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A strong ubiquitous promoter–enhancer for development and aging of *Drosophila melanogaster*

Ruedi Ackermann* and Christine Brack

Biozentrum, Department of Cell Biology, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

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One of the major advantages in using *Drosophila* for developmental as well as for aging studies, is the possibility to generate transgenic flies to study the expression of specific genes of interest. Both for tissue-specific expression and for ubiquitous expression of a transgene (e.g., a housekeeping gene), expression vectors with strong promoters are desirable. So far, no strong promoter is available for ubiquitous expression in *Drosophila*. The most frequently used constructs contain either the heat shock protein 70 (hsp70) promoter (1) or the actin5C promoters (2), both of which have disadvantages. Strong expression from the hsp70 promoter requires heat induction, a treatment with negative effects in long-term studies like aging. The actin5C promoters, on the other hand, show some developmental specificity (3).

In our previous studies we have shown that the *Drosophila melanogaster* peptide synthesis elongation factor EF-1α F1 (EF) mRNA is expressed in all stages of development, including also old flies (4). Here we report that the *Drosophila* EF promoter–enhancer can be used for the ubiquitous expression of transgenes in flies. This new powerful promoter–enhancer for *Drosophila* shows strong activity in all cells and all stages of development. It is also able to drive the expression of a reporter gene in *Drosophila* cell culture.

As shown in Figure 1, a 5 kb genomic fragment of the *Drosophila* EF gene (5) including 3.5 kb of upstream sequences, the transcription start site, the complete intron and part of the second exon with 150 bp of the coding region was fused in frame to a bacterial β-galactosidase (LacZ) gene containing the hsp70 polyadenylation signal and cloned into the pUChsneo P-element vector (6) to yield P[hsneo; EFPLacZ].

First, this P-element was tested in tissue culture cells (Schneider S2 cells). The strength of the EF promoter–enhancer was tested by measuring the LacZ activity. The activity of the copia promoter—which is considered to be a very strong promoter in Schneider cells (8)—was set at 100%. The EF promoter showed an activity of 114.3 ± 25%, Bluescript as a negative control 7.5 ± 14.1% compared with the copia promoter. The same construct was used for P-element mediated germline transformation of *Drosophila*. Using the quantitative LacZ activity assay (9), the activity of the EF promoter–enhancer was measured in extracts from embryos, larvae, pupae and adult flies (Fig. 2A), as well as in extracts of young and old flies (Fig. 2B). The 5 kb EF promoter–enhancer fragment was able to direct the LacZ expression in all stages of development and also in aging flies.

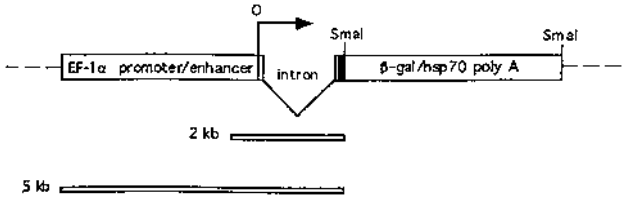

Figure 1. The structure of the P[hsneo; EFPLacZ] P-element construct. The 5 kb *Xba*I–*Xho*I EF-lα (EF) promoter–enhancer fragment was ligated into the pUChsneo (2) (dotted lines). In order to get an inframe fusion, a 3.8 kb *Sma*I LacZ fragment containing the hsp70 polyadenylation signal was cloned 3′ of the promoter–enhancer fragment. The 2 kb EF promoter fragment is indicated below the P[hsneo; EFPLacZ] P-element construct. O: Transcription start site of the EF gene. The intron and part of the second exon are indicated. The 50 amino acids of the EF coding region are shown as a filled box. The LacZ gene is flanked by *Sma*I restriction sites.

