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Review

Sex-specific regulation of aging and apoptosis

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Abstract

Genetic analysis of Drosophila, mice and humans indicates that gene alleles, mutations and transgenes that affect life span tend to do so differently depending on the sex of the organism. The likely reason for this is that the sexes are different genotypes (e.g., X/X vs. X/Y) and face quite different environments: e.g., to reproduce, males have to mate with females while females have to mate with males. Genes are subject to different genetic interactions and different gene-by-environment effects in male vs. female. The consequence is that through evolution certain genes are differently selected and optimized for each sex. Both the mitochondrial genome and the X chromosome are asymmetrically inherited in Drosophila and mammals; through evolution these genes spend relatively more time under selection in females and are therefore expected to be better optimized for function in the female than in the male. Consistent with this the Drosophila X chromosome has been found to be a hotspot for sexually antagonistic fitness variation. Old Drosophila and old mammals exhibit apoptosis—an observation consistent with the idea that the mitochondria are less functional during aging due to maternal-only inheritance. One feature of aging that is common to Drosophila and mammals is that females tend to live longer than males, and this may be due in part to sub-optimal mitochondrial function in males. The data support the conclusion that a significant part of the aging phenotype is due to antagonistic pleiotropy of gene function between the sexes. Liberal application of Occam's razor yields a molecular model for the co-regulation of sex, apoptosis and life span based on the on/off status of a single gene: *Sxl* in *Drosophila melanogaster* and *Xist* in humans. Aging may simply represent an ancient and conserved mechanism by which genes re-assort.

Keywords: Game theory; *dIAP2*; Replicator; Endosymbiosis; ESS

1. Introduction

Recent and classic observations suggest an important and ancient role for the mitochondria in the determination of the germ-line/soma distinction and sexual identity. A defining feature (and I would argue, the defining feature) of the oocyte is its asymmetric inheritance of the mitochondrial genomes. Asymmetric inheritance of mitochondrial genes and sex chromosome genes promotes the evolution of sexually antagonistic gene functions. Genes exhibiting such antagonistic pleiotropy (compromised function in one sex or both) are expected to contribute preferentially to the aging phenotype. In this review I try to relate these diverse topics and create a logical framework through which our embryonic understanding of gametogenesis and mitochondrial segregation can be used to predict which genes and molecular processes are most likely to regulate the functional life span of the organism.

2. Aging and life span

Aging in living organisms is more correctly termed senescence, and is generally described as a cumulative, irreversible process resulting in decreased function and increased risk of death. Aging of some kind appears to affect all living organisms, from bacteria to humans (Ackermann et al., 2003; Stewart et al., 2005). How long an individual lives – its life span – is characteristic of different species (Finch, 1990): Drosophila can live 100 days, humans can live 100 years. Within each species life span is typically quite variable—even among individuals who are nearly genetically identical. Comparisons of life spans between groups are therefore often reported as mean and maximum life spans for the group or cohort. In cold-blooded (poikilothermic) animals like Drosophila, life span scales with temperature across a broad range in both sexes. This suggests that there is an irreversible,

Abbreviations: SG, switch gene; Sex determination gene; DR, dietary restriction

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cumulative damage that leads to increased risk of death of the organism. In addition, certain interventions such as dietary restriction (DR) (Mair et al., 2003; Partridge et al., 2005) or a mild heat stress (Tatar et al., 1997) can cause a rapid and reversible shift in a population from a higher mortality rate to a lower one, demonstrating more acute mechanisms regulating survival. The molecular nature of these mortality mechanisms is unknown, however oxidative stress, hydrolytic stress, and toxic metabolite stress have each been implicated (Busuttil et al., 2003; Hekimi and Guarente, 2003; Gems and McElwee, 2005; Landis and Tower, 2005; Wallace, 2005). The characteristic life spans of different species, and the variable life spans of individuals within a species are determined by how their unique genetic make-ups and environments make them more or less susceptible to these mortality mechanisms.

3. Evolutionary theory of aging

Like all things biological, aging and life span are shaped by genes and evolution (Kirkwood and Austad, 2000). Natural selection efficiently removes deleterious mutations from the population. However, a mutation that causes a problem only at late ages is not efficiently removed. For example, a human gene allele that predisposes individuals to Alzheimer's or Parkinson's disease is not efficiently removed from the population because by the time the disease is manifested the gene has usually already been passed on to the next generation (Finch and Sapolsky, 1999). The idea that such late-acting mutations accumulate in the genome and create the aging phenotype constitutes the ''mutation accumulation'' theory of aging. The ''antagonistic pleiotropy'' model suggests that gene alleles with late-acting deleterious effects are maintained in the population by active selection because these same gene alleles have benefits during the developmental and/or reproductive stages. Significant experimental evidence exists in support of both mechanisms (Hughes and Reynolds, 2005). For example, the life span of Drosophila can be increased in the laboratory by selecting populations for late-life reproduction (Luckinbill et al., 1984; Rose, 1984; Rauser et al., 2006).

4. Asymmetric gene inheritance—a battle of the sexes

Antagonistic pleiotropy, as described above, refers to gene alleles that are beneficial at an early age and deleterious at a late age. Another type of antagonistic pleiotropy is between the sexes: a gene allele that benefits one sex of the species can be relatively deleterious to the other sex (Rice, 1992, 1998; Chippindale et al., 2001). This is possible because the sexes have different genotypes (e.g., X/X vs. X/Y), different environments (e.g., unique genital tract microbial fauna) and different selective pressures (e.g., childbirth). These sexually antagonistic genes are expected to contribute preferentially to the aging phenotype: all other things being equal, a gene allele optimized for function in both sexes is less likely to cause a problem during aging than a gene allele that functions sub-optimally in one or both sexes.

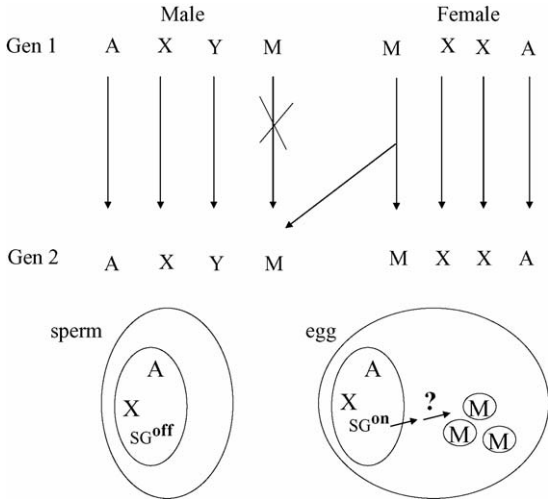

Fig. 1. Asymmetric segregation of the X and Y chromosome and mitochondrial genome (M). Drosophila and human males in generation 1 do not pass mitochondrial genes (M) on to their offspring in generation 2, rather these genes come from the female parent (maternal inheritance). Asymmetric segregation or maternal inheritance of the mitochondrial genome means the female gamete or egg contributes the functional mitochondrial genomes to the embryo while the male gamete or sperm does not. A, autosomes; M, mitochondrial genomes. SG = switch gene and sex-determination gene.

