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Expression and functional analysis of a novel Fusion Competent Myoblast specific GAL4 driver

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Abstract

In the *Drosophila* embryo, body wall muscles are formed by the fusion of two cell types, Founder Cells (FCs) and Fusion Competent Myoblasts (FCMs). Using an enhancer derived from the *Dmef2* gene ([C/D]*), we report the first GAL4 driver specifically expressed in FCMs. We have determined that this GAL4 driver causes expression in a subset of FCMs and, upon fusion, in developing myotubes from stage 14 onwards. In addition, we have shown that using this *Dmef2-5x[C/D]*-GAL4* driver to express dominant negative Rac in only FCMs causes a partial fusion block. This novel GAL4 driver will provide a useful reagent to study *Drosophila* myoblast fusion and muscle differentiation.

Keywords: Muscle; Fusion Competent Myoblast; Myotube; *Drosophila*; Dmef2; Lmd; Rac; Myoblast; Fusion; GAL4

1. Results and discussion

Dmef2 is the sole *Drosophila* member of the Myocyte Enhancing Factor 2 (MEF2) family of transcription factors (Nguyen et al., 1994). *Dmef2* is required for the differentiation of mesodermal tissues within the developing *Drosophila* embryo, specifically the body wall, visceral and cardiac muscles (Bour et al., 1995; Lilly et al., 1995; Lin et al., 1996; Ranganayakulu et al., 1995). During the early stages of mesoderm development, Dmef2 is expressed in all mesodermal cells and its expression is controlled by another mesodermal transcription factor, Twist (Twi) (Cripps et al., 1998). Dmef2 expression continues in somatic, visceral and cardiac progenitors and is maintained throughout the development of these tissues (Bour et al., 1995; Lilly et al., 1994; Ranganayakulu et al., 1995). Previous studies identified enhancers responsible for the expression of *Dmef2* in the two cell types that form body wall muscles, the Founder Cells (FCs) and Fusion Competent Myoblasts (FCMs) (Nguyen and Xu, 1998). FCs determine the identity of individual muscles. Upon fusion to FCs, FCMs become entrained to the FCs individual developmental program (Baylies et al., 1998; Beckett and Baylies, 2006a). Further dissection of an FCM-specific enhancer (I-E) identified a 55 bp sequence ([C/D]*) that is necessary and sufficient to direct expression of a lacZ reporter gene in FCMs. This [C/D]* enhancer is bound directly by the FCM-specific transcription factor Lame duck (Lmd, also known as Myoblasts incompetent and Gleeful), requires *lmd* for its expression and therefore mediates FCM-specific

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expression of *Dmef2* (Duan et al., 2001; Furlong et al., 2001; Ruiz-Gomez et al., 2002). We have cloned multiple copies of this [C/D]* enhancer element upstream of the GAL4 gene to create a novel GAL4 driver (*Dmef2-5x[C/D]*-GAL4*) that we characterize here.

We crossed flies homozygous for the *Dmef2-5x[C/D]*-GAL4* driver to flies carrying a *UAS-GFP* transgene and analyzed GFP expression in the resulting embryos. We detected mesoderm-specific GFP expression from stage 14 onwards (10.5 h after egg laying [AEL]; Fig. 1). GFP expression is first detected during early to mid stage 14 in a small number of ventral mesodermal cells (Fig. 1A). This initiation of reporter gene expression is later than that observed using a direct lacZ fusion (Duan et al., 2001) and presumably is due to the delay caused by the GAL4/UAS system. From late stage 14 and throughout stage 15 (11.5–13 h AEL), GFP expression is detected along the dorsal–ventral (D–V) axis of the embryo in both growing myotubes and surrounding cells (Fig. 1B). Based on their location, it is likely that these surrounding cells are FCMs. GFP expression is also detected in the final body wall muscles at stage 16 (13–16 h AEL; Fig. 1C–D). However, when we analyzed these embryos we found that GFP expression was not detected in all muscles and GFP levels varied between muscles. This differential muscle expression is variable between hemisegments and embryos, and is quantified in Table 1. For example, Fig. 1D shows that GFP is not expressed in all LT muscles, and in those in which it is expressed, it is not expressed at the same level. We also found that GFP expression is rarely detected in the VT1 and VA3 muscles. These muscles are both small in size, with the VT1 muscle, for example, containing 2–4 nuclei at stage 16 (Bate, 1990; Beckett and Baylies, 2007). The absence of GFP expression could be due to the late initiation of GFP expression in FCMs as well as the small number of fusion events occurring in these muscles. The expression of GFP in mesodermal cells surrounding the growing myotubes and the varying levels of GFP expression in the final muscles suggested that this *Dmef2-5x[C/D]*-GAL4* driver is expressed in FCMs and that, upon fusion to myotubes, it is also expressed in myotubes. The variation in GFP levels in the final muscles between hemisegments and embryos also suggested that the *Dmef2-5x[C/D]*-GAL4* driver is not expressed in all FCMs, consistent with the temporal delay caused by the GAL4/UAS system, and that an individual FCM does not always fuse to the same muscle.

