I give permission for public access to my thesis and for copying to be done at the
discretion of the archives’ librarian and/or the College library.

Signature_________________        Date________
The Role of $\beta$FTZ-F1 in the Innervation of the Abdominal Muscles in *Drosophila*

By

Rizwana Islam

A paper presented to the

Faculty of Mount Holyoke College in

Partial Fulfillment of the Requirements for

The Degree of Bachelors of Arts with

Honor

Department of Biological Sciences

South Hadley, MA 01075

May, 2005
This paper was prepared under the direction of Professor Craig Woodard For 8 credits
ACKNOWLEDGEMENTS

I would like to thank Dr. Craig Woodard for his help, support, kindness and understanding. I would also like to thank Marian Rice, who helped work with the fluorescence microscope, Tina Fortier for her advice and help, Marc Boucher for his generosity and the entire Fly Lab for their encouragement. Finally, I would like to thank the Biology Department for providing me with the opportunity to do research.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Figures</td>
<td>v</td>
</tr>
<tr>
<td>Abstract</td>
<td>viii</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Drosophila as a model organism</td>
<td>2</td>
</tr>
<tr>
<td>Drosophila life cycle</td>
<td>2</td>
</tr>
<tr>
<td>Drosophila metamorphosis</td>
<td>5</td>
</tr>
<tr>
<td>The hierarchy of gene regulation during metamorphosis</td>
<td>9</td>
</tr>
<tr>
<td>$\beta$FTZ-F1</td>
<td>12</td>
</tr>
<tr>
<td>Musculature</td>
<td>17</td>
</tr>
<tr>
<td>Motor neurons</td>
<td>21</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>33</td>
</tr>
<tr>
<td>Methods and materials</td>
<td>34</td>
</tr>
<tr>
<td>Results</td>
<td>51</td>
</tr>
<tr>
<td>Discussion</td>
<td>64</td>
</tr>
<tr>
<td>References</td>
<td>75</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>The life cycle of <em>Drosophila melanogaster</em></td>
<td>4</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Ecdysone concentration in the fly body plotted against time (in hours) relative to puparium formation</td>
<td>14</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Flies carrying a loss of function mutation of the $\beta$FTZ-F1 gene show distinguishable defects during metamorphosis</td>
<td>16</td>
</tr>
<tr>
<td>Figure 4</td>
<td>The abdominal musculature of the third instar larva</td>
<td>19</td>
</tr>
<tr>
<td>Figure 5</td>
<td>The abdominal body wall muscles of one half segment in <em>Drosophila</em></td>
<td>20</td>
</tr>
<tr>
<td>Figure 6a</td>
<td>Details of the central nervous system in <em>Drosophila</em></td>
<td>25</td>
</tr>
<tr>
<td>Figure 6b</td>
<td>The ventral nerve cord</td>
<td>26</td>
</tr>
<tr>
<td>Figure 7</td>
<td>The ventral nerve cord</td>
<td>27</td>
</tr>
<tr>
<td>Figure 8</td>
<td>The abdominal body wall muscles and their innervation patterns in a single body wall segment of a fruit fly at the larval stage</td>
<td>31</td>
</tr>
<tr>
<td>Figure 9a</td>
<td>$w; P[w^+, Nrv2-GAL4+UAS-GFP] $;+ males</td>
<td>39</td>
</tr>
<tr>
<td>Figure 9b</td>
<td>$w;+; TM3,Sb e/TM6B,Tb Hu e$ virgin females</td>
<td>40</td>
</tr>
<tr>
<td>Figure 10a</td>
<td>$w;P[w^+, Nrv2-GAL4+UAS-GFP];TM3, Sb e flies</td>
<td>41</td>
</tr>
<tr>
<td>Figure 10b</td>
<td>$w;P[w^+, Nrv2-GAL4-UAS-GFP];TM6B, Tb Hu e flies</td>
<td>42</td>
</tr>
<tr>
<td>Figure 11a</td>
<td>$w; P [w^+, Nrv2-GAL4+UAS-GFP]; TM3,Sb e/TM6B, Tb Hu e$ flies</td>
<td>43</td>
</tr>
<tr>
<td>Figure 11b</td>
<td>$w;+; ex17/TM6B, Hu e$ flies</td>
<td>44</td>
</tr>
</tbody>
</table>
Figure 12a: w; P [w⁺, Nrv2-GAL4+UAS-GFP]; TM3, Sb e/TM6B, Tb Hu e flies

Figure 12b: w; +; DfCat/TM6B, Hu e flies

Figure 13a: w; P [w⁺, Nrv2-GAL4+UAS-GFP]; ex17/TM6B, Tb Hu e flies

Figure 13b: w; P [w⁺, Nrv2-GAL4+UAS-GFP]; DfCat/ TM6B, Tb Hu e flies

Figure 14a: GFP fluorescence in the abdominal region of the w; P[w⁺, Nrv2-GAL4-UAS-GFP]++; animal (control fly) at 0 hour after puparium formation

Figure 14b: GFP fluorescence in the abdominal region of a w; P [w⁺, Nrv2-GAL4-UAS-GFP]; ex17/DfCat animal (experimental fly) at 0 hour after puparium formation

Figure 15a: GFP fluorescence in the abdominal region of a w; P[w⁺, Nrv2-GAL4-UAS-GFP]++; animal (control fly) at 6 hours after puparium formation

Figure 15b: GFP fluorescence in the abdominal region of a w; P [w⁺, Nrv2-GAL4-UAS-GFP]; ex17/DfCat animal (experimental fly) at 6 hours after puparium formation

Figure 16a: GFP fluorescence in the abdominal region of a w; P[w⁺, Nrv2-GAL4-UAS-GFP]++; animal (control fly) at 12 hours after puparium formation

Figure 16b: GFP fluorescence in the abdominal region of a w; P [w⁺, Nrv2-GAL4-UAS-GFP]; ex17/DfCat animal (experimental fly) at 12 hours after puparium formation

Figure 17a: GFP fluorescence in the abdominal region of a w; P[w⁺, Nrv2-GAL4-UAS-GFP]++; animal (control fly) at 14 hours after puparium formation
Figure 17b: GFP fluorescence in the abdominal region of a w; P[w+, Nrv2-GAL4-UAS-GFP]; ex17/DfCat animal (experimental fly) at 14 hours after puparium formation
ABSTRACT

In *Drosophila melanogaster*, a gene called βFTZ-F1 mediates the genetic and developmental responses to the steroid hormone 20-hydroxyecdysone (ecdysone). Loss of function mutations of the βFTZ-F1 gene gives rise to pupal lethality and causes defects in these processes that occur during the prepupal-pupal transition. In the wild type flies, head eversion and wing and leg extension are brought by contractions of the abdominal muscles, which generate hydrostatic pressure to drive these events. In the βFTZ-F1 mutants, the muscular contractions are not strong enough to generate enough pressure to cause head eversion and wing and leg extension. My hypothesis is that the βFTZ-F1 gene plays a key role in the innervation of the abdominal muscles by motor neurons. Thus a mutation in the βFTZ-F1 gene causes a defect in the innervation pattern of these muscles, causing them to be unable to produce strong enough contractions to drive morphogenesis.

I tested the hypothesis that the defects observed in βFTZ-F1 mutants are due to abnormalities in the innervation of abdominal muscles in *Drosophila*. I compared the innervation pattern of abdominal muscles in fly stocks called Nrv2-GAL4+UAS-GFP, which express green fluorescent protein (GFP) in the nervous system, which also carried mutations in the βFTZ-F1 gene. I observed a change in the morphology of the innervating motor neurons from 6 hours after puparium formation. The change was more pronounced in flies aged to 12 hours or more after puparium formation. In the βFTZ-F1 mutants, the two bundles of longitudinal nerves collapse to form one single bundle. The segmental nerves no longer project toward the periphery and innervate muscles at each segment but spread out together near the posterior end of the animals. These results support my hypothesis that βFTZ-F1 plays a role in the innervation of abdominal muscles in *Drosophila*. 
INTRODUCTION

The fruit fly *Drosophila melanogaster* is used in this study as a model to trace the developmental events that occur during metamorphosis of the fly. Of particular interest in this case is a gene called \( \beta \text{FTZ-F1} \), whose product is an orphan nuclear receptor with no known ligand. This gene mediates the responses of a key hormone in the fruit fly called 20-hydroxyecdysone, which is a steroid hormone. It plays a very important role in metamorphosis or the transformation of the body plan from the larval to the adult form. Animals carrying loss of function mutations of the \( \beta \text{FTZ-F1} \) gene display several developmental defects, the most prominent of which are defects in head eversion and wing and leg extension. These processes are brought about by the contractions of the abdominal muscles of the fly body wall and are influenced by the \( \beta \text{FTZ-F1} \) gene (Fortier et al., 2003).

In the mutants, the contractions are less vigorous and consistent than in the adult and are thus insufficient to generate enough hydrostatic pressure within the prepupal body to cause head eversion and wing and leg extension, which are processes that mark the prepupal-pupal transition. This inconsistency and weakened force of contractions may be due to improper or insufficient innervation of the abdominal muscles by motor neurons, which drive these contractions. The other possibility is that a change in the morphology of the muscles themselves occurs and they can no longer respond as efficiently to neuronal stimulation to undergo regular contractions. In this study, I examined the
former possibility that a defect in the innervation pattern of the abdominal
muscles is responsible for the observed defects in the mutants.

**DROSOPHILA AS A MODEL ORGANISM**

*Drosophila melanogaster*, the fruit fly, has been used as a model system
for studying the molecular mechanisms involved in development for about a
century (Manning, 2004). The advantages of using the small insect of about 3mm
lie firstly in its size, which allows handling of large numbers with relative ease.
Secondly, it has a short life cycle of about ten to twelve days, and it is relatively
cheap to maintain under laboratory conditions. Moreover, the availability of
mutant strains of flies, with defects in any of several thousand genes, makes
biological processes easier to study in them. Much is known about its life cycle
and behavior as it has been extensively studied for a long time. Finally, its entire
genome has been sequenced (Manning, 2004). All these factors contribute
towards the popularity of *Drosophila* as a model system.