In both Drosophila and humans females are genotype X/X and males are X/Y. (Fig. 1). Since the Y is inherited only through the male, it means that the Y chromosome genes are optimized for function only in males. Consistent with this, genes on the Y are generally involved in male-specific functions such as spermatogenesis and male sexual differentiation (Charlesworth and Charlesworth, 2005). In contrast, because females have two copies of every X chromosome gene and males have only one copy, it means that through evolution X chromosome genes spend more time under selection in females than they do in males.

Table 1
Distribution of genes in various GO categories among Drosophila chromosomes (source: Flybase (Drysdale and Crosby, 2005))

Gene category	X	2	3	Total	Skew?
Total	2309 (16%)	5688 (40%)	6302 (44%)	14384	(Base)
Sex determination	11 (30%)	13 (35%)	13 (35%)	37	X
Oogenesis	61 (30%)	66 (33%)	76 (37%)	203	X
Spermatogenesis	9[a] (11%)	48 (57%)	27 (32%)	84	A
Mitochondrial	43 (17%)	91 (36%)	120 (47%)	254	(None)
Apoptosis/cell death	28 (11%)	107 (43%)	112 (45%)	247	A
Anti-apoptosis	3 (7%)	19 (45%)	20 (47%)	42	A

[a] The 11-copy *stellate* locus was counted as one gene.

This suggests that there might be some skew in the distribution of genes with sex-specific functions between the X chromosome and the autosomes, and this prediction has been fulfilled by data from the genome projects. In Drosophila the X chromosome is about half the size of the 2nd and 3rd chromosomes. Consistent with this the estimated number of genes is 2309 on the X (16%), 5688 on the 2nd (40%), and 6302 on the third (44%), out of a total of about 14384. On the Drosophila X genes involved in oogenesis appear over-represented and genes involved in spermatogenesis under-represented as might be expected for a chromosome better optimized for the female (Table 1). The same skew exists for genes showing female-biased or male-biased expression at the RNA level (Arbeitman et al., 2002; Parisi et al., 2003, 2004; Oliver and Parisi, 2004). The X chromosome was also found to be a hotspot for sexually antagonistic fitness variation—meaning that naturally occurring X chromosomes often contain gene alleles that benefit one sex more than the other in terms of traits like male reproductive success and female fecundity (Gibson et al., 2002).

5. Mitochondrial asymmetric inheritance

Mitochondria are asymmetrically inherited (maternal inheritance only), meaning natural selection acting on the mitochondrial genome (mitogenome or M) and the mitogenome-nuclear genome interactions is effective only in females (Fig. 1) (Rand et al., 2001, 2006; Rand, 2005). In other words, the mitochondrial genome is optimized for function in the female. The male is therefore inherently less fit because the highly beneficial mitochondrial genome is not optimized for his genome. This predicts that mitochondrial malfunction should contribute to the aging phenotype, and perhaps do so in males more than females. This is one possible explanation for the observation that in many species, including Drosophila and humans, males tend to live less long than females.

Does maternal inheritance of the mitochondria skew the distribution of mitochondria-related gene functions between the X and autosomes? Genes involved in general mitochondrial function were found to be pretty evenly distributed (17% vs. 36% and 47%, respectively; Table 1). However, it is interesting to note that genes involved in programmed cell death are reduced in abundance on the X, especially genes with anti-apoptotic function. Out of the 28 genes involved in programmed cell death found on the X chromosome (Table 2), pro-apoptotic functions outnumber anti-apoptotic functions 25 vs. 3. The observation that (anti)apoptotic genes are preferentially on the autosomes (like spermatogenesis

Table 2
Drosophila X chromosome programmed cell death genes sorted by function

Gene name	Anti-apoptosis	Apoptosis	Induction of Apoptosis	Apoptotic program	Autophagic cell death	Autophagy	Salivary gland cell death	Germ cell programmed death	Retinal cell death	Positive regulation of retinal programmed cell death
Myb	X									
Sk1	X									
CG32703	X									
Tak1		X								
wgn		X								
CG17754		X								
Dredd		X		X						
Atg5/CG1643		X			X	X	X			
Ing3/CG6632		X	X							
CG10990		X	X							
Corp/CG10965			X							
mnb			X							
Traf2			X							
Tao-1/CG14217			X							
CG32666			X							
Appl			X	X						
kirre				X						
rst				X					X	
ec/CG2904									X	
N/CG3936									X	
lz/CG1689										X
br						X	X			
Cyp1					X		X			
l(1)G0148					X		X			
CG5254					X		X			
CG7860					X		X			
CG10992					X		X			
usp/CG4380					X					
Tre1/CG3171								X		

genes) suggests some degree of sexual antagonism with regard to apoptosis regulation.

6. Apoptosis

In addition to energy production, mitochondria have another critical function. Apoptosis is a form of active cellular suicide involving characteristic morphological changes such as membrane blebbing. It functions to remove cells that are otherwise unwanted, such as in the developmental sculpting of human fingers and Drosophila gut, or dangerous, such as virally infected cells (Baehrecke, 2002, 2003; Cashio et al., 2005; Yin and Thummel, 2005). An evolutionarily conserved set of cysteine-proteases called the caspases carry out most of the cellular self-destruction (Abraham and Shaham, 2004). The caspases exist in a relatively inactive state in virtually all cells of eukaryotes where they are regulated by a balance of specific activators and inhibitors. The mitochondria regulate apoptosis by releasing cytochrome C and other pro-apoptotic factors into the cytosol in response to various ''death signals'' (Adams, 2003). These signals include nuclear DNA damage, p53 activation and the balance of Bcl-family member activities. The released cytochrome C binds to Apaf-1 protein which promotes assembly of a multiprotein complex called the apoptosome and activates the initiator caspase-9 and a ''caspase cascade'' (Adams and Cory, 2002; Arama et al., 2006). Since apoptosis is essential to the development and function of both male and female animals, both sexes must be capable of regulating a basic cellular apoptotic machinery.

7. The mitochondria and apoptosis in gametogenesis

Mitochondria exhibit striking behaviors in germ cells that have generally been interpreted to be a result of the requirement for transmitting healthy mitochondria to the next generation, and the presumed high energy demand of sperm motility and the dramatic transformations associated with spermatogenesis. However, the role of the mitochondria in male and female gametogenesis extends far beyond the mere production of energy.

