Development 126, 1495-1504 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
DEV7705

Requirement for the *Drosophila* COE transcription factor Collier in formation of an embryonic muscle: transcriptional response to Notch signalling

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Accepted 11 January; published on WWW 3 March 1999

SUMMARY

During *Drosophila* embryogenesis, mesodermal cells are recruited to form a stereotyped pattern of about 30 different larval muscles per hemisegment. The formation of this pattern is initiated by the specification of a special class of myoblasts, called founder cells, that are uniquely able to fuse with neighbouring myoblasts. We report here the role of the COE transcription factor Collier in the formation of a single muscle, muscle DA3$^{[A]}$(DA4$^{[T]}$). Col expression is first observed in two promuscular clusters (in segments A1-A7), the two corresponding progenitors and their progeny founder cells, but its transcription is maintained in only one of these four founder cells, the founder of muscle DA3$^{[A]}$. This lineage-specific restriction depends on the asymmetric segregation of Numb during the progenitor cell division and involves the repression of *col* transcription by Notch signalling. In *col* mutant embryos, the DA3$^{[A]}$ founder cells form but do not maintain *col* transcription and are unable to fuse with neighbouring myoblasts, leading to a loss-of-muscle DA3$^{[A]}$ phenotype. In wild-type embryos, each of the DA3$^{[A]}$-recruited myoblasts turns on *col* transcription, indicating that the conversion, by the DA3$^{[A]}$ founder cell, of 'naive' myoblasts to express its distinctive pattern of gene expression involves activation of *col* itself. We find that muscles DA3$^{[A]}$ and DO5$^{[A]}$ (DA4$^{[T]}$ and DO5$^{[T]}$) derive from a common progenitor cell. Ectopic expression of Col is not sufficient, however, to switch the DO5$^{[A]}$ to a DA3$^{[A]}$ fate. Together these results lead us to propose that specification of the DA3$^{[A]}$ muscle lineage requires both Col and at least one other transcription factor, supporting the hypothesis of a combinatorial code of muscle-specific gene regulation controlling the formation and diversification of individual somatic muscles.

Key words: Somatic myogenesis, *collier*, *Drosophila melanogaster*, COE protein, Asymmetric cell division, Notch signalling, Cell fate

INTRODUCTION

The formation of the embryonic musculature of *Drosophila melanogaster* is a highly dynamic and genetically regulated process (see reviews by Bate, 1993; Abmayr et al., 1995; Baylies et al., 1998). A standard arrangement of 30 somatic muscles develops in each of the abdominal hemisegments A2-A7, with minor variations in more anterior and posterior trunk segments (Bate, 1993). Each muscle fiber is an individual syncytium that can be distinguished by its position, shape, epidermal attachment sites and, ultimately, pattern of innervation. Thus, while all muscles share a same general muscle differentiation program and general properties such as the presence of contractile proteins, receptors for neurotransmitters etc., each muscle has unique properties, which are manifested as each muscle precursor differentiates to form a muscle fibre.

Recent evidence has accumulated to suggest that the acquisition of muscle-specific properties depends on the prior specification of a special class of mesoderm-derived myoblasts, called founder cells, at particular locations in the somatic mesoderm (Rushton et al., 1995; Baylies et al., 1998, for review). The mechanisms involved in this specification process bare a striking analogy to those involved in the specification of neuroblasts. Groups of equivalent cells competent to form myoblasts, the promuscular clusters, can be recognised early in the myogenic program by their expression of the proneural gene *lethal of scute* (*l'sc*) (Carmena et al., 1995). In a process reminiscent of neuroblast segregation, specification of one (sometimes two) progenitor cell from each cluster depends on the process of lateral inhibition mediated by the activity of the neurogenic genes (Corbin et al., 1991; Bate et al., 1993; Carmena et al., 1995). Each muscle progenitor then undergoes an asymmetric division, which plays a crucial role for the diversification of the muscular fate. As in neural lineages, this asymmetric division involves the segregation into one daughter cell of the Numb (Nb) protein (Rhyu et al., 1994; Spana et al., 1995; Ruiz-Gomez et al., 1997; Carmena et al., 1998), which has been proposed to bias Notch signalling between the two sibling cells, presumably via a physical interaction between Numb and the cytoplasmic domain of Notch (Guo et al., 1996). The two cells resulting from the asymmetric division of the muscle progenitor, the 'founder cells', are uniquely able to recruit neighbouring 'naive' myoblast cells and fuse with these to form syncytial muscle precursors (Bate, 1990; Dohrman et al., 1990; Abmayr

et al., 1995; reviewed by Baylies et al., 1998). The current view is that the properties specific to each muscle result from the selective expression, in each founder cell, of one or several putative muscle identity genes encoding transcription factors. Several such genes, expressed in subsets of founder cells, have been described, including *S59* (Dohrman et al., 1990; Carmena et al., 1995), *apterous (ap)* (Bourguoin et al., 1992*), vestigial (vg)* (Bate and Rushton, 1993; Bate et al., 1993*), even-skipped (eve)* (Frasch et al., 1987; Ruiz-Gomez and Bate, 1997), *krüppel (kr)* (Ruiz-Gomez et al., 1997*), nautilus (nau)* (Michelson et al., 1990), *muscle segment homebox* (*msh*) (d'Alessio and Frasch, 1996; Nose et al., 1998) and *ladybird (lb)* (Jagla et al., 1998). Loss of function or ectopic expression of *ap* (Bourgouin et al., 1992), *kr* (Ruiz-Gomez et al., 1997), *msh* (Nose et al., 1998), *lb* (Jagla et al., 1998) and *nau* (Keller et al., 1997, 1998) support this model as they alter the properties of at least a subset of muscle precursors in which these transcription factors are expressed. However, the low expressivity of these phenotypes suggested that either each factor plays a minor and/or redundant role in muscle patterning, or that the majority of muscles fibres are specified by a combination of transcription factors rather than a single one (Ruiz-Gomez et al., 1997; Nose et al., 1998). Altogether, these studies provide a framework for considering early aspects of muscle patterning. However, except for rare cases, the mesodermal lineages have not been elucidated and the molecular mechanisms utilised for the assignation of a specific cell fate to each muscle remain enigmatic.

We describe here the role of the COE transcription factor Collier in the formation of a single somatic muscle per hemisegment, muscle DA3$^{[A]}$ (in the abdominal segments, DA4$^{[T]}$ in the thoracic segments T2 and T3). *col* is initially transcribed in two promuscular clusters per abdominal segment A1-A7 and one cluster in thoracic T2 and T3. This expression becomes restricted to the derived progenitor cells, under the control of neurogenic genes but independent of *l'sc*. *col* remains transiently transcribed on in each of the derived founder cells, but is then switched off, except in the founder cell for muscle DA3$^{[A]}$ (DA4$^{[T]}$). We show that the selective maintenance of *col* transcription in the DA3$^{[A]}$ founder cell is dependent upon an asymmetric cell division and involves a transcriptional response of *col* to Notch signalling. The mesodermal *col* mutant phenotype is the specific loss of muscle DA3$^{[A]}$ (DA4$^{[T]}$). This highly penetrant phenotype results from the failure of the mutant DA3$^{[A]}$ (DA4$^{[T]}$) founder cell to recruit and fuse with surrounding myoblasts. In wild-type embryos, this fusion results in the activation of *col* transcription in the nucleus of each recruited myoblast. The patterns of expression of *col* and a *col-lacZ* reporter gene show that muscles DA3$^{[A]}$/DO5$^{[A]}$ (DA4$^{[T]}$/DO4$^{[T]}$) derive from a common progenitor cell. Ectopic expression experiments indicate that Col alone is not sufficient to switch the DO5$^{[A]}$ to a DA3$^{[A]}$ cell fate, suggesting that Col is one of several transcription factors required for the muscle DA3$^{[A]}$-specific differentiation program.

MATERIALS AND METHODS

Drosophila strains

The following *Drosophila* flies used: *white* (*w*) as the 'wild-type' reference; $wg^{1.7}$, hh^{15} (Lindsley and Zimm, 1992); nb^{1}, a null allele of *nb* (Uemura et al., 1989); two alleles of *inscuteable*, $insc^{P72}$ and $insc^{P49}$ (Kraut et al., 96); null alleles of *Notch*, N^{55e11} and *Delta*, Dl^{L562}; a strong allele of *sanpodo*, $spdo^{K433}$ (Salzberg et al., 1994); *Df(1)260-1*, a complete deletion of the *AS-C* complex (Garcia-Bellido, 1979). col^{1}, col^{2} and col^{3} behave as null alleles of *col* in formation of the embryonic head and musculature (M. C., unpublished data).

