Characterization of the intergenic RNA profile at *abdominal-A* and *Abdominal-B* in the *Drosophila* bithorax complex

Esther Bae*†, Vincent C. Calhoun*, Michael Levine*, Edward B. Lewis‡, and Robert A. Drewell*‡§

*Department of Molecular and Cell Biology, Division of Genetics and Development, 401 Barker Hall, University of California, Berkeley, CA 94720; and ‡Division of Biology, California Institute of Technology, Pasadena, CA 91125

Contributed by Edward B. Lewis, November 4, 2002

The correct spatial expression of two *Drosophila* bithorax complex (BX-C) genes, *abdominal-A* (*abdA*) and *Abdominal-B* (*AbdB*), is dependent on the 100-kb intergenic *infraabdominal* (*iab*) region. The *iab* region is known to contain a number of different domains (*iab2* through *iab8*) that harbor cis-regulatory elements responsible for directing expression of *abdA* and *AbdB* in the second through eighth abdominal segments. Here, we use *in situ* hybridization to perform high-resolution mapping of the transcriptional activity in the *iab* control regions. We show that transcription of the control regions themselves is abundant and precedes activation of the *abdA* and *AbdB* genes. As with the homeotic genes of the BX-C, the transcription patterns of the RNAs from the *iab* control regions demonstrate colinearity with the sequence of the *iab* regions along the chromosome and the domains in the embryo under the control of the specific *iab* regions. These observations suggest that the intergenic RNAs may play a role in initiating cis regulation at the BX-C early in development.

In *Drosophila*, thoracic and abdominal segmental identities are specified by genes in the bithorax complex (BX-C) (1). The BX-C contains >300 kb of genomic DNA but codes for only three homeotic (*Hox*) transcription factors: *Ultrabithorax* (*Ubx*), *abdominal-A* (*abdA*), and *Abdominal-B* (*AbdB*) (2). Transcription of each of the three protein-coding genes is regulated by an extensive region of cis DNA (1, 3–5). In the cases of *abdA* and *AbdB*, the cis-regulatory DNA required for accurate spatial and temporal expression during development lies predominantly in the 100-kb intergenic region (6–8) (Fig. 1*A*). This region contains an organized array of genetically defined domains: *infraabdominal* (*iab*) regions *iab2* through *iab8* (Fig. 1*A*). Mutations in any given *iab* region disrupt the development of a corresponding abdominal segment; *iab3* mutations affect abdominal segment 3 (more precisely, parasegment 8, which is composed of the posterior part of abdominal segment 2 and the anterior part of abdominal segment 3), *iab4* mutations affect abdominal segment 4, and so on (9, 10).

Previous studies have shown that the *abdA* and *AbdB* transcripts are not the only RNAs produced from this region of the BX-C, because the *iab* regions also are transcribed in the early embryo (11–13). However, the resolution of the mapping was limited in these studies and, therefore, unable to characterize a specific function for the intergenic transcripts. In this study, we have performed high-resolution *in situ* hybridization mapping to more accurately analyze endogenous intergenic transcription in the *iab* regions. In blastoderm-stage embryos, these RNAs are abundant and their transcription patterns show spatial modulation along the anteroposterior axis of the embryo, exhibiting a colinear expression pattern correlating with the *iab* domain from which they originate. We discuss these findings with regard to regulation of *abdA* and *AbdB* expression.

