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Short communication

The steroid hormone receptor EcR finely modulates Drosophila lifespan during adulthood in a sex-specific manner

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ABSTRACT

The steroid hormone ecdysone influences Drosophila lifespan. Longevity is extended in mutants deficient for ecdysone synthesis or mutants of the ecdysone receptor (EcR). However, the underlying mechanisms remain unclear. Here we conditionally inactivated EcR by RNA interference or expression of dominant negative forms, using the RU486 inducible system. A mild ubiquitous inactivation of EcR during adulthood was sufficient to slow the aging of male flies, whereas a stronger EcR inactivation decreased longevity. Surprisingly, ubiquitous inactivation of EcR strongly decreased female lifespan. This deleterious effect was suppressed in sterile ovo^{D1} mutant females, suggesting that EcR represses a negative signal for lifespan produced in ovaries. These results reveal a complex adult and sex-specific control of lifespan by steroid signalling in Drosophila.

Hormonal signals influence lifespan in many species as best illustrated by the widely conserved insulin/IGF-1 signalling pathway (IIS) (Tatar et al., 2003; Kleemann and Murphy, 2009; Toivonen and Partridge, 2009). Steroid/steroid-like hormones have also been identified as lifespan modulators. In *Caenorhabditis elegans*, bile acid-like steroids called dafachronic acids activate DAF-12, a nuclear receptor that regulates dauer diapause, reproductive development and lifespan (Antebi et al., 1998, 2000; Gerisch et al., 2001; Held et al., 2006; Motola et al., 2006). In *Drosophila melanogaster*, ecdysteroids are the only class of steroid hormones. Ecdysone is the precursor of 20-hydroxyecdysone (20E), required during developmental transitions and metamorphosis (King-Jones and Thummel, 2005). 20E binds to a heterodimer complex of two nuclear receptors, EcR and ultraspiracle (USP), which are orthologues of the vertebrate farnesoid X receptor (FXR) or liver X receptor (LXR), and RXR receptors, respectively. Ecdysone signalling regulates lifespan in Drosophila (Simon et al., 2003). Indeed, lifespan is extended in both males and females heterozygous for mutations in EcR. Moreover, ecdysteroid deficient *DTS-3* mutant females are long-lived. Little is known however about the roles of ecdysone signalling in adults. In female, ecdysteroids seem to be mainly produced in the ovary and are essential for oogenesis (Riddiford, 1993; Buszczak et al., 1999; Carney and Bender, 2000). In males, ecdysteroids are required for spermatogenesis and regulate the courtship reproductive behaviour (Wismar et al., 2000; Ganter et al., 2007) and their site of production is unknown. In this study, we further investigated the mechanisms involved in steroid control of lifespan in Drosophila.

We used the RU486 (RU) inducible-GeneSwitch system (Osterwalder et al., 2001) to inactivate EcR. The GeneSwitch protein (GS) is a GAL4 modified protein that recognizes and activates UAS-dependent transgenes only in the presence of RU added into Drosophila food. In the following, RU *X* denotes the concentration of $X\,\mu g\,ml^{-1}$ in the food medium. First, we generated a new ubiquitously expressed GeneSwitch driver, da-GS. This driver exhibits a broad RU-dependent expression pattern of *lacZ* and *GFP* reporter genes (Supplementary Figure 1). In particular, expression was detected in all tissues known to influence lifespan in Drosophila and other species, including gut, fat body, muscles, brain and gonads. Quantitative analysis was performed by measuring luciferase activity of *da-GS/UAS-luciferase* flies. Expression level is highly correlated to the RU concentration in the media (Supplementary Figure 2). Expression is stable throughout age in females (30 days versus 10 days), and increases

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with age in males. Expression is thus 8.5-fold higher in 10-day-old females than in males, but only threefold higher in 30-day-old flies. We also checked that *da-GS*/+ fly lifespan is not affected by RU treatment (Supplementary Figure 3). The da-GS driver was used to drive expression of a UAS-dependent RNA interference construct targeting all EcR isoforms (UAS-EcR-RNAi) (Colombani et al., 2005). Reduction of EcR levels was observed on Western-blots for both males and females (Supplementary Figure 4). Compared to control flies (RU 0), male flies exhibit a decrease in EcR level of about twofold under RU 50 treatment and more than fourfold under RU 200 treatment. The decrease in females ranged from 1.25-fold (RU 50) to fivefold (RU 200).