Transgenic lines and heat-shock treatment

The P[col5-lacZ] construct was constructed by inserting a 5 kb *col* fragment that extends in the protein coding region of *col* (560 nucleotides of the 5′ part of *col* cDNA, Crozatier et al., 1996), into the pCaSpeRβ-gal vector. Transgenic lines were generated by injection of *w* embryos according to published procedures (Rubin and Spradling, 1982). One homozygous viable line carrying an insert on the third chromosome was consistently used in this study.

The *HScol* construct was constructed by inserting the 3.8 kb *col* cDNA under control of the hsp70 promoter in the PCaSpeR-hs vector. The line called HScol 36-8, which carries homozygous viable HS-col insert on the 3rd chromosome, was preferentially used in this study. Similar results were obtained with another line (HScol 17-1), that carries an homozygous lethal insert on the same chromosome. The transgenic HScol embryos were aged at 25°C and heat-shock treated, at different times of development (indicated in the text), for 30 minutes at 37°C.

Histochemistry

Labelling embryos with antibody staining and/or in situ hybridisation was as referenced in Crozatier et al. (1996). The *col* intronic probe is a 2.5 kb unspliced cDNA that contains the first *col* exon (693 bp) and about 1.8 kb of intronic sequences (M. C., unpublished data). Incubations with primary antibodies were carried out at 4°C overnight at the following dilutions: rabbit anti-Col (1/400), rabbit anti-MHC (1/500, generously provided by D. Kiehardt), mouse anti-β-gal (1/1000, purchased from Promega). Colour reactions were developed using diaminobenzidine alone (brown colour) or with 0.8% $NiCl_2$ (black colour) as substrates.

Generation of anti-Col antibodies

The *col* cDNA was cloned into pGex vector to express the full-length Col protein. Polyclonal rabbit antisera were prepared by Eurogentec. The rabbit anti-Col antibodies were purified by affinity-chromatography using a GST-Col fusion as a bait. In col^{1} mutant embryos, the Col protein cannot be detected with anti-Col antisera, correlating with the prediction of a truncated protein, based on sequence analysis of the mutant DNA (M. C., unpublished data).

RESULTS

Col is required for muscle DA3 $^{[A]}$ formation

The *col* gene was first characterised for its role in embryonic head patterning, correlating with its expression at the early gastrula stage in a single stripe of lateral cells corresponding to the mitotic domain MD2 and possibly parasegment 0 (Crozatier et al., 1996). The recent isolation of several independent *col* mutations (M. C., unpublished data) led us to investigate its potential role in mesoderm formation, since in situ hybridisation experiments indicated that, after gastrulation, *col* is expressed in specific muscle precursors as well as subsets of neurons of both the PNS and CNS (Crozatier et al., 1996, and see below). The *cis*-acting regulatory region responsible for *col* expression in the mesoderm was identified by generating reporter transgenes expressing a Col/β-galactosidase (*lacZ*) fusion protein under the control of genomic *col* DNA fragments; a transgene containing 5 kb of

col upstream DNA (P[*col5-lacZ*]) reproduces the *col* expression pattern in the head and in the mesoderm (Figs 1, 2, and M. C., unpublished data). The greater stability of the β-galactosidase compared to that of *col* mRNA allowed us to trace the mesodermal cells in which *col* is expressed until stage 16, when each muscle can be identified by its position and shape. Double immunostaining of embryos with anti-β-gal and anti-MHC antibodies indicated that *col* is specifically expressed in the cells generating a single muscle per hemisegment, muscle DA3$^{[A]}$ in abdominal segments A1-A7 and muscle DA4$^{[T]}$ in thoracic segments T2 and T3, according to the nomenclature proposed by Bate (1993) (Figs 1, 2). No muscle precursor expresses *col-lacZ* in segment T1, consistent with the absence of a muscle analogous to DA4$^{[T]}$ in this segment. In the segment A8, Col is expressed in four different muscles: ventral transverse 1 (VT1), ventral transverse 2 (VT2), gut suspension 3 (GS3) and terminal oblique 2 (TO2) (Fig. 1B, C and see Bate, 1993); this segment-specific pattern will not be considered further in our analysis. We next determined whether *col* activity was necessary for formation of the muscles in which it is specifically expressed by examining *col* mutant embryos carrying a copy of the P[*col5-lacZ*] transgene double-stained with MHC and β-gal antibodies (Fig. 1D,F). For each of the three *col* mutations recently isolated, the same result was observed, i.e., muscle DA3$^{[A]}$/DA4$^{[T]}$ is missing or, in a few cases, reduced to a thinned muscle (see Fig. 1F). This loss-of-muscle phenotype is both highly penetrant and specific: in *col* mutant embryos, DA3$^{[A]}$/DA4$^{[T]}$ is missing in 95% of the hemisegments, whereas no other muscle is either missing or atrophied, including the neighbouring muscles DO5$^{[A]}$, DO4$^{[A,T]}$ and DT1$^{[A]}$ (see below and Fig. 1D,F). We used a *col* transgene composed of the 5 kb *col* upstream region fused to the full-length *col* cDNA P[*col5-cDNA*] in phenotypic rescue experiments (M. C., unpublished data). A single copy of this transgene fully rescued the formation of the DA3$^{[A]}$/DA4$^{[T]}$ muscle (not shown), confirming that *col* activity is specifically required for its formation.

Col expression during muscle development

To investigate in more detail the mesodermal function of *col*, we performed a detailed analysis of its expression during myogenesis, using a combination of in situ hybridisation, immunostaining with anti-Col antibodies and anti-β-gal antibodies on P[*col5-lacZ*] transgenic embryos (Fig. 2). At stage 10, the Col protein is expressed in a single cluster of mesodermal cells in thoracic T2 and T3 and two clusters in abdominal A1-A7 hemisegments, respectively. This expression becomes rapidly restricted to the progenitor cells singled out from each cluster (stage 11). Col is also present in the two founder cells derived from these progenitor cells (stage 12). Col staining further allows to follow the recruitment of myoblasts by these founder cells, since it is detected in muscle precursor nuclei during myoblast fusion (stage 13). We relied on Col staining at this stage to identify the Col-expressing muscle precursors, based on their relative position and morphology (Fig. 2B; Bate, 1993). Col is expressed in two dorsal muscles in each of the T2 and T3 thoracic segments, the dorsal acute muscle 4 (DA4$^{[T]}$) and the dorsal oblique muscle 4 (DO4$^{[T]}$), and four muscles in each abdominal segment A1-A7, the dorsal acute muscle 3 (DA3$^{[A]}$), the dorsal oblique muscle 5 (DO5$^{[A]}$), the dorsal oblique 4 (DO4$^{[A]}$) and the dorsal transverse muscle 1 (DT1$^{[A]}$) (Fig. 2B). After stage 13, the Col protein is detected only in muscle DA3$^{[A]}$/DA4$^{[T]}$, where it persists up to stage 15, whereas it is absent from the differentiated muscle fibre. Expression of the P[*col5-lacZ*] transgene precisely reproduces the Col expression pattern during muscle formation, except that β-gal staining is not detected prior to specification of the progenitor and persists in muscles DA3$^{[A]}$/DA4$^{[T]}$ at stage 16 (see Fig. 1).

