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Genetics

Fruit flies with additional expression of the elongation factor EF-1α live longer

(*Drosophila melanogaster*/aging/protein synthesis)

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ABSTRACT **In *Drosophila melanogaster*, the decrease in protein synthesis that accompanies aging is preceded by a decrease in elongation factor EF-1α protein and mRNA. Here we show that *Drosophila* transformed with a *P*-element vector containing an EF-1α gene under control of *hsp70* regulatory sequences have a longer life-span than control flies.**

A common feature of many types of aging organisms is a progressive decline of protein synthesis, which becomes a potential danger to physiological function (1). In *Drosophila melanogaster*, the step in protein synthesis most impaired by age appears to be the binding of aminoacyl-tRNA to ribosomes (2), a reaction requiring elongation factor EF-1α. Furthermore, a sharp decline has been found in the rate of synthesis of the EF-1α protein, starting early in adult life and preceding by several days the decline in total protein synthesis (3). This corresponds to a decrease in the amount of mRNA for EF-1α occurring at about the same time. As measured by translation in a rabbit reticulocyte system (4) and by hybridization (5), this mRNA is reduced in 15 days at 20°C to a few percent of its amount in newly hatched flies. The present experiments give a strong indication that a supplementary supply of EF-1α mRNA, provided by a gene inserted into the *Drosophila* genome under independent transcriptional control, can lengthen the mean lifetime of the flies by maintaining protein synthesis longer.

D. melanogaster has two copies, F1 and F2, of the EF-1α gene with different time controls of expression during development (6, 7). Whereas F2 is transcribed mostly at the pupal stage, F1 is suspected to be the housekeeping gene needed in all growing cells and is the gene used in this investigation. The germ line of rosy-506 (ry^{506}) mutant flies (8) was transformed by using the *P*-element plasmid construct shown in Fig. 1*b*. This consists of the ry^+ gene as marker, together with cDNA of the whole coding sequence of F1, placed under the control of the *Drosophila* 70-kDa heat shock protein (*hsp70*) promoter and termination sequences. The *hsp70* promoter provided expression of the gene in all types of cells and with no known age-specific control. It has been found to give a basal level of expression below the heat shock onset temperature (12) and the levels of expression have been measured in cell cultures (13). Of the EF-1α-transformed fly lines obtained, four (E1–E4) were balanced and then made homozygous. For comparison and control, in addition to the original ry^{506} line, three lines (C1–C3) were available, obtained by transforming ry^{506} with a construct containing the ry^+ gene but not the EF-1α insert (Fig. 1*a*).

Comparative measurements of mean lifetime for male flies were made under carefully controlled conditions (see Fig. 2 legend), always using a sample of 100 newly hatched flies and counting the survivors each day. The first observation made

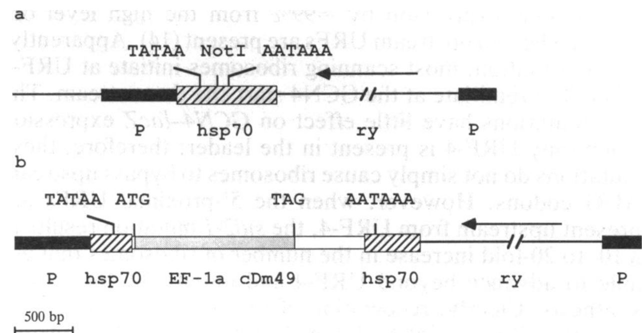

FIG. 1. Structure of the *hsp70*–EF-1α fusion plasmids. (*a*) Vector plasmid pNHT4. This plasmid is derived from plasmid pHT4, a plasmid constructed (9) by inserting the *hsp70* promoter and termination–processing sequences into Carnegie 20, a vector that contains the ry^+ gene flanked by the ends of the *P* transposon. The single *Kpn* I site of pHT4 has been replaced by a *Not* I site: the *Kpn* I site was blunt-ended with phage T4 DNA polymerase, *Not* I linkers were added, and, after digestion with *Not* I, the plasmid was recircularized, resulting in pNHT4. (*b*) Final construct pNHTEF. The 2.0-kilobase *Hin*dIII–*Xba* I fragment of the cDNA clone cDm49, containing the whole open reading frame of EF-1α (6), was cloned in a vector having *Not* I sites on both ends of the polylinker and then was cut out as a 2.0-kilobase *Not* I fragment and cloned into vector pNHT4. This plasmid was then coinjected with pπ25.7 w.c. helper plasmid (10) into ry^{506} recipient eggs, essentially by the method of Rubin and Spradling (11). bp, Base pairs.

