Canada

THE CHARACTERIZATION OF THE ANTI-FTZ CUTICULAR
PHENOTYPE IN *DROSOPHILA MELANOGASTER*

by:

Beata J. Blachuta

Department of Zoology

Molecular Biology Program

l

Submitted in partial fulfillment

of the requirements for the degree of

Master of Science

Faculty of Graduate Studies

The University of Western Ontario

London, Ontario

December, 1999

ABSTRACT

Fushi tarazu (FTZ) activity is essential for proper *Drosophila* segmentation. Null *ftz* mutantss have a ftz cuticular phenotype; embryos lack even-numbered parasegments. When FTZ is ectopically expressed, the opposite, anti-ftz, cuticular phenotype is observed; embryos lack odd-numbered parasegments. It has been proposed that endogenous FTZ expressed in the even-numbered parasegmented primordia may activate a signalling pathway that represses ectopic FTZ activity in the odd-numbered parasegments (Argiropoulos, 1997). My aim was to identify factors that are components of this signaling pathway; however, my attempts were unsuccessful.

Previous observations suggested that the tyrosine residues in the terminal regions of FTZ are important in the generation of the anti-ftz phenotype (Percival-Smith, unpublished). However, ectopic expression of two FTZ derivatives with a 32 tyrosine tail, resulted in ftz and wg cuticular phenotypes. Furthermore, the frequency of these phenotypes was affected by the duration of heat shock. EN and *ftz* enhancer expression was also repressed when these FTZ derivatives when ectopically expressed. Therefore, I propose that $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ are behaving as dominant negative molecules that repress FTZ regulated genes.

Key Words: *fushi tarazu*, FLP-DFS technique, anti-ftz phenotype, pair-rule genes, segmentation

Now this is not the end. It is not even the beginning of the end. But it is perhaps, the end of the beginning.

Winston Churchill (1874-1965)

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

αFTZ-F1	αFTZ F1 protein
αFtz-F1	*αFtz-F1* gene
AEL	after egg laying
CxD	Dichaete phenotype
CyO	Curly of Oster phenotype
en	*engrailed* gene
EN	Engrailed protein
e^s	ebony phenotype
FLP	FLP recombinase
FLP-DFS	FLP mediated Dominant Female Sterile Technique
FRT	FLP recombinase target site
ftz	*fushi tarazu* gene
ftz	fushi tarazu phenotype
FTZ	Fushi tarazu protein
FTZ^{dn}	dominant negative FTZ protein
HD	Homeodomain
PCR	Polymerase Chain Reaction
PRD	Paired protein
Sb	Stubble phenotype
Sco	Scutoid phenotype
Sp	sternopleural phenotype
w	*white* gene
wg	*wingless* gene
wg	wingless phenotype
WG	Wingless protein
y	*yellow* gene
YT	tyrosine tail

INTRODUCTION

1.1 Developmental Genetics

After fertilization and nuclear fusion, the single celled zygote begins

the process of developing into a complex multicellular organism. All of the

information necessary for generating the many cell types, tissues, organs, and

organ systems is contained within the maternal and zygotic genomes.

Therefore, the process of development requires a highly, spatially and

temporally, regulated expression of genes.

Before the differentiation of specific structures occurs, a three-

dimensional body plan is determined. The process of segmentation in the

fruit fly, *Drosophila melanogaster*, embryo provides an excellent model for

studying the molecular controls necessary for the patterning of the body plan.

1.2 *Drosophila melanogaster* as a Developmental Model

Drosophila melanogaster has successfully been used as a model

organism for the study of development for many years. Since the eggs are

easily harvested, each stage of its life cycle, from fertilization to adult, has

been well characterized. Embryogenesis has been described in great detail,

both morphologically and at a cellular level (reviewed in Campos-Ortega and

Hartenstein, 1985). Extensive study has provided a broad knowledge base of

Drosophila genetics and the available molecular tools unique to *Drosophila*

make it an excellent model for the study of development, as well as other

biological processes.

When considering a model organism for the study of development, an important consideration is the accessibility of the embryos needed for investigation. *Drosophila* females are sexually active just 12 to 14 hours after eclosion, and a single mating can result in sufficient insemination for the female to lay fertile eggs for 6 to 8 days. A female can lay up to 100 eggs per day, and as many as 3000 in a lifetime (reviewed in Ashburner, 1989). These numbers, coupled with the fact that the eggs are laid externally, allow for the rapid collection of a large number of eggs.

The *Drosophila* life cycle has been thoroughly characterized (reviewed in: Campos-Ortega and Hartenstein, 1985; Bate and Martinez-Arias, 1993). After fertilization, during the syncytial blastoderm stage, the embryo undergoes 13 synchronous nuclear divisions. At approximately the tenth division, a group of nuclei migrate to the posterior pole of the embryo and cellularize, giving rise to the germline, or pole cells. Later, the remaining nuclei migrate to the periphery of the embryo and cellularize. It is during the cellular blastoderm stage that segmentation of the embryo begins. At the end of the cellular blastoderm stage (almost 3 hours after fertilization), the basic body plan of the embryo is determined; all three body axes are established, and the signals determining the segmentation pattern are active. The embryo then undergoes gastrulation, during which time the three germ layers are formed through the movement of the blastoderm cells to new positions in the embryo. Organs are formed during the stages of germband extension and retraction. Embryogenesis ends approximately 24 hours after fertilization with the hatching of a first instar larva. After two subsequent larval stages (second and third instar), the *Drosophila* enters a pupal stage during which

metamorphosis takes place, and an adult fly emerges approximately 5 days later.

Through extensive investigation, using classical genetic analysis, mutant alleles in thousands of *Drosophila* genes have been identified (FlyBase. 1999). With the aid of balancer chromosomes. mutant alleles in known genes. as well as gross chromosomal abnormalities, have been easily maintained in *Drosophila* stocks. Non-lethal mutations identified throughout the genome provide geneticists with a myriad of genetic markers. Also available to *Drosophila* researchers, is a detailed cytogenetic map developed using the polytene chromosomes found in the salivary glands of third instar larva.

One of the most interesting milestones in *Drosophila* research is the determination of the complete DNA sequence of the euchromatic regions of the *Drosophila* genome (Berkeley *Drosophila* Genome Project. unpublished). This knowledge will allow the study of the coordinate function of the complete genome in *Drosophila* biology. In addition to the sequence of the genomic DNA. the sequence of the transcribed regions and the sequence of the insertion sites of P-element induced lethal alleles are also being determined (FlyBase, 1999). These sequencing projects will result in genome-wide tools for the study of *Drosophila* development.

P-element mediated transformation of the germline is one of the most important molecular tools available to *Drosophila* geneticists, allowing for the stable reintroduction of DNA into the *Drosophila* germline (Rubin and Spradling, 1982). P-element mediated transformation permits the study of the

effect of ectopic expression, both spatially and temporally, of known genes and the study of recombinant genes *in vivo*.

1.3 Segmentation

The proper development of *Drosophila melanogaster* depends on the early segmentation of the embryo into metameric units called parasegments. Parasegments include cells that will give rise to the posterior part of one larval/adult segment, and the anterior part of the next posterior larval/adult segment (Martinez-Arias and Lawrence, 1985). The establishment of the segmental body plan in *Drosophila* requires the coordinate function of the protein products encoded by segmentation genes (Carroll and Scott, 1986). The action of various sets of segmentation gene products involved in the determination of the anterior-posterior axis of the embryo is organized in a hierarchy. This hierarchy begins with the action of the protein products encoded by the maternal coordinate genes *bicoid* and *nanos*, which are involved in the establishment of the basic anterior-posterior axis of the embryo (Driever and Nusslein-Volhard, 1989; Driever *et al.* 1989; Wang and Lehmann, 1991). The products of the maternal coordinate genes regulate the expression of a subset of segmentation genes known as the gap genes , such as *giant*, *hunchback*, *Krüppel*, *knirps*, and *tailless* (Eldon and Pirrotta, 1991; Lehmann, 1984; Wieschaus *et al.* 1984; Knipple *et al.* 1985; Heder *et al.* 1998; Nauber *et al.* 1988; Liaw and Lengyel, 1993). These genes are expressed in broad domains across the anterior-posterior axis of the embryo, dividing the embryo into sections which will give rise to more than one segment. Loss-of-function mutations of gap genes result in the loss of

contiguous groups of segments. The protein products encoded by gap genes regulate the expression of the pair-rule genes, such as *even-skipped, fushi tarazu, hairy, paired,* and *runt* (Goto *et al,* 1989; Harding *et al,* 1989; Hafen *et al.* 1984; Carroll and Scott, 1986; Howard *et al.* 1988; Kilchherr *et al.* 1986; Gergen and Butler, 1988). These genes are expressed in bands of cells, one segment wide, that divide the embryo into fourteen segments. Through this on/off expression pattern, pair-rule gene products are responsible for pattern formation in pairs of segments. Loss-of-function mutations in pair-rule genes result in the loss of sections of every other segment. Pair-rule gene products regulate the expression of segment polarity genes. which include *wingless* and *engrailed* (Baker. 1987; Kornberg *et al.* 1985; DiNardo *et al.* 1985). These genes are expressed in a portion of each segment, and assign polarity to the fourteen segments. Loss-of-function mutations in segment polarity genes affect polarity within each segment, leading to deletions, duplications and reversals of polarity. In addition to the establishment of the segmented body plan, segmentation genes are also required for the specification of the identity of each segment (reviewed by Akam, 1987).

1.4 Pair-Rule Genes

Nusslein-Volhard and Wieschaus (1980) first identified the pair-rule class of segmentation genes, which includes *even-skipped, fushi tarazu, odd-skipped, paired, odd-paired, sloppy paired, hairy* and *runt.* These genes and their protein products are expressed and required in single segment wide domains during morphogenesis. The domains of pair-rule gene requirement were deduced from each gene's mutant phenotype; loss-of-function mutations

of pair-rule genes result in defects of every other segment (Nusslein-Volhard and Wieschaus, 1980). Pair-rule genes have been extensively studied, and found to encode transcription factors (reviewed in Bate and Martinez-Arias, 1993). Initially, it was proposed that only the gap genes regulated the expression of these transcription factors (Nusslein-Volhard and Wieschaus, 1980). Similarly, the sole targets of the pair-rule gene products were thought to be segment polarity genes. However, further studies have shown that a "primary" subset of pair-rule genes (*even-skipped, hairy*, and *runt*) respond to gap gene regulation, and in turn regulate "secondary" pair-rules (*fushi tarazu, odd skipped, paired, odd paired*, and *sloppy paired*) (Pankratz and Jackle, 1993). Combinations of pair-rule gene products then regulate the expression of segment polarity genes, such as *engrailed*. (DiNardo and O'Farrell, 1987)

1.5 *fushi tarazu*: Structure and Function

The name of the pair-rule gene *fushi tarazu* (*ftz*), originates from a Japanese phrase which means segment deficient, and describes the gene's loss-of-function mutant phenotype (Wakimoto and Kaufman, 1981). Since its identification in 1980 (Nusslein-Volhard and Wieschaus 1980; Wakimoto and Kaufman, 1981), the pair-rule gene *fushi tarazu* has been cloned and its molecular structure has been characterized. The DNA sequence of *ftz* contains a 1239 bp open reading frame, interrupted by a 150 bp intron (Laughon and Scott, 1984). Translation of the FTZ open reading frame results in a 43 kDa protein consisting of 413 amino acids. The DNA sequence of *ftz* contains a homeobox, that, when translated, encodes a

homeodomain (HD). A homeodomain is a 60 amino acid DNA binding protein domain which contains a helix-turn-helix motif found in many DNA binding proteins (Laughon and Scott, 1984). Other domains of the FTZ protein include a protein degradation site, the PEST sequence, and an αFTZ-F1 binding site (Kellerman *et al.* 1990; Guichet *et al.* 1997). In addition, the FTZ protein is heavily phosphorylated suggesting that signal transduction pathways play a role in directing tissue-specific FTZ activities (Krause and Gehring, 1989; Dong *et al.* 1998). Furthermore, the phosphorylation of FTZ residue threonine 263 by protein kinase A, is necessary for normal segmentation (Dong *et al.* 1998). Phosphorylation of this threonine residue, in the N-terminal arm of the homeodomain of FTZ, is not necessary for proper FTZ function in the CNS (Dong and Krause, 1999).