**DROSOPHILA LIFE CYCLE**

Within its short life cycle, *Drosophila melanogaster* goes through seven
different stages (Figure 1). It starts its life as an embryo or egg, which is about
half a millimeter long (Manning, 2004). The embryo develops or hatches into a
larva, called the first instar larva, about one day after fertilization. The larva is
able to feed and grows continuously to molt approximately a day later into the
second larval instar, which also lasts for one day. The separation between these two stages is marked by the cuticular molts that the animals undergo. The larval cuticle is shed in response to increase in body size in two steps. Initially, the older cuticle undergoes apolysis or separation from the epithelium and a new cuticle is formed by secretions from the epithelial cells. This is followed by the actual shedding of the older cuticle (Chihara et al., 1982). The larva molts into the third instar larva at the end of the second instar larval stage. It remains in this form for two days and leaves the food source in search of a solid surface to which it attaches itself. Thus the end of the third larval instar is called the “wandering” stage. The larva then undergoes a process known as puparium formation when the larval cuticle is modified to form the pupal case (Chihara et al., 1982). The pupal case retains the white color of the larval cuticle for a brief period of about 15 minutes, after which it tans to a darker shade of brown and simultaneously hardens. The larva thus undergoes transition from a motile organism into the immobile prepupa, in which form it spends the next 12 hours. At the end of that period, the prepupal-pupal transition takes place, which is marked by head eversion and leg and wing extension. During the four days between puparium formation and the end of the pupal stage, the body is completely remodeled to give rise to the adult form, which becomes fertile within the ensuing 12 hours (Manning, 2004).
Figure 1: The life cycle of *Drosophila melanogaster*. The number within each section denotes the duration of each stage in days. The embryonic and the first two larval stages last for one day each, while the third instar larval stage is 2 days long. The prepupal stage lasts for 0.5 days or 12 hours and the pupal stage about 3.5 days. At the end of the pupal stage, the adult flies eclose and emerge from the pupal case. Metamorphosis lasts from the beginning of the prepupal stage to the end of the pupal stage (modified from Weigmann et al., 2003).
**DROSOPHILA METAMORPHOSIS**

The transformation of the body plan from larva to adult is called metamorphosis, which begins with the formation of the puparium (pupal case) between the third instar larval and prepupal stages and is brought about by a pulse of a steroid hormone called 20-hydroxyecdysone (hereafter referred to as ecdysone) (Riddiford et al., 1993). This pulse is referred to as the late larval ecdysone pulse. A pulse is the increase in concentration of the hormone at a specific time during development of the fly. Steroid hormones such as ecdysone are derivatives of cholesterol and are able to cross the lipid bilayer of cell membranes to bind to specific receptors within the cell. The hormone-receptor complex then enters the nucleus and binds to DNA at specific loci to induce or repress the transcription of specific target genes. Ecdysone thus regulates the expression of target genes and brings about a diverse range of developmental changes that occur during metamorphosis. Metamorphosis involves the destruction of larval structures by programmed cell death and their replacement with adult tissues developing from progenitor cells called imaginal cells. Pulses or brief periods of high titers of ecdysone trigger the major postembryonic developmental changes in *Drosophila* (Riddiford et al., 1993).

Puparium formation (pupariation) is accompanied by shortening and broadening of the animal and eversion of the anterior spiracles used for gas exchange. In the next 15 minutes after puparium formation, the animal is called a "white prepupa" as it still has a white cuticle. This stage is referred to as the 0
hour prepupa stage. Following this period, the animal cuticle tans and hardens, forming a protective pupal case (Chihara et al., 1982).

About 2 to 4 hours after puparium formation, a “gas bubble” forms in the mid abdominal cavity of the prepupa (Fortier et al., 2003). The gas bubble expulsion occurs from inside the fly to the posterior end of the puparium (Chadfield and Sparrow, 1985).

During the mid-prepupal stage or about 6 hours after puparium formation, the ecdysone titer dips. At this point, the larval cuticle begins apolysis or retraction from the underlying epidermis, starting from the anterior of the fly (Fetchel et al., 1989). Simultaneously, the pupal cuticle is secreted by both larval and imaginal cells. The anterior part of the cuticle is secreted by imaginal cells in the head and the thorax, while the posterior cuticle is secreted by larval cells in the abdomen. The cuticle can be divided into the exterior epicuticle and an interior procuticle, consisting of chitin. The inner cuticle can be further differentiated into an endo- and exocuticle (Fetchel et al., 1989). The deposition of the pupal cuticle is accomplished by the end of the prepupal stage. The cuticle layer fuses to form a homogeneous sheet and this fusing as well as deposition are timed very precisely to allow adult structures to form.

About 9 hours after puparium formation, imaginal cells that give rise to the leg and wings of the fly become visible in the thorax of the developing prepupa. Imaginal cells can be of three different types: histoblast nests, imaginal rings and imaginal discs. Histoblast nests are made up of about 12 cells that
remain inactive during embryonic and larval development but start to divide during metamorphosis and migrate to replace the larval epidermis with the adult epidermis. These cells also play a role in the formation of the adult midgut. The imaginal rings multiply by division during the larval stages and give rise to structures such as the adult foregut, hindgut and salivary glands (Madhavan and Schneiderman, 1977). The imaginal discs, on the other hand, are made up of thousands of cells that multiply during the larval and early pupal and prepupal stages of development. At the onset of the prepupal stage, the imaginal discs fuse together to form precursors to the head, wings and legs in the thoracic region. At the same time, the imaginal discs also divide and differentiate in order to generate components of the pupal cuticle (Fetchel et al., 1988).

An ecdysone pulse about 10 to 12 hours after puparium formation, called the prepupal ecdysone pulse, triggers head eversion and leg and wing extension, and defines the prepupal-pupal transition (Riddiford et al., 1993). Head eversion is the pushing out of the head from the thoracic region to the anterior-most part of the fly. The head everts into the gas bubble that is translocated from the posterior end to the anterior by wave-like contractions of the abdominal muscles (Fortier et al., 2003). Simultaneously, the prepupa moves towards the posterior allowing a space to be formed at the anterior end and also shortens its body size (Fortier et al., 2003). The muscular contractions cause an increase in the internal hydrostatic pressure, which aids head eversion as well as inflation and extension of the appendages such as the legs and wings (Chadfield and Sparrow, 1985).
Simultaneously, apolysis of the larval cuticle takes place and the mouth hooks used for the consumption of food during the larval stages separate from the developing body but remain attached to the interior of the pupal case as the cells attaching them to the body are destroyed by programmed cell death (Demerec, 1994).

After prepupal-pupal transition, the eyes begin to develop (Roseland and Schneiderman, 1979). The adult legs and wings continue to develop throughout the pupal stage (Roseland and Schneiderman, 1979). At about 24 hours after puparium formation, the apolysis of the pupal cuticle begins, which is followed by the formation of the adult cuticle, secreted by imaginal cells, a day later or 48 to 50 hours after puparium formation (Chihara et al., 1982; Fechtel et al., 1989). Thus the epidermis of the fly remains free of an outer cuticle layer for about 18 to 24 hours between the apolysis of the pupal cuticle and formation of the adult cuticle. However, the fly remains covered by the pupal case during this period. This delay allows cells to migrate to proper regions and imaginal discs to evaginate in order to form the wings and legs of the adult fly as well as bristles and hair on the apical surfaces of the epidermal cells (Fristrom and Fristrom, 1993). The adult cuticle is formed while the fly is still enclosed within the pupal case and functions to protect the fly from adverse environmental conditions as it emerges out of the pupal case. The adult cuticle tans as the pupa develops into the pharate adult (Fristrom and Fristrom, 1993; Bainbridge and Bownes, 1981). After the formation of the adult cuticle, the eyes develop pigmentation and geometric
patterning while the legs and wings darken in color and bristles and hair develop on the epidermal cells. These processes take place within the remaining 48 hours of the pupal stage. At the end of the three and a half days as a pupa, the adult fly pushes its way out of the pupal cuticle and the pupal case with its legs (Andres and Thummel, 1992). The adult fly emerges from the anterior region of the pupal case. The process of the adult fly emerging from its pupal case is known as eclosion.

**THE HIERARCHY OF GENE REGULATION DURING METAMORPHOSIS**

The steroid hormone ecdysone plays a key role in the regulation of metamorphosis by the activation or repression of specific genes at specific times during metamorphosis. Ecdysone is present in various forms in the fruit fly. Ecdysone is secreted by the larval prothoracic gland and then quickly converted to 20-hydroxyecdysone. This happens during the embryonic stage, at the larval molts and also at the end of the third larval instar stage. 20-hydroxyecdysone is the only functional form of the hormone during metamorphosis (Borst et al., 1974). The receptor for ecdysone is known as the Ecdysone Receptor-Ultraspireacle (EcR-Usp), which is a heterodimer complex. The receptor has a ligand binding domain as well as a DNA binding domain and binds to DNA at sites upstream of the promoter sequences. As the hormone binds to the receptor complex, it activates or represses the transcription of the target genes. Different
isoforms of the receptor are expressed in different tissues and allow regulation of tissue-specific events (Talbot et al., 1993).

Varying the concentration of ecdysone at different stages during development also controls temporal or stage-specific expression of genes. The late larval ecdysone pulse at the end of the third larval instar induces the expression of a set of genes called the “early genes”, which in turn regulate the activation of a larger set of genes called “late genes”. Three of the very important early genes are the $E74A$, $E75A$ and the $BR-C$ or Broad-Complex genes, which control events leading to pupariation or entry into metamorphosis. These early genes regulate their own expression levels and are only expressed briefly at specific times during development (Ashburner, 1967; Richards, 1976).

The $E74$ gene encodes two major gene products: $E74A$ and $E74B$. $E74A$ plays a key role in puparium formation and in the activation of late genes in combination with the $BR-C$ gene products (Fletcher et al., 1995). $E74B$ also functions in puparium formation and in processes such as head eversion during the prepupal-pupal transition, by preventing premature histolysis of larval muscles, which drive these processes (Fletcher et al., 1995). The $BR-C$ gene codes for several DNA-binding proteins with zinc-finger domains (DiBello et al., 1991). These proteins regulate evagination and fusion of imaginal discs (Kiss et al., 1988), histolysis of salivary glands, optic lobe organization (Restifo and White, 1991) and development of the thoracic muscles (Restifo and White, 1992). The $E75$ gene encodes 3 different proteins, two of which are members of the nuclear
hormone receptor family. One of the gene products, E75A, represses its own expression while activating the expression of the late genes (Hill et al., 1993).

