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Genetics

Structural relationships among genes that control development: Sequence homology between the Antennapedia, Ultrabithorax, and fushi tarazu loci of *Drosophila*

(homoeosis/protein domains/gene evolution)

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ABSTRACT **Genes that regulate the development of the fruit fly *Drosophila melanogaster* exist as tightly linked clusters in at least two cases. These clusters, the bithorax complex (BX-C) and the Antennapedia complex (ANT-C), both contain multiple homoeotic loci: mutations in each locus cause a transformation of one part of the fly into another. Several repetitive DNA sequences, including at least one transposon, were mapped in the ANT-C. DNA from the 3′ exon of Antennapedia (*Antp*), a homoeotic locus in the ANT-C, hybridized weakly to DNA from the 3′ exon of Ultrabithorax (*Ubx*), a homoeotic locus in the BX-C. DNA from each of these 3′ exons also hybridized weakly to DNA from the fushi tarazu locus of the ANT-C. The fushi tarazu (*ftz*) locus controls the number and differentiation of segments in the developing embryo. Sequence analysis of the cross-hybridizing DNA from the three loci revealed the conservation of predicted amino acid sequences derived from coding parts of the genes. This suggests that two homoeotic loci and a "segment-deficient" locus encode protein products with partially shared structures and that the three loci may be evolutionarily and functionally related.**

The Antennapedia complex (ANT-C) of *Drosophila* is a cluster of genes that regulate differentiation and pattern formation in the developing fly (1, 2). Some of the ANT-C loci are homoeotic: mutations lead to switches of cell fates from one developmental pathway to another. One such locus is Antennapedia (*Antp*), which normally functions in each of the three thoracic segments, in the abdominal segments, and in the humeral disc (3–6). Abnormal *Antp* function caused by certain mutations can lead to the transformation of antennae into legs or of second and third legs into first legs (7, 8). Thoracic development is also controlled by genes in the bithorax complex (BX-C), in particular by the Ultrabithorax (*Ubx*) locus (9–13). *Ubx* mutations lead to transformations to third thoracic segment structures into second thoracic segment structures. The homoeotic loci of the ANT-C and BX-C work coordinately to control developmental pathways. Lewis (9, 14, 15) has proposed that the homoeotic genes of the BX-C may have evolved from a common ancestral gene, diversifying to control segment-specific developmental processes. This report presents evidence that suggests an extension of Lewis' idea to relationships between genes of the ANT-C and genes of the BX-C.

In addition to homoeotic loci, the ANT-C includes a locus (fushi tarazu, *ftz*) that controls the number of segments formed (2, 4) and their differentiation. The relationship of homoeotic loci, which affect the type of segment that forms, to the "segment-deficient" loci, which affect the number and pattern of segments, is not well understood.

Recent molecular analyses of the BX-C (refs. 16 and 17; R. Saint, M. Goldschmidt-Clermont, P. A. Beachy, and D. S. Hogness, personal communication) and the ANT-C (18–20) have revealed that the *Ubx* and *Antp* loci are extraordinarily large functional units of 73 kilobases (kb) and 103 kb, respectively. Both loci encode multiple RNA species. In contrast to *Antp* and *Ubx*, the *ftz* locus appears to be a simpler transcription unit contained within a 2-kb region of the genome (ref. 18; unpublished data). It is not known whether any of the *Antp*, *Ubx*, or *ftz* RNA molecules encode proteins.

To learn more about the DNA organization of the ANT-C, repetitive DNA sequences have been mapped. Some of the repetitive sequences are in the coding parts of *Antp* and *ftz*. The investigation of repetitive DNA revealed related sequences in the *Antp*, *ftz*, and *Ubx* loci. The sequences shared at the three loci include conserved amino acid coding sequences.

METHODS

Methods for DNA purification, gels, blots, and nick-translation are described in a previous report (18). Genomic DNA probes were prepared by nick-translation to a specific activity of $\approx 10^7$ dpm/μg. All hybridizations were performed in 4× concentrated SET buffer (SET buffer = 0.15 M NaCl/20 mM Tris·HCl/1 mM EDTA, pH 8.0), 2× concentrated Denhardt's solution [2× concentration = 0.04% (each) bovine serum albumin, polyvinylpyrrolidone, and Ficoll], and 100 μg of sheared, denatured salmon sperm DNA per ml at 65°C. Filters were washed in 0.15 M NaCl/0.015 M sodium citrate/0.1% sodium dodecyl sulfate at 65°C. For DNA sequencing, restriction fragments were cloned into M13mp8, M13mp9, and M13mp10 (21) and sequenced by using the procedure of Biggin *et al.* (22).