Expression of Col in segments T2 and T3 suggests that muscles DA4$^{[T]}$ and DO4$^{[T]}$ share a common progenitor, specified from the only promuscular cluster that expresses Col in these two segments. By analogy, it suggests that the abdominal muscles DA3$^{[A]}$ and DO5$^{[A]}$ derive also from a common progenitor cell homologous to the DA4$^{[T]}$/DO4$^{[T]}$ progenitor in thoracic segments. Our results also suggest that

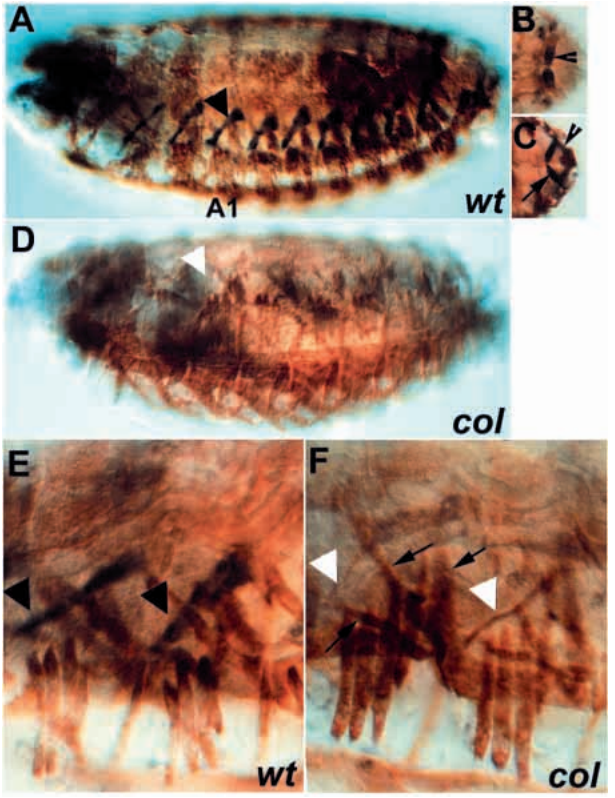

Fig. 1. *col* requirement for muscle DA3$^{[A]}$ (DA4$^{[T]}$) formation, revealed by expressing a *col-lacZ* transgene. Stage 16 wild-type (A-C,E) and *col* mutant (D,F) embryos carrying a P[*col5-lacZ*] transgene stained with anti-Myosin Heavy Chain (brown) and anti-β-galactosidase (black) antibodies to identify the *col*-expressing muscles; laterodorsal views except in B and C. (A) The black arrowhead points to muscles DA4$^{[T]}$ and DA3$^{[A]}$ present in each T2-T3, and A1-A7 segment, respectively. (B,C) In the A8 segment, *col* is expressed in four muscles: VT1 and VT2 seen in one optical section (B), GS3 (arrow) and TO2 (arrowhead) seen in another section (C). (D) In *col* mutant embryos muscles DA4$^{[T]}$ and DA3$^{[A]}$ (white arrowhead) are missing. (E,F) Enlarged views of two adjacent abdominal segments. Muscle DA3$^{[A]}$ (white arrowhead) is either missing or reduced to a thinned cell in *col* mutant embryos, whereas other muscles are not affected. Black arrows point to muscles DO5$^{[A]}$, DO4$^{[A]}$ and DT1$^{[A]}$ which also express P[*col5-lacZ*] at stage 13 (see Fig. 2).

muscles DO4[A] and DT1[A] (which has no homolog in the thoracic segments) derive from the second progenitor that expresses Col in segments A1-A7 and is specified later than, and posterior to, the DA3[A]/DO5[A] progenitor. Since, by all experimental criteria examined, DA4[T] behaves as DA3[A], we restrict below our analysis to DA3[A] in the abdominal segments.

col transcription is maintained only in the DA3[A] founder cell and is activated in the nuclei of all myoblasts recruited in the DA3[A] muscle syncytium

Since *col* activity is specifically required for muscle DA3[A] formation, its expression in other founder cells was intriguing. The syncytial structure of muscle precursors made it possible that the presence of the Col protein was due to translation of a pre-existing *col* mRNA accumulated in founder cells prior to myoblast fusion, rather than de novo *col* transcription. In order to distinguish between these two possibilities, we performed in situ hybridisation with a *col* intron probe that specifically labels *col* nascent transcripts in nuclei (Figs 2C, 3). The results show that all four Col-positive founder cells present in each abdominal hemisegment transcribe *col* immediately after division of the progenitors (Fig. 3). Soon after (late stage 12), however, *col* transcription becomes restricted to a single founder cell, the founder cell seeding muscle DA3[A] (Fig. 3). During the process of myoblast recruitment by this founder cell, and as fusion proceeds, *col* transcription is activated in the nuclei of myoblasts newly incorporated in the DA3[A] muscle precursor (Fig. 2C). At stage 14, all 14 nuclei incorporated in the DA3[A] syncytium and only these nuclei actively transcribe *col*. This shows that the conversion, by the DA3[A] founder cell, of 'naive myoblasts' to express its distinctive pattern of gene expression occurs cell by cell, at the transcriptional level.

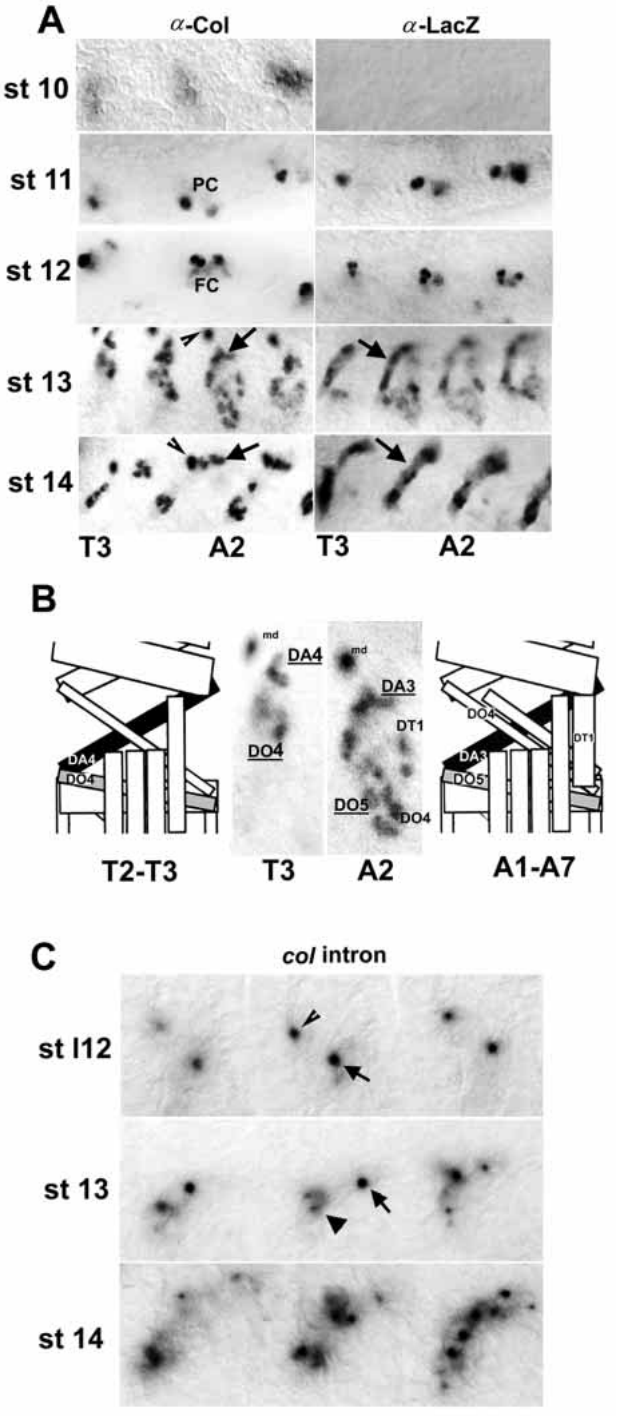

Fig. 2. Dynamics of Col expression in the developing muscles. All embryos are oriented dorsal up and anterior to the left. (A) Embryos stained with anti-Col antibodies (left) or anti-β-gal antibodies (P[col5-lacZ] transgenic embryos) (right). Only segments T3 to A2 are shown. At the early stage 10, expression of Col protein is detected in one and two clusters of mesodermal cells in T3 and A1-A2 segments, respectively. No expression of P[*col5-lacZ*] can be detected. At stage 11, Col expression is restricted to the progenitor cells (PC) (one in T3, two in A1-A2) singled out from each Col-positive promuscular cluster. From that stage, expression of P[*col5-lacZ*] reproduces that of the endogenous *col* gene. By stage 12, Col expression is detected in the two founder cells (FC) resulting from the division of each progenitor. At stage 13, the Col protein remains present in two (T3) or four (A1-A2) muscle precursors. The black arrowhead points to a dorsal multidendritic neuron (md) which also expresses *col*, but not the P[*col5-lacZ*] transgene. At stage 14, Col expression is only detected in the DA3[A] muscle precursor (black arrow), correlating with active *col* transcription in nuclei of all myoblasts recruited by the DA3[A] founder cell (see C). (B) Enlarged view of the Col-expressing muscle precursors in segment T3 and segment A2 in an embryo at stage 13. Schematic patterns of the dorsal muscles of one thoracic (T2, T3) and one abdominal segment (A1-A7), viewed from the exterior, are represented. The Col-expressing muscles are dorsal acute 3 (or 4) DA3[A] (or DA4[T]) in black; dorsal oblique 5 (or 4) DO5[A] (or DO4[T]) in grey; dorsal transverse 1 DT1[A]; dorsal oblique 4, DO4[A]. (C) Wild-type embryos labelled by in situ hybridization with a *col* intron probe. At late stage 12 (l12), *col* transcription is only detected in one founder cell (black arrow) per hemisegment, the DA3[A] founder cell. The arrowhead points to a dorsal md. At stage 13, during the process of myoblast recruitement by the founder cell (arrow), and as fusion proceeds, *col* transcription is activated in the nucleus of each newly incorporated myoblast (arrowhead). At stage 14, all the nuclei incorporated in the DA3[A] muscle precursor actively transcribe *col*.