During the mid-prepupal stage, about 6 hours after puparium formation, the ecdysone levels decrease and the βFTZ-F1 gene is expressed. Ecdysone has been shown to repress the transcription of βFTZ-F1 in vitro by Woodard et al. (1994). Thus the highest concentration of βFTZ-F1 transcripts are found at about 4 to 6 hours after puparium formation since ecdysone has a low titer ranging from 3 to 8 hours after puparium formation (Andres et al., 1993).

A second high titer pulse of ecdysone occurs about 10 to 12 hours after puparium formation. Early genes such as E74 and E75 are reinduced at this stage and play a role in head eversion and appendage formation, which mark prepupal-pupal transition (Fletcher et al., 1995; Fletcher and Thummel, 1995). The early genes are induced at this later stage of development as βFTZ-F1 provides them with the competence to respond to the high ecdysone titer (Woodard et al., 1994; Broadus et al., 1999). In addition, other genes such as E93 are also expressed at this stage but are not found during the ecdysone pulse at the beginning of puparium formation. This is because βFTZ-F1 expressed before the later ecdysone pulse induces stage-specific genes such as E93 and provides them with the competence to respond to the high ecdysone titer (Woodard et al., 1994; Broadus et al., 1999). E93 sets up a hierarchy of events in the salivary gland, which leads to its destruction by programmed cell death during the prepupal-pupal transition (Baehrecke and Thummel, 1995).
Some of the other genes induced by the prepupal ecdysone pulse are ones that encode the pupal cuticle proteins. The exocuticle component of the procuticle is formed by low molecular weight pupal cuticle proteins that are transcribed about 8 to 10 hours after puparium formation (Doctor et al., 1985; Fristrom et al., 1986; Wolfgang et al., 1986). From 12 to 20 hours after puparium formation, high molecular weight pupal cuticle proteins are transcribed, that form the endocuticle, starting from the anterior and continuing to the posterior (Doctor et al., 1985).

Another gene called *EDG-84A* encodes a low molecular weight pupal cuticle protein secreted by imaginal cells (Fechtel et al., 1989). The expression of *EDG-84A* may also be regulated by \( \beta FTZ-F1 \) (Yamada et al., 2000).

**\( \beta FTZ-F1 \)**

The *fushi tarazu factor 1* or *FTZ-F1* gene encodes two proteins called \( \alpha FTZ-F1 \) and \( \beta FTZ-F1 \), which are both members of the nuclear hormone receptor superfamily that bind to DNA and regulate transcription (Lavorgna et al., 1991). The coding sequence of the two transcripts has a long overlapping region or common coding region as well as individual sequences specific to each transcript. \( \alpha FTZ-F1 \) regulates segmentation during the embryonic stage of the fly and its expression occurs very early during development in 1.5 hour to 4 hour old embryos (Ueda et al., 1990). \( \beta FTZ-F1 \) is expressed during late embryogenesis in 16 to 22 hour embryos (Lavorgna et al., 1991), during larval molts and at the mid
prepupal stage between the larval and prepupal ecdysone pulses (Baehrecke et al., 1993) (Figure 2).

The βFTZ-F1 protein plays a significant role during metamorphosis of adult body structures in *Drosophila*. βFTZ-F1 represses its own transcription and its transcription is also repressed by ecdysone, which ensures that during metamorphosis, its expression is limited to the mid prepupal period (Woodard et al., 1994; Broadus et al., 1999). βFTZ-F1 also mediates the genetic and developmental responses to ecdysone by providing specific genes with the competence to be induced by the hormone at specific times. In addition, βFTZ-F1 influences several developmental events that occur during metamorphosis such as controlling the muscular contractions that drive morphogenetic events at the prepupal-pupal transition in *Drosophila* (Fortier et al., 2003). The βFTZ-F1 protein induces or regulates the stage-specific expression of genes such as *E93* (Woodard et al., 1994; Broadus et al., 1999). Ectopic expression of βFTZ-F1 from a transgene increases the induction of the early genes such as *BR-C, E74A* and *E75A* after an ecdysone pulse and also causes *E93* to be expressed early in third larval instar salivary glands after induction by ecdysone (Woodard et al., 1994; Broadus et al., 1999).
Figure 2: Ecdysone concentration in the fly body plotted against time (in hours) relative to puparium formation.

Figure 2: Ecdysone concentration in the fly body plotted against time in hours relative to puparium formation. The ecdysone has two peaks or pulses, first at 0 hours after puparium formation, called the late larval ecdysone pulse and then at about 10-12 hours after puparium formation, known as the prepupal ecdysone pulse. During the late larval ecdysone pulse, the early genes such as BR-C, E74A and E75A are expressed. At around the mid-prepupal period, about 6 hours after puparium formation, βFTZ-F1 is expressed as the ecdysone titer falls to a trough. Finally, during the prepupal pulse of ecdysone, the early genes are reinduced and stage-specific early genes such as E93 are expressed (Woodard et al., 1994).
Loss of function mutations in $\beta$FTZ-F1 result in pupal lethality and defects in programmed cell death of the larval salivary glands (Lee et al., 2002) as well as in the expression of the BR-C, E74A, E75A and E93 genes in the late prepupa (Broadus et al., 1999). Flies with mutations in the $\beta$FTZ-F1 gene display a number of defects, the most prominent of which are defects observed in head eversion and in wing and leg extension (Fortier et al., 2003). Head eversion takes 10 times as long in the mutant animals as in the wild type. Flaws are also seen in the retraction of the prepupal body to the posterior end and in the movement of the gas bubble to the posterior from the mid abdominal region. The gas bubble in the mutants does not completely translocate to the posterior but residual gases remain in the abdominal area and are visible as a translucent region. The subsequent movement of the gas bubble to the anterior is also slower and less efficient than in the wild type. The $\beta$FTZ-F1 mutant adult flies still have properly segmented legs but they are much shorter than in the wild type and are bent and extend much later. The legs in the wild type are aligned with eye-antennal imaginal discs before the final extension but in the mutants, the legs are not drawn far enough towards the anterior to reach the imaginal discs. The mutants also do not shorten their bodies as much as the wild type flies. These $\beta$FTZ-F1 mutants are unable to contract their abdominal muscles vigorously and consistently to generate enough internal hydrostatic pressure to drive morphogenetic processes such as head eversion and wing and leg extension and hence display the observed defects (Fortier et al., 2003) (Figure 3).
Figure 3: Flies carrying a loss of function mutation of the \( \beta FTZ-F1 \) gene show distinguishable defects during metamorphosis.

Figure 3: Flies carrying a loss of function mutation of the \( \beta FTZ-F1 \) gene show distinguishable defects during metamorphosis. The figure on the left shows a fly wild type for \( \beta FTZ-F1 \) while the one on the left shows a mutant. In the mutant, head eversion does not occur normally and defects are also observed in leg and wing extension (modified from Broadus et al., 1999).
MUSCULATURE

The contractions of the abdominal muscles bring about the shortening of the prepupal body, the translocation of the mid-abdominal gas bubble to the posterior end of the puparium and the subsequent movement of the bubble to the anterior to create space for the head to evert (Robertson, 1936) and finally the building up of hydrostatic pressure within the fly body to aid head eversion as well as leg and wing extension (Fortier et al., 2003). The abdominal muscles thus play a very important role during metamorphosis. These muscles are larval in origin and a subset of them persists long enough after puparium formation to drive these morphogenetic events. Most other larval muscles are histolysed during the prepupal or early pupal stages and replaced by adult musculature originating from myoblasts or muscle precursor cells (Hartenstein, 1993).

Each abdominal hemisegment is made up of 30 muscle fibers arranged in a stereotypic pattern (Bate, 1990) (Figure 4, Figure 5). The different types of muscles present include the different dorsal muscles that run in an anterior-posterior direction at the dorsal end of the animal. The dorsal muscles consist of the dorsal external oblique muscles and the dorsal internal oblique muscles, which run obliquely along the body of the animal. The dorsal muscles also include the three dorsal acute muscles, which are in parallel orientation to each other and seem to converge with the oblique muscle. A single dorsal transverse muscle completes the list of dorsal muscles. This muscle is set almost at a right angle to
the dorsal oblique muscles. There are four lateral transverse muscles, divided into two groups, the lateral external transverse muscles and the lateral internal transverse muscles. These run parallel to the dorsal transverse muscle and the segment border muscles (SBM), of which there are two in each segment. A lateral longitudinal muscle runs perpendicular to the lateral transverse muscles. A lateral external oblique muscle is also present that runs obliquely near the latter muscle in the anterior-posterior direction. All the lateral muscles are present in the middle of the abdominal segment and are also called pleural muscles. Finally, there are a large number of ventral muscles. There are six different types of ventral oblique muscles divided into three different subsets: the ventral external oblique muscles, the ventral internal oblique muscles, and the ventral superficial oblique muscles. These muscles are found at the ventral end of the abdominal segment as suggested by their names and run obliquely to the anterior-posterior direction, exactly parallel to the dorsal oblique muscles. Four ventral longitudinal muscles are arranged in parallel and very close to each other, placed next to the ventral oblique muscles. Three ventral acute muscles and a ventral transverse muscle that runs parallel to the SBM complete the set of 30 abdominal body wall muscles that make up each abdominal hemisegment (Bate, 1990).
Figure 4: The abdominal musculature of the third instar larva. A subset of these muscles survives the histolysis during the early stages of metamorphosis and actively drives morphogenetic events. The dorsal pharyngeal musculature (dpm) is seen as a unique set of muscles at the dorsal end; the dorsal external oblique muscles (de) and the dorsal internal oblique muscles (di) run obliquely to the anterior-posterior axis; the pleural external transverse muscles (pet), the pleural internal transverse muscles (pit) pleural longitudinal muscles (pl) and the pleural external oblique muscles (po) are the lateral muscles; finally the ventral muscles, the ventral external oblique muscles (ve) ventral internal oblique muscles (vi) and the ventral superficial oblique muscles (vs) are found at the ventral end of the animal (Hartenstein, 1993).
Figure 5: The abdominal body wall muscles of one half segment in

_Drosophila_

Figure 5: The abdominal body wall muscles of one half segment in _Drosophila_. The internal muscles are colored red and the external muscles, green. Muscles are referred to by their positions (D, dorsal; L, lateral; V, ventral), followed by their orientation (A, acute; L, longitudinal; O, oblique; T, transverse). The figure also shows the SBM (segment border muscle) (Landgraf et al., 2003a).
MOTOR NEURONS

There are approximately 40 motor neurons in each abdominal hemisegment that innervate the array of the 30 somatic muscles (Thor and Thomas, 2002; Zito et al., 1999). Motor neurons are specialized nerve cells that associate with muscles, control their contractions and thus play a major role in movement (Thor and Thomas, 2002). Of the 40 motor neurons, 31 have been identified and characterized by Landgraf et al. (1997). Most motor neurons and large interneurons of the insect adult nervous system are of embryonic origin (Hartenstein, 1993). To this set of embryonically born neurons, a large number of neurons are added during larval and early pupal stages. However, in the posterior abdominal segments, the number of postembryonic neuroblasts or neuronal precursor cells is very small (Hartenstein, 1993).