Whole-Mount *in Situ* Hybridization

Probes from the Bithorax complex were PCR-amplified by using *Drosophila yw*^{67} adult genomic DNA as a template. The DNA probes were cloned into pGEMT-Easy (Promega). Sense and antisense riboprobes (relative to the direction of *abdA* and *AbdB* transcription; see Fig. 1) were prepared by using a digoxigenin (DIG) RNA-labeling kit (Roche, Gipf-Oberfrick, Switzerland). PCR primer sequences and positions in BX-C (14) were as follows: BPP s, 5′-TATTATTCGTCTCCAGTCGC-3′ (47980); BPP as, 5′-CTCAGATTGATGGTGGTGGTGG-3′ (49031); Bexon s, 5′-GAACAAGAAGAACTCACAGC-3′ (53954); Bexon as, 5′-TAGGCATAGGTGTAGGTGTAGG-3′ (55566); 8E s, 5′-CAAGTGTTGCCATCGTGG-3′ (59940); 8E as, 5′-CATTCCGTCCAGCAATAGAACC-3′ (61783); 7E s, 5′-AAGGCGACCATTATTAGAGTGC-3′ (66156); 7E as, 5′-TTGAAGTCACACAGATGAACGG-3′ (68096); 7-1 s, 5′-GCCACACTCATCGTTATTCTCC-3′ (71024); 7-1 as, 5′-TTGGAGTAGGAGAAGAAGAAGG-3′ (72858); 7-2 s, 5′-GACATCTAACTCTCCTTCAACC-3′ (76879); 7-2 as, 5′-TTATGAAGTCGTAGTTGTCGGC-3′ (78772); 6-1 s, 5′-ATTATGACGGACTGATTGGC-3′ (89455); 6-1 as, 5′-TTGCTGTTGTTGCTACACTACG-3′ (91210); 6-2 s, 5′-AGCAACCACTATGGCAGTCTGG-3′ (96681); 6-2 as, 5′-ATCCGCCTGATAAGGTTCCTCG-3′ (97937); 5-1 s, 5′-TTCCTCTGACCGTGCTCATTGG-3′ (99668); 5-1 as, 5′-AGTGTGTGGTCCGCAATACAGC-3′ (101631); 5-2 s, 5′-ATTGGAATGGAGACTCGCAGCC-3′ (101688); 5-2 as, 5′-ATTCCTTACTATTCGGTACACC-3′ (103688); 5E s, 5′-CAAGATGCTCGCTCGTAACG-3′ (103787); 5E as, 5′-GAAGGTGTGGATAGTTCAGTC C-3′ (105773); 5-3 s, 5′-CGCTGTCTGAATCTTGGC-3′ (106763); 5-3 as, 5′-AAGACACCTGCTTACTAACC-3′ (108463); MCP-1 s, 5′-GCCATTAGTCTGCTCTGAGG-3′ (110002); MCP-1 as, 5′-GACGATGACGATGACGAAGACC-3′ (112089); MCP-2 s, 5′-TTGAGTATTCCACTTACGCTCC-3′ (113068); MCP-2 as, 5′-CGGAGATAACGAATGGCG-3′ (114879); MCP-3 s, 5′-CACTCGCCATTCGTTATCTCCG-3′ (114858); MCP-3 as, 5′-ACCAGGAACGACAATGCC-3′ (116782); MCP-4 s, 5′-TCAATCTCCGTCCTCATTATCG-3′ (117013); MCP-4 as, 5′-TGCGCACTGAACGAATGC-3′ (118783); 4-1 s, 5′-GTATTAGGTGGTCCTGACAGCG-3′ (120611); 4-1 as, 5′-GGTAAGTGTGCCAGATGC-3′ (122366); 4-2 s, 5′-GGCAGCGAATGTTCAAGG-3′ (123505); 4-2 as, 5′-TCGGTATCGGTATCTCCAGTGC-3′ (125457); 4-3 s, 5′-TCACCACCTCCTTCTCATCG-3′ (125733); 4-3 as, 5′-GTCTTATGTGACAAGTGCTGGC-3′ (127486); 4-4 s, 5′-ATGATTGCGATAACCACAGACG-3′ (127544); 4-4 as, 5′-ACTGCTCCTTCTTGTGGTCC-3′ (129275);

Abbreviations: BX-C, bithorax complex; A–P, anteroposterior; APP, *abdA* promoter-proximal probe; BPP, *AbdB* promoter-proximal probe.

†Present address: Division of Biology, Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 91125.

§To whom correspondence should be addressed. E-mail: rdrewell@caltech.edu.

