flies in these species and that steroid hormones interact with the gonad to affect lifespan.

1. Introduction

Endocrine mechanisms coordinate development and physiology in response to environmental cues and internal signals, and this regulatory plasticity is critical for adjusting and maintaining survival and reproduction in the face of environmental change (Finch and Rose, 1995; Tatar and Yin, 2001; Flatt et al., 2005; Fielenbach and Antebi, 2008). While traditionally research in endocrine physiology has focused on the effects of hormones on growth, development, and physiology, increasing evidence suggests that hormones and mutations in genes involved in endocrine signaling can also have pervasive effects on whole organism life history traits, in particular longevity (Tatar et al., 2003; Tu et al., 2006; Toivonen and Partridge, 2009; Fielenbach and Antebi, 2008). One such central endocrine pathway, for example, the insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS) pathway, has been found to be a major regulator of lifespan and other life history traits in worms, flies, and mice (Tatar et al., 2003; Giannakou and Partridge, 2007; Fielenbach and Antebi, 2008). However, while most current research is focused on understanding the details whereby this evolutionarily conserved peptide hormone receptor pathway influences life history and aging, much less is known about the effects of other endocrine pathways.

Recent work in the roundworm (*Caenorhabditis elegans*) and the fruit fly (*Drosophila melanogaster*) has now begun to reveal that nuclear hormone receptors (NHRs) and their lipophilic ligands profoundly affect aging and related life history traits (Tatar et al., 2003; Flatt et al., 2005; Rottiers and Antebi, 2006; Tu et al., 2006; Fielenbach and Antebi, 2008; Toivonen and Partridge, 2009). Here we review the recent findings about how lipophilic steroid hormones and their receptors affect lifespan and related life history traits in *C. elegans* and *D. melanogaster*. Based on the available data, we argue that the steroid regulation of lifespan and associated life history traits is governed by parallel endocrine principles in worms and flies.

2. Steroid hormones as master regulators of development and physiology

Steroid hormones are small, fat soluble, cholesterol-derived molecules that can pass through the cell membrane into the cytoplasm where they activate signaling by binding to cytosolic or nuclear receptor proteins. Steroid hormone receptors are members of the large superfamily of nuclear hormone receptors (NHRs), a group of transcription factors that bind lipophilic hormones (e.g., steroids, retinoids, thyroid hormones, bile-acid like hormones, and fatty acids)

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and that regulate the transcription of many target genes (Gronemeyer and Laudet, 2001). By controlling transcription, NHRs coordinate many aspects of development, physiology, and metabolism (Chawla et al., 2001; Francis et al., 2003; King-Jones and Thummel, 2005; Flatt et al., 2006; Magner and Antebi, 2008).

NHRs are typically defined by a ligand-binding domain (LBD), which can also bind co-activators and co-repressors, and by an evolutionarily conserved N-terminal DNA-binding domain (DBD). Different genomes vary dramatically in the number of loci that encode NHRs: *Drosophila*, for example, has only 18, and humans have 48, whereas the *C. elegans* genome contains 248 NHRs (Gronemeyer and Laudet, 2001; Gissendanner et al., 2004; King-Jones and Thummel, 2005; Robinson-Rechavi et al., 2005). Although for many NHRs, especially those of vertebrates, ligands have been identified, many are so-called "orphan" receptors without known ligand. Furthermore, several NHRs can function without ligand (Mangelsdorf and Evans, 1995; Gronemeyer and Laudet, 2001).

Among the functionally best understood NHRs are the steroid hormone receptors DAF-12 (dauer formation 12) in *C. elegans* and EcR (ecdysone receptor) in *Drosophila*. Both receptors activate highly pleiotropic signaling pathways that regulate a multitude of developmental and physiological processes and that are crucial for coordinating life history in response to environmental and internal inputs.