The axons of the individual motor neurons leave the CNS or the central nervous system and the motor nerve cords and project towards the periphery along six main branches known as the intersegmental nerve (ISN), the transverse nerve (TN) and the 4 segmental nerve branches (SN) called SNa, SNb, SNC and SNd, which arranged in this sequence from the dorsal to the ventral region (Landgraf et al., 1997). Thus *Drosophila* motor neurons can thus be divided into six major types according to their axon projections (Thor and Thomas, 2002). Almost all of the adult motor neurons in *Drosophila* arise from functional larval motor neurons. Even though the larval muscles are histolyzed during
metamorphosis, the efferent (motor) axons persist and give rise to new terminal branches that associate with the adult muscles (Tissot et al., 1998).

Each motor neuron occupies a specific position in the neuromere and makes an invariant set of connections with target muscles (Landgraf et al., 1997). A neuromere or encephalomere is a transient segment of the embryonic brain. The motor neurons have characteristic dendritic trees, which often overlap in case of functionally related motor neurons. The TN is seen to project along the borders of the abdominal segments and is comprised of a few motor axons (Bodmer and Jan, 1987; Gorczyca et al., 1994). The ISN is divided into two roots, the posterior and the anterior roots, made up of axons, the motor neurons of which lie in the same segment as the muscles they innervate, and the in the segment anterior to it, respectively (Landgraf et al., 1997). SNa and SNc consist of axons from motor neurons located in the same segment as the muscles they innervate. These axons leave the CNS through the segmental nerve root. In contrast, SNb and SNd consist of the axons of the motor neurons from the next anterior segment that project from the anterior root of the ISN. These branches also contain an axon of a segmental motor neuron, called a VUM or ventral unpaired median neuron, which innervates a particular subset of muscles. The VUM neuron exits through the posterior root of the ISN and projects through both the SN and ISN nerve branches. Thus SNb and SNd are made up of axons that extend through the anterior root of the ISN before separating from the ISN in the peripheral region (Landgraf et al., 1997). In some texts, SNb and SNd are thus referred to as ISNb
and ISNd. ISN motor neurons innervate internal muscles while the SN and the TN motor neurons innervate external muscles. The internal muscles each span the length of one segment, going from the anterior to the posterior direction. External muscles on the other hand are usually transverse (Landgraf et al., 2003a).

The ISN and SN motor neurons expand their dendrites in distinct regions of the neuropile (Landgraf et al., 1997). The neuropile is the complex net of axonal, dendritic, and glial branches, in which the nerve cell bodies are embedded. In *Drosophila*, the neuropile is located within the ventral nerve cord (Landgraf et al., 2003b) (Figure 6). The neuropile consists of long axon tracts or the longitudinal nerves and the terminal axonal or dendritic arborizations carrying synapses (Younossi-Hartenstein, 2003). The longitudinal nerves (longitudinal connectives) are composed of interneurons that connect the sensory and the motor neurons, which innervate the muscles, with the brain (Landgraf et al., 2003a). There are few, if any, monosynaptic connections between sensory and motor neurons in the abdominal ventral nerve cord of *Drosophila*. In the ventral nerve cord, motor neuron dendrites form in the dorsal-most region of the neuropile, between the longitudinal glia and the motor axons (Landgraf et al., 2003a). Midline glial cells are found between the longitudinal axons of the ventral nerve cord while exit glia surround the point where the ganglionic branch cells of the tracheal system switch from the ISN to the SN at the ventral nerve cord (Hidalgo, 2001; Englund, 1999) (Figure 7). Longitudinal glial cells are found running parallel to the longitudinal axons (Hidalgo, 2001). Dendrites of the ISN motor
neurons occupy a domain extending posteriorly from the posterior part of one neuromere into the anterior part of the next (Langraf et al., 2003). SN motor neuron dendrites on the other hand, occupy a domain that lies between the domains of ISN motor neuron arbors (Langraf et al., 2003a).

The cell bodies and the dendritic arborizations of the motor neurons that innervate the abdominal muscles lie in or around the anterior commissure (AC) or posterior commissure (PC) of the CNS (Landgraf et al., 1997). There is a series of commissures or nerve fiber tracks that connect the two symmetric halves of the nervous system or the two bundles of the neuropile (Schnorrer and Dickson, 2004). Many of the motor neurons that innervate the different types of abdominal muscles have not yet been named and the nomenclature of the musculature itself differs slightly in different texts. Thirty one out of the 40 motor neurons that innervate the abdominal muscles have been mapped completely while the position and characteristics of 9 others still remain elusive. Recent texts, however, report the total number of motor neurons that innervate the abdominal muscles to be 36 (Landgraf et al., 2003a).

The anterior root of the ISN consists of axons of motor neurons that innervate the dorsal acute muscles DA1 and DA3, the dorsal oblique muscles DO1, DO2, DO3, DO4 and DO5, the dorsal transverse muscle DT1 and the lateral longitudinal muscle LL1 (Landgraf et al., 1997) (Figure 8). DA1 is innervated by
Figure 6a: Details of the central nervous system in *Drosophila*

Figure 6a: Details of the central nervous system in *Drosophila*. The interneurons (IN) and efferent neurons (EN) or motor neurons are found in the cortex (CX) of the CNS, while sensory neurons (SN) are found in the periphery. All neurons send processes towards the synaptic neuropile (NP). Efferent motor neurons project through segmental nerves (N) towards muscles (M) where they form neuromuscular junctions (NMJ). Black arrows indicate anterior (Landgraf et al., 2003b).
Figure 6b: The ventral nerve cord

Figure 6b: The ventral nerve cord (VNC). The cell bodies of neurons in the intersegmental nerves (ISN), segmental nerves (SN) and transverse nerves (TN) lie around the VNC. The SN, TN and ISN all synapse with neurons in the longitudinal nerves. The anterior commissure (AC) and the posterior commissure (PC) are also shown (modified from Landgraf et al., 2003a)
Figure 7: The ventral nerve cord

Figure 7: The ventral nerve cord showing the bundles of longitudinal nerves that make up the longitudinal connectives, the midline and the longitudinal glia, and the anterior and posterior commissures (modified from Hidalgo, 2001)
a motor neuron called aCC, which has its cell body in the PC. DO1, DO2 and LL1 are innervated by U neurons which have their cell bodies in the AC. DA3 is also innervated by a U motor neuron, with its cell body near the PC. DT1, DO3, DO4 and DO5 are innervated by unnamed motor neurons. The posterior root of the ISN projects an axon from a motor neuron called RP-2 that innervates DA2, whose cell body lies near AC (Landgraf et al., 1997).

The SNa is made up of a group of axons from motor neurons that innervate the laterally placed muscles, which are the LT1, LT2, LT3 and LT4 or the lateral transverse muscles, the LO1 or lateral oblique muscle and the SBM or segment border muscle (Landgraf et al., 1997) (Figure 8). These axonal projections are all part of unnamed neurons and form part of the segmental nerve. The SNb consists of motor neurons that innervate the ventral longitudinal muscles, VL1, VL2, VL3 and VL4 and also the ventral oblique muscles, VO1, VO2 and VO3. While motor neurons called V and RP5 both innervate the VL1 muscle, RP1 and RP4 innervate VL2. Another motor neuron called RP3 innervates both VL3 and VL4. The oblique muscles are innervated by unnamed neurons. These motor neurons exit the CNS through the anterior root of the ISN and not the SN, unlike the SNa motor neurons. The cell bodies of the motor neurons innervating the ventral longitudinal muscles lie close to the AC in all cases except for in RP4. The other ventral oblique muscles, VO4, VO5 and VO6 are innervated by unnamed motor neurons whose axons are part of the SNd and exit the CNS with the anterior root of the ISN. The ventral acute muscles, VA1,
VA2 and VA3 on the other hand, are innervated by axons in the SNC, which exit the CNS through SN (Landgraf et al., 1997).

Certain VUM neurons also innervate what are referred to as VUM muscles. The VUM dorsal muscle and the VUM ventral muscle are innervated by motor neurons, which are considered part of the ISN and SNb or SNd respectively and exit the CNS with the posterior root of the ISN (Landgraf et al., 1997). In contrast, the VUM neuron that innervates the lateral VUM muscle is part of SNa, leaving the CNS with SN. Finally, the ventral transverse muscle, VT1 is innervated by a motor neuron whose axon projects through the transverse nerve (Landgraf et al., 1997).