Through deletion analysis Hiromi and Gehring (1985) identified three *cis*-acting regulatory elements of *ftz*. The first is the zebra element, which is sufficient to drive *ftz* expression in eight evenly spaced stripes. Second, the neurogenic element is responsible for normal *ftz* expression in the developing nervous system. Finally, the upstream element is required for FTZ auto-regulatory activity. The upstream element contains two independent enhancers: the distal enhancer directs expression of seven mesodermally restricted stripes, and the proximal *ftz* enhancer directs expression in stripes that span both the ectoderm and the mesoderm (Pick *et al.* 1990). The proximal enhancer is required for stripe establishment prior to the onset of *ftz* autoregulation. Direct interaction between the *ftz* enhancer and the FTZ protein is required for autoregulatory *ftz* enhancer activity (Schier and Gehring, 1992; Hyduk and Percival-Smith, 1996). However, it has been

demonstrated that a FTZ polypeptide with a deletion of the homeodomain is still able to activate the *ftz* enhancer, although it does not rescue the ftz cuticular phenotype in *ftz* null mutants (Hyduk and Percival-Smith, 1996; Furukubo-Tokunaga *et al.* 1992). Hyduk and Percival-Smith (1996) proposed a model whereby the FTZ protein has two activities that operate at different times during embryogenesis. Initially FTZ HD-dependent transcriptional activation upregulates *ftz* expression through an autoregulatory mechanism mediated through the *ftz* enhancer. However, by the end of the cellular blastoderm stage, and during gastrulation. FTZ activates FTZ-dependent Engrailed (EN) expression and establishes FTZ-dependent cuticle, through HD-independent FTZ activity. HD-independent FTZ activity suggests that other proteins are involved in regulating the activity of FTZ. Protein-protein interactions between FTZ and the pair-rule protein Paired (PRD) have been shown to have a synergistic effect on regulation of an *engrailed*-driven promoter (Ananthan *et al.* 1993). In addition, a direct interaction between FTZ and PRD is required for the repression of *wingless* (Copeland *et al.* 1996). A member of the steroid receptor superfamily, αFTZ-F1 interacts specifically and directly with FTZ, through the αFTZ-F1 binding domain of FTZ (Guichet *et al.* 1997). The deletion of the αFTZ-F1 binding domain inactivates the FTZ protein, and null *αFtz-F1* alleles produce a ftz-like phenotype (Guichet *et al.* 1997). Thus, αFTZ-F1 is a FTZ cofactor. A modified yeast two hybrid screen was used to identify a novel FTZ cofactor candidate, tentatively named Ftz Interacting Protein 2 (Yu *et al.* 1999). It is currently in the process of being characterized.

1.6 The role of *fushi tarazu* in *Drosophila* Development

Normal *ftz* gene expression requires the activities of the gap genes *Krüppel, knirps, hunchback, giant,* and *tailless,* and the pair-rule genes *hairy, runt,* and *even-skipped* (Carroll and Scott, 1986; Ingham and Martinez-Arias, 1986; Hiromi and Gehring, 1987). A recent modified yeast two hybrid screen performed by Yu and colleagues (1999) identified several other candidate *ftz* regulators. Two of these regulators are novel genes, yet to be characterized. The first of the known proteins, Sloppy paired, was shown to directly repress *ftz* transcription. The second, Tramtrack, was shown to have a potential to activate transcription via the proximal enhancer in yeast cells. This finding is opposite to previous evidence suggesting that Tramtrack is a repressor of *ftz* gene expression (Harrison and Travers 1990; Brown and Wu, 1993). These conflicting findings suggest that Tramtrack may be either activating the transcription of a *ftz* repressor, or it may have a dual function of repression and activation of transcription, based on its interaction with other proteins (Yu *et al.* 1999).

Once transcribed and translated, *FTZ* mRNA and FTZ protein first accumulate in a broad domain at the syncytial blastoderm stage (Hafen *et al.* 1984). During the cellular blastoderm stage, the broad domain of FTZ expression is resolved into seven stripes that correspond to the even-numbered parasegments. These even-numbered parasegments are missing in *ftz* embryos, demonstrating the importance of *ftz* expression in their development (Hafen *et al.* 1984; Hiromi *et al.* 1985). Furthermore, reciprocal shift experiments of the temperature-sensitive allele of *ftz* (*ftz^{J17ts}*) show that

the temperature-sensitive period of FTZ requirement corresponds to the cellular blastoderm and gastrulation stages.

The absence of FTZ activity in an embryo homozygous for an embryonic lethal null *ftz* allele results in the deletion of even-numbered parasegments. This deletion by reflected by the absence of alternating segments in the first instar larval cuticle (Wakimoto and Kaufman, 1981). The thoracic denticle belt T2, and the abdominal denticle belts A1, A3, A5, and A7 are missing in *ftz* mutant first instar larval cuticles; these larval segments are derivatives of the even-numbered parasegmental primordia. Furthermore, it has been observed, that when FTZ is ectopically expressed, the result is a reciprocal pair-rule phenotype (with denticle belts T1, T3, A2, A4, A6, and A8 missing), termed the anti-ftz phenotype (Struhl, 1985). These observations suggested that the on/off pattern of FTZ expression is important for segmentation.

1.7 *fushi tarazu* Regulates Engrailed Expression

FTZ target genes include *engrailed, wingless, Ultrabithorax, Tenascin major,* and *teashirt* (Duncan, 1986; DiNardo and O'Farrell, 1987; Ish-Horowich *et al.* 1989; Baumgartner *et al.* 1994; Core *et al.* 1997). The role FTZ plays in the regulation of the segment polarity gene *engrailed* is very well characterized. During the late cellular blastoderm stage, the EN protein is expressed in fourteen evenly spaced stripes along the anterior-posterior axis of the *Drosophila* embryo. These EN stripes mark the primordia of the anterior part of each parasegment. In the absence of *ftz* expression, the even-numbered EN expression stripes are lost, giving rise to a ftz EN expression

pattern (DiNardo and O'Farrell, 1987). Conversely, when FTZ is expressed throughout the embryo, or ectopically expressed, a 1-2 cell anterior widening of the FTZ-dependent EN stripes, the anti-ftz EN expression pattern, is observed (Ish-Horowicz *et al*. 1989). Since the even-numbered stripes of EN expression are FTZ dependent, the presence or absence of these bands is a widely used indicator of the presence or absence of *ftz* gene expression and/or FTZ protein activity (DiNardo and O'Farrell, 1987).

1.8 The Analysis of the Anti-ftz Phenotype: Rationale and Objectives

The interaction between FTZ ectopically expressed from a *Tubulin α1 promoter/ftz* fusion gene $(P\{w^-.ftz^{i-413}<Tub\ \alpha1\})$, and endogenous FTZ was previously studied by B. Argiropoulos and A. Percival-Smith (Argiropoulos, 1997). The analysis of the phenotypes caused by the expression of various fusion genes from the *Tubulin α1 promoter* resulted in a model for the interaction between endogenous FTZ activity expressed in even-numbered parasegments, and FTZ activity expressed ectopically in the odd-numbered parasegments (Figure 1). The *Tubulin α1 promoter* was used because it is a constitutive promoter, active in all cells; Tubulin α1 is a component of microtubules (Robert and Hyams, 1979).

It was proposed that endogenous FTZ activity, expressed in the even-numbered parasegments, activates the expression of a non-cell-autonomous signaling pathway that represses FTZ activity synthesized from the *Tubulin α1 promoter/ftz* fusion gene in the odd-numbered parasegments (Argiropoulos, 1997). This model for the generation of the anti-ftz phenotype is based on two observations. First, when two copies of the FTZ fusion gene are present in an otherwise wild-type fly, only a low frequency of anti-ftz

Figure 1. The model for the generation of the anti-ftz phenotype as proposed by Argiropoulos (1997). Endogenous FTZ (blue) is expressed in even-numbered parasegments only, whereas ectopic FTZ is expressed in every cell of the embryo. Endogenous FTZ, in even-numbered parasegments, is required for the expression of a secreted signalling factor that represses ectopic FTZ activity in odd-numbered parasegments.

EVEN-NUMBERED PARASEGMENT   ODD-NUMBERED PARASEGMENT
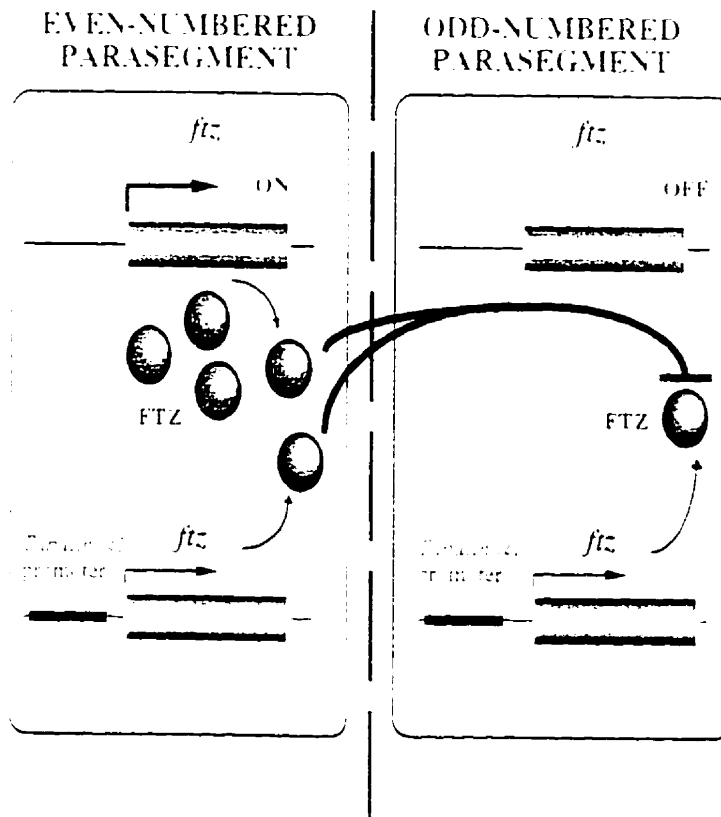
phenotypes was observed. However, when two copies of $P\{w^-, ftz^{1-413}<Tub$ $\alpha 1\}$ and only one copy of the endogenous ftz gene are present, the anti-ftz phenotype was fully penetrant. Hence, the reduction of endogenous FTZ activity resulted in an increase in ectopically expressed FTZ activity. Second, ectopic expression of $FTZ^{\Delta 151-209}$ from the *Tubulin $\alpha 1$ promoter* generates a strong anti-ftz phenotype, suggesting that the deleted region is responsible for negative regulation of ectopic FTZ activity by endogenous FTZ activity (Argiropoulos, 1997). My aim was to identify the component(s) of the cell-non-autonomous signalling pathway by which endogenous FTZ represses ectopic FTZ. I screened zygotic lethal maternal effect mutant alleles using the FLP mediated Dominant Female Sterile (FLP-DFS) technique for possible candidate genes whose products function downstream of endogenous FTZ in the pathway responsible for the repression of ectopically expressed FTZ activity.

Secondly, a systematic deletion analysis of the FTZ polypeptide has been performed by Percival-Smith and colleagues (unpublished). These deletions of FTZ were assayed for their ability to induce the anti-ftz phenotype. The analysis showed that the FTZ-F1 binding site was essential for the generation of the anti-ftz phenotype (Guichet et al. 1997). In addition, these experiments suggested that the terminal regions of the FTZ polypeptide played an important role in the generation of the anti-ftz phenotype. The ectopic expression of full length FTZ from a *heat shock promoter* results in the anti-ftz phenotype, as does the expression of $FTZ^{\Delta 1-50}$, $FTZ^{\Delta 51-100}$, and $FTZ^{\Delta 380-413}$ (Figure 2 A-D). However, when more than one of these sections is deleted, as in the case of $FTZ^{\Delta 1-100}$, $FTZ^{\Delta 1-50, 380-413}$, $FTZ^{\Delta 51-100, 380-413}$, $FTZ^{\Delta 316-413}$, $FTZ^{\Delta 1-100, 380-413}$, $FTZ^{\Delta 1-100, 151-413}$ (Figure 2 E-J), the anti-ftz