Table 1
Expression of *Dmef2-5x[C/D]*-GAL4 > UAS-GFP* in final larval body wall muscles

Muscle	% GFP positive (*n*)	Muscle	% GFP positive (*n*)	Muscle	% GFP positive (*n*)
DA1	97 (36)	LT1	74 (46)	VL1	87 (52)
DA2	83 (36)	LT2	76 (49)	VL2	58 (53)
DA3	88 (43)	LT3	76 (49)	VL3	62 (52)
DO1	100 (36)	LT4	53 (45)	VL4	62 (53)
DO2	100 (41)	LO1	73 (45)	VO1	95 (57)
DO3	85 (53)	SBM	82 (45)	VO2	95 (57)
DO4	98 (53)	VA1	87 (55)	VO3	93 (57)
DO5	50 (52)	VA2	76 (55)	VO4	61 (49)
DT1	77 (53)	VA3	11 (53)	VO5	100 (47)
LL1	91 (47)	VT1	8 (55)	VO6	83 (46)

To confirm that the *Dmef2-5x[C/D]*-GAL4* driver is expressed in FCMs and, subsequently, upon fusion, in myotubes, we used confocal microscopy. We crossed *Dmef2-5x[C/D]*-GAL4* flies to flies carrying a *UAS-lacZ* transgene and stained the resulting embryos with antibodies against β-galactosidase (βgal) and Krüppel (Kr), an FC identity transcriptional regulator (Fig. 2A; Ruiz-Gomez et al., 1997). We detected βgal expression in a subset of mesodermal cells from stage 14, similar to the results that we obtained using the GFP reporter described above (Fig. 2A). Kr is expressed in the nuclei of a subset of FCs and developing myotubes (Ruiz-Gomez et al., 1997).

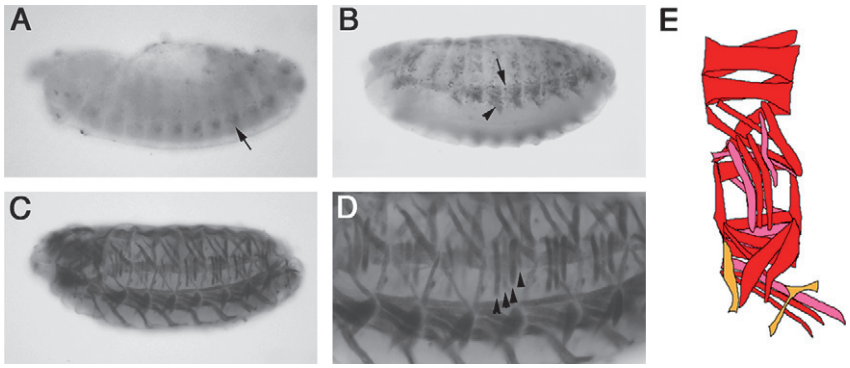

Fig. 1. The *Dmef2-5x[C/D]*-GAL4* driver causes expression of a GFP reporter in the somatic mesoderm and body wall muscles. *Dmef2-5x[C/D]*-GAL4 > UAS-GFP* embryos were stained with an antibody against GFP. Lateral views of stage 14 (A), 15 (B) and 16 (C and D) embryos are shown. (D) A close up of the embryo in (C). (A) In contrast to the direct enhancer-reporter LacZ construct (Duan et al., 2001), GFP expression is first detected in a subset of cells in the ventral somatic mesoderm at during stage 14 (black arrow, A). (B) During stage 15, GFP expression is detected in growing myotubes (black arrowhead, B) and surrounding cells (black arrow, B). (C and D) GFP expression is detected in the final body wall muscles at stage 16. GFP is not detected in all muscles or at uniform levels. Black arrowheads highlight differing levels of GFP expression in LTs1-4. (E) Schematic showing quantification of GFP expression in individual muscles from Table 1. Red = 75–100%, pink = 50–74% and orange ⩽ 50%.