The trend or pattern of innervation that is seen here is that the dorsal muscles are mostly innervated by motor neurons that project their axons through ISN while lateral muscles are innervated by axons in the SNa. The ventral muscles are innervated by axons that project through all other nerve branches, SNb, SNC and SNd. Only one muscle is found to be innervated by the transverse nerve in this case. A few of the muscles are multiply innervated while most are innervated by a single motor neuron. The motor neurons that innervate neighboring muscles have overlapping dendritic trees (Landgraf et al., 1997). The motor neurons may be derived from a common neuroblast, in which they are found to be clustered. They may also originate from different neuroblasts, in which case their dendritic arbors still overlap even though their cell body
positions and their axonal projections may differ. The muscles they innervate, however, have similar positions and thus may function similarly during muscular contractions (Landgraf et al., 1997). The neuromuscular junctions formed between all 30 abdominal body wall muscles and motor neurons are described here. However, 9 other motor neurons are also present whose axonal projections have not yet been identified. These may be involved in multiply innervating muscles as at least one neuromuscular junction has been found for each of the 30 abdominal muscles. In fact, many of these muscles have been discovered to be multiply innervated in the third instar larval stage (Keshishian et al., 1993).
Figure 8: The abdominal body wall muscles and their innervation patterns in a single body wall segment of a fruit fly at the larval stage (these muscles and nerves survive metamorphosis and show the same pattern in prepupal and pupal stages). The neuromuscular junctions form in the late larval stages and each muscle is innervated at a characteristic position. Anterior is up and ventral midline is left. Muscles are referred to by their positions (D, dorsal; L, lateral; V, ventral), followed by their orientation (A, acute; L, longitudinal; O, oblique; T, transverse) and SBM. The nerve branches shown are intersegmental nerves, ISN with internal muscle targets; segmental nerves, SN with external muscle targets and transverse nerves, TN (Landgraf et al., 1997).
Muscular contractions of the abdominal body wall muscles drive the morphogenetic processes of the prepupal-pupal transition (Fortier et al., 2003). The $\beta$FTZ-F1 gene is required for these contractions. Thus in animals with mutant copies of the $\beta$FTZ-F1 gene, several developmental and morphogenetic defects are observed. The defects include but are not limited to improper or incomplete head eversion and faulty wing and leg extension. These are the most prominent of the defects and can be visualized even through the pupal case of mutants after they go through puparium formation or metamorphosis. The muscles whose contractions drive metamorphosis are innervated by a number of specific motor neurons. A loss of function mutation of the $\beta$FTZ-F1 gene in Drosophila may be responsible for abnormalities in the innervation of these muscles, which can no longer generate enough hydrostatic pressure by contraction to drive the developmental events. Thus the hypothesis that I tested here is that the developmental and morphogenetic defects observed in the $\beta$FTZ-F1 mutants occur due to a defect in the pattern of innervation of the abdominal body wall muscles.
METHODS AND MATERIALS

DROSOPHILA STOCKS

In order to visualize the motor neurons and the innervation patterns of the abdominal muscles, I have used a stock of flies called Nrv2-GAL4+UAS-GFP, which express GFP only in the nervous system. Nervana 2 is the Drosophila Na\(^+\), K\(^+\) ATPase β-subunit gene (Sun et al., 1999). The 5' flanking DNA of the gene consists of transcriptional regulatory elements. They are fused to the yeast transcriptional activator GAL4, which binds specifically to the upstream activating sequence or UAS. The Nrv2-GAL4 transgenes are genetically recombined with UAS-GFP (S65T) transgenes in Drosophila in the same chromosome to form the Nrv2-GAL4+UAS-GFP. The transgene product is expressed in the nervous system in embryos, larvae, pupae and adults. In Drosophila, the Na\(^+\), K\(^+\) ATPase β-subunit genes are called the Nervana 1 and 2 genes, which are expressed in functionally active neurons and glia, as they are required to maintain ionic gradients (Sun et al., 1999). The S65T mutation in GFP incorporated in the Nrv2-GAL4+UAS-GFP flies speeds up the appearance of fluorescence.

To look at whether mutations in the \(\beta FTZ-F1\) gene change the innervation pattern of muscles in Drosophila, a stock of flies carrying alleles with two different mutations in the \(\beta FTZ-F1\) gene were examined. The desired mutations in
the flies are a hypomorphic defect in the $\beta FTZ-F1$ gene, known as $FTZ-F1^{17}$ (referred to as $ex17$ in the text) and a deletion of the entire $\beta FTZ-F1$ gene, called $Df(3L)Cat^{DH104}$ (hereafter referred to as $DfCat$), which results in lethality during metamorphosis (Broadus et al., 1999). A hypomorphic defect is one in which the expression of the mutant gene is much reduced or weakened when compared to the wild type. $DfCat$ is a large deletion in the third chromosome of the fruit fly, which removes the $\beta FTZ-F1$ gene completely along with several other genes. The stock of flies that carried both mutations was obtained after several crosses from stocks that carried the $ex17$ and $DfCat$ mutations over $TM6B$, $Tb$ $Hu$ $e$. $TM6B$ is a balancer chromosome with several mutations. This ensures that the other mutations carried on this chromosome are not lost during crossing of the flies and are retained in the progeny.

The desired flies thus have both the $Nrv2$-GAL4+UAS-GFP transgene insert and the $ex17$ over $DfCat$ mutations. This stock of flies expresses GFP in the nervous system and also carries the two mutant alleles of the $\beta FTZ-F1$ gene, which causes the mutant flies to die at the pupal stage. The hypomorphic defect in the $\beta FTZ-F1$ gene ensures that some $\beta FTZ-F1$ protein is still produced, allowing the flies to live into metamorphosis. However, these flies still show the defects that occur due to a loss of function mutation of the $\beta FTZ-F1$ gene and are thus ideal for use in this experiment.
These flies are generated through a number of crosses, beginning with the stocks carrying GFP tags in proteins expressed in the nervous system or the Nrv2-GAL4+UAS-GFP stock of flies. The crosses are as outlined below:

Parental generation:
Cross 1: w; P [w+, Nrv2-GAL4+UAS-GFP]; + males (Figure 9a) x w; +; TM3, Sb e/TM6B, Tb Hu e females (virgin) (Figure 9b)

The males in this cross are from the w+, Nrv2-GAL4+UAS-GFP stock w or white mutation, which results in white eyes instead of a bright red, which is the wild type color. However, this mutant phenotype is masked in the Nrv2-GAL4+UAS-GFP flies as they carry the w+ or wild type allele in a P element insert in the second chromosome, which results in a yellow to red pigmentation in the eyes. These flies are crossed with virgin females with white eyes, which also carry three homozygous lethal mutations: Sb or Stubble that results in short bristles, Hu or humeral that gives rise to mis-patterned humeral bristles and Tb or tubby, which results in a shorter and fatter body. These flies also carry a mutation in the e or ebony gene, which gives rise to a darker body color when it is homozygous for both alleles. These flies carry the white mutation in the first chromosome. The TM3 and TM6B (T-third chromosome; M-multiple inversions) are balancer chromosomes present in these flies, which have multiple inversion mutations that function to prevent the loss of the ex17 or DfCat alleles from the original chromosome by cross over.
The F1 or first filial generation consists of the flies:

F1: \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM3, Sb e \) (Figure 10a) and \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM6B, Tb Hu e \) (Figure 10b)

These flies are of two types: the \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM3, Sb e \) flies and the \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM6B, Tb Hu e \) flies. The \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM3, Sb e \) flies have red pigmented eyes, stubble or short uncrossed bristles and a tan or wild type body color. The \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM6B, Tb Hu e \) flies also have red eyes but are \( Stubble^+ \) for bristles or have long bristles that cross as well as mispatterened \( Humeral \) bristles. A second cross is carried out between the progeny, where male flies carrying the \( Stubble \) mutation are crossed with female flies carrying the \( Humeral \) mutation and vice versa.

From the F2 or second filial generation, the following flies are collected:

\( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM3, Sb e/TM6B, Tb Hu e \) (Figure 11a, 12a)

These flies have both balancer chromosomes in them as well as the transgene \( Nrv2-GAL4+UAS-GFP \) and its marker. They thus have red eyes and a dark body color as they are homozygous for the \( ebony \) mutation. They also carry both the \( Stubble \) and \( Humeral \) mutations and display both phenotypes. The third cross is set up between these F2 flies and flies carrying either the \( ex17 \) mutation or the \( DfCat \) mutation. The \( ex17 \) flies have white eyes and a tan body while the \( DfCat \) flies have white eyes but an \( ebony \) or dark body color as they carry two copies of the \( ebony \) gene. The crosses are outlined below:
Cross 3i) \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM3, Sb e/TM6B, Tb Hu e \)
\( x w; +; ex17/TM6B, Tb Hu e \) (Figure 11b)

ii) \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM3, Sb e/TM6B, Tb Hu e \)
\( x w; +; DfCat e/TM6B, Tb Hu e \) (Figure 12b)

The progeny from these two crosses carry either the P element insert containing the GFP marker or the transgene \( Nrv2-GAL4+UAS-GFP \) and either the \( ex17 \) or \( DfCat \) mutation. The cross 3i generates the flies \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; ex17/TM6B, Tb Hu e \), which have red eyes that confirm that the transgene insert is present and tan bodies, as well as the \( ex17 \) mutation of the \( \beta FTZ-F1 \) gene. The second cross gives rise to the flies \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; DfCat/ TM6B, Tb Hu e \), which have red eyes, dark bodies and the \( DfCat \) mutation. The progeny are then crossed together in the fourth cross of the experiment, which is outlined below:

Cross 4: \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; ex17/TM6B, Tb Hu e \) (Figure 13a)
\( x w; P[w^+, Nrv2-GAL4+UAS-GFP]; DfCat/ TM6B, Tb Hu e \) (Figure 13b)

The resultant progeny makes up the desired stock of flies carrying the \( FTZ-F1^{17}/Df(3L)Cat^{DH104} \) genotype and the \( Nrv2-GAL4+UAS-GFP \) transgene. These flies thus express very little \( \beta FTZ-F1 \) protein and die during the pupal stage as expected. They express GFP in their nervous system and thus allow examination of the innervation of abdominal muscles using pre-existing positional information. The genotype of the progeny is shown below.

Progeny: \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; ex17/DfCat \)
These animals do not live to reach their adult stages and have to be identified at the prepupal stage from among progeny with different genotypes generated by cross 4. This is possible due to the Tubby marker in the flies not carrying the ex17/DfCat mutation in this generation. The Tubby individuals have shorter and fatter bodies and are easily distinguishable from the longer and thinner ex17/DfCat flies.

