Repression by Notch is required before Wingless signalling during muscle progenitor cell development in *Drosophila*

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The larval muscles of *Drosophila* arise from the fusion of muscle founder cells, which give each individual muscle its identity, with myoblasts (reviewed in [1]). Muscle founder cells arise from the asymmetric division of muscle progenitor cells, each of which develops from a group of cells in the somatic mesoderm that express *lethal of scute* [2]. All the cells in a cluster can potentially form muscle progenitors, but owing to lateral inhibition, only one or two develop as such [2–5]. Muscle progenitors, and the subsequent founder cells, then express transcription factors such as Krüppel, S59 and Even-skipped, which confer identity on the muscle [6–8]. Definition of some muscle progenitors, including three groups that express S59, depends on Wingless signalling [9]. Lateral inhibition requires Delta signalling through Notch and the transcription factor Suppressor of Hairless [3–5]. As the Wingless and lateral-inhibition signals are sequential [8], one might expect that muscle progenitors would fail to develop in the absence of Wingless signalling, regardless of the presence or absence of lateral-inhibition signalling. Here, we examine the development of the S59-expressing muscle progenitor cells in mutant backgrounds in which both Wingless signalling and lateral inhibition are disrupted. We show that progenitor cells failed to develop when both these processes were disrupted. Our analysis also reveals a repressive function of Notch, required before or concurrently with Wingless signalling, which is unrelated to its role in lateral inhibition.

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Results and discussion

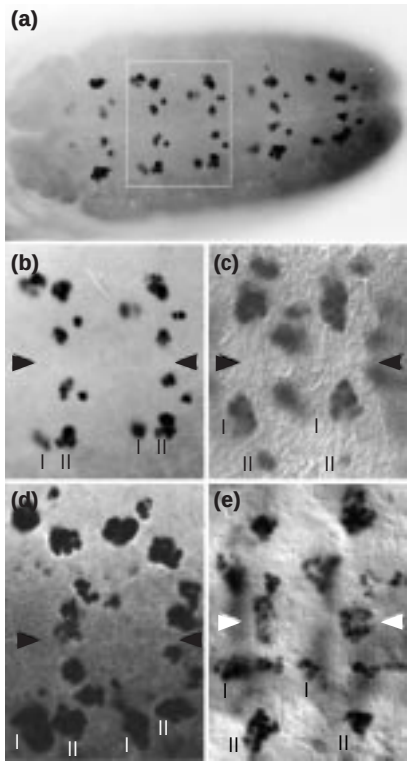
During wild-type development, expression of S59 is first seen during stage 10 in a single muscle progenitor cell either side of the midline in every segment [2,7]. By stage 11, this pattern has evolved in abdominal segments such that S59 expression is seen both in the nervous system and in two groups of muscle progenitor cells (Figure 1a,b). During stage 12, a third muscle progenitor cell starts to express S59. These muscle progenitor cells give rise to three muscle founder cells that maintain the expression of S59. Fusion of these founder cells with myoblasts results in the S59-expressing muscles seen in late stages of embryogenesis (Figure 2a) [2,7].

Disruption of lateral-inhibition signalling, in either *Notch* (*N*) germline-clone, *suppressor of Hairless* (*Su(H)*) germline-clone or *Delta* (*Dl*) zygotic mutant embryos, increases the number of cells expressing S59 compared with wild type at stage 11 (Figure 1c–e) [5]. Because of general degeneration of these embryos during germ-band retraction, however, it is difficult to examine the expression of S59 after stage 11, but the mesoderm clusters that can be identified are expanded (data not shown).

Unlike the disruption of lateral-inhibition signalling, attenuation of Wingless signalling, by removing either *wingless* (*wg*) or *dishevelled* (*dsh*) function, blocks the expression of S59 in the mesoderm (Figure 3a) [9]. On the other hand, increasing Wingless signalling, either by overexpressing the Wingless protein in the mesoderm using the GAL4/UAS system (*twist–GAL4*>UAS*wg* embryos) [10], or by removing *shaggy* (*sgg*) function (sgg^{m11} germline-clone embryos), leads to enlarged groups of S59-expressing muscle progenitor cells during stage 11 (Figure 2b,d). During germ-band retraction, however, the groups are reduced in size. In the *twist*–GAL4>UAS*wg* embryos the reduction in cluster size leads to a largely normal set of three muscles (Figure 2c), whereas in the sgg^{m11} embryos the reduction is more extreme and leads to the loss of S59-expressing muscles (Figure 2e).