RESULTS

Repetitive DNA Sequences. The physical structure of a 320-kb region of the ANT-C (18) has been previously reported. To identify repetitive DNA sequences within the cloned region, total ^{32}P-labeled genomic DNA was hybridized to Southern blots of restriction digests of the cloned DNA sequences. Repetitive cloned sequences yield stronger signals because of their more abundant representation in the probe. Nine regions of the ANT-C DNA showed elevated levels of hybridization. Fig. 1 depicts the range of signal intensities of

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Abbreviations: ANT-C, Antennapedia complex; BX-C, bithorax complex; kb, kilobase(s).

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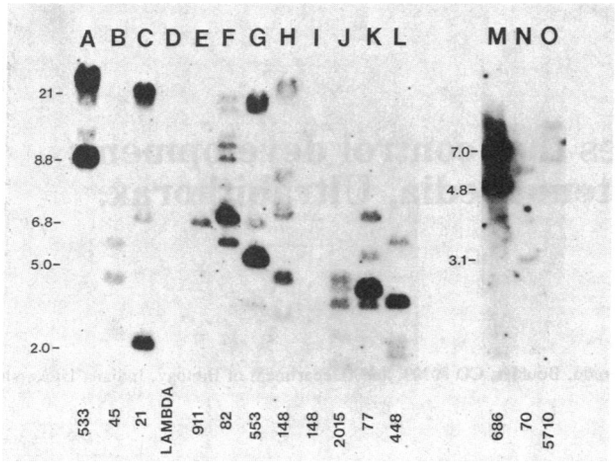

FIG. 1. Repetitive DNA in the ANT-C; Southern blots of restriction enzyme-digested cloned DNA were probed with 45 × 10^6 cpm of nick-translated genomic Canton S DNA. Approximately equal amounts of DNA were loaded into each lane, except lane I, which has only 1/5 as much DNA as lane H. Lanes M–O are from a separate gel. The strong signals in lane M correspond to fragments containing sequences from the F element transposon. Fragment sizes are indicated in kilobase pairs. The locations of repetitive sequences are indicated. The lanes contain the following phage DNAs (refer to Fig. 2), digested with the indicated restriction enzyme: A, A533 (*Eco*RI); B, A45 (*Eco*RI); C, A21 (*Hin*dIII); D, wild-type λ (*Hin*dIII); E, A911 (*Hin*dIII); F, A82 (*Eco*RI and *Xba* I); G, A553 (*Hin*dIII); H, A148 (*Hin*dIII); I, A148 (*Hin*dIII and *Sal* I); J, 2015 (*Eco*RI); K, A77 (*Eco*RI); L, A448 (*Eco*RI); M, A686 (*Eco*RI); N, A70 (*Eco*RI and *Xba* I); O, 5710 (*Eco*RI).

this subset of cloned segments, after hybridization to adult fly DNA and autoradiography. The results with embryonic DNA probe are the same (data not shown). The weakest signals are presumed to represent single-copy or predominantly single-copy DNA (e.g., Fig. 1, lanes N and O). The strength of the repetitive DNA signal may depend on the number of copies of the sequence, on the length of the repetitive sequence, and on the degree of conservation of a family of related sequences. The genomic DNA probe does not hybridize to DNA from wild-type λ phage under these conditions (Fig. 1, lane D).

The locations of repetitive DNA sequences in the −100 to +220 region of the ANT-C are indicated on a map (Fig. 2), the coordinates of which correspond to the coordinates used in previous work (18). Many of the repetitive DNA sequences are close to or at previously mapped sites of homology with embryonic transcripts (18). One of the repetitive elements (Fig. 1, lane M) is the previously reported (18) transposable F element (23) at position +162 (within *Antp*) in the Canton S genome (Fig. 2, clone 686). Also within the *Antp* locus region are two other strongly repetitive signals at +110 to +113 and +127 to +130 (Fig. 1, lanes K and L). In experiments using subclones (not shown), the latter two repeats have been mapped more precisely to +112 to +112.5