was that the ry^+ control strains C had much longer lifetimes than the ry^{506} mutant. Evidently the lack of xanthine dehydrogenase coded by the rosy locus, although not lethal to the flies, has a strong negative effect on their viability. Second, the E strains with the additional EF-1α gene were observed at 25°C to have on average a significantly longer lifetime than the control strains C ($P < 0.001$), but there were variations in lifetime between the individual lines of the C and E strains. These can be understood in terms of different positions of *P*-element insertion into the genome, possibly mutating a nonessential gene or encountering transcriptional enhancing effects from neighboring genomic sequences. It is also possible that some genetic background differences affecting viability could derive from the balancer strains used. These effects were eliminated from a detailed comparison between the C and E strains by taking two specific lines, C3 and E4, and comparing their relative lifetimes at 25°C and 29.5°C. Insertion positions and genetic background then remain unaltered in the comparison and only the effect of additional transcription of the EF-1α gene at 29.5°C should be seen. (By hybridization on polytene chromosome squashes these insertions were seen on the third chromosome at positions 65A and 79F, respectively, for the C3 and E4 flies. The structure

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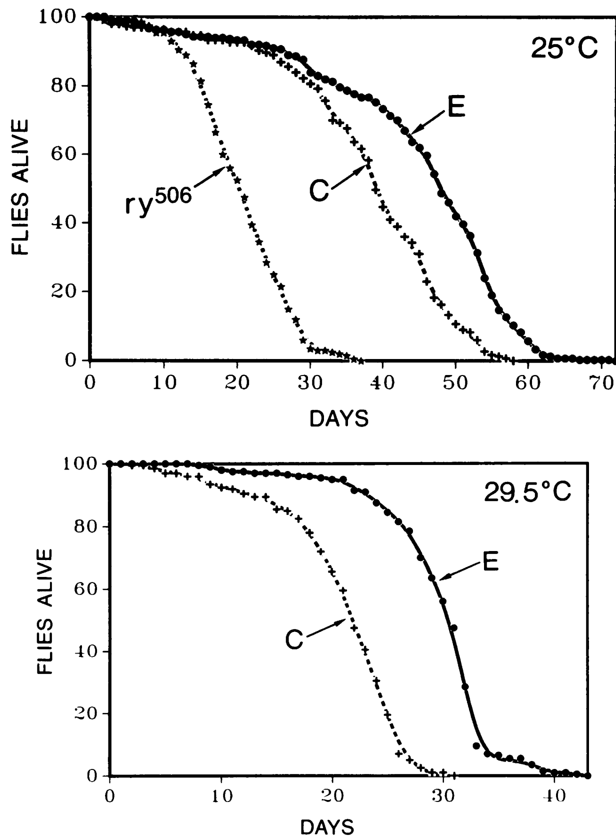

FIG. 2. (*Upper*) Survival curves for male ry^{506}, C3, and E4 flies at 25°C. One hundred freshly hatched flies were used for each test and placed in vials (3 in × 1 in; 1 in = 2.54 cm), 10 flies to each vial, at constant temperature, 60% humidity, and identical light conditions with a 12-hr/12-hr light/dark cycle. The food used was a standard cornmeal/sugar/agar medium with baker's yeast, containing Nipagin M as mold inhibitor. The flies were moved to fresh vials every 3½ days; a short pulse of carbon dioxide was given to immobilize the flies when they were sorted or moved. The pre-imaginal temperature was 25°C for all test flies and the larval density was high in the vials (3 in × 1 in) used for development. The number of flies still alive was determined at 24-hr intervals. (*Lower*) Survival curves for male C3 and E4 flies at 29.5°C. The conditions were otherwise as given above.

of both inserts was also checked by genomic Southern blot analysis.)

The survival curves at 25°C for the C3 and E4 lines (means of three measurements) and of the ry^{506} mutant line (two measurements) are shown in Fig. 2 *Upper*. The mean age for ry^{506} was 20.7 days with a standard deviation (SD) of 6.4 days, whereas for C3 the mean age was almost doubled to 38.2 days (SD 11.5). The mean survival time for E4 was 45.1 days (SD 13.6), very significantly ($P < 0.001$) more than that for C3. At 29.5°C (Fig. 2 *Lower*), the lifetimes of both C3 and E4 were shorter (two measurements), as expected from the known decrease in survival time with increasing temperature (14). The mean lifetime for E4 was 29.8 days (SD 5.4), even more significantly ($P << 0.001$) greater than that of 21.1 days (SD 5.5) determined for C3. Thus the proportional increase of mean lifetime for the lines with the extra copy of the EF-1α gene was about 18% at 25°C, whereas it more than doubled to 41% at 29.5°C, a temperature at which more transcription from the *hsp70* promoter of the EF-1α insert occurs (12).

These experiments with the *Drosophila* germ line transformed with the EF-1α gene suggest a possible important aging control exerted at the transcriptional level by this gene. Since a similar drop in the supply of mRNA for EF-1α has also been observed in the livers of aging mice (5), and the activity of EF-1α decreases toward the end of the life-span of serially passaged cultures of human fibroblasts (15), further investigation of the control elements for this gene could have important implications for gerontological research.

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