As Wingless signalling is required for the initiation of S59 expression in the mesoderm and lateral-inhibition signalling is required for the subsequent restriction of S59 expression to one or two cells within each cluster, it is expected that in the absence of Wingless signalling S59 will not be expressed, even if lateral-inhibition signalling is also blocked. This appears to be the case in $wg^{S107.5};Dl^{FX3}$ zygotic and $wg^{S107.5},Su(H)^{SF8}$ germline-clone embryos (Figure 3b and c, respectively). In contrast, we observed mesodermal S59 expression in $Df(1)N^{81k1},dsh^{v26}$ (Figure 3d) and $Df(1)N^{81k1};wg^{CX4}$ (data not shown) germline-clone embryos, in which Wingless signalling is

Figure 1



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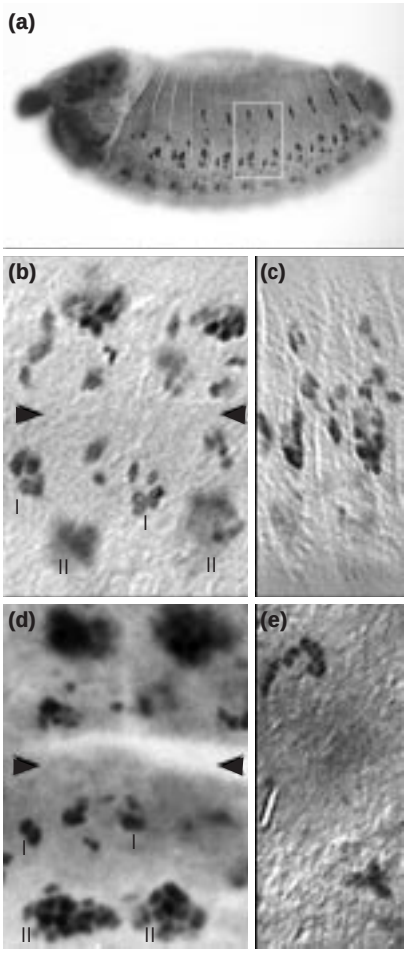
Loss of lateral inhibition leads to an increase in the number of S59-expressing cells within the mesoderm. (a) Dorsal view of a late stage 11 wild-type embryo showing expression of S59 (dark stained cells) in the abdominal segments. The boxed region containing two abdominal segments is enlarged in (b), where the midline is marked by black arrowheads and the positions of the first two groups of muscle progenitor cells expressing S59 are marked by roman numerals. (c–e) Dorsal view of two abdominal segments from late stage 11 (c) *Su(H)^{SF8}* germline-clone, (d) *Dl^{FX3}* zygotic and (e) *Df(1)N^{81k1}* germline-clone mutant embryos. As in (b), the positions of the first two groups of muscle progenitor cells expressing S59 are marked by roman numerals and the position of the midline is marked by black arrowheads except in the *Df(1)N^{81k1}* germline-clone embryo in (e), where the position at which the midline ought to develop is marked by white arrowheads. In each mutant, there are more S59-expressing cells in both the mesoderm and the nervous system compared with wild-type embryos. Also, the absence of the midline in the *Df(1)N^{81k1}* embryo leads to the fusion of S59 expression in the nervous system cells across the midline.