Mitochondria play a central role in the differentiation of the gametes in Drosophila and other species. Mitochondrial rRNA appears to be an essential component of the germ plasm—the maternal, cytoplasmic determinant of germ-cell fate (Amikura et al., 2005; Kobayashi et al., 2005). During Drosophila oogenesis ribosomal RNAs encoded by the mitochondria are transported out of the mitochondria into the cytosol of the oocyte where they are required for formation of the morphologically distinct germ plasm. The cells that inherit the germ plasm during the development of (male or female) embryos give rise to the germ cells in the adult. This is consistent with an ancient role for the mitochondria in the evolution of sexual differentiation and germ-line/soma distinctions.

The observation that mitochondrial genes are almost never inherited through the male means one or both of two things (Nishimura et al., 2006): (i) there exists a male-specific mechanism for destruction of functional mitochondrial genomes, or (ii) the mitochondrial genomes that are delivered to the oocyte from sperm are non-viable in the oocyte or embryo environment. In either event we can state that there is a female-specific mechanism for mitochondrial inheritance, and males either lack or for some other reason do not express this mechanism (Fig. 1).

What is the molecular and cellular nature of the mechanisms for asymmetric mitochondrial inheritance? The dramatic behavior of mitochondria in germ cells offers some clues: oogenesis in many species is characterized by a morphologically distinct aggregate of mitochondria and other cytoplasmic material called the Balbiani body (Kloc et al., 2004; Wilk et al., 2005). For example, the developing Drosophila oocyte is connected to its sister germ-line cyst cells via cytoplasmic bridges, and the Balbiani body and a specialized actin structure called the fusome mediate early movement of mitochondria into the oocyte (Cox and Spradling, 2003). Perhaps it is only this early population of oocyte mitochondria that are incorporated into the germ plasm to be inherited by the next generation? Later the cyst cells dump more mitochondria into the oocyte cytoplasm prior to – or coincident with – undergoing a form of programmed cell death (Buszczak and Cooley, 2000). Subsequently, a significant number of oocytes and eggs may be destroyed and reabsorbed in the Drosophila ovary, in a process modulated by insulin-like signaling (Drummond-Barbosa and Spradling, 2001; Flatt et al., 2005). Germ-line cysts, mitochondrial transport mechanisms and multiple apoptotic-like processes appear to function in mammalian oogenesis as well (Pepling and Spradling, 2001; Hussein, 2005). In adult mammals most female germ cells are destroyed prior to fertilization in an apoptosis-like process called atresia. It has been hypothesized that atresia is one mechanism to remove oocytes carrying mutant mitochondria and thereby ensure the inheritance of functional mitochondria by the next generation (Krakauer and Mira, 1999). In summary, female inheritance of mitochondrial genomes appears to be accomplished by active transport and concentration of (probably a subset) of mitochondria into the oocyte, and perhaps the destruction of cells containing unwanted mitochondria.

In the male germ line the mitochondria undergo a series of dramatic transformations linked to the morphological development of the sperm, and ultimately give rise to highly derivative structures containing only a small fraction of the starting mitochondrial DNA: for example, the mammalian sperm midpiece and the insect sperm ''nebenkern''. In Drosophila an apoptosis-like process has been found to be essential for normal sperm development, in particular the spermatid individualization step in which most of the cytoplasm and the majority of the mitochondria are eliminated from the developing spermatids (Fabrizio et al., 1998; Arama et al., 2003, 2006; Cashio et al., 2005). Ectopic expression of the baculovirus caspase-inhibitor gene *p35* in the testes, or mutation of the Drosophila homologs of cytochrome C, Apaf or Caspase-9 caused severe defects in this process. These essential apoptosis events are attractive as a possible mechanism for sperm-specific destruction of mitochondrial genomes. In the

unicellular alga *C. reinhardii* and the Japanese pet fish *O. latipes* the fate of the mitochondrial DNA has been examined in detail, and in each case what little male mitochondrial DNA makes it to the egg is actively destroyed just after fertilization (Nishimura et al., 2006).

8. Evolution—the benefit of battle

The asymmetric inheritance of the M, X and Y chromosomes creates abundant opportunities for antagonistic pleiotropy of gene function between the sexes. This sets up a situation of balancing competition and selection between the male and female that is thought to benefit both because it promotes genetic diversity—sometimes called a Red Queen situation (Nowak and Sigmund, 2004). It is possible to envision this situation as a driving force in eukaryotic evolution: the male is inherently less fit because he receives an M that is not optimized for his genome. Since selection cannot act in the male to improve M gene function, it acts to improve the fit of the male genome to the M. Because of this there is strong selection in the male for mutation of the X to compensate for his lack of fitness—as opposed to the autosomes, which they share equally. This leads to hypermutation of the X in the male: such X-linked mutations will be heterozygous in his daughters and might benefit him and his grandsons. Since the male is characterized by suboptimal mitochondrial function, it predicts the hypermutation of the X in the male might proceed primarily through oxidative mechanisms.

There is predicted to be strong selective pressure acting on the male to make spermatogenesis and sperm success as dependent upon the mitochondria as possible, e.g., motile sperm, regulated apoptosis and the elaborate morphological changes. In this way the male ‘‘forces’’ the female to give him as good M genes as possible. In other words, the males have created a limit to the extent to which the female can make the mitochondria sub-optimal for his genome, because if she makes it worse her eggs will not get fertilized. This is the likely explanation for that fact that across species males are characterized by large numbers of sperm, all of which appear to be marginally functional. Natural selection in the male and female will act on the X and on X-autosome interactions to create ever-more distinct gametogenesis mechanisms—in order to ‘‘force’’ the opposite sex into providing them with as good a set of genes as possible: natural selection acts in the female to make oogenesis as dependent upon the X and X-autosome interactions as possible to try to force males into giving her as good (and un-mutated) X chromosomes as possible. This dance between the male and female through time drives the evolution of multicellularity and the separation of germ-line and soma. For example, natural selection acts in the male to make spermatogenesis as dependent upon the mitochondria as possible, including the elaboration of a separate and disposable soma that houses and supports spermatogenesis and is highly dependent on mitochondrial function.

The same general rules can be extended to mate choice for several species. For example female Drosophila select males based primarily on energetic (mitochondria-dependent) traits—he chases her. In turn males are predicted to select females based primarily on potential for maternal contribution (e.g., size of reproductive tissues) and X chromosome genetic diversity (e.g., odor). Consistent with this idea, X chromosome gene expression appears to be especially variable in human females (Carrel and Willard, 2005).

The evolutionary considerations make predictions as to what types of genes are more likely to exhibit antagonistic pleiotropy and be involved in limiting the life span of flies and mammals—specifically, genes controlling mitochondrial function and sex-specific functions such as gametogenesis, sex-determination, sex-specific differentiation, behavior and metabolism. To what extent have these predictions been born out? A number of approaches have been used to try to identify genes and pathways that regulate life span in various organisms. While male/female comparisons have not always been made, some trends are already apparent.