**MAINTENANCE OF DROSOPHILA STOCKS**

The flies were cultured in plastic vials and provided with fly food containing yeast, cornmeal, malt and agar. The stocks were maintained in incubators at constant temperatures of either 25°C or 18°C. At 18°C, the rate of fly development is slowed down to half and this phenomenon has been tapped into to collect virgin females for setting up each cross. All other experiments were carried out at 25°C. Stocks of flies carrying the Nrv2-GAL4-UAS-GFP marker and the balancer chromosomes with additional markers, TM3 Sb e and TM6B Tb Hu e had been established and maintained for future experiments. Similarly, w; P [w+, Nrv2-GAL4+UAS-GFP]; ex17/TM6B, Tb Hu e flies and w; P [w+, Nrv2-GAL4+UAS-GFP]; DfCat/TM6B, Tb Hu e fly stocks had also been generated and maintained. The vials were cleared regularly once a week and the flies transferred to a new vial with fresh food, starting from about two weeks after the cross had been initially set up. A light microscope was used to visualize the phenotypic differences between the different stocks before setting up appropriate crosses.
Figure 9a: \( w; P/w^+, Nrv2\text{-GAL4}\text{+UAS-GFP}\text{ } J^+ \text{ males} \)

Figure 9a: \( w; P/w^+, Nrv2\text{-GAL4}\text{+UAS-GFP}\text{ } J^+ \text{ males}. \) They have red eyes due to the \( w^+ \) genotype in the P element insert and wild type features: \( Sh^+ \) bristles, which cross, \( Hu^+ \) bristles, which are three in number, a wild type body shape and tan coloration.
Figure 9b: $w^+; TM3,Sb \ e/TM6B,Tb \ Hu \ e$ virgin females

Figure 9b: $w^+; TM3,Sb \ e/TM6B,Tb \ Hu \ e$ virgin females. They have white eyes and carry the $Sb$ mutation and have bristles that do not cross. They also have mispatterned and numerous humeral bristles because of the $Hu$ mutation, a short fat body due to the $Tb$ mutation and a dark coloration due to the $e$ mutation.
Figure 10a: \( w^{+}/w, \text{Nrv2-GAL4+UAS-GFP};TM3, Sb \) flies

Figure 10a: \( w^{+}/w, \text{Nrv2-GAL4+UAS-GFP};TM3, Sb \) flies. They have red eyes due to the \( w^{+} \) genotype in the P element insert and the \( Sb \) mutation or bristles that do not cross, wild type body shape and tan coloration.
Figure 10b: *w;P[w+, Nrv2-GAL4-UAS-GFP];TM6B, Tb Hu e flies*

Figure 10b: *w;P[w+, Nrv2-GAL4-UAS-GFP];TM6B, Tb Hu e flies*. They have red eyes due to the *w*% genotype in the P element insert and carry the *Hu* mutation and therefore mispatterned and numerous humeral bristles; they are *Sb*% and have a wild type body color.
Figure 11a: \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM3,Sb e/TM6B, Tb Hu e \) flies

Figure 11a: \( w; P[w^+, Nrv2-GAL4+UAS-GFP]; TM3,Sb e/TM6B, Tb Hu e \) flies. They have red eyes due to the \( w^+ \) genotype in the P element insert and the \( Sb \) mutation or bristles that do not cross, and dark body coloration due to homozygous \textit{ebony} mutation.
Figure 11b: $w; +; ex17/TM6B, Hu$ flies

Figure 11b: $w; +; ex17/TM6B, Hu$ flies. They have white eyes and carry the Hu mutation and therefore have mispatterned and numerous humeral bristles; they also have a wild type body color.
Figure 12a: w; P [w+, Nrv2-GAL4+UAS-GFP]; TM3,Sb e/TM6B, Tb Hu e flies. They have red eyes due to the w+ genotype in the P element insert and the Sb mutation or bristles that do not cross, and dark body coloration due to homozygous ebony mutation.
Figure 12b: \( w; +; Df\text{Cat}/TM6B, Hu e \) flies. They have white eyes and carry the \( Hu \) mutation and therefore have mispatterned and numerous humeral bristles; they also have an \( ebony \) or dark body color.
Figure 13a: $w; P/w^+, Nrv2$-GAL4+UAS-GFP/; $ex17/TM6B, Tb Hu e$ flies

Figure 13a: $w; P/w^+, Nrv2$-GAL4+UAS-GFP/; $ex17/TM6B, Tb Hu e$ flies. They have red eyes due to the $w^+$ genotype in the P element insert and the $Hu$ mutation and therefore mispatterned and numerous humeral bristles and tan coloration.
Figure 13b: $w; P[w^+, Nrv2-GAL4+UAS-GFP]; DfCat/ TM6B, TbHu e$ flies

Figure 13b: $w; P[w^+, Nrv2-GAL4+UAS-GFP]; DfCat/ TM6B, TbHu e$ flies. They have red eyes due to the $w^+$ genotype in the P element insert and carry the Hu mutation and therefore mispatterned and numerous humeral bristles; they have an ebony body color.
OBSERVATION OF THE INNERVATION PATTERNS OF ABDOMINAL MUSCLES IN CONTROL AND \textit{\textbeta FTZ-F1} MUTANTS

Animals of the appropriate genotype were collected at 0 hours after puparium formation and placed in a moist chamber made from a modified culture dish. The flies were then aged to 6, 12, 14, 18, 20 and 22 hours after puparium formation in the incubator at 25°C. The animals were examined under a compound fluorescent microscope using the blue filter to visualize GFP fluorescence. The Software MetaView was used to captures images, processing the images and short videos. All videos were made over a period of 10 minutes and 60 frames of images. The images were taken at a 250x magnification and focused mainly on the abdomen of the animals. A stereoscope was also used to capture images of the distinguishing features of each stock of flies.
RESULTS

The $w; P[w^+, Nrv2-GAL4+UAS-GFP]/\text{ex17}/DfCat$ flies (flies with the \(\beta\)FTZ-F1 mutation) did not live beyond the pupal stage as expected. The control flies with the genotype $w; P[w^+, Nrv2-GAL4+UAS-GFP]/+$ did survive the pupal stage and went on to form fully functional adults. This observation was made from examining 10 vials of experimental and control flies each.

Bright GFP fluorescence was observed in all the experimental and control animals at the prepupal and pupal stages. The intensity of the fluorescence was high enough to visualize and monitor morphological changes in the nervous system through the pupal case and no dissection was necessary. The fluorescence was, however, more clearly visible through the ventral sides of the animals, making it easy to see the abdominal nerves. At the prepupal and early pupal stages, bright GFP fluorescence was observed in the brain and the ventral ganglion, from which the ventral nerve cord arises (Figure 14, 15, 16 and 17). Also clearly visible at this stage were the longitudinal nerves within the ventral nerve cord and run across the abdominal segments in an anterior-posterior direction. The segmental nerves projected off the longitudinal nerves and branched into fine nerve fibers at the distal end. The nerve fibers at the anterior end of the animals could also be clearly seen. However, the intersegmental nerves, ISN, and the transverse nerves, TN, which run along the segmental
boundaries of the abdomen, were not visible in any of the animals. Large bright structures were seen at the end of the segmental nerves, which might have been peripheral glia. Glial cells make up the sustentacular tissue that surrounds and supports neurons in the central nervous system. However, the longitudinal glia and midline glia found along the ventral nerve cord were not visible in the animals. Also, most of the peripheral sensory neurons did not show any fluorescence at any point.

As metamorphosis proceeded, the nerves began to appear wavy and frayed and showed much reduced levels of fluorescence. The intensity of fluorescence of the nerve fibers and cell bodies disappeared altogether in pupa older than 14 hours. However, structures of the adult central nervous system began to appear beyond that point, replacing the larval central nervous system. The brain, optic lobes and thoracico-abdominal ganglion could be seen in the animals that were aged beyond 18 hours after puparium formation. Some peripheral nervous system structures could be observed at this point, which were the antennae, labial palps, maxillary palps and leg nerves. Since the neurons innervating the abdominal muscles could not be resolved at this point, the data collected from animals aged beyond 14 hours after puparium formation were not considered in this study.

At 0 hour after puparium formation, no difference was observed in the patterning of the motor neurons that innervated the abdominal somatic muscles between the control and the mutant flies (Figure 14a, 14b; n = 18 control animals and 11 mutant animals examined). In both cases, the longitudinal nerves were
found to be arranged in two precise bundles that together formed the ventral nerve cord. The space between the two bundles consisted of transverse fibers from the anterior and posterior commissures and midline glial cells. These structures however, did not express any GFP and thus did not show any fluorescence. The segmental nerves were visible in both control and mutant animals, projecting from the ventral nerve cord towards the periphery in each abdominal hemisegment. Clusters of glia were seen at the ends of the segmental branches in each case.

About 6 hours after puparium formation, there were some distinct changes in the longitudinal nerves of the ventral nerve cord from which the segmental nerves project in the w; P[w+, Nrv2-GAL4-UAS-GFP]; ex17/DfCat animals (Figure 15b; n = 3 animals observed). The control flies did not exhibit any of these changes (Figure 15a; n = 12 animals examined). In the mutant animals, the distance between the longitudinal nerve bundles seemed to be much reduced and the integrity of the two separate bundles lost. All the nerves seemed to be frayed and wavy in the mutants when compared to those in the control flies. There was however, no observable difference between the segmental nerves in the control and the mutant flies. At this stage, movies made to compare the activity of the nerves in each case showed no difference. The nerves in the mutant animals showed a regular twitching movement in approximately the same rate as that in the control animals. The nerves showed very little movement in each case, moving only about less than a couple of degrees approximately on either side of where they were located.
The differences between the longitudinal nerves of the ventral nerve cord in the control and the mutant flies became more pronounced at 12 hours after puparium formation. The distance between the two nerve bundles of the ventral nerve cord completely disappeared at this stage in the w; P[w+; Nrv2-GAL4-UAS-GFP]; ex17/DfCat flies (Figure 16b; n = 5 animals examined). The segmental branches did not branch off at regular intervals as in the control flies but branched off all together where the two bundles of the longitudinal nerves seemed to separate. In the control animals, the longitudinal nerves were found in two distinct bundles and each segmental nerve projected away from them towards the periphery at each hemisegment (Figure 16a; 11 animals examined). A movie was made to record the movements of the nerves visible in each case. The movies showed that in the mutant, the rate of movement of the nerves was much slower than that of the control flies. This was confirmed by prolonged observation of the live pupa for over two hours from 12 hours after puparium formation to 14 hours after puparium formation.

The same pattern was observed in the mutant flies aged to 14 hours after puparium formation. As the animals aged, the distance between the nerve bundles increased in the control animals (Figure 17a; n = 7 animals examined). The nerve fibers seemed to become more frayed at these later hours after the formation of the pupal case. The nerve fibers in the mutants also became frayed and wavy (Figure 17b; n = 5 animals examined). However, the longitudinal nerves in the mutants did not separate into two bundles as in the control flies but merged to
form a single bundle. Segmental nerves that innervate muscles in the posterior abdominal segments appeared to be mispatterened as well. Instead of projecting individually towards each hemisegment, the segmental nerves formed two bundles near the posterior end. Thus some of the abdominal segments near the posterior end appeared not to be innervated by segmental nerves while the posterior-most abdominal segments were multiply innervated by them.
Figure 14a: GFP fluorescence in the abdominal region of the w; P[w+,Nrv2-GAL4-UAS-GFP]/+ animal (control fly) at 0 hour after puparium formation.