We first analyzed the lifespan of male flies in which EcR was ubiquitously inactivated only during adulthood by RNA interference with the da-GS driver (Fig. 1A). Complete data relative to longevity experiments are presented in Supplementary Tables S1 and S2. A twofold decrease in EcR levels (RU 50 compared with RU 0) led to a strong longevity increase. The median and maximum lifespan were increased respectively by 26% and 45%. A greater decrease in EcR expression levels (RU 100 condition) had no effect on the median and only increased the maximum lifespan by 17%. Moreover, a further reduction of EcR levels (RU 200 condition) had significant deleterious effects on longevity with the median lifespan 14% lower than that of the control. To confirm this phenotypic behaviour, we used UAS-dependent dominant negative (DN) forms of EcR (UAS-EcR-F645A and UAS-EcR-W650A, referred collectively as UAS-EcR-DN) (Cherbas et al., 2003). EcR-F645A binds ligand while EcR-W650A does not. Both mutants dimerize with USP, bind DNA, but fail to activate target gene expression and hence compete with endogenous EcR. Since the phenotype of EcR depleted flies is strongly dependent on EcR level, we first compared these two EcR-DN transgenes with the UAS-EcR-RNAi construct for their ability to induce developmental lethality when driven by da-GS at different RU concentrations (Supplementary Figure S5). The minimal RU concentration associated with absence of adult escapers is at least 10-fold lower for dominant negative constructs than the one required for RNAi inactivation. This incited us to test low RU concentration to express DN forms