9. Life span QTLs

Quantitative trait loci (QTLs) are regions of the chromosome that are associated with differences in a scalable phenotype such as bristle number or life span. Life span QTLs can be identified based on the general strategy of crossing a short-lived strain with a long-lived strain, deriving sub-strains of varying life span, and correlating specific chromosomal genetic markers with shorter or longer life span across strains. This strategy works quite well in organisms such as Drosophila, *C. elegans* and mouse and QTLs affecting life span have been identified in several labs (Nuzhdin et al., 1997, 2005; Leips and Mackay, 2000; Vieira et al., 2000; Jackson et al., 2002; Mackay, 2002; Ayyadevara et al., 2003; Valenzuela et al., 2004; Wang et al., 2004; Hsu et al., 2005). One of the most striking observations from these studies is the degree of sex-specificity of the QTLs—many or most of the life span QTLs identified in both Drosophila and mouse are sex-specific, and their effects can be modified by mating (Reiwitch and Nuzhdin, 2002). This has led to the conclusion that antagonistic pleiotropy of gene function between sexes and developmental stages shapes life span (Vieira et al., 2000; Leips et al., 2006). It is difficult to go from QTL to a specific gene, but there have been some successes (De Luca et al., 2003; Miller, 2005). For example the Drosophila gene Ddc catalyzes the final step in the synthesis of the neurotransmitters dopamine and serotonin and affects both courtship behavior and life span.

10. Life span mutations and transgenes

A number of single-gene mutations have been identified that can increase Drosophila life span (Helfand and Rogina, 2003; Ford and Tower, 2006). Where tested most appear to affect both male and female, although there is often a bias in effect for one sex or the other (Burger and Promislow, 2004).

The antioxidant enzyme Cu/ZnSOD is found in the cytoplasm and outer mitochondrial space in most eukaryotic cells (Landis and Tower, 2005). Ubiquitous over-expression of Cu/ZnSOD in Drosophila was found to increase life span in

both male and female flies (Sun and Tower, 1999). Over-expression of human Cu/ZnSOD (huCu/ZnSOD) preferentially in Drosophila motorneurons was also found to increase life span in males and females, using two independent huCu/ZnSOD transgenes (Parkes et al., 1998). Interestingly, a recent analysis of one of those huCu/ZnSOD transgenes in several long-lived genetic backgrounds found life span extension primarily in females (Spencer et al., 2003). This might indicate some sex bias in the mechanism of life span extension by huCu/ZnSOD over-expression in Drosophila motorneurons, or might simply represent a sex bias in the expression of that one particular transgene insertion.

A striking example of what could be sexually antagonistic gene function is a seminal fluid protein (produced in the Drosophila male) that may help his sperm compete against other male's sperm-yet at the same time shortens the life span of the inseminated female (Wolfner, 2002). The fact that genes can be expressed in one sex but function in the other sex, either through insemination or maternal contribution to the embryo, provides ample opportunities for the evolution of sexually antagonistic gene functions.

A conserved insulin-like signaling pathway has been identified that negatively regulates life span in *C. elegans*, Drosophila and mice (Bartke and Brown-Borg, 2004; Kenyon, 2005). In Drosophila, inhibition of the insulin-like pathway or transgenic over-expression of the target transcription factor dFOXO increases life span preferentially in females (Clancy et al., 2001; Tatar et al., 2001; Hwangbo et al., 2004). This suggests that in Drosophila insulin-like signaling normally limits life span more in females than in males. Why might this be? Perhaps because this pathway regulates reproduction and metabolism and females invest more metabolic resources in reproduction than do males. Dietary restriction (DR) also increases life span to a greater extent in Drosophila females than it does in males (Magwere et al., 2004). A mild stress applied early in life can sometimes increase the life span of an animal, an effect called hormesis (Cypser and Johnson, 2003). In Drosophila, mild heat and other hormetic stresses tend to benefit males more than females (Vieira et al., 2000; Burger and Promislow, 2004).

There are a small number of interventions and genes that have been shown to increase life span in rodents (Miller, 2005). DR increases both male and female life span, but may do so more in females (Masoro, 2005). Ames dwarf mouse, Snell dwarf mouse, and Little dwarf mouse represent mutations in the insulin-like signaling and growth hormone pathways and increase life span in both sexes, again with a preference for females (Bartke, 2005). Strikingly, in the Ames dwarf mouse, extension of life span correlates with an almost complete loss of gender dimorphism in the gene expression patterns observed in the liver (Amador-Noguez et al., 2005). This was interpreted to suggest that a reduction in costly physiological investments in reproduction contributes to extended longevity.

Genes known to regulate human life span are still more rare (Perls and Terry, 2003; Martin, 2005). Importantly, a key regulator of mitochondrial-dependent apoptosis pathways, *p53*, is implicated in life span regulation in Drosophila, mice and humans (Tyner et al., 2002; Gaspari et al., 2003; Maier et al., 2004; Bauer et al., 2005; van Heemst et al., 2005; Gatza et al., 2006).

Regulation of life span by the insulin-like pathway in the hermaphrodite nematode *C. elegans* correlates with levels of oxidative stress resistance (Larsen, 1993). Life span extension occurs in the adult and is mediated by a set of genes including small heat shock proteins and ones similar to the classic Phase II response involved in detoxification and excretion of lipophilic metabolites (Walker et al., 2001; Lee et al., 2003a; Murphy et al., 2003; An et al., 2005; Gems and McElwee, 2005). Interestingly, one of the major targets of reduced insulin-like signaling is the mitochondrial antioxidant MnSOD (Honda and Honda, 1999)—which has been shown to be sufficient to increase life span in adult flies (Sun et al., 2002).

The ability to inhibit specific gene expression in *C. elegans* by simple feeding of dsRNA has allowed for genome-wide screens for negative regulators of life span, and the assessment of when during the life cycle these genes function to inhibit life span. In addition to the insulin-like signaling pathway a major class of genes identified were ones with mitochondrial functions (Dillin et al., 2002; Lee et al., 2003b). The data suggest that a large number of mitochondrial genes, and presumably the mitochondria itself, function during *C. elegans* development to limit the life span of the subsequent adult. Taken together, the data suggest that in *C. elegans* the mitochondria can function during development to limit subsequent adult life span, and can function in the adult to promote life span. So far there is no indication that these life span effects involve apoptotic-like mechanisms. Virtually all experiments were done in hermaphrodites, so few male/female comparisons are available (McCulloch and Gems, 2003).

In summary the genetic and transgenic studies clearly support a role for mitochondria-related genes and functions in aging and life span regulation across species, with hints of important sex-specific differences. The predicted importance of other sex-specific genes and pathways is indicated by the QTL studies, but remains to be confirmed by the identification of specific genes with differing effects on male and female life span. The trends that appear so far are that female life span may be more limited by the insulin-like signaling pathway and DR, while male life span may be more limited by (oxidative) stress.