Figure 2



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Increasing Wingless signalling enlarges the groups of cells expressing S59 during stage 11 but this does not lead to more or larger S59-expressing muscles at later stages. (a) Lateral view of a wild-type stage 14 embryo showing the mesodermal expression of S59. An area similar to the boxed area is shown in (c) and (e). (b,d) Dorsal view, similar to that shown in Figure 1b, of two abdominal segments from late stage 11 (b) *twist–GAL4*>UAS*wg* and (d) *sgg^{m11}* germline-clone embryos. As in Figure 1b, the position of the first two groups of muscle progenitor cells expressing S59 is marked by roman numerals and the midline is marked by arrowheads. The hyperactivation of Wingless signalling leads to an increase in the number of S59-expressing cells in both mesodermal groups. (c,e) Lateral view of two abdominal segments of stage 14 (c) *twist–GAL4*>UAS*wg* and (e) *sgg^{m11}* germline-clone embryos. Although hyperactivation of Wingless signalling leads to an enlarged groups of muscle progenitor cells initially, extra or larger S59-expressing muscles fail to develop. In the *twist–GAL4*>UAS*wg* embryos, the final pattern of muscles is very similar to the wild type, whereas many muscles are actually absent from the *sgg^{m11}* germline-clone embryos. The cells that stop expressing S59 probably revert to the fusion-competent myoblast fate.

blocked and Notch function is removed. Finally, as with the single-mutant embryos, the double-mutant embryos degenerate during germ-band retraction, making it difficult to examine S59 expression after stage 11.

Our results first confirm that Wingless signalling is required for the initiation of S59 expression (Figure 2) [9] and that a Delta-initiated lateral-inhibition signal is required for the restriction of S59 expression to one or two cells of each initial cluster (Figure 1) [3–5]. They also confirm the prediction that, in the absence of a Wingless signal, S59 is not expressed, regardless of whether lateral-inhibition

signalling is occurring (Figure 3); similar results have been obtained by others for the single, dorsally positioned, *even-skipped*-expressing muscle founder cell [8]. There are two surprising results, however. First, S59 expression is observed in *Df(1)N^{81k1},dsh^{v26}* and *Df(1)N^{81k1};wg^{CX4}* germline-clone embryos (Figure 3 and data not shown). Second, even though hyperactivating Wingless signalling leads to initially enlarged groups of S59-expressing muscle progenitor cells, a reasonably normal muscle pattern is obtained (Figure 2).

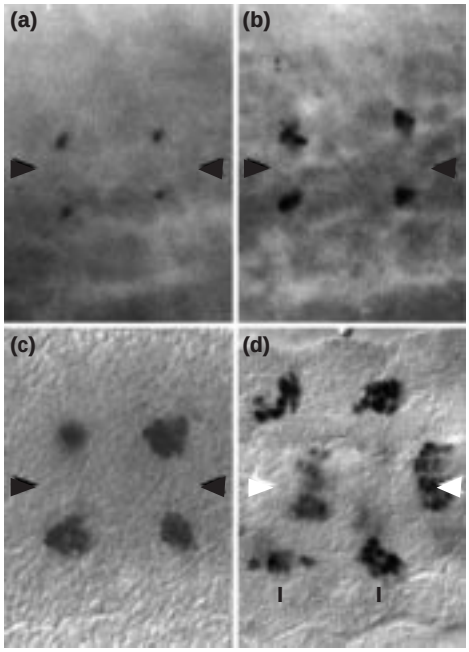
The observed S59 expression in *Df(1)N^{81k1},dsh^{v26}* and *Df(1)N^{81k1};wg^{CX4}* embryos can be explained if it is assumed that *Notch* has a repressive function that precedes Wingless signalling. In this situation, removal of *Notch* function will lead to the derepression of S59 expression before Wingless signalling. Consequently, whether Wingless signalling occurs or not does not matter. This repressive function cannot be related to Delta signalling, however, as the removal of *Delta* or *Su(H)* function in embryos where Wingless signalling is not occurring does not result in S59 expression. The repressive function of Notch uncovered in our experiments must therefore be distinct from its repressive role during lateral inhibition.