3. DAF-12 is a key coordinator of *C. elegans* life history

C. elegans DAF-12, a homolog of the vertebrate vitamin D receptor (VDR) and the liver X receptor (LXR), plays multiple roles in regulating development and physiology in response to environmental conditions, including major effects on developmental timing, dauer formation, reproductive maturation, metabolism, and lifespan (Antebi et al., 2000; Snow and Larsen, 2000; Gerisch et al., 2001; Rottiers and Antebi, 2006; Gerisch et al., 2007). Thus, DAF-12 appears to be a hormonal master coordinator of *C. elegans* life history (Fielenbach and Antebi, 2008; Magner and Antebi, 2008).

In functional terms, DAF-12 signaling is best understood for its effects on developmental timing and reproductive maturation, especially with regard to dauer formation (Antebi et al., 2000; Gerisch et al., 2001; Rottiers and Antebi, 2006; Fielenbach and Antebi, 2008). Under benign environmental conditions *C. elegans* develops through four larval stages to a reproductive adult in 3–5 days, but in response to adverse conditions such as starvation, high temperature, or crowding worms enter an alternative third larval stage, the so-called "dauer", a non-feeding, stress-resistant diapause stage that can live up to 3–6 months. Once favorable conditions have returned, dauer larvae resume development and progress into reproductively mature adults with normal adult lifespan. Null mutations in *daf-12*, which typically affect the DBD, cause a "dauer formation-defective" (Daf-d) phenotype that bypasses the dauer state irrespective of environmental conditions, whereas lesions in the LBD typically induce a "dauer formation-constitutive" (Daf-c) phenotype that always enters the dauer state (Rottiers and Antebi, 2006; Fielenbach and Antebi, 2008). Certain dauer formation mutants of DA/DAF-12 can also bypass the larval dauer stage and become long-lived, stress-resistant adults (Rottiers and Antebi, 2006; Fielenbach and Antebi, 2008; see discussion later).

Although for a long time DAF-12 was an "orphan" NHR without known ligand, recent work has identified several DAF-12 ligands and begun to elucidate their biosynthesis and effects upon dauer formation and other phenotypes. Several genes that act upstream of DAF-12 are known to encode enzymes required for ligand production: the *C. elegans* Niemann-Pick type C1 (NPC) homologs *ncr-1* and *ncr-2* which are important for cholesterol trafficking and sterol homeostasis (Sym et al., 2000; Li et al., 2004; Motola et al., 2006); the cytochrome P450 (CYP450) gene *daf-9* related to steroidogenic hydroxylases (Gerisch et al., 2001; Jia et al., 2002; Mak and Ruvkun, 2004); *daf-36* which encodes a Rieske-like oxygenase (Rottiers et al., 2006); and *hsd-1*, a 3-β-hydroxysteroid dehydrogenase family member (Patel et al., 2008). These enzymes form part of a biosynthesis pathway that leads to the production of two 3-keto bile-acid like steroids, Δ-4 and Δ-7 dafachronic acid (DA), that specifically bind and activate DAF-12 (Motola et al., 2006). Furthermore, a related bile-acid, 25S-cholestenoic acid (CA), has also been found to bind DAF-12 (Held et al., 2006). Supplementation with DA rescues dauer and other phenotypes of *ncr-1*, *ncr-2*, *daf-9*, and *daf-36* mutants, and application of CA rescues *daf-9* mutants (Held et al., 2006; Motola et al., 2006; Rottiers et al., 2006; Gerisch et al., 2007; Sharma et al., 2009). A common vertebrate steroid, pregnenolone, might also act as a DAF-9 produced ligand of DAF-12 (Broué et al., 2007), but since pregnenolone cannot activate DAF-12 *in vitro* this molecule might only represent a ligand precursor (Motola et al., 2006).