11. Oxidative stress and apoptosis in old animals

A large body of data demonstrates a correlation between mitochondrial misfunction, oxidative stress and aging across species (Walter et al., 1998; Hekimi and Guarente, 2003; Fridovich, 2004; Landis and Tower, 2005; Wallace, 2005). During aging oxidatively damaged macromolecules and abnormal mitochondria increase in concentration and oxidative stress-response genes are expressed in tissue-specific patterns. These observations appear to apply generally to both males and females of Drosophila, rodents and humans. As specific molecular markers for apoptosis have become available, it has become apparent that apoptosis is also occurring during aging in tissue-specific patterns in Drosophila and mouse; however,

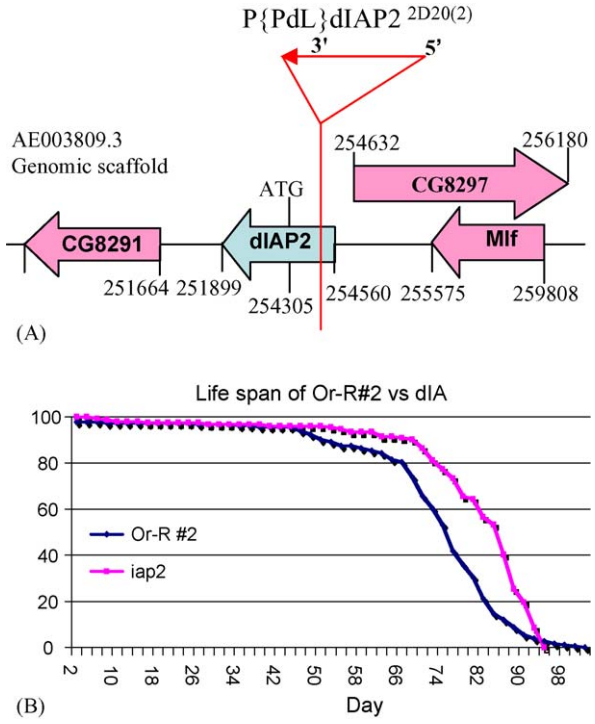

Fig. 2. Identification of *dIAP2* mutation life span extension in male Drosophila. (A) Diagram of *PdL* insertion in the *dIAP2* gene. (B) *dIAP2* mutation effect on life span relative to Oregon R WT control chromosome. Survival curve for male flies of control genotype *y ac w*; +/+; $rtTA^{(3)E2}$/+ and experimental genotype *y ac w* ; PdL^{dIAP2}/+; $rtTA^{(3)E2}$/+ passaged on food containing 60 μg/ml doxycycline from day 4 onwards, data re-plotted from (Landis et al., 2003). *dIAP2* mutant mean life span = 84.305 days, variance = 265.367, Std. Dev. = 16.290, St. Err. = 1.182. Or-R control #2 mean life span = 72.457 days, variance = 286.086, Std. Dev. = 16.914, St. Err. = 1.247. Mean difference = 11.849, unpaired, two-sided *t*-test, $P < 0.0001$, percent change = 100(84.3–72.45)/72.45 = +16%.

there has been little if any comparison of male vs. female patterns (Kujoth et al., 2005; Zheng et al., 2005).

12. Does apoptosis limit or regulate life span?

The observation of apoptotic events in old animals begs the question of whether this process limits life span. Results of a genetic screen support a role for apoptosis in Drosophila life span regulation (Fig. 2). Previously, 10,000 male flies were generated where each fly had at least one new insertion of an engineered P transposable element called *PdL* (Landis et al., 2003). *PdL* contains an outwardly directed, doxycycline(-DOX)-regulated promoter at its 3′ end, that can drive over-expression of a gene downstream of the insertion site. The longest-lived of the 10,000 males contained a single *PdL* insertion causing over-expression of *dIAP2*—a known anti-apoptotic caspase inhibitor with conserved function in humans (Fig. 2A). One-hundred nine strains were derived from the longest-lived flies and the strains were re-tested for life span in cohorts of ∼400 male flies ± DOX. *dIAP2* over-expression in the presence of DOX yielded the second-longest life span of all 109 lines and a life span increase relative to the control chromosome of +16% (Fig. 2B). The *dIAP2* mutation had not previously been pursued because there was only a small difference between the +DOX and −DOX life spans (Landis et al., 2003). It now appears that this is due to the leaky nature of the mutation and the potency of the gene product for life span effects (Yishi Li and J.T., unpublished observations). A similar screen for life span-extending mutations in Drosophila identified the *dPOSH* gene, which may also be involved in apoptosis regulation (Aigaki et al., 2002).

Preliminary data suggests that the apoptosis regulators *p53* and baculovirus *p35* also regulate adult Drosophila life span (M. Waskar et al, in prep.). Finally, Seroude and coworkers have recently found that inhibiting apoptosis in *Drosophila* muscle tissue by over-expression of caspase inhibitors *dIAP1* or baculovirus *p35* increases both muscle function and life span (Personal communication: Tissue-specific inhibition of apoptosis extends *Drosophila* life span, J. Zheng, J. Yeung, L. Seroude, submitted). Taken together the data suggest that, in Drosophila at least, apoptotic-like mechanisms act in tissue- and developmental stage-specific ways to regulate life span. However, it should be noted that other studies indicate that *p53* can affect Drosophila life span via a mechanism other than apoptosis (Bauer et al., 2005). Some preliminary data suggest intriguing sex-specific differences in the way apoptotic regulators affect Drosophila life span (Waskar et al., unpublished observations) and this should be a particularly interesting area for future research.

13. A binary switch model for sex determination, apoptosis and life span

A molecular model consistent with the data and evolutionary theories can be constructed using a binary switch—the on/off status of a gene that regulates mitochondrial genome maintenance. The mitochondrial genome is asymmetrically inherited—meaning that some mechanism exists to ensure that mitochondrial genomes are present and inherited through the cytoplasm of the oocyte and are (almost) never inherited through the sperm, as discussed above. Therefore, asymmetric segregation is accomplished by a mitochondrial inheritance system expressed in the oocyte that is not expressed in the sperm, i.e., a mitochondrial inheritance system downstream of the female germ-line sex determination pathway (Fig. 1). What might be the molecular nature of this female-specific mitochondrial inheritance mechanism? One possibility is that only the mitochondrial genomes present in the oocyte are licensed for replication—therefore, any mitochondrial genes coming in from the male would be diluted out, as appears to be the case. Similarly, the mitochondria in the oocyte could be protected by a female-specific anti-apoptotic mechanism. Mitochondria actively turn-over in many cell types, and apoptosis is reported to be the default state for the mitochondria (Jones, 2000; Brookes, 2005)—meaning that in the absence of some anti-apoptotic signal the mitochondria and its genome will tend to self-destruct. Regardless of the precise molecular nature of the female-specific mitochondrial inheritance mechanism, it represents a mitochondrial maintenance signal downstream of the female-sex determination pathway (Fig. 1),

