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Short Contribution

Plasticity within the lateral somatic mesoderm of *Drosophila* embryos

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ABSTRACT Each of 30 *Drosophila* larval somatic muscles has its individual shape, insertion sites and innervation. From the very beginning, the formation of individual muscles is controlled by a set of muscle identity genes. The four lateral transverse muscles (LT1-LT4) are thought to be specified by the combinatorial activity of *Krüppel (Kr), apterous (ap)* and *muscle specific homeobox (msh)* genes whilst the activity of the *ladybird (lb)* genes is required for proper formation of the neighbouring segmental border muscle (SBM). We have recently shown that ectopic expression of *lb* changes the identity of *Kr*-expressing lateral muscle precursors and recruits them to form enlarged or duplicated SBMs. Here we report that loss of *msh* function leads to a similar transformation resulting in the overproduction of SBMs. Inversely, in *msh* gain of function embryos, the prospective SBM myoblasts change their identity resulting in the formation of enlarged lateral transverse muscles. These data indicate a key role for the *msh* and *lb* genes in the specification and diversification of myoblast lineages from the lateral domain, and reveal a plasticity of cell fate within the somatic mesoderm of *Drosophila.*

KEY WORDS: *msh, ladybird, identity genes, muscle, Drosophila*

Drosophila larval somatic muscles arise from muscle progenitor cells (Carmena *et al.*, 1995). Each muscle progenitor divides asymmetrically (Ruiz Gomez and Bate 1997; Carmena *et al.*, 1998) to produce a pair of sibling cells: either the founders (Bate, 1990; Dohrmann *et al.*, 1990) of two distinct larval muscles or a founder and the precursor of adult muscles (Ruiz Gomez and Bate, 1997). The founder cells recruit neighbouring myoblasts, fuse with them and form syncytial muscle fibres while the precursors of adult muscles do not fuse with neighbouring myoblasts and, during embryogenesis, behave as non-differentiated somatic mesodermal cells. The distinct fates of founders, derived from the same progenitor, are specified by the asymmetric segregation of the membrane-associated Numb (Nb) protein (Ruiz Gomez and Bate, 1997; Carmena *et al.*, 1998). The presence or absence of Nb, in a given founder cell, is essential for maintenance of lineage-specific expression of identity genes and the formation of a proper muscle pattern. As was recently shown (Ruiz Gomez and Bate, 1997), ectopic expression of *nb* in a founder cell normally devoid of *nb* leads to the activation of a *nb*-dependent muscle identity gene resulting in the loss of *nb*-negative and duplication of *nb*-positive muscle fibres. The opposite losses/duplications observed in gain and loss of *nb* function mutants (Ruiz Gomez and Bate, 1997) indicate a *nb*-orchestrated plasticity which reflects the close relations between muscle fibres derived from the sibling founder cells.

Within the lateral somatic mesoderm one can distinguish a group of four lateral transverse muscles (LT1-LT4) and the SBM lying under the segmental furrow. The LT1, LT2 and LT3, LT4, most likely, (Ruiz Gomez and Bate, 1997) derive from two pairs of sibling founders, whilst the SBM and lateral adult precursors (LaPs) originate from another pair of founders (Jagla *et al.*, 1998). The LT muscles express a LIM homeodomain protein Ap (Bourgouin *et al.*, 1992), a homeodomain protein Msh (Lord *et al.*, 1995; D'Alessio and Frasch, 1996; Nose *et al.*, 1998) and a zinc-finger transcription factor Kr (Ruiz Gomez *et al.*, 1997), whereas the homeodomain proteins (Ladybird early)

Abbreviations used in this paper: SBM, segmental border muscle; msh, muscle segment homeobox gene.

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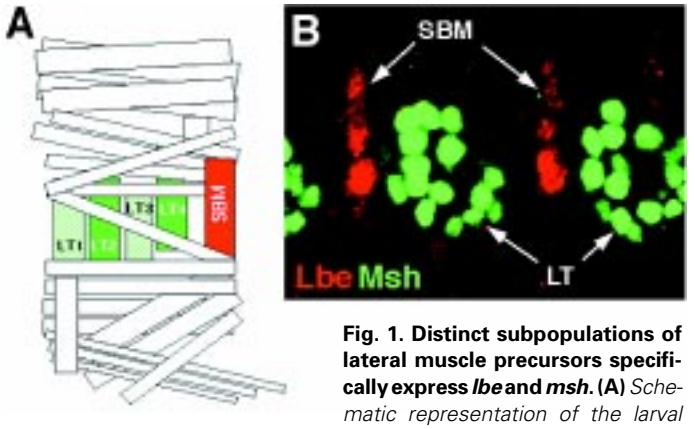