DA/DAF-12 signaling interacts with several other endocrine pathways known to affect dauer formation such as cGMP, TGF-β, and IIS (Fielenbach and Antebi, 2008). Epistasis analyses position DAF-12 signaling downstream of cGMP, TGF-β, and IIS, with *daf-12* null mutations suppressing Daf-c mutant phenotypes in these pathways (Riddle et al., 1981; Vowels and Thomas, 1992; Thomas et al., 1993). For example, insulin-like peptides (ILPs) that bind to the insulin-like receptor DAF-2 are thought to be required for DAF-9/DAF-36 activity and DA production (Rottiers and Antebi, 2006; Fielenbach and Antebi, 2008), and epistasis experiments place DAF-16/FOXO, a forkhead transcription factor downstream of IIS, upstream of DAF-9 and DAF-12 (Gerisch et al., 2001; Jia et al., 2002). Conversely, DA, HSD-1, DAF-9 and DAF-12 can exert feedback effects on DAF-16/FOXO activity (Matyash et al., 2004; Gerisch et al., 2007; Dumas et al., 2010), and DAF-12 and DAF-16/FOXO interact in co-immunoprecipitation assays (Dowell et al., 2003). In addition to its interactions with DAF-16/FOXO, DAF-12 also interacts with DAF-2 (Gems et al., 1998; Larsen et al., 1995). The DA/DAF-12 and IIS/DAF-2/DAF-16 pathways thus intersect in multiple and complex ways (Gems et al., 1998; Fielenbach and Antebi, 2008).

The data at hand suggest a unifying model of how steroid hormones regulate *C. elegans* life history: under favorable conditions, activation of IIS and TGF-β pathways stimulates the production of DA (and possibly other ligands) and liganded DAF-12 promotes reproductive growth and adult development, whereas unliganded DAF-12 induces a program that promotes dauer development, survival, and stress resistance (Rottiers and Antebi, 2006; Fielenbach and Antebi, 2008). Interestingly, as we will discuss next, insects such as *Drosophila* coordinate development, physiology, and life history by employing a steroid hormone pathway that is similar to DAF-12/DA signaling.

4. EcR signaling orchestrates *Drosophila* development and physiology

In the fruit fly and other insects, the ecdysone receptor (EcR), a homolog of the vertebrate farnesoid X receptor (FXR) and liver X receptor (LXR), mediates signaling by steroid hormones called ecdysteroids. Like DAF-12, EcR signaling coordinates development and physiology by eliciting various cell autonomous and non-autonomous transcriptional responses in many target tissues (Riddiford, 1993; Riddiford et al., 2000; Kozlova and Thummel, 2000; Thummel, 2001a; King-Jones and Thummel, 2005; Spindler et al., 2009).

The hormonally active ligand of EcR, 20-hydroxy-ecdysone (20E), is produced from a cholesterol precursor derived from dietary yeast ergosterol or plant sterols; thus, like *C. elegans*, *Drosophila* is a cholesterol auxotroph (Gilbert et al., 2002; Kurzchalia and Ward, 2003). Ecdysteroids are produced in response to prothoracicotropic hormone (PPTH) and nutritional inputs integrated and relayed by IIS (McBrayer et al., 2007; Colombani et al., 2005). The first step is the conversion of cholesterol into 7-dehydro-cholesterol (7-DHC) by a Rieske-like oxygenase encoded by *neverland* (*nvd*; Yoshiyama et al.,

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2006) in steroidogenic tissues (larval prothoracic gland, adult gonad), and 7-DHC is subsequently transformed by cytochrome P450 enzymes (e.g., encoded by "Halloween" genes such as *phantom*, *disembodied*, *shadow*) into several intermediate products. The final step is the conversion of the prehormone ecdysone (E) into 20E by an intracellular mono-oxygenase encoded by *shade* in peripheral tissues such as epidermis, gut, fat body (equivalent to mammalian liver and adipose tissue), and Malphigian tubules (equivalent to the kidney) (Gilbert et al., 2002; Petryk et al., 2003; Gilbert and Warren, 2005). At the target tissues, 20E activates signaling by binding to a heterodimer that consists of EcR and another NHR, ultraspiracle (USP), a homolog of vertebrate retinoid X receptor (RXR) (Koelle et al., 1991; Yao et al., 1993; King-Jones and Thummel, 2005), with well-known downstream signaling effects (Thummel, 1996; Kozlova and Thummel, 2000). However, other signaling modes of 20E that do not involve EcR, for example non-genomic actions, or signaling via heterodimers consisting of *Drosophila* hormone receptor 38 (DHR38) and USP, have been reported as well (Baker et al., 2003; Srivastava et al., 2005).

