Regulation of Gene Expression Is Linked to Life Span in Adult Drosophila

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ABSTRACT

Examination of gene expression and aging in adult Drosophila reveals that the expression of some genes is regulated by age-dependent mechanisms. Genetic mutations, Hyperkinetic and Shaker, which are known to shorten life span through an acceleration of the aging process, were used to study the expression of an enhancer trap marked gene. The temporal pattern of expression for such a marked gene shows scaling with respect to life span; it is altered in direct proportion to the life expectancy of the adult animal. This demonstrates that expression of this gene is controlled through mechanisms coupled to physiologic as opposed to chronologic age. Results provide direct evidence for linkage between the regulation of gene expression and life span and establish a model system for the genetic analysis of aging.

The success of molecular genetics in exploring the early developmental events of vertebrates and invertebrates has established a powerful paradigm of how a genetic program is translated into a complex series of biological events. The process of aging, a similarly complex biological phenomenon, is also known to be genetically determined, and, as with early development, models of aging that incorporate the role of programmed genetic events have been proposed (reviewed by Finch 1990). Despite these and other conceptual parallels between development and aging, there has been little direct evidence linking specific genetic elements to aging or life span (ARKING et al. 1993; KEN YON et al. 1993; SUPAKAR et al. 1993; ORR and SOHAL 1994; LARSEN et al. 1995). An important element that has been missing in applying molecular genetic techniques to understanding the process of aging is a suitable phenotype. The careful description and examination of such a phenotype would provide information on the characteristics of the aging process itself as well as establish a model amenable to direct genetic analysis of the aging process.

The adult fruit fly, Drosophila melanogaster, provides a good system for examining gene expression during aging. Except for cells in the gonads and some cells in the gut, there is no cell mitosis in the adult fly (BOZUCK 1972; ITO and HOTTA 1992). Examination of the number of cells in a subset of the visual (LEONARD et al. 1992) and olfactory systems (S. HELFAND, unpublished results) with aging has shown that there is very little loss in their numbers over the course of adult life. Therefore the adult fly may be viewed as consisting of a set of synchronously aging cells (ARKING and DUDAS 1989). Examination of macromolecular synthesis has shown that many of the cells of the adult antenna and retina continue to make a β-galactosidase (β-gal) reporter protein throughout life and that degradation of the β-gal molecule in the adult fly occurs within several hours in both young and old animals (S. HELFAND, unpublished data). The enhancer trap technique, used in the analysis of early development in the Drosophila embryo, provides an indirect means of determining the transcriptional activity of a particular gene, by measuring the amount of an expressed reporter gene such as β-gal (O’KANE and GEHRING 1987; BIER et al. 1989; FREEMAN 1991). Thus, the relationship between gene expression and the process of aging can be examined.

Using genes marked with the reporter protein β-gal, it has recently been demonstrated that the expression of many different genes is modulated with age in adult D. melanogaster (HEL FAND et al. 1995). This modulation is nonrandom; each gene follows its own distinct temporal pattern of expression over the life span of the adult fly. When life span is altered using ambient temperature, the temporal pattern of expression of a number of these genes was found to change in a manner that was related to life span (HEL FAND et al. 1995). This suggests that in some instances the control of gene expression is set by biological or physiological time and not simply chronological or calendar time. Thus, these genes appear to be regulated by age-dependent mechanisms. One objection to this interpretation is that ambient temperature may affect gene expression through a direct thermodynamic effect on the expression of the genes themselves, independent of life span. A nonthermodynamic method of changing life span through use of genetic mutations should provide further insight into relationships between gene expression and aging.
When life span is altered using ambient temperature, life span independent of thermodynamic considerations shortens life span and an acceleration of the process of aging results in a compensatory decrease in their overall life span. From this they concluded that the kinetics of death of *D. melanogaster* were characterized by a log-arithmically increasing mortality rate previously described by Gompertz as a hallmark of normal senescence. Since these two different mutations each result in a shortening of life span and an acceleration of the process of aging, we selected them as a means of altering life span independent of thermodynamic considerations in a study of adult gene expression. The enhancer trap line 1085 was used. This line’s marked gene has previously been shown to scale with respect to life span when life span is altered using ambient temperature (Helfand et al. 1995).