A DA3[A] founder cell forms in *col* mutant embryos but does not maintain *col* transcription and is unable to recruit surrounding myoblasts

To study this lack of muscle DA3[A] phenotype of *col* mutant embryos in more detail, we looked at *col* transcription in these embryos, using the *col* intron probe (Fig. 3). No difference was

observed with respect to wild-type embryos, up to early stage 12: four founder cells (in A1-A7, two in T2 and T3) transcribe *col* (Fig. 3A,D). At late stage 12, however, and unlike wild-type embryos, *col* transcription is not maintained in the DA3[A] founder cell (Fig. 3B,E). Immunostaining of stage 13 *col* mutant embryos with anti-Col antibodies shows that, contrary to the other founder cells, the DA3[A] founder cell has not fused with surrounding myoblasts, although it has started to elongate (Fig. 3C,F and not shown). Furthermore, Col staining fades from this unfused founder cell as quickly as from the DO5[A] or DO4[A] and DT1[A] muscle precursors. These data indicate that in *col* mutant embryos, the DA3[A] founder cell forms but does not maintain *col* transcription and is unable to fuse with neighbouring myoblasts.

Mesodermal and epidermal cues required for the segregation of *col*-expressing progenitor cells

The *col*-expressing promuscular clusters and progenitor cells have a distinctive position, as defined relative to morphological landmarks and ectodermal Engrailed (En) expression (Fig. 4A). The DA3[A]/DO5[A] progenitor cell lies underneath the anterior epidermal compartment whereas the DT1[A]/DO4[A] progenitor cell lies on the anterior edge of the posterior compartment, consistent with mapping of the primordium for the somatic mesoderm (see Azpiazu et al., 1996). Since Wingless (Wg) and Hedgehog (Hh) signalling have been shown to be required for mesoderm segmentation and formation of a subset of muscle founder cells (Baylies et al. 1995; Azpiazu et al. 1996), we analysed *col* expression in *wg* and *hh* mutant embryos. At stage 10, both mutant embryos show changes in mesodermal *col* expression: rather than being restricted to specific clusters in the anterior compartment, it appears almost continuous along the anteroposterior axis (Fig. 4B,D,F). Therefore, both *wg* and *hh* signallings appear to restrict *col* transcription to specific clusters. Lack of Wg or Hh activity does not seem, however, to impede specification of the

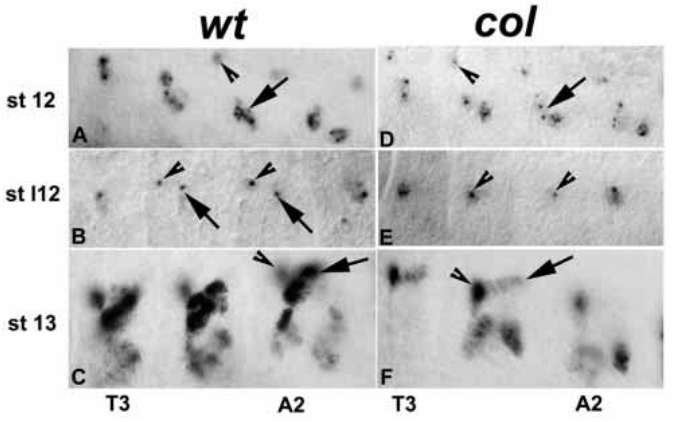

Fig. 3. In *col* mutant embryos, the DA3[A] founder cell does not maintain *col* transcription and does not recruit myoblasts. In situ hybridisation with a *col* intron probe was performed on wild-type (A,B) or *col* mutant (D,E) embryos, same orientation and segments as in Fig. 2. At early stage 12 (A,D), *col* transcription is detected in two, or four, founder cells per thoracic and abdominal (arrows) segment, respectively; the arrowhead points to a md. At late stage 12, only the DA3[A] founder cell (arrows) keeps transcribing *col* (B). This expression is lost in *col* mutant embryos (E). (C,F) Immunostaining with anti-Col antibodies of wild-type (C) and *col* mutant embryos (F) at stage 13. (C) Two or four muscles per thoracic and abdominal segment, respectively, do express Col. Muscle DA3[A] is indicated by an arrow in A2. (F) In *col* mutant embryos the DA3[A] muscle precursor is present, but as a single cell which tends to elongate (arrow in A1) and does not recruit other myoblasts.

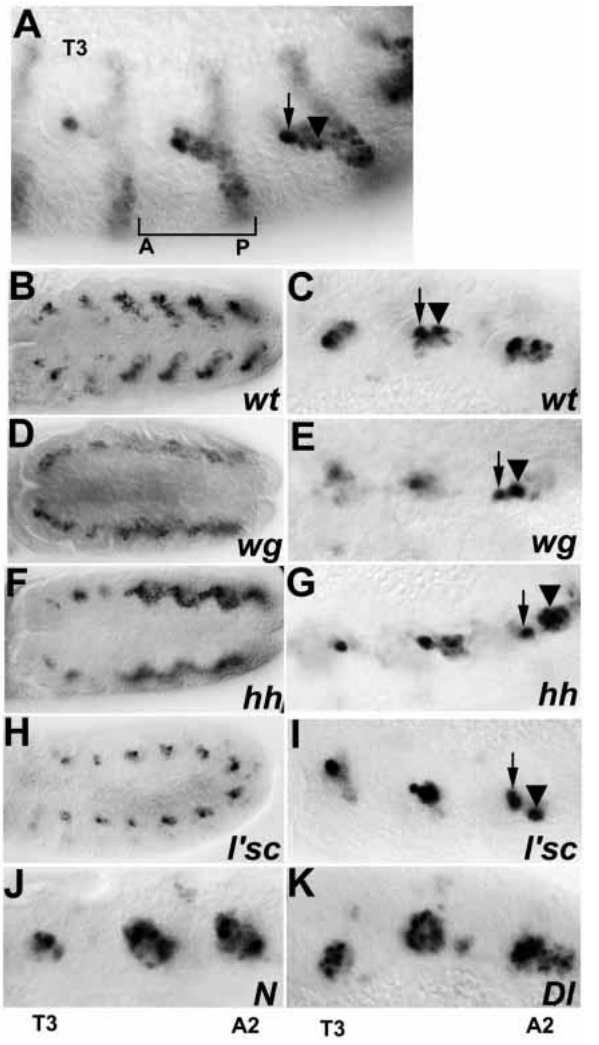

Fig. 4. Specification of the Col progenitor cells occurs normally in *wg* and *l'sc* mutant embryos and is dependent upon Notch/Delta signalling. (A) Detailed lateral view of a wild-type embryo double stained with anti-Col and anti-En antibodies. The DA3[A]/DO5[A] progenitor (arrow) lies underneath the anterior epidermal compartment (noted A) and the DT1[A]/DO4[A] progenitor (arrowhead) lies at the anterior edge of the posterior compartment (noted P). (B-K) Col staining of wild-type and mutant embryos, as indicated on the bottom of each panel. Stage 10 embryos, dorsal (B,D,F) or lateral (H) view. In *wg* and *hh* mutant embryos, Col expression in the mesoderm appears abnormally spread along the A-P axis (compare to the expression in discrete promuscular clusters in wild-type embryos (B). (C,E,G,I-K) Enlarged lateral views of segments T3-A2 of stage 11 embryos, Both the DA3[A]/DO5[A] (arrow) and DT1[A]/DO4[A] (arrowhead) progenitor cells are normally singled out in either *wg* or *l'sc* mutant embryos whereas only the DA3[A]/DO5[A] progenitor cell is singled out correctly in *hh* mutant embryos. In either *N* (J), or *Dl* (K) embryos, Col expression is not restricted to progenitor cells but remains in all cells of the promuscular cluster.

DA3[A]/DO5[A] progenitor, which is singled out as in wild-type embryos (Fig. 4C,E,G). We noticed, however, that, while the DA3[A]/DO5[A] progenitor appears to be specified normally, more than one cell is singled out from the DT1[A]/DO4[A] cluster in *hh* mutant embryos (Fig. 4G).

