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Molecular and genetic organization of *Drosophila melanogaster* polytene chromosomes: evidence for two types of interband regions

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Abstract

The 3A and 60E regions of *Drosophila melanogaster* polytene chromosomes containing inserted copies of the P{lArB} transposon have been subjected to an electron microscopic (EM) analysis. We show that both inserts led to formation of new bands within the interband regions 3A4/A6 and 60E8-9/E10. This allowed us to clone DNA of these interbands. Their sequences, as well as those of DNA from other four interbands described earlier, have been analyzed. We have found that, with the exception of 60E8-9/E10 interband, all other five regions under study corresponded to 5′ or 3′ ends of genes. We have further obtained the evidence for 60E8-9/E10 interband to harbor the 'housekeeping' *RpL19* gene, which is transcribed in many tissues, including salivary glands. Based upon the genetic heterogeneity of the interbands observed a revised model of polytene chromosome organization is discussed.

Introduction

It has been long proposed that the differential condensation of eukaryotic interphase chromatin could be considered as an important mechanism regulating gene activity, recombination, and DNA replication. Such condensation can be observed directly in *Drosophila* salivary gland polytene chromosomes as a reproducible pattern of densely packed bands and less packed puffs and interbands. Extensive studies of changes in polytene chromosome morphology in the course of fly development have clearly established the link between the decondensation of bands into puffs and the activation of genes in corresponding regions (reviewed in: Zhimulev, 1999). However, the functional organization of interbands and the nature of banding pattern in general still remain enigmatic. According to different estimates, the size of interband DNA ranges in length from 0.5 to 3.8 kb and it constitutes about 5% of euchromatic DNA (Beermann, 1972; Sorsa, 1984; for review: Zhimulev, 1996). Features of interbands such as localization of RNA polymerase II (Jamrich, Greenleaf & Bautz, 1977; Sass & Bautz, 1982), incorporation of [^{3}H] uridine (Semeshin, Zhimulev & Belyaeva, 1979), presence of DNA:RNA hybrids (Vlassova et al., 1985) and products of transcription – RNP granules (Skaer, 1977; Mott & Hill, 1986) – led to a widely accepted hypothesis that interbands contain permanently transcribed 'housekeeping' genes (Gersh, 1975; Zhimulev & Belyaeva, 1975). Other chromosomal models suggesting that a band and an interband may form a functional unit (gene coding part plus its regulatory region) have also been proposed (Crick, 1971; Paul, 1972; Sorsa, 1984). More recent data pointed out to the interesting possibility for the interbands to represent boundary elements that delimit chromosomal domains. Thus, a special chromatin structure (scs') is thought to restrict

decondensation of a distal part of the 87A7 band after induction of the *hsp70* gene located within it (Udvardy, Maine & Schedl, 1985). The scs' is able to block enhancer-promoter communications in transgenic assays (Kellum & Schedl, 1992; Vazquez & Schedl, 1994) and is capable to bind BEAF-32 protein (Zhao, Hart & Laemmli, 1995). BEAF-32, which seems to be essential for the scs' function (Zhao, Hart & Laemmli, 1995; Cuvier, Hart & Laemmli, 1998), was shown to be localized to hundreds of interbands in polytene chromosomes, suggesting that these regions have the same functions as scs' (Zhao, Hart & Laemmli, 1995). Later Vazquez & Schedl (2000) gave more support to this hypothesis by demonstrating that the DNA fragment removed by the deletion fa^{swb}, which impairs *Notch* function and is located, most probably, inside of the 3C6/C7 interband (Rykowski et al., 1988), also contains an element capable of blocking enhancer-promoter communications.

To distinguish between the above-mentioned hypotheses, the analysis of many interbands is required. The approach based on EM analysis of polytene chromosomes carrying P transposons makes this possible. Indeed, it has been shown that P element constructs of different molecular organization tend to insert predominantly into interbands (Semeshin et al., 1986, 1989). This finding provides an opportunity to clone DNA fragments from interband regions, either by constructing genomic libraries from transformed strains and probing them with the insert DNA or by using the plasmid rescue assay (O'Kane & Gehring, 1987). Nucleotide sequences of three interbands have thus been determined (Demakov, Semeshin & Zhimulev, 1993; Schwartz, Demakov & Zhimulev, 1998). Primary analyses of these sequences revealed that they are unique in the *Drosophila melanogaster* genome, lack putative protein-coding reading frames and known genes. They were shown to contain numbers of AT-rich regions, polypurine/polypyrimidine, and purine-pyrimidine tracts, which promote specific association of interband DNA with the nuclear matrix *in vitro* (Schwartz et al., 1999). However, it is not clear whether other interbands share the same features.