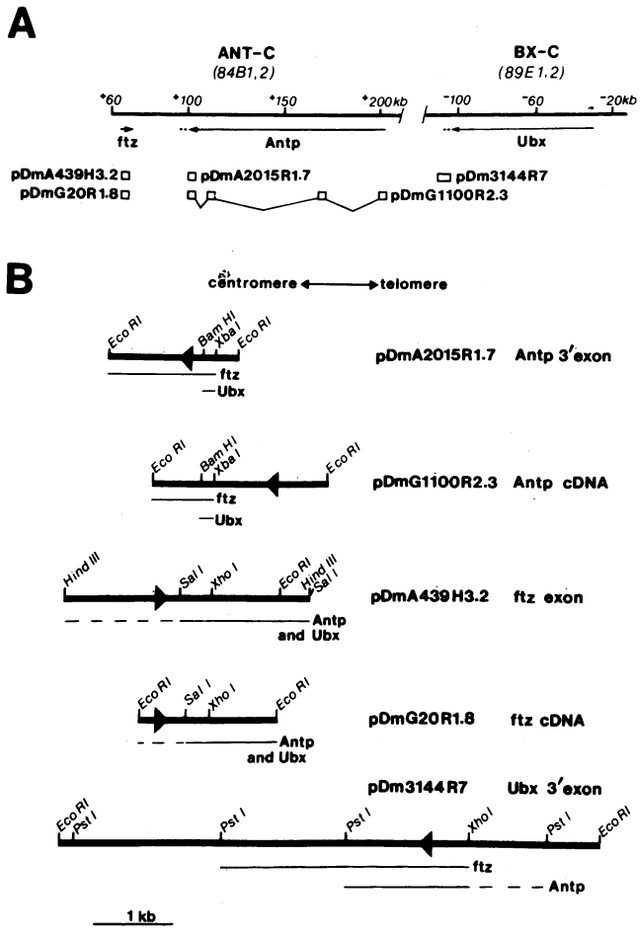

FIG. 3. Subclones used to analyze *Antp*, *ftz*, and *Ubx* homologies. (*A*) Origins of subclones in the ANT-C and BX-C. The parts of the ANT-C and BX-C shown are located in polytene chromosome bands 84B1,2 and 89E1,2, respectively. The coordinates (in kilobase pairs) are those of ref. 18 for the ANT-C and ref. 17 for the BX-C. The arrows indicate transcription units and the directions of transcription (arrowheads are the 3′ ends). The pDmA439H3.2, pDmA2015R1.7, and pDm3144R7 subclones are genomic fragments; the pDmG20R1.8 and pDmG1100R2.3 subclones are cDNA clones derived from the indicated genomic sequences. The centromeric-telomeric orientation is as shown. (*B*) Restriction maps of the subclones and locations of cross-hybridizing sequences. The centromeric-telomeric orientation is the same as in *A*, and the directions of transcription are indicated by the large arrowheads. The *Eco*RI sites at the ends of the cDNA clones are derived from linkers used in the construction of the cDNA library (except the site at the right end of pDmG20R1.8, which may be a genomic *Eco*RI site). The *Sal* I site shown at the right end of the pDmA439H3.2 subclone is a pUC8 vector site. All other sites correspond to sites in the *Drosophila* genome. Below each restriction map, the fine lines indicate the parts of each subclone to which the probes from the other loci hybridize (see Fig. 4). Dashed lines indicate weaker hybridization.

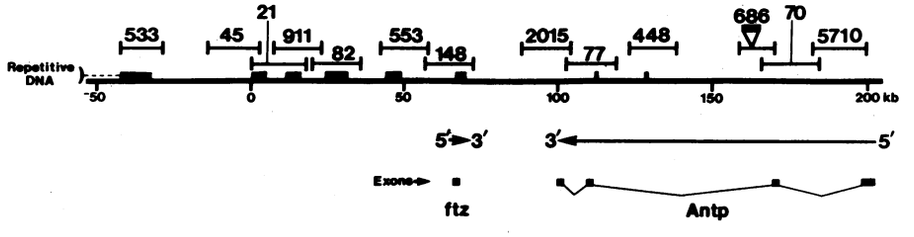

FIG. 2. Locations of repetitive DNA on the physical map of the ANT-C. Regions containing repetitive sequence are indicated by thickened bars. Bracketed lines above the restriction map are the cloned segments of DNA, isolated in phage vectors, that were used to construct the map and to perform the experiment presented in Fig. 1. The triangle at +162 is an F element transposon (18). The *ftz* locus is located between +66 and +69.2; the *Antp* locus is between +100 and +203. The precise limits of these loci, and others in the ANT-C, are not established as yet.

and +128.5 to +129. The repetitive DNA near coordinate 0 is probably close to the Deformed (*Dfd*) gene, a gene involved in the development of the head (ref. 18; unpublished data).