Following establishment of the promuscular clusters, specification of the progenitors is controlled by lateral inhibition, a cell-cell interaction process mediated by the neurogenic genes *Notch* (*N*) and *Delta* (*Dl*) (Corbin et al., 1991; Bate et al., 1993). In both *N* and *Dl* mutant embryos, promuscular Col expression is initiated normally but fails to become restricted to a single cell per cluster, similar to observations previously made for the expression of *l'sc* (Fig. 4J,K). As a consequence, an hyperplasic expression of Col is observed from stage 11 (see also Fig. 5). Since it is expressed in promuscular clusters and segregating muscle progenitors, *l'sc* has been proposed to play a role in muscle progenitor selection similar to the role of *achaete* and *scute* in neuroblast specification (Carmena et al., 1995). However, in embryos lacking *l'sc* activity, selection of the Col-expressing progenitors occurs normally at stage 11 and muscle DA3[A] forms as in wild type (Fig. 4H,I and data not shown).

Muscles DA3[A] and DO5[A] share a common progenitor

A key event in the generation of the muscle diversity is the asymmetric division of progenitor cells (Carmena et al., 1995). The distinction between sibling muscle founder cells depends upon the differential distribution of the membrane-associated protein Numb (Nb), under the control of *inscuteable (insc)* (Ruiz-Gomez and Bate, 1997; Carmena et al., 1998; Park et al., 1998). One proposal for the action of Nb in determining differences in cell fate is that it biases the N-mediated cell-cell interactions by inhibiting Notch activity, so that this interaction becomes, in turn, asymmetric (Guo et al., 1996). We analysed the formation of muscles DA3[A] and DO5[A] in *insc*, *nb* and *N* mutant embryos, using the expression of P[*col5-lacZ*] to follow the DA3 cell fate (Fig. 5). We found that, in *insc* mutant embryos, the DA3[A] muscle is duplicated at the expense of DO5[A] in most of the segments (Fig. 5C). Although not 100% penetrant, the DA3[A] duplication phenotype always correlates with the absence of DO5[A], indicating a transformation of DO5[A] into DA3[A] (the incomplete expressivity of the *nb* and *insc* phenotypes has been previously discussed, Carmena et al., 1998). The reciprocal phenotype was observed in *nb* embryos: muscle DA3[A] is missing whereas muscle DO5[A] is duplicated (Fig. 5B). By analogy with the sensory organ precursor (SOP) lineage (Guo et al., 1996), this finding suggests that the DA3[A] founder cell is the cell that inherits Nb. Absence, or duplication, of DA3[A] in *nb* and *insc* mutant embryos, respectively, indicate that Nb function is required for specifying the DA3[A] cell fate. This conclusion is supported by the DA3[A] duplication phenotype observed in embryos mutant for *sanpodo* (*spdo*), another gene that acts antagonistically to *nb* in the *Notch*-mediated determination of alternative cell fates and encodes a tropomodulin-like protein (data not shown; Salzberg et al., 1994; Dye et al., 1998; Skeath et Doe, 1998). The question was then raised of how *nb* and *col* functions relate to each other in specifying DA3[A]. We thus examined *col* transcription in *insc* and *nb* mutant embryos, using the *col* intronic probe.

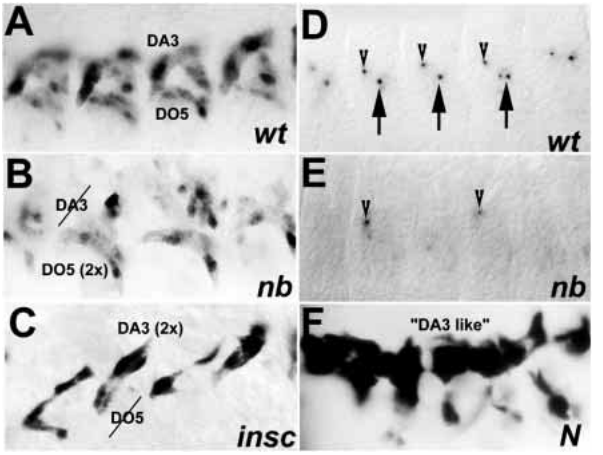

Fig. 5. DA3[A] to DO5[A], and reciprocal transformation, in *nb* and *insc* mutant embryos, respectively. (A-C,F) Anti-β-gal immunostaining of stage 13 (A,B) or stage 14 (C,F) wt (A) or mutant (B,C,F) embryos carrying a copy of P[*col5-lacZ*]. Lateral views of four adjacent abdominal segments are shown. In *nb* embryos (B), the DO5[A] muscle is duplicated at the expense of DA3[A]. Conversely, DA3[A] is duplicated at the expense of DO5[A] in *insc* mutants (C). In *Notch* (*N*) embryos, muscles are not formed properly (F). (D,E) In situ hybridisation with a *col* intron probe on late stage 12 embryos; *col* transcription is specifically maintained in the DA3[A] founder cell (arrows) in wild-type embryos (D) but is lost from this cell in *nb* mutant embryos (E). The arrowhead points to the expression of *col* in a multidendritic neuron (md), which is variably affected by the *nb* mutation. The maintenance of *col-lacZ* expression in a large number of 'muscle precursor cells' suggests their DA3[A] identity.

col transcription is controlled by Notch signalling in the establishment of the DA3[A]/DO5[A] lineage

In wild-type embryos at late stage 12, only one founder cell (DA3[A]) maintains *col* transcription (Fig. 5D, see also Fig. 3B,E); in *insc* mutant embryos, two cells do so (data not shown). Conversely, no founder cell continues to transcribe *col* in *nb* mutant embryos (Fig. 5E). These data indicate that Nb determines the choice between the DA3[A] and DO5[A] cell fates, by allowing *col* transcription to persist in the DA3[A] founder cell. In *N* mutant embryos, a large disruption of the muscle pattern occurs, as a result of the cumulative effects of overproduction of muscle progenitor cells, lack of myoblast fusion, disorganisation of the muscle epidermal attachment sites as well as, possibly, lack of a signal to the mesoderm emanating from the epiderm (Bate, 1993; Fuerstenberg and Giniger, 1998). Despite this cumulative phenotype, we used *N* mutant embryos to analyse the role of N in establishing the DA3[A] cell fate, taking advantage of the perdurable expression of the *col-lacZ* transgene. In *N* embryos, there is a large increase in the number of muscle cells that express high levels of Col and β-gal at stage 11, resulting from the defective progenitor selection (see Fig. 4J). β-gal expression persists in these cells up to stage 16, suggesting that they have adopted a DA3[A] fate (Fig. 5F). All together, and based on the recent finding that Notch is required to maintain progenitor-specific gene expression in one sibling founder cell and repress it in the other (Ruiz-Gomez et al., 1997; Carmena et al., 1998), a comparison of the patterns of *col* expression between wild-type and *insc*, *nb* or *N* mutant embryos indicates that the restriction

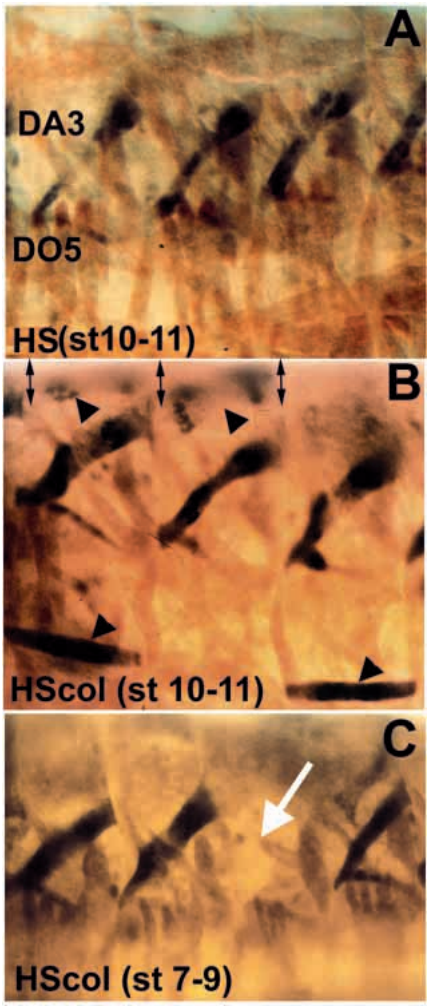

Fig. 6. Ectopic expression of *col* activates *col-lacZ* expression in specific muscles but does not alter the muscle pattern. Stage 15 embryos carrying the P[*col5-lacZ*] (A) plus *HScol* transgene (B,C) stained with anti-MHC (brown) and anti-β-gal (black); four adjacent abdominal segments are shown. (A,B) Ectopic *col* expression induced by heat-shock treatment at stage 10-11 (5-7 hours AEL), after selection of the progenitor cell, does not affect the muscle pattern but results in ectopic β-gal expression in a specific subset of muscles, muscles DA2$^{[A]}$ and VL1$^{[A]}$ (arrowheads). The segmental limits are indicated by double arrows. (C) Heat-shock treatment performed at stage 7-9 (4-5 hours AEL), i.e., before the selection of the progenitor cell, leads to the specific loss of muscles DA3$^{[A]}$ and DO5$^{[A]}$ (white arrow), in one segment of about half of the embryos.