To further extend the study of the molecular and genetic organization of interband regions, we have used the EM approach and cloned the DNA from two additional interbands, namely, 3A4/A6 and 60E8-9/E10. We have analyzed these and the earlier obtained interband sequences (Demakov, Semeshin & Zhimulev, 1993; Schwartz, Demakov & Zhimulev, 1998), as well as the putative interband sequence from 3C6/C7 region of the X-chromosome (Keppy & Welshons, 1977; Ramos et al., 1989), for the presence of coding regions and transcription activity in salivary glands.

Materials and methods

Flies

D. melanogaster lines 148 and 55, containing P{lArB} construct (O'Kane & Gehring, 1987) with genotypes *P{lArB}3A4-6*; ry^{506} and *P{lArB}60E7-10*; ry^{506}, respectively, were kindly provided by L. Omel'yanchuk. $Df(1)fa^{swb}$ stock was generously provided by S. Artavanis-Tsakonas. The Oregon-R stock used in these studies has been maintained in our laboratory for many years.

Cytology

Preparations of *D. melanogaster* polytene chromosome squashes for EM analysis were done as previously described (Semeshin et al., 1982).

DNA clones

A *Drosophila* genomic library in λDASHII phage vector was provided by M. Noll. Sets of cosmid clones containing DNA fragments from 3A and 60E regions were kindly provided by the European *Drosophila* Genome Project (EDGP) (Siden-Kiamos et al., 1990). The LD02836 cDNA clone was provided by D. Harvey (Berkeley *Drosophila* Genome Project (BDGP) EST collection). The 60E-2,1S and 61C-3,8HB DNA clones in a plasmid vector pBluescript II-KS (pBS-KS) (Stratagene) as well as pWhiteRabbit DNA (constructed by Nicholas Brown) as a *white*-specific probe were used for Northern blot analysis.

Nucleic acid purification and analysis

All standard DNA and RNA manipulations were performed as described elsewhere (Sambrook, Fritsch & Maniatis, 1989). Total RNA was extracted and poly $(A)^+$ RNA was purified on oligo-(dT) cellulose, according to the same man-

ual, from embryos (0–12 h), first-instar (24–48 h) and second-instar (48–72 h) larvae, early pupae (0–1 h after puparium formation), late third-instar larval salivary glands, late third-instar larvae, and adult flies. The procedure of plasmid rescue was carried out as described earlier (Schwartz, Demakov & Zhimulev, 1998).

Analysis and alignment of DNA sequences

P2827 primer (5′-TCCTTTCACTCGCACTT-3′) complementary to the 3′ end of the P element and T3 primer from pBS-KS were used for sequencing of plasmid rescued DNA clones. Primers specific for the cDNA clone LD02836 were used for sequencing of this DNA. All sequences were determined for both strands on ABI310-Prism automated sequencing system.

Sequences from interband regions were compared to the BDGP database of *Drosophila* genomic sequences, using BLASTN program (Altschul et al., 1997; http://www.flybase.net/blast). Sequence analysis was performed with MAR-Finder (Singh, Kramer & Krawetz, 1997; http://www.futuresoft.org/MAR-Wiz) and SMARTest (Frisch et al., 2000; http://genomatix.gsf.de/cgi-bin/smartest_pd/smartest.pl). Table 1 shows the coordinates of nucleotide sequences of genomic scaffolds (Adams et al., 2000), where the interband sequences and P insertions analyzed were detected.

