INTRODUCTION

During metamorphosis the larval muscles of Drosophila are broken down and a new set of muscles, which allow the adult to perform a more complex repertoire of behaviors, is built. The adult muscles are formed from pools of twist-expressing myoblasts, which are set aside in the embryo and are present in the larva (Bate et al., 1991; Currie and Bate, 1991). In the abdomen the precursors of the adult muscles are closely associated with nerves. This association is established in the embryo (Bate et al., 1991) and is maintained throughout adult abdominal muscle development (Currie and Bate, 1991). In the larval thorax, myoblasts are associated both with the imaginal discs (Ursprung et al., 1972; Lawrence, 1982; Reed et al., 1975) and with nerves (Bate et al., 1991; Fernandes and VijayRaghaven, 1993). Despite the association between nerves and myoblasts in both the abdomen and thorax, the role (if any) which innervation plays in adult muscle development is not understood. There is however evidence that points to a critical role for innervation in the development of one aspect of the adult abdominal muscle pattern, namely, the male specific muscle (msm) also known as the Muscle of Lawrence (Gailey et al., 1991). This muscle, which forms uniquely in the dorsal part of abdominal segment 5 (A5), was first described by Lawrence and Johnston (1984). It develops from a characteristic aggregation and overgrowth of 3 to 5 adjacent muscles in the dorsal muscle set and forms a prominent structure, which is longer and more substantial than neighboring muscles in the same segment. Posteriorly, the msm attaches at the level of the segment boundary between A5 and A6 (Taylor, 1992; Currie, 1991). The anterior attachment site is more variable but is always well in front of the other dorsal muscles and occasionally reaches as far as the A5/A4 boundary. This muscle also contains more nuclei than other dorsal muscles (Currie, 1991; Taylor and Knittel, 1995). In addition, the msm is unique in that it is the only muscle in A5 that expresses the actin isoform actin 79B (Courchesne-Smith and Tobin, 1989). The function of this muscle is unclear, and, although it has been suggested that it is used during copulation, mutant flies which lack the msm are not sterile (Gailey et al., 1991). Using nuclear transplantations to generate gynandromorphic mosaic animals, Lawrence and Johnston (1986) showed that it is the genotype of the innervation, not the genotype of the myoblasts or the epidermis to which the muscle attaches, which controls the development of the msm. However, their mosaic analysis could not exclude the possibility that it is other, non-neuronal cells, close in origin to the neurons, which might dictate the development of the msm. In addition, the timing of the inferred interaction between muscle and nerve, which is apparently necessary for msm development, is unknown, and it is not clear whether innervation plays a more general role in the establishment of the rest of the adult muscle pattern in Drosophila.

In this paper, we report the results of a direct test of the function of innervation in the formation of the overall adult abdominal muscles in Drosophila melanogaster.

SUMMARY

The adult abdominal muscles in Drosophila are generated de novo during metamorphosis and form a simple and characteristic pattern. Throughout adult abdominal development there is a close association between nerves and myoblasts. However, the role of innervation in adult myogenesis is unclear. In males there is an additional muscle, which is unique to abdominal segment 5 (A5). This male specific muscle forms from the same pool of myoblasts as other dorsal muscles in A5 but develops several distinctive characteristics. Previous work indicates the genotype of the innervation of this male specific muscle may play a crucial role in its proper development, although the part played by innervation in the development of other muscles is unknown. Here we test directly the function of innervation in adult myogenesis in general and for the development and differentiation of the male specific muscle in particular. After denervation at the onset of metamorphosis, muscle growth is impaired although the overall muscle pattern continues to develop. Uniquely, the male specific muscle fails to form. Our results indicate that there is an essential role for innervation during the period of metamorphosis for the formation of a full complement of abdominal muscles and for muscle growth. Furthermore, innervation is absolutely required for the formation of the male specific muscle and the development of its special characteristics.