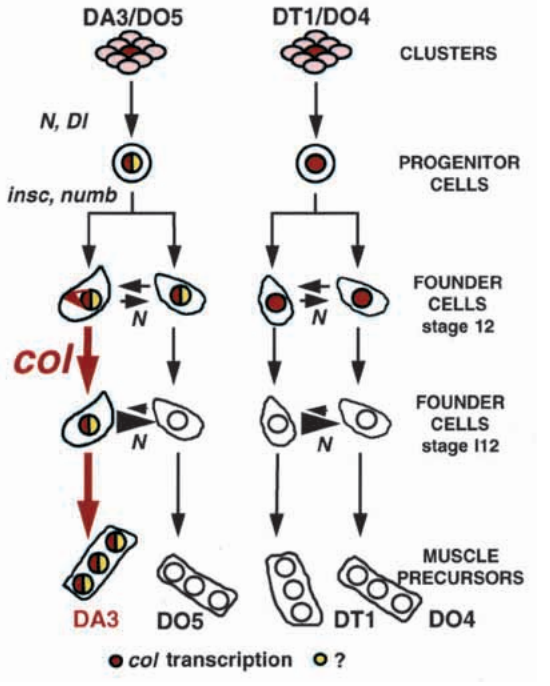

Fig. 7. Diagram showing *col* transcription and function requirement in the development of muscles DA3$^{[A]}$/DO5$^{[A]}$ and DT1$^{[A]}$/DO4$^{[A]}$. Light- and dark-red indicates levels of *col* transcription. The yellow staining indicates the expression of the other putative transcription factors(s) expressed in the DA3$^{[A]}$/DO5$^{[A]}$ but not in the DT1$^{[A]}$/DO4$^{[A]}$ lineages. The red arrows indicate at which steps *col* function is required for DA3$^{[A]}$ formation. Autoregulation of *col* transcription is indicated by a circular arrow in the DA3$^{[A]}$ founder cell. Levels of Notch (N) signalling are represented by horizontal black arrows.

of *col* transcription to a single founder cell is under the control of Notch signalling, at two successive levels. Notch activity is first required for restricting *col* expression to a single cell per cluster during the progenitor selection process. Second, Notch signalling is necessary to restrict *col* transcription to only one of two sibling founder cells and distinguish between the DA3$^{[A]}$ and DO5$^{[A]}$ fates.

Ectopic expression of Col is not sufficient to switch from DO5$^{[A]}$ to DA3$^{[A]}$ cell fate

While *col* activity is absolutely required for the formation of muscle DA3$^{[A]}$, it remained uncertain whether it is sufficient to convert the DO5$^{[A]}$ into a DA3$^{[A]}$ muscle. To address this question, we ectopically expressed Col at different time points during embryonic development, using a heat-shock *col* construct, and followed the DA3$^{[A]}$ fate with P[*col5-lacZ*] expression. The A2-A7 pattern of muscles of heat-shock-treated embryos was visualised by double immunostaining for β-gal and MHC at stage 16 (Fig. 6). We found that ectopic Col expression induced at 4-5 hours AEL (stage 7-9), i.e., before singling out of the progenitor cell has occurred, does not alter much the final muscle pattern. We only observed the specific loss of muscles DA3$^{[A]}$ and DO5$^{[A]}$, on average in one segment in half of the embryos (Fig. 6A,C). Examination of these heat-shocked embryos at an earlier stage (stage 11) indicated that this muscle loss, which is not observed in control heat-shocked embryos, is likely due to a defect in singling out the DA3$^{[A]}$/DO5$^{[A]}$ progenitor (data not shown). Embryos heat-treated between 5 and 7 hours AEL (stage 10-11), i.e, after the selection of the progenitor cell, show a normal pattern of larval muscles. We noticed, however, an ectopic expression of P[*col5-lacZ*], in a specific subset of muscles, DA2$^{[A]}$, VL1$^{[A]}$ and VL2$^{[A]}$, which all share with muscle DA3$^{[A]}$ the expression of *vestigial* (*vg*) (Fig. 6B). Finally, no change in the final embryonic muscle pattern was observed following UAS-driven *col* expression using different mesodermal drivers (24B-gal4 or twist-gal4). Considered together, these data indicate that ectopic *col* expression is not sufficient by itself to either switch

cell fate between DO5[A] and DA3[A] or change the cell fate of other muscle precursors.

DISCUSSION

We discuss below the role of Collier, the *Drosophila* member of a small family of non-basic HLH transcription factors, the COE proteins (for Col/Olf-1/EBF), during embryonic muscle formation. Our finding that Col is transcribed in the promuscular cluster, progenitor, founder cell and syncytial precursor of a single somatic muscle, muscle DA3[A]/DA4[T] and that this muscle is specifically missing in *col* mutant embryos provides us with a paradigm to address the issue of how a muscle lineage is established in the *Drosophila* embryo.

Two independent steps control Col expression in specific muscle progenitors

The first sign of patterning in the somatic mesoderm is the formation of promuscular clusters, defined by the expression of the proneural gene *l'sc*. In addition, each cluster may express a characteristic combination of 'muscle-specific' transcription factors (Carmena et al., 1995; 1998; Ruiz-Gomez et al., 1997; Jagla et al., 1998). *col* is one such factor, whose expression is restricted to a single cluster in the thoracic segments T2 and T3 and two clusters in the abdominal segments A1-A7, from which are selected the DA4[T]/DO4[T] progenitor, and DA3[A]/DO5[A] and DO4[A]/DT1[A] progenitors, respectively. For simplicity, we will consider in the discussion only the abdominal segments, since the DA4[T] and DA3[A] muscles are probably homologs. The appearance of mesodermal clusters at precise times and positions has previously been shown to be, in part, under the control of positional signals emanating from the overlying neuroectoderm and mediated by the secreted factors Wg and Hh (Bate and Rushton, 1993; Baylies et al., 1995; Azpiazu et al., 1996). Selection of the DA3[A]/DO5[A] progenitor (as visualised by high levels of Col expression) takes place normally in *wg* and *hh* mutant embryos, despite the fact that Col expression is not initiated in specific clusters of cells but appears more like a continuous band along the AP axis. This suggests that two successive informations define the position of Col-expressing progenitors. An early signal, requiring both *wg* and *hh* activities, restricts Col expression to specific promuscular clusters in the anterior compartment of each segment. A second information ensures that Col expression is maintained in only one cell from each cluster, leading to the formation of uniquely specified progenitors in segments T2-A7. At least in case of the DA3[A]/DO5[A] progenitor, this second information must be either insensitive or barely sensitive to *wg* and *hh* signalling and possibly responds to intrinsic patterning properties of the mesoderm, such as the activity of pair-rule and homeotic genes (Michelson, 1994; Azpiazu et al., 1996). The existence of two largely independent, successive steps in restricting muscle-identity gene expression to specific muscle progenitors is supported by the pattern of expression of the *col-lacZ* reporter gene. In contrast to Col itself, *col-lacZ* expression was not found to initiate in promuscular clusters but directly in progenitors, suggesting that separate *cis*-regulatory elements control each of these two steps. Further evidence is provided by Col expression in segment T1. In this segment, as in T2 and T3, *col* is expressed at stage 10 in a single cluster of mesodermal cells (not shown) but, contrary to T2 and T3, this cluster does not generate a Col-expressing progenitor. Accordingly, *col-lacZ* is not expressed in T1.