Table 1. Positions of P transposons inserted in interbands analyzed within scaffolds of genomic DNA sequences from BDGP database

Interbands	Scaffold	Insertion site[a]
3A4/A6	AE003424	..aatcaatatc 5′-3′ gctccgccgc.. (231917)
60E8-9/E10	AE003465	..ctttaggtat 5′-3′ aaacgaacta.. (276286)
61C7/C8	AE003469	..caagatgaaa 5′-3′ gatcggcgca (12171)
85D9/D10	AE003682	..tacgctaggc 3′-5′ gatttctaca.. (102633)
86B4/B6	AE003687	..tctcctttgg 3′-5′ cgtgcacgtc.. (3380)

[a] Exact positions of inserts on the sequences are given in parenthesis. Also 5′ and 3′ ends of P transposons and genomic sequences flanking them are represented.

Results

EM analysis of interband regions 3A4/A6, 3C5-6/C7 and 60E8-9/E10

We examined by EM the 3A and 60E chromosomal regions (strains 148 and 55, respectively), in which the P{lArB} transposon was found to be integrated, as determined by *in situ* hybridization with a *lacZ* DNA probe (not shown). The banding pattern of the 3A subdivision in the untransformed Oregon R strain differed from that on Bridges' map (1938) (Figure 1(A)) as follows: the 3A1-2 doublet of Bridges' map corresponds to two singlets, and the bands denoted by Bridges as 3A5 and 3A10 are absent (Figure 1(B)). In the transformed strain 148, a new band appears between the 3A4 and 3A6 bands (Figure 1(C)) and it is not seen in the untransformed strain (Figure 1(B)). In subdivision 60E, the banding pattern of the Oregon R strain (Figure 1(E)) slightly differs from that on Bridges' map (Figure 1(D)). Thus, 60E1-2, 60E5-6 and 60E8-9 bands should be considered as singlets and 60E3 and 60E7, two single bands in Bridges' map, were not revealed by EM. Insertion of the transposon into the region results in the appearance of a new band within the 60E8-9/60E10 interval (Figure 1(F)). Note that the new bands are morphologically indistinguishable from the adjacent bands 3A6 and 60E8-9 (Figure 1(C),(F)). In the case of strain 148, EM *in situ* hybridization indicated that the insert correspond to the band located to the left of 3A6 (Semeshin et al., 1998). In strain 55, according to our molecular data presented below, the transposon is located approximately 1 kb distally to the *RpL19* gene (Figure 2). Using complementation analysis of deletions that affect the 60E region, Hart Klein and Wilcox (1993) mapped this gene to the interval 60E8-9/60E10. Thus, we conclude that the insertions of P{lArB} in lines 148 and 55 occurred within the 3A4/A6 and 60E8-9/E10 interbands, respectively.

Under the light microscope the fa^{swb} deficiency appears as a fusion of bands 3C5-6 and 3C7, which was hypothesized to result from the elimination of the interband material between them (Keppy & Welshons, 1977). However, light microscopy did not permit determining how complete the loss of the interband material in this mutant was. Here we performed the EM analysis of the 3C region in