GENETICS

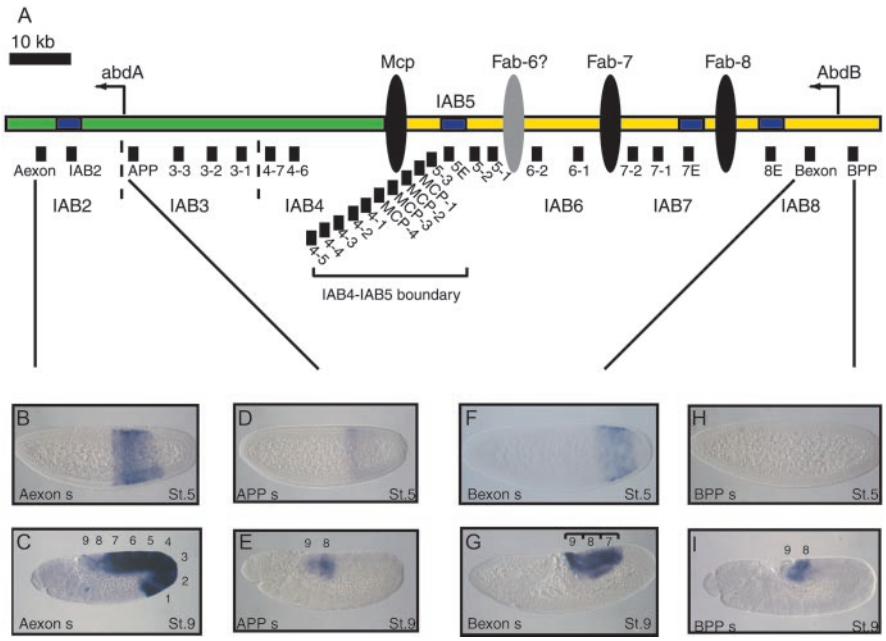

Fig. 1. Intergenic transcription at the *abdA-AbdB* locus. (*A*) Summary of *abdA-AbdB* locus. The *abdA* and *AbdB* transcription start sites are indicated by leftward arrows. The intergenic region is ≈100 kb in length. The *iab* regions that control expression of the two *Hox* genes are indicated (IAB2 to IAB8). IAB2, IAB3, and IAB4 (shown in green) regulate expression of *abdA*. IAB5, IAB6, IAB7, and IAB8 (shown in yellow) direct *AbdB* expression. The insulator DNAs that separate the different *iab* regions are indicated (black ellipses). The presumptive Fab6 insulator (gray ellipse) has yet to be identified. Characterized enhancers within the *iab* regions are shown as blue rectangles. The locations of probes used for *in situ* hybridization analysis in this study are shown as black bars under the locus. (*B–I*) Transcription patterns detected with *in situ* hybridization probes. Embryos are orientated with anterior to the left and dorsal up. Probes against the *abdA* (*B* and *C*) and *AbdB* (*F* and *G*) coding regions detect the expected distribution of transcripts (see text). An APP probe detects transcription at blastoderm stage 5 (*D*) and later in development (*E*), unlike a BPP probe, which detects transcription in abdominal segments 8 and 9 only later in embryonic development (*H* and *I*).

4-5 s, 5′-ACCACAAGAAGGAGCAGTCG-3′ (129258); 4-5 as, 5′-GCACTCTCACCTACACGAATGC-3′ (131,319); 4-6 s, 5′-CGACAGCAACATCAGCAATCGC-3′ (135,904); 4-6 as, 5′-ATGCGGTCACCATTGCTCTTCG-3′ (137,616); 4-7 s, 5′-GTCTGCTGTTGAATGTTGACCG-3′ (138,200); 4-7 as, 5′-GAAGTTCTATTGTGTAGTGGCG-3′ (139,391); 3-1 s, 5′-CATAGATACGAACTCACAGACG-3′ (140,638); 3-1 as, 5′-TATTCCGCCATTCCGTTGGACC-3′ (142,398); 3-2 s, 5′-GTGACATTCTGTTGAGCCGACC-3′ (143,635); 3-2 as, 5′-TTATGCTGCGGATTATCTTGGC-3′ (144,635); 3-3 s, 5′-GGAATAGACGAAGATGCTCAGC-3′ (146,932); 3-3 as, 5′-CGCCATCTGTATTCCGTTCG-3′ (148628); APP s, 5′-GTGGTAGCAACAACATAAGG-3′ (150762); APP as, 5′-CTATTGCTCTCATCCTCCTTCG-3′ (152745); IAB2 s, 5′-TCTACCTATCTTCTTCTGCTCC-3′ (171019); IAB2 as, 5′-TAAGACGGTGTCAGACGG-3′ (172988); Aexon s, 5′-CACCAACAGCAGCAACAACAGC-3′ (173566); and Aexon as, 5′-CATTGTATTCAAGCGTTGGC-3′ 174756.

In situ hybridizations were carried out on 2- to 4-h and 0- to 10-h yw^{67} embryos as described previously (15). *In situ* hybridizations were repeated at least three times. Expression patterns in blastoderm embryos were measured by photographing at least 10 embryos and calculating the mean domain of expression as a percentage of the total embryo length (0 = anterior tip, 100 = posterior tip).

Results

Intergenic RNAs at the BX-C. A comprehensive series of 1- to 2-kb probes that span the *iab* intergenic region between *abdA* and *AbdB* was generated (see Fig. 1*A*) and used for *in situ* hybridizations in *Drosophila* embryos. Almost all of these intergenic probes show distinct transcription patterns that are spatially modulated along the anteroposterior (A–P) axis of the blastoderm embryo.

In midstage 5 blastoderm embryos, probes mapping to the exons of the *abdA* (Fig. 1*B*) and *AbdB* genes (Fig. 1*F*) detect the expected patterns of expression (16–18). *abdA* is expressed from 53.5% to 81.4% along the A–P axis of the embryo, whereas *AbdB* is more posterior (66.6–91.1%). Additional transcription is detected with an *abdA* promoter-proximal probe (APP), which terminates ≈650 bp 5′ of the start site of the *abdA* transcription unit (14). This probe detects transcription in a sense orientation relative to *abdA* and *AbdB* expression and is restricted more toward the posterior of the embryo than the *abdA* transcript (54.9–86.6%) (Fig. 1*D*). However, a promoter-proximal probe upstream of the *AbdB* transcription unit (BPP) fails to detect any expression in blastoderm-stage embryos (Fig. 1*H*). Taken together, these results indicate that early transcription at this chromosomal location is tightly restricted to the intergenic region and the protein-coding *Hox* genes themselves. At later developmental stages, *abdA* is expressed strongly in abdominal segments 1–7 and more weakly in segment 8 (Fig. 1*C*), whereas *AbdB* is detected predominantly in segments 7–9 (Fig. 1*G*) and more weakly in segments 5 and 6 (data not shown). The APP (Fig. 1*E*) and BPP (Fig. 1*I*) probes detect similar patterns of late expression in the most posterior abdominal segments, 8 and 9. No transcription could be detected with any of these probes in the antisense orientation (data not shown).