Key words: Drosophila, innervation, muscle development, male specific muscle
abdominal muscle pattern and the development of the male specific muscle. We use a new technique which allows us to cut nerves to individual hemisegments at the onset of metamorphosis, forcing the muscles to develop in the absence of functional innervation. We find that after denervation the basic muscle pattern still develops, however, strikingly, the MSM fails to form. We conclude that while innervation during metamorphosis is essential for the development of the MSM and its unique characteristics, it is not required for the formation of other elements of the muscle pattern.

**MATERIALS AND METHODS**

**Fly stocks**

Three transformant strains of flies were used for these experiments. One strain has the *lacZ* gene linked to the myosin heavy chain (MHC) promoter. Staining for β-galactosidase activity in these flies reveals all larval and adult muscles. This stock was provided by Dr S. Bernstein and Dr N. Hess. The second strain has the *lacZ* gene under the control of the actin 79B promoter (Courchesne-Smith and Tobin, 1989). This stock was provided by Dr S. Tobin. The third stock is a *twist lacZ* line which has the *lacZ* gene under the control of the *twist* promoter. This stock was provided by Drs C. Thiess and B. Thiess. All stocks were raised on an artificial diet (Ashburner, 1989) at 25°C.

**Nerve cutting operation**

White prepupae (0 hours after pupariation; APF), or wandering third instar larvae, were collected, washed and placed on a sylgard base under *Drosophila* saline (Roberts, 1986) in a Petri dish. In cases where wandering third instar larvae were used they were first anaesthetized with ether. Glass micropipettes used for the operation were pulled on a Kopf micropipette puller and the tips broken off. The micropipette was attached via a length of plastic tubing to a mouth-held plastic Gilson pipette tip.

Under a dissecting microscope, the prepupa was held in place, ventral surface up, with forceps. The micropipette was pierced through the body wall at a site just to the left or right of the ventral midline in the third abdominal segment of ventral denticles in the region of the abdominal nerves. Suction was applied until one or more of the abdominal nerves was sucked up into the micropipette. Suction was maintained while the micropipette was drawn out of the prepupa, bringing with it a loop of the intact nerve still within the pipette. The micropipette was then withdrawn leaving a loop of nerve outside the prepupa. This loop was cut on both sides, close to the pupal case, with iridectomy scissors, thereby removing a length of the nerve rather than simply severing it. Prepupae were then removed from the saline solution, placed on moist filter paper in a Petri dish and allowed to complete adult development in an incubator at 25°C. Each nerve cut in this way only innervates one hemisegment, the development of other, unoperated hemisegments serves as an internal control.

**Immunohistochemistry**

Animals were usually dissected 2-10 days after eclosion. The flies were anaesthetized with ether for 5-7 minutes and then dissected under saline and flat preparations of their abdomens were made by cutting along the ventral midline, pinning them out and removing the gut.

Staining for β-galactosidase activity was performed as follows. Dissected abdomens were briefly prefixed for 5 minutes in 4% paraformaldehyde in phosphate-buffered saline (PBS). They were then washed in PBS for at least 45 minutes prior to developing. The PBS was then replaced with the developing solution containing X-gal according to the recipe of Fernandes et al. (1991). The preparations were left in the developing solution overnight at room temperature, postfixed for 30 minutes in 4% paraformaldehyde, dehydrated through an alcohol series and cleared in cedar wood oil prior to mounting in DPX (BDH).

