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Gene expression pattern

GFP-tagged balancer chromosomes for *Drosophila melanogaster*

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Abstract

We constructed green fluorescent protein (GFP)-expressing balancer chromosomes for each of the three major chromosomes of *Drosophila melanogaster*. Expression of GFP in these chromosomes is driven indirectly by a *Kruppel* (*Kr*) promoter, via the yeast GAL4-UAS regulatory system. GFP fluorescence can be seen in embryos as early as the germ band extension stage, and can also be seen in larvae, pupae, and adults. We show the patterns of GFP expression of these balancers and demonstrate the use of the balancers to identify homozygous progeny. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: GFP; *Drosophila*; Balancer chromosomes

1. Results

Among diploid model organisms, *Drosophila melanogaster* is uniquely blessed with balancer chromosomes. These balancers have been generated for each of the major chromosomes, and they have three properties that facilitate genetic analysis: (1) they suppress recombination with their homologs; (2) their presence is signaled by dominant markers affecting adult and (occasionally) larval morphology; and (3) they have recessive mutations that cause lethality or reduced fecundity in homozygotes. These properties make it possible to maintain and preserve stable populations of mutants as balanced heterozygotes.

Another use of balancer chromosomes is to identify homozygous mutant progeny. In a balanced cross, mutants can be selected by the absence of the markers that are present on the balancer chromosomes. Originally, balancers had markers with adult phenotypes only, but more recently, markers such as β-galactosidase, *Tubby*, *Black cells* or *yellow*$^+$ have been added (for instance, see Kania et al., 1990; Nambu et al., 1990; Goriely et al., 1991; Bourgouin et al., 1992; Panzer et al., 1992; Schweisguth and Posakony, 1994; Davis et al., 1996). These mutants can be scored at larval and embryonic stages. However, since these markers either do not express or are not manifested in early embryos, or because they can be scored only in fixed specimens, we created a new set of balancers that express green fluorescent protein (GFP), which can be recognized in live, gastrulating embryos. We modified *FM7c* (Merriam, 1972), *CyO* (Oster, 1956), and *TM3* (Tinderholt, 1960) balancer chromosomes by transposing P elements carrying fusion genes consisting of *Kr*-GAL4 (Brand and Perrimon, 1993) and UAS-GFP (Chalfie et al., 1994). The GFP fluorescence in these 'green balancers' can be scored in living embryos, larvae, and adults. This report describes the construction and characterization of these new chromosomes. A similar set of GFP-expressing balancers has been reported in which the GFP gene is driven directly by the Actin A5C promoter (Reichhart and Ferrandon, 1998). Although GFP expression cannot be scored until late in embryogenesis with these balancers, they have comparable properties.

1.1. GFP expression

The yolk in *Drosophila* embryos is brightly fluorescent, but the yolk fluorescence is yellow and can be distinguished from GFP green fluorescence with an appropriate long-pass emission filter. We followed the changes in the distribution of the yolk that mark the successive stages of embryogenesis. Yolk is uniform in the early embryo (not shown), but recedes from the periphery at cellularization, stages 4–5 (Fig. 1A) and continues to shrink and change shape as gastrulation proceeds (Fig. 1B). We did not detect maternally-derived expression of GFP, and did not detect GFP fluorescence at stage 4, when endogenous *Kr* expression

