CHAPTER 5

Genetics

5.1. INTRODUCTION

Genetic influences on aging and longevity are now added to the discussion of environmental influences on nutrition and host defense mechanisms from prior chapters. Arterial disease, diabetes, obesity, and Alzheimer disease may be modified by some of the same alleles of lipoprotein and cytokine genes. In some respects, inflammatory processes in these chronic diseases are amplifications of processes ongoing throughout life in the absence of specific diseases. Environmental causes of infectious and inflammatory processes and pharmacological interventions may also be sensitive to allelic variations. Diet restriction (DR) and obesity-diabetes represent opposing degrees of inflammation. DR shows anti-inflammatory effects whereas obesity and diabetes are proinflammatory. In each condition, the relation to inflammation alters the progression of arterial and Alzheimer disease in rodent models. Developmental influences on adult disease processes include nutrition and infection. Enhanced fetal growth from maternal obesity and diabetes is increasingly recognized in association with insulin-like growth factors that are also gene candidates for longevity. The fly and worm mutants give genetic clues to processes that broadly regulate development and aging. Before delving into details of these gene variants, I outline a perspective about the limitations of genetic determination in the phenotypes of aging and then consider sex determination, which is the largest genetic effect on human longevity.

5.2. SOURCES OF INDIVIDUAL VARIATIONS IN AGING AND LIFE SPAN

The genetic analysis of life span has recognized the extensive non-heritable individual variations within a species. In the inbred fly, mouse, and worm, the heritability (V_H) of life span ranges 20–50% (Finch and Tanzi, 1997; Finch and Kirkwood, 2000). Twin V_H for life span is about 25% (Skytthe, 2003; Christensen et al, 2006). According to the hypothesis that the reproductive schedule is a major factor in the evolution of life span (Section 1.2.8), it is cogent that age at menopause shows a higher range of V_H, 45–86% (Towne et al, 2005; Kirk et al, 2001; Murabito et al, 2005; de Bruin et al, 2004). Other life history traits fall closer to the V_H of lifespan: age at menarche,V_H of 45–50% (Towne et al, 2005; Kirk et al, 2001; Snieder et al, 1998). Birth weight, which is a determinant of adult health (Chapter 4.10) has V_H 33–46% (Svensson et al, 2006), slightly less than menarche. The balance of the variance in life span, 50–80%, is usually considered 'environmental (V_E), and attributed to chance variations from external and internal factors.

Even in the highly protected lab environment, worms and other experimental models display great individual variability in aging. In a theory developed with Tom Kirkwood, a portion of the non-heritable variance in life span arises from chance developmental variations (non-heritable constitutional variability, V_C) (Finch and Kirkwood, 2000, p. 11; Kirkwood et al, 2005). For example, highly inbred self-fertilizing worms hatched in the same culture dish differ individually (constitu-

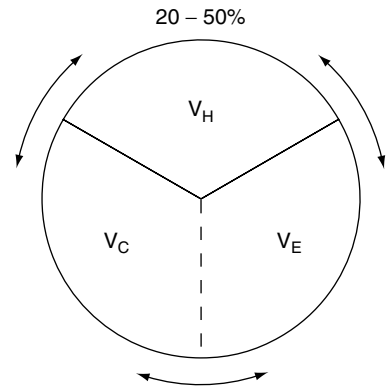

FIGURE 5.1 Variance in life span may be partitioned into three components: V_H, V_E, and V_C (Finch and Kirkwood, 2000, p. 11). My original figure. The heritable component of life span V_H is 20–50% in flies, worms, and human twins (Finch and Tanzi, 1997). The majority of the variance in individual life span is non-heritable and may be partitioned as mortality due to the external environment V_E (environment in classical quantitative genetics) and V_C (random individual constitutional factors that arise from chance variations at a cell and molecular level). An unknown proportion of the non-heritable variations is due to developmental noise. The phenotype also diversifies during aging through structural DNA changes in somatic cells (mutations, telomere shortening) and epigenetic changes (loss of imprinting, derepression) that progressively accumulate during aging.

tionally) in the rates of feeding, locomotion, and egg laying, and in their life spans, over a 2- to 3-fold range. Surprisingly, the coefficient of variation[1] for worm life span (34%) in the homogeneous culture dish is almost 2-fold larger than for identical twin life spans (19%) in the open world (Finch and Kirkwood, 2000, p. 15). We do not have good estimates of the random developmental contribution to life spans because of interactions between V_C and V_E (Fig. 5.1). The classic quantitative genetics model subsumes V_C under V_E (Falconer, 1996),[2] which may obscure important independent classes of interactions with V_H and V_E. Nonetheless, individual genotypes determine the range of reactions and responses.

[1]The coefficient of variation (COV) is a dimensionless number that normalizes the variance to the mean. For life span, *X*, the COV is calculated as the standard deviation, *SD/X*.

[2]"[L]arge amount of variability is . . . due neither to heredity nor to tangible environmental conditions" (Wright, 1920, p. 328). "Some of the intangible variation . . . may arise from 'developmental' variation . . . which can not be attributed to external circumstances, but is attributed . . . to accidents or errors of development" (Falconer, 1996, p. 135).

Chance external factors include accidents, infections, poor diet, predation, weather, etc. (Fig. 5.1). Chance internal factors include bystander damage from free radicals to cells and molecules, chance variations in cell numbers during development, and stochastic variations in gene expression. For example, chance variations in a worm heat shock gene transcription (hsp-16.2) present in young worms predict individual variations in life span, over a 2-fold range (Rea et al, 2005). The molecular origins of these differences could involve random variations in the assembly of multi-component transcription factors and their movements on DNA, which are described by the stochastics of diffusion (Brownian motion) (Finch and Kirkwood, 2000, pp. 58–65; Chang et al, 2006; Hu et al, 2006).

Variations of neuronal connections arise during development in *C. elegans*, despite the invariance of cell numbers as shown in two detailed studies. Motor-interneurons "might have gap junctions in one animal and chemical synapses in another . . . often synapses on the dorsal side of the nerve ring appear more sloppy." (Albertson and Thompson, 1976, p. 319). I suggest that the individual variations in pharyngeal pumping and food ingestion at hatching could arise from such neurodevelopmental variations in the pharynx. Some worms may be developmental phenocopies of the *eat* mutants described below, which have different pharyngeal pumping rates that correlate with life span. The ventral nerve cord also shows individual variations in dendritic processes and synaptic connections that could influence locomotion (White et al, 1976). Advances in optical techniques can access this level of variation in cell ultrastructure and gene expression over the life span of individual worms.