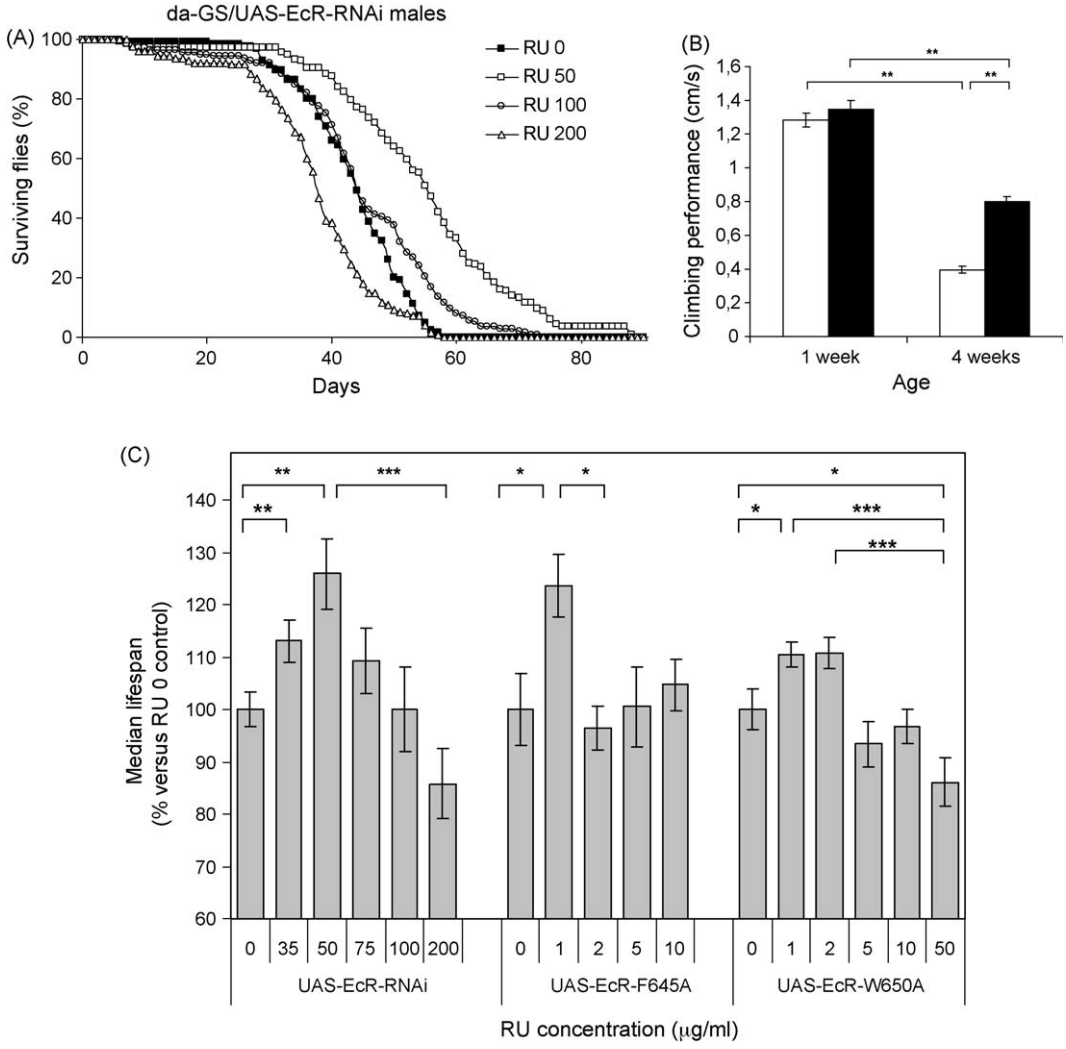

Fig. 1. Mild ubiquitous EcR inactivation during adulthood increases male lifespan and locomotor activity. (A) Survival experiments on *da-GS/UAS-EcR-RNAi* male flies treated with increasing amounts of RU (50, 100, 200 μg of RU per ml of food). All logrank test p values are based on comparisons between treated and untreated flies. The mean lifespan values are as follows: RU 0, 42 days ($n = 124$); RU 50, 54 days ($n = 136$) $p < 0.0001$; RU 100, 44 days ($n = 138$) $p = 0.003$; RU 200, 36 days ($n = 128$) $p < 0.001$. (B) Measurement of the locomotor activity of flies by rapid iterative negative geotaxis (RING) assays. Climbing performance (±SEM) of 1-week- and 4-week-old *da-GS/UAS-EcR-RNAi* male flies treated (black box) or not (white box) with RU 50. For both treated and not treated flies significant ($^{**}p < 0.0001$) decrease in climbing ability are observed for aged flies compared to young ones. A significant rescue of this phenotypic decline is observed for RU treated flies at 4 weeks. (C) Median lifespan of *da-GS/UAS-EcR-RNAi*, *da-GS/UAS-EcR-F645A*, and *da-GS/UAS-EcR-W650A* male flies treated with increasing amounts of RU. All values are percentages based on RU 0 control (±SEM). Significant statistical differences between different conditions of RU treatment: $^{*}p < 0.03$, $^{**}p < 0.01$, $^{***}p < 0.005$.

during adulthood in the following experiments. Expression of EcR-F645A and EcR-W650A under the RU 1 condition increased median lifespan of 24% and 10%, respectively (Fig. 1C and Supplementary Figure S6). Higher expression abolished this positive effect and even leads to deleterious effect for EcR-W650A (14% decrease of median lifespan under the RU 50 condition). Thus, by three independent means, we observed a bell-shaped lifespan response to levels of EcR activity. We further examined males under the RU 50 condition of RNAi inactivation. Their fertility and body weight were not affected (data not shown) but their locomotor activity was significantly improved (Fig. 1B). Indeed, 4-week-old flies exhibited an ability to climb that was more than twofold greater than that of untreated flies. No statistically significant differences were observed between 1-week-old flies and controls. So, a mild EcR inactivation during adulthood strongly delays the age-related decline in the climbing performance of male flies.

Our findings provide further insights into data reported by Simon et al., who implicated EcR in an extension of longevity and an improvement in the locomotor activity of old flies (Simon et al., 2003, 2006). Here, populations (RU-treated or untreated flies) were from the same crosses, thus definitely excluding an effect of the genetic background. Moreover, our findings showed that only a twofold reduction of EcR increased lifespan, a condition that potentially mimics the level of active EcR in heterozygous EcR mutant flies. However, EcR is required during adulthood, as a stronger reduction of EcR has deleterious effects. Furthermore, a developmental reduction of EcR is not necessary to modulate male adult lifespan.

We then investigated which adult tissues require EcR for lifespan modulation. We first inactivated EcR in the central nervous system (CNS) using the elav-GS line (Roman et al., 2001; Latouche et al., 2007). We could not detect any significant effect on lifespan under this condition (Supplementary Table S1). Then we turned to the S_1106 GeneSwitch line, which is mainly expressed in the adult fat body, a critical tissue for insulin signalling control of lifespan (Roman et al., 2001; Giannakou et al., 2004; Hwangbo et al., 2004), but also around testes and in the digestive system (Poirier et al., 2008). EcR inactivation in *S_1106/UAS-EcR-RNAi* male flies increased their mean and median lifespan over the full range of RU concentrations tested. Indeed, the mean lifespan of males was up to 25% greater than controls, under the RU 100 condition ($p < 0.01$) (Supplementary Table 1 and Figure S7). However, expression of both dominant negative constructs did not increase lifespan under the conditions tested here (RU1 and RU 5). Discrepancy between these results may arise from inadequate range of RU treatments for the EcR-DN expression in this tissue. Alternatively, mechanisms dependent of EcR protein levels but not mimicked by the DN competing isoforms may be involved in the targeted tissues. In particular, EcR-DN has been shown to compete with endogenous EcR for transcriptional activation, but it is unclear if they also interfere with EcR-mediated transcriptional repression. Thus additional experiments will be required in the future to definitely conclude about how EcR inactivation affects lifespan in the S_1106 targeted tissues.

We then analyzed lifespan of female flies in which EcR was ubiquitously inactivated. Surprisingly, we observed a strong deleterious effect of EcR inactivation in females. Expression of the dominant negative constructs decreased median lifespan by more than 80% under the RU 10 condition (Fig. 2A, B and D). Moreover, using the RNA interference construct, female median lifespan was 43% lower under the RU 50 condition (Fig. 2C). We tested a wide range of RU concentrations (RU 1 to RU 75 for the RNAi construct), and all led to a lower mean lifespan than RU 0 (even though this effect was not statistically significant for the lowest concentrations). We then investigated whether this effect was reversible. Thus, flies expressing the RNAi construct were exposed to RU 50 until they were 30 days old, and then exposed to RU 0 media. Immediately after the switch, the survival curves of these females diverged from those of continuously induced flies (Fig. 2C). This indicates that restoring normal EcR levels at an advanced age improves the death rate.