In this report we show that the temporal pattern of gene expression scales with respect to life span when life span is altered using genetic mutations that are known to reduce longevity through an acceleration of the aging process. The results confirm the existence of age-dependent mechanisms of gene regulation, define a linkage between genetic elements and aging, and describe a model system suitable for the exploration of the mechanistic relationship between genetics and aging.

**MATERIALS AND METHODS**

**Fly stocks:** The description of the 1085 enhancer trap line can be found in Helfand et al. (1995). Genetic analysis has shown that the 1085 gene is on the third chromosome (S. L. Helfand, unpublished results). *Hyperkinetic* (Hk) and *Shaker* (Sh) fly stocks were obtained from the Stock Center in Indiana. All animals used in this study, wild type and mutant, possess one copy of the 1085 enhancer trap-marked chromosome. Males hemizygous for the Hk or Sh mutations exhibit the anesthesia-induced “shaking” phenotype, while females heterozygous for either of these two mutations appear wild type and do not show the anesthesia-induced shaking phenotype (Lindsley and Zimmer 1992).

**Fly culturing:** All flies were kept in plastic vials containing a standard corn meal agar medium with several grains of yeast added (Ashburner 1989). Approximately 30 flies were in each vial; flies were passed to fresh vials every 7 days. All flies were cultured in humidified temperature-controlled environmental chambers at 25°C throughout development. Adult flies were collected without anesthesia within 2 hr of emergence from the pupal case and put in humidified temperature-controlled environmental chambers set at 18 or 25°C.

**Life span:** Life span studies were carried out using methods of collection and culture noted above except that the flies were passed into fresh vials every other day at which time the number of dead males and females were recorded. Over 400 male and female flies were scored for each life span study. Life spans for heterozygote females with one copy of Hk or Sh that served as additional controls were similar to wild-type males (data not shown).

**Quantitation of β-gal expression in whole mount adult antennae:** The amount of β-gal expressed in the antennae of the 1085 enhancer trap line is too low to be detected by standard chromogenic assays such as chlorophenol red-β-D-galactopyranoside (CPRG) (Glaser and Lis 1990). We made use of an optically based computer-assisted video microscopy system for the quantification of β-gal that uses X-gal-reacted whole mounts and has been detailed in (Blake et al. 1995; Helfand et al. 1995).

At least 20 antennae were sampled at each time point, using methods previously described (Blake et al. 1995; Helfand et al. 1995). Standard procedures for X-gal staining were followed (Ashburner 1989; Blake et al. 1995). Quantification of levels of β-gal expression in the third segment of the antennae, as measured by amount of staining, was performed using computer-assisted video microscopy (Blake et al. 1995; Helfand et al. 1995).

**FIGURE 1.—Survivorship curves for wild-type (Canton-S) (△), HkΔ (●), and ShΔ (□) adult male Drosophila at 25°C (A) and 18°C (B).** Survivorship curves are based on a sample size of >400 animals. All animals, wild type and mutant, possess one copy of the 1085 enhancer trap-marked chromosome.
which were collected from the same vials and aged together with the hemizygous mutant males, served their life span was similar to wild type (data not shown). Percentage of life span was determined using the maximal life span days of adult life (A) or first 12% of wild type life span (B) for adults maintained at 25°C. All animals possess one copy of the 1085 enhancer trap-marked chromosome. Females heterozygous for the recessive life shortening Hk' (X) or Sh' mutations, which were collected from the same vials and aged together with the hemizygous mutant males, served as additional controls. Their life span was similar to wild type (data not shown). Percentage of life span was determined using the maximal life span of each genotype from the survivorship curves. Error bars, SEM.

FAND et al. 1995). The reliability of the system is such that capture of the same image at different times shows <2% difference in values.

