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Review

dSir2 and longevity in *Drosophila*

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ABSTRACT

The silent information regulator 2 (Sir2 or Sirtuin) family of proteins is highly conserved and has been implicated in the extension of longevity for several species. Mammalian Sirtuins have been shown to affect various aspects of physiology including metabolism, the stress response, cell survival, replicative senescence, inflammation, the circadian rhythm, neurodegeneration, and even cancer. Evidence in *Drosophila* implicates Sir2 in at least some of the beneficial effects of caloric restriction (CR). CR delays age-related pathology and extends life span in a wide variety of species. Here we will review the evidence linking *Drosophila* Sir2 (dSir2) to longevity regulation and the pathway associated with CR in *Drosophila*, as well as the effects of the Sir2 activator resveratrol and potential interactions between dSir2 and p53.

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1. Introduction

Silent information regulator 2 (Sir2) proteins, or Sirtuins, are members of a highly conserved family of proteins that act as either nicotinamide adenine dinucleotide (NAD^+) -dependent protein deacetylases or mono-ADP-ribosyltransferases (Imai and Guarente, 2010; Haigis and Sinclair, 2010). The founding member of this family is yeast Sir2, but Sirtuins are found in a variety of species ranging from bacteria to humans. Yeast Sir2 regulates silencing of the mating type loci, homologous recombination at the rDNA loci (Brachmann et al., 1995), and silencing of subtelomeric regions. Aging in yeast has been measured by counting the number of times a mother cell can divide and produce a daughter cell, usually called replicative life span. The role of Sir2 in yeast longevity was discovered when it was found that having an extra copy of the *sir2* gene extended replicative life span while *sir2* mutants have a shorter life span (Kaeberlein et al., 1999). Since that time Sirtuins in multicellular organisms have been linked to multiple physiological processes including metabolism, stress responses, cell survival, replicative senescence, inflammation, circadian rhythm, neurodegeneration, cancer, and others (Imai and Guarente, 2010; Haigis and Sinclair, 2010).

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There are seven members of the Sirtuin family in mammals, SIRT1 to SIRT7. While they all have highly conserved catalytic and NAD+ binding domains, sequences vary at their two termini (Frye, 2000). Furthermore, they differ with regard to intracellular location and deacetylation targets, and consequently are involved in different physiological processes (Haigis and Sinclair, 2010). There are five members of the Sir2 family in *Drosophila melanogaster*. Based on sequence similarity, dSir2 is the *Drosophila* homologue of yeast Sir2 and human SIRT1. *Drosophila* has been instrumental in confirming the role of Sir2 in regulating the longevity of model organisms.

2. dSir2 enzymatic activity and cofactors

Sir2 as a NAD+ dependent deacetylase targets histone as well as non-histone targets. Sirtuins catalyze deacetylation of the substrates and release nicotinamide (NAM) and O-acetyl-ADP-ribose. NAM is a strong non-competitive Sir2 inhibitor (Haigis and Sinclair, 2010). In yeast, worms and flies NAD+ is regenerated in a 4-step process, starting with the enzyme pyrazinamidase/nicotinamidase 1 (PNC1). Overexpression of *PNC1* in yeast increases longevity by decreasing the levels of nicotinamide, thereby decreasing direct inhibition of Sir2, as well as by increasing the NAD+/NADH ratio (Anderson et al., 2003). Yeast PNC1 levels and activity are induced in response to CR and mild stress, and PNC1 is required for yeast longevity extension caused by CR (Anderson et al., 2003). Knockdown of worm *pnc1* decreases survivorship, however, overexpression of worm *pnc1* increases oxidative stress resistance but not longevity in a Sir2-dependent manner (van der Horst et al., 2007). It has been recently reported that the *Drosophila* homologue of PNC1, D-NAAM (*Drosophila* nicotinamide amidase), has a similar beneficial effect on fly longevity (Balan and Miller, 2008). Overexpression of D-NAAM results in up to a 30% extension of the mean life span and 20% extension of maximal life span. Overexpression D-NAAM in a *dSir2* mutant background did not extend fly life span, suggesting that the longevity effect of D-NAAM requires the presence of dSir2 (Balan and Miller, 2008). The levels of D-NAAM are affected by oxidative stress but CR, heat shock, and target of rapamycin (Tor) inhibitors do not affect the levels of D-NAAM in S2-cells (Balan and Miller, 2008), in contrast to the findings in yeast. However, in human neuroblastoma cells resistance to oxidative stress, induced by NOC-9, is increased by overexpression of D-NAAM. Furthermore, this resistance is Sir2-dependent. Although these data imply an important role of dSir2 in mediating the beneficial effects of D-NAAM, there is still a possibility that other targets may be involved. For instance, it was found that the nicotinamide effect on yeast longevity is not solely mediated by sirtuins (Tsuchiya et al., 2006).