Pulses of 20E orchestrate the development from the embryo through the three larval instars into the reproductively mature adult, in particular developmental timing and metamorphosis (Riddiford, 1993; Thummel, 1996; Kozlova and Thummel, 2000; Truman, 2005). In brief, 20E regulates a wide range of developmental and cellular processes such as cell polarity (Romani et al., 2009), cell cycle (Fallon and Gerenday, 2010), and cell migration (Bai et al., 2000), as well as cell and tissue proliferation, differentiation, histolysis, and apoptosis (Thummel, 1996, 2001b). In the adult, 20E affects, among other things, courtship behavior (Ishimoto et al., 2009), vitellogenesis and egg production (Bownes, 1982; Richard et al., 1998, 2001; Buszczak et al., 1999; Carney and Bender, 2000), adult reproductive diapause (Richard et al., 2001), innate immunity (Flatt et al., 2008b), as well as stress resistance and lifespan (discussed later).

The similar nature and range of developmental, physiological, and whole organism life history traits influenced by 20E/EcR and DA/DAF-12 suggests that there are striking parallels between the steroid hormone pathways of worms and flies. It is thus tempting to speculate that 20E/EcR and DA/DAF-12 might in fact represent functionally homologous endocrine systems.

5. Parallels between DA/DAF-12 and 20E/EcR signaling

Several important facts lend support to the notion that DA/DAF-12 and 20E/EcR signaling are functionally equivalent (see Fig. 1). Perhaps most importantly, as we have pointed out previously, 20E/EcR and DA/DAF-12 share many similar regulatory functions in development, physiology, and life history, in particular in terms of developmental rate and timing, reproductive maturation, and diapause-like survival states. For example, the endocrine regulation of the stress-resistant, long-lived larval dauer stage in *C. elegans* has been likened to that of adult reproductive diapause (dormancy) in *Drosophila* and other insects, a non-reproductive, stress-resistant and long-lived alternative phenotype that is elicited by low temperature and shortened photoperiod (Tatar and Yin, 2001).

Consistent with the view that 20E/EcR and DA/DAF-12 represent homologous pathways, the LBD of DAF-12 has 34% sequence similarity to that of EcR (Antebi et al., 2000). The DAF-12 LBD is also closely related to that of *Drosophila* hormone receptor 96 (DHR96; 40% similarity), a NHR that binds cholesterol and regulates cholesterol homeostasis and xenobiotic metabolism (King-Jones et al., 2006; Horner et al., 2009); however, since DAF-12 and EcR share many biological functions, EcR seems to be a more promising candidate for the DAF-12 ortholog. Parallels between DA/DAF-12 and 20E/EcR also apply to ligand production. While NCR-1 and NCR-2 are required for DA production in the worm, the *Drosophila* homolog of NCR-1 (NPC-1) is necessary for 20E synthesis, and *npc*-1 mutants can be rescued by 20E application (Huang et al., 2005, 2007; Fluegel et al., 2006). Similarly, the Rieske-like oxygenase NVD required for 20E production in *Drosophila* represents the homolog of DAF-36 required for DA synthesis in *C. elegans* (Yoshiyama et al., 2006). Thus, there exist fundamental homologies between DA/DAF-12 and 20E/EcR signaling, both in terms of hormone biosynthesis and receptor-mediated signaling.