Within the *ftz* locus region at +67 to +69 (ref. 18; unpublished data), a weakly repetitive signal is observed (Fig. 1, lane H). If a *ftz* probe is hybridized to genomic DNA at low stringency, several bands are observed (data not shown), in keeping with the results shown here. To examine the possibility of *ftz*-related sequences occurring more than once within the ANT-C, a probe containing *ftz* sequences (+66 to +69.2) was hybridized to DNA from phage clones spanning the cloned ANT-C region. Three sites of hybridization other than *ftz* itself were detected (data not shown). Two of these sites are located to the left of *ftz* in regions (+3 to +5, +26 to +31) where the genes have not yet been precisely mapped. Another site of *ftz* probe hybridization is at +100 to +101.7, the location of the 3′ *Antp* exon (18). In contrast to the *ftz* locus, the *Antp* 3′ exon was not detectably repetitive in experiments using genomic DNA probes (Fig. 1, lane J), which suggests that the *ftz* locus is homologous to more sequences in the genome than is the *Antp* exon.

The *Antp* and *Ubx* loci are similar both in the large size of their transcription units, 103 kb and 73 kb, respectively, and in their function in the regulation of segmental differentiation. Thus, it seemed possible that the two loci might have evolutionary or structural relationships. Indeed, a 7-kb subclone containing the 3′ exon of *Ubx* was found to cross-hybridize with *ftz* and *Antp* sequences. To map the DNA sequence homologies between *Antp*, *ftz*, and *Ubx* more precisely, five subclones were used. The subclones contain genomic and cDNA sequences and are shown in Fig. 3.

The inserts were excised from each of the three exon subclones, purified, labeled with ^{32}P, and hybridized to a series of restriction digests of the other subclones. Hybridization conditions were moderately stringent (see *Methods*). The ethidium bromide-stained gels and autoradiograms of hybridization to their corresponding blots (Fig. 4 *A–C*) demonstrate that cross-hybridization occurs among all three probes and that the homologous sequences are also present in *Antp*