Staining procedures for antibodies were as reported earlier (Currie and Bate, 1991). Flat preparations were fixed for 45 to 60 minutes in 4% paraformaldehyde in phosphate-buffered saline (PBS) and then washed three or four times in phosphate-buffered saline with 0.3% Triton X-100 (PBS-TX). They were then incubated in primary antibody (anti-HP antibody (Coppel) at 1:250 dilution, anti 22C10 provided by Prof. S. Benzer at 1:500, antibody RC5 provided by Prof. S. Benzer at 1:400, and anti-β-galactosidase from Prof. N. Patel at 1:2000) overnight at 4°C. Subsequently, the primary antibody was removed, abdomens were washed four times over a period of 1 hour in PBS-TX and then incubated for 1 hour in a 1:50 dilution of goat serum. After a quick wash, they were incubated in a 1:200 concentration of biotinylated secondary antibody (Vector Laboratories) for 1 hour at room temperature. After four washes in PBS-TX they were exposed to a peroxidase-linked avidin/biotin complex (Vectahals ABC) for 30 minutes. The preparations were thoroughly washed and then the peroxidase label was revealed in a reaction medium consisting of PBS, diaminobenzidine (Sigma), nickel chloride and hydrogen peroxide under a microscope to determine the end point. The reaction was stopped by replacing the reaction medium with 30% ethanol. The preparations were dehydrated, cleared in cedar wood oil and mounted in DPX.

Specimens were examined on a Zeiss microscope with Nomarski optics. Birefringence of muscles was observed using crossed polaroids.

**RESULTS**

**Identification of denervated hemisegments**

We concentrated on the effect of denervation on the dorsal abdominal muscles which form a simple parallel array of about 18 fibres in each hemisegment (Miller, 1950). In a small number of the cases in which only one nerve was cut it was possible to identify the affected hemisegment by the contraction of muscles in that hemisegment at the time of transection. In such cases the muscles in the denervated hemisegment formed but were both thinner and less numerous than their counterparts in the contralateral, unaffected, control hemisegment (Fig. 1). In these cases there was never more than one affected hemisegment which had thinner muscles. As decreases in muscle thickness and number were caused by nerve transection these characteristics were used as the criteria...
Innervation is essential for male muscle to identify denervated hemisegments in cases where they could not be identified reliably at the time of transection.

To confirm the denervated status of hemisegments with thinner and fewer muscles we stained a number of the abdomens with an anti-HRP antibody which recognizes a neural antigen (Jan and Jan, 1982). The normally innervated muscles in unoperated control hemisegments are thick and the neuromuscular junctions (NMJs) are clearly visible (Fig. 2A). This adult innervation is somewhat different from that of the larval muscles. The site of contact of the innervation tends to be near the mid-point of the muscle. The innervation occurs as a single process which runs parallel with the long axis of the muscle and does not extend along its whole length. The nerve process is almost always unbranched and has a series of varicosities of around 2 μm in diameter, which are smaller than the large type I varicosities on the larval muscles (Johansen et al., 1989). They are also less punctate and closer together than the varicosities in the larva. Occasionally, much narrower processes extend beyond the end of the varicose NMJs.

The thinner muscles in denervated hemisegments showed one of two different anti-HRP staining phenotypes. In a few cases there was no positive staining on any of the muscles in a denervated hemisegment, indicating a complete absence of innervation (Fig. 2B). However in the majority of denervated hemisegments, while most of the muscles were unstained, some of the fibres were positively stained. Of 506 muscles that could be scored in over 50 denervated hemisegments 350 had no innervation at all. Of the remainder, 102 had abnormal innervation (see below) and 54 had innervation that could not be distinguished from wild type. The morphology of the abnormal staining was characteristic and different from that of normally innervated muscles in control hemisegments. One example of such staining is shown in Fig. 2C. At a gross level, the pattern of staining is somewhat similar to that seen on normally innervated muscles, although the varicosities can be more variable in size than on control muscles. However, there is a striking difference in the levels of staining of the varicosities. On normally innervated muscles the varicosities stain strongly throughout. However, on muscles in denervated hemisegments, the staining appears to be only around the surface of the varicosities, and the staining inside the varicosities is so weak that they appear almost hollow (Fig. 3C, compare with Fig. 3A). These muscles appeared no more robust than their neighbours which had no trace of innervation. In all but one of the cases of such positive staining, where the innervation could be traced back (n=4), it connected with the main nerve trunk, which is composed of all the sensory axons in that hemisegment. There was only one case in which there was evidence of inappropriate innervation coming from an adjacent segment.