Figure 2. Diagrammatic representation of FTZ derivatives. All these derivatives have been ectopically expressed from a *heat shock promoter* fusion gene. Solid block indicates the position of the homeodomain, and superscript numbers indicate the amino acid number. The ability to induce the anti-ftz phenotype is shown in the right hand column.
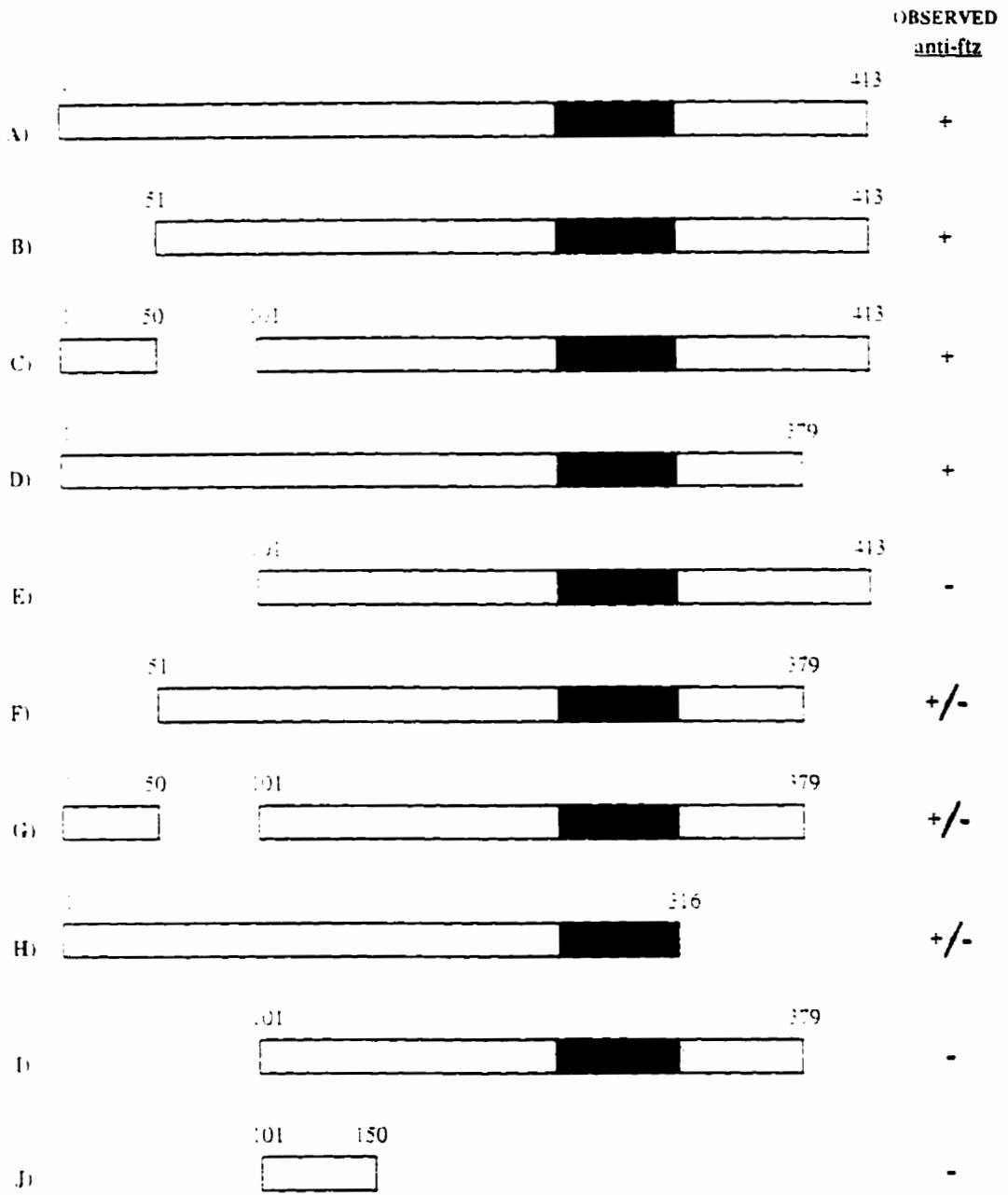
phenotype is not detected. Since these regions are tyrosine rich as compared to the rest of the FTZ protein (Figure 3), it has been proposed that the number of tyrosine residues at the N and/or C terminal regions of FTZ is important for the induction of the anti-ftz phenotype (A. Percival-Smith, personal communication). Tyrosine richness has also been conserved in the *Drosophila virulus* FTZ homologue, which has been shown to rescue FTZ activity in *Drosophila melanogaster*. Ectopic expression of the *Tribolium* FTZ homologue, which has a significantly lower tyrosine content than FTZ, does not result in the anti-ftz phenotype (Krause and Percival-Smith, unpublished result). This observation indicates the importance of tyrosines in the generation of the anti-ftz phenotype. By ectopically expressing FTZ constructs with a tyrosine residue tail (YT), my objective was to establish whether the tyrosines in the FTZ terminal regions are important for the generation of the anti-ftz phenotype.

Figure 3. Amino acid sequence of FTZ. The functionally redundant regions important for the induction of the anti-ftz phenotype: 1-50 (blue), 51-100 (green), 316-379 (cyan), and 380-413 (purple). Region 1-50 contains 10 tyrosine residues. Therefore 20% of the region is composed of tyrosine (Y) residues. Region 51-100 contains 6 tyrosine residues (12% Y). Region 316-379 contains 5 tyrosine residues (8% Y). Region 380-413 contains 6 tyrosine residues (18%). The remainder of the protein (black) contains only 10 tyrosine residues (less than 5%). Tyrosine residues are shown in red.

```
1      MATTNSQSHY SYADNMNMYN MYHPHSLPPT YYDNSGSNAY YQNTSNYHSY

51     QGYYPQESYS ESCYYYNNQE QVTTQTVPPV QPTTPPPKAT KRKAEDDAAS

101    IIAAVEERPS TLRALLTNPV KKLKYTPDYF YTTVEQVKKA PAVTTKVTAS

151    PAPSYDQEYV TVPTPSASED VDYLDVYSPQ SQTQKLKNGD FATPPPTTPT

201    SLPPLEGIST PPQSPGEKSS SAVSQEINHR IVTAPNGAGD FNWSHIEETL

251    ASDCKDSKRT RQTYTRYQTL ELEKEFHFNR YITRRRRIDI ANALSLSERQ

301    IKIWFQNRRM KSKKDRTLDS SPEHCGAGYT AMLPPLEATS TATTGAPSVP

351    VPMYHHHQTT AAYPAYSHSH SHGYGLLNDY PQQQTHQQYD AYPQQYQQQC

401    SYQQHPQDLY HLS
```

MATERIALS AND METHODS

2.1 Fly Strains and Maintenance

Table 1 lists the genotypes of all of the fly stocks used in this study, as well as their source. Flies were maintained at 25°C and 80% humidity, on standard cornmeal agar media supplemented with Baker's yeast (Sang, 1978).

2.2 Using the FLP-DFS Technique to Study the Epistatic Relationship Between Two Alleles

The development of the FLP-DFS technique has led to the identification of new loci whose gene products are required maternally for proper segmentation (Perrimon *et al.* 1996). The FLP-DFS technique was used to screen 16 of these loci found on autosomes, and two loci on the X chromosome for candidate genes involved in the repression of ectopic FTZ activity by endogenous FTZ, and/or the generation of the anti-ftz phenotype (Figure 4). Eggs derived from egg chambers homozygous for one of the 18 loci were fertilized with sperm from one of the following: wild type males, males carrying full-length *ftz* fused to the *Tubulin αl promoter* (BA103), or males carrying *ftz* with a deletion of amino acids 151 to 209 fused to the *Tubulin αl promoter* (BA108). If a gene product coded for by one of the 18 loci screened is required for the repression of ectopic FTZ activity, or generation of the anti-ftz phenotype, then the phenotype of its loss-of-function will be altered by ectopic expression of FTZ^{1-413} or $FTZ^{\Delta151-209}$.

Table 1. Names, genotypes and origins of fly stocks used in this study. The genotypes are written in the nomenclature described in FlyBase (1999).

Stock	Genotype	Origin
oreR	wild type	A. Percival-Smith
ry	ry^506	A. Percival-Smith
DH502	P{UbiIZ50H ry^+}; TM3, P{Ubb-lacZ}bbS w^+} l fiz^13 ry^506 e^s	Hyduk & Percival-Smith (1996)
β2-Tub FLP	P{β-Tubulin-FLP}	G. Struhl
BA103	y w; P{w^+, ftz^+} ftz^13 313, Tubal? P{w^+, ftz^+} ftz^13 313, Tubal?	B. Argiropoulos (1997)
BA108	y w; P{w^+, ftz^+} ftz^-M51 209, y^+, Tubal?	B. Argiropoulos (1997)
BJB101	ry^506; P{hsp ftz^+} ftz^13 313, ry^+ ry^+}	This Work
BJB102	ry^506; P{hsp ftz^+} ftz^-M51 209, y^+, ry^+}	This Work
X-linked Stock (1813)	((l))Dx, v f w ovo^D1} v^21 P{mini w^+}; FRT^101 v; P{ry^+}; FLP}^38 P{ry^+}; FLP}^38	Chou & Perrimon (1996)
Flipase on 2nd (1929)	y w, P{ry^+}; FLP}^2; C(y)(Sco	Chou & Perrimon (1996)
Flipase on 3rd (1970)	y w, P{ry^+}; FLP}^3; TM3, Sb C(y)	Chou & Perrimon (1996)
P{ovo^D1}^21 FRT^21 (2121)	P{mini w^+; ovo^D1}^21 FRT^21 P{hs neo; ry^+}; FRT^21 40A S;Sp Ms(2)M1	Chou & Perrimon (1996)
P{ovo^D1}^3R FRT^3R (2125)	P{mini w^+; ovo^D1}; FRT}^3R G13 P{mini w^+; ovo^D1}^3R FRT^3R 79D S;Sp Ms(2)M1	Chou & Perrimon (1996)
P{ovo^D1}^3L FRT^3L (2139)	w; P{mini w^+; ovo^D1}^3L FRT}^3L ^3A ru h st P{ub85J}D ss e^s TM3, Sb	Chou & Perrimon (1996)
P{ovo^D1}^3R FRT^3R (2149)	w; P{hs neo; ry^+}; FRT^3R 82B P{mini w^+; ovo^D1}^3R FRT^3R ru h st P{ub85J}D ss e^s TM3, Sb	Chou & Perrimon (1996)

Figure 4. Summary of the FLP-DFS technique. A genotype is produced that carries the lethal mutant on one chromosomal arm, and ovo^{D1} on the homologous arm. Also carried in the genotype is the FLP gene fused to a *heat shock promoter*. FLP recombinase binds to the FRT sites located near the centromeres, and mediates site specific recombination at the FRT sites. Recombination results in the production of two germ cell populations. The first cell population is homozygous for the dominant female sterile allele ovo^{D1}. The second population is homozygous for the lethal allele and does not have the ovo^{D1} allele. This population of cells forms eggs which are fertilized by sperm carrying *Tubulin α1 ftz* fusion genes.
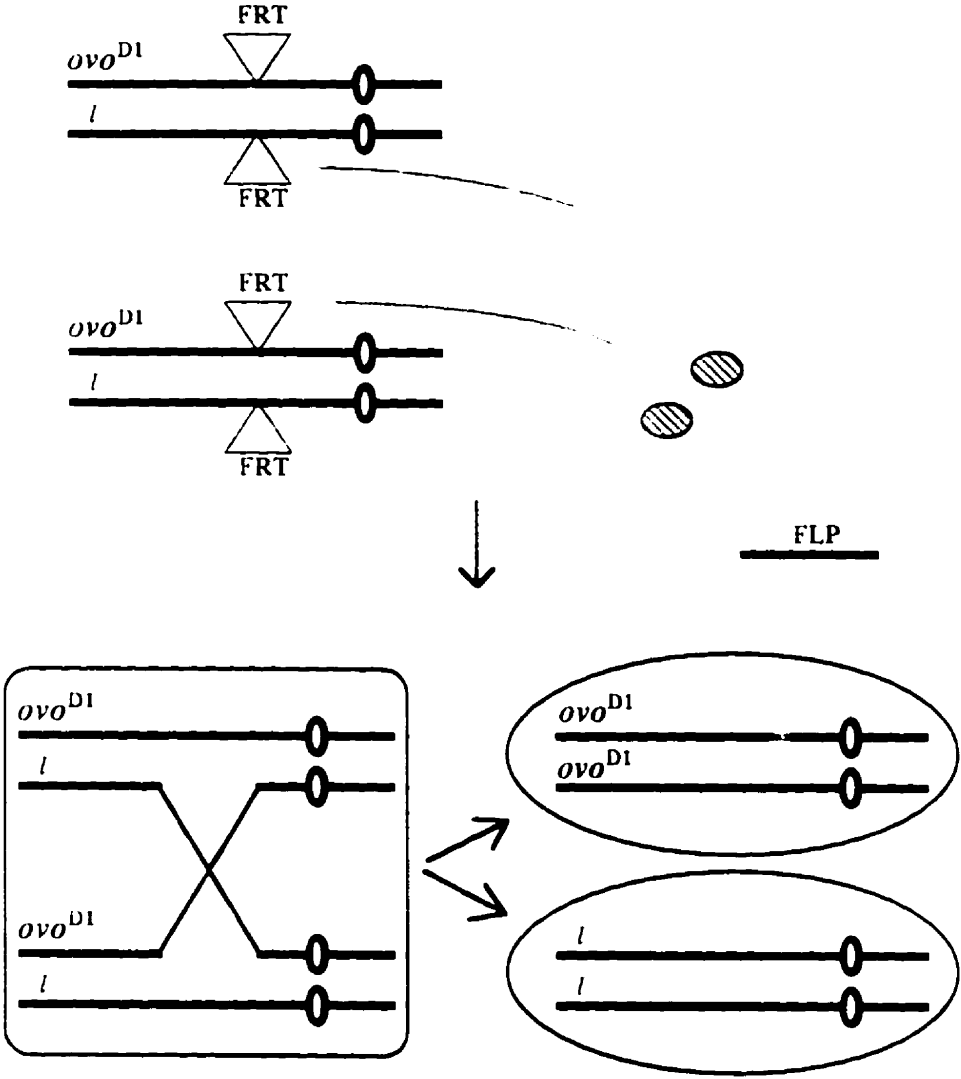
Since $P\{w^-.ftz^{1\to13}<Tub\ \alpha1\}$ can be maintained as a stable stock, males from BA103 were collected. However, ectopic expression of FTZ$^{\Delta151\text{-}209}$ is lethal. Hence, generation of sperm carrying $\{w^-.ftz^{\Delta151\text{-}209}<Tub\ \alpha1\}$ required the separation of FTZ$^{\Delta151\text{-}209}$ from the *Tubulin* $\alpha1$ *promoter* by the *yellow* gene flanked by two FLP recombinase target (FRT) sites. Crossing virgin females from the stock containing $P\{w^-.ftz^{\Delta151\text{-}209}<y^-<Tub\ \alpha1\}$ (BA108) to males containing $P\{\beta_2\ Tubulin\text{-}FLP\}$ results in the excision of the *yellow* gene and the expression of FTZ$^{\Delta151\text{-}209}$ by the *Tubulin* $\alpha1$ *promoter*. Since the β-*Tubulin promoter* is sperm specific, the expression of FLP recombinase and subsequent excision of the *yellow* gene will occur only during spermatogenesis in the males without the need of heat shock. When an egg is fertilized with the resulting sperm, every cell of the embryo will ectopically express the FTZ$^{1\to13}$ or the FTZ$^{\Delta151\text{-}209}$.