The spatial pattern of transcription in the intergenic region is restricted in very specific anteroposterior patterns throughout early embryonic development. Distinct patterns are detected from probes mapping to each *iab* region (Fig. 2*A*). For example, probes from the *iab3* region detect transcription extending from a position slightly more posterior than the

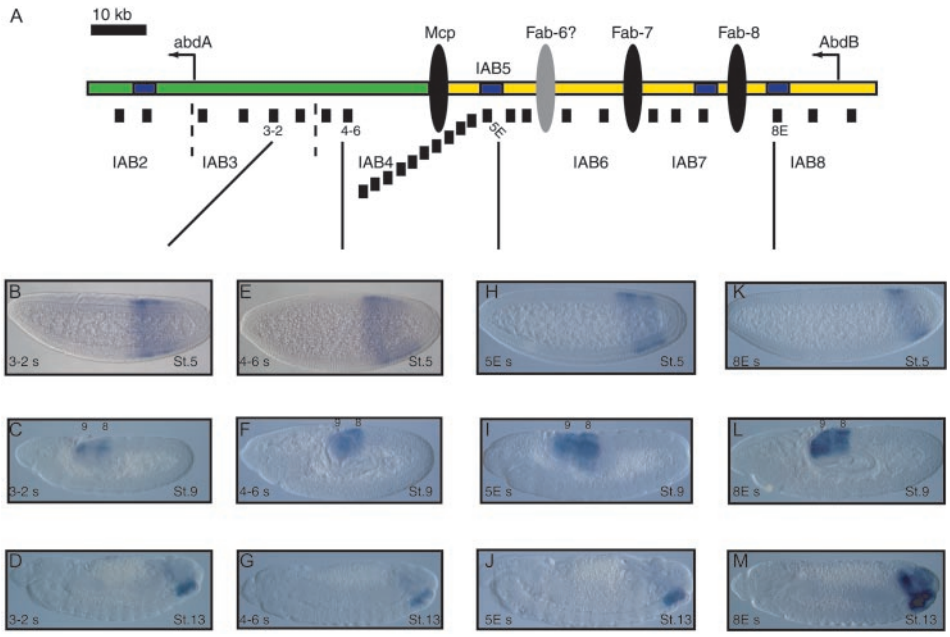

Fig. 2. Transcription in the *iab* regions. (*A*) The location of probes at the *abdA-AbdB* locus. (*B–M*) Patterns of sense transcription detected by *in situ* hybridization RNA probes. At stage 5, transcripts from the different *iab* regions show distinct distribution patterns along the A–P axis of the embryo. A probe from the *iab3* region detects expression (*B*) extending farther toward the anterior of the embryo than *iab4* (*E*). Expression patterns become increasingly restricted toward the posterior of the embryo in the *iab5* region (*H*) and *iab8* region (*K*). From stage 9 of development, transcription from all *iab* regions is restricted to the two most posterior abdominal segments, 8 and 9 (*C*, *F*, *I*, and *L*). This pattern persists through stage 13 of development (*D*, *G*, *J*, and *M*).

anterior limit of the *abdA* expression domain toward the posterior of the embryo (56.6–90.6% embryo length) (Fig. 2*B*). Accordingly, probes from the other *iab* regions detect anterior limits of expression increasingly restricted more toward the posterior pole of the blastoderm embryo as they get closer in location to *AbdB* on the chromosome. Specifically, *iab4* probes detect expression from 59.6% to 90.4% (Fig. 2*E*); *iab5* probes, from 66.1% to 89.9% (Fig. 2*H*); *iab6* probes, from 68.1% to 91.3%; *iab7* probes, from 70.2% to 90.8%; and an *iab8* probe, from 71.9% to 91.2% (Fig. 2*K*). Interestingly, in many of the embryos examined, the probes detect stronger expression at the anterior region of their expression domain when compared to the signal at the posterior region (Figs. 1 and 2). Furthermore, no significant differences are seen between probes from within the same *iab* region, even though some of the probes map to known cis-regulatory elements (for example, enhancers 5E, 7E, and 8E), whereas others are from intergenic sequences of currently unknown function. The IAB2 enhancer is located in an intron of the *abdA* transcription unit and, accordingly, gives the same pattern of expression as the probe against the *abdA* exon.