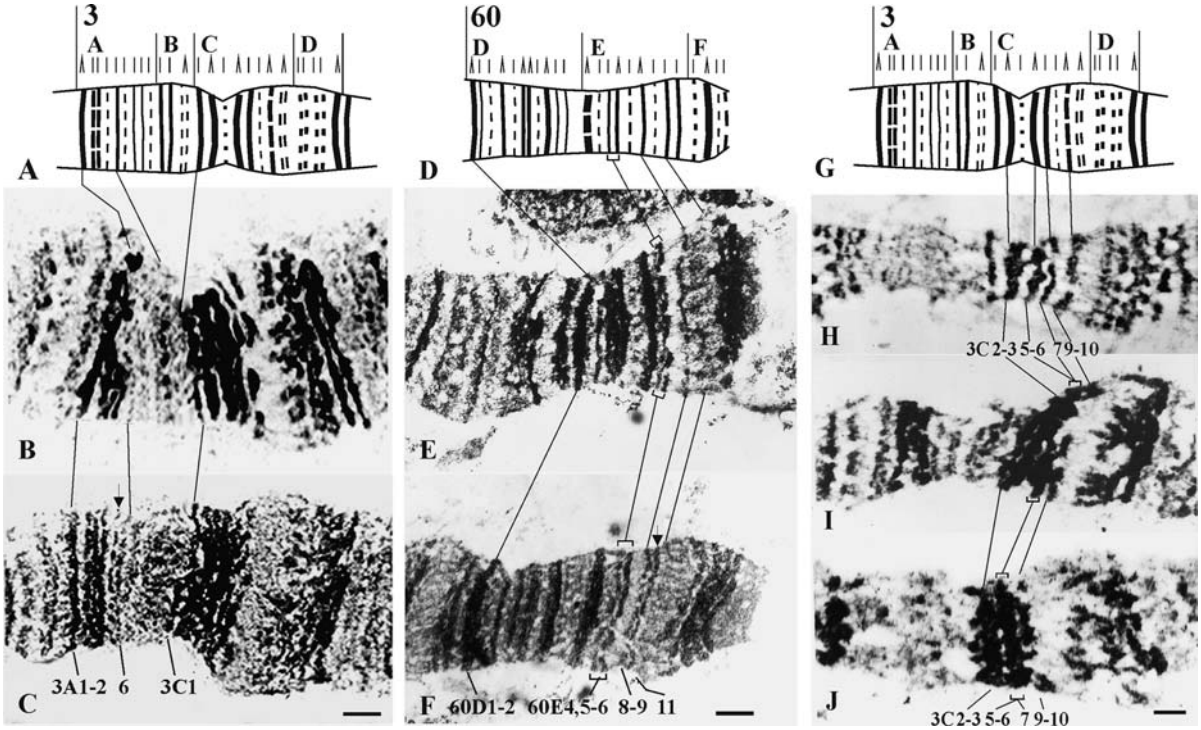

Figure 1. EM maps of the 3A and 3C regions of the X-chromosome and the 60E region of the 2R-chromosome. A, D, G Bridges' map; B, E, H wild-type Oregon-R strain; C transformed line 148; F transformed line 55; I, J *Df(1)fa*^{swb} line. Arrows point to the new bands resulting from the P{lArB} insertions. Bars represent 1 μm.

males carrying the deficiency. Male X chromosomes were used for EM analysis since these are more easily stretched and provide better resolution than female chromosomes. According to Bridges' map, four of the 12 bands in this subdivision are doublets (Figure 1(G)). In the Oregon R strain used as a control, all the bands appeared as singlets (Figure 1(H)). We failed to detect thin single bands 3C4 and 3C8, which are indicated on Bridges' map. It should also be noted that the 3C11-12 doublet is often decondensed and usually it is not visible in EM preparations (Figure 1(H)–(J)). It contains the *sgs4* gene, which is active in salivary glands (Korge et al., 1980). So, the 3C1/3C10 interval contains five single bands including 3C5-6 and 3C7, which are separated by tiny interbands (Figure 1(H)). A similar banding pattern was observed in the strain homozygous for the *fa*^{swb} deletion. The only difference was related to the bands 3C5-6 and 3C7, which appeared fused and formed a solid band without any decompacted material within it (Figure 1(I) and (J)). Thus, the deletion causes a complete removal of the 3C6/C7 interband.

Cloning of 3A4/A6 and 60E8-9/E10 interband DNA

To clone the DNA of interbands 3A4/A6 and 60E8-9/E10 DNAs we obtained the genomic sequences adjacent to the 3' end of the P{lArB} transposon (clones 3A-5S and 60E-2.1S, Figure 2) by the plasmid rescue technique (O'Kane & Gehring, 1987). The sequences of the transposon insertion sites were determined (Table 1) and DNA fragments overlapping these sites in transgenic flies were subcloned (Figure 2). The subclones permitted to determine the sequences of interband regions of approximately 2 kb at either side of the insertion site. These sequences were compared with the *Drosophila* genome database (Altschul et al., 1997; Adams et al., 2000). They had high level of similarity to those found in scaffolds AE003424 (3A5/A6) and AE003465 (60E8-9/E10). No significant similarity with other *D. melanogaster* genomic sequences was found for the DNA from the 3A4/A6 and 60E8-9/E10 interband regions.