Stocks carrying the 18 maternal effect mutations linked to an FRT site were obtained from N. Perrimon (1996). Virgin females from stocks with the mutation on the X chromosome (*zeste white 3* and *hopscotch*) were crossed to males from stock 1813. The progeny of the cross were heat shocked at 36.5°C for 1 hr on day 3 and day 4 after egg laying (AEL), which corresponds to the third instar larval stage. Virgin females with Bar eyes were then collected and crossed to wild type ($y\ w$) males, or males that produced sperm carrying either $P\{w^-.ftz^{1\to13}<Tub\ \alpha1\}$ or $P\{w^-.ftz^{\Delta151\text{-}209}<Tub\ \alpha1\}$. First instar larval cuticles were prepared from these final crosses.

In the case of mutant alleles on the second chromosome, virgin females from the FRT stocks carrying the mutations (P944, P936, P1407, and P1469)

were crossed to males from the stock 1929. Virgin Scutoid (Sco) females with curly wings (CyO), having a mutation located on the left arm (2L) of the second chromosome (P944 and P936), were crossed to CyO, non-Sternopleural (Sp) males from the stock 2121. When the mutation was located on the right arm (2R) of the second chromosome (P1407 and P1469), the virgin Sco, CyO females were crossed to CyO, non-Sp males from the stock 2125. The progeny from these crosses were heat shocked as previously described. Virgin non-CyO females were collected and crossed to the same set of 3 males as for the X chromosome. First instar larval cuticles were prepared from these final crosses.

In the case of mutations on the third chromosome, virgin females from the FRT stocks carrying the mutations (P1633, P1572, P1598, P1639, P1590, P1605, P1594, P1539, P1711, P2144, P1681, and P1523) were crossed to males from the stock 1970. Virgin non-Dichaete (CxD) females with a mutation on the left arm (3L) of the third chromosome (P1633, P1572, P1598) were crossed to Stubble (Sb), non-ebony (e^5), males from the stock 2139. In stocks with the mutation located on the right arm (3R) of the third chromosome (P1639, P1590, P1605, P1594, P1539, P1711, P2144, P1681, and P1523), the virgin non-CxD females were crossed to Sb, non-e^5, males from the stock 2149. The progeny from these crosses were heat shocked as described above. Virgin non-Sb females were collected and crossed to the same set of 3 males as for the X chromosome. First instar larval cuticles were prepared from these final crosses.

2.3 Preparation of First Instar Larval Cuticle

After a 30-minute interval, embryos were collected and allowed to develop for 24-26 hours at 25°C and 80% humidity. The first instar larvae were dechorionated in a 3% sodium hypochlorite solution for 1 minute, and devitellinized by shaking in a 1:1 mixture of heptane and methanol. The larvae were then mounted in 1:1 Hoyer's mountant:lactic acid, and incubated at 60°C until the soft tissue was digested leaving the cuticle (24-48 hours) (Van der Meer. 1977). The resulting cuticles were visualized using darkfield optics.

2.4 Generating FTZ Polypeptide Constructs

Four *ftz* constructs were generated for ectopic expression experiments (Figure 5). Three constructs contain a 32 amino acid tyrosine tail (YT). The YT was constructed by multiple rounds of Linker Tailing of the tyrosine rich double stranded sequence

5'GTACCCGGGGTAGTAGTAGTAGTAGTAGTAGTAGTAGTA

(Vetrogen). In the first round the double stranded oligonucleotide, which contains phosphates only in phosphodiester linkages, was ligated into the *Smal* restriction enzyme site of pBC SK (Stratagene). The ligated plasmid DNA was purified from the unligated oligonucleotides using sepherose column chromatography (Spectra/Gel A6, Spectrum). The purified plasmid DNA was heated to 96°C in a heat block, then allowed to reanneal by turning off the heat block and cooling it slowly to room temperature. The annealed product was then transformed into *E. coli* cells (HB101, Gibco). A colony containing the plasmid and insert (in the correct orientation) was selected,

Figure 5. Diagrammatic representation of FTZ derivatives constructed for this study. The black box indicates the position of the homeodomain, and superscript numbers indicate the FTZ amino acid number. The gray box indicates the attachment of the tyrosine tail (YT) to the FTZ sequences. Ectopic expression of each construct was expected to result in anti-ftz cuticular phenotype . The sequence of the YT is as follows: TSGSPYY YYYYYYYYPYYYYYYYYYYYPYYYYYYYYYYPGYGYRPLV.

EXPECTED CUTICULAR
PHENOTYPE

FTZ $^{516+YT}$

anti-ftz

FTZ $^{101-470+YT}$

anti-ftz

FTZ $^{101-150+YT}$

YT

anti-ftz

FTZ $^{1-470+1-100}$

anti-ftz
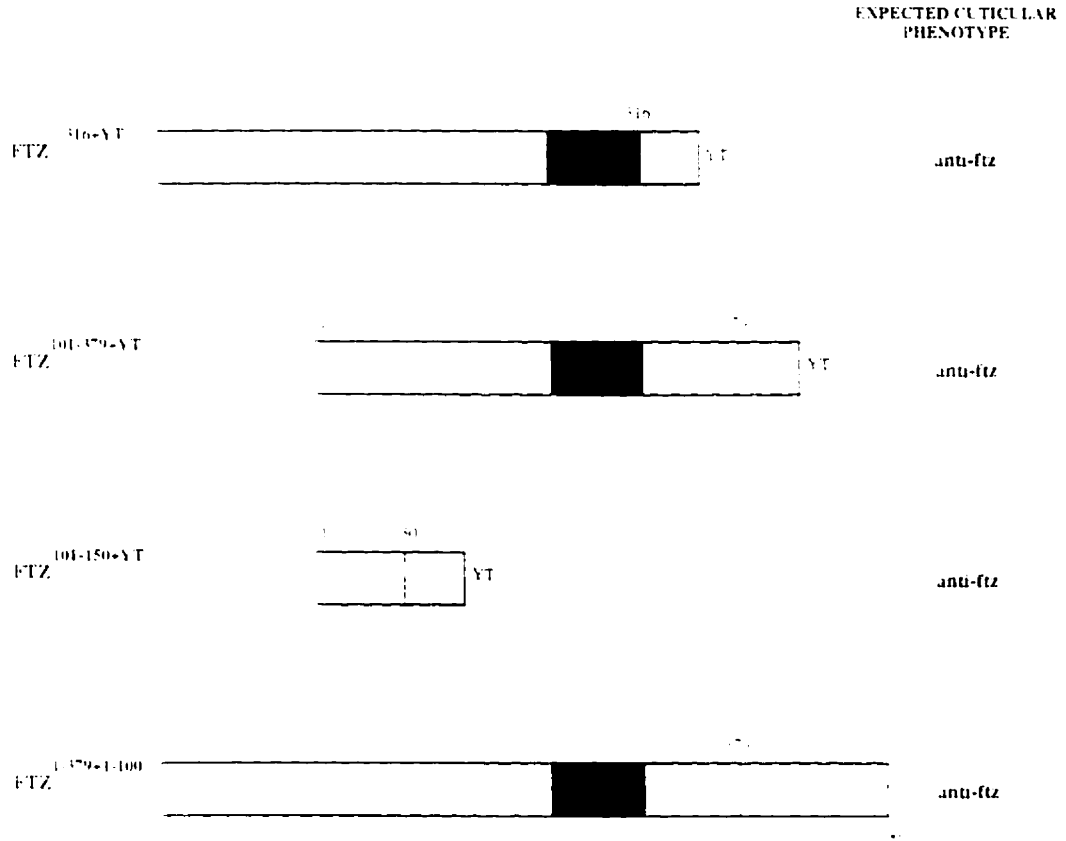
propagated, and the plasmid was purified (Maxi DNA prep, Qiagen). The

*Sma*I site of pBC SK was destroyed during the ligation of the first

oligonucleotide into the plasmid, however, an additional *Sma*I site in the

insert allowed for a second round of Linker Tailing in the same manner as

noted above. The second round resulted in a plasmid containing two copies

of the tyrosine rich oligonucleotide linked in the same orientation. This

procedure was then repeated to link a third tyrosine rich sequence to the first

two, leading to the generation of a DNA fragment containing 32 tyrosine

codons.

Four constructs (Figure 5) were made using polymerase chain reaction

(PCR)-mediated mutagenesis (Fitzpatrick *et al.* 1992). High fidelity *Pfu*

DNA polymerase (Stratagene) was used in all PCR reactions. Primer

sequences are listed in Table 2. Primers APS18 and BJBY3, APS35 and

BJBY5, or APS35 and BJBY7 were used in PCR reactions containing the *ftz*

open reading frame to amplify DNA fragments encoding the amino acids

FTZ^{1-316}, $FTZ^{101-379}$ or $FTZ^{101-150}$ (Figure 6). At the 5' end of each of these

DNA fragments, a *Not*I restriction enzyme site was introduced during the

PCR. Similarly, at the 3' end of each of these DNA fragments, a sequence of

9 nucleotides complementary to the YT was introduced during the PCR.

DNA fragments containing the polytyrosine stretch were amplified in PCR

reactions containing tyrosine construct DNA and primer BJBY1 plus primer

BJBY2, BJBY4, or BJBY6 (Figure 6). At the 5' end of the DNA fragment, a

9-nucleotide sequence complementary to the *ftz* DNA was included during

the PCR. At the 3' end of each of these YT DNA fragments, a *Not*I

restriction enzyme site was introduced during the PCR. The *ftz* DNA

Table 2. Sequences of PCR primers used in the generation of the *ftz* fusion genes encoding the FTZ derivatives made for this study.

Primer	Sequence (5'-3')
BJBY1	GGATCCAGTGGCGGCCGCTACCCGTACCCGGGTAG
BJBY2	AAGGATCGCACTAGTGGGATCCCCCTAC
BJBY3	TCCACTAGTGGGATCCTTCTTCGGACTTC
BJBY4	CTCAATGATACTAGTGGATCCCCCTAC
BJBY5	TCCACTAGTATCATTGAGGCAGGCCATAAC
BJBY6	ACCGGCAGGCACTAGTGGGATCCCCCTAC
BJBY7	TCCACTAGTGGCTGGGGTGACCTTGGTG
BJBY8	AAGGATCGCATGGCCACCACAAACAGG
BJBY9	GGTGGGCCATGGCGATCCTTCTTCGGACTTC
BJBY10	CGCAGATCTGCGGCCGCGAAGGCAGCATCATCTTCG
BJBY11	CGCAGATCTGCGGCCGCGG
APS18	GCTATGGGCCGCCGCAGGAGGAAGGCTAGCTATAAAC
APS35	GCTATGGGCCGCCGAAACATGGATCATCGGCCGGTGGGAGG

Figure 6. Diagrammatic representation of the PCR strategy used to construct

the fusion genes that encode the FTZ derivatives used in this study. Primer

names/numbers are circled, and primer direction and target sequence is

shown with arrows. The *Not*I sites used to clone the constructs into the P-

element carrying vector pNMT4 are also shown. The black box indicates the

position of the homeobox, and superscript numbers indicate the amino acid

number. The gray box indicates the attachment of the tyrosine tail (YT) to

the *ftz* sequence.