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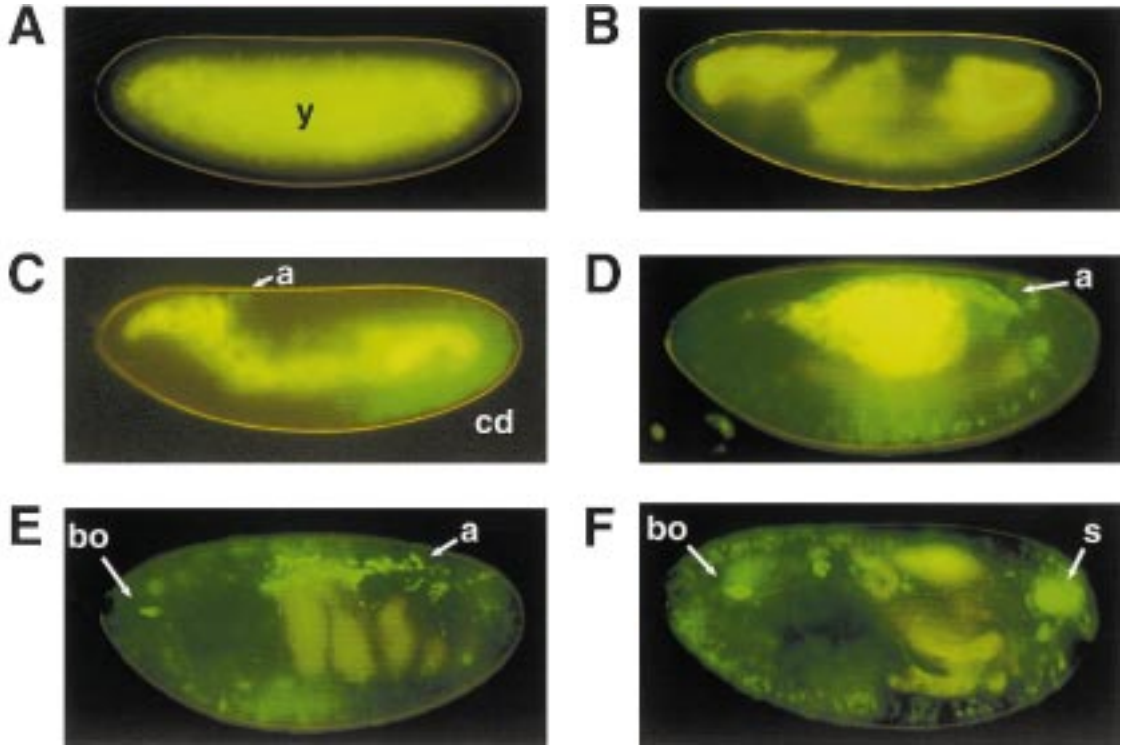

Fig. 1. *Drosophila* embryos with balancer *FKG34* at various stages of embryonic development are oriented anterior to the left, dorsal side up. The yellow fluorescence derives from embryonic yolk and gut, while the green is due to GFP expression. (A) Cellularization, stages 4–5. (B) Early germ band extension, before GFP expression, stage 8. (C) Germ band extension, stages 9–12, with *Kr* central domain and amnioserosal GFP expression indicated. (D) Germ band retraction, stage 13–14. (E) Embryo, stage 16, with Bolvic's organ (bo) marked. (F) Late embryo, stage 17, just before hatching, with spiracles (s) marked. Abbreviations: y, yolk; a, amnioserosa; cd, central domain; bo, Bolvic's organ; s, spiracles.

begins (Knipple et al., 1985). GFP fluorescence was first detected in early stage 9 embryos as a green swath at the posterior end of the embryo (Fig. 1C). This stripe appears to correspond to the medial stripe of *Kr* expression. GFP fluorescence was also present in the amnioserosa. Expression faded markedly during germ band retraction, stages 13 and 14 (Fig. 1D), but increased again at the end of embryogenesis, culminating in strong expression in the Bolvic's organs, the posterior spiracles, and to a lesser extent in the cuticle (Fig. 1E,F). In addition, GFP fluorescence was observed in macrophages which distribute in a punctate pattern (Fig. 1E). This fluorescence may be due to acquisition of GFP after the macrophages engulf amnioserosa cells (V. Hartenstein, pers. commun.).

Expression of GFP in first instar larvae was similar to that of stage 17 embryos (Fig. 2A). In second instar larvae, GFP expression was prominent primarily in the salivary glands (Fig. 2B). Expression in late third instar larvae and pupae was significantly different in appearance. At these stages, fluorescence in the fat bodies dominated (Fig. 2C,D). Fluorescence in late pupae and young adults was predominantly in the abdominal regions (Fig. 2E,F). Abdominal expression was also prominant in young adults, but it quickly diminished and became restricted to internal structures near the thorax (Fig. 2G).