Mammals also have individual variations in cell numbers that influence outcomes of aging (Finch and Kirkwood, 2000, pp. 83–91). An instructive example is ovarian cell variations, which impact on reproductive senescence and related pathology. Ovaries of inbred mice vary 3-fold between individuals at birth in the number of primary follicles and oocytes (Gosden et al, 1983). Thus, inbreeding does not suppress individual variability of reproductive and other phenotypes (Phelan and Austad, 1994). The variations within a strain approximate the variations between strains in the average oocyte number and in the timing of reproductive senescence (Finch, 1990, p. 330; vom Saal et al, 1994). Human ovaries from unrelated stillbirths had the same COV as inbred mice (Finch and Kirkwood, 2000, p. 24). The ovarian oocyte pool was shown to determine the age of reproductive senescence in elegant experiments with graded partial ovariectomy (Nelson and Felicio, 1986). Variations in age at menopause also have a non-heritable component. The heritability of coronary disease and osteoporotic fractures could include a component from ovarian cell variations during development, which determines the number of estrogen-producing follicles surviving to mid-life. Recall from Section 2.10.4 that estrogen may be protective for atherosclerosis. Similarly, the number of follicles at birth may determine the risk of later-life genito-urinary tract infections, because the composition of microbial flora is highly sensitive to estrogen deficits (Devillard et al, 2004; Heinemann and Reid, 2005; King et al, 2003).

Developmental variations in the cerebral arterial bed warrant attention in relation to stroke damage. Identical twins can be discordant for cerebral aneurysms (Astradsson and Astrup, 2001; Puchner et al, 1994). Inspection of the angiograms of the non-involved parts of the carotid gives the impression of considerable variation in branching. A similar range of variations is seen in sketches of the orbito-frontal arteries of unrelated individuals (Whitaker and Selnes, 1975). Hypothetically, the same thrombosis in the varying sub-branches would cause different degrees of infarction and circulatory blockage in these individuals. A topological analysis of twin angiograms for branching properties (Pries et al, 1996) might give further insights into cognitive difference between twins, currently attributed variations in neuronal circuit architecture. The extensive differences in onset age of Alzheimer disease could also have a component in the arterial bed architecture: Identical twins in concordant pairs differ by an average of 4.5 years in dementia onset, with the range up to 16 years (Gatz et al, 1997).

The levels of adipose tissue at birth also vary between individuals. Differences in the expression of the imprinted genes MEST and BMP3 in fat depots of C57BL/6J mice predict the level of obesity in response to high fat diets (Koza et al, 2006). Besides the variations in gene expression that may arise autonomously, as in the heat shock gene of worms described above, mammals with multiple fetuses show an additional source of variation from fetal neighbor interactions. As analyzed by vom Saal, the sex of the neighboring fetus influences sex steroid levels during development that account for individual differences in reproductive and neuroendocrine system development (Finch and Kirkwood, 2000, pp. 158–171; vom Saal, 1989; vom Saal et al, 1994). The 3-fold differences between identical twins in weight gain on high fat diets (Bouchard et al, 1990) could represent other fetal interactions.

Adding to these developmental variations, individuals further differentiate during aging due to epigenetic drift or somatic cell variations in an individual from changes in the levels of gene expression (Martin, 2005). The imprinted *Igf2* autosomal gene, which is repressed in young female mice (Section 4.9), becomes activated during aging with tissue specificity (Bennett-Baker et al, 2003). The X-linked *Atp7a* showed similar progressive activation. These changes are not correlated between tissues in the same animal, implying that they arise sporadically (cell autonomously) in each tissue. DNA methylation, which is a major mechanism of imprinting, is also altered during aging. Blood leukocytes of identical twins show much greater differences in DNA methylation with aging in CpG islands in the promoter of C14orf162 and in chromosomal methylation patterns (Fraga et al, 2005). The differences with aging were greater in twins that had different lifestyles and environments. Mosaic features of somatic cell aging are also shown in the diploid cell models of senescence (Chapter 1). The scale of these epigenetic changes may be 100-fold greater than somatic DNA mutations, as discussed by Bennet-Baker et al (2003).

I'm sure to have tried the patience of some readers in deliberating on individual variance before addressing the proper 'genetics' of aging. But the genetics

of longevity must consider the different types and levels of variance in the interactions of gene-environment (G *x* E) and gene-gene (G *x* G) during aging. These interactions are qualitatively different than in younger organisms because of two major postnatal divergences in the genome: 'epigenetic drift,' as just discussed, and DNA structural instability. Somatic cell genotypes diversify within each individual during aging because of DNA instability in mitochondria and chromosomes, and especially in the telomeric shortening that also alters gene expression (Bahar et al, 2006) (Chapter 1). These findings suggest that genetic influences have some intrinsic upper limit due to epigenetic and structural variations in individual genomes during aging that would arise even if the environment was ideally invariant.

5.3. SEX DIFFERENCES IN LONGEVITY

In humans, the sex chromosomes are *the* major genetic determinant of longevity differences within populations. This simple fact is often overlooked in discussions of genetic influences on aging. In most countries, women have greater life expectancy (LE) (Gavrilov and Gavrilova, 1991), whether measured at birth (total LE), or LE after age 1 (Teriokhin et al, 2004), or as years lived in full health (DALE, or disability adjusted LE equivalent to the 'healthy life expectancy') (Mathers, 2001). Currently, women live an average of 3.9 y longer than men (late 20th century global average from 227 countries and territories) (Mathers et al, 2001). The human female has lower mortality during all phases of life (Crimmins and Finch, 2006b; Newman and Brach, 2001; Suthers et al, 2003).

In general, the female advantage scales in proportion to the mean (Fig. 5.2A), which plots male and female life spans against the mean life span of both sexes for each country (Teriokhin et al, 2004). Some extremes are shown in Table 5.1 and Fig. 5.2B, C. At the low extreme, Zimbabwean women live 2.81 y *less* than men. At the high extreme, Lithuanian women live 12.05 y longer. African men had only 37 y of health (DALE), whereas western European women averaged 70 y, a 2-fold difference (Mathers et al, 2001). For populations with life expectancies >80 y, women live ≥ 5 y longer.

The female advantage is present at birth and holds across most ages, as shown for female:male mortality ratios in Sweden, mid-18th century to present (Drevenstedt et al, in prep.) and in many other countries (Gravrilov and Gavrilova, 1991; Horiuchi, 1999; Teriokhin et al, 1994). Despite fluctuations, some explicable by wars, the baseline age-specific sex mortality ratios are soon regained. The Gompertz acceleration of mortality (ascending line after age 40) has a relatively constant slope across these historical Swedish periods, despite the 10-fold range of mortality at each age (Fig. 2.7) (Drevenstedt et al, in prep.). Although other countries and periods may show greater variations in the Gompertz slope (Gavrilov and Gavrilova, 1991), in nearly all populations the female mortality curves are displaced to lower values. That is, aging in demographic terms appears to begin later in women.