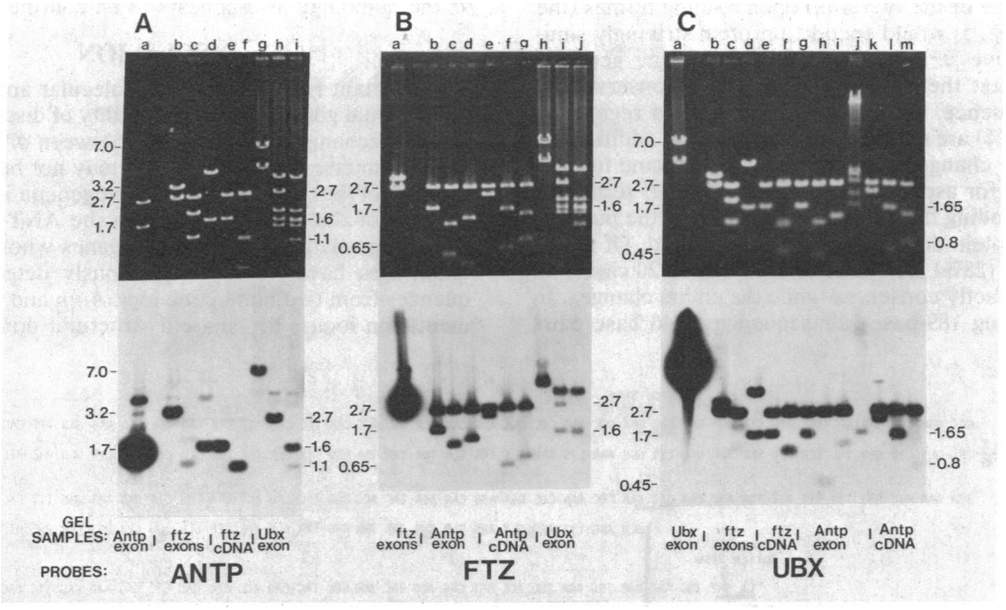

FIG. 4. Cross-hybridization of *Antp*, *ftz*, and *Ubx* DNA sequences. Subclones (maps shown in Fig. 2) were digested with restriction enzymes as listed below. The DNA was transferred to nitrocellulose and hybridized to nick-translated ^{32}P-labeled DNA probes made from purified DNA fragments excised from the vectors. Probes: *Eco*RI 1.7-kb fragment from pDmA2015R1.7, 1.3×10^7 cpm (the *Antp* 3′ exon) (*A*); *Hin*dIII 3.2-kb fragment from pDmA439H3.2, 5×10^6 cpm (the *ftz* exon) (*B*); *Eco*RI 7.0-kb fragment from pDm3144R7, 3×10^7 cpm (the *Ubx* 3′ exon) (*C*). The clones are all in the vector pUC8 (2.7 kb; ref. 24), except pDm3144R7 is in the vector pBR322 (4.3 kb). Upper panels, ethidium bromide-stained gels; lower panels, autoradiograms of blots, each exposed for 1 hr. (*A*) Lane a, pDmA2015R1.7 cut with *Eco*RI: lanes b–d, pDmA439H3.2 cut with *Hin*dIII, *Hin*dIII + *Xho* I, *Sal* I; lanes e and f, pDmG20R1.8 cut with *Eco*RI, *Eco*RI + *Sal* I; lanes g–i, pDm3144R7 cut with *Eco*RI, *Pst* I, *Pst* I + *Xho* I. (*B*) Lane a, pDmA439H3.2 cut with *Hin*dIII; lanes b–d, pDmA2015R1.7 cut with *Eco*RI, *Eco*RI + *Bam*HI, *Eco*RI + *Xba* I; lanes e–g, pDmG1100R2.3 cut with *Eco*RI, *Eco*RI + *Bam*HI, *Eco*RI + *Xba* I; lanes h–j, pDm3144R7 cut with *Eco*RI, *Pst* I, *Pst* I + *Xho* I. (*C*) Lane a, pDm3144R7 cut with *Eco*RI; lanes b–d, pDmA439H3.2 cut with *Hin*dIII, *Hin*dIII + *Xho* I, *Sal* I; lanes e and f, pDmG20R1.8 cut with *Eco*RI, *Eco*RI + *Sal* I; lanes g–i, pDmA2015R1.7 cut with *Eco*RI, *Eco*RI + *Bam*HI, *Eco*RI + *Xba* I; lane j, A2015R1.7 cut with *Eco*RI; lanes k–m, pDmG1100R2.3 cut with *Eco*RI, *Eco*RI + *Bam*HI, *Eco*RI + *Xba* I. The *Antp* probe: The 1.7-kb *Antp* exon fragment hybridizes to a 1.7-kb *Sal* I fragment of the *ftz* exon (*A*, lane d) and to the corresponding 1.3-kb *Sal* I–*Eco*RI fragment of the *ftz* cDNA clone (*A*, lane f). Weaker hybridization to the remaining part of the *ftz* exon is detectable (*A*, lane d). The *Antp* probe also hybridized to the *Ubx* 3′ exon, primarily to a 2.6-kb *Pst* I fragment (*A*, lane h) that is within the transcribed part of *Ubx* (R. Saint, P. A. Beachy, and D. S. Hogness, personal communication). When this *Pst* I fragment is subdivided by an *Xho* I site, the *Antp* probe hybridizes to both subfragments, but more strongly to the larger one (*A*, lane i). The *ftz* probe: The 3.2-kb *Hin*dIII fragment containing the *ftz* exon hybridizes to the *Antp* 3′ exon, strongly to the 1.2-kb *Eco*RI–*Bam*HI fragment and the 1.37-kb *Eco*RI–*Xba* I fragment and more weakly to the 0.45-kb *Bam*HI–*Eco*RI fragment (*B*, lanes c and d). The *ftz* probe also hybridizes to the corresponding region of the 3′ *Antp* cDNA clone (*B*, lanes e–g; Fig. 3 *A* and *B*). The *ftz* probe hybridizes to the adjacent 1.6-kb and 2.6-kb *Pst* I fragments of the 3′ *Ubx* (*B*, lane i). A *Pst* I–*Xho* I double digest reveals that the *ftz*–*Ubx* homology in the 2.6-kb *Pst* I fragment is mostly in the 1.6-kb *Pst* I–*Xho* I subfragment, which is adjacent to the 1.6-kb *Pst* I fragment (*B*, lane j; Fig. 3*B*). The *ftz* probe contains a small amount of contaminating pUC8 vector, which accounts for the hybridization to pUC8 (2.7 kb) and pBR322 (4.3 kb) on the blots. Control experiments (not shown) reveal no cross-hybridization between pUC8 and any of the *Drosophila* sequences analyzed here. The *Ubx* probe: The 7-kb *Eco*RI fragment containing the *Ubx* 3′ exon hybridizes to the *ftz* exon and cDNA subclones in the same way that the *Antp* exon does (compare *A*, lanes b–f, to *C*, lanes b–f), except that the *Ubx* probe contains a small amount of contaminating pUC8 DNA. The hybridization of the *Ubx* probe to the *Antp* exon is primarily to the 1.4-kb *Eco*RI–*Xba* I fragment and the 0.45-kb *Bam*HI–*Eco*RI fragment (*C*, lanes h and i). The corresponding pattern of hybridization is seen in the *Antp* cDNA clone (*C*, lanes k–m). The *Ubx* probe hybridizes to the *Antp* exon cloned into a phage vector as well (*C*, lane j).