The general process of singling out progenitors from groups of competent myoblasts involves lateral inhibition mediated by the neurogenic genes *N* and *Dl* (Corbin et al., 1991; Carmena et al., 1995). The formation of at least a subset of founder cells was also shown to be dependent upon *l'sc* activity. Specification of the Col-expressing progenitors requires the activities of *N* and *Dl* but not that of *l'sc*, indicating that another 'promuscular' gene other than *l'sc* mediates Notch signalling during this process. *col* cannot be itself this promuscular gene, since the DA3[A]/DO5[A] and DT1[A]/DO4[A] progenitors form in *col* mutant embryos. Nevertheless, ectopic expression of Col early during mesoderm patterning, leads with low frequency to a defective segregation of the DA3[A]/DO5[A] progenitors and consecutive loss of muscles DA3[A] and DO5[A]. Therefore, although Col activity in promuscular clusters is not required for segregation of the progenitor, artificially maintained high levels of Col activity could interfere with the progenitor selection.

Col is strictly and specifically required for formation of muscle DA3[A] (DA4[T])

During establishment of the DA3[A] and DO5[A] sibling lineages, *col* transcription is maintained only in the DA3[A] founder cell. Each of the DA3[A]-recruited myoblasts, in turn, activates *col* transcription. These results indicate that a specific reprogramming of gene transcription occurs in nuclei of recruited myoblasts after fusion, that conveys all nuclei of a forming muscle precursor to express the founder-cell-specific transcription factor(s). The conversion of fused myoblasts to express *col* may allow the propagation of the specific recruitment properties of the DA3[A] founder cell at the same time as the expression by all DA3[A] nuclei of the same muscle differentiation program. One way such a cell-by-cell selective conversion can be efficiently achieved is positive autoregulation. The observation that *col* transcription is not maintained in *col* mutant DA3[A] founder cells suggests that, indeed a *col*-positive autoregulatory loop is established at this step in wild-type embryos. In *col* mutant embryos, the DA3[A]/DO5[A] progenitor segregates normally, but the DA3[A] founder cell does not maintain *col* transcription and does not fuse with the neighbouring myoblasts, leading to the lack of muscle DA3[A]. These data indicate that Col activity is strictly and specifically required for the DA3[A] founder cell to retain its ability to recruit surrounding myoblasts. In contrast with what has been observed for mutations in other muscle-specific transcription factors, such as Nau, Kr or Ap, in *col* mutant embryos the loss of DA3[A] phenotype is almost completely penetrant. In only some rare cases, a narrow (probably mononucleate) DA3[A] muscle differentiates, at a normal position and orientation. Therefore, the process that is primarily affected by *col* mutations is the recruitment of myoblasts by the DA3[A] founder cell. Altogether, these data suggest that *col* may serve as a muscle-specific 'fusion-promotion factor'. The most puzzling question raised by this phenotype remains as to whether the low level of Col expression displayed by all cells of the DA3[A]/DO5[A] promuscular cluster is required for this highly reproducible and stereotyped recruitment.

col transcription links asymmetric cell division to the DA3[A]/DO5[A] sibling cell fates

The alternative DA3[A] and DO5[A] fates correlate with *col* transcription persisting in one sibling muscle precursor and not the other, a process depending upon Nb activity during the progenitor cell division. Examination of the DA3[A]/DO5[A] lineage in embryos mutant for *N*, *insc* or *nb* further indicates that DA3[A] is the primary fate. These data support the proposal, based on studies of Kr expression during establishment of the VA1/VA2 lineage (Ruiz-Gomez et al., 1997), that Nb blocks the N-mediated repression of progenitor identity gene expression. The use of a *col* intronic probe that specifically labels nascent transcripts allowed us to establish that this N-mediated selective repression occurs at the transcriptional level: in *nb* mutants, *col* transcription is repressed in both the DA3[A] and DO5[A] founder cells (and no DA3[A] muscle forms). Conversely, in *insc* mutants, where Nb is presumably segregated in both founder cells, *col* transcription is maintained in these two sibling cells (not shown) (and two DA3[A] muscles form). These data identify *col* as a transcriptional target of Notch signalling in controlling the muscle lineage process. While transacting factors mediating the transcriptional response to Notch in controlling binary cell fate decisions in the SOP lineage start to be identified (Gho et al., 1996; Guo et al, 1996), those mediating Notch signalling in the myogenic program are at present unknown. However, the identical patterns of *col* and *col-lacZ* expression in the mutants examined indicates that all *cis*-regulatory elements necessary for the transcriptional response of *col* to Notch signalling are contained within the 5 kb *col* upstream region.

The *nb* versus *col* mutant phenotypes support a combinatorial action of several muscle-identity genes in controlling DA3[A] formation

Removing Col activity does lead to loss of muscle DA3[A] whereas the loss of *col* expression in the DA3[A] founder cells, due to mutations in *nb*, correlates with duplication of DO5[A] at the expense of DA3[A]. Conversely, generalised expression of Col does not switch DO5[A] into DA3[A] whereas this switch occurs in *insc* embryos. How can one reconcile all these data? Our working model of a regulatory pathway controlling muscle DA3[A] formation postulates the combined activity of Col and at least one other ‘ muscle-identity ’ factor (Fig. 7). Together, these factors would act to maintain the transcription of *col*, which is required for the myoblast recruitment process, and the DA3[A]-specific muscle differentiation program. This model postulates that, similar to *col*, the expression of this (these) other muscle identity factor(s) must be maintained in the DA3[A] and not the DO5[A] founder cell, in response to Notch signalling. Although expressed in the DO4[A]/DT1[A] progenitor, *col* transcription is not maintained in either the DO4[A] or the DT1[A] founder cells, suggesting that, contrary to Col, the other DA3[A] factor(s) is (are) not expressed in the DO4[A]/DT1[A] lineage (Fig. 7). What might this (these) factor(s) be? Two muscle ‘identity’ genes have been reported to be expressed in the DA3[A] as well as other muscle founders, *vg*, which encodes a novel protein and *nau*, which encodes a b-HLH transcription factor related to vertebrate MyoD (Wiliams et al., 1991; Michelson et al., 1990). The recent characterisation of the *nau* loss-of-function phenotype has shown that *nau* is not required for the formation of muscle precursors but rather plays a role in their differentiation into mature muscle fibers (Keller et al., 1998). Nau may thus correspond to a factor acting in combination with Col in directing the unique DA3[A] differentiation program. The *vg* muscle phenotype has not been reported so far, precluding a comparison with the *col* mutant phenotype. Nevertheless, suggestive evidence that *vg* might be involved in regulation of *col* expression is provided by the ectopic *col-lacZ* expression observed in conditions of heat-shock-induced ubiquitous *col* expression. It is interesting to note that all three muscles in which *col-lacZ* is ectopically activated (muscles DA2[A], VL1[A], VL2[A]) also express *vg*. All together, our results support the involvment of a combinatorial code of muscle-identity genes expressed in muscle progenitors and controlling the diversification of the somatic muscles (Bate, 1993). How Col interacts with the myogenic pathway in controlling formation of the DA3[A] muscle, or in other terms, what are the specific targets of Col in this process, remains a challenging question. Col belongs to a small family of non-basic HLH transcription factors, the COE proteins, which are highly conserved during evolution (Crozatier et al., 1996; refs in Bally-Cuif et al., 1998). We have recently shown that one *Xenopus* member of this family, *XCoe2*, is involved in the specification of primary neurons and that *Xcoe2* activity is subject to feed-back regulation by lateral inhibition (Dubois et al., 1998). The present report raises the interesting possibility that, beyond an apparent diversity of function, regulation of *Xcoe2* expression during primary neuron formation and *col* during embryonic muscle formation reflect the existence of an evolutionary conserved pathway linking Notch signalling and *col*/*Xcoe2* function in binary cell decisions in vertebrates and invertebrates.

We are grateful to William Chia, Michel Vervoort and the Bloomington and Umea Drosophila Stock Centers for mutant strains, and Dan Kiehart for the anti-muscle Myosin antibody. We also thank Michael Bate for its help in identifing muscle DA3, Rolf Bodmer for communication of results prior to publication, François Schweisguth, Marc Haenlin and Julian Smith for their comments on the manuscript. We also wish to thank Claude Ardourel and Marie-Josephe Guinaudy for excellent technical assistance. This study was supported by the Centre National de la Recherche Scientifique and Human Science Frontier Organisation.

REFERENCES

Abmayr, S.M., Erickson, M. S. and Bour, B. A. (1995). Embryonic development of the larval body wall musculature of *Drosophila melanogaster*. *Trends Genet.* **11**, 153-159.