The temporal pattern of expression detected by almost the entire series of probes is consistent. In blastoderm embryos, transcription is detected from late stage 4/early stage 5 (19), before the completed cellularization of the blastoderm. The initiation of intergenic transcription therefore precedes activation of either *abdA* or *AbdB*, which, in agreement with the observations of earlier studies (12), is first detectable from midstage 5 onward. To confirm this observation, embryos were cohybridized with probes against the *iab4s* transcript and the segment polarity gene *engrailed*. Early in development, the distinct stripes of the *engrailed* pattern are seen initially as anterior stripes in stage 5 embryos (20, 21). In late stage 4 embryos, *iab4s* transcription is already detectable in posterior regions, before *engrailed* expression (Fig. 3*A*). By early stage 5, transcription is detected in the posterior *iab4s* pattern and in the emerging anterior *engrailed* stripes (Fig. 3*B*).

Colinear Transcription Program. The anterior expression limit of the sense-orientation transcripts in the embryo correlates with their arrangement on the chromosome, demonstrating that the colinearity of expression previously characterized for the protein-coding *Hox* genes at the BX-C (22) is maintained throughout the intergenic *iab* region. This colinear relationship is demonstrated clearly when the domains of transcription are plotted on a graph (Fig. 3*C*). Probes from each *iab* region have distinct anterior limits that become restricted more toward the posterior of the embryo as the *iab* region is located closer to AbdB on the chromosome. Intriguingly, probes from *iab5*, *iab6*, *iab7*, and *iab8*, the regions known to regulate *AbdB* expression, detect transcription patterns that are restricted to the domain of *AbdB* expression, whereas probes from *iab3* and *iab4*, from regions known to direct expression of *abdA*, detect transcription extending into the domain of *abdA* expression (Fig. 3*C*). The distinct transcription patterns observed indicate that the different transcripts may be firing from discrete promoters within each *iab* region.

Only four of our intergenic probes fail to detect early sense transcription by *in situ* hybridization: 4-5, 4-4, MCP-4, and 6-1. However, probes 4-5, 4-4, and 6-1 detect transcription in the antisense orientation. Their transcription patterns are presented in the following section. All other probes fail to detect transcription in the antisense orientation. Later in embryonic development, all probes detecting sense transcripts, except those against *abdA* and *AbdB*, detect uniform transcription only in the two most posterior abdominal segments, 8 and 9. During germband extension (stage 9), expression is in both the ectoderm and mesoderm (Fig. 2). By stage 13, the transcripts are expressed strongly in the ventral nerve cord and more weakly in other posterior tissues (Fig. 2).

Antisense Transcripts in *iab* Regions. A further level of complexity in the cis-regulatory program of the *iab* regions was revealed with probes capable of detecting antisense transcription at the intergenic region. Almost no antisense transcription is detect-

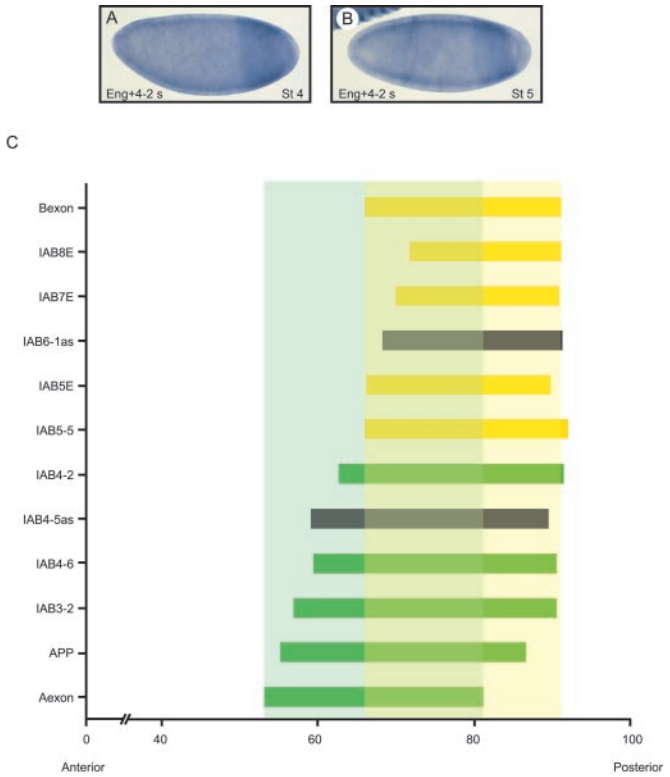

Fig. 3. Temporal activation of sense *iab* transcripts. (*A*) At late stage 4 in embryos hybridized with probes against *engrailed* and *iab4s* transcripts, expression is detected only in the posterior *iab4* domain, before the appearance of anterior *engrailed* expression. (*B*) In early blastoderm stage 5 embryos, sense transcripts can be detected in the *iab4* domain and the anterior *engrailed* stripe 2. (*C*) Colinear distribution of transcripts in the *iab* regions. Measure of transcript distribution is shown as a percentage of embryo length. The anterior tip of the embryo corresponds to 0 and the posterior tip corresponds to 100. The probes are listed according to their order on the chromosome (see Fig. 1*A*), with the exception of probes against the two *Hox* genes, Aexon (green) and Bexon (yellow), which are shown at the bottom and the top of graph, respectively. Colinearity between the chromosomal order of probes and their transcription patterns is observed. The anterior limits of transcription for probes from the *iab5*, *iab6*, *iab7*, and *iab8* regions are restricted within the *AbdB* domain of expression (pale yellow), whereas probes from *iab3* and *iab4* regions detect patterns extending into the *abdA* expression domain (pale green). The distribution of the antisense transcripts detected in *iab4* and *iab6* are also colinear with their chromosomal locations and are shown in black.