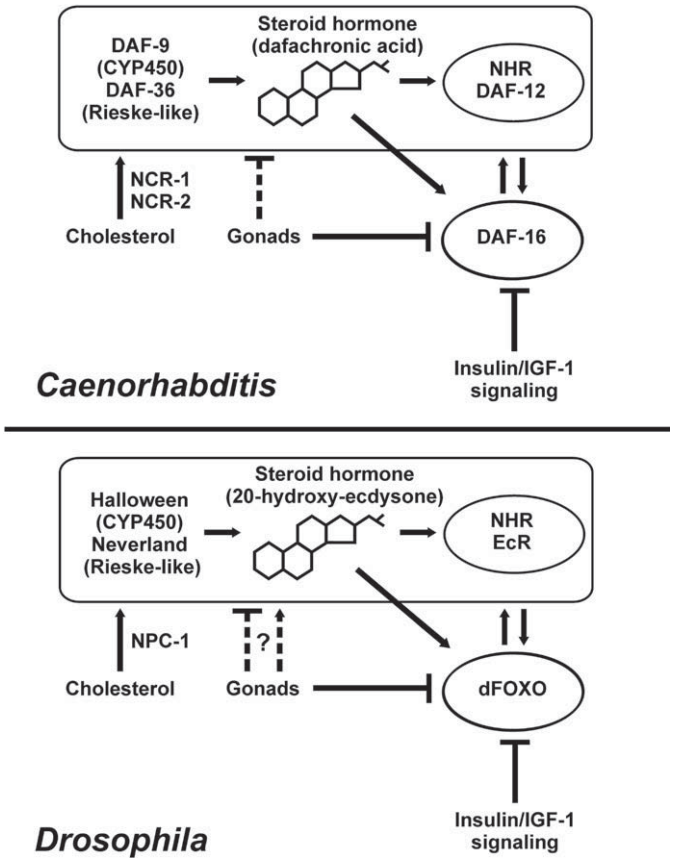

Fig. 1. Models of functional endocrine parallels in the steroid hormone regulation of life history traits, especially lifespan, between (A) *C. elegans* and (B) *D. melanogaster*. See text for details.

A comparison of worms and flies further reveals similar regulatory wiring of interactions between steroid hormone signaling and IIS. In the nematode, DAF-9/DAF-36 activity and DA production require IIS, and in *Drosophila* 20E production depends upon IIS as well (Tu et al., 2002; Colombani et al., 2005; Walkiewicz and Stern, 2009). For example, mutants of the *Drosophila* insulin receptor (dInR) exhibit decreased ecdysteroid levels (Tu et al., 2002). Similarly, decreased IIS in the prothoracic gland of the fly reduces expression of the Halloween genes *disembodied* and *phantom* and decreases 20E production, whereas upregulation of IIS has the opposite effect (Colombani et al., 2005). IIS is therefore required for steroid hormone production in worms and flies, as is also observed in other species, such as mosquitoes (Riehle and Brown, 1999), blowflies (Maniere et al., 2004) and mammals (L´Allemand et al., 1996). Interestingly, steroid hormones can also have feedback effects upon IIS. For example, DA/DAF-12 modulates the activity of DAF-16/FOXO in the nematode, and 20E/EcR regulates the activity of the fly homolog of DAF-16, dFOXO (Colombani et al., 2005). The recent observations in *Drosophila* by Colombani et al. (2005) are particularly interesting in view of the well-known genetic interactions between DAF-12 and IIS/DAF-2/DAF-16 in *C. elegans* previously mentioned further.

Taken together, the current evidence suggests that DA/DAF-12 and 20E/EcR are homologous endocrine systems. These parallels also appear to extend to the regulation of lifespan (Fig. 1).

6. Similarities in the steroid hormone regulation of lifespan between worms and flies

In addition to the well-known effects on the longevity of IIS and pathways, recent experiments in *C. elegans* and *D. melanogaster* have also identified several steroid and other lipophilic hormones as important regulators of longevity, including DA in worms and 20E in flies (Tatar et al., 2003; Flatt et al., 2005; Flatt and Kawecki, 2007; Rottiers and Antebi, 2006; Tu et al., 2006; Russell and Kahn, 2007; Fielenbach and Antebi, 2008; Toivonen and Partridge, 2009).

