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MECHANISMS OF DEVELOPMENT

The *Him* gene inhibits the development of *Drosophila* flight muscles during metamorphosis

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ABSTRACT

During *Drosophila* metamorphosis some larval tissues escape the general histolysis and are remodelled to form adult tissues. One example is the dorso-longitudinal muscles (DLMs) of the indirect flight musculature. They are formed by an intriguing process in which residual larval oblique muscles (LOMs) split and fuse with imaginal myoblasts associated with the wing disc. These myoblasts arise in the embryo, but remain undifferentiated throughout embryogenesis and larval life, and thus share characteristics with mammalian satellite cells. However, the mechanisms that maintain the *Drosophila* myoblasts in an undifferentiated state until needed for LOM remodelling are not understood. Here we show that the *Him* gene is expressed in these myoblasts, but is undetectable in developing DLM fibres. Consistent with this, we found that *Him* could inhibit DLM development: it inhibited LOM splitting and resulted in fibre degeneration. We then uncovered a balance between *mef2*, a positive factor required for proper DLM development, and the inhibitory action of *Him*. *Mef2* suppressed the inhibitory effect of *Him* on DLM development, while *Him* could suppress the premature myosin expression induced by *mef2* in myoblasts. Furthermore, either decreased *Him* function or increased *mef2* function disrupted DLM development. These findings, together with the co-expression of Him and Mef2 in myoblasts, indicate that *Him* may antagonise *mef2* function during normal DLM development and that *Him* participates in a balance of signals that controls adult myoblast differentiation and remodelling of these muscle fibres. Lastly, we provide evidence for a link between *Notch* function and *Him* and *mef2* in this balance.

1. Introduction

In *Drosophila* there are two phases of myogenesis (Bate, 1993). The first occurs in the embryo to produce the larval musculature, which then largely disappears during metamorphosis. The second initiates in larval life to produce the muscles of the adult fly. The larval somatic musculature develops from myoblasts that derive from a subset of mesodermal cells that express Twist at a high level (Bate, 1993; Borkowski et al., 1995; Baylies and Bate, 1996; Riechmann et al., 1997). Each individual muscle is seeded by a single specialised myoblast, called a founder cell, which then fuses with other nearby myoblasts to form the syncytial fibre (reviewed in Baylies et al., 1998; Frasch, 1999; Taylor, 2002; Chen and Olson, 2004). The adult muscles also develop from Twist-expressing cells (Bate et al., 1991; Currie and Bate, 1991). In the case of the flight and leg muscles, these adult muscle precursor cells are associated with wing and leg imaginal discs. These cells, known as imaginal myoblasts or adepithelial cells, are set aside during embryogenesis, and maintained in an undiffer-

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entiated state throughout embryo and larval life (Lawrence, 1982; Bate et al., 1991; Broadie and Bate, 1991; Soler et al., 2004; Maqbool et al., 2006).

During metamorphosis most larval tissues, including muscles, are histolysed and adult tissues are formed *de novo* from imaginal cells. However, some larval tissues escape from this histolysis and through association with imaginal cells are remodelled to form adult tissues. For example, both the tracheal system and part of the nervous system undergo pruning and elimination of some of their branches and then are remodelled (Truman, 1990; Manning and Krasnow, 1993; Kuo et al., 2005; Marin et al., 2005; Weaver and Krasnow, 2008). In the musculature, there are two subsets of larval muscles that escape histolysis: the abdominal intersegmental muscles, which are later transformed into the temporary dorsal oblique muscles (Kimura and Truman, 1990; Wasser et al., 2007); and, in each hemithorax, the three larval oblique muscles (LOMs) that give rise to six indirect flight muscles called the dorso-longitudinal muscles (DLMs) (Fernandes et al., 1991; Fernandes and Keshishian, 1996). The DLMs are formed by fusion between the LOMs and imaginal myoblasts associated with the wing disc. About 8 h after puparium formation (APF), these myoblasts migrate to surround the three LOMs. Then, between 12 and 20 h APF, the LOMs become vacuolated and split into the six DLMs, as myoblasts fuse with them. By 30 h APF most myoblasts have fused with the newly formed DLMs (Fernandes et al., 1991). Although the LOMs escape from histolysis, in normal development they show some signs of degeneration, for example vacuoles. Interestingly, in the absence of imaginal myoblasts the LOMs do not split and they eventually degenerate (Roy and VijayRaghavan, 1998). This indicates that these myoblasts are required for LOM remodelling and are also necessary for subsequent muscle survival. They also share some characteristics with mammalian adult satellite cells, which similarly are maintained in a committed, but undifferentiated, state, and can be triggered to enter myogenesis to make or repair muscle at a later time (Seale and Rudnicki, 2000; Shi and Garry, 2006; Kuang et al., 2008; Zammit, 2008). Thus, the development of the DLMs, with its remodelling dependent on a population of committed, but undifferentiated myoblasts, is both distinct from *Drosophila* embryo myogenesis and also an intriguing system to investigate remodelling and growth from undifferentiated cells, with its implications for understanding tissue repair and regeneration.