Fig. 1. Distinct subpopulations of lateral muscle precursors specifically express *lbe* and *msh*. (A) *Schematic representation of the larval muscle fibre architecture. White and green hatched boxes indicate the lateral transverse muscles LT1 and LT3 derived from the msh-positive founder cells. Green boxes show LT2 and LT4 fibres derived from muscle founders expressing both msh and Kr genes. The red box indicates the lb-positive SBM.* **(B)** *Confocal scan of a stage 14 msh-lacZ (rH96) embryo stained with anti-Lbe antibody (red) and anti-lacZ antibody (green) revealing msh expression. The lateral epidermal patches of msh expression are out of focus. Notice that lacZ labels a cluster of msh-positive lateral mesodermal cells comprising the myoblasts forming LT muscles.*

Lbe and (Ladybird late) Lbl are specifically expressed in the SBMs (Jagla *et al.,* 1998). In the *nb* mutant embryos, the loss of *Kr*-expressing LT2 and LT4, *lb*-expressing SBM (Ruiz Gomez and Bate, 1997; Jagla *et al.*, 1998) as well as the duplication of LT1, LT3 and LaPs reveal a *nb*-dependent plasticity.

Interestingly, alteration of muscle identity gene function can also provoke muscle transformations. For example, the ubiquitous mesodermal expression of *lb* leads to the loss of the majority of LT muscles and the overproduction of SBMs (Jagla *et al.*, 1998) indicating a larger field of plasticity, independent, in this case, on *nb* activity. This raises the possibility that LT and the SBM-forming founders have closely related fates and that the muscle identity genes specific for the lateral domain may recruit them to the alternative pathways.

To verify this hypothesis, we decided to deregulate one of the LT-specific muscle identity genes. Since the *msh* mutants (Nose *et al.*, 1998), in comparison with *ap* mutants, (Bourgouin *et al.*, 1992) display a more pronounced muscle phenotype, we have chosen to analyse *msh* loss and gain of function mutant embryos. Using double LacZ/Lbe staining, we first compared the wild-type *msh* and *lb* expression domains in rH96 embryos (see Experimental Procedures and Nose *et al.*, 1998). As expected, both genes are expressed in the non-overlapping lateral subdomains. *lbe* specifically labels SBM (Fig. 1; see also Jagla *et al.*, 1998), while *msh* is active in the adjacent group of myoblasts forming the LT1, LT2, LT3 and LT4 precursors. These data together with previous phenotypic analyses of embryos lacking *msh* gene function and those with ectopic *msh* expression (Nose *et al.*, 1998) indicate that *msh* may play an essential role in the formation of LT muscles.

To test cell fate specification in *msh* mutant embryos we have stained myoblasts from the lateral domain using both the SBM-specific anti-Lbe antibody and the anti-Kr antibody which specifically labels the LT2 and LT4 precursors (Fig. 2). Since, in these experiments, the number of *Kr*-positive LT precursors is dramatically reduced (Fig. 2E; Table 1) and the neighbouring SBMs are enlarged or duplicated (Fig. 2B, see also Table 1), our data clearly show that in the absence of *msh* gene function the prospective LT founder cells change their identity. This muscle phenotype is reminiscent of that observed in embryos ectopically expressing *lb* (Jagla *et al.*, 1998) and indicate that similar changes of muscle identities are induced by gain of *lb* and loss of *msh* gene function.

Opposite alterations in the activity of muscle identity genes (Fig. 2C,F and Table 1) and in the pattern of muscle fibres (Nose *et al.*, 1998, and data not shown) appear in the *24B-Gal4/UAS-msh* embryos ubiquitously expressing *msh* in the mesoderm. The *lb*-positive, SBM-forming myoblasts are completely absent (Fig. 2C, Table 1) suggesting that they are recruited to form other muscle fibres. Indeed, as indicated by the enlarged *Kr* staining (Fig. 2F), these myoblasts, influenced by *msh* activity may adopt an LT fate and participate in the formation of supernumerary or enlarged lateral transverse muscles. Since, the *lb*-positive myoblasts disappear from the majority of hemisegments while the supernumerary *Kr*-positive fibres form in only some of them (see Table 1), we speculate that in the *UAS-msh* embryos the prospective SBM myoblasts are committed to either the *Kr*-positive and *Kr*-negative fates. The *msh*-induced shifts of muscular fates may be due to negative transregulation exerted by the Msh and other identity gene products. This possibility is supported by the presence of eh1/TN-like repression domain (Smith and Jaynes, 1996) in both the Msh and the Lbe proteins.

Altogether, our results indicate that the LT and SBM muscle precursors derive from the closely related muscle founder cells belonging to a *msh/lb*-dependent plasticity field. To understand how this field is determined, further cell lineage studies have to be correlated with extrinsic signalling pathways and intrinsic information provided by the network of identity genes.

Experimental Procedures

Drosophila strains

The following *Drosophila* strains were used: the *msh-lac-Z* line rH96 (Nose *et al.*, 1998), a *msh* null allele, $msh^{\Delta 68}$ and the *UAS-msh-m25-m1* line, exhibiting a high level of ectopic *msh* expression. These lines, kindly provided by A. Nose, were recently described by Isshiki *et al.* (1997).