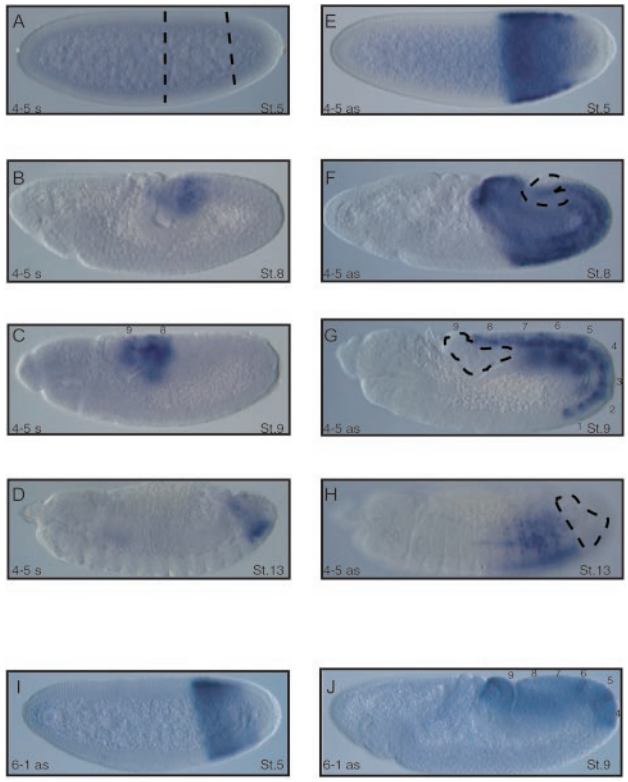

Fig. 4. Antisense transcripts in the *iab* regions. (*A–D*) Sense transcription pattern at *iab4* region during embryonic development detected by probe 4-5. No transcript is detectable at stage 5 (*A*). Transcription is detectable at stage 8 and, by stage 9, is restricted to abdominal segments 8 and 9. Transcription persists in the two most posterior abdominal segments through stage 13 (*D*). (*E–H*) The *iab4as* transcript (23) also is detected by probe 4-5, although the pattern is distinct from that of sense transcription. At blastoderm stage 5, the antisense transcript is expressed strongly in the *iab4* domain (*E*) and persists in abdominal segments 1–7 through developmental stages 8 (*F*), 9 (*G*), and 13 (*H*), but is excluded from the most posterior segments. The distributions of the *iab4as* and *iab4* sense transcripts appear to be mutually exclusive. (*I* and *J*) A novel antisense transcript is detected by probe 6-1. At blastoderm stage 5, the transcript is restricted to the *iab6* domain (*I*) and, by stage 9, is expressed strongly in abdominal segments 4, 5, and 6 and more weakly in 7, 8, and 9 (*J*). The sense transcription detected by probe 6-1 (data not shown) is mutually exclusive to the *iab6as* pattern.

able throughout the intergenic region. However, our panel of *in situ* probes does detect the previously characterized *iab4as* transcript (probes 4-5 and 4-4; see Fig. 1*A*) (23) as well as a previously unidentified antisense transcript in the *iab6* region (probe 6-1; see Fig. 1*A*). Careful analysis of the *in situ* hybridization signal in embryos shows that the expression patterns for the antisense transcripts are distinct from those of the sense transcripts already described.

The *iab4as* transcript is expressed in blastoderm-stage embryos in the same domain as the sense transcript detected by the adjacent 4-6 probe (Figs. 4*E* and 2). However, the *iab4as* transcript appears to preclude sense transcription from the opposite strand on the chromosome, because no early sense transcription is detected with the 4-5 probe (Fig. 4 *A* and *E*). Sense transcripts can be detected around stage 8 of embryonic development in posterior abdominal segments 8 and 9 (Fig. 4*B*). Intriguingly, the broadly expressed *iab4as* transcript is absent from these segments (Fig. 4*F*). The mutually exclusive expression patterns of *iab4as* and *iab4s* become even more apparent slightly later in development; the *iab4* sense transcription persists in abdominal segments 8 and 9, whereas the *iab4as* transcript is restricted predominantly to abdominal segments 2–7 (Fig. 4 *C* + *G* and *D* + *H*).