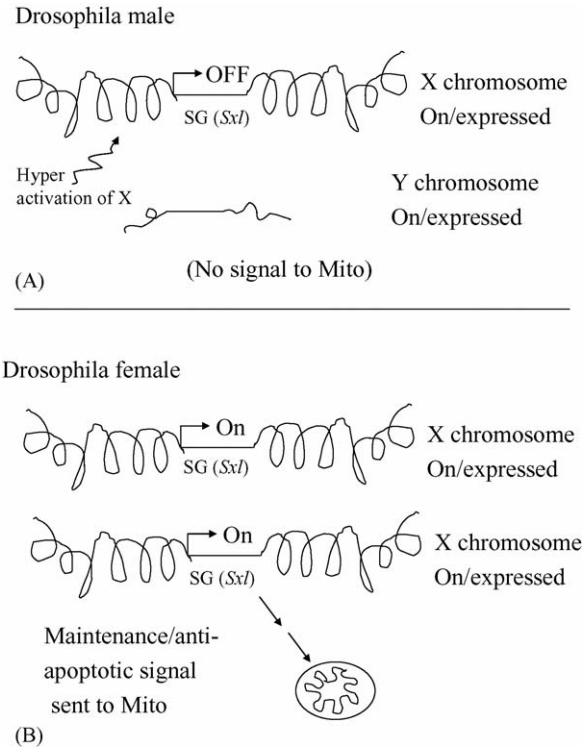

Fig. 3. Diagram of Drosophila dosage compensation and sex determination.

and is most simply thought of as an anti-apoptotic signal sent to the mitochondria.

In Drosophila both germ-line and somatic sex determination as well as dosage compensation are controlled by the on/off status of the *Sxl* (*Sex lethal*) gene (Birchler et al., 2003; Graham et al., 2003; Bhadra et al., 2005). *Sxl*-on controls female differentiation and therefore production of this theoretical anti-apoptotic signal (Fig. 3). How then are mitochondria maintained in the male soma and in sperm precursor cells in the absence of this anti-apoptotic signal? There are two possibilities: The first, and simplest, is maternal contribution of the anti-apoptotic signal. The female would deposit in the egg enough of the anti-apoptotic signal to support male development and spermatogenesis; however, the male is incapable of synthesizing the signal. The second possibility is expression of the anti-apoptotic signal (or some compensatory signal) in the male soma and sperm precursor cells—but this requires another pathway for production of the signal and it is not clear why the male would not accomplish the same thing in the sperm. The first model seems most consistent with female control over mitochondrial gene function. Perhaps the most intriguing prediction of this model is that to a significant degree male mitochondrial function and life span in flies (and humans) might be determined by the amount of anti-apoptotic signal that he inherits maternally. A number of Drosophila gene products are maternally supplied in quantities sufficient to perdure and function in the resulting adult animals—as evidenced by maternally rescued mutations (Table 3). These genes are good candidates for encoding the anti-apoptotic signal and include *Sxl* itself, the *Sxl* target gene *daughterless* and the anti-apoptotic gene *Akt1*. Interestingly, Drosophila genes with maternally rescued phenotypes appear enriched on the X chromosome (Table 3), and maternal-effect genes have recently been proposed to participate in sexual conflict in species using ZW sex determination (Miller et al., 2006).

Table 3
Some Drosophila genes with maternally rescued phenotypes[a]

Abbreviation		Gene name	Chromosome
1	abo	abnormal oocyte	2
2	Akt1	Akt1	3
3	arm	armadillo	X
4	cos	costa	2
5	da	daughterless	2
6	Dfd	Deformed	3
7	dl	dorsal	2
8	Dl	Delta	3
9	dpp	decapentaplegic	2
10	dsh	disheveled	X
11	Dsor1	Downstream of raf1	X
12	ecd	ecdysoneless	3
13	fliI	flightless I	X
14	fu	fused	X
15	Gβ13F	G protein β-subunit 13F	X
16	hb	hunchback	3
17	hep	hemipterous	X
18	N	Notch	X
19	ncd	non-claret disjunctional	3
20	oc	ocelliless	X
21	Pu	Punch	2
22	pum	pumilio	3
23	retn	retained	2
24	RpII15	RNA polymerase II 15kD subunit	3
25	Sce	Sex combs extra	3
26	sgg	shaggy	X
27	smo	smoothened	2
28	Stat92E	Signal-transducer and activator of transcription protein at 92E	3
29	Su(fu)	Suppressor of fused	3
30	Sxl	Sex lethal	X
31	tkv	thickveins	2

[a] Genes listed in Flybase with phenotype descriptors matching key words *maternally* AND *rescued* AND *lethal*.

In humans there exists a gene that, like Drosophila *Sxl*, is on only in females and that controls dosage compensation—the *Xist* gene (Fig. 4) (Chow et al., 2005). *Xist* (or some other human female-specific gene) could control an analogous female-specific anti-apoptotic pathway for mitochondrial maintenance. The human female hormone estrogen has anti-apoptotic properties and could be part of such a mechanism (Nilsen and Brinton, 2004; Vina et al., 2005), and interestingly the mitochondrial enzyme 17-β-estradiol dehydrogenase shows up in several model organism studies of aging (Landis et al., submitted).

14. Why is apoptosis the default state for the mitochondria?

A variety of genes, both nuclear and mitochondrial, co-exist in the cell to their mutual benefit and thereby optimize their survival, replication and transmission to the next generation.

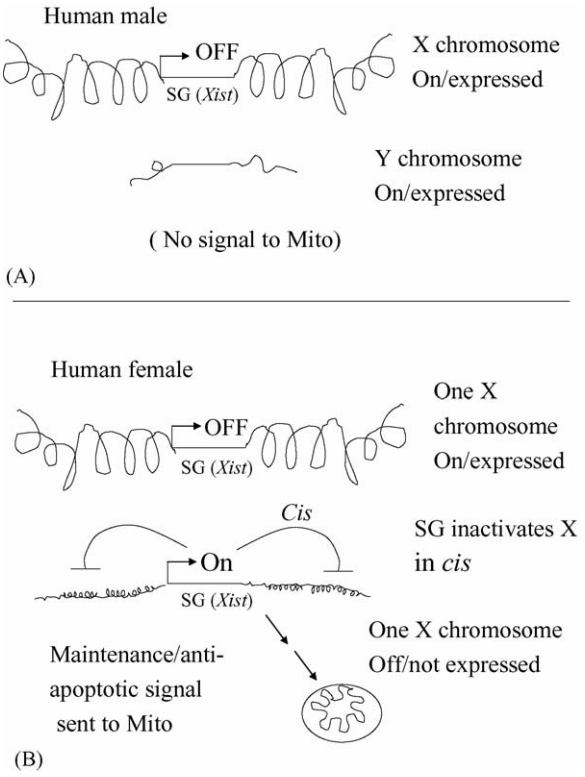

Fig. 4. Diagram of human dosage compensation and proposed sex determination.