RESULTS

Hk' and Sh' show a shortened life span at 25 and 18°C: Fly stocks were mated to place the marked 1085 gene into the Hk' or Sh' mutant backgrounds. The effect Hk' and Sh' mutations have on life span is shown in Figure 1. At 25 and 18°C both Hk' and Sh' show a decrease in life span as compared to matched controls. The decrease in life span is more pronounced for Hk' than for Sh' as noted by TROUT and KAPLAN (1970).

The onset of temporal pattern of expression of the 1085-marked gene scales to life span: At the same time that life span or survivorship studies were being done, a separate cohort of animals derived from the mating of the marked 1085 gene stock and the Hk' or Sh' mutant stocks were aged, individuals were extracted at periodic intervals throughout the adult life span, and the level of β-gal activity in the antennae was determined and plotted against age in days or percentage of life span. In the case where a single copy of the β-gal reporter protein is present, the normal temporal pattern of the 1085 gene, as measured by β-gal expression, begins at day 6 of adult life for males at 25°C, rises rapidly to its peak by 25-35 days, and then declines thereafter (Figures 2 and 3). When placed into the background of the Hk' or Sh' mutations, the normal temporal pattern of expression of the marked 1085 gene was found to be accelerated in concert with a shortening in life span.

The initiation of the expression of β-gal is earlier in animals expressing the Hk' and Sh' age-accelerating phenotype. This effect is shown for animals living at 25°C in Figure 2. Males that express the recessive Hk' phenotype show an early and very pronounced increase in the expression of the β-gal reporter protein as compared to wild-type male or heterozygous Hk' female controls. For example, expression is seen by day 2 in Hk' males and not until day 6 in wild-type males or day 7 for Hk' heterozygous female controls (Figure 2A). In our previous study the animals being studied had two copies of the 1085-marked gene and onset of expression in wild type under these conditions was found to be on day 3 at 25°C (HELFLAND et al. 1995). The effect of the Sh' mutation on the initial expression of the 1085 gene at 25°C, like its effect on life span, is intermediate between Hk' and control animals; expression is first seen on day 3.

The entire adult temporal pattern of expression of the 1085-marked gene scales to life span: While Hk' and Sh' accelerate the initiation of gene expression in chronological or calendar time, when gene expression is examined as a function of life span or biological time/physiological age, gene expression for each of the four different genotypes is found to coincide (Figure 2B). Over the entire life span of the adult fly, the expression of β-gal in Hk' and Sh' animals maintained at 25°C peaks and declines earlier than in matched controls when examined in relation to chronological or calendar time (Figure 3A). However, when examined in relation to the percentage of each genotype's life span, the temporal pattern of gene expression appears to be nearly superimposable. This is particularly true for the periods of expression from the onset of gene expression through peak expression (Figure 3B). Animals maintained at 18°C show a similar effect (Figure 4), with greater disparity between mutants and control strains than at 25°C. The temporal pattern of expression of β-gal in females heterozygous for the recessive life shortening phenotype of Hk' or Sh' is comparable to that seen for the wild-type male control at both 25 and 18°C (data not shown).
DISCUSSION

Although it is generally believed that the genome plays a significant part in the process of aging, few genetic mechanisms have been uncovered that reveal how the genome affects aging (Arring et al. 1993; Kenyon et al. 1993; Supakar et al. 1993; Orr and Sohal 1994; Larsen et al. 1995). In part this may be due to the difficulty of assessing the process of aging in a manner that is amenable to genetic analysis. Physiological age, as opposed to chronological age, is often difficult to determine. The gold standard for determining the rate of aging is through mortality rates derived from survivorship data. Survivorship data are obtained from the analysis of populations of organisms and thus cannot be used to assess events at the level of individuals. Furthermore, since mortality or age at death is the endpoint being measured, these data do not provide information on the dynamic changes that are occurring during life.