and *ftz* cDNA clones. The fragments of each subclone that cross-hybridized are shown in Fig. 3*B*. The *Antp* and *Ubx* probes hybridized to the same parts of the *ftz* subclones. However, the *ftz* and *Antp* probes hybridized differently to the *Ubx* restriction fragments, and the *ftz* and *Ubx* probes hybridized differently to the *Antp* restriction fragments. Therefore, the conserved sequences are not identically arranged at the three loci. The details of the cross-hybridization results are presented in the legend for Fig. 4.

Sequence Analysis. The DNA sequences of parts of the three cross-hybridizing regions of the *Antp*, *ftz*, and *Ubx* loci suggest that the homology between the loci includes sequence homology not only at the DNA level but also at the protein level (Fig. 5). The hybridization results presented in Fig. 4 suggest that the homologies extend beyond the sequenced parts of the three loci. The direction of transcription of each locus is known (refs. 16 and 18; R. Saint, M. Goldschmidt-Clermont, and D. S. Hogness, personal communication; unpublished data) and is indicated in Figs. 2 and 3. In the *Antp* sequence, two reading frames in the correct orientation are open. Only one reading frame is open in the *ftz* sequence. One of the two *Antp* open reading frames (the one shown in Fig. 5) would encode a protein strikingly similar to the putative *ftz* product. The *Antp* and *ftz* genomic sequences suggest the existence of a highly conserved 63 amino acid sequence. Within the 63 amino acid region, 51 amino acids (81%) are exactly conserved and an additional 6 amino acids are changed conservatively (isoleucine for leucine, glutamate for aspartate, lysine for arginine, serine for threonine). Including the conservative changes, the putative *ftz* and *Antp* protein domains are 90% conserved. Of the 63 amino acids, 18 (28%) are lysine or arginine. In 20 cases, an amino acid is exactly conserved while the codon changes. In the corresponding 189-base-pair sequences, 146 base pairs are precisely conserved (77%). Two of the possible reading frames are also open in the *Ubx* sequence, and again one of them encodes an amino acid sequence very similar to the corresponding parts of *Antp* and *ftz*. The *Ubx* and *ftz* amino acid sequences are each closer to the *Antp* sequence than they are to each other.

The sequence of the *Antp* cDNA is exactly colinear with the genomic exon sequence throughout the 63 amino acid homology region. However, the cDNA sequence diverges from the genomic sequence ≈44 base pairs upstream of the *Xba* I site common to both clones. The presence of the 5′ C-A-G-G 3′ sequence in the genomic *Antp* sequence suggests that the divergence between the *Antp* cDNA and genomic sequences is due to an RNA splicing event (25). The lysine codon in the *Antp* cDNA sequence (AAG) agrees with the consensus sequence for an exon–intron splice junction (24). Apart from the lysine, the homology between *ftz* and *Antp* appears to be confined to the 3′ *Antp* exon contained in pDmA2015R1.7. The sequence of the *ftz* cDNA clone pDmG20R1.8 (unpublished data) reveals the existence of a small *ftz* intron just upstream of the homology region, so in *ftz* the homology also appears to be confined to a 3′ exon.