The second observation suggests that in response to increased Wingless signalling there is a linked increase in lateral-inhibition signalling. This would mean that increased Wingless signalling will only lead to a significant increase in the number of muscle progenitors if lateral inhibition cannot occur. The difference we observe in the final muscle pattern between *twist–GAL4*>UAS*wg* and *sgg^{m11}* embryos is probably due to the difference in how Wingless signalling is activated in the different embryos. In the *twist–GAL4*>UAS*wg* embryos, Wingless signalling is activated only transiently and is restricted to the mesoderm (see Supplementary material published with this paper on the internet). In contrast, Wingless signalling is activated globally and throughout embryogenesis in *sgg^{m11}* germline-clone embryos. This difference, along with the proposed linkage between Wingless signalling and lateral inhibition would mean that lateral inhibition is much greater in the *sgg^{m11}* embryos. This situation would explain the greater reduction in the size of the groups of S59-expressing muscle progenitor cells observed in the *sgg^{m11}* embryos and the loss of muscles if the restriction is too great.

The link between Wingless signalling and lateral inhibition could occur in a number of ways. For example, Wingless signalling may directly alter a component of the Delta signalling pathway, which increases its ability to transduce the Delta signal. Alternatively, it could affect Delta signalling by altering the transcription of one of the components of the pathway. Either of these mechanisms would allow the organism to generate a lateral-inhibition signal appropriate to the input signal: a strong Wingless signal would lead to a strong lateral-inhibition signal and prevent unnecessary and unwanted development, whereas a weak Wingless signal would lead to a weak lateral-inhibition signal that allows development to proceed even though the input signal is weak. This would allow normal development to occur even if there are fluctuations in the input signal.

It is likely that the repressive function of Notch that we have described here is related to that identified by Rusconi and Corbin [5]. Their results together with our own observations suggest that the muscle progenitor cells develop from a large pool of developmentally equivalent cells that is refined through two steps to produce one

Figure 3



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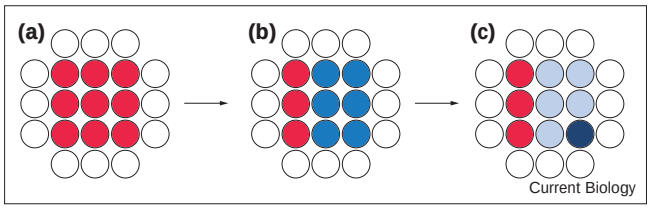
Removal of *Notch* function but not *Su(H)* or *Delta* function in embryos lacking Wingless signalling leads to the expression of S59 in the mesoderm. (a–d) Dorsal view, similar to that shown in Figure 1b, of two abdominal segments from early stage 11 mutant embryos: (a) *dsh^{v26}* germline-clone; (b) *wg$^{S107.5}$;Dl^{FX3}* zygotic; (c) *wg$^{S107.5}$,Su(H)^{SF8}* germline-clone; (d) *Df(1)N^{81k1},dsh^{v26}* germline-clone. The positions of the first groups of muscle progenitor cells expressing S59 are marked by roman numerals and the position of the midline is marked by black arrowheads except in the *Df(1)N^{81k1},dsh^{v26}* embryo (d) where the position at which the midline ought to develop is marked by white arrowheads. In the *dsh^{v26}* embryos (a), as in *wg$^{S107.5}$* embryos (data not shown) [9], S59 expression is lost from the mesoderm and only two neural cells per segment express S59. S59 expression is also lost from the mesoderm of *wg$^{S107.5}$;Dl^{FX3}* germline-clone (b) and *wg$^{S107.5}$,Su(H)^{SF8}* germline-clone (c) embryos. Mesodermal S59 expression is, however, observed in *Df(1)N^{81k1},dsh^{v26}* germline-clone embryos (d); as this is an early stage 11 embryo only one group of muscle progenitor cells is seen (see text) [2,7]. Finally, the absence of the midline in the *Df(1)N^{81k1},dsh^{v26}* germline-clone embryo leads to the fusion of S59 expression in the nervous system across the midline.