A phenotype linked to the aging process and capable of providing dynamic information reflecting the physiological age of individuals would be most valuable for molecular and genetic analyses. We have previously found that some genes in the adult D. melanogaster are regulated by time-dependent mechanisms. Through the use of ambient temperature to alter life span and the rate of aging, we showed that some of these genes appear to be regulated by age-dependent mechanisms (Helfand et al. 1995). We proposed that these genes, being regulated by mechanisms that are keyed to life span rather than simply chronology, reflect the physiological age of the adult and provide a suitable phenotype for examining the role gene regulation plays in the process of aging. One proviso to this interpretation, however, is that ambient temperature may have an effect on gene expression unrelated to its effect on life span. In this report we sought to investigate this objection by examining the effect two different genetic mutations, Hk' and Sh', which are known to shorten life span through an acceleration of the aging process (Trout and Kaplan 1970), have on the temporal patterns of gene expression for one of our marked genes, 1085.
As can be seen from our results, the temporal pattern of gene expression of the marked 1085 gene scales with respect to life span when life span is altered using the genetic mutations, \( Hk' \) and \( Sh' \). Despite the differences in life span seen with the wild-type, \( Hk' \) and \( Sh' \) animals, the overall pattern of expression for the 1085-marked gene throughout the life of the animal is similar (i.e., the shape of the curve is unchanged). It is the rate at which this predetermined pattern of gene expression progresses with calendar time that is altered when life span or the rate of aging changes. With respect to biological time or physiological age the timing of gene expression is unaltered. This can be further illustrated by comparing the temporal pattern of expression for the two most extreme examples: the \( Hk' \) animal at 25° and the wild-type control at 18°. Despite a two-and-one-half-fold difference in life span [Figure 1, A and B (~48-day maximal life span for \( Hk' \) male at 25° and ~120-day maximal life span for wild-type male at 18°)] and a dramatic difference in the chronological timing of gene expression (compare \( Hk' \) male in Figure 3A to wild-type male in Figure 4A) when the level of \( \beta \)-gal expression is represented as a proportion of the life span, the temporal patterns are nearly superimposable (compare \( Hk' \) male in Figure 3B and wild-type male in Figure 4B). Peak expression, for example, is timed to ~30–40% of the animal’s expected life span.

From a historical perspective, the means we have employed to alter life span (i.e., ambient temperature, \( Hk' \), and \( Sh' \)) all share a common feature. They change the metabolic activity of the animal in concert with their effect on life span. The association between metabolic activity and longevity is one of the hallmarks of “the rate of living hypothesis” reviewed in SOHAL (1986). The work of TROUT and KAPLAN on \( Hk' \) and \( Sh' \) was designed to test this hypothesis. They concluded that the effect of mutant shakers on life span was consistent with the rate of living hypothesis (TROUT and KAPLAN 1970). Our data showing that gene expression scales with respect to life span is also consistent with the rate of living hypothesis, but at present provides no further data to prove or disprove it. What is missing from the rate of living hypothesis is the mechanism through which an alteration in metabolic activity leads to a change in rate of aging and longevity. The linkage between gene expression and life span provides an opportunity to define the mechanisms regulating age-dependent gene expression. The identification of these mechanisms may provide information on how alterations in metabolic rate result in changes in gene expression and perhaps life span. Is metabolic activity translated by each individual cell, as might happen in a truly poikilothermic animal with all cells being influenced relatively equally by ambient temperature? Or is the metabolic activity of a specialized subset of cells responsible for setting the rate of living? This model suggests a centralized control mechanism, which is supported by TROUT and KAPLAN’s mosaic fate mapping analysis, that indicates a focus in the nervous system is responsible for the effect of \( Hk' \) and \( Sh' \) on longevity (TROUT and KAPLAN 1981).

The finding of genes whose pattern of expression scales to life span demonstrates a linkage between gene expression and aging. This linkage provides a genetic correlate with physiological, as opposed to chronological, aging. Linkage between the control of gene expression and rate of aging provides a phenotype for aging that now makes it amenable to direct genetic and molecular analysis.

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LITERATURE CITED


O’KANE, K., and W. QHILLING, 1987 Detection in situ of genomic


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