Genetic variation and selection are the basis for evolution as we know it. For variation and selection to occur, genes must give rise to new alleles and these alleles must in turn segregate or otherwise re-assort—i.e., come apart and re-unite in different combinations. From the point of view of any given gene in the cell (Dawkins, 1976), it is beneficial for its partners to vary, i.e., leave and return, so that natural selection can act to optimize its set of partners. The genes collaborating in the nucleus have evolved an elegant and rather egalitarian mechanism to accomplish this based on the spindle: independent assortment and recombination. But how do the genes in the nucleus accomplish this segregation relative to the genes in the mitochondria, and vice-versa? Natural selection has acted to create a different mechanism by which the genes in the nucleus and the genes in the mitochondria separate and re-unite over evolutionary time–sex and asymmetric inheritance: in the sense of natural variation and selection, the mitochondrial genes and nuclear genes are together in the female and apart in the male.

Several observations demonstrate that the mitochondria and mitochondrial genomes generally have a shorter functional half-life than does the nucleus or cell. First, mitochondria are known to actively turn over in many non-dividing cell types (Spees et al., 2006). Second, as discussed above, the male germ line initially has cells with abundant functional mitochondria, but ultimately gives rise to cells where the mitochondria are absent or non-functional in terms of inheritance. Clearly modern-day mitochondria are dependent upon the nucleus and cellular milieu for growth and replication, but why should mitochondrial apoptosis (self-destruction) be the default state? From the point of view of the eukaryotic female cell this may be the simplest way to control mitochondrial abundance and gene inheritance—to engineer the mitochondria with an apoptotic mechanism and ration the antidote. In other words, engineering the mitochondria with a shorter half-life and rationing a survival/growth factor. From the point of view of the mitochondria, this may be the simplest way to accomplish two things: first to be maintained in the cell, and second, to be maintained in the cell as an entity separate from the nucleus. In general the current state of affairs can be thought of in terms of game theory as an evolutionarily stable strategy (ESS) for the cooperation of the nucleus and the mitochondria (Nowak and Sigmund, 2004; Burt and Trivers, 2006). As is typical of many game-theory strategies for cooperation, one party (in this case the mitochondria) must sometimes leave or defect. What does leaving or defecting amount to in a biological context? Cellular apoptosis, organellar apoptosis, and asymmetric segregation (a failure to be inherited) seem to be likely mechanisms. With regard to inheritance and function, the mitochondria defects in the male.

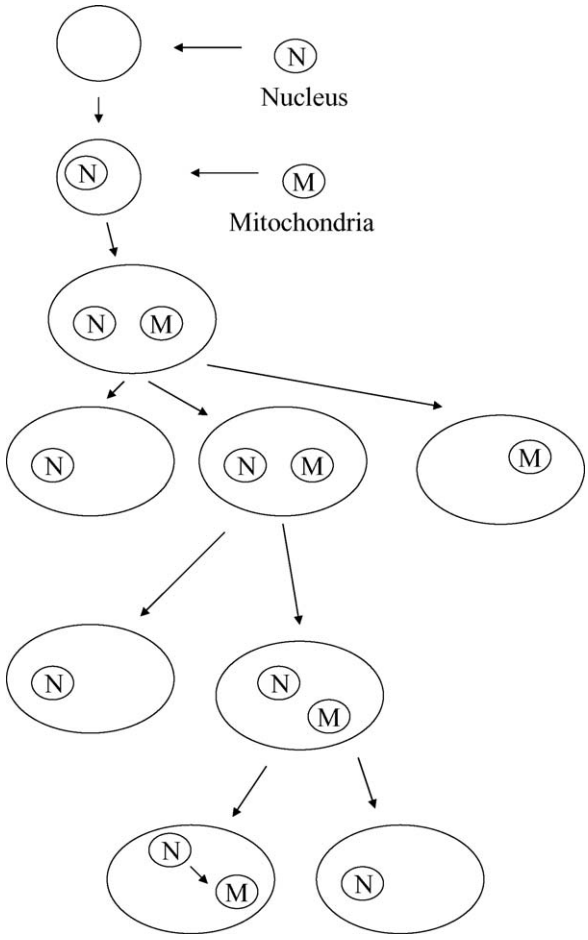

Fig. 5. Invasion of the eukaryotic cell by the mitochondria. When the mitochondria (M) invaded the eukaryotic cell it created competition for inheritance between the mitochondria and the nucleus (N). The only way the M could be maintained is if it provided some advantage to the cell. In turn, the only way M could be maintained as a entity separate from N is to have a finite half life, i.e., be lost at some rate by segregation or apoptosis—this is the same thing as asymmetric segregation. The simplest way to accomplish this is two states in N, one state that prevents M loss, and one that does not.

When the mitochondria invaded the eukaryotic cell (Gray et al., 1999; Lang et al., 1999; Searcy, 2003; Timmis et al., 2004) it created competition between the nucleus and the mitochondria for inheritance, as well as the potential opportunity for mutually beneficial cooperation (Fig. 5). This sets up a situation similar to a ''prisoner's dilemma'' in game theory (Nowak and Sigmund, 2004; Burt and Trivers, 2006): If the mitochondria always stays with the nucleus the nucleus will absorb the mitochondria and it will cease to be a separate, multi-copy entity. However, if the mitochondria sometimes leaves – i.e., is lost at some finite rate, either by segregation or apoptosis – the nucleus must actively maintain the mitochondria and this allows it to remain an independent entity. In other words, shorter half-life and asymmetric inheritance for the mitochondria represents an evolutionarily stable strategy (ESS) for the co-operation of the nucleus and mitochondria. The only way the mitochondria can be maintained as a separate entity from the nucleus is to have a finite half-life, i.e., be lost at some rate by segregation or apoptosis. This is the same thing as asymmetric segregation: there are two states of the nucleus, with functional mitochondria (e.g., egg) and without (e.g., sperm). This requires the existence (or drives the evolution) of two states in the nucleus-one state that prevents mitochondria loss and one that does not (e.g., SG-on/off). In other words, the powerful selective advantage of the mitochondria creates the sex determination gene and chromosome in the nucleus: a successful and continued infection by the mitochondria would require the existence of SG-on/off to establish and maintain the ESS. In this model any asymmetrically inherited gene(s) with a large selective advantage (like the mitochondrial genome) would define the female (more fit), and the male (less fit).

