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Cell death regulates muscle fiber number

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ABSTRACT

Cell death can have both cell autonomous and non-autonomous roles in normal development. Previous studies have shown that the central cell death regulators *grim* and *reaper* are required for the developmentally important elimination of stem cells and neurons in the developing central nervous system (CNS). Here we show that cell death in the nervous system is also required for normal muscle development. In the absence of *grim* and *reaper*, there is an increase in the number of fibers in the ventral abdominal muscles in the Drosophila adult. This phenotype can be partially recapitulated by inhibition of cell death specifically in the CNS, indicating a non-autonomous role for neuronal death in limiting muscle fiber number. We also show that FGFs produced in the cell death defective nervous system are required for the increase in muscle fiber number. Cell death in the muscle lineage during pupal stages also plays a role in specifying fiber number. Our work suggests that FGFs from the CNS act as a survival signal for muscle founder cells. Thus, proper muscle fiber specification requires cell death in both the nervous system and in the developing muscle itself.

1. Introduction

Programmed cell death is a prevalent and important cell fate in the development of multicellular organisms (Arya and White, 2015). Accurate regulation of both proliferation and cell death is essential to control cell number in developing tissues. The selective death of cells is also necessary for the precise organization of tissues, such as matching neuronal inputs to their targets, or to pattern sensory organs such as the eye. Although the presence of apoptotic cells is detected in many developing tissues, the importance of cell death in the normal development of most tissues has not been extensively studied.

Drosophila provides a particularly strong model system to examine the role of apoptosis in development, as the central regulators of cell death in development are known, and their activity can be manipulated genetically (Arya and White, 2015). Here we focus on the role of cell death in Drosophila adult muscle development. Loss of muscle tissue is an important pathology in human disease, and studies in Drosophila have the potential to provide mechanistic insights into how muscle death is regulated (Piccirillo et al., 2014).

Muscle development in Drosophila occurs in two stages (Schejter and Baylies, 2010). In the embryo, muscles consist of single multinucleated fibers that form in a stereotyped pattern. Each muscle originates with a founder cell (FC), which determines muscle identity. Fusion-competent myoblasts fuse with the founder cell, resulting in elongation and formation of the mature larval muscles. Adult muscle development occurs in the pupae. In contrast to larval muscles, adult muscles consist of multiple fibers. Adult muscle precursors, set aside during embryonic life, proliferate to become the myoblasts of the adult musculature (Broadie and Bate, 1991). Adult muscles are specified in two ways (Atreya and Fernandes, 2008). Some adult muscles are templated by pre-existing larval muscles. These muscles grow through the fusion of additional myoblasts during pupal life. Other muscles, such as the body wall muscles, are formed de novo from adult specific FCs. In these muscles, the formation of each fiber is initiated by a single FC, which then fuses with additional myoblasts to form a mature fiber (Dutta et al., 2004).

Innervation plays a differing role in embryonic and adult muscle development. In the embryo, muscle formation precedes and occurs independently of innervation (Bate, 1990). In contrast, formation of adult body wall muscles occurs simultaneously with innervation, and myoblasts migrate to their final destination along nerve pathways (Bate et al., 1991). In the absence of innervation, patterning of most adult muscles is normal. However, the number and size of fibers is reduced (Currie and Bate, 1995).

Major muscle groups in the adult include appendage muscles, thoracic flight muscles, and abdominal body wall muscles. The repetitive and simple organization of the abdominal muscles provides a particularly tractable model for examining alterations

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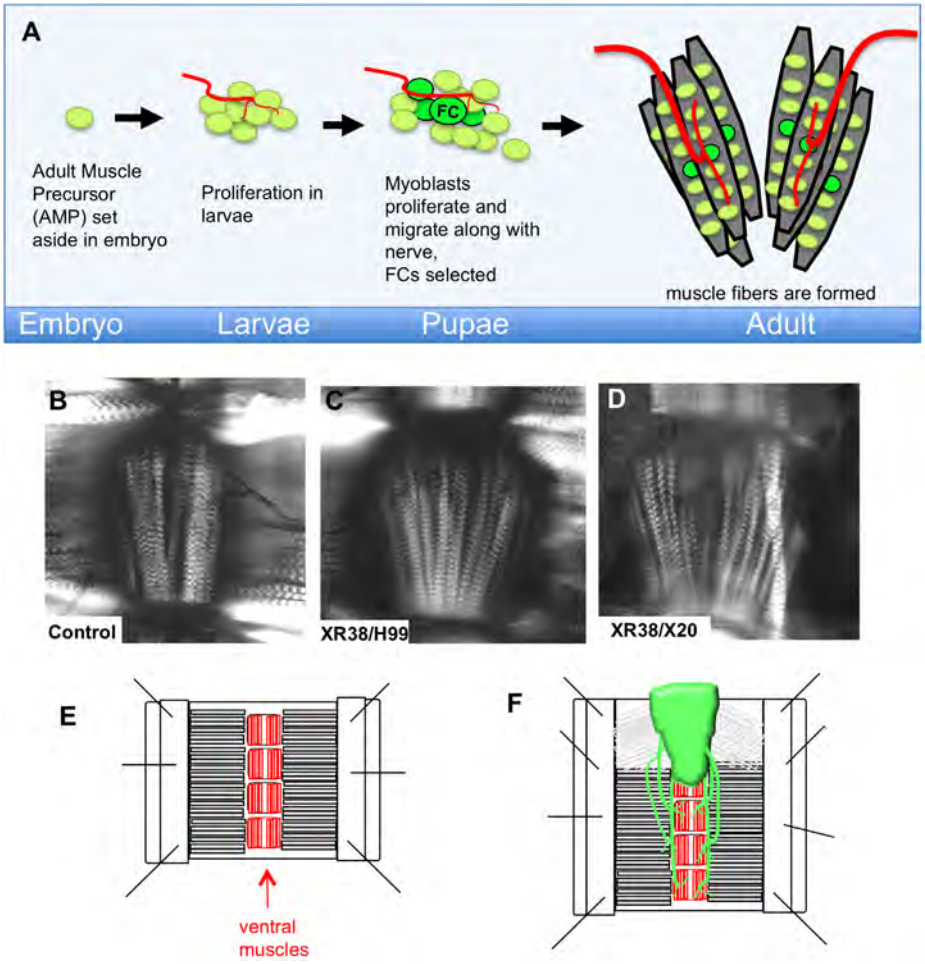