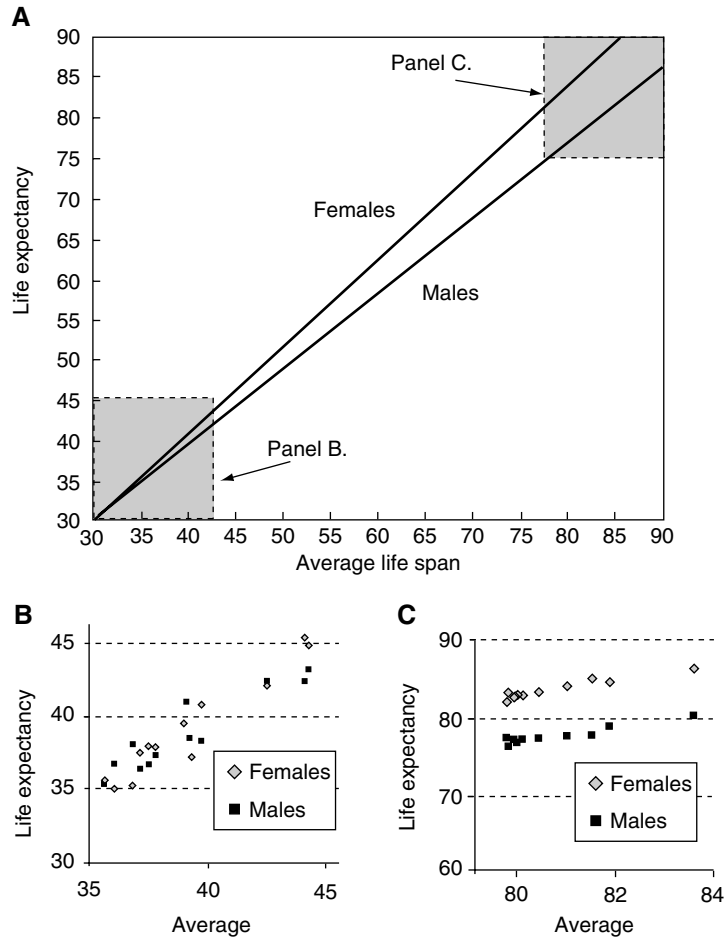

FIGURE 5.2 Human life spans from global average from 227 countries and autonomous territories (late 20th century public data). Redrawn from (Teriokhin et al, 2004). Calculated as life expectancy after age 1. A. The female advantage of life span increase with greater life expectancy. Male and female life expectancy graphed against the mean life span (unweighted mean of both sexes, male-female mean life span half-sum). B. Life expectancy <45 y: Female:male ratio 1.003 ± 0.014; calculated from data in (Teriokhin, et al, 2004). C. Life expectancy >79 y: Female:male ratio 1.086 ± 0.004.

TABLE 5.1 Sample of Extreme National Demographic Profiles

	Life Span (LS) Calculated as Life Expectancy After age 1 Year[a]			Environmental Mortality per Year (Gompertz-Makeham Coefficient, *A*)			Healthy Life Span (disability Adjusted Life Expectancy, DALE) Average of Both Sexes[b]
	Female	Male	Difference/ Ratio	Female	Male	Difference/ Ratio	
Andora	86.61	80.62	5.99/1.07	0	0	0.0004/5.00	72.3
Japan	84.28	77.76	6.52/1.08	0	0.001	0.0001/1.10	74.5
Lithuania	75.69	63.64	12.05/1.19	0	0.006	0.0025/0.59	64.1
Sweden	82.66	72.22	10.44/1.14	0	0.001	0.0005/1.45	73
U.S.A.	80.25	74.55	5.70/1.08	0	0.002	0.0002/1.08	70
Gambia	56.39	52.45	3.94/1.08	0.011	0.0116	−0.0005/0.96	48.3
Malawi	37.57	36.5	1.07/1.03	0.015	0.015	−0.0001/0.99	29.4
Mozambique	35.1	36.76	−1.66/0.95	0.025	0.023	0.002/1.09	34.4
Zambia	37.97	37.4	0.57/1.02	0.023	0.0224	0.0006/1.03	30.3
Zimbabwe	35.31	38.12	−2.81/0.95	0.025	0.0218	0.0032/1.15	32.9

a. Examples reformatted from (Teriokhin et al, 2004). The environmental mortality is calculated as the Makeham term M in the Gompertz-Makeham equation for mortality as a function of age x: $m(x) = M + I\exp(\alpha x)$, where the age-independent first term M represents environmental causes of death, I is the initial mortality rate, and α is the Gompertz constant of mortality rate acceleration (Section 1.2.1, Table 1.3). Sex differences in life span are explained by sex differences in A (Teriokhin et al, 2004), which approximates the initial mortality rate I (Table 1.3) and (Finch, 1990, pp. 663–666). The Gompertz slope coefficient α for birth cohorts is relatively stable across human environments (Finch and Crimmins, 2004; Jones, 1956) (Fig. 2.7A).
b. (Mathers et al, 2001).

The female advantage in life expectancy at birth in industrialized countries was about 2–3 around 1900, with further progressive increases. However, later in the 20th century, the female advantage shrank in some countries (Horiuchi, 1999). These trends are attributable to changes between the sexes in behavioral risks, mostly cigarette smoking, but also to alcohol consumption and occupational hazards (Horiuchi, 1999; Pampel, 2002; Waldron, 1985). Nonetheless, the female advantage culminates in the dominance of women among centenarians (Robine and Vaupel, 2001; Robine et al, 2006). The growth of the oldest age group beyond 90 is a demographic novelty that emerged after 1850 and continues growing remarkably in health-rich populations. Centenarians are increasing exponentially, doubling every 6 years, faster than any other age group. Sex differences of life span in late 20th century countries are strongly associated with sex differences in the Gompertz equation 'initial mortality rate' coefficient, I (Table 5.1, legend; Table 1.3) (Teriokhin et al, 2004). The female advantage persists across the 20-fold range of environmental mortality in different countries (Gompertz-Makeham coefficient M) (Table 5.1).

However, at life spans <45 y, the female longevity advantage is less robust and wobbles (Fig. 5.2B) (Table 5.1). The age 45 cut-off is crucial to under-

standing the evolution of the human life span, because life spans are typically 30–40 y (Fig. 1.1A) in pre-industrial countries (Oeppen and Vaupel, 2002) and in the remaining small groups of forager-hunter gatherers (Gurven and Kaplan, 2007). For example, Ache foragers living in the forest before contact had lower female mortality and longer life expectancy at age 20 (female 39.8 y, male 34.2 y) (Hill and Hurtado, 1996, p. 196), but few other groups have been described. Birth rates are also much higher in these health-poor populations (Easterlin and Crimmins, 1985; Teriokhin et al, 2004), where infections are major causes of death. Only when early mortality from infectious disease has been greatly diminished do we see large proportions of adults surviving to post-reproductive ages, with increasing female advantage in longevity. Evidently, the lower prevalence of infections in industrialized nations has diminished a major 'natural' cause of mortality. Thus, we may consider that the recently growing female advantage at later ages is a post-Darwinian phenomenon that cannot be attributed to natural selection of genotypes for superiority in reproduction. However, health-poor populations with high mortality remain under greater Darwinian selection because of highly prevalent gastro-enteritis, HIV, malaria, worms and other parasites, TB, and other chronic and acute infections that are major causes of mortality (Chapter 4).