Control animals were pierced with a micropipette as white prepupae but no nerves were removed or cut. In these animals the morphology and pattern of muscles were unaffected.

**Denervation and the adult myoblasts**

The adult myoblasts are closely associated with the larval...
nerves and migrate over them to the sites of adult muscle formation (Currie and Bate, 1991). We therefore checked the effect that the nerve transection operation has on the larval nerves and twist-expressing myoblasts, to eliminate the possibility that nerve transection was indirectly affecting adult muscles by disrupting the migration of the myoblasts. We cut nerves to individual hemisegments in wandering third instar larvae carrying a twist-lacZ construct, fixed them as flat preparations after they had recovered from the anaesthetic, and stained them with antibody 22C10 which labels the innervation and an antibody to β-galactosidase to reveal the twist-expressing adult myoblasts (Fig. 3). We were particularly interested in the two branches from the intersegmental nerve (ISN), on either side of muscle 3 (for muscle nomenclature, see Bate, 1990), which project to dorsal sensory structures on the epidermis. It is along these branches that the dorsal myoblasts migrate to reach the site of adult muscle formation (Currie and Bate, 1991). In each of 27 hemisegments with transected nerves neither of these branches was disrupted and the twist-expressing groups of myoblasts appeared to be normal (Fig. 3B). There was, however, an occasional disruption of another branch from the ISN. This branch contacts the posterior face of muscle 8 and was disrupted in 3 of 27 hemisegments counted. Disruption of this branch would not be expected to affect the dorsal muscles, as myoblasts associated with this branch contribute only to the lateral muscles (Currie and Bate, 1991). The rest of the innervation in hemisegments where nerves were transected appeared normal. Thus, the nerve cutting operation itself does not appear to affect directly either the dorsal myoblasts or their route for migration.

**Effect of denervation on muscle size**

Denervated hemisegments in adult flies were identified by the presence of clearly thinner muscles than in control hemisegments in the same animal. This was a more reliable indicator than counts of muscle numbers, which were complicated by the difficulty of dissecting preparations where all dorsal muscles were both intact and visible. In order to quantify the effect of denervation on muscle thickness we concentrated on the three dorsal muscles closest to the dorsal midline in each hemisegment of A5 (for muscle position in relation to the dorsal midline see Fig. 1). These fibres do not contribute to the male specific muscle. We have found that there are always at least three, and usually five, dorsal muscle fibres medial to the first male specific muscle fibre ($n$=19). We measured muscle thickness in denervated and contralateral innervated hemisegments of A5 in 11 abdomens in which only one hemisegment of A5 was denervated (Fig. 4). Measurement of the posterior region of these muscles in denervated and contralateral control hemisegments shows that there is a marked difference in size between normal, innervated muscles and their denervated counterparts in A5 (Fig. 4).

**Effect of denervation on the general muscle pattern**

Denervation has a number of effects on the development of the dorsal muscles, but the effect on the msm is very different from the effects on all other muscles and will be dealt with in the next section. In all denervated hemisegments ($n$>90), the fundamental pattern of parallel aligned adult abdominal muscles still forms (Fig. 1). There are, however, two clear differences between muscles formed in denervated hemisegments and those in hemisegments where the innervation is intact. Although the pattern of muscles and attachment sites is normal, there are fewer muscles and those that form are thinner than those in control hemisegments (Figs 1, 2 and 4). The denervated muscles do show signs of normal differentiation. They span the correct region of the segment and, as demonstrated by the staining in the MHCB gal line they, like their normally innervated counterparts, express β-galactosidase (Fig. 1), indicating that the myosin heavy chain gene is being expressed.
Furthermore, the denervated muscles are birefringent in polarized light, indicating that they have developed the highly ordered arrangement of myofibrils characteristic of normal adult muscle.