In both worms and flies steroid hormone deficiency seems to extend lifespan. In *C. elegans*, strong *daf-9* mutations extend adult lifespan and increase stress resistance, and this effect depends on DAF-12 and the DAF-12 co-repressor DIN-1 (Gerisch et al., 2001; Jia et al., 2002; Ludewig et al., 2004). In contrast, the hypomorphic mutant alleles of *daf-9 and daf-36* have no or only little effect upon longevity (Gerisch et al., 2001; Jia et al., 2002; Rottiers et al., 2006). Because DAF-9 is required for DA synthesis, DA deficiency might promote longevity through the unliganded DAF-12 receptor. Consistent with this model, DA supplementation suppresses the lifespan extension of DA-deficient *daf-9* mutants (Gerisch et al., 2007). As in the nematode, steroid hormone deficiency also extends lifespan in *Drosophila*. Temperature-sensitive female mutants of *DTS-3*, a gene encoding a protein with Krüppel Zn-finger domains but whose molecular identity remains unknown, have a 50% reduction in ecdysteroid titers, are infertile, and long-lived (Walker et al., 1987; Simon et al., 2003). Like the effects of DA supplementation in *daf-9* mutants, 20E supplementation restores lifespan of 20E deficient *DTS-3* mutants to wild-type levels (Simon et al., 2003). Similarly, 20E deficient mutants of *dare*, a gene encoding an adrenoxin reductase required for 20E biosynthesis (Freeman et al., 1999), are long-lived (Simon and Krantz, 2005). Thus, in both worms and flies DA and 20E promote aging, whereas hormone deficiency promotes longevity.

Striking parallels between *C. elegans* and *Drosophila* are also found when we compare the longevity effects of the steroid hormone receptors. Certain mutants of *daf-12* can affect lifespan, but the magnitude and direction of the effects depend on the mutant allele and on sex (Fisher and Lithgow, 2006; McCulloch and Gems, 2007). *daf-12* (*rh61rh411*), a putative Daf-d null allele with nonsense mutations affecting both the DBD and LBD, shortens lifespan, but *daf-12* (*rh273*), a Daf-c mutation thought to cause ligand insensitivity, extends lifespan (Fisher and Lithgow, 2006). Sex-specific effects of DAF-12 are seen with the Daf-d mutant allele *daf-12* (*m20*) that weakly extends lifespan in males, but shortens it in hermaphrodites (McCulloch and Gems, 2007). DAF-12 thus exhibits "janus"- or "chimera"-like behavior with regard to its effects on aging (Fisher and Lithgow, 2006; Rottiers and Antebi, 2006). A similar situation might apply to the effects of EcR on lifespan in *Drosophila*. Whereas several constitutive mutations in the EcR LBD and DBD have been reported to cause lifespan extension in both females and males (Simon et al., 2003), a recent study has found that mild adult-specific inactivation of EcR by RNAi increases lifespan in males, but decreases it in females (Tricoire et al., 2009). Like DAF-12 signaling, EcR might thus have context-dependent effects on aging.

The complex interactions between DA/DAF-12 and IIS provide another illustration of such "chimera"-like behavior of steroid hormone signaling in longevity. For example, while *daf-12* null mutants synergistically increase the longevity of strong (class II) *daf-2* (insulin-like receptor) mutants, lifespan extension of weak *daf-2* mutants are suppressed by class III *daf-12* mutants (Larsen et al., 1995; Gems et al., 1998). Furthermore, the increased longevity phenotype of *daf-2* mutants, but not that of *daf-9* mutants, requires DAF-16/FOXO and is fully suppressed by mutations in *daf-16/foxo*, suggesting that DAF-9 and DAF-16/FOXO function independently to affect longevity (Gerisch et al., 2001; Jia et al., 2002). However, although DA treatment is unable to suppress longevity in *daf-2* mutants, it can in fact enhance the longevity of *daf-2* (*e1368*) mutants (Gerisch et al., 2007). In some contexts, therefore, DA/DAF-12 might act to maximize IIS/DAF-2/DAF-16 dependent longevity (Gerisch et al., 2007). Whether interactions between EcR and IIS/dInR/dFOXO also affect *Drosophila* lifespan is currently unknown, but the fact that 20E is able to modulate dFOXO activity in larval flies (Colombani et al., 2005) makes this an attractive possibility.