These results were confirmed by qualitative staining of whole mount developmental stages and adult flies (data not shown). The LacZ staining experiment resulted in ubiquitous staining of all cells in all tissues, indicating that the 5 kb fragment contains all the information required for position-independent activity. In comparison, a vector containing a genomic 2 kb fragment consisting only of 500 bp of the EF promoter region (see Fig. 1) gave strong expression with varying tissue-specificity depending on the insert positions. The additional 3 kb upstream sequence thus contributes enhancer functions needed for position-independent full transcriptional activity (7). The 5 kb EF promoter–enhancer fragment was successfully used to rescue a lethal EF mutant (7).

Of particular interest to us was the confirmation that, although the activity of the 5 kb EF promoter declines within the first 5 days of adult life, its activity remains almost constant in old flies. The LacZ quanatations are directly comparable with the quantitation of the EF mRNA on Northern blots (4). This promoter–enhancer will thus be very useful for ubiquitous transgene expression in aging flies. In combination with the regulatable tetracycline repression or transactivation vectors (10), this powerful ubiquitous promoter–enhancer should become a useful tool also for the selective expression of ‘toxic’ genes (e.g., genes with temporal negative effects).

* To whom correspondence should be addressed

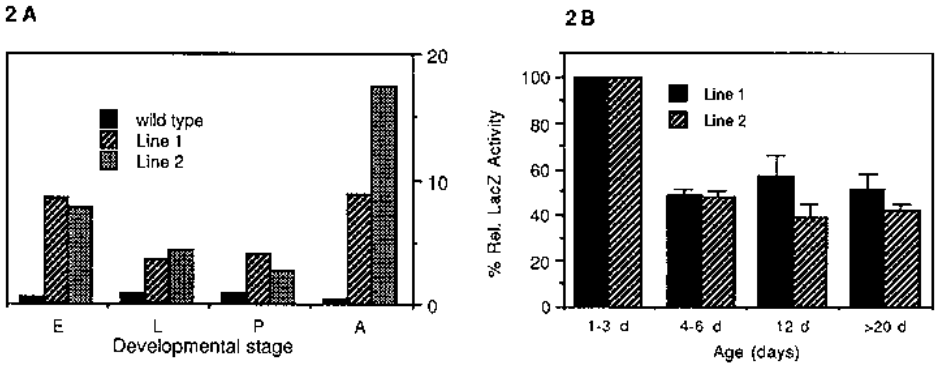

Figure 2. Quantitation of β-galactosidase activity. (**A**) Extracts of developmental stages and adult flies of two independent P[hsneo; EFPLacZ] lines were assayed for LacZ activity as described in (9). For background activity, wild-type flies were used. The activity was calculated per mg of protein measured using the BIORAD system. E: embryos, L: larvae, P: pupae, A: adult flies. (**B**) The LacZ activity in extracts of aging flies of two independent P[hsneo; EFPLacZ] lines was measured as described in (9). The values represent the means of two independent experiments with the standard deviations. The values of the youngest flies were set at 100%. The ages of the flies used in these experiments are indicated.

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REFERENCES

1 Klemenz,R., Weber,U. and Gehring,W.J. (1987) *Nucleic Acids Res.*, **15**, 3969–3974.
2 Thummel,C.S., Boulet,A.M. and Lipshitz,H.D. (1988) *Gene*, **74**, 445–456.
3 Bond,B.J. and Davidson,N. (1986) *Mol. Cell. Biol.*, **6**, 2080–2088.
4 Shikama,N. and Brack,C. (1996) *Gerontology* **550**, in press.
5 Walldorf,U., Hovemann,B. and Bautz,E.K.F. (1985) *Proc. Natl Acad. Sci. USA*, **82**, 5795–5799.
6 Steller,H. and Pirrotta,V. (1985) *EMBO J.* **41**, 167–171.
7 Ackermann,R. and Brack,C., manuscript in preparation.
8 Sinclair,J.H., Burke,J.F., Ish-Horowicz,D. and Sang,J.H. (1986) *EMBO.J.*, **5**, 2349–2354.
9 Ashburner,M. (1989) *Drosophila. A Laboratory Manual.* Cold Spring Harbor University press, Cold Spring Harbor, NY.
10 Gossen,M. and Bujard,H. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 5547–5551.