Figure 14a: GFP fluorescence in the abdominal region of a w; P[w+,Nrv2-GAL4-UAS-GFP]/+ animal (control fly) at 0 hour after puparium formation. The longitudinal nerves (ln) are in parallel to the length of the body and arranged in two distinct bundles. Each segmental nerve (sn) projects from the ventral nerve cord, where it synapses with neurons in the longitudinal nerves, towards individual abdominal hemisegments. Bright fluorescence is seen at the ends of the segmental nerves from peripheral glia. Anterior is left and dorsal is up. (Bar = 40µm)
Figure 14b: GFP fluorescence in the abdominal region of a w; P[w+ , Nrv2-GAL4-UAS-GFP]/; ex17/DfCat animal (experimental fly) at 0 hour after puparium formation.

The same pattern of arrangement of nerve fibers is observed in the mutants as in the control flies. The longitudinal nerves (In) are in parallel to the length of the body and arranged in two distinct bundles and each segmental nerve (sn) projects from the ventral nerve cord towards individual abdominal hemisegments. Anterior is left and dorsal is up. (Bar = 40µm)
Figure 15a: GFP fluorescence in the abdominal region of a $w; P^{w+}, Nrv2$-GAL4-UAS-GFP/+ animal (control fly) at 6 hours after puparium formation.

Figure 15a: GFP fluorescence in the abdominal region of a $w; P^{w+}, Nrv2$-GAL4-UAS-GFP/+ animal (control fly) at 6 hours after puparium formation. The longitudinal nerves (ln) are in parallel to the length of the body and arranged in two distinct bundles. Each segmental nerve (sn) projects from the ventral nerve cord, where it synapses with neurons in the longitudinal nerves, towards individual abdominal hemisegments. Bright fluorescence is seen at the ends of the segmental nerves from peripheral glia (pg). Anterior is left and dorsal is up. (Bar = 40µm)
Figure 15b: GFP fluorescence in the abdominal region of a *w; P[w+, Nrv2-GAL4-UAS-GFP]; ex17/DfCat* animal (experimental fly) at 6 hours after puparium formation.

Figure 15b: GFP fluorescence in the abdominal region of a *w; P[w+, Nrv2-GAL4-UAS-GFP]; ex17/DfCat* animal (experimental fly) at 6 hours after puparium formation. The integrity of the longitudinal nerves (In) seems to be lost and there is a smaller gap between the two bundles of longitudinal nerves. The segmental nerves (sn) appear to have no difference with ones in the control flies. Anterior is left and dorsal is up. (Bar = 40µm)
Figure 16a: GFP fluorescence in the abdominal region of a \( w; P_{w^+,Nrv2-}\text{-GAL4-UAS-GFP}\_\text{+/+} \) animal (control fly) at 12 hours after puparium formation.

Figure 16a: GFP fluorescence in the abdominal region of a \( w; P_{w^+,Nrv2-}\text{-GAL4-UAS-GFP}\_\text{+/+} \) animal (control fly) at 12 hours after puparium formation. The longitudinal nerves (ln) are in parallel to the length of the body and arranged in two distinct bundles. Each segmental nerve (sn) projects from the ventral nerve cord, where it synapses with neurons in the longitudinal nerves, towards individual abdominal hemisegments. Anterior is left and dorsal is up. (Bar = 40\( \mu \text{m} \))
Figure 16b: GFP fluorescence in the abdominal region of a w; P[w+, Nrv2-GAL4-UAS-GFP]; ex17/DfCat animal (experimental fly) at 12 hours after puparium formation.

Figure 16b: GFP fluorescence in the abdominal region of a w; P[w+, Nrv2-GAL4-UAS-GFP]; ex17/DfCat animal (experimental fly) at 12 hours after puparium formation. The two bundles of longitudinal nerves (ln) collapse on each other while the segmental nerves (sn) do not appear to innervate muscles in some of the posterior hemisegments but seem to multiply innervate muscles in the posterior-most abdominal hemisegments. Anterior is left and dorsal is up. (Bar = 40μm)
Figure 17a: GFP fluorescence in the abdominal region of a $w; P/w^+, Nrv2$-GAL4-UAS-GFP/+ animal (control fly) at 14 hours after puparium formation.

Figure 17a: GFP fluorescence in the abdominal region of a $w; P/w^+, Nrv2$-GAL4-UAS-GFP/+ animal (control fly) at 14 hours after puparium formation. The longitudinal nerves (ln) are in parallel to the length of the body and arranged in two distinct bundles. Each segmental nerve (sn) projects from the ventral nerve cord, where it synapses with neurons in the longitudinal nerves, towards individual abdominal hemisegments. Bright fluorescence is seen at the ends of the segmental nerves from peripheral glia. Anterior is left and dorsal is up. (Bar = 40µm)
Figure 17b: GFP fluorescence in the abdominal region of a w; P{w+, Nrv2-GAL4-UAS-GFP}; ex17/DfCat animal (experimental fly) at 14 hours after puparium formation. The two bundles of longitudinal nerves (ln) collapse on each other while the segmental nerves (sn) do not appear to innervate muscles in some of the posterior hemisegments but seem to multiply innervate muscles in the posterior-most abdominal hemisegments. Anterior is left and dorsal is up. (Bar = 40µm)

DISCUSSION
At 0 hour APF, no difference is found in the innervation patterns of the abdominal muscles between the control and the $\beta$FTZ-F1 mutant or experimental flies. This is as expected because the $\beta$FTZ-F1 gene is not expressed at this point. $\beta$FTZ-F1 gene expression reaches its peak at about 6 hours APF, which is when differences begin to appear. The abnormalities in the innervation patterns in the mutant animals become more apparent as time elapses and can be distinctly identified in the animals aged to 12 hours APF or beyond. The segmental nerves that innervate a subset of the abdominal muscles are found to be mispatterned in the $\beta$FTZ-F1 mutants. The longitudinal connectives or bundles of longitudinal nerves consisting of interneurons that synapse with neurons in the SN, TN and ISN collapse on each other in the mutants. The motor neurons in the SN and TN innervate the remaining abdominal muscles. Even though the motor neurons in the ISN and TN could not be visualized in the mutants, they might also be mispatterned since the interneurons they synapse with are mispatterned. The $\beta$FTZ-F1 gene thus plays a role in determining the innervation patterns of the abdominal muscles. The $\beta$FTZ-F1 gene may affect the neurons directly or have an impact on other genes or structures, which in turn are responsible for the mutant phenotypes observed.
EFFECT OF \( \beta FTZ-F1 \) MUTATION ON THE MIDLINE GLIAL CELLS
AND NEURONS IN THE VENTRAL NERVE CORD

The results indicate that a loss of function mutation of the \( \beta FTZ-F1 \) gene causes the longitudinal nerve bundles in the ventral nerve cord to collapse together in the region where midline glia and commissural neurons are found. The midline glial cells and the commissural neurons do not express any GFP fluorescence in the stocks of flies used and thus their fate cannot be conclusively determined in this study. The longitudinal nerves might have developed over the glial and commissural cells or these cells might be absent in the mutant animals. The motor neurons innervating the abdominal muscles have ipsilateral axons (affecting the same side of the body) and the only axons that cross the midline of the ventral nerve cord are the commissural axons (Schnorrer and Dickson, 2004). In the mutant animals in the later stages (post 12 hours APF), the ipsilateral axons are positioned next to each other. In control flies, the ipsilateral axons never cross the midline while the commissural axons cross it only once (Schnorrer and Dickson, 2004). The ability of axons to cross is dependent on their sensitivity to the midline repellent Slit, conferred by the Slit receptor Roundabout (Robo). All neurons express Robo in their growth cones but commissural neurons express it only after having crossed the midline. Before crossing, an intracellular sorting receptor called Commissureless (Comm), active only in the commissure neurons, collects the newly synthesized Robo protein at the Golgi and delivers it to lysosomes, where it is degraded (Schnorrer and Dickson, 2004). Thus in the
mutant flies, the expression of the Robo or Slit genes might have been affected by the mutation in the βFTZ-F1 gene. In flies with functional copies of the βFTZ-F1 gene, the midline glial cells express Slit, which binds to Robo in the commissural and ipsilateral axons of the neurons in the longitudinal nerve cord. These neurons synapse with the segmental neurons that innervate abdominal muscles. These muscles contract during prepupal-pupal transition to drive head eversion and leg and wing extension. In the βFTZ-F1 mutants, Robo and/or Slit may not be expressed in sufficient amounts or not expressed at all. This would cause neurons in the longitudinal and segmental nerves to cross the midline. Thus the separation between the two distinct bundles of the longitudinal nerves would disappear and the segmental nerves would not reach their appropriate target muscles in specific abdominal hemisegments. This mispatterning of innervation might lead to a decrease in force generated by contraction of the abdominal muscles. Hence, the defects observed in βFTZ-F1 mutants, such as defects in head eversion and wing and leg extension would arise.

The midline glial cells also seem to be affected by the βFTZ-F1 mutation. The glial cells usually function to support and guide axonal growth by acting as substrates for developing neurons (Bastiani and Goodman, 1986; Hidalgo et al., 1995; Booth et al., 2000). Studies carried out with flies carrying mutations in a gene called glial cells missing (gcm), which causes a defect in glial cell differentiation, showed that the structure of the nervous system is disrupted in these animals (Landgraf et al., 2003a). Thus the βFTZ-F1 gene may play a role in
the expression of gcm, which may cause defects in glial differentiation, which may ultimately be responsible for defects in innervation of the abdominal muscles and the resultant defects observed in the $\beta FTZ-F1$ mutants.

The glial cells play a number of very important roles in axon guidance (Hidalgo, 2001). If midline glial cells are ablated early during development, the commissural axons never cross the midline to connect the two segments of the ventral nerve cord. If the midline cells are ablated later on, the longitudinal axons collapse over the midline, revealing a midline-dependent repulsive function, as discussed earlier. Moreover, ablation of the longitudinal glia in the CNS affects the initial formation of the longitudinal fascicles (bundle of nerve fibers) and subsequent fasciculation and defasciculation events. Finally, ablation of the exit glia in Drosophila disrupts guidance of the motor axons as they exit the CNS and of sensory axons as they approach the CNS (Hidalgo, 2001). The $\beta FTZ-F1$ mutants show a collapse of the longitudinal nerves on the midline, which could thus be a consequence of the absence of midline glial cells. Thus the $\beta FTZ-F1$ gene may play an important role in the maintenance of the midline glial cells.