The *iab6as* transcript demonstrates a similar mutual exclusivity of expression with sense transcription from its chromosomal location. The *iab6as* transcript is expressed early in the embryo in the *iab6* domain (Figs. 4*I* and 2) and becomes predominantly restricted to abdominal segments 4–7 later in development (Fig. 4*J*). Further characterization of the *iab6as* transcript is needed. Exon prediction software fails to identify any significant ORFs in the *iab6* region, suggesting the *iab6as* transcript is a noncoding RNA, as is the case for the *iab4as* transcript. The role of these enigmatic antisense transcripts will be discussed later.

Discussion

Colinear Transcription Program at BX-C *iab* Regions. Previous studies have shown that the *iab* regions of the BX-C are transcribed, although the resolution provided by these studies was unsuitable to accurately assess the functional potential for these RNAs

(11–13). Here, we show that transcription through these cis-regulatory regions is abundant and subject to a highly ordered developmental program. The early expression of sense transcripts (relative to the direction of *abdA* and *AbdB* expression) from the different *iab* regions is organized into sequential domains along the A–P axis of the developing embryo. This organization is reminiscent of the colinearity exhibited by the BX-C homeotic genes themselves, which are expressed in the same order along the A–P axis of the embryo as they are organized along the chromosome (1). The intergenic transcripts follow the same rule, because there is colinearity between the location of the *iab* regions on the chromosome and the anterior limit of transcription in the blastoderm-stage embryo. In this way, regions increasingly closer to *AbdB* are expressed in increasingly more posterior domains in the embryo, with transcripts from each individual *iab* region showing unique, spatially restricted patterns of expression (see Fig. 2). The pattern of transcription from each *iab* region corresponds to the segmental domain of the embryo that is affected by mutations in each particular *iab* region (10). Therefore, it is conceivable that the early sense transcripts could define the domains of activity for cis-regulatory elements within each *iab* region.

The timing of expression in the intergenic region is also significant. If the sense transcripts indeed are capable of defining the domains of activity for cis-regulatory elements in the *iab* regions, then it would be expected that they are transcribed before the time at which the cis-regulation is required. The *iab* transcripts in fact are detectable by late stage 4/early stage 5 of embryonic development, before the time at which expression is seen from the *abdA* or *AbdB* genes (16–18). Earlier studies also noted this temporally restricted order of transcription (12). The spatial and temporal distribution of the sense transcripts represents the earliest known response of the BX-C to the hierarchical positioning information inherited in the embryo from the gap and pair-rule genes (11, 24, 25). It appears that the early transcripts represent an initial primed state of the BX-C and, therefore, could act to define the domains of activity for the *iab* regions in the embryo. This activity has some parallels with the intergenic transcription that has been characterized at mammalian genes. For example, in the *Ig* genes, germ-line transcription through cis-regulatory elements is thought to activate interactions with regulatory proteins that are necessary to direct the switching of the class of *Ig* gene expressed in individual cells (26, 27). The *iab* transcripts may play a similar role in regulating *abdA* and *AbdB* gene expression.

Interplay of Transcription with Cis-Regulatory Elements. Later in development, all of the intergenic sense transcripts are restricted to expression in the two most posterior abdominal segments. Why the transcription persists late in development is unclear. It is possible that once the domains of activity for the *iab* regions are established by the early transcripts, other factors may maintain the *iab*-regulated expression of the target homeotic genes. The transcriptional regulatory proteins of the Polycomb group and Trithorax group are good candidates for this role because they are known to be necessary to maintain expression states for the homeotic genes in the BX-C (28–30). They act through cis-regulatory elements in the *iab* regions, which have been identified as Polycomb response elements or cellular memory modules (31), to promote either a silenced or activated state throughout development by generating stable, higher-order chromatin structures (32). It is possible that the transcription we detect through the Polycomb response elements early in development primes their segment-specific activity, which is heritable through future cell divisions. Once the Polycomb response elements are activated, they are able to maintain the regulation of the homeotic gene-expression patterns, and, consequently, the *iab* transcripts are no longer required. One prediction of this model is that ectopic transcription through the *iab* Polycomb response elements later in development may interfere with Polycomb-mediated silencing. The continued expression of the *iab* transcripts in abdominal segments 8 and 9 late in development may indicate that homeotic gene expression in these segments is not regulated by the Polycomb or Trithorax group proteins and that the functional role for the transcripts consequently persists.

The potential role of the antisense transcripts remains enigmatic. The previously characterized *iab4as* transcript (23) is known to be processed, although it appears to have no protein-coding potential. Our identification of an additional antisense transcript in the *iab6* domain may suggest a shared function. One possibility is that the antisense transcripts contribute to the inhibition of the spreading of the sense transcripts from one *iab* region to another, because they prevent transcription from the opposite DNA strand (Fig. 4 *A–J*). Their chromosomal locations, relatively close to the insulator elements *Mcp* and *Fab-7*, are consistent with this notion. It is possible that the antisense transcripts may be processed and function to inhibit the sense transcripts by an RNA interference mechanism, similar to the silencing characterized in *Schizosaccharomyces pombe* (33). However, despite extensive attempts, we have failed to identify antisense transcripts in the *iab5* or *iab7* regions. Further molecular characterization of the sense and antisense transcripts and analysis of genetic mutations at the BX-C will be necessary to further facilitate elucidation of their *in vivo* functional activities.