Gender differences in mortality are seated in the genetics of sex determination. The male phenotype depends strictly on the Y-chromosome sex-determining locus (SRY), which encodes a transcription factor required for testes development (Barsoum and Yav, 2006). In the absence of a fetal testis, mammalian female organs develop as the default phenotype. Of the genes carried by the two X chromosomes present in females, one of the two alleles is usually randomly inactivated during development (X-inactivation is different from gene imprinting; Section 4.10). However, about 15% of X-linked genes escape inactivation and are expressed to some extent in adult cells (Carrel and Willard, 2005). Thus, female and male genomes differ in the following major attributes: Y-chromosome genes that are absent from the X chromosome; the heterozygote advantage of women for harmful recessive alleles that are not offset in males; and, the expression in women of both copies of some X-linked genes.

Sex differences in postnatal mortality are not obviously explained by sex hormone levels and involve the cultural complexes described as gender differences. The prenatal sex difference in mortality is less subject to cultural influences because the fetal sex is not clearly recognizable by the mother without modern technology. However, ultrasonic imaging indicates greater male wakefulness (Robles de Medina et al, 2003) and earlier female oral-motor development (Miller et al, 2006a). Humans experience irreducible gender effects on mortality risk through their social roles and status at all ages. Gender-dangers include childbirth in women and fighting in males. Independent of these risk situations, sex differences are recognized in morbidity that are the result of differences in organ structure during development. Osteoporotic fractures begin to increase during aging 5–10 y earlier in women than in men. Why heart

disease mortality also lags behind in women is still obscure, despite sex differences in many vascular event risk factors, including blood lipid and blood pressure regulation. Males are more vulnerable to infections in many studies. During the first year, male infants were 20% more likely to die from infections than females (U.S. births, 1983–1987) (Read et al, 1997). Other studies, pro and con, are summarized by (Wells, 2000). Whatever the 'intrinsic' role of biological sex, the gender issues in health from culture and behavior are always present and hard to resolve, especially in health-poor populations. Despite these uncertainties, we may anticipate a convergence of the physiological and genetic basis for the sex differentials in mortality.

Many other species have major sex differences in life span (Finch, 1990; Kirkwood, 2001; Tower, 2006). A comprehensive analysis of more than 1000 groups of rodents showed 5% greater maximal longevity of the female in rats, but no sex difference in mice (Rollo, 2002). Sex differences in longevity may also be influenced by the resistance to infections: In general, female rodents survive most pathogenic infections better than males (Pasche et al, 2005). Infections in the early era of husbandry may have contributed to some sex differences in life span of mouse strains (Storer, 1966). Among five species of *Drosophila*, the females had small differences in mortality acceleration rates (Gompertz slopes), whereas male interspecific differences include both the slope and baseline mortality rate (Promislow and Haselkorn, 2002). Metabolic rates differ 3-fold between species and tend to be higher in the females, but do not correlate highly with life span. Sex effects on age-specific mortality differ between fly lines and are influenced by population density (Khazaeli et al, 1995).

Sex differences in rodent and fly mortality are associated with reproductive stresses (costs) (Chapman et al, 1998; Phelan and Rose, 2005) that are minor in modern human populations (Aiello and Key, 2002; Hrdy, 2000). As predicted by evolutionary theory. Virgin flies live longer than mated females, even though egg production was unaltered (Fowler and Partridge, 1989). Fighting during mate selection increases male mortality, while production of large numbers of eggs is energetically costly. In the worm, germ cell ablation increased life span, discussed below (Fig. 5.4), whereas in the fly, germ cell ablation may decrease life span (Barnes et al, 2006). Of course, the absence of germ cells can alter endocrine functions in other ways. Generalizations are premature.

Many sex-specific cases of trade-offs in immunity and reproduction can be interpreted in terms of antagonistic pleiotropy (Tower, 2006). Contact with seminal fluid shows a trade-off in flies; while being toxic to females, seminal fluid prevents mating with other males. One toxin in seminal fluid is the protease inhibitor Acp62F (Wolfner, 2002). Seminal fluid also contains antimicrobial peptides that may protect from venereal transmitted infections, as seen in other insects (see below) (Khurad et al, 2004). Sex differences in nutrition-infection interactions may be anticipated, because of sex differences in response to diet restriction in flies (see below, Fig. 5.6) and the sex differences in vulnerability to infections discussed above. The selective vulnerability of male flies in *Drosophila*

bifasciata and *D. psuedoobscura* to the bacterium *Wolbachia* might be a good model (Veneti et al, 2004). Many fly strains carry this rickettsial bacterium as an intracellular symbiont, which is transmitted maternally. *Wolbachia* may participate in reproductive isolation mechanisms in natural populations, mediated by male-specific mortality.

Furthermore, transgenic manipulations of life span are influenced by the sex of the genetic host in fly strains and differ by background genotype. Targeted overexpression of human superoxide dismutase (SOD) in fly motorneurons generally increased life span, with sex-specific effects that differed between long-lived wild-caught fly strains (Landis and Tower, 2005; Spencer et al, 2003). Insulin-producing neurons contribute to the sex differences in motor activity (Belqacem and Martin, 2006). Some mutants that inhibit insulin-like pathways increase the female life span more than the male (Tu et al, 2002a).

5.4. METABOLISM AND HOST-DEFENSE IN WORM AND FLY

Metabolic genes influence longevity from single-celled yeast to worms, flies, and mice (Fig. 1.3A) (Kenyon, 2005; Finch and Ruvkun, 2001; Longo and Finch, 2003). Many of these genes belong to systems of hormone signaling, including insulin-like peptides, insulin-like growth factors (IGF), and sterols. Variants of insulin-like genes also influence inflammation. In some cases, these hormones enhance survival during states of reduced metabolism, which are coupled to increased stress resistance. Depending on the species, development may be arrested in hypometabolic states.

5.4.1. Metabolic Gene Signaling

All animals have multiple insulin-like peptides that mediate growth during development and postnatal metabolism and feeding behaviors (Nassel, 2002; Wu and Brown, 2006). The invertebrate insulins are almost exclusively neurosecretions, whereas vertebrate neuronal insulin expression is minor relative to pancreatic ß-cells. IGF-1 is widely expressed in brain, liver, and other tissues (Bondy and Cheng, 2004). The invertebrate insulins have many specialized functions, and some conserved activities in glucose regulation. The fly has glucose receptors in the corpora cardiaca, which receives insulin as a neurosecretion and responds to hypoglycemia, as does the pancreas (Kim and Rulifson, 2004). Genetic ablation of the insulin-secreting medial neurosecretory cells in fruit flies increased hemolymph glucose 3-fold, as expected (Broughton et al, 2005).