Our results are in apparent contradiction with previous data showing that females heterozygous for EcR mutations have a longer lifespan than their wild-type counterparts (Simon et al., 2003). Given the broad levels of EcR inactivation we have tested, this discrepancy is unlikely to be linked to different levels of EcR activity between the two studies. Indeed, the RU 50 condition that led to a 1.25-fold decrease in EcR protein level (Supplementary Figure S4) was strongly deleterious for female flies. Thus, in females but not males, an EcR reduction may be required during development to increase lifespan. Alternatively, as the RNA interference and dominant negative constructs used here were not expressed in female germ line cells (Rorth, 1998), an EcR reduction in the germ line may be required to extend female lifespan. To investigate which tissue is responsible for the deleterious effect observed here, we first inactivated EcR in the CNS using the elav-GS driver. Expression of the dominant negative constructs led to a less than 15% median lifespan decrease (Supplementary Table 1), which is far from the 80% decrease observed with the da-GS driver at the same RU concentration. Thus, the CNS is unlikely to be the main tissue responsible for the deleterious effect. We also inactivated EcR using the S_1106 driver and could not observe any significant positive or negative effect on female lifespan in this context (Supplementary table 1 and Figure S7).

We then focused on ovarian effects of EcR inactivation and ovary involvement in EcR-dependent modulation of female lifespan. Indeed, EcR is normally expressed in both germ line and somatic cells of the ovary and is required during oogenesis (Buszczak et al., 1999; Carney and Bender, 2000; Hackney et al., 2007). We observed ovarian defects associated with ubiquitous somatic inactivation of EcR induced by RNA interference. A few days after starting RU treatment, the number of eggs laid per female was lower than that laid by untreated females and was dependent on the concentration of RU in the medium (Supplementary figure S8A). Most of the remaining eggs did not develop and this phenotype was partially reversible (Supplementary figure S8B). Egg defects resemble those reported by others, namely, the formation of wide, branched dorsal appendage and thin egg shells. We dissected ovaries from RU 50 and RU 200-induced females and only observed defects from stage 10 of oogenesis, notably delayed border cell migration and abnormal dorsal appendage morphogenesis. However, germ line development in the germarium (as evidenced by immunostaining for Hts/adducing) and early stages of oogenesis occurred normally and the number of follicules per ovariole was also similar to that in non-induced females (data not shown). Therefore, germ line cell survival in general does not seem to be affected. To investigate whether the deleterious effect on longevity of EcR downregulation was linked to the ovary, we analyzed the effects of ubiquitous somatic inactivation of EcR with the da-GS driver in sterile ovo^{D1} females in which egg chambers exhibit arrested development and degenerate prior to stage 5 of oogenesis (Oliver et al., 1987). Longevity of flies carrying or not the ovo^{D1} mutation could not be directly compared because they are not in the same genetic background. Moreover, the ovo^{D1} mutation itself has been shown to increase lifespan (Sgro and Partridge, 1999). So, we compared RU-treatment effect on lifespan in the different genotypes. RU treatment decreased median lifespan by 39% in *da-GS > UAS-EcR-RNAi* but only by 20% in ovo^{D1}; *da-GS > UAS-EcR-RNAi* females (Fig. 3B and C and Supplementary Table 2). Moreover, RU treatment decreased median lifespan of *da-GS > UAS-EcR-DN* female flies by more than 60% but is neutral in