Although cellular and genetic fundamentals of DLM development have been established (Roy and VijayRaghavan, 1999), many gaps remain in our understanding. For example, both the genetic control of the remodelling process and the factors that govern imaginal cell differentiation are incompletely understood. One inhibitory signal is Notch, which is widely used to influence cell differentiation (Artavanis-Tsakonas et al., 1999). Expression of activated Notch during adult *Drosophila* myogenesis induces DLM degeneration (Anant et al., 1998). Interestingly, as well as inhibiting *Drosophila* DLM development, Notch can also inhibit mammalian satellite cell differentiation (Conboy and Rando, 2002). On the other side of the balance, a positive factor is Mef2, a MADS box transcription factor that plays a critical role in *Drosophila* myogenesis, including in the development of the DLMs (Bour et al., 1995; Lilly et al., 1995; Ranganayakulu et al., 1995; Cripps et al., 1998; Sandmann et al., 2006). Various combinations of hypomorphic *mef2* mutant alleles affect the LOM splitting process and the final pattern of the DLMs (Ranganayakulu et al., 1995; Cripps et al., 1998; Nguyen et al., 2002; Baker et al., 2005). Mef2 is first expressed in the wing associated myoblasts from the very late third instar (Ranganayakulu et al., 1995; Cripps and Olson, 1998; Lovato et al., 2005), many hours before the splitting process which starts at 14 h AFP, and the activation of target genes. Moreover, over-expression of *mef2* can induce premature muscle gene expression and differentiation in the myoblasts of the third larval instar (Lovato et al., 2005). This led to the conclusion that tight control of Mef2 function is required in these cells to prevent premature muscle differentiation (Lovato et al., 2005), but it is not known how Mef2 activity is restrained during this phase.

Here we analysed the role of *Him* in DLM development and have addressed this question. *Him* encodes a novel inhibitor of *Drosophila* embryo muscle development that we recently characterised and which can downregulate the transcriptional activity of Mef2 (Liotta et al., 2007). In this study we analysed *Him* expression during adult muscle development and its capacity to affect the DLMs. We found, consistent with its expression pattern, that *Him* could inhibit DLM development and that this phenotype could be suppressed by *mef2*. Furthermore, *Him* could suppress the premature myosin expression induced by *mef2* in disc-associated myoblasts. Together these results and other findings we present indicate that *Him* can antagonise *mef2* and is involved in a balance of signals that control the differentiation of adult myoblasts and influence muscle remodelling. We also provide evidence that links *Notch* function to *Him* and *mef2* in this balance, and in sum have furthered understanding of how the maintenance and differentiation of these adult precursor cells is controlled.

2. Results

2.1. *Him expression during DLM development*

Him (CG15064) RNA has previously been shown to be expressed in third instar wing imaginal discs (Rebeiz et al., 2002; Butler et al., 2003). Here we used confocal microscopy and a *Him-GFP* mini-gene to analyse the expression of Him throughout DLM development from first instar larvae to 30 h after puparium formation (APF). This mini-gene construct comprises approximately 3.8 kb of upstream genomic sequence, GFP fused to the *Him* coding sequence, and the *Him* 3′UTR (Liotta et al., 2007). Its pattern of expression closely resembles that of the endogenous *Him* gene in both larval and adult muscle development ((Liotta et al., 2007); Supplemental Fig. 1). Expression in cells associated with the wing imaginal disc is first detected during the middle of the third larval instar (96 h after egg laying (AEL)). These cells are identified as myoblasts by co-expression with Twist (Fig. 1A–C). Increased Him-GFP expression is then detected in the late third instar (96–120 h AEL) (Fig. 1D–F). Subsequently, high levels of Him-GFP are maintained in these cells during the early stages of pupal development.