Figure 4



A pictorial representation of the formation of S59-expressing muscle progenitor cells. A small field of representative cells (circles) is shown. (a) Initially, a large group of cells is defined (red) that can express S59. The cells within the cluster are prevented from expressing S59, however, by the repressive function of Notch described in this paper. (b) The repressive function of Notch is alleviated by Wingless signalling within a few cells of the cluster, leading to the expression of S59 (mid blue). The most likely source of Wingless for this signal is the ectodermal stripes [9]. This is likely to form an anterior–posterior gradient of Wingless across the somatic mesoderm. If the concentration of Wingless has to be above a certain threshold to generate a signal strong enough to block repression by Notch, the repression will only be blocked within those cells that are near the source of Wingless. (c) The process of lateral inhibition acting on the cells that express S59 selects one muscle progenitor cell (dark blue) that maintains S59 expression and divides to produce two muscle founder cells. The other cells (light blue) stop expressing S59 and become fusion-competent myoblasts.

muscle progenitor cell (Figure 4). A very large group of cells is initially defined that have the potential to become muscle progenitor cells but are prevented from doing so by the novel function of Notch identified here and by Rusconi and Corbin [5]. Wingless signalling then alleviates this repressive function of Notch within a few cells of the cluster to establish an equivalence group. This triggers the process of lateral inhibition, which subsequently selects a single cell to become a muscle progenitor. In this situation, overexpressing Wingless or constitutively activating Wingless signalling will alleviate the initial repressive function of Notch in all the cells as observed, revealing the larger extent of the initial cluster (Figure 2). The linked increase in lateral-inhibition signalling, however, ensures that the normal number of muscle progenitor cells develop.

This model contrasts with others in which Wingless signalling is instructive and defines the position at which muscle progenitor cells will develop [8], but can explain why overexpressing Wingless leads to the development of S59-expressing muscles in their normal position [9]. In this model the Wingless signal is permissive and not instructive: it does not define where S59 will be expressed but merely reveals places defined by earlier mechanisms. Finally, our data suggest that the loss of S59 expression in the absence of a Wingless signal is due to the early repression mediated by Notch.

Materials and methods

Germline clones were generated using the dominant female-sterile/flippase system [11,12]. Expression of S59 in the embryo was detected using a polyclonal rabbit antibody raised against the S59 protein [9] and stainings were done using standard techniques [13].

Supplementary material

Details of the *Drosophila* strains used in this work are published with this paper on the internet.

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Supplementary material

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Supplementary materials and methods

The mutant *Drosophila* strains used in this paper are detailed in Table S1. This gives a summary of the mutant stocks used in this study, indicating the exact stock used, the type of allele, and the reference in which the allele was first described. In addition to these stocks, the *C(1)DX/w,ovo^{D1},[FRT101w^{+}]/Y;FLP^{38}* stock [S1] was used for generating germline clones of chromosome I mutants; the *y,w,hspf/p^{22};Sp/CyO;MKRS/TM2,ry* and *[w^{+};ovo^{D1}]$^{13\times3}$[FRT40A]/Sp,S,bw^{D},Ms(2)/CyO* stocks [S2] were used for generating germline clones of mutants on the left-hand arm of chromosome II; and the *w,twist*–GAL4;*twist*–GAL4 [S3] and *w;UAStwg^{E1}* [S4] stocks were used to overexpress Wingless in the mesoderm between stages 8 and 14.

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Table S1

Mutant *Drosophila* strains used in these experiments.

Stock	Type of allele	Reference
Df(1)N^{81k1},v,[FRT101w^{+}]/FM7c	Null allele of *Notch*	[S5]
Df(1)N^{81k1},dsh^{v26},[FRT101w^{+}]/FM7c	Double-mutant chromosome carrying null alleles of *Notch* and *dishevelled*	This paper
y,w,dsh^{v26},[FRT101w^{+}]/FM6,f	Null allele of *dishevelled*	[S6]
sgg^{m11},w^{a},sn^{3},[FRT101w^{+}] FM6,f	Null allele of *shaggy*	[S7]
wg$^{S107.5}$,pr,cn/CyO	Null allele of *wingless*	[S8]
w;wg$^{S107.5}$,[w^{+};lac]$^{A1–29}$,Su(H)^{SF8},[FRT40A]/CyO	Double-mutant chromosome carrying null alleles of *wingless* and *Suppressor of Hairless*	This paper
w;[w^{+};lac]$^{A1–29}$,Su(H)^{SF8},[FRT40A]/CyO	Null allele of *Suppressor of Hairless*	[S9]
Dl^{FX3}/TM3,Ser,Sb	Null allele of *Delta*	[S10]
Oregon R	Wild-type strain	[S11]