The reduced size of muscles in denervated hemisegments could be due to an effect on muscle growth or on the number of myoblasts which fuse to form the muscles. In order to establish which of these factors was involved, we stained abdomens with the antibody 8C5, which recognises a general nuclear antigen, to reveal the nuclei in muscles from control and denervated muscles (Fig. 5). In control muscles, the number of nuclei per muscle varies somewhat across the hemisegment, those muscles nearer the dorsal midline having more nuclei than those situated more laterally. Despite this normal level of variation, it is clear that denervation reduces the number of myoblasts which form the dorsal muscles. However, there are signs that denervation must also affect muscle growth. In some cases, we found muscles in denervated hemisegments which had as many nuclei as some of the more lateral muscles in control hemisegments and yet these denervated muscles were still substantially thinner. Similarly, the persistent larval muscles (which in normal development pass through a cycle of partial loss of structure and subsequent regrowth; Currie, unpublished data) in denervated hemisegments are much thinner than in control hemisegments. As these muscles incorporate very few, if any, new nuclei (Currie, 1991; Taylor and Knittel, personal communication) this effect must be due to a reduction in muscle growth. Thus, in *Drosophila* as in some Lepidoptera (Nüesch, 1985), a normal pattern of most adult muscles can develop in the absence of innervation, although the muscles are reduced in size and number. These effects appear to be due both to a reduction in the number of myoblasts which fuse to form the muscles, and to a reduction in growth itself.

**Denervation prevents the formation of the male specific muscle**

The msm forms uniquely in the dorsal part of A5 and is located 3-5 muscle fibres in from the dorsal midline (Fig. 6A). Denervating A5 has a striking effect on the development of the msm. In every case where the nerve to a hemisegment in A5 in a male is cut, the msm in that hemisegment fails to form (n=52). It is important to note that in a denervated A5, although no msm forms, other dorsal muscles can form in the location normally occupied by the msm. Where the nerve to only one hemisegment in A5 is cut, the msm in the contralateral hemisegment develops normally (Fig. 6B). Denervation of other segments has no effect on the development of the msm. In denervated A5 hemisegments, none of the remaining dorsal muscles form.
muscles, including those that form in the location normally occupied by the msm, express any of the male specific characteristics. They do not span beyond the normal level of attachment for typical dorsal muscles, nor are they any more substantial than their denervated neighbors. We stained denervated male A5 hemisegments (n=16) with anti-HRP and found that there was no evidence of msm development in hemisegments where there was no positive staining on any of the muscles in the region which would give rise to the msm (n=4) or in hemisegments where there was some abnormal positive stain on one or two of the muscles (n=12). There are no special effects in A5 in operated females; denervation simply causes fewer, thinner muscles to develop than in controls.

To confirm that none of the muscles in A5 develops any of the known characteristics of the msm in the absence of innervation, the nerve cutting experiments were repeated on a strain of flies carrying a P element insert with the gene for β-galactosidase under the control of the promoter for the actin 79B gene. In unoperated flies of this strain, β-galactosidase is selectively expressed in A5 in the msm (Fig. 7A this study and Courchesne-Smith and Tobin, 1989). In denervated A5 hemisegments, however, there is no β-galactosidase expression in any of the remaining muscles (n=12) (Fig. 7B). Thus, the local activation of the actin 79B promoter, as judged by the expression of the construct, appears to be innervation dependent.

DISCUSSION

The differentiation of denervated muscle

The results of this study demonstrate that when adult muscles develop in denervated hemisegments, they differentiate relatively normally. They span the correct region, express myosin heavy chain and are birefringent under polarised light, indicating that some aspects of their development, including the formation of attachment sites and the assembly of a contractile apparatus are independent of innervation. These observations agree both with those previously made for the development of denervated adult muscles in Lepidoptera and those made for the embryonic development of larval muscles in Drosophila. In Antheraea polyphemus, thin muscles, which are formed in denervated segments, also have normal cross striations and the ability to contract when stimulated, although at much higher voltages than required by normal muscle (Nüesch, 1968). In these animals ultrastructural development seems normal but markedly slower than in control muscles. Broadie and Bate (1993) found that larval muscles in Drosophila embryos can differentiate apparently normally despite the absence of innervation. These non-innervated muscles express myosin heavy chain and can contract vigorously when electrically stimulated (Broadie and Bate, 1993). Although these aspects of muscle differentiation also occur in denervated hemisegments in the developing adult fly, it is clear that, unlike the situation in the embryo, other aspects of muscle development are profoundly affected by loss of innervation. Thus, the number of muscles and their size is substantially reduced.