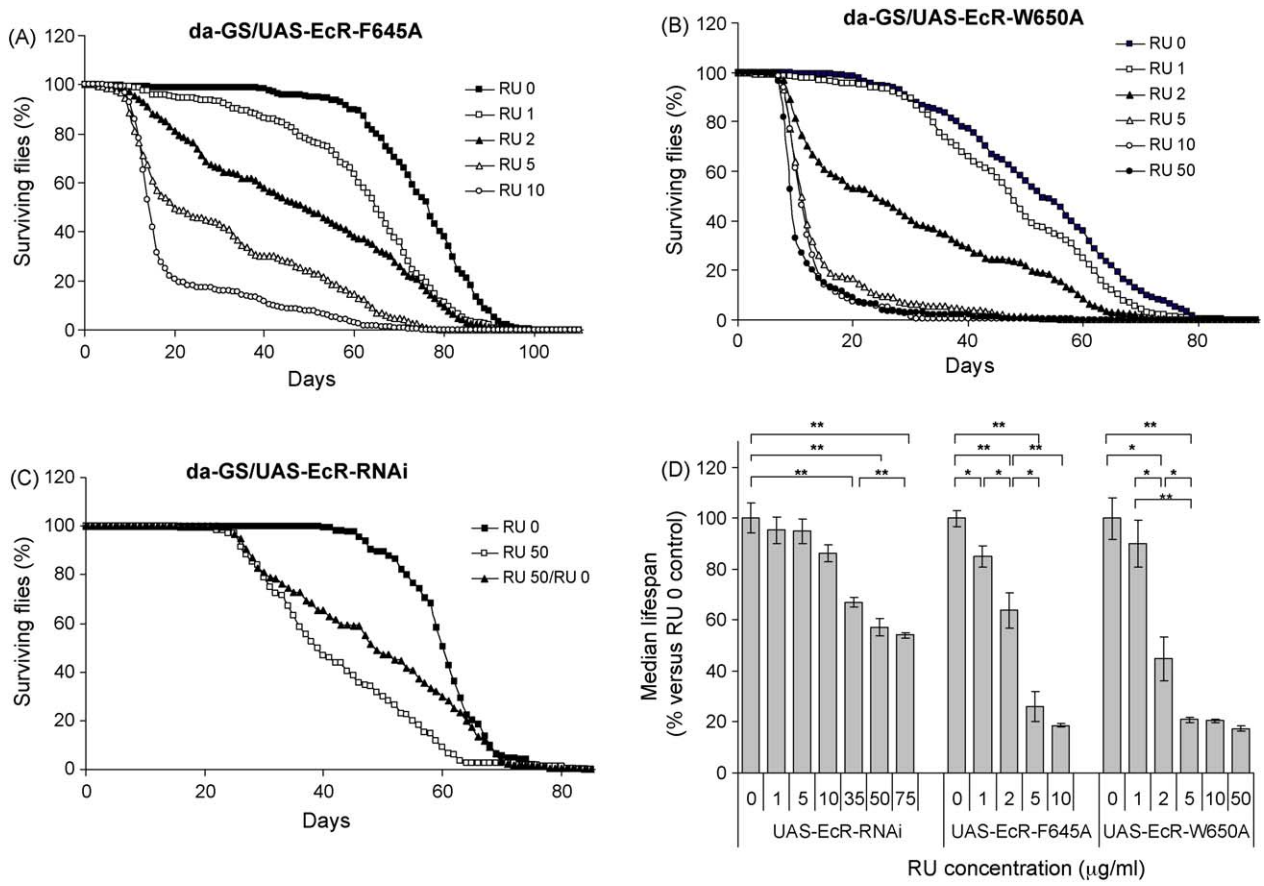

Fig. 2. Ubiquitous EcR inactivation decreases female lifespan in a dose-dependent and reversible manner. (A) Survival experiments on *da-GS/UAS-EcR-F645A* female flies treated with increasing amounts of RU (1, 2, 5 and 10 μg of RU per ml of food). The mean lifespan values are as follows: RU 0, 74 days ($n = 223$); RU 1, 61 days ($n = 221$) $p < 0.0001$; RU 2, 47 days ($n = 234$) $p < 0.0001$; RU 5, 30 days ($n = 223$) $p < 0.0001$; RU 10, 19 days ($n = 227$) $p < 0.0001$. All logrank test p values are based on comparisons between treated and untreated flies. (B) Survival experiments on da-*GS/UAS-EcR-W650A* female flies treated with increasing amounts of RU (1, 2, 5, 10 and 50 μg of RU per ml of food). The mean lifespan values are as follows: RU 0, 51 days ($n = 232$); RU 1, 47 days ($n = 202$) $p < 0.0001$; RU 2, 28 days ($n = 196$) $p < 0.0001$; RU 5, 14 days ($n = 203$) $p < 0.0001$; RU 10, 12 days ($n = 209$) $p < 0.0001$; RU 50, 11 days ($n = 175$) $p < 0.0001$. All logrank test p values are based on comparisons between treated and untreated flies. (C) Survival experiments on *da-GS/UAS-EcR-RNAi* female flies (Trial#3 in Supplementary Table S1). Flies were untreated (RU 0), treated continuously with 50 μg/ml of RU (RU 50), or treated with RU 50 which was switched to RU 0 at 30 days of age (RU 50/RU 0). The mean lifespan values are as follows: RU 0, 59 days ($n = 181$); RU 50, 41 days ($n = 164$) $p < 0.0001$ (compared with the RU 0 condition); RU 50/RU 0, 48 days ($n = 180$) $p < 0.0001$ (compared with the RU 50 condition). (D) Median lifespan of *da-GS/UAS-EcR-RNAi* (Trial#1 and 2 in Supplementary Table S1), *da-GS/UAS-EcR-F645A*, and *da-GS/UAS-EcR-W650A* female flies treated with increasing amounts of RU. All values are percentages based on RU 0 control (±SEM). Significant statistical differences between different conditions of RU treatment: $^{*}p < 0.01$, $^{**}p < 0.001$.

ovo^{D1}; *da-GS > UAS-EcR-F645A* and even moderately extend lifespan of *ovo*^{D1}; *da-GS > UAS-EcR-W650A* flies (Fig. 3A and C and Supplementary Table 2). So *ovo*^{D1} mutation suppressed, at least partially, the deleterious effect of EcR inactivation on female lifespan. We thus propose that EcR is required to repress an ovarian signal that is deleterious to lifespan, and that degenerated *ovo*^{D1} ovarioles have partially lost the capacity to produce this signal. The effect of *ovo*^{D1} mutation in *ovo*^{D1}; *da-GS > UAS-EcR-W650A* female flies could even reveal a positive effect on lifespan of EcR inactivation otherwise masked by the deleterious ovarian signal.