Current models of insulin/IGF-like signaling in nematodes include the sequence of biochemical steps from the insulin-like receptor DAF-2 to the transcription factor DAF-16 (Fig. 5.3). Sensory neurons secrete an insulin-like peptide that activates an insulin-like receptor (DAF-2), followed by a phosphorylation cascade through phosphatidylinositol (PI) from AGE-1 (a PI-3OH kinase, Fig. 1.3). After further steps,

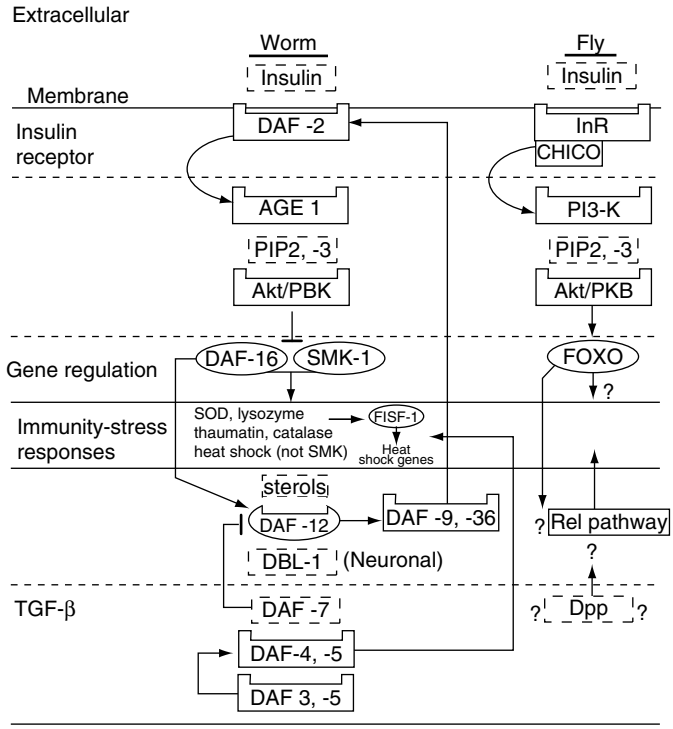

FIGURE 5.3 Metabolic signaling and immunity in worm (*Caenorhabditis*) and fly (*Drosophila*), based on mutant effects. Adapted from graphs and information in (Gerisch, 2004; Hertweck, 2004; Kurz and Tan, 2004; Libina et al, 2003; Mak and Ruvkun, 2004). Note that most of these predicted pathways are based on mutant effects and the orthologous proteins have not been isolated and analyzed biochemically. Both fly and worm have insulin-like peptides which activate insulin-receptors with ensuing kinase cascades and downstream effects on transcription. These pathways are known in more detail for worm than fly. The TGF-β pathway in worm (DAF-7 peptide) converges with insulin signaling and sterols at DAF-12. The fly orthologue of TGF-β is the Dpp peptide in fly, which interacts with the Rel pathway during early development. Because Rel/NfKB homologues are major transcriptional regulators of immune genes in the fly, it is plausible to consider Dpp-Rel interactions in adult immunity and aging. The GATA transcription factor (not shown here) regulated the expression of anti-microbial peptide genes independently of the Toll system (Senger et al, 2006).

the transcription factor DAF-16 translocates to the cell nucleus to modulate genes that are hypothesized to enhance longevity by increasing stress resistance (Morley and Morimoto, 2004). The phosphorylation of DAF-16 inhibits its translocation to the nucleus, e.g., in loss of function mutant AKT-1 kinases. Mutants impairing DAF-

16 activation are shorter-lived. DAF-16 nuclear translocation is highly sensitive to a narrow range of phosphorylation so that genetic defects can have widely different consequences ranging from extended longevity to developmental arrest (Imae et al, 2003). Other longevity mutants are found in the sirtuins, mTOR, and other gene systems that may serve as intra-cellular sensors of individual cell energy.

Despite this great progress, only one invertebrate insulin has been isolated and directly studied. An insulin cross-reacting peptide was semipurified from the brain of *Calliphora* (blowfly) and acted like vertebrate insulin in increasing glucose uptake in two assays: *in vitro* glucose uptake by rat fat cells and *in vivo* uptake from *Calliphora* hemolymph (Duve et al, 1979; Duve et al, 1982). Unexpectedly, in fly embryo cells, human insulin inhibited glucose uptake and glycogen synthesis but stimulated the pentose phosphate pathway (Ceddia et al, 2003). No worm insulin has been isolated and directly tested for functions. The mechanisms deduced for all the worm and fly longevity mutants by indirect evidence may be revised as the biochemistry becomes further developed.

Relationships among the ancient metabolic systems of insulins, sirtuins, and target of rapamycin (TOR) are outlined in Fig. 1.3A and Fig.3.9. Additionally, the AMP kinases (AMPK), the Per-Arnt-Sim protein domain kinases (PASK) and the hexosamine pathway could be involved because of their sensitivity to glucose and other nutrients. The yeast 'silent information regulator-2' ('sir-2') gene family (whence *sir-2–uins*) was recognized to repress the mating-type loci and extend the reproductive life span (Hekimi and Guarante, 2003). Sirtuins are NAD+-dependent deacetylases with many protein substrates (Blander and Guarante, 2004). The NAD-dependence couples sirtuin activity to cell nutritional status through the [NAD]/[NADH] ratio (Lin and Guarante, 2003). SIR2 increases chromatin condensation (heterochromatinization) by deacetylating local histones and interacting with proteins bound to 'silencer' DNA sequences (Blander and Guarante, 2004; Rusche et al, 2003). SIR2 is induced by resveratrol (a polyphenolic sirtuin activator and anti-oxidant) (Cohen et al, 2004; Longo and Kennedy, 2006). Resveratrol also shows anti-fungal activity, consistent with its presence in grapes (Jung et al, 2005; Sinclair, 2005), and is an immunosuppressant in transplantation experiments. Sirtuin activators are referred to as STACs.

SIR2 may have different roles in the longevity model of yeast aging (Section 1.2.6) (Longo and Kennedy, 2006). A current controversy concerns the role of SIR2 in the increase of yeast life span by diet restriction. SIR2 functions differ between the experimental yeast model (replicative senescence vs. aging; Section 1.2.5), which may be due to the different levels of media glucose used by the experimenters.

Human SIRT1 deacetylates histones and a remarkable range of key regulators, including ku70 and other mediators of apoptosis and DNA repair, the transcription factors FoxO[4] and p53, and even cytoskeletal α-tubulin (Blander and

[4]FoxO (forkhead) genes have alternate names: FoxO1, also FKHR; FoxO3, also FKHRL1; FoxO4, also AFX. FOXO is the mammalian forkhead orthologue of worm DAF-16.