Expression patterns of GFP in animals with the different green balancer chromosomes were largely similar. The most significant variations noted were with *TKG4*, which drove stronger expression in the amnioserosa during germ band extension. In addition, *CKG19* produced a stronger GFP signal than the others, especially in the cuticle late in embryogenesis.

1.2. Sorting embryos by GFP expression

To determine if GFP expression driven from these balancers can serve as a reliable embryonic marker, we sorted embryos at germ band extension (stage 9). For *FKG34*, we crossed *FKG34/Y* males to *yw* females. We selected 171 embryos lacking green fluorescence and 172 green fluorescent embryos, and then allowed them to develop to adulthood. All 141 of the surviving green fluorescent animals enclosed as females and carried the balancer; all 125 of the surviving non-fluorescent embryos were males and did not carry the balancer. For the second chromosome, we sorted 168 stage 9 embryos from a cross of smo^{Q}*FRT40A/CKG19* males and smo^{Q}*FRT40A/FRT40A* females in which germ line clones of smo^{Q} homozygous gametes had been induced. Eighty-two (49%) did not express GFP, indicating that these eggs had been fertilized by a mutant-bearing sperm. Seventy-nine of these embryos developed smo^{-} cuticles (Alcedo et al., 1996; van den

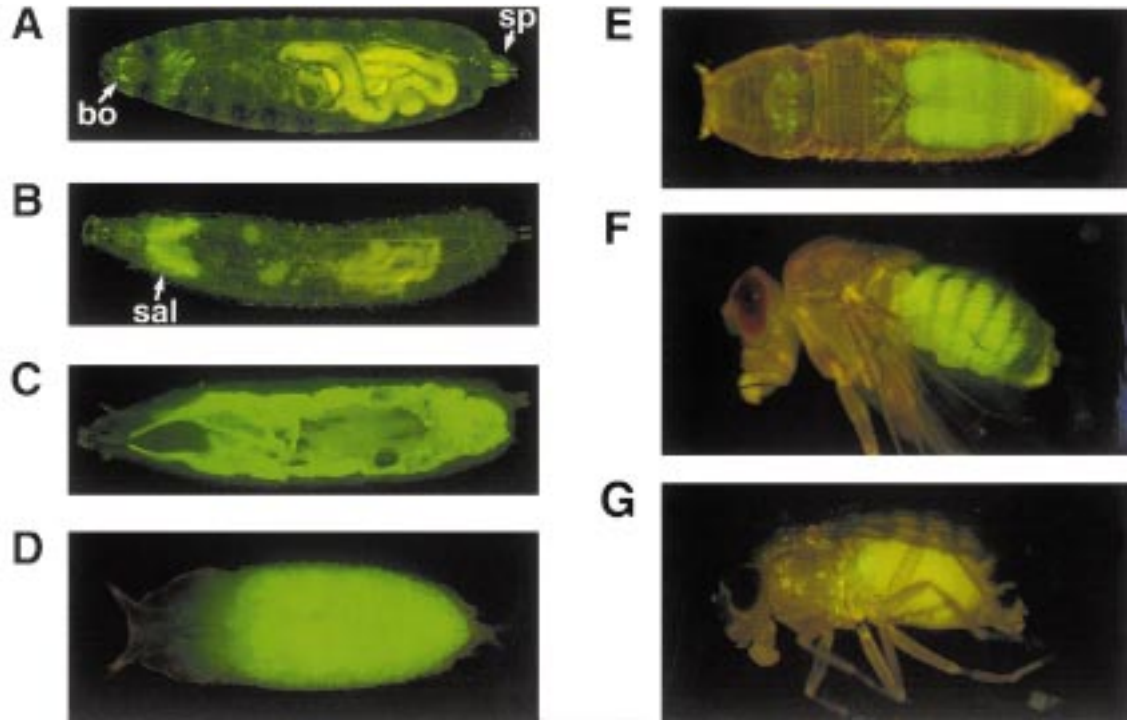

Fig. 2. Larvae, pupae and adult *Drosophila* with Balancer *FKG34*. (A) First instar larva. (B) Second instar larva. (C) Third instar larva. (D) Early pupa. (E) Late pupa, pharate adult. (F) Young adult, approximately 6-h-old. (G) Adult, approximately 2-days-old. sal, salivary gland; b.o., Bolvic's organ; sp, spiracles.