Fig. 1. Cell death mutants have increased fibers in abdominal ventral muscles. (A) In the early embryo, a small number of adult muscle precursors (AMPs) are born from muscle progenitors, but remain quiescent until larval life, when they begin to proliferate (Figeac et al., 2010). In pupal life, these myoblasts proliferate further and migrate along nerves towards their eventual locations. Founder Cell (FC) selection takes place in the abdomen starting at 24 h APF (Dutta et al., 2004). Fusion competent myoblasts then fuse with the FCs to form muscle fibers. (B–D) Ventral abdominal muscles from young females were examined by polarized light (shown as focal plane composites). Increased muscle fiber number is readily obvious in, XR38/H99 (C) and XR38/X20 (D) cell death defective adults compared to control (B). (E and F) Diagram of adult abdomen filet preparations used to visualize muscles. Abdomens were opened at the dorsal midline, pinned, fixed and stained. Ventral nerve cords (VNCs) were retained in some preps (F).

in adult myogenesis (Hebbar et al., 2006). The tissue is accessible in both pupae and adults, and extensive studies have described the normal development of these muscles (Bate et al., 1991; Currie and Bate, 1995; Dutta et al., 2004). Adult abdominal body wall muscles consist of dorsal muscles, ventral longitudinal muscles and lateral muscles.

Our studies focus on the abdominal ventral muscles. These muscles form at the ventral midline, comprising clusters of 5–6 fibers in each central abdominal hemisegment (our data and Bate et al., 1991; Broadie and Bate, 1991). In the embryo, a single ventral adult muscle precursor (AMP) is set aside in each abdominal hemisegment (Bate et al., 1991; Broadie and Bate, 1991; Figeac et al., 2010, Fig. 1A). The proliferation of this precursor in larval life results in a cluster of about 8 myoblasts per abdominal hemisegment at the end of larval stages (Bate et al., 1991; Broadie and Bate, 1991). These myoblasts are closely associated with abdominal nerves (Bate et al., 1991; Dutta et al., 2004). In early pupal life, myoblast numbers increase, and the cells migrate out along the nerves. By 24 h after puparium formation (APF) a small population of these cells begins to express high levels of the founder cell (FC) marker *dumbfounded/Kirre (duf)* (Dutta et al., 2004). Each FC initiates the formation of a muscle fiber and fuses with additional myoblasts to form mature muscle fibers. Thus, FC number corresponds to the final fiber number.

The selection of FCs requires the Fibroblast Growth Factor (FGF) pathway (Dutta et al., 2005). This pathway is required for mesoderm spreading and differentiation in the embryo (Kadam et al., 2009). In early pupal life, the *heartless* FGF receptor (*htl*) is expressed in myoblasts. Expression of dominant negative *htl* decreases the number of founders, while increased expression of *htl* increases founder number and the number of lateral abdominal fibers. The source of the FGF ligands to activate *htl* has not been identified. In addition to *htl* in the FCs, the presence of the innervating motor neurons is also required for correct fiber number (Currie and Bate, 1995; Fernandes and Keshishian, 2005).

The genes *reaper* (*rpr*), *hid, grim* and *sickle* (*skl*) (the RHG genes) regulate the majority of somatic cell deaths in Drosophila (Grether et al., 1995; Kurada and White, 1998; Tan et al., 2011; Garcia-Hughes et al., 2015). We have described significant alterations in CNS cell death when *grim* and *rpr* are disrupted (Peterson et al., 2002; Tan et al., 2011; Arya et al., 2015). Neural stem cells or neuroblasts (NBs) that normally die in the central abdominal segments of the embryonic ventral nerve cord (VNC) survive inappropriately in *grim rpr* mutants. The surviving stem cells continue to divide, resulting in massive enlargement of the abdominal VNC. In the course of our characterization of RHG mutants, we also identified an alteration in the number of fibers in the ventral abdominal muscles. Here we show that inhibition of cell death in the

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