Guarante, 2004; Borra et al, 2004). FoxOs regulate glucose metabolism and resistance to oxidative stress. FoxO is acetylated by CBP (cAMP response element-binding protein-binding protein) and deacetylated by SIRT1, which coactivates FoxO1 (Daitoku et al, 2004). The FoxOs respond to diet and insulin; e.g., fasting increased FoxO4 by 35% in muscle (Imae et al, 2003). FoxO3 and FoxO4 increased in muscle of aging rats (Furuyama et al, 2002).

TOR (target of rapamycin) acts as a nutrient sensor in mammalian and yeast cells (Gray et al, 2004; Long et al, 2004; Lorberg and Hall, 2004; Powers et al, 2006a; Proud, 2004). Rapamycin is a natural antifungal and immunosuppressing antibiotic. TOR nutrient sensing depends on its complex with RAPTOR (regulatory associated protein of TOR). Deficits of leucine or other amino acids activate TOR and in turn attenuate protein synthesis and metabolic quiescence. Rapamycin-treated cells appear starved. TOR kinase inhibits protein synthesis by phosphorylating the elongation factor (eEF2), which is activated by insulin. In flies, TOR signaling through elongation factor binding protein (4E-BP) also acts as a metabolic brake to control fat metabolism (Teleman et al, 2005). Unlike the fly, the worm *C. elegans* is insensitive to rapamycin. This insensitivity may be adaptive because rapamycin is produced by a soil bacterium *Streptomyces hygroscopicus* (Long et al, 2002).

5.4.2. Immunity and Metabolism

Innate immune defenses to infections are highly specialized and vary widely between phyla (Fig. 5.4) (Brennan and Anderson, 2004; Martinelli and Reichhart, 2005; Mylonakis and Aballay, 2005). Four key functions are involved in host defenses: (I) pathogen recognition; (II) activation of cell and systemic machinery to inactivate, isolate, or remove the pathogen; (III) activation of somatic repair processes; and (IV) mobilization of energy, as needed for acute or chronic responses. The ergonomics of host defense (Fig. 1.2B) has not received much attention in current molecular studies of invertebrate immunity. Many common pathogens are detected and bound by specialized pathogen receptor proteins that recognize 'pathogen associated molecular patterns' (PAMPS) (Section 1.3) (Medzhitov and Janeway, 2002). These complex interactions are represented in Fig. 5.4 as black triangles interacting with abstract pathogen receptors.

Nematode immunity is very different from that in insects. Lacking a pumped circulation, nematodes do not rapidly deploy the mobile phagocytes and anti-microbial proteins of insects and vertebrates. Nematode bacteriocidal agents are presumed but have not been described. Insect bacteriocidal mechanisms include induction of anti-microbial peptides, e.g., defensin, among more than 20 others with specificity for various bacteria and fungi (Table 5.2). Many of these peptide genes show increased expression during aging in flies (Landis et al, 2004; Pletcher

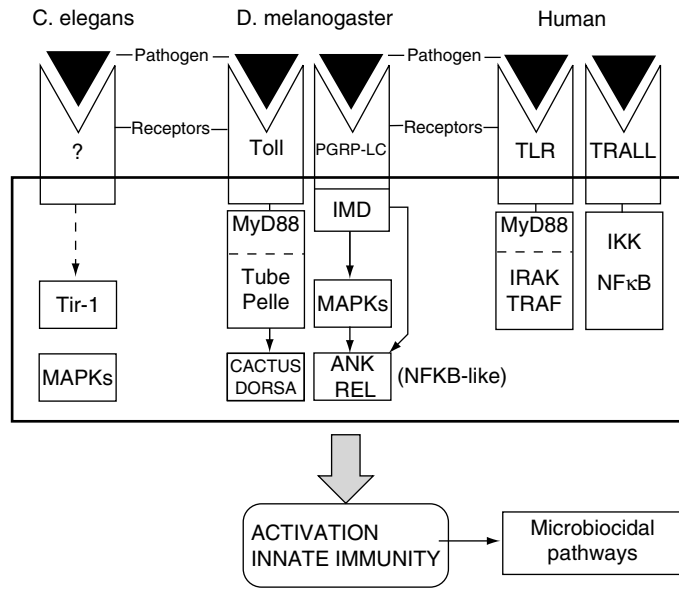

FIGURE 5.4 Elements of innate immunity, vastly oversimplified, representing *C. elegans, D. melanogaster* and human. Adapted from (Martinelli and Reichhart, 2005; Mylonakis and Aballay, 2005; Oda and Kitano, 2006). The pathogen ligands are represented as black triangles, which are bound by the membrane receptors on macrophages or other cells that are front-line defenses. The outlined box encompasses myriad signal transduction machinery that engages complex kinase interactions, e.g., MyD88 in the fly and mammals, but absent from worm. The output activates various microbicidal pathways that induce acute phase proteins, anti-microbial peptides, free radicals, phagocytosis, and cell death. At least 10 regulatory layers can be conceptualized in mammals, with modules that allow exquisite conditional regulation of immune output according to information on the physiological state: energy reserves, burden of tissue damage, and pathogen load. IKK, IκB kinase; Imd, immune deficiency pathway; IRAK, interleukin 1 receptor-associated kinase; MAPK, mitogen-activated protein kinase; MyD88, myeloid differentiation primary response gene 88, which recruits and activates IRAK and other kinases; NF-κB, nuclear factor κB, transcription factor that regulates many immune system genes; Pelle, kinase with roles in embryogenesis and adult immunity; PGRP-LC, peptidoglycan recognition proteins; Tir-1, Toll/interleukin-1 resistance domain protein (*C. elegans*) and orthologue of human SARM; TLR, Toll-like receptors (11 in mammals), which bind diverse pathogenic ligands; *TRAF*, tumor necrosis factor receptor-associated factor; Tube, an adaptor protein downstream of Toll, recruits MyD88 and Pelle (kinase).