FTZ$^{1-316+YT}$

FTZ$^{101-379+YT}$

FTZ$^{101-150+YT}$

FTZ$^{1-379+1-100}$
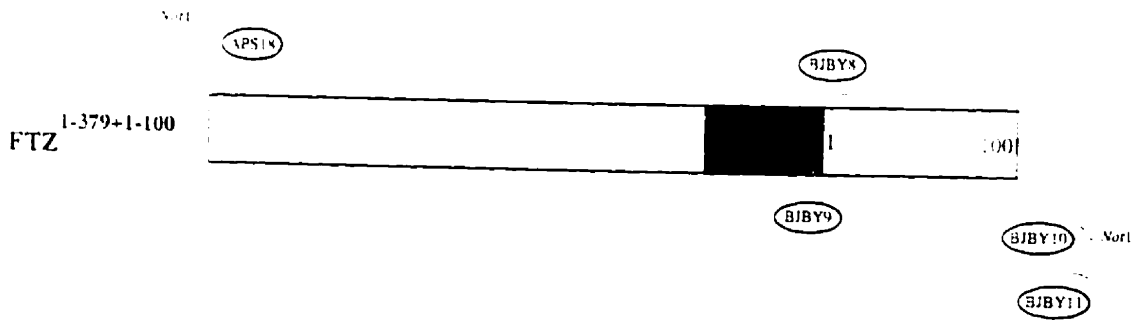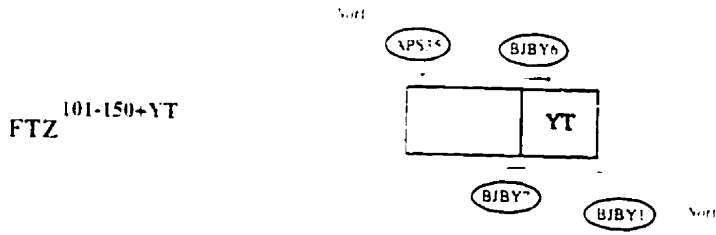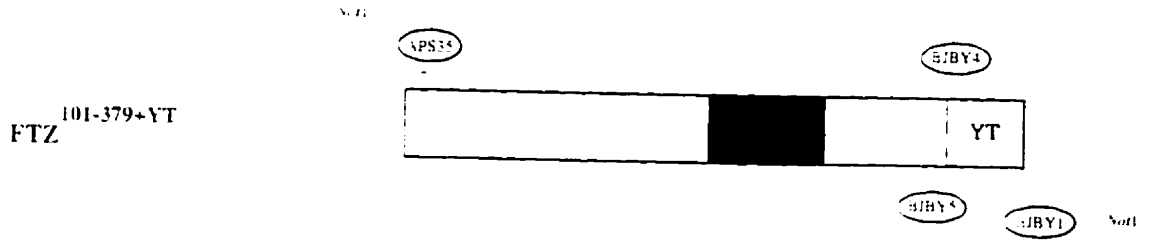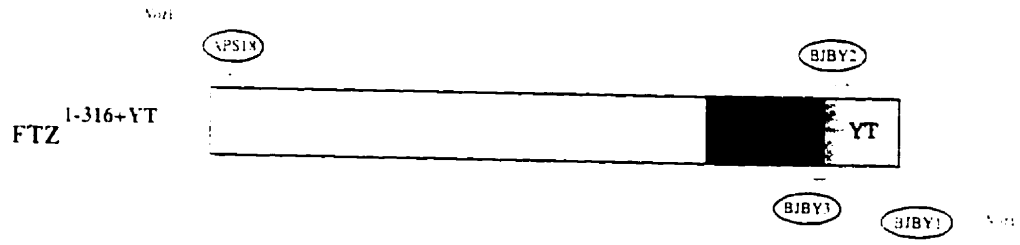
fragments were joined to the YT in PCR reactions containing the two

fragments to be joined, the primer used to amplify the 5' of the FTZ fragment,

and the primer used to amplify the 3' of the YT fragment in previous reactions

(Figure 6). These constructs were then digested with NotI, gel isolated, and

cloned behind the heat-shock promoter of the expression vector pNMT4

(Schneuwly et al. 1987).

A fourth construct was made to test the functional redundancy of the

terminal regions of the FTZ polypeptide through the substitution of amino

acids 316-416 by the amino acids 1-100 (Figure 5). A PCR reaction

containing primers APS18 and BJBY9, and the ftz open reading frame, was

used to amplify the DNA encoding the amino acids FTZ^{1-316} (Figure 6). At

the 5' end of this DNA fragment a NotI restriction enzyme site was

introduced during the PCR. Similarly, at the 3' end of this DNA fragment,

the 9 nucleotides that encode the first 3 amino acids of FTZ were introduced

during the PCR. A PCR reaction containing primers BJBY8 and BJBY10

and the ftz open reading frame was used to amplify the DNA encoding the

amino acids FTZ^{1-100} (Figure 6). At the 5' end of this DNA fragment, a 9-

nucleotide sequence encoding the last 3 amino acids of the FTZ

homeodomain was introduced during PCR. Similarly, at the 3' end of this

DNA fragment, a NotI restriction enzyme site was introduced during the PCR

(Figure 6). The two FTZ DNA fragments were then joined together in a PCR

reaction containing the two fragments to be joined, and the primers BJBY11

and APS18. This fourth construct was also subsequently digested with NotI,

gel isolated, and cloned behind the heat-shock promoter of the expression

vector pNMT4 (Schneuwly et al. 1987).

Two of the fusion genes, FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ (Figure 5) were then successfully transformed into the genome of ry^{506} flies by P-element mediated transformation (Rubin and Spradling, 1982). Successful transformation was verified by PCR amplification of the constructs from genomic DNA (Figure 7).

2.5 Embryo Collection and Heat Shock

Embryos were collected at 30-minute intervals on apple juice plates, and incubated at 25°C and 80% humidity (Wieschaus and Nusslein-Volhard, 1986). At 2:55 hours AEL, the embryos were collected from the plates onto a mesh screen. The embryos were heat shocked at 37°C for 15 minutes by placing the screen into a 2 ml microcentrifuge tube and submerging it into a circulating waterbath. After heat shock, the embryos were incubated at 25°C and 80% humidity until they were fixed, or until cuticles were prepared. For experiments assaying the dosage effect, embryos were treated as above, but heat shocked for 5, 7.5, 10, 12.5, and 15 minute intervals.

2.6 *ftz* Enhancer Activation

To test the effect of ectopic expression of the FTZ constructs on the pattern *ftz* enhancer activation, males from the stocks BJB101, BJB102, BA103 or OreR were crossed with virgin females from the stock DH502 (Hyduk and Percival-Smith, 1996) (Table 1). A P-element inserted on the first chromosome of the DH502 stock contains a FTZ-dependent enhancer element fused to a bacterial *lacZ* gene. Activation of the enhancer results in the expression of β-galactosidase. Embryos collected from this cross were

Figure 7. PCR detection of *heat shock promoter/ftz* fusion gene constructs inserted into the *Drosophila* genome. Lane 1 contains $ftz^{1-316+YT}$ DNA (expected length is 1072bp) amplified from genomic DNA using primers 18 and BJBY1. Lane 2 contains $ftz^{101-379+YT}$ DNA (expected length is 990bp) amplified from genomic DNA using primers 35 and BJBY1. The molecular size standard is the 100bp ladder (Pharmacia). The bright band in the standard is 800bp in length.
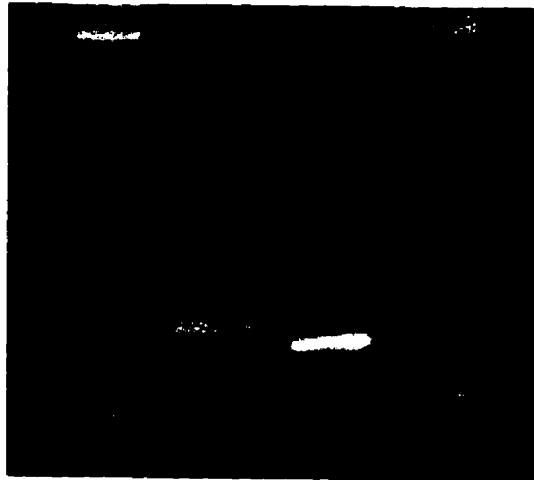
heat shocked as previously described, and the chromogenic substrate X-gal (5-bromo-4-chloro-3-indolyl-D-galactosidase) was used to detect β-galactosidase activity in the embryos (Bellen et al., 1989). At the germband extension stage (4.5 hours AEL), the embryos were dechorionated in a 3% sodium hypochlorite solution for 1 minute and fixed in a scintillation vial containing 5 ml heptane, 4.5 ml PBS (35 mM sodium phosphate, 120 mM sodium chloride, pH 7.4), and 0.5 ml 37% formaldehyde for 20 minutes. Subsequently, the embryos were stained in 0.5 ml of staining buffer containing 12.5 μl of 8% X-gal in dimethyl sulfoxide. After staining, the embryos were devitellinized in a 1:1 mixture of heptane and methanol, mounted on slides using 80% glycerol in 20 mM Tris (pH 8.0), and visualized under brightfield illumination.

2.7 Immunolocalization of Engrailed

To detect and localize the expression of Engrailed protein, embryos were dechorionated and fixed at 4.5 hours AEL as described above, and immunostained with a 3-fold dilution of a mouse anti-Engrailed monoclonal antibody (Mab 4D9: Patel et al. 1989). The embryos were then treated with a secondary antibody (rabbit anti-mouse antibody conjugated to biotin; Vector Laboratories) at a 1:200 dilution. The resulting antibody complex was detected with a 1:100 dilution of avidin bound to biotinylated horseradish peroxidase (ABC reaction), which was developed with 0.1 mg/ml diaminobenzidine tetrahydrochloride (DAB) in 50 mM tris, pH 7.2, 0.4% nickel chloride and 0.01% hydrogen peroxide. Kellerman and colleagues (1990) describe the protocol indicated above in detail.

RESULTS

3.1 The Gene(s) Involved in the Pathway Responsible for Repression of Ectopic FTZ Activity were not Identified Using the FLP-DFS Technique

In yeast, FLP recombinase catalyzes site specific recombination between two 600 bp FLP recombination target (FRT) sites (Broach and Hicks, 1980). This site specific recombination system is used with the dominant female-sterile (FLP-DFS) technique to generate germ-line mosaics in *Drosophila* (Chou and Perrimon, 1992; Chou and Perrimon, 1996). The FLP-DFS technique is summarized in Figure 4. The FLP-DFS technique allows the identification and study of the maternal effect of recessive zygotic lethal mutations on the autosomes and X chromosomes. In the FLP-DFS technique, flies are heterozygous for the zygotic lethal mutation, which allows them to live to adulthood. On the other homologous chromosome, female adult flies carry the ovo^{D1} mutation, which results in dominant female sterility due to an inability to produce eggs. Both homologous chromosomes carry FRT sites closely linked to the centromere. Induction of FLP recombinase expression from the *hsp*-FLP fusion gene results in site specific recombination at the FRT sites, causing exchange of the chromosome arm. In the germ-line, this exchange produces cells homozygous for the lethal mutation and ovo^-. These homozygous germ cells form egg chambers whose germ line derivatives, the oocyte and nurse cells, are homozygous for the lethal mutation. If the lethal mutation has a maternal effect phenotype, it will be observed after the egg is fertilized and laid.

B. Argiropoulos (1997) proposed a model in which endogenous FTZ expressed in the even-numbered parasegments represses FTZ activity ectopically expressed in the odd-numbered parasegments from the *Tubulin* $\alpha 1$ *promoter-ftz* fusion gene. This model suggests that FTZ$^{\Delta 151\text{-}209}$ gives a strong anti-ftz phenotype, not observed with FTZ$^{1\text{-}413}$, because a regulatory region, which mediates the negative regulation by endogenous FTZ activity, is missing in FTZ$^{\Delta 151\text{-}209}$. Hence, a strong anti-ftz phenotype should result from the ectopic expression of FTZ$^{1\text{-}413}$ if a mutation removes a component of the cell non-autonomous signaling pathway that mediates the repression of ectopically expressed FTZ by endogenous FTZ expressed in the even-numbered parasegments. The FLP-DFS technique was used to test whether any of the genes required for normal segmentation patterning, identified by Perrimon and colleagues (1996), are involved in the pathway which mediates endogenous FTZ repression of ectopic FTZ activity. Eggs derived from mutant egg chambers were fertilized with wild-type (*y w*) sperm, or sperm containing either *P{w⁻.ftz$^{1\text{-}413}$<Tub α1}* or *P{w⁻.ftz$^{\Delta 151\text{-}209}$<Tub α1}*. Fertilization with *P{w⁻.ftz$^{1\text{-}413}$<Tub α1}* containing sperm should provide a positive result, represented by the modification of the segmental phenotype of the mutant, to an anti-ftz phenotype, thereby identifying a maternal effect mutation of a gene involved in the proposed pathway. Also of interest is the possible modification, by maternal effect mutations, of the anti-ftz phenotype produced when the eggs are fertilized with *P{w⁻.ftz$^{\Delta 151\text{-}209}$<Tub α1}* containing sperm.