DISCUSSION

An important reason for using molecular approaches to developmental genetics is the possibility of discerning structural and mechanistic relationships between different genes or gene products, relationships that may not be apparent from the phenotypes of mutants or from genetic interactions. An analysis of the repetitive DNA in the ANT-C has revealed surprising relationships between genes whose structural relationships have not been previously detected. DNA sequences from two homoeotic loci, *Antp* and *Ubx*, and a segmentation locus, *ftz*, share a structural domain. There are

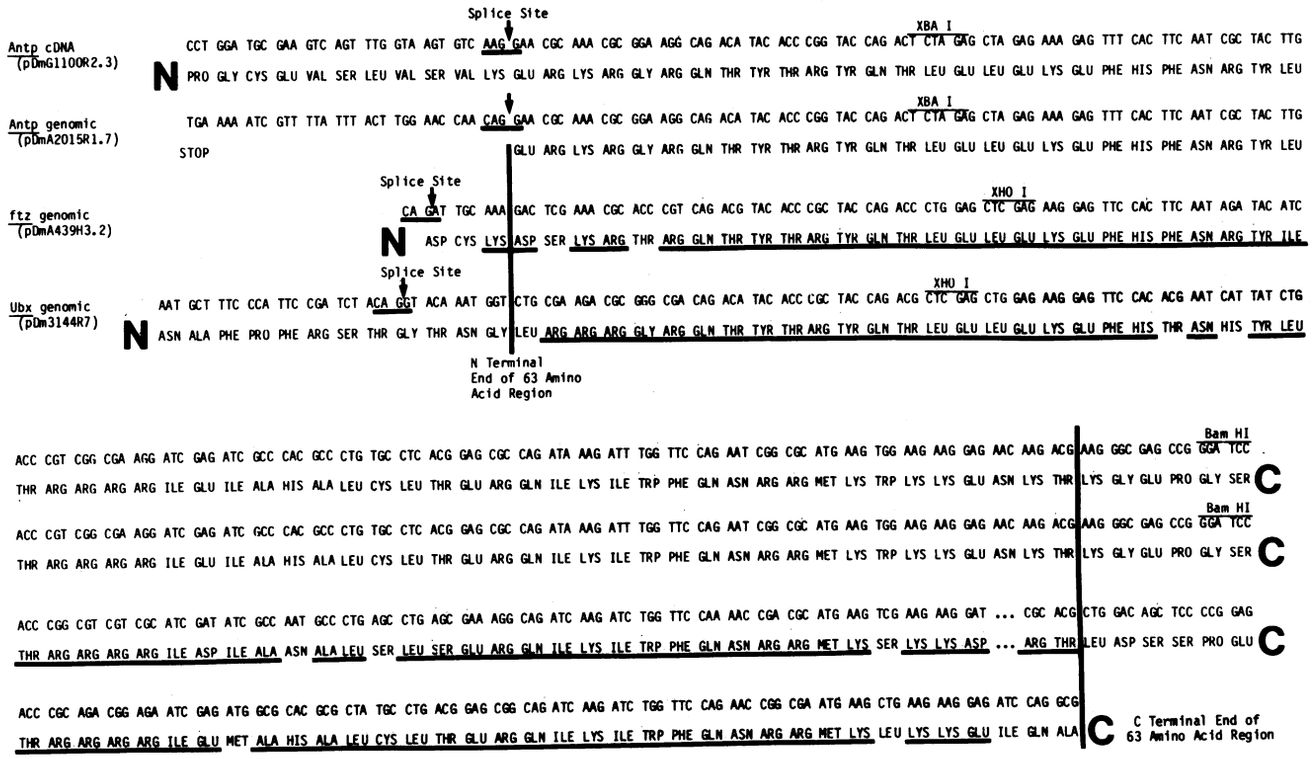

FIG. 5. Sequence homologies between *Antp*, *ftz*, and *Ubx*. The sequence shown here may be aligned with the subclone restriction maps of Fig. 3 by using the restriction sites indicated above each DNA sequence. The *Antp* and *Ubx* sequences are inverted relative to their chromosomal orientations to bring them into the conventional 5′ to 3′, left to right orientation. The underlined amino acids in the *ftz* and *Ubx* sequences are those that are homologous to the *Antp* sequence (including conservative changes). The 3′ end of a *ftz* intron is located at the splice site indicated for the *ftz* genomic sequence. A *Ubx* splice site is also indicated (see text).