In summary, in this study we provide evidence for a striking sex-specific effect of the inactivation of the steroid receptor EcR on Drosophila longevity. While mild inactivation of EcR increases lifespan in males, it shortens female lifespan. This situation is reminiscent of the sex-specific action of the key nuclear receptor DAF-12 on *C. elegans* lifespan (McCulloch and Gems, 2007). Indeed, a strong *daf12* mutation decreases hermaphrodite lifespan but increases male lifespan. Interestingly, DAF-12 has been involved in the complex signalling that couples the *C. elegans* gonad status to its lifespan. Several lines of evidence indicate that a signal, issued from the germ cells, represses lifespan in a DAF-12 dependent manner and is relayed by the intestine (also a fat tissue) (Hsin and Kenyon, 1999; Berman and Kenyon, 2006). Our results suggest that, in male drosophila, the S_1106 targeted tissues including the digestive system and the fat body (considered to be the fly equivalent of the mammalian liver and white adipose tissue), are candidate tissues for the integration by EcR of a signal that negatively regulates male lifespan. Additional experiments are required to investigate the role of EcR in these tissues and to determine if EcR may relay a signal from the gonad. In female flies, we used the *ovo*^{D1} mutant to demonstrate that functional ovaries are required for an EcR dependent decrease of lifespan. Since *ovo*^{D1} ovarioles degenerate prior to stage 5, it is likely that somatic follicular cells and/or nurse cells beyond stage 4 are required to produce a negative signal on lifespan in EcR depleted females. Whether these cells are responsible for the signal production in a cell autonomous manner, or whether they relay an initial signal produced elsewhere in the body remains to be determined. The nature of the signal is also still unknown. However, since ecdysone is produced (and probably at least partially converted in 20E) in follicular and/or nurse cells of the ovary, an attractive hypothesis would be that EcR inhibits ovarian ecdysone synthesis and that overproduction of Ecdysone/20E is responsible for the reduced lifespan in EcR deficient female flies. This would explain why EcR inactivation decreased lifespan in this study, while the 20-E deficient *DTS-3* mutant females are long lived (Simon et al., 2003).