The results of the screen are shown in figure 8. As expected, the cuticular phenotype of the maternal effect mutation was observed when the

Figure 8. Cuticular phenotypes of embryos homozygous for a maternal effect lethal mutation, and ectopically expressing one of three FTZ proteins. These embryos were generated with the use of the FLP mediated Dominant Female Sterile Technique (Chou and Perrimon, 1992; Chou and Perrimon, 1996). Each row represents one lethal mutation carried by the egg. This maternal effect lethal mutation is shown to the left of each row. The maternal effect mutant eggs were fertilized by sperm carrying *Tubulin* α1:*ftz* fusion genes. The *Tubulin* α1:*ftz* fusion genes that the eggs were crossed to are shown at the top of each column.

	wt	$P\{w^{+}.ftz^{1-413}<Tub\alpha1\}$	$P\{w^{+}.ftz^{\Delta151-209}<Tub\alpha1\}$
zw3			
hop			
l(2)00632 P944			
l(2)00255 P936			
l(2)05428 P1407			
l(2)06214 P1469			
l(3)04556 P1633			
l(3)02619 P1572			
Ftz-F1 P1598			

	wt	P{$w^+.ftz^{1-413}$<$Tub\alpha1$}	P{$w^+.ftz^{\Delta151-209}$<$Tub\alpha1$}
l(3)04837 P1639			
l(3)03463 P1590			
l(3)03719 P1605			
l(3)03550 P1594			
l(3)01618 P1539			
l(3)07013 P1711			
l(3)15A6 P2144			
mrl P1681			
l(3)01164 P1523			
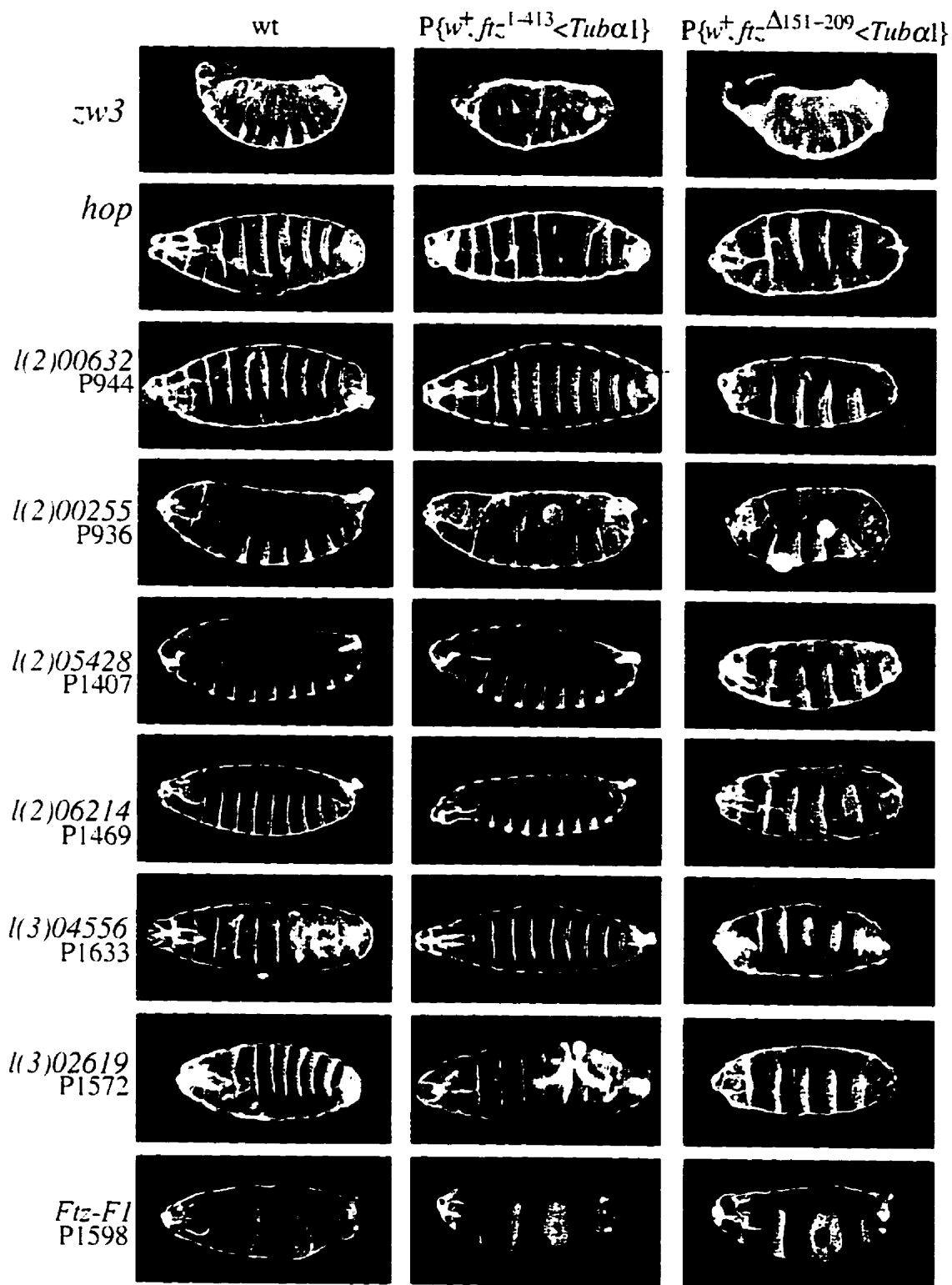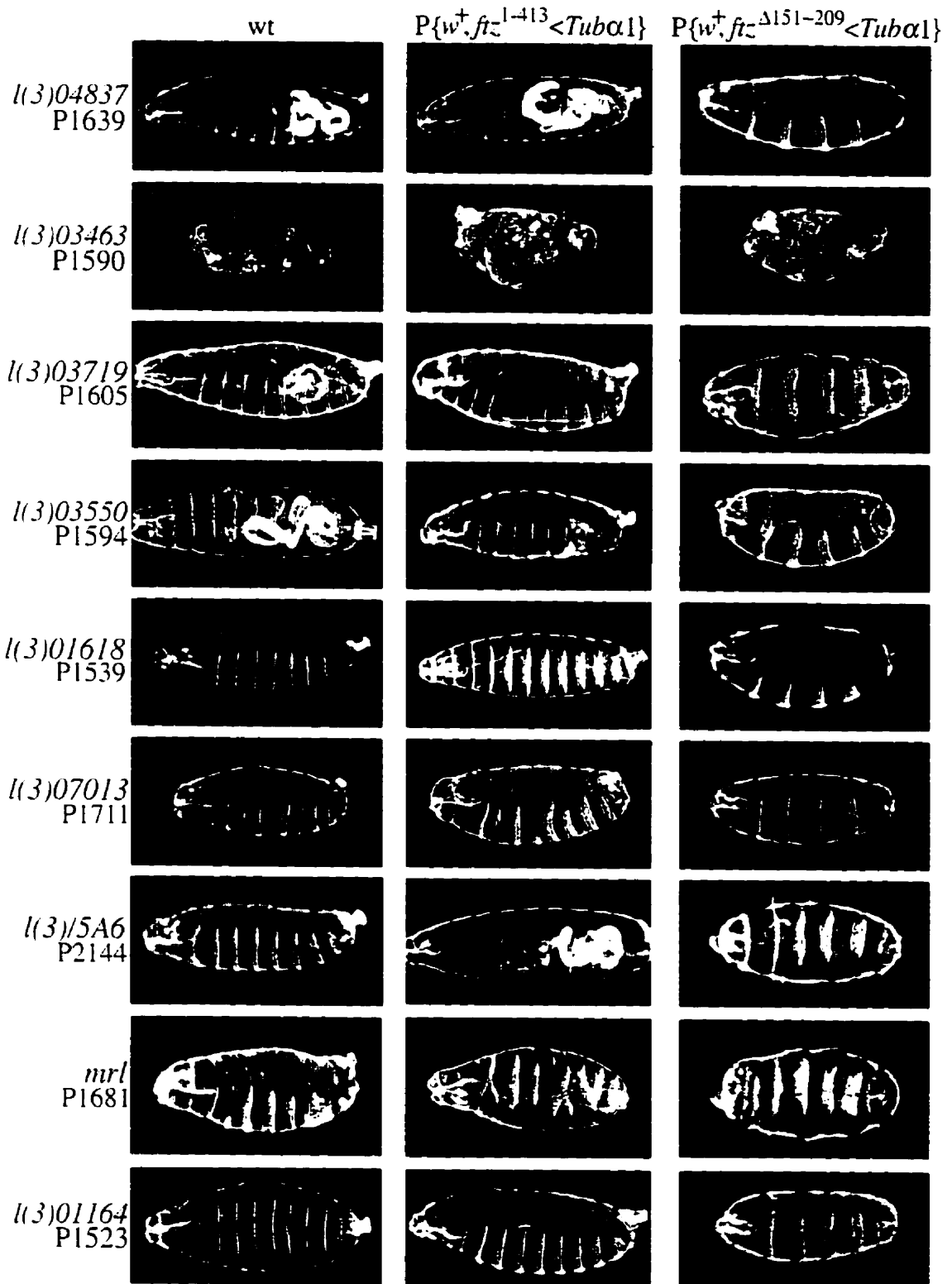
eggs were fertilized with wild type sperm (Perrimon *et al.* 1996). All 18 mutants showed no change in phenotype when the eggs were fertilized with sperm carrying a P-element containing *Tubulin α1 promoter* driven ftz^{1-413} ($P\{w^-, ftz^{1-413}<Tub\ α1\}$). The anti-ftz phenotype which is observed when $ftz^{\Delta151-209}$ is expressed in a wild type background, and also observed in first instar larval cuticles derived from egg chambers homozygous for 17 of the 18 lethal mutations. The only exception was a null mutant allele in the *αFtz-F1* gene, which resulted in a ftz phenotype, even when $ftz^{\Delta151-209}$ was ectopically expressed. Guichet and colleagues (1997) observed similar results in their previous analysis of αFtz-F1. However, ectopic expression of $FTZ^{\Delta151-209}$ in a $Ftz-F1^{209}$ background, caused rescue of the Ftz-F1 phenotype in a previous study (Argiropoulos, 1997). These observations suggest that unlike the *αFtz-F1* allele used in this study the $αFTZ-f1^{209}$ alleles are hypomorphic. Consequently, the 18 loci required for proper segmentation, isolated by Perrimon and colleagues (1996), are not involved in the pathway responsible for the repression of ectopic FTZ activity.

3.2 Ectopic Expression of $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ Did Not Result in the Anti-ftz Phenotype

It has been proposed that the number of tyrosine residues at the N and/or C terminal regions of FTZ is important for the induction of the anti-ftz phenotype (A. Percival-Smith, personal communication). The ectopic expression of full length FTZ results in the anti-ftz phenotype, as does the expression of $FTZ^{\Delta1-50}$, $FTZ^{\Delta51-100}$, and $FTZ^{\Delta380-413}$. However, when more than one of these sections is deleted, as in the case of $FTZ^{\Delta1-100}$, $FTZ^{\Delta1-50, 380-}$

$^{-13}$, FTZ$^{\Delta 51-100, 380-413}$, FTZ$^{\Delta 316-413}$, FTZ$^{\Delta 1-100, 380-413}$, and FTZ$^{\Delta 1-100, 151-413}$, the anti-ftz phenotype is not detected (Figure 2). Also, ectopic expression of the *Tribolium* FTZ homologue, which has a significantly lower tyrosine content, does not result in the anti-ftz phenotype (H.M. Krause & A. Percival-Smith, unpublished result). In order to address these observations, *ftz*$^{1-316+1-100}$ was constructed to test the redundancy of the terminal regions of FTZ. In order to test whether the generation of the anti-ftz phenotype is dependent on the tyrosines in the N and C terminal regions of FTZ, the following *ftz* constructs were made with the addition of a tail of 32 tyrosine residues (YT): *ftz*$^{1-316+YT}$, and *ftz*$^{101-379+YT}$, *ftz*$^{101-150+YT}$. The latter construct has the ability to test whether the αFTZ-F1 binding site and the tyrosine tail are sufficient for generating the anti-ftz phenotype. The *ftz* constructs were fused behind a *heat shock promoter* and two of them $P\{w^-, ftz^{1-413+YT} <Tub\ \alpha 1\}$ and $P\{w^-, ftz^{101-379+YT} <Tub\ \alpha 1\}$, were integrated into the *Drosophila* genome. If the tyrosine residues are required for the generation of the anti-ftz phenotype, the addition of the tyrosine tail (YT) to the FTZ constructs should result in a strong anti-ftz phenotype (Figure 5).

When FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ were ectopically expressed, the anti-ftz cuticular phenotype was not observed (Figure 5). Three independent transformant lines of FTZ$^{1-316+YT}$ and two independent transformant lines of FTZ$^{101-379+YT}$ were tested. The ectopic expression of FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ resulted in ftz and wingless (wg) cuticular phenotypes (Figure 9 E, F, H, and I). The ftz cuticular phenotype is characterized by the deletion of the denticle belts T2, A1, A3, A5, and A7 (Figure 9 E and H), while the wg cuticular phenotype is characterized by a complete lack of segmentation. The