TABLE 5.2 Host-Defense Genes with Increased Expression[a] in Aging *Drosophila*

Agent	Activity[a]
attacin	anti-bacterial, Gram-negative[b,c]
cecropins	anti-bacterial, Gram-negative[c]
defensin	anti-bacterial, Gram positive[b,c]
diptericin	anti-bacterial, Gram-negative[d]
drosocin	anti-bacterial, Gram-negative[b]
drosomycin	anti-bacterial, Gram neg. and pos.; anti-fungal[b]
Hsp70	in the endocytic complex; associated with Toll-like receptors[b]
metchnikowin	anti-bacterial, Gram-positive and anti-fungal[b]
Pgrp-lc, peptidoglycan recognition proteins	pathogen receptors for Gram-negative bacteria; activate Relish/imd pathways and phagocytosis; 13 genes and splice variants[c,d]
relish	NF-κB-like transcription factors required for induction of anti-microbial genes[c]
Toll receptors	signal transduction downstream of pathogen receptors (Pgrp-1); mammalian orthologues bind microbial peptides, e.g., Toll-4 binding of LPS[c]

a. The relative size of age changes is generally not provided by the cited papers, due to the uncertainty in microarray analysis of background estimates. RNA blot images give the impression of many-fold increase during aging in whole body extracts. b. (Landis et al, 2004); c. (Pletcher et al, 2002); d. (Seroude et al, 2002).

et al, 2002) (Section 1.8, Fig. 1.24). These defenses are also integral to successful mating because pathogens are transmitted venereally in insects (Khurad et al, 2004). Seminal fluid also contains anti-microbial peptides that protect from venereal infections. The sex-peptide of male *Drosophila* seminal fluid rapidly induces defensins and other peptides that are at low levels in virgin female flies (Peng et al, 2005a). In male flies, sexual activity decreased the clearance of pathogenic *E. coli* in proportion to the frequency of female contacts, suggesting trade-offs for reproduction at the expense of life expectancy (McKean and Numey, 2001).

The pathogen receptor machinery differs between fly, worm, and human, but does share some elements of subcellular signaling machinery (Fig. 5.4). The insect immunity gene responses employ transcription factor binding sites resembling those of vertebrate immunity, including GATA srp-like, NF-κB/Rel-like, and Stat (Wertheim et al, 2005). The ancient Toll receptors are widely used core regulators of immune responses. Toll activation in flies induces numerous host defenses, including genes encoding anti-microbial peptides (Table 5.2) and gene mediating phagocytosis, hemolymph coagulation, and production of free radicals used for cell signaling and cytotoxicity. In fly embryos, the Toll pathway is critical to establishing the embryonic dorsoventral axis. I suggest that the employment of Toll in early development may also activate host defense genes to protect the energy-rich larvae.

Toll-like receptors (TLRs) differ widely between phyla. In mammals, TLR4 directly binds LPS endotoxin, whereas the fly primary pathogen receptors are the peptidoglycan recognition proteins (PGRPs) and Toll is downstream. In the worm, the only Toll-like receptor (TOL-1) is not on a known pathogen-stimulated signaling pathway. While TOL-1 deletion did not alter resistance to pathogenic bacteria, TOL-1 deletion did slightly shorten life span of worms fed on the mildly pathogenic *E. coli* OP50 strain (Kim and Ausubel, 2005). The worm also lacks NF-κB-like transcription factors and the MyD88 effector but does share with flies and mammals the PMK-1 (p38 mitogen-activated protein kinase) in its immune circuits.

Kim and Ausuble (2005) propose that the ancestral metazoan immune signaling system was a PMK-1/p38 element that interacts with TIR-1/SARM, another immune element of arthropods, nematodes, and the vertebrates. From a genomic perspective, the conserved PMK signaling elements in immune circuits are a regulatory 'kernel' of ancient signaling functions in host defenses for 600 million years or more (Davidson, 2006; Davidson and Erwin, 2006). It would not be surprising if other nematodes had different roles of Toll-like receptors and employed NF-κB and MyD88.

Heat shock-induced proteins (HSPs), which increase resistance to oxidation and other stress, are also active in host defense through chaperoning of phagocytosed pathogens. In mammals, HSP chaperone functions extend to anti-bacterial host defense mechanisms for HSP70, which is part of a complex of proteins that interact with the Toll-receptors that remove bacterial fragments by endocytosis (TLR2, gram-positive bacteria; TLR4, gram-negative bacteria) (Asea, 2003; Triantafilou and Triantafilou, 2003). HSP-TLR interactions have not been shown in flies or worms. Because temperature (behavioral fever) is used as a host defense to inhibit microbial infections in insects (Blanford and Thomas, 1999), as in vertebrates, HSP responses may be protective for fever-induced protein denaturation.

Energy reserves are fundamental to immunity. Host defenses are usually energy demanding and require rapid and possibly long-term mobilization (Section 1.3.3). Energy allocation during host defense may trade off short-term survival at the expense of growth (Fig. 1.2B and Section 4.6). At a cell level, macrophage production of superoxide is dependent on glucose transport and oxidation (Kiyotaki et al, 1984), and activation of macrophages induces glucose transporters and increases glucose and fructose transport (Malide et al, 1998). At the physiological level, diet restriction (DR) attenuates NF-κB dependent gene expression changes, which are widely used in host defense (Pletcher et al, 2002). In mammals, DR has anti-inflammatory effects, and moreover, can impair instructive immunity and decrease resistance to infections (Section 3.2.4). Leptin, which is secreted by adipocytes as a metabolic hormone and regulator of feeding behavior, is also major immunoregulator (Section 1.3.3), e.g., mice starved to impair immune responses to *Streptococcus* (Klebsiella) had rapid immune restoration by leptin injection (Mancuso et al, 2006).

Immune and fat cells may be ancient partners. Fat depots accumulate macrophages, which may be a source of the increased plasma CRP and IL-6 associated with obesity (Chapters 1 and 4). Specialized adipocytes in lymph nodes are resistant to starvation and may supply the follicular dendritic cells of instructive immunity with fatty acids (Pond, 2005). Moreover, the insect fat body is the major site of anti-microbial peptide production and secretion (Brennan and Anderson, 2004; Mylonakis and Aballay, 2005). Connections of immunity through MAPK signaling in lipolysis (Carmen and Victor, 2005) may be integrated in inflammatory responses to produce the energy need for biosynthesis and fever. TNFα-induced lipolysis, for example, involves MAPK activation (Souza et al, 2003). Insect fat body oenocytes may also be connected to immunity.

Trade-offs from energy allocation to immune functions are well known in human growth (Fig. 1.2B; Chapter 4). Many invertebrates also show these trade-offs by reduction in fecundity and lifespan, when anti-microbial defenses are activated (Zerofsky et al, 2005). Overexpression of the pathogen receptor protein PGRP (Fig. 5.4) in the fat body shortened life span of both sexes by about 20% (Libert et al, 2007). PRGP overexpression also increased anti-microbial peptide levels and resistance to pathogenic bacteria. In young flies, the induction of antimicrobial peptides by heat-killed bacteria also decreased egg production. This may be attributed to decreased yolk protein synthesis from resource re-allocation for synthesis of antimicrobial peptides, which may reach 5 μg/ml in the haemolymph. The *relish* fly mutant, which lacks the NF-κB required for induction of genes encoding antimicrobial peptides, maintained egg production during immune stimulation (Kim et al, 2001). Fly larva are vulnerable to wasp 'parasitoid' infections. Eggs laid by wasps inside larvae are encapsulated and killed by blood haemocytes. Selection for wasp resistance increased the density of circulating hemocytes in adult flies, which can attack the parasite eggs (Kraaijeveld et al, 2001). However, resistant lines had poorer survival during diet restriction (competition for food), suggesting a trade-off for host defense (Kraaijeveld and Godfray, 1997). Parasitoid-resistance was also indirectly selected by rearing in crowded conditions (Sanders et al, 2005). High larval density limits food (density-dependent diet restriction), but also increases exposure to excreta.