3. *Drosophila* Sir2: expression, localization, and role as a transcriptional modulator

Drosophila Sir2 (dSir2) is expressed widely in the early embryo but becomes restricted to the nervous system by late embryogenesis (Newman et al., 2002), showing both a nuclear and cytoplasmic localization that shifts over the course of embryogenesis (Newman et al., 2002; Rosenberg and Parkhurst, 2002). After embryogenesis expression of dSir2 protein has been determined for whole bodies, with low levels in larvae and moderate levels in pupae and adults (Rosenberg and Parkhurst, 2002; Cho et al., 2005). When adults were examined using immunolocalization, dSir2 protein was present in the nuclei of neurons and in the nuclei and cytoplasm of fat body cells (Rogina and Helfand, 2004).

dSir2 localizes to numerous euchromatic and heterochromatic sites on salivary gland chromosomes (Newman et al., 2002; Rosenberg and Parkhurst, 2002). dSir2 chromatin binding sites have been mapped in KC cells (of embryonic origin) using an *in situ* methylation assay (van Steensel et al., 2001). This also shows an association of dSir2 with multiple euchromatic and heterochromatic sites. dSir2 mutations have a modest effect on position-effect variegation, a form of heterochromatin-mediated silencing (Newman et al., 2002; Astrom et al., 2003). There is evidence for genetic interactions between dSir2 and transcription factors such as Foxo (Griswold et al., 2008), Hairy (Rosenberg and Parkhurst, 2002) and dp53 (Bauer et al., 2009), and evidence of direct physical interactions with Hairy and p53 (Rosenberg and Parkhurst, 2002; Bauer et al., 2009). Overexpression of dSir2 in S2 cells (of embryonic origin) changes the expression of several hundred euchromatic genes (approximately 100 genes are upregulated and 215 genes are down-regulated) (Cho et al., 2005). Mammalian SIRT1 interacts with 34 known deacetylation targets and six binding partners (Baur, 2010a), so additional dSir2 targets may be identified in the future.

Flies with homozygous *dSir2* null mutations are fully viable, fertile, and develop at normal rates (Newman et al., 2002; Astrom et al., 2003). Homozygous null mutants have reduced life span compared to controls (Astrom et al., 2003), whereas null flies that are transheterozygotes for two different null mutations have only slightly reduced life spans (Newman et al., 2002). No effect on life span was observed in flies heterozygous for a null mutation in *dSir2* (Pallos et al., 2008). When dSir2 expression was reduced using RNAi transgenes, ubiquitous inhibition of dSir2 mediated by the actin 5C Gal4-driver resulted in severely reduced viability while inhibition only in the nervous system gave normal viability (Kusama et al., 2006). Life span was examined under the latter condition and found to be reduced. Discrepancies between the results with RNAi and null mutations might be explained by pleiotropic effects of the RNAi transgenes.

4. Effects of dSir2 on *Drosophila* longevity

Overexpression of Sir2 extends longevity in yeast and worms (Kaeberlein et al., 1999; Tissenbaum and Guarente, 2001). Similarly, flies that overexpress dSir2 live longer compared to genetically-matched controls (Rogina and Helfand, 2004; Bauer et al., 2009). Using three different UAS lines ($dSir2^{EP2300}$, $dSir2^{EP2384}$ and $dSir2^{EY03602}$) and five different GAL-4 drivers, it was found that overexpression of dSir2 increases fly longevity in both male and female flies but the magnitude of the longevity effects depends on the timing, place and levels of dSir2 overexpression. The biggest increase in mean life span, 57%, was observed when tubulin-GAL4 was used to drive ubiquitous overexpression of dSir2 using the $dSir2^{EP2300}$ line. Under these conditions dSir2 expression was 4-fold increased compared to controls. The mean increase in longevity for ubiquitous overexpression, averaged across all three UAS lines, was 29% for females and 18% for males (Rogina and Helfand, 2004). No effect on longevity was observed when the weak armadillo-GAL4 driver was used. Life span can still be increased in male and female flies when dSir2 is expressed pan-neurally throughout development. When there is pan-neural expression only during adult life, median and maximal life spans were extended in females but only maximal life span was extended in males. Another study has confirmed life span extension when dSir2 expression is induced in the $dSir2^{EP2300}$ line (Pallos et al., 2008). A report examining developmental defects caused by dSir2 overexpression in the eye imaginal disc showed overexpression of both *dSir2* and the adjoining gene *dnaJ-H* when the $dSir2^{EP2300}$ line was crossed to the *gmr-GAL4* driver (Griswold et al., 2008). However, when the same transgenic line, $dSir2^{EP2300}$, was crossed to an inducible pan-neural driver and *dSir2* was overexpressed only in adults there was life span extension but no overexpression of *dnaJ-H* (Bauer et al., 2009). Two concentrations of inducer were used, causing 3-fold and 5-fold increases in dSir2 expression, and greater life span extension was obtained when there was higher overexpression. At both concentrations of inducer there was barely any change in *dnaJ-H* expression compared to controls, indicating life span extension was solely due to *dSir2* (Bauer et al., 2009). It is likely that the other transgenic lines used in Rogina and