Figure 9. Ectopic expression of FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ from the *heat shock promoter* result in ftz and wingless phenotypes. **A,** Wild-type first instar larval cuticles have 3 thoracic and 8 abdominal denticle belts. **B,** 14 stripes of EN expression were observed in wild-type germband extended embryos. **C** and **D,** Embryos ectopically expressing FTZ show the anti-ftz phenotype. Cuticles have only T2, A1, A3, A5, and A7 denticle belts. The even-numbered EN stripes are widened. **E** and **F,** Ectopic expression of FTZ$^{1-316+YT}$ results in a ftz cuticular phenotype (the cuticle has only T1, T3, A2, A4, A6, and A8 denticle belts), or a wingless cuticular phenotype (one broad patch of denticles, and no naked cuticle), respectively. **G,** Ectopic expression of FTZ$^{1-316+YT}$ results in a loss of even-numbered EN stripes. **H** and **I,** Ectopic expression of FTZ$^{101-379+YT}$ results in a ftz cuticular phenotype, or a wingless cuticular phenotype, respectively. **J,** , Ectopic expression of FTZ$^{101-379+YT}$ results in incomplete even-numbered EN stripes.
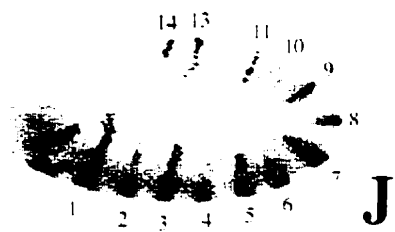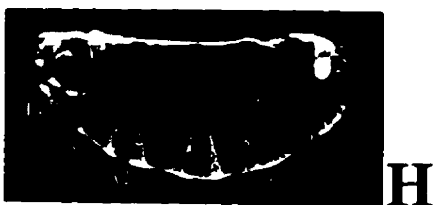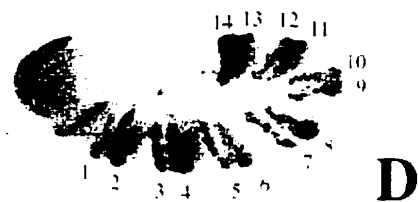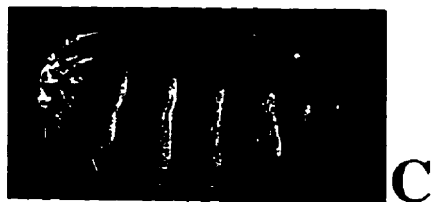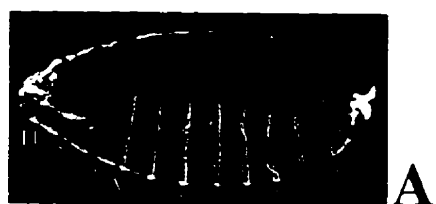
wg first instar larval cuticles have a patch of denticles and no naked cuticle (Figure 9 F and I) (Nusslein-Volhard and Wieschaus, 1980). The frequency of ftz and wg phenotypes observed, was dependent on the duration of heat shock (Table 3 and 4). At shorter heat shock durations, ectopic $FTZ^{1-316+YT}$ activity resulted in a ftz cuticle (Figure 9 E). However, higher heat shock durations resulted in a phenotype similar to the wg phenotype (Figure 9 F). Similarly, the cuticular phenotypes resulting from ectopic expression $FTZ^{101-379+YT}$ were also affected by the duration of heat shock (Table 4). The activity of $FTZ^{101-379+YT}$ was weaker than that of $FTZ^{1-316+YT}$; longer heat shocks were necessary to generate similar phenotypes. When $FTZ^{101-379+YT}$ was ectopically expressed, ftz cuticular phenotypes were observed at low heat shock times, and wg cuticular phenotypes were observed at high heat shock times (Figure 9 H and I, respectively). Unlike ectopic FTZ expression (Table 5), the ectopic expression of $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ at higher heat shock durations, resulted in a failure to deposit cuticle (Table 3 and 4).

3.3 Effect of Ectopic $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ on the Expression of Engrailed

The segment polarity gene *engrailed* (*en*) is expressed during gastrulation in fourteen 1 to 2 cell wide stripes, perpendicular to the anterior-posterior axis of the embryo (DiNardo and O'Farrell, 1987) (Figure 9 B). In embryos lacking FTZ activity, EN expression in the even-numbered parasegments is lost; EN expression in the even-numbered parasegments is dependent on the presence of FTZ activity. Furthermore, ectopic FTZ expression results in the anti-ftz EN expression pattern, which is a 1 to 2 cell

Table 3. Distribution of the cuticular phenotypes observed when $FTZ^{1-316+YT}$ was ectopically expressed from a *heat shock promoter* after 5, 7.5, 10, 12.5, and 15 minutes of heat shock.

	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
wt	82 (70%)	69 (56%)	55 (47%)	39 (41%)	40 (35%)
ftz	33 (28%)	42 (34%)	30 (26%)	27 (28%)	16 (13%)
wg	2 (2%)	10 (8%)	19 (16%)	17 (18%)	29 (25%)
no cuticle	0	3 (2%)	13 (11%)	12 (13%)	31 (27%)
total	117	124	117	95	116

Table 4. Distribution of the cuticular phenotypes observed when FTZ[101-379-YT] was ectopically expressed from a *heat shock promoter* after 5, 7.5, 10, 12.5, and 15 minutes of heat shock.

	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
wt	110 (85%)	90 (75%)	86 (56%)	50 (41%)	76 (52%)
frz	16 (12%)	22 (18%)	56 (36%)	42 (34%)	36 (25%)
wg	0	0	0	2 (2%)	6 (4%)
no cuticle	4 (3%)	8 (7%)	12 (8%)	28 (23%)	28 (19%)
total	130	120	154	122	146

Table 5. Distribution of the cuticular phenotypes observed when FTZ[1-413] was ectopically expressed from a *heat shock promoter* after 5, 7.5, 10, 12.5, and 15 minutes of heat shock.

	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
wt	83 (78%)	101 (71%)	64 (53%)	42 (41%)	59 (42%)
anti-ftz	24 (22%)	42 (29%)	56 (47%)	61 (59%)	81 (58%)
no cuticle	0	0	0	0	0
total	107	143	120	103	140

anterior widening of the FTZ-dependent EN stripes (Ish-Horowich *et al.* 1989) (Figure 9 E). Ectopic expression of FTZ$^{1-316+YT}$ resulted in a lack of EN expression in even-numbered parasegments, which is similar to the EN expression pattern in a *ftz* embryo (Figure 9 G). Ectopic expression of FTZ$^{101-379+YT}$ also resulted in a decrease in EN expression in even-numbered parasegments (Figure 9 J). In addition, the ectopic expression of both FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ resulted in a significant proportion of embryos that did not express EN (Table 6).

3.4 Activity of *ftz* Enhancer in Embryos Ectopically Expressing FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$

Autoregulatory activation of FTZ expression is required for the maintenance of the seven bands of high FTZ expression at the late cellular blastoderm stage, (Hiromi and Gehring, 1987). The *ftz* enhancer mediates this autoregulatory FTZ activity. Activation of the *ftz* enhancer occurs in seven stripes along the anterior-posterior axis of a wild-type germband extended embryo (Figure 10 A). These stripes are broader in embryos which are ectopically expressing FTZ (Figure 10 B). Activity of the *ftz* enhancer is not detected in *ftz* null mutants (Hiromi and Gehring, 1987). Ectopic expression of FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ resulted a significant decrease, or complete absence of *ftz* enhancer activity (Figure 10 C and D; Table 7).

Table 6. Distribution of embryonic phenotypes, assessed by immunolocalization of the EN protein, observed when $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ were ectopically expressed.

	wild type	ftz	anti-ftz	none	total
control	84 (100%)	0	0	0	84
FTZ (1-413)	55 (52%)	0	51 (48%)	0	106
FTZ (1-316+YT)	44 (54%)	18 (22%)	0	19 (24%)	81
FTZ (101-379+YT)	75 (65%)	20 (17%)	0	21 (18%)	116

Figure 10. Ectopic expression of $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ results in a decrease in *ftz* enhancer activation. **A.** In wild-type germband extended embryos the *ftz* enhancer is activated in seven bands of cells. **B.** Ectopic FTZ expression results in seven broad bands of *ftz* enhancer activation. C and **D.** Ectopic expression of $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ results in a decrease in *ftz* enhancer activation.

A

B

C

D

Table 7. Distribution of embryonic phenotypes, assessed by detection of *ftz* enhancer activity, observed when FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ were ectopically expressed.

	wild type	partial	none	anterior	total
control	68 (63%)	0	0	40 (37%)	108
FTZ (1-413)	106 (59%)	0	0	73 (41%)	179
FTZ (1-316+YT)	0	17 (27%)	18 (29%)	27 (44%)	62
FTZ (101-379+YT)	0	25 (34%)	10 (14%)	39 (52 %)	74

DISCUSSION

4.1 Suppression of Ectopic FTZ Activity by Endogenous FTZ

A previous study has suggested that endogenous FTZ, which is specifically expressed in even-numbered parasegments, plays a role in the repression of ectopically expressed FTZ activity in odd-numbered parasegments (Argiropoulos, 1997). This model for the repression of ectopic FTZ activity is based on the observation that when FTZ is ectopically expressed from two copies of a *Tubulin αl promoter/ftz* fusion gene ($P\{w^-$, $ftz^{1-413}<Tub$ $\alpha l\}$) in an otherwise wild-type fly, the result is a low frequency of anti-ftz phenotypes. However, when two copies of $P\{w^-, ftz^{1-413}<Tub$ $\alpha l\}$ and only one copy of the endogenous *ftz* gene are present, the anti-ftz phenotype is fully penetrant. Furthermore, ectopic expression of $FTZ^{\Delta 151-209}$ from the *Tubulin αl promoter* generates a strong anti-ftz phenotype, suggesting that the region deleted in the FTZ derivatives is responsible for negative regulation of ectopic FTZ activity by endogenous FTZ activity (Argiropoulos, 1997). The eighteen loci screened did not contain a candidate gene required for the repression of ectopic FTZ activity. The loss-of-function phenotypes of these maternal effect genes were not altered by ectopic expression of FTZ^{1-413}. Furthermore, the gain-of-function phenotype (anti-ftz) caused by ectopic expression of $FTZ^{\Delta 151-209}$ was not altered in the null maternal effect mutant background of any of the seventeen out of eighteen genes. The ftz-like phenotype of the *αFtz-F1* mutation was not rescued by ectopic $FTZ^{\Delta 151-209}$, as was previously observed by Argiropoulos (1997),

suggesting that the *αFtz-F1* mutation used to make this observation was a hypomorphic allele.

4.2 FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ Are Dominant Negative Molecules

The N and/or C terminal regions of FTZ are important for the induction of the anti-ftz phenotype (Percival-Smith, personal communication). The ectopic expression of full length FTZ results in the anti-ftz phenotype, as does the expression of FTZ$^{\Delta1-50}$, FTZ$^{\Delta51-100}$, and FTZ$^{\Delta380-413}$. However, when more than one of these sections is deleted, as in the case of FTZ$^{\Delta1-100}$, FTZ$^{\Delta1-50, 380-413}$, FTZ$^{\Delta51-100, 380-413}$, FTZ$^{\Delta316-413}$, FTZ$^{\Delta1-100, 380-413}$, and FTZ$^{\Delta1-100, 151-413}$, the anti-ftz phenotype is not detected. Also, the ectopic expression of the *Tribolium* FTZ homologue, with its significantly lower tyrosine content, does not result in the anti-ftz phenotype (Krause & Percival-Smith, unpublished result). Therefore, the anti-ftz phenotype was expected when FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ were ectopically expressed. However, the ectopic expression of FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ resulted in a ftz-like phenotype at short durations of heat shock, and a wg-like phenotype at long durations of heat shock. Therefore, I propose that FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ are dominant negative molecules. My proposal is based on the observation that null loss-of-function *ftz* alleles result in a ftz phenotype, whereas gain-of-function *heat shock promoter:ftz* fusion alleles result in the opposite, or anti-ftz, phenotype. Ectopic expression of FTZ$^{1-316+YT}$ and FTZ$^{101-379+YT}$ resulted in a ftz-like phenotype; the phenotype observed with null *ftz* alleles.

FTZ has both positive and negative regulatory activities; it is responsible for EN expression and WG repression in even-numbered

parasegments (DiNardo and O'Farrell, 1987; Duncan, 1986). It appears that FTZ^{1-316} and $FTZ^{101-379}$ are inactive due to the loss of N and C terminal regions of the protein, including the positive and negative regulatory regions. The addition of the YT to both of these polypeptides results in the addition of only the negative regulatory activity. The relationship between the number of tyrosine residues and negative regulatory activity is visible when the embryonic effects of $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ are compared. $FTZ^{1-316+YT}$, which has a total of 57 tyrosines, has more dramatic effects on the developing embryo, than $FTZ^{101-379+YT}$, which has a total of 47 tyrosine residues.

Both $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ were ectopically expressed in wild-type flies that have a wild-type ftz gene. The possibility that the $FTZ^{1-316+YT}$ and $FTZ^{101-379+YT}$ molecules could be inhibiting FTZ expression, has not been ruled out. However, this model does not explain the observed wg phenotypes. It is more likely that these FTZ dominant negative molecules (FTZ^{dn}) bind to αFTZ-F1, and in turn this protein complex binds to the regulatory elements of FTZ regulated genes and shuts off their expression. Both αFTZ-F1 and FTZ^{dn} are expressed in every cell of the embryo. However, from examination of EN expression, it appears that the FTZ^{dn} molecule has more negative regulatory effect on even-numbered EN expression, than on odd-numbered EN expression. This ftz-like EN expression pattern, and the ftz-like cuticular phenotype suggest that FTZ regulated genes in the even-numbered parasegments are repressed preferentially. This preferential repression may be responsible for the ftz-like phenotype observed at short heat shock durations. However, higher heat

shock durations resulted in a wg-like phenotype. Higher durations of heat shock presumably lead to higher expression levels of the FTZ^{dn} molecules. At higher levels of FTZ^{dn}, these molecules are able to shut off FTZ regulated genes in both the even-numbered, and the odd-numbered parasegments, resulting in a wg-like phenotype. Further experiments are required to show that αFTZ-F1 is required for induction of the wg phenotype, and that FTZdn does not affect endogenous FTZ expression.

LITERATURE CITED

Akam, M. (1987) The molecular basis for metameric pattern in the Drosophila embryo. **Development** *101*, 1-22.

Ananthan, J., Baler, R., Morrissey, D., Zuo, J., Lan, Y., Weir, M., and Voellmy, R. (1993) Synergistic activation of transcription is mediated by the N-terminal domain of Drosophila Fushi tarazu homeoprotein and can occur without DNA binding by the protein. **Mol. Cell. Biol.** *13*, 1599-1609.