Heuvel and Ingham, 1996), while three arrested development prior to cuticle formation. We also tested the third chromosome balancer *TKG4* by sorting stage 9 embryos from a cross of hh^{AC}/*TKG4* and found that of 27 sorted non-fluorescent embryos, all 27 developed hh^{-} cuticles (Lee et al., 1992). We conclude that the balancers for each of the three chromosomes provides an unequivocal identification that can be used to select embryos.

We anticipate that the GFP-expressing green balancer chromosomes will be useful for a number of purposes. As we have shown, they can be used to identify the presence of these balancers in living embryos and larvae. Embryos lacking these balancers can be identified at any stage of development after embryonic germ band extension and can be used for developmental, biochemical, histological, and in vitro studies of genetically defined material. We hope that these balancers will also facilitate the production of genetically defined cell lines.

There are a few caveats to the general utility of these chromosomes. They cannot be used to identify embryos prior to germ band extension. Also, since they drive GFP via GAL4, they cannot be used in conjunction with chromosomes carrying UAS constructs where misexpression is lethal. This second problem can be avoided by using a different set of green balancers in which GFP is driven directly by the Actin A5c promoter (Reichhart and Ferrandon, 1998). These balancers were derived from *FM7c*, *CyO*, and *TM3*, Ser^{1}, and GFP expression is first visible at stage 14.

2. Methods

2.1. Strains

Stocks carrying P elements were: $w : \mathrm{P}\{w^{+mC} = tw\text{-}GAL4\}$ (III); $w : \mathrm{P}\{w^{+mC} = A5c\text{-}GAL4\}$; $w : \mathrm{P}\{w^{+mC} = Kr\text{-}GAL4\}TL22/CyO$; $w : + : \mathrm{P}\{w^{+mC} = Kr\text{-}GAL4\}$ (III). $w : \mathrm{P}\{w^{+mC} = UAS\text{-}GFPS65T\}T2$ (II) and $w : \mathrm{P}\{w + mC = UAS\text{-}GFPS65T\}T10$ (III). The following stocks were used as a source of P element transposase: *TMS*, $\mathrm{P}\{ry^{+t7.2} = \Delta 2\text{-}3\}99B/TM6$, *Tb** and *yw*: *CyO*, $\mathrm{H}\{w^{+mC} = \mathrm{P}\Delta 2\text{-}3\}Hop2.1/Bc^{1}Egfr^{E1}$. The *smo* stock was: smo^{Q}, $\mathrm{P}\{ry^{+t7.2} = neoFRT\}40A/CyO$. All crosses and transpositions were carried out at 19–23°C.

GFP-tagged *FM7c*, *CyO* and *TM3* balancers were selected for the presence of both $\mathrm{P}\{w^{+mC} = Kr\text{-}GAL4\}$ and $\mathrm{P}\{w^{+mC} = UAS\text{-}GFPS65T\}$ and chosen for their bright GFP fluorescence. They are designated *FKG10*, *FKG34*, *CKG19*, *CKG30*, and *TKG4* and *TKG5*, respectively, and are available from the Bloomington Stock Center.

2.2. GFP detection

The embryonic chorion, plastic dishes, and crude grades of agar exhibit considerable auto-fluorescence. Therefore, embryos were dechorionated and placed on a bed of 1.2% Sea Chem LE Agarose (FMC Corp.) in a watch glass. GFP expression was scored using a Leica Fluorescent Dissecting Microscope with a long-pass GFP Endow filter cube

(Chroma cat. #41018). Use of a long-pass rather than a band-pass GFP filter is essential to distinguish the green fluorescence of GFP from the yellow fluorescence of the endogenous embryo yolk. Sorting embryos based on GFP expression was facilitated by tightly staged embryo collections of approximately 1 h.

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