The reduction in the number of muscles in denervated hemisegments may simply be the result of a depletion in the pool of myoblasts from which the muscles are formed. Reduction of the number of adult myoblasts by exposure to HU during larval life (Broadie and Bate, 1991; Taylor and Knittel, 1995) results in a reduction of the number of adult muscles formed.

The effect on muscle size appears to be due, in part at least, to an effect on muscle growth. This might be caused by the lack of excitation, or possibly by the absence of a trophic factor, either of which could be required for muscle growth. The dependence of muscle growth and atrophy on innervation in vertebrates has been demonstrated (Engel and Stonnington, 1974). Denervation in adult insects also causes muscle atrophy (Rees and Usherwood, 1972). However, muscles in denervated hemisegments also contain fewer nuclei and this could in some
The role of nerves in muscle patterning

Since the muscle pattern is largely completed by 41 hours APF (Currie and Bate, 1991) and as there are fewer muscles in denervated hemisegments, the effect of denervation on muscle number must occur before this time, although the muscle pattern itself remains intact.

The majority of the muscles in denervated hemisegments completely lack innervation. However there are some muscles that show positive staining with anti-HRP indicating that in some way a limited number of nerve contacts can be formed in hemisegments where nerves are cut at the onset of metamorphosis. The abnormal staining on such innervated muscles could be due to the remnants of dead or dying innervation which had managed some degree of outgrowth after nerve transection. In cockroaches signs of degenerating axon terminals can still be found on the muscle surface up to 12 days after denervation (Wood and Usherwood, 1979). In the case of our experiments however, this explanation seems unlikely because growing nerves would be unlikely to migrate across a hemisegment after being severed from their cell bodies. This then leaves the possibilities of regeneration from the cut ends of axons or innervation coming in from adjacent segments, either of which would be a significant event. If regeneration occurs, it would have to be both rapid and accurate if axons were to find their appropriate hemisegment. If innervation from adjacent segments is involved then there would have to be a mechanism by which neurons could detect uninnervated muscles in adjacent segments and respond by growing towards them and a mechanism which under normal circumstances would prevent outgrowth from one segment onto innervated muscles in the next. In the embryo, if normal innervation of a muscle is prevented ectopic innervation by passing axons from the same or adjacent segments can occur. The striking aspect of the case for denervated adult muscles described here is that if innervation comes from another segment then the distances involved are far greater than is the case in the embryo and the neurons are growing into regions they would not normally occupy. So far we are unable to account for the positive staining which we find in some denervated hemisegments. Perhaps the simplest explanation we can offer is that nerve transection delays the outgrowth of axons from motoneurons which normally innervate the dorsal abdominal muscles but does not completely prevent it. Some axons succeed in reaching their targets. Nonetheless, this delayed innervation does not completely prevent it. Some axons succeed in reaching their targets. Nonetheless, this delayed innervation cannot rescue the consequences of early muscle denervation with the result that the innervated, like the uninnervated muscles, are smaller and contain fewer nuclei. Several corollaries follow if this is true. First there must be a critical period during which nerves exert an effect on myoblast division and/or muscle growth. Second, the muscle pattern is independent of innervation for its formation. Third it might be the case that muscles with an innervation which persists from the larva would not receive appropriate innervation after the nerves had been cut. It is in fact the case that we hardly ever observe inner-
vation of the persistent larval muscles in hemisegments where the nerves have been transected.