Argiropoulos, B. (1997) Characterization of Ectopic Expression of Fushi tarazu Activity using the Yeast Flip Recombinase Ectopic Expression System. Master's Thesis. Faculty of Graduate Studies. University of Western Ontario.

Ashburner, M. (1989) Drosophila. A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Baker, N.E. (1987) Molecular cloning of sequenceds from *wingless*, a segment polarity gene in *Drosophila*: the spatial distribution of a transcript in embryos. **EMBO J.** *6*, 1765-1777.

Bate, M., and Martinez-Arias, A. (1993) The development of *Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Baumgartner, S., Martin, D., Hagios, D., and Chiquet-Ehrismann, R. (1994) *Ten-m*, a Drosophila gene related to tenascin, is a new pair-rule gene. **EMBO J.** *13*, 3728-3740.

Bellen, H.J., O'Kang, C.J., Wilson, C., Grossniklaus, U., Pearson, R.K., and Gehring, W.J. (1989) P-element-mediated enhancer detection: a versatile method to study development in Drosophila. **Genes Dev.** *3*, 1288-1300.

Broach, J.R., and Hicks, J.B. (1980) Replication and recombination functions associated with the yeast plasmid, 2μm plasmid. **Cell** *21*, 501-508.

Brown, J.L., and Wu, C. (1993) Repression of *Drosophila* pair-rule segmentation genes by ectopic expression of *tramtrack*. **Development** *117*, 45-58.

Campos-Ortega, J. and Hartenstein, V. (1985) The embryonic development of *Drosophila melanogaster*. Springer-Verlag, New York.

Carroll, S.B., and Scott, M.P. (1986) Localization of the *fushi tarazu* protein during Drosophila embryogenesis. **Cell** *43*, 47-57.

Fitzpatrick, V.D., Percival-Smith, A., Ingles, C.J., and Krause, H.M. (1992) Homeodomain-independent activity of the *fushi tarazu* polypeptide in Drosophila embryos. **Nature** *356*, 610-612.

Chou, T., and Perrimon, N. (1992) Use of a yeast site-specific recombinase to produce female germline chimeras in Drosophila. **Genetics** *131*, 643-653.

Chou, T., and Perrimon, N. (1996) The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. **Genetics** *144*, 1673-1679.

Copeland, J.W.R., Nasiadka, A., Dietrich, B.H., and Krause, H.M. (1996) Patterning of the Drosophila embryo by a homeodomain-deleted FTZ polypeptide. **Nature** *379*, 162-165.

Core, N., Charroux, B., McCormick, A., Vola, C., Fasano, L., Scott, M.P., and Kerridge, S. (1997) Transcriptional regulation of the *Drosophila* homeotic gene *teashirt* by the homeodomain protein Fushi tarazu. **Mech. Dev.** *68*, 157-72.

DiNardo, S., and O'Farrell, P. H. (1985) Embryonic pattern in *D. melanogaster* as revealed by accumulation of the nuclear *engrailed* protein. **Cell** *43*, 59-69.

DiNardo, S., and O'Farrell, P. H. (1987) Establishment and refinement of segmental pattern in the Drosophila embryo: spatial control of *engrailed* expression by pair-rule genes. **Genes Dev.** *1*, 1212-1225.

Dong, J., Hung, L.H., Strome, R., and Krause, H.M. (1998) A phosphorylation site in the ftz homeodomain is required for activity. **EMBO J.** *17*, 2308-2318.

Dong, J., and Krause, H.M. (1999) Tissue-specific requirements for a phosphorylation site in the Fushi tarazu homeodomain. **Dev. Genes Evol.** *209*, 427-31.

Driever, W., and Nusslein-Volhard, C. (1989) The bicoid protein is a positive regulator of hunchback transcription in the early Drosophila embryo. **Nature** *337*, 138-143.

Driever, W., Thoung, G., and Nusslein-Volhard, C. (1989) Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. **Nature** *340*, 1037-1046.

Duncan, I. (1986) Control of the Bithorax complex functions by the segmentation gene *fushi tarazu* of *D. Melanogaster.* **Cell** *47*, 297-309.

Eldon, E.D. and Pirrotta, V. (1991) Interaction of the Drosophila gap gene *giant* with maternal and zygotic pattern-forming genes. **Development** *111*, 367-378.

FlyBase (1999) The FlyBase database of the Drosophila genome projects and community literature. Available from http://flybase.bio.indiana.edu/ **Nuc. Acid Res.** *27*, 85-88.

Furukubo-Tokunafa, K., Muller, M., Affolter, M., Pick, L., Klotter, U., and Gehring, W.J. (1992) *In vivo* analysis of the helix-turn-helix motif of the Fushi tarazu homeodomain of *Drosophila melanogaster.* **Genes Dev.** *6*, 1082-1096.

Gergen, J.P. and Butler, B.A. (1988) Isolation of the Drosophila segmentation gene *runt* and analysis of its expression during embryogenesis. **Genes Dev.** *2*, 1179-1193.

Goto, T., Macdonald, P., and Maniatis, T. (1989) Early and late periodic patterns of *even-skipped* expression are controlled by distinct regulatory elements that respons to different spatial cues. **Cell** *57*, 413-422.

Guichet, A., Copeland, J.W.R., Erelyi, M., Hlousek, D., Zavorszky, P., Ho, J., Brown, S., Percival-Smith, A., Krause, H.M., and Ephrussi, A. (1997) The nuclear receptor homologue Ftz-F1 and the homeodomain protein Ftz are mutually dependent cofactors. **Nature** *385*, 548-552.

Hader, T., La Rosse, A., Ziebold, U, Busch, M, Taubert, H, Jackle, H., and Divera-Polmar, R. (1998) Activation of posterior pair-rule stripe expression in response to maternal *caudal* and zygotic *knirps* activities. **Mech. Dev.** *71*, 177-186.

Hafen, E., Kuroiwa, A., and Gehring, W.J. (1984) Spatial distribution of transcripts from the segmentation gene *fushi tarazu* during Drosophila embryonic development. **Cell** *37*, 833-841.

Harding, K., Hoey, T., Warrior, R., and Levine, M. (1989) Autoregulatory and gap gene response elements of the *even-skipped* promoter of Drosophila. **EMBO J.** *8*, 1205-1212.

Harrison, S.D., and Travers, A. (1990) The *tramtrack* gene in *Drosophila* encodes a zinc finger protein that interacts with the *ftz* transcriptional regulatory region and shows a novel embryonic expression pattern. **EMBO J.** *9*, 207-216.

Hiromi, Y., and Gehring, W.J. (1987) Regulation and Function of the Drosophila segmentation gene *fushi tarazu*. **Cell** *50*, 963-974.

Hiromi, Y., Kuroiwa, A., and Gehring, W.J. (1985) Control elements of the Drosophila segmentation gene *fushi tarazu*. **Cell** *43*, 603-613.

Howard, K., Ingham, P., and Rushlow, C. (1988) Region-specific alleles of the *Drosophila* segmenation gene *hairy*. **Genes Dev.** *2*, 1037-1046.

Hyduk, D., and Percival-Smith, A. (1996) Genetic characterization of the homeodomain-independent activity of the Drosophila *fushi tarazu* gene product. **Genetics** *142*, 481-492.

Ingham, P., and Martinez-Arias, A. (1986) The correct activation of Antennapedia and Bithorax complex genes requires the *fushi tarazu* gene. **Nature** *324*, 592-597.

Ish-Horowicz, D., Pinchin, S.M., Ingham, P.W., and Gyurkovics, H.G. (1989) Autocatalytic *ftz* activation and metameric instability induced by ectopic *ftz* expression. **Cell** *57*, 223-232.

Kellerman, K.A., Mattson, D.M., and Duncan, I. (1990) Mutations affecting the stability of the *fushi tarazu* protein of Drosophila. **Genes Dev.** *14*, 1936-1950.

Kilchherr, F., Baumgartner, S., Bopp, S., Frei, E., and Noll, M. (1986) Isolation of the *paired* gene of Drosophila and its spatial expression during early embryogenesis. **Nature** *321*, 493-499.

Knipple, D.C., Seifert, E., Rosenberg, U.B., Preiss, A., and Jaeckle, H. (1985) Spatial and temporal patterns of *kruppel* gene expression in early Drosophila embryos. **Nature** *317*, 40-44.

Kornbeg, T., Siden, I., O'Farrell, P., and Simon, M. (1985) The *engrailed* locus of Drosophila: *in situ* localization of transcripts reveals compartment-specific expression. **Cell** *40*, 45-53.

Krause, H.M. And Gehring, W.J. (1987) Stage-specific phosphorylation of the *fushi tarazu* protein during Drosophila development. **EMBO J.** *8*, 1197-1204.

Laughon, A., and Scott, M. (1984) Sequence of a Drosophila segmentation gene: protein structure homology with DNA-binding proteins. **Nature** *310*, 25-30.

Lehmann, R. And Nusslein-Volhard, C. (1984) Hunchback, a gene required for segmentation of an anterior and posterior region of the embryo. **Dev. Biol.** *119*, 402-417.

Liaw, G.J., and Lengyel, J.A. (1993) Control of *tailless* expression by *bicoid*, *dorsal*, and synergistically interacting terminal system regulatory elements. **Mech. Dev.** *40*, 47-61.

Martinez-Arias, A., and Lawrence, P. (1985) Parasegments and compartments in the Drosophila embryo. **Nature** *313*, 639-642.

Nauber, U., Pankratz, M.J., Kienlin, A., Seifert, E., Klemm, U., and Jackle, H. (1988) Abdominal segmentation of the Drosophila embryo requires a hormone receptor-like protein encoded by the gap gene *knirps*. **Nature** *336*, 489-492.

Nüsslein-Volhard, C., and Wieschaus, E. (1980) Mutations affecting segment number and polarity in Drosophila. **Nature** *287*, 795-801.

Nüsslein-Volhard, C., and Wieschaus, E. (1986) Looking at embryos. In: *Drosophila, a practical approach*. Roberts, D.B., ed. IRL Press, Oxford, pp.199-228.

Patel, N.H., Martin-Blanco, E., Coleman, K.G., Poole, S.J., Ellis, M.C., Kornberg, T.B., and Goodman C.S. (1989) Expression of *engrailed* proteins in arthroposd, annelids, and chordates. **Cell** *58*, 955-968.

Perrimon, N., Lanjuin, A., Arnold, C., and Noll, E. (1996) Zygotic lethal mutations with maternal effect phenotypes in *Drosophila melanogaster*. II. Loci on the second and third chromosomes identified by P-element-induced mutations. **Genetics** *144*, 1681-1692.

Pankratz, M.J., and Jackle, J. (1993) Blastoderm segmentation. In: *The Development of Drosophila melanogaster*. M. Bate, and A.M. Arias, eds. Cold Spring Harbor Laboratory Press, Vol. 1. pp. 467-516.

Pick, L., Schier, A., Affolter, M., Schmidt-Glenewinkel, T., and Gehring, W. J. (1990) Analysis of the *ftz* upstream element: germ layer-specific enhancers are independently regulated. **Genes Dev.** *4*, 1224-1239.

Rubin, G.M. and Spradling, A.C. (1982) Genetic transformation of Drosophila with transposable element vectors. **Science** *218*, 348-353.

Sang, S. (1978) The nutirtional requirements of Drosophila. In: *The genetics and biology of Drosophila*. Ashburner, M., and Wright, T.R.F., eds. Academic Press, New York, Vol. 2a, pp. 159-192.

Schier, A.F., and Gehring, W.J. (1993) Functional specificity of the homeodomain protein *fushi tarazu*: the role of DNA-binding specificity *in vivo*. **PNAS** *90*, 1450-1454.

Schneuwly, Klemanz, R., and Gehring, W.J. (1987) Redesigning the body plan of *Drosophila* by ectopic expression of the homeotic gene *Antennapedia*. **Nature** *233*, 816-818.

Struhl, G. (1985) Near-reciprocal phenotypes caused by inactivation or indiscriminate expression of the Drosophila segmentation gene *ftz*. **Nature** *318*, 677-680.

Van der Meer, J. (1977) Optical clean and permanent whole mount preparation for phase contrast microscopy of cuticular structures of insect larvae. **D. I. S.** *52*, 160.

Wakimoto, B.K., and Kaufman, T.C. (1981) Analysis of larval segmentation in lethal genotypes associated with the Antennapedia gene complex of *Drosophila melanogaster*. **Dev. Biol.** *81*, 51-64.

Wang, C., and Lehmann, R. (1991) Nanos is the localized posterior determinant in Drosophila. **Cell** *66*, 637-647.

Robert, K, and Hyams, J.S. (1979) Microtubules. Academic Press, New York.

Wieschaus, E.C., Nusslein-Volhard, C., and Jurgens, G. (1984) *Kruppel*, a gene whose activity is required early in the zygotic genome for normal embryonic segmentation. **Dev. Biol.** *104*, 172-186.

Yu, Y., Yussa, M., Song, J., Hirsch, J., and Pick, L. (1999) A double interaction screen identifies positive and negative *ftz* gene regulators and Ftz-interacting proteins. **Mech. Dev.** *83*, 95-105.