The mechanisms underlying the janus-like behavior of DAF-12 and EcR signaling with regard to lifespan are currently unknown and await future study. For instance, it has been suggested that this signaling behavior might be due to the fact that DAF-12 can switch between an activator and repressor state that depends on the availability of co-repressors or co-activators (Rottiers and Antebi, 2006). In *Drosophila*, EcR signaling is similarly modulated by co-activators (taiman; Bai et al., 2000) or co-repressors (SMRTER or Alien; Tsai et al., 1999; Dressel et al., 1999), and it would be interesting to examine the effects of such co-factors on lifespan regulation. Moreover, both DAF-12 and EcR have several receptor isoforms (Bender et al., 1997; Antebi et al., 2000; Mouillet et al., 2001), and it is possible that distinct isoforms might have different effects on lifespan (Snow and Larsen, 2000).

Clearly, in both *C. elegans* and *Drosophila* steroid hormones have comparable effects on lifespan, thereby further supporting the notion that DA/DAF-12 and 20E/EcR signaling are equivalent (Fig. 1). Moreover, in *C. elegans* – and possibly also in *Drosophila* – steroid hormone signaling interacts with the gonad to regulate lifespan, as we discuss below (Fig. 1).

7. In worms and flies the longevity effects of steroid hormones depend on the gonad

In the nematode, ablation of germline precursor cells extends lifespan by about 60%, while simultaneous ablation of the somatic gonad abolishes this effect, suggesting that the germline and somatic gonad produce signals with opposing effects on longevity (Hsin and Kenyon, 1999; Yamawaki et al., 2008). The lifespan extending effect of germline ablation requires the activity of several genes involved in IIS and DA/DAF-12 signaling (Fielenbach and Antebi, 2008). In the context of IIS, germline ablation is unable to extend lifespan in mutants of *daf-16/Foxo* (Hsin and Kenyon, 1999). Remarkably, in the case of strong *daf-2* mutants the somatic gonad is dispensable for germline ablation to extend lifespan by activating DAF-16/FOXO (Yamawaki et al., 2008). This suggests that the somatic gonad normally provides factors that suppress IIS below a critical threshold, but that these factors are no longer necessary for germline ablation to extend lifespan when the levels of IIS are sufficiently low (Yamawaki et al., 2008). With regard to DA/DAF-12 signaling, HSD-1 is dispensable for lifespan extension caused by germline ablation (Dumas et al., 2010), but germline ablation is unable to extend lifespan in *daf-12* mutants (Hsin and Kenyon, 1999; Motola et al., 2006; Gerisch et al., 2007). Similarly, longevity of germline-ablated worms is also abrogated in mutants of *daf-9* and *daf-36*, suggesting that DA/DAF-12 is required for germline ablation to promote longevity (e.g., Gerisch et al., 2001, 2007; Gerisch and Antebi, 2004; Rottiers et al., 2006). While germline ablation cannot extend lifespan in these DA-deficient mutants, longevity can be restored by application of DA (Gerisch et al., 2007). In contrast, the lifespan of *daf-12* mutants and germline-ablated *daf-12* mutants is not affected by DA, suggesting strict dependence of the ligand on DAF-12 function (Gerisch et al., 2007). Interestingly, both germline ablation and DA supplementation activate DAF-16/FOXO (Gerisch et al., 2007), suggesting regulatory crosstalk between DA/DAF-12 and IIS/DAF-16 (also see previous discussion, and Section 3). Interestingly, germline ablated worms have elevated pregnenolone, and application of pregnenolone to germline ablated *daf-9* mutants can restore their longevity (Broué et al., 2007); however, it remains unclear whether

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pregnenolone represents a bona fide ligand of DAF-12 (Motola et al., 2006).