We thank Katharine Arney, Andrew Dowsett, and Joanne Topol for comments on the manuscript and Mark Stapleton for providing the *engrailed* probe. This work was supported by grants from the National Institutes of Health to M.L. (GM34431) and E.B.L. (HD06331). R.A.D. was funded by a Wellcome Trust Prize Traveling Fellowship.

1. Lewis, E. B. (1978) *Nature* **276,** 565–570.
2. Sanchez-Herrero, E., Vernos, I., Marco, R. & Morata, G. (1985) *Nature* **313,** 108–113.
3. Duncan, I. (1987) *Annu. Rev. Genet.* **21,** 285–319.
4. Lewis, E. B., Knafels, J. D., Mathog, D. R. & Celniker, S. E. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8403–8407.
5. Morata, G., Sanchez-Herrero, E. & Casanova, J. (1986) *Cell Differ.* **18,** 67–78.
6. Sanchez-Herrero, E. (1991) *Development (Cambridge, U.K.)* **111,** 437–449.
7. Boulet, A. M., Lloyd, A. & Sakonju, S. (1991) *Development (Cambridge, U.K.)* **111,** 393–405.
8. Celniker, S. E., Sharma, S., Keelan, D. J. & Lewis, E. B. (1990) *EMBO J.* **9,** 4277–4286.
9. Lewis, E. B. (1996) in *Les Prix Nobel 1995* (Nobel Foundation, Stockholm), pp. 235–260.
10. Karch, F., Weiffenbach, B., Peifer, M., Bender, W., Duncan, I., Celniker, S., Crosby, M. & Lewis, E. B. (1985) *Cell* **43,** 81–96.
11. Casares, F. & Sanchez-Herrero, E. (1995) *Development (Cambridge, U.K.)* **121,** 1855–1866.
12. Sanchez-Herrero, E. & Akam, M. (1989) *Development (Cambridge, U.K.)* **107,** 321–329.
13. Zhou, J., Ashe, H., Burks, C. & Levine, M. (1999) *Development (Cambridge, U.K.)* **126,** 3057–3065.
14. Martin, C. H., Mayeda, C. A., Davis, C. A., Ericsson, C. L., Knafels, J. D., Mathog, D. R., Celniker, S. E., Lewis, E. B. & Palazzolo, M. J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8398–8402.
15. Ohtsuki, S., Levine, M. & Cai, H. N. (1998) *Genes Dev.* **12,** 547–556.
16. Karch, F., Bender, W. & Weiffenbach, B. (1990) *Genes Dev.* **4,** 1573–1587.
17. Kuziora, M. A. & McGinnis, W. (1988) *EMBO J.* **7,** 3233–3244.
18. DeLorenzi, M., Ali, N., Saari, G., Henry, C., Wilcox, M. & Bienz, M. (1988) *EMBO J.* **7,** 3223–3231.
19. Bownes, M. (1975) *J. Embryol. Exp. Morphol.* **33,** 789–801.
20. Tabata, T., Eaton, S. & Kornberg, T. B. (1992) *Genes Dev.* **6,** 2635–2645.

21. Drees, B., Ali, Z., Soeller, W. C., Coleman, K. G., Poole, S. J. & Kornberg, T. (1987) *EMBO J.* **6,** 2803–2809.
22. McGinnis, W. & Krumlauf, R. (1992) *Cell* **68,** 283–302.
23. Cumberledge, S., Zaratzian, A. & Sakonju, S. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3259–3263.
24. Harding, K. & Levine, M. (1988) *EMBO J.* **7,** 205–214.
25. Ingham, P. W. & Martinez-Arias, A. (1986) *Nature* **324,** 592–597.
26. Laurencikiene, J., Deveikaite, V. & Severinson, E. (2001) *J. Immunol.* **167,** 3257–3265.
27. Xu, M. & Stavnezer, J. (1992) *EMBO J.* **11,** 145–155.
28. Karch, F., Galloni, M., Sipos, L., Gausz, J., Gyurkovics, H. & Schedl, P. (1994) *Nucleic Acids Res.* **22,** 3138–3146.
29. Busturia, A. & Bienz, M. (1993) *EMBO J.* **12,** 1415–1425.
30. Hagstrom, K., Muller, M. & Schedl, P. (1997) *Genetics* **146,** 1365–1380.
31. Cavalli, G. & Paro, R. (1999) *Science* **286,** 955–958.
32. Pirrotta, V. (1998) *Cell* **93,** 333–336.
33. Volpe, T. A., Kidner, C., Hall, I. M., Teng, G., Grewal, S. I. & Martienssen, R. A. (2002) *Science* **297,** 1833–1837.