Social insects show further complexities in trade-offs with immunity that come from their coexistence with a complex microbial biome (Evans and Armstrong, 2006; Evans and Pettis, 2005). The hive is a constant target of invasive colonization by bacteria and fungi by its warmth and nutrients. The cost of immune response to colonies infected by the 'foulbrood' *Paenibacillus* was indicated by reduced larval production in proportion to the induction of the antibacterial gene abaecin (Evans, 2004). As another example, immune activation of worker bees by endotoxin (LPS) shortened life span 50% if bees were starved, but had little effect with well-fed bees (Moret and Schmid-Hempel, 2000). Long-lived queens are fed royal jelly, which is the major nutrient source needed for yolk production. The royal jelly includes the defensin-like royalisin among its 'swarm' of antimicrobial peptides (Fontana et al, 2004).

Vitellogenin in the hemolymph is also associated with immunity. In worker bees, hemolymph vitellogenin co-varies with the number of the phagocytic hemocytes and resistance to oxidant stress (Amdam and Omhott, 2002; Amdam et al, 2004). Oxidative protein damage varied inversely with levels of vitellogenin, whether constitutive or manipulated (Seehuus et al, 2006). Major changes in immunity arise during foraging activities when mortality is accelerated due to environmental hazards and possibly from increased bacterial load (Amdam et al, 2005). During foraging, vitellogenin and hemocytes decrease sharply. These major changes are reversed by forcing workers to revert to hive tasks. This striking finding suggests that in the high-energy demanding phase of foraging, immunity is suppressed as a life history trade-off. Again, we see the critical role of energy reserves in trade-offs of host defense and longevity. A worm experiment described below also suggests a cost for vitellogenin production.

The antioxidant activity of vitellogenin may be mediated by an SOD-like domain with homology to bovine Cu/Zn SOD (Tokishita et al, 2006). Vitellogenins and the vertebrate apolipoproteins belong to an ancient class of large lipid transfer proteins (LLTP) that branched prior to the divergence of protostomes (nematode-insect lineage) and deuterostomes (chordate lineage) (Babin et al, 1999). There may be shared mechanisms of lipoproteins in the interactions of immune and vascular functions with longevity.

5.5. THE WORM

5.5.1. Overview

In the lab worm *Caenorhabditis elegans*, cell aging changes are less characterized than those of development (Section 1.2.4). During its relatively long postreproductive phase, feeding and movement gradually slow, and mortality rates accelerate. Wads of partially digested bacteria accumulate in the pharynx and gut (Section 2.3.2, Fig. 2.5C) (Herndon et al, 2002). Bacterial clouds that form around body orifices of aging worms suggest a weakening of anti-microbial defenses (Vanfleteren, 1998). Many anti-microbial genes are also induced. When food is limited during development, worms may arrest in non-feeding larval stages (*dauer* larvae) that also extend life span. Diet restriction (DR) by strongly decreasing the titer of bacterial food increases adult worm life span by up to 60% (Houthoofd, 2006; Klass, 1977). DR worms are thin and less fecund. The longest life spans are achieved by growth on liquid axenic culture, which causes a thin DR-like appearance. Axenic cultured worms are more resistant to stress and temperature and have higher catalase and SOD activities that resemble responses to DR (Houthoofd et al, 2002).

Age-1, the first gerontogene identified on a chromosome, increased life span by about 50%, together with delayed slowing of movements and slower acceleration of mortality (Gompertz slope) (Johnson, 1990). *age-1* and other mutants that

cause obligatory dauer larval arrest also have longer adult phases. Although the chromosomes that carried *age-1* and other mutants were identified, these strong genetic effects on longevity were skeptically received. Until recently, many doubted that single mutations could cause much increase in life span by actually slowing aging, because of assumptions that aging results from complex interactions among numerous genes (Johnson, 2005). The strength of these single gene effects on longevity may depend on the highly protected lab conditions.

Mutants in more than 50 genes are known to modify *C. elegans* life span (Hekimi and Guarente, 2003; Lee et al, 2003; Olsen et al, 2006). Many of the mutants alter the basic dyad of metabolism and innate immunity. Many of these genes are shared in eukaryotic aerobic organisms, whether single-celled or multi-cellular tissue grade (Barbieri et al, 2003; Gems and Partridge, 2001; Finch and Ruvkun, 2001; Kenyon, 2001; Lee et al, 2003; Longo and Finch, 2003; Tatar et al, 2003). Several neuroendocrine pathways with vertebrate orthologues influence worm life spans: 'insulin/insulin-like growth factor (IGF-like)'(metabolism), TOR pathway (nutrient sensing); and 'TGF-β-like' (inflammation). These pathways are associated with transduction of sensory signals that can induce dauer larval stages and converge on the orphan nuclear receptor DAF-12, which binds steroid-like ligands (Fig. 5.3) (Motola et al, 2006), discussed further below. This convergence of insulin and TOR in regulating both the dauer state and life span implies deep links between metabolism, host defense, and life span. The TGF-β-like pathway could join insulin and TOR in regulating aging processes through its role in innate immunity (Kurz, 2004; Nicholas and Hodgkin, 2004).

Several concerns are noted. The interpretation of the mutant effects is largely based on sequence similarity to mammalian genes, and the biochemistry and physiology of these pathways is not directly known. Moreover, the lab diet *E. coli* OP50, a mammalian enterobacteria, may be a confound (Section 2.3.2) (Garigan et al, 2002; Lithgow, 2003). *E. coli* strain OP50 as a food shortens life span, relative to a diet of heat-killed bacteria (Garsin et al, 2003; Mallo et al, 2002), or culture on liquid media (Houthoofd et al, 2002). Some long-lived mutants are resistant to the mild toxicity of *E. coli* OP50, as discussed below. Some consider that *E. coli* is not an optimal food and should be considered 'mildly toxic' (Section 2.3.2). However, these toxic effects do not emerge until later ages. In humans, chronic infections cause oxidative stress with mitochondrial dysfunctions, e.g., in blood leukocytes and myocardium during trypanosome infections (Chagas' disease) (Wen et al, 2006). It will be important to resolve whether the toxicity of *E. coli* OP50 is restricted to later ages because such an effect might interact with the other bacterial pathogens being studied (Scott Pletcher, pers. comm.). Given the intense artificiality of lab models, with 'wild-type' strains selected for rapid maturation and high fecundity than in the real wild-type of wild-caught mice (Section 2.3), it seems to me that delayed effects of lab diets on aging processes and life span are an important concern in experimental design and interpretation of mutant effects.