In the fly, we do not currently know whether germline ablation and steroid hormone signaling converge to affect lifespan, yet several lines of evidence suggest that the gonad might interact with 20E/EcR signaling to affect fly lifespan. This possibility is particularly intriguing since EcR is not only expressed in the ovary in both germline and somatic cells and required for oogenesis (Buszczak et al., 1999; Carney and Bender, 2000), but also because the gonad is the major site of ecdysteroid production (Gilbert et al., 2002). Similar to *C. elegans*, we have recently found that ablation of germline stem cell can extend lifespan and modulate IIS/dFOXO in *Drosophila*, suggesting that the gonadal regulation of aging might be evolutionarily conserved (Flatt et al., 2008a). Germline-less, long-lived flies also exhibit increased expression of the secreted fly insulin/IGF-binding protein IMP-L2 (Flatt et al., 2008a), and IMP-L2 is an IIS antagonist (Honegger et al., 2008) known to be induced by 20E (Natzle et al., 1986; Osterbur et al., 1988; Andres et al., 1993). It is therefore an interesting possibility that in flies germline ablation extends lifespan by interacting with IIS and 20E/EcR signaling, as is the case in the nematode. Direct evidence for an interaction between EcR signaling and the gonad in modulating lifespan comes from a recent study by Tricoire et al. (2009): the authors found that EcR inactivation decreases female lifespan, but this effect is rescued in sterile female mutants of ovo^{D1}, a mutation that causes developmental arrest of egg chambers prior to or at stage 4 (Oliver et al., 1987). Although it remains unclear whether ovo^{D1} mutants have defective germline stem cell proliferation (cf. Flatt et al., 2008a), or whether they are 20E deficient (the ovary is the major 20E producing tissue in the female fly), this important result shows that 20E/EcR signaling can interact with the gonad to modulate lifespan. Another interesting observation is that sterile, long-lived mutants of dInR exhibit ovarian ecdysteroid deficiency – and 20E deficiency might thus contribute to lifespan extension upon reduced IIS (Tu et al., 2002). Finally, adult *Drosophila* females undergoing reproductive diapause *Drosophila* exhibit ovarian (vitellogenic) arrest, increased stress resistance, and greatly improved adult survival (Tatar and Yin, 2001; Schmidt and Paaby, 2008), and diapause is associated with reduced ovarian ecdysteroid levels, a condition that can be rescued by 20E application (Richard et al., 1998, 2001). In addition, natural variation in diapause incidence has been mapped to the *couch potato* locus, a gene encoding an ecdysteroid-responsive RNA binding protein that is expressed in the peripheral nervous system and the ring gland, a composite larval endocrine organ that contains the ecdysteroidogenic prothoracic gland (Schmidt et al., 2008; P. S. Schmidt, personal communication). Thus, although the evidence from *Drosophila* discussed above is circumstantial, the data suggest intricate connections among the gonad, 20E/EcR, IIS, and longevity that deserve further investigation (Fig. 1).

8. Conclusions

The recent discoveries in the nematode and the fruit fly reviewed previously clearly suggest that DA/DAF-12 and 20E/EcR represent functionally equivalent steroid hormone pathways with similar effects on life history, in particular on longevity. Intriguingly, in both species the gonad appears to interact with steroid hormone signaling to modulate lifespan. Together with the observation that transplanted ovaries from young donor mice can increase the life expectancy of old recipient mice (Cargill et al., 2003; Mason et al., 2009), these findings suggest that the gonad regulation of lifespan might be evolutionarily conserved. By virtue of functional and sequence homologies in several aspects of endocrine regulation among species we speculate that the mechanisms described in worms and flies might be evolutionarily conserved and thus also play a role in mammals.

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