TABLE 1

NUMBER OF LATERAL *KR*-POSITIVE AND *LB*-POSITIVE MUSCLE PRECURSORS PER HEMISEGMENT IN LOSS AND GAIN OF FUNCTION *MSH* MUTANT EMBRYOS

Genotype	wild-type	*msh* $^{\Delta 68}$ (loss of function)	*UAS-msh* (gain of function)
LT2 +LT4	2	0.75	2.3
SBM	1	1.6	0.1

Muscle fibers were counted in 100 wild-type, 57 $msh^{\Delta 68}$ and 61 *UAS-msh* hemisegments.

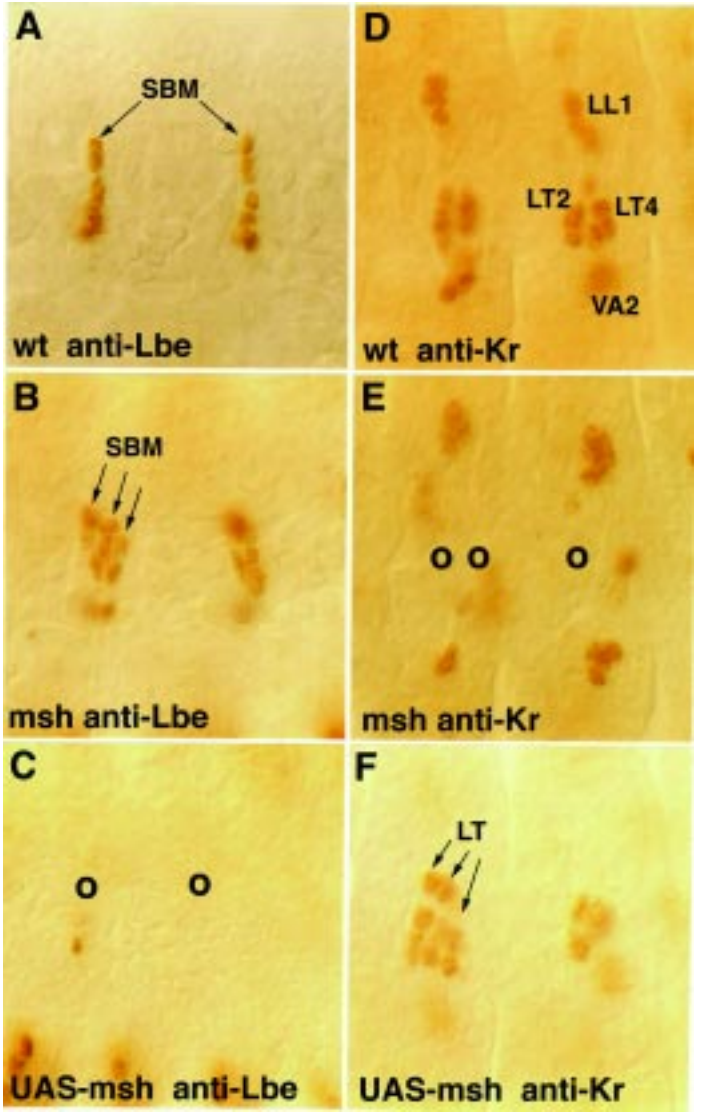

Fig. 2. Plasticity in the lateral mesoderm of the *Drosophila* embryo. **(A,B,C)** *Lateral views of stage 15 embryos stained with anti-Lbe and* **(D,E,F)** *stage 13 embryos stained with anti-Kr antibody.* **(A)** *Ib-positive SBMs and* **(D)** *Kr-expressing lateral muscles (LT2 and LT4) in the wild-type embryo.* **(B,E)** *In the msh null mutants,* **(B)** *the SBMs are strongly enlarged and* **(E)** *almost all Kr-positive lateral muscle precursors (LT2 and LT4) are absent (see also Table 1).* **(C,F)** *In embryos ectopically expressing msh,* **(C)** *the SBM-forming myoblasts are absent and* **(F)** *the number of Kr-expressing lateral muscles increases.*

Ectopic expression and immunocytochemistry

The uniform ectopic expression of *msh* in the mesoderm was induced using the *Gal4-UAS* system (Brand and Perrimon, 1993). Virgin females, from the mesoderm-specific effector line *24B-Gal4* were crossed with *UAS-msh-m25-m1* males and the embryos stained with the following primary antibodies: monoclonal anti-Lbe (1:1), rabbit anti-β-galactosidase (1:5000) and rabbit anti-Kr (1:2000) kindly provided by P. Carrera and G. Vorbrüggen. Labelled cells were detected using the ABC-Elite-peroxidase kit (Vector Laboratories) with diaminobenzidine as a substrate. To determine the position of *lb*-positive myoblasts with respect to *msh*-positive lateral muscle precursors, we have used secondary antibodies conjugated to Cy3 or Cy2 (Jackson Immuno-Research). Whole-mount embryos were photographed on the Axiophot microscope under Nomarski optics or scanned using a Leica confocal microscope.

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