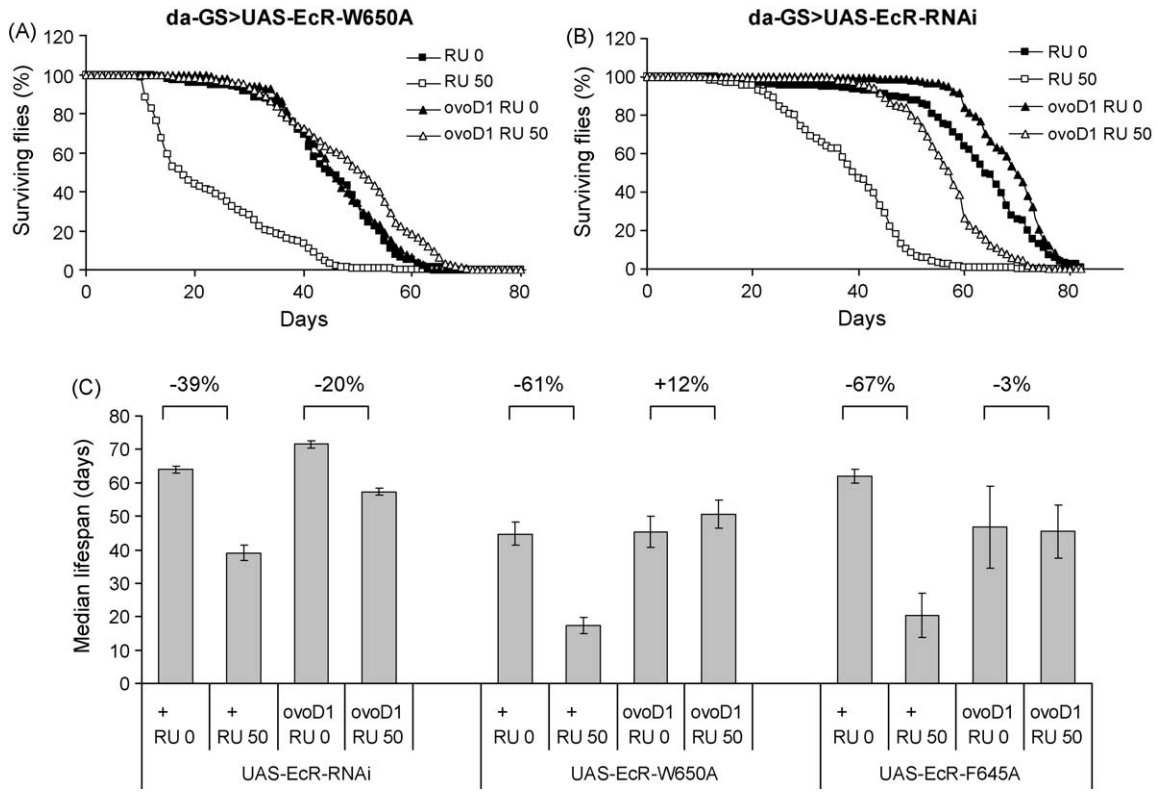

Fig. 3. *Ovo^{D1}* mutation suppresses the deleterious effect of EcR inactivation on female lifespan. (A) Survival experiments on *da-GS/UAS-EcR-W650A and ovo^{D1}/+; da-GS/UAS-EcR-W650A* female flies. Survival curves for *da-GS/UAS-EcR-W650A* females flies that were untreated (RU 0) or treated with 50 mg/ml of RU (RU 50) were compared with those for untreated (ovoD1 RU 0) or treated with RU (ovoD1 RU 50) *ovo^{D1}/+; da-GS/UAS-EcR-W650A* female flies. The median lifespan values are as follows: RU 0, 45 days (n = 95); RU 50, 17 days (n = 166); ovoD1 RU 0 45 days (n = 80); ovoD1 RU 50, 51 days (n = 161). (B) Survival experiments on *da-GS/UAS-EcR-RNAi* and *ovo^{D1}/+; da-GS/UAS-EcR-RNAi* female flies. Survival curves for *da-GS/UAS-EcR-RNAi* females flies that were untreated (RU 0) or treated with 50 mg/ml of RU (RU 50) were compared with those for untreated (ovoD1 RU 0) or treated with RU (ovoD1 RU 50) *ovo^{D1}/+; da-GS/UAS-EcR-RNAi* female flies. The median lifespan values are as follows: RU 0, 64 days (n = 150); RU 50, 39 days (n = 145); ovoD1 RU 0 72 days (n = 234); ovoD1 RU 50, 57 days (n = 229). (C) Median lifespan (±SEM) of *da-GS/UAS-EcR-RNAi*, *da-GS/UAS-EcR-F645A*, *da-GS/UAS-EcR-W650A*, *ovo^{D1}/+; da-GS/UAS-EcR-RNAi*, *ovo^{D1}/+; da-GS/UAS-EcR-F645A*, and *ovo^{D1}/+; da-GS/UAS-EcR-W650A* female flies treated with RU 50 or untreated. In all three cases the significant ($p < 0.0003$) deleterious effect observed after RU treatment is strongly attenuated by the presence of the *ovo^{D1}* mutation. In *ovo^{D1}/+; da-GS/UAS-EcR-RNAi* female flies, RU treatment still results in a significant ($p < 0.0003$) decrease in lifespan while in *ovo^{D1}/+; da-GS/UAS-EcR-F645A*, and *ovo^{D1}/+; da-GS/UAS-EcR-W650A* female flies, no significant difference can be observed between treated and not treated animals (p = 0.46 and 0.23, respectively).

Impaired ovarian ecdysone synthesis has been reported in insulin receptor mutants (Tu et al., 2002). Moreover, inactivation in the larval prothoracic gland of the phosphatidylinositol-3 kinase (PI3K), the major effector of IIS, decreased ecdysteroid titers, and EcR inhibits PI3K in the larval fat body (Colombani et al., 2005). Thus, interactions between EcR and IIS could be involved in regulation of ovarian ecdysone synthesis. Another potential signalling molecule expressed in the ovary is the DILP5 insulin-like peptide. DILP5 is expressed in ovarian follicular cells, where its function remains unknown (Ikeya et al., 2002). Whether it is regulated by EcR should be investigated. All together, our data and others suggest that, at several levels inside the body, ecdysone signalling and IIS may be connected to synchronize growth, reproductive ability and lifespan. Moreover, the striking positive effect on lifespan of young somatic gonadal tissue in mice (Cargill et al., 2003), suggests that lifespan regulation by gonadal signals may be evolutionary conserved. Thus, one may expect that better understanding these complex mechanisms in invertebrates will also shed new lights on the control of longevity in mammals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mad.2009.05.004.

References

Antebi, A., Culotti, J.G., Hedgecock, E.M., 1998. daf-12 regulates developmental age and the dauer alternative in *Caenorhabditis elegans*. Development 125, 1191–1205.

Antebi, A., Yeh, W.H., Tait, D., Hedgecock, E.M., Riddle, D.L., 2000. daf-12 encodes a nuclear receptor that regulates the dauer diapause and developmental age in *C. elegans*. Genes Dev. 14, 1512–1527.

Berman, J.R., Kenyon, C., 2006. Germ-cell loss extends *C. elegans* life span through regulation of DAF-16 by kri-1 and lipophilic-hormone signaling. Cell 124, 1055–1068.

Buszczak, M., Freeman, M.R., Carlson, J.R., Bender, M., Cooley, L., Segraves, W.A., 1999. Ecdysone response genes govern egg chamber development during mid-oogenesis in Drosophila. Development 126, 4581–4589.

Cargill, S.L., Carey, J.R., Muller, H.G., Anderson, G., 2003. Age of ovary determines remaining life expectancy in old ovariectomized mice. Aging Cell 2, 185–190.

Carney, G.E., Bender, M., 2000. The Drosophila ecdysone receptor (EcR) gene is required maternally for normal oogenesis. Genetics 154, 1203–1211.

Cherbas, L., Hu, X., Zhimulev, I., Belyaeva, E., Cherbas, P., 2003. EcR isoforms in Drosophila: testing tissue-specific requirements by targeted blockade and rescue. Development 130, 271–284.

Colombani, J., Bianchini, L., Layalle, S., Pondeville, E., Dauphin-Villemant, C., Antoniewski, C., Carre, C., Noselli, S., Leopold, P., 2005. Antagonistic actions of ecdysone and insulins determine final size in Drosophila. Science 310, 667–670.

Ganter, G.K., Walton, K.L., Merriman, J.O., Salmon, M.V., Brooks, K.M., Maddula, S., Kravitz, E.A., 2007. Increased male–male courtship in ecdysone receptor deficient adult flies. Behav. Genet. 37, 507–512.