Nonetheless, in our experiments, the majority of muscles have no innervation and yet still establish the basic muscle pattern. If this fundamental pattern is innervation independent then it must require other sources of information. Indeed there is good evidence that both the epidermis and the mesoderm itself play a role in the patterning of the muscles. Cautery experiments by Haget (1953) and Bock (see Seidel et al., 1940) demonstrated that in embryos of other insects the epidermis is required for the correct development of the larval muscles. The epidermis also clearly plays a role in specifying attachment sites for the thoracic pattern of adult muscle (Michelson, 1994). Thus, the requirement for autonomous homeotic gene expression in the mesoderm was thought to be out of register with expression in the ectoderm. Both of these experiments indicate that segmental patterns in the mesoderm are autonomously regulated by mesodermal expression of homeotic genes. In addition, the pattern of differentiation of thoracic muscles in flies where the epidermis of T3 is homeotically transformed towards T2 (Fernandes et al., 1994) suggests that there is a requirement for autonomous homeotic gene expression in the mesoderm both for the histolysis of the larval muscles and for the formation of the correct adult muscles. A requirement for autonomous homeotic gene expression in the mesoderm was also suggested by Hooper (1986) who based her ideas on the finding that muscle patterns are altered in mutants of Ubx and on earlier findings that showed Ubx expression in the mesoderm to be out of register with expression in the ectoderm (Akam, 1983). Thus, the nerve independent patterning of the adult muscles could be influenced by both epidermal and mesodermal cues.

The male specific muscle

The all or none effect of innervation on the development of the male specific muscle is in striking contrast to the lesser effect that denervation has on the rest of the dorsal abdominal muscles. Given the essential part played by innervation in the formation of this muscle, it may be significant that in wild-type flies not only is the morphology of the msd very different from that of its neighbors in A5, but its innervation is also unique. The innervation of the muscle fibres which make up the msd is more extensive than that of the other dorsal muscles in this, or any other segment (Currie, 1991, B. J. Taylor pers. comm.). The nerves that contact the msd branch more on the muscle fibres and spread along a greater proportion of the muscle length.

It may well be that one role of this special innervation is to select from a previously specified dorsal muscle set (Broadie and Bate, 1991) a group of myoblasts whose differentiation will be unique. Despite the fact that the msd differs markedly in its size from the other dorsal muscles in the same segment, evidence from clonal analysis (Lawrence and Johnston, 1984) shows that the myoblasts which form this muscle are not segregated from those that form the other, smaller dorsal muscles in A5 early in the embryo.

In addition to its size, another unique aspect of the differentiation of the msd, which fails in the absence of innervation, is the expression of the actin 79B isoform. This isoform is also expressed in a number of other muscles in the adult including muscles of the male genitalia and certain tubular muscles in the thorax. In vertebrates, it has been shown that innervation is important for the normal progress of expression of both actin (Shimizu et al., 1988) and myosin (Van Horn and Crow, 1989; Gauthier, 1987) isotypes during skeletal muscle development. The results reported here suggest that innervating neurons may have similar functions in regulating actin expression during the development of the msd in Drosophila. Taylor and Knittel (1995) have recently found that reduced nuclear numbers may also affect the expression of actin 79B in the msd, muscles with only 9 nuclei staining very weakly. As nuclei were not counted in denervated male A5 in the present study the possibility that the absence of detectable staining could be due to the reduction in nuclear numbers cannot be ruled out. Experiments are now under way to establish whether there is a general requirement for innervation for the expression of actin 79B.

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We conclude that innervation is essential for the normal development of the male specific muscle. By generating gynandromorphs using nuclear transplantation, Lawrence and Johnston (1986) found that even in flies where both the muscle and the epidermis to which it attached were female, a msd would still form if the innervation was male. They were, however, unable to test directly the implied role for innervation. Here we verify experimentally their conclusion that innervation might determine this element of the muscle pattern. We have further shown that it is during the period of metamorphosis that the necessary interaction between nerve and developing muscle must occur. We also demonstrate that innervation plays a significant but different role in the development of the other dorsal abdominal muscles.

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