It is possible to see X chromosome hypermutation in the male as an attempt by the male to either activate or destroy the gene (SG) that limits mitochondrial gene inheritance to the female, a process that might in turn be hypothesized to drive the dynamic deterioration and evolution of sex chromosomes (Charlesworth and Charlesworth, 2005; Graves, 2006). Consistent with this idea the gene at the top of the sex determination pathway appears to mutate rapidly and change in identity often through evolution (Graham et al., 2003) (*Sxl* in *Drosophila melanogaster*, *tra* in *Ceratitis capitata*, *Xist?* in humans), and these genes are predicted to exhibit antagonistic pleiotropy and function in regulating life span. A change in the identity of the SG might be a handy mechanism for speciation.

Interesting parallels can be seen between this model and what happens when a largely detrimental genome such as the intracellular parasite *Wolbachia pipientis* infects the Drosophila egg cytoplasm (Fry and Rand, 2002; Starr and Cline, 2002; Fry et al., 2004)—successful infection can be dependent on the particular allele of *Sxl*.

15. Asymmetric segregation of genes as an evolutionary force

The general strategy of finite half-life creating asymmetric segregation could be an ancient and important one in evolution. Consider a primordial gene *A* that encodes a replicator molecule

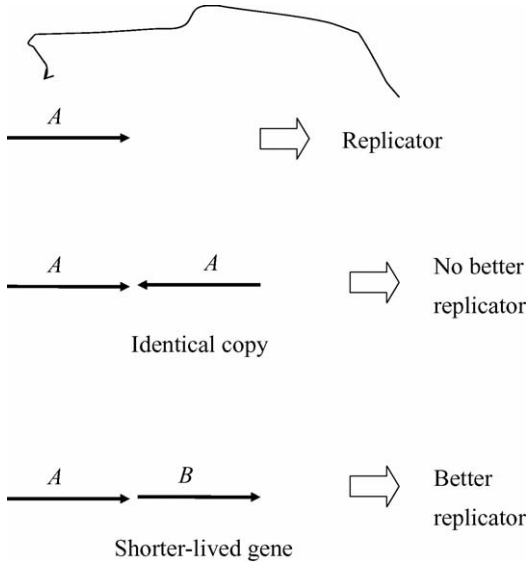

Fig. 6. Genes and replicators. Gene *A* encodes a single-subunit replicator that replicates gene *A*. A perfectly identical and symmetric copy of gene *A* has no selective advantage because it encodes the same replicator (by definition). One way a second gene *B* (such as an imperfect copy of *A*) can be maintained as a separate entity is if it has a shorter half-life than *A* (i.e., is lost at some rate). By definition this creates two states for *A*: *A* alone and *A* + *B*, which is the same thing as asymmetric segregation. To be maintained *A* + *B* must encode a better replicator (i.e., have greater fitness).

that replicates gene *A* (Fig. 6). *A* and its product might be floating around free in the primordial soup, or be surrounded by the membrane of a proto-cell (Szathmary, 2000; Hogeweg and Takeuchi, 2003; Scheuring et al., 2003; Line, 2005). Another gene *B* could cooperate with and be linked to *A* (either covalently or by inclusion in the same cell) but to be selected for and maintained *A* + *B* must have greater fitness than *A* alone, such as by encoding a better replicator. It is easy to see how this might work, but if *A* and *B* are always linked together they are not separate genes. How can *A* and *B* cooperate yet still exist and evolve as separate entities? As mentioned above, for evolution to occur, a genes' partner(s) must somehow vary as a function of time. One simple way for this to be accomplished is if gene *B* has a shorter half-life than *A* (i.e., is lost at some finite rate, i.e., ages). By definition this creates two states for *A*: *A* by itself and *A* + *B*.

In summary, a beneficial new gene with a shorter half-life by definition creates asymmetric segregation, and asymmetric segregation by definition creates increased complexity of the system. This ESS model suggests that finite half-life (aging or senescence) is the consequence of natural selection for increased complexity (evolution).

Is there any evidence that genes exist in such an ESS today? It is interesting to note in this regard that the gene sequences with the longest half-lives (i.e., most conserved through evolution) include many polymerases, translation components, motor molecules and transporters—perhaps representing the ancient master replicators. In contrast the most rapidly evolving genes include ones involved in reproduction, especially male gametogenesis (Good and Nachman, 2005; Nielsen et al., 2005; Richards et al., 2005). It also seems possible that the DNA-end replication problem (Olovnikov, 1973; Ohki et al., 2001)

represents a strategy by which the (ancient) DNA polymerase gene ensures that more distal genes on the chromosome have a shorter half-life.

16. The mitochondrial apple?

In Biblical history the snake tempts Eve into eating an apple from the forbidden tree of knowledge. Adam and Eve become aware of their nakedness and in retribution God casts them out of the Garden of Eden forever. When the proto-eukaryotic female ingested the highly beneficial mitochondrial genome and maintained it through asymmetric inheritance, she introduced an asymmetry in fitness between the sexes. The resultant antagonistic pleiotropy of gene function between male and female helped drive the evolution of multicellularity and ultimately self-awareness, but came at a cost of aging phenotypes and limited life span.

Models for the co-evolution of sex and asymmetric inheritance are not new, and include fascinating ones where sperm dynamics represent the vestiges of the movement of the mitochondria's Rickettsia-like ancestor from one cell to another (Fabrizio et al., 1998; Bazinet and Rollins, 2003; Bazinet, 2004). Space considerations preclude discussion of prokaryotic toxin/antitoxin systems (Gerdes et al., 2005) which seem eerily similar to the systems for mitochondrial inheritance discussed here, or Honeybees—where expression of mitochondrial genes distinguishes the long-lived Queen from the genetically identical short-lived workers (Corona et al., 2005).

Apoptotic cell death is implicated in many human aging-related diseases, such as Alzheimer's disease and Parkinson's disease. However, apoptosis has sometimes been discounted as a likely species-general mechanism of aging based on the lack of detectable apoptotic cell death in old *C. elegans* and the lack of effect of critical apoptosis genes such as *ced-3* caspase on *C. elegans* life span (Garigan et al., 2002; Herndon et al., 2002). The current results suggest that those conclusions should be re-examined in light of the fact that *C. elegans* is a hermaphrodite, and predict that apoptosis might limit life span in *C. elegans* males.

In the past I have generally assumed the fact that mitochondria and oxidative stress are so consistently implicated in life span regulation (Fridovich, 2004) must mean that oxidative damage is inherently more toxic than the many other damages and challenges cells suffer over time. However, after researching this article I conclude the reason is because mitochondrial genes are asymmetrically inherited and therefore mitochondrial functions are prone to antagonistic pleiotropy. I wonder what aging would look like if it were the Golgi genes or spindle genes that were asymmetrically inherited instead of mitochondrial genes? Perhaps oxidative stress and mitochondrial malfunction would not be so relevant.

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