EFFECT OF $\beta FTZ-F1$ MUTATION ON MYOTOPIC MAP FORMED BY DENDRITIC ARBORS ON THE NEUROPILE
The dendrites of the motor neurons, rather than their cell bodies, are arranged in such a way that their positions in the neuropile correlate with the distribution of their respective target muscles (Landgraf et al., 2003a). Thus, motor neuron dendritic fields are organized as a myotopic map, which represents centrally the array of body wall muscles in the periphery. The dendritic arbors of the SN motor neurons that innervate muscles of similar anteroposterior positions, such as the ventral acute muscle and the segment border muscle lie in a common region of the neuropile. The internal muscles found in the dorsoventral axis are innervated by the ISN and are represented centrally by three dendritic domains that reflect their different locations in the periphery. Motor neurons that innervate the ventral internal muscles expand their dendritic arbors in the anterior half of the ISN dendritic domain while the motor neurons with dorsolateral internal muscle targets (lateral longitudinal 1, dorsal acute 3, and dorsal oblique 3–5) put their arbors into the posterior part of the ISN dendritic domain. Finally, the dendritic arbors of the neurons innervating the dorsal muscles lie between those representing ventral and dorsolateral internal muscle groups. The domains occupied by the dendrites of their innervating motor neurons are parasegmental even though the muscles are segmental in their organization (Landgraf et al., 2003a). The gcm mutants retain the characteristic positions of the dendritic arbors of each type of neuron in the neuropile even though other abnormalities occur in the nervous system. Thus the patterning of the neuropile into distinct motor neuron dendritic domains is intrinsic to the motor neurons. To draw conclusions
on whether individual motor neurons are affected by the \( \beta FTZ-F1 \) mutation, the
dendritic arbors may be examined carefully to see if there is any change in the
myotopic map in the \textit{ex17/DfCat} mutants.

**FLUORESCENCE PATTERNS IN NEURONS INNERVATING
MUSCLES INVOLVED IN METAMORPHOSIS**

The abdominal muscles are innervated by motor neurons whose axons
project through the SN, ISN and TN. However, in the animals examined, only the
SN shows any fluorescence. The SN innervates only the external muscles which
differ from internal muscles (innervated by ISN) in that they require \textit{wingless} (\textit{wg}) signalling for their specification (Baylies et al., 1995). They also express the
cell adhesion molecule (CAM) Connectin (Landgraf et al., 2003a). The neurons
that innervate these external muscles also express this molecule. The only external
muscle that is an exception to this rule is the ventral transverse 1 muscle (Nose et
al., 1992; Meadows et al., 1994; Prokop et al., 1996). In the mutant animals aged
12 hours APF, the SN does not branch off at each segment but the branches
project out together at the posterior of the animals this may be because of a
change in the expression of Connectin, brought about by the \( \beta FTZ-F1 \) mutation.

The abdominal muscles are not the only muscles whose contractions bring
about the changes observed during metamorphosis in the control animals. The
dorsal pharyngeal muscles also survive metamorphosis and actively contribute to
the morphogenetic events (Fortier et al., 2003). The neurons innervating the
pharyngeal muscles are located at the anterior portion of the animals. Due to intense fluorescence exhibited by the brain, these neurons could not be identified. The $\beta FTZ-F1$ mutation might have affected these neurons and their innervation patterns as well.

**EFFECT OF ECDYSONE ON THE MOTOR NEURONS**

The $\beta FTZ-F1$ gene mediates the effects of ecdysone. Experiments carried out on motor neurons innervating abdominal muscles showed that ecdysone reduced the amplitude of the excitatory junction potentials of the motor neurons and caused them to release fewer synaptic vesicles for each stimulation (Ruffner et al., 1999). The effects of ecdysone were shown to be presynaptic. The rapid effects of ecdysone are thought to contribute to the quiescent behavior associated with molts in *Drosophila*. During metamorphosis, rearrangement of muscles and nerves occur, and neurons become very sensitive to the neuromodulatory effects of ecdysone. Thus muscular contractions might be suppressed to avoid damaging the developing pupa (Ruffner et al., 1999). The effects of ecdysone in this case are completely non-genomic, that is they do not involve the activation or repression of genes. These responses are brought about by direct binding of the hormone to membrane bound receptors, which results in the activation of various cellular processes (Ruffner et al., 1999). The non-genomic processes include activation of the inositol triphosphate (IP$_3$), guanosine 3,5-cyclic monophosphate (cGMP) and tris (hydroxymethyl) aminomethane signaling pathways and increased release of
calcium inside cells (Thummel, 1996; Wehling, 1995). βFTZ-F1 mediates the responses of various genes to ecdysone by providing them with the competence to be induced by the hormone (Woodard et al., 1994; Broadus et al., 1999). Since the effects of ecdysone are non-genomic in this case, the βFTZ-F1 gene may not have any effect on the response of the motor neurons due to high titers of ecdysone. Thus it is unlikely that the abnormal innervation pattern of the βFTZ-F1 mutants is due to a differential response of the motor nerves to ecdysone.

CONCLUSIONS AND FURTHER EXPERIMENTS

The results obtained show that a loss of function mutation of the βFTZ-F1 gene affects the innervation pattern of abdominal muscles in Drosophila. The longitudinal nerves in the ventral nerve cord form synapses with the segmental nerves that innervate the external muscles in the abdominal body wall segments. A βFTZ-F1 mutation affects these longitudinal nerves and also the pattern of the segmental nerves.

The stocks of flies used to examine the difference in the pattern of innervation of the abdominal muscles express GFP only in a subset of the motor neurons. The innervation pattern of individual muscles at each hemisegment could not be observed in the flies expressing the Nrv2-GAL4-UAS-GFP marker. Thus using another stock of flies that express GFP in the neuromuscular synapses would be useful in looking at characteristic innervation patterns of each muscle. One such stock of flies is the MHC-CD8-GFP-Sh (line 1A - a homozygous second
chromosome insert), which is described in Zito et al. (1999). The CD8-GFP-Sh is a chimeric membrane protein, tagged with the S65T mutant form of the fluorescent label GFP, which localizes to the postsynaptic specialization through its PDZ interaction domain and is expressed in muscles only (Zito et al., 1999). The GFP fluorescence is visible through the translucent cuticle of the developing pupae and allows visualization of the neuromuscular synapses. The CD8-Sh or CD8-Shaker chimeric protein consists of the extracellular and transmembrane domains of the human T lymphocyte protein CD8 and the cytoplasmic C-terminal sequences of the Shaker potassium channel. It is targeted to the postsynaptic membrane of the NMJ or glutamatergic neuromuscular junction by association with the PDZ protein Discs-large (Dlg). The CD8-GFP-Sh protein is under the control of the MHC or myosin heavy chain promoter, which drives expression in all muscles (Zito et al., 1999).

Using a confocal microscope instead of a compound fluorescent microscope would allow examination of the nerves in much greater detail and discover any other possible impacts of the \( \beta FTZ-F1 \) mutation on the neurons innervating the abdominal muscles. The muscles could also be examined individually by antibody staining with anti-Fascicilin II antibodies. This antibody is specific to axons since the levels of Fas II, a synaptic cell adhesion molecule, is involved in controlling synaptic growth (Zito et al., 1999).

Antibody staining of the midline glial cells and the commissural neurons would yield more information about their fates in \( \beta FTZ-F1 \) mutants. Using
antibodies against Robo, Slit or Comm proteins would allow comparison of their expression levels in wild type and $\beta$FTZ-F1 mutant flies. This would help establish whether the $\beta$FTZ-F1 gene has an impact on the expression of Robo, Slit or Comm. These genes are involved in directing axons to their proper positions by preventing them from crossing the midline of the ventral nerve cord. Thus reduction of the level of expression of these genes in the $\beta$FTZ-F1 mutants would indicate that mutation of the $\beta$FTZ-F1 gene leads to mispatterning of motor neurons by affecting these genes. Staining with antibodies against the gcm protein would help establish if glial differentiation is affected by the mutation in the $\beta$FTZ-F1 gene.

Mutation of the $\beta$FTZ-F1 gene may affect the innervation pattern of abdominal muscles that drive metamorphosis in a number of ways. The contractions of the abdominal muscles generate hydrostatic pressure, which forces the head to evert and the appendages such as wings and legs to extend (Fortier et al., 2003). These muscles are innervated by motor neurons whose axons are found in the segmental, intersegmental and transverse nerves. The $\beta$FTZ-F1 mutants examined in this study showed that many of the segmental nerves do not reach their target muscles. In addition, the two bundles of longitudinal nerves, with which the motor neurons in the intersegmental and transverse nerves synapse, collapse on each other. The bundles of longitudinal axons are found on either side of the midline in the ventral nerve cord and neurons in the longitudinal nerves as well as the ISN and TN are ipsilateral in wild type flies. In the $\beta$FTZ-F1 mutants,
the neurons may not be ipsilateral and thus innervation of abdominal muscles by appropriate motor neurons may not happen. As a result, the contractions of the muscles do not generate enough force to drive the morphogenetic events and the defects observed in the mutants appear.

$\beta$FTZ-$F1$ may thus play a role in defining the structure and function of motor neurons directly or it may affect other genes, which in turn affect motor neurons. No direct role of the $\beta$FTZ-$F1$ gene in neuronal structure or function has been implied in any earlier study. Further studies need to be carried out to establish how $\beta$FTZ-$F1$ affects motor neurons and their innervation patterns. Techniques such as DNA microarrays may prove to be extremely useful for such studies to yield data about which genes are affected by a $\beta$FTZ-$F1$ mutation. The results obtained in this study support the hypothesis that the developmental and morphogenetic defects observed in the $\beta$FTZ-$F1$ mutants occur due to a defect in the pattern of innervation of the abdominal body wall muscles. Further research needs to be carried out before the underlying mechanism of how $\beta$FTZ-$F1$ controls the innervation of abdominal muscles can be understood. These analyses will help elucidate the genetic and molecular mechanisms underlying control of development in Drosophila.

REFERENCES