two important implications of this finding. First, it is likely that these loci encode proteins. The functions of loci such as *Antp*, *ftz*, and *Ubx* may of course not be limited to protein products, and proof of the existence of the protein products must await further experiments. A second implication is that products of the homoeotic loci, and perhaps of a number of other loci involved in regulating development, share a structural component. The concept of such a related set of genes was first proposed by Lewis (9, 14, 15) for the homoeotic loci of the BX-C. The BX-C genes regulate differentiation of thoracic and abdominal segments. Lewis suggested that a series of tandemly arranged genes in the BX-C had evolved from a common ancestral gene to function in different segments of modern insects. Kaufman and co-workers (1, 3) proposed the designation of another cluster of homoeotic genes as the ANT-C. Loci of the ANT-C (including *Antp* and *ftz*) affect development of the head, thoracic, and abdominal segments. The homology between loci in the two clusters supports Lewis' proposal of related structure and shared evolution among homoeotic loci.

Both *Antp* and *Ubx* are loci involved in the differentiation of segments, and both appear to be extraordinarily large transcription units with multiple RNA products. The homologous regions described here are located similarly in both the *Ubx* and *Antp* proteins, close to the putative COOH terminus (unpublished data; P. A. Beachy and D. S. Hogness, personal communication). An intriguing feature of the amino acid sequence homology region is the high proportion of basic amino acids. The predominance of basic residues is suggestive of interactions with nucleic acids or acidic proteins. The 5′ end of the *Antp* homology region coincides with a splice junction (Fig. 5) joining the *Antp* 3′ exon to an exon about 11 kb away. An in-frame stop codon is observed in the genomic sequence 11 codons upstream from the splice site. These sequences provide direct evidence that the *Antp* exons are joined by RNA splicing, although the occurrence of DNA rearrangements as well has not been ruled out. A splice junction sequence is also present in the *Ubx* sequence (Fig. 5) and is in fact used, as evidenced by a cDNA clone sequence (R. Saint, P. A. Beachy, and D. S. Hogness, personal communication). The homologous regions of all three loci may correspond, at least approximately, to exon coding units.

In homozygous *ftz* embryos the number of segments is halved and the remaining segments show a change in the pattern of cuticular structures (2, 4). Similar phenotypes result from mutations in a set of loci collectively referred to as "pair-rule" loci (26). It has been suggested that *ftz* may be distinguished from at least some of the other pair-rule loci because in *ftz/ftz* embryos, the "deletion" of alternate segments may actually represent a transformation of the cells of missing segments into the posterior part of the next more anterior segment (2). Such transformations can be viewed as pairwise homoeotic conversions and suggest that *ftz* function is closely integrated with homoeotic gene function (2). The direction of transcription of *ftz* is opposite to that of *Antp*. If the *ftz* locus and the 3′ exon of *Antp* arose from a common ancestral gene, the present chromosomal arrangement in *D. melanogaster* could have been achieved, for example, by a tandem duplication followed by an inversion.

Note Added in Proof. McGinnis *et al.* (27) have recently reported results in agreement with those described here. Some of the unpublished data regarding *ftz*, mentioned above, are in press (28).

The research reported here was initiated in the laboratories of Thomas Kaufman and Barry Polisky, and we would like to express our appreciation to them for encouragement and guidance. We thank Robert Laymon for excellent technical assistance. Allen Laughon provided unpublished data on the sequence of the *ftz* cDNA and genomic subclones. We also thank Philip Beachy and David Hogness for unpublished information and helpful discussions; Welcome Bender for clones and useful discussions; John Spieth, Debra Peattie, Barbara Quarantillo, and Allen Laughon for help with DNA sequencing; and Larry Gold for the use of sequencing facilities. We are grateful to Susan Strome and Dan Stinchcomb for their comments on the manuscript and to Cathy Inouye and Karen Brown for help in its preparation. The research was supported by grants from the National Institutes of Health to Thomas C. Kaufman (GM 29709) and to M.P.S. (HD 18163-01).

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