Gerisch, B., Weitzel, C., Kober-Eisermann, C., Rottiers, V., Antebi, A., 2001. A hormonal signaling pathway influencing *C. elegans* metabolism, reproductive development, and life span. Dev. Cell 1, 841–851.

Giannakou, M.E., Goss, M., Junger, M.A., Hafen, E., Leevers, S.J., Partridge, L., 2004. Long-lived Drosophila with overexpressed dFOXO in adult fat body. Science 305, 361.

Hackney, J.F., Pucci, C., Naes, E., Dobens, L., 2007. Ras signaling modulates activity of the ecdysone receptor EcR during cell migration in the Drosophila ovary. Dev. Dyn. 236, 1213–1226.

Held, J.M., White, M.P., Fisher, A.L., Gibson, B.W., Lithgow, G.J., Gill, M.S., 2006. DAF-12-dependent rescue of dauer formation in *Caenorhabditis elegans* by (25S)-cholestenoic acid. Aging Cell 5, 283–291.

Hsin, H., Kenyon, C., 1999. Signals from the reproductive system regulate the lifespan of *C. elegans*. Nature 399, 362–366.

Hwangbo, D.S., Gershman, B., Tu, M.P., Palmer, M., Tatar, M., 2004. Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature 429, 562–566.

Ikeya, T., Galic, M., Belawat, P., Nairz, K., Hafen, E., 2002. Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila. Curr. Biol. 12, 1293–1300.

King-Jones, K., Thummel, C.S., 2005. Nuclear receptors—a perspective from Drosophila. Nat. Rev. Genet. 6, 311–323.

Kleemann, G.A., Murphy, C.T., 2009. The endocrine regulation of aging in *Caenorhabditis elegans*. Mol. Cell. Endocrinol. 299, 51–57.

Latouche, M., Lasbleiz, C., Martin, E., Monnier, V., Debeir, T., Mouatt-Prigent, A., Muriel, M.P., Morel, L., Ruberg, M., Brice, A., Stevanin, G., Tricoire, H., 2007. A conditional pan-neuronal Drosophila model of spinocerebellar ataxia 7 with a reversible adult phenotype suitable for identifying modifier genes. J. Neurosci. 27, 2483–2492.

McCulloch, D., Gems, D., 2007. Sex-specific effects of the DAF-12 steroid receptor on aging in *Caenorhabditis elegans*. Ann. N. Y. Acad. Sci. 1119, 253–259.

Motola, D.L., Cummins, C.L., Rottiers, V., Sharma, K.K., Li, T., Li, Y., Suino-Powell, K., Xu, H.E., Auchus, R.J., Antebi, A., Mangelsdorf, D.J., 2006. Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans*. Cell 124, 1209–1223.

Oliver, B., Perrimon, N., Mahowald, A.P., 1987. The ovo locus is required for sex-specific germ line maintenance in Drosophila. Genes Dev. 1, 913–923.

Osterwalder, T., Yoon, K.S., White, B.H., Keshishian, H., 2001. A conditional tissue-specific transgene expression system using inducible GAL4. Proc. Natl. Acad. Sci. U.S.A. 98, 12596–12601.

Poirier, L., Shane, A., Zheng, J., Seroude, L., 2008. Characterization of the Drosophila gene-switch system in aging studies: a cautionary tale. Aging Cell 7, 758–770.

Riddiford, L.M., 1993. Hormones and *Drosophila* development. The Development of *Drosopila melanogaster*, vol. 2. Cold spring Harbor Laboratory Press, NY, pp. 899–939.

Roman, G., Endo, K., Zong, L., Davis, R.L., 2001. P[Switch], a system for spatial and temporal control of gene expression in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S.A. 98, 12602–12607.

Rorth, P., 1998. Gal4 in the Drosophila female germline. Mech. Dev. 78, 113–118.

Sgro, C.M., Partridge, L., 1999. A delayed wave of death from reproduction in Drosophila. Science 286, 2521–2524.

Simon, A.F., Liang, D.T., Krantz, D.E., 2006. Differential decline in behavioral performance of *Drosophila melanogaster* with age. Mech. Ageing Dev. 127, 647–651.

Simon, A.F., Shih, C., Mack, A., Benzer, S., 2003. Steroid control of longevity in *Drosophila melanogaster*. Science 299, 1407–1410.

Tatar, M., Bartke, A., Antebi, A., 2003. The endocrine regulation of aging by insulin-like signals. Science 299, 1346–1351.

Toivonen, J.M., Partridge, L., 2009. Endocrine regulation of aging and reproduction in Drosophila. Mol. Cell. Endocrinol. 299, 39–50.

Tu, M.P., Yin, C.M., Tatar, M., 2002. Impaired ovarian ecdysone synthesis of *Drosophila melanogaster* insulin receptor mutants. Aging Cell 1, 158–160.

Wismar, J., Habtemichael, N., Warren, J.T., Dai, J.D., Gilbert, L.I., Gateff, E., 2000. The mutation without children(rgl) causes ecdysteroid deficiency in third-instar larvae of *Drosophila melanogaster*. Dev. Biol. 226, 1–17.