Azpiazu, N., Lawrence, P. A., Vincent, J. P. and Frasch M. (1996). Segmentation and specification of the *Drosophila* mesoderm. *Genes Dev.* **10**, 3184-3194.

Bally-Cuif, L., Dubois, L. and Vincent, A. (1998). Molecular cloning of *Zcoe2*, the zebrafish homolog of *Xenopus Xcoe2* and mouse *EBF-2*, and its expression during primary neurogenesis. *Mech. Dev.* **77**, 85-90.

Bate, M. (1990). The embryonic development of larval muscles in *Drosophila*. *Development* **110**, 791-804.

Bate, M. (1993). The mesoderm and its derivatives. In The development of *Drosophila melanogaster*, vol. 2, (ed. M. Bate and A. Martinez-Arias). pp.1013-1090. Cold Spring Harbor, New York: CSH Laboratory Press.

Bate, M. and Rushton, E. (1993). Myogenesis and muscle patterning in *Drosophila*. *C. R. Acad. Sci. Paris* 316, 1055-1061.

Bate, M., Rushton, E. and Frasch, M. (1993). A dual requirement for

neurogenic genes during *Drosophila* myogenesis. *Development* **Supplement**, 149-161.

Baylies, M. K., Martinez-Arias, A. and Bate, M. (1995). *wingless* is required for the formation of a subset of muscle founder cells during *Drosophila* embryogenesis. *Development* **121**, 3829-3837.

Baylies, M., Bate, M. and Ruiz-Gomez, M. (1998). Myogenesis: A view from *Drosophila. Cell* **93**, 921-927.

Bourgouin, C., Lundgren, S. E. and Thomas, J. B. (1992). *apterous* is a *Drosophila* LIM domain gene required for the development of a subset of embryonic muscles. *Neuron* **9**, 549-561.

Carmena, A., Bate, M. and Jimenez, F. (1995). *lethal of scute*, a proneural gene, participates in the specification of muscle progenitors during *Drosophila* embryogenesis. *Genes Dev*. **9**, 2373-2383.

Carmena, A., Murugasu-Obei, B., Menon, D., Jimenez, F. and Chia, W. (1998). *inscuteable* and *numb* mediate asymmetric muscle progenitor cell division during *Drosophila* myogenesis. *Genes Dev*. **12**, 304-315.

Corbin, V., Michelson, A. M., Abmayr, S. M., Neel, V., Alcamo, E., Maniatis, T. and Young, M. W. (1991). A role for the *Drosophila* neurogenic genes in mesoderm differentiation. *Cell* **67**, 311-323.

Crozatier, M., Valle, D., Dubois, L., Ibnsouda, S. and Vincent, A. (1996). *collier*, a novel regulator of *Drosophila* head development, is expressed in a single mitotic domain. *Curr. Biol.* **6**, 707-718.

D'Alessio, M. and Frasch, M. (1996). *msh* may play a conserved role in dorsoventral patterning of the neuroectoderm and mesoderm. *Mech. Dev*. **58**, 217-231.

Dohrmann, C., Azpiazu, N. and Frasch, M. (1990). A new *Drosophila* homeobox gene is expressed in mesodermal precursor cells of distinct muscles during embryogenesis**.** *Genes Dev***. 4**, 2098-2111.

Dubois, L., Bally-Cuif, L., Crozatier, M., Moreau, J., Paquereau, L. and Vincent, A. (1998). Xcoe2, a transcription factor of the Col/Olf-1/EBF family involved in the specification of primary neurons in *Xenopus*. *Curr. Biol*. **8,** 199-209.

Dye, C. A., Lee, J. K., Atkinson, R. C., Breuster, R., Han, P. L. and Bellen, H. J. (1998). The *Drosophila sanpodo* gene controls sibling cell fate and encodes a tropomodulin homolog, an actin/tropomyosin-associated protein. *Development* **125**, 1845-1856.

Fuerstenberg, S. and Giniger, E. (1998). Multiple role for Notch in *Drosophila* myogenesis. *Developmental Biology* **201**, 66-77.

Frasch, M., Hoey, T., Rushlow, C., Doyle, H. J. and Levine, M. (1987). Characterisation and localization of the *even-skipped* protein of *Drosophila*. *EMBO J*. **6**, 749-759.

Garcia-Bellido, A. (1979). Genetic analysis of the *achaete-scute* system of *Drosophila melanogaster*. *Genetics* **91**, 491-520.

Gho, M., Lecourtois, M., Geraud, G., Posakony, J. W. and Schweisguth, F. (1996). Subcellular localization of Suppressor of Hairless in Drosophila sense organ cells during Notch signalling. *Development* **122**, 1673-1682.

Guo, M., Jan, L. Y. and Jan, Y. N. (1996). Control of daughter cell fates during asymmetric division, interaction Numb and Notch. *Neuron* **17**, 27-41.

Jagla, T., Bellard, F., Lutz, Y., Dretzen, G., Bellard, M. and Jagla, K. (1998). *ladybird* determines cell fate descisions during diversification of *Drosophila* somatic muscles. *Development* **125**, 3699-3708.

Keller, C. A., Erickson, M. S. and Abmayr, S. M. (1997). Missexpression of *nautilus* induces myogenesis in cardioblasts and alters the pattern of somatic muscle fibers. *Dev. Biol*. **181**, 197-212.

Keller, C. A., Grill, M. A. and Abmayr, S. M. (1998).A role for *nautilus* in the differentiation of muscle precursors. *Developmental Biology* **202**, 157-171.

Kraut, R., Chia, W., Jan, Y. N. and Knoblich, J. A. (1996). Role of *inscuteable* in orienting asymmetric cell divisions in *Drosophila*. *Nature* **383**, 50-55.

Lindsley, D. and Zimm, G. (1992). The genome of *Drosophila melanogaster*. Academic Press I. NC.

Michelson, A. (1994). Muscle pattern diversification in *Drosophila* is determined by the autonomous function of homeotic genes in the embryonic mesoderm. *Development* **120**, 755-768.

Michelson, A. M., Abmayr, S. M., Bate, M., Martinez-Arias, A. and Maniatis, T. (1990). Expression of Myo-D family member prefigures muscle pattern in *Drosophila* embryos. *Genes Dev*. **4**, 2086-2097.

Nose, A., Isshiki, T. and Takeichi, M. (1998). Regional specification of muscle progenitors in *Drosophila*: the role of the *msh* homeobox gene. *Development* **125**, 215-223.

Park, M., Yaich, L. and Bodmer, R. (1998). Mesodermal cell fate decisions in *Drosophila* are under the control of the lineage genes *numb*, *Notch* and *sanpodo*. *Mech. Dev*. **75**, 117-126.

Rhyu, M., Jan, L. and Jan, Y. N. (1994). Asymmetric distribution of Numb protein during division of the sensory organ precursor confers distinct fates to daughter cells. *Cell* **76**, 477-491.

Rubin, G. and Spradling, A. (1982). Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**, 348-353.

Ruiz-Gomez, M. and Bate, M. (1997). Segregation of myogenic lineages in *Drosophila* requires Numb. *Development* **124**, 4857-4866.

Ruiz-Gomez, M., Romani, S., Hartmann, C., Jäckle, H. and Bate, M. (1997). Specific muscle identities are regulated by Krüppel during *Drosophila* embryogenesis. *Development* **124**, 3407-3414.

Rushton, E., Drysdale, R., Abmayr, S, Michelson, A. and Bate, M. (1995). Mutations in a novel gene, *myoblast city*, provide evidence in support of the founder cell hypothesis in *Drosophila* muscle development. *Development* **121**, 1979-1988.

Salzberg, A., D' Evelyn, D., Schulze, K. L., Lee, J., K., Strumpf, D., Tsai, L. and Bellen, H. J. (1994). Mutations affecting the pattern of the PNS in *Drosophila* reveal novel aspects of neuronal development. *Neuron* **13**, 269-287.

Skeath, J. B. and Doe, C. Q. (1998). Sanpodo and Notch act in opposition to Numb to distinguish sibling neuron fates in the *Drosophila* CNS. *Development* **125**, 1857-1865.

Spana, E., Kopczynski, C., Goodman, C. and Doe, C. (1995). Asymmetric localisation of Numb autonomously determines sibling neuron identities in the *Drosophila* CNS. *Development* **121**, 3187-3195.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L. Y. and Jan, Y. N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. *Cell* **58**, 349-360.

Wiliams, J. A., Bell, J. B. and Caroll, S. B. (1991). Control of Drosophila wing and haltere development by the nuclear *vestigial* gene product. *Genes Dev*. **5**, 2481-2495.