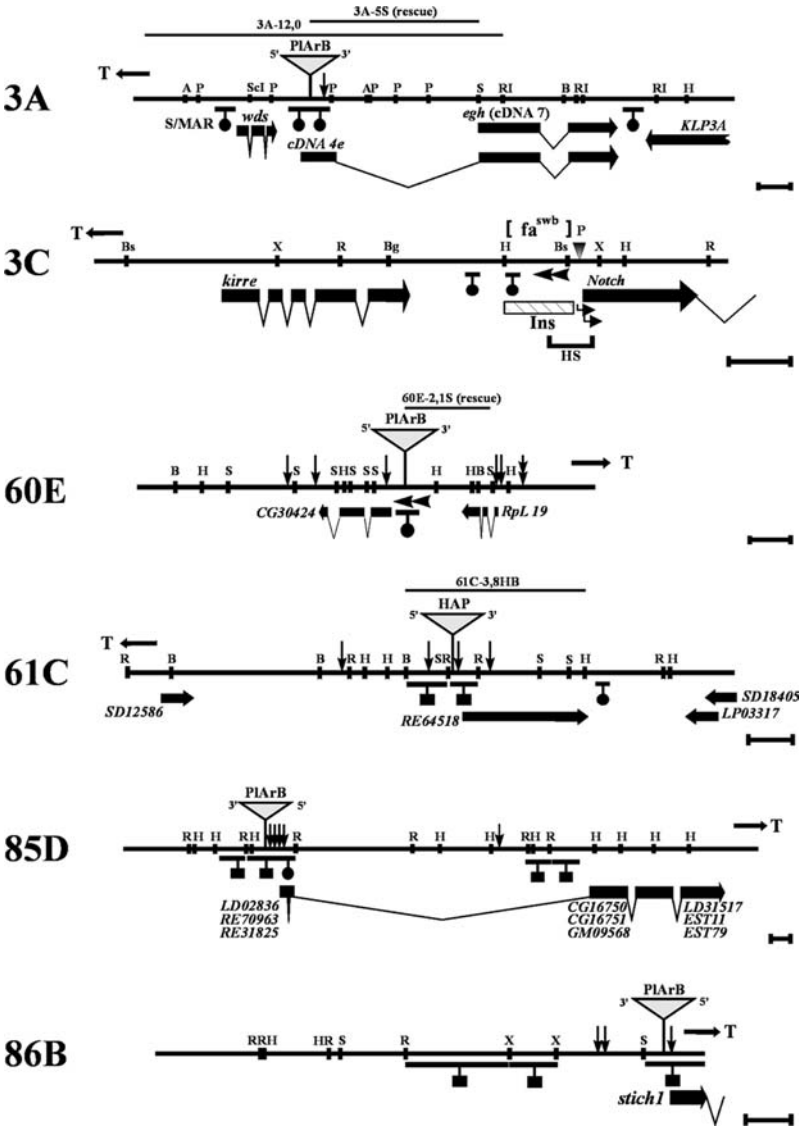

Figure 2. Molecular and genetic maps of the 3A, 3C, 60E, 61C, 85D and 86B regions. On top: *Triangles* show the transposons used for interband DNA cloning. Localization of P element insertions according to FlyBase is marked by *vertical arrows*. *Horizontal lines* show DNA fragments used in this study. For the 3C region, 'hot' spot for P insertions is represented by an *arrowhead*; *square brackets* delimit deficiency fa^{swb}. On center: Restriction maps of the regions. Restriction sites nomenclature: A, *Apa*II; B, *Bam*HI; Bg, *Bg*lI; Bs, *Bss*HII; H, *Hind*III; R, *Eco*RI; P, *Pst*I; S, *Sal*I; Sc, *Sac*I; X, *Xho*I. *Horizontal arrows* point toward the telomere (T). On bottom: Positions of known genes and transcripts are marked by *filled arrows* (thick lines, exons; thin lines, introns). S/MAR fragments identified experimentally and predicted are represented in *squares* and *circles,* respectively. For 3C and 60E regions, *arrowheads* mark direct repeats. INS and HS show insulator and nuclease hypersensitive areas, respectively (Vazquez & Schedl, 2000). Bars represent 1 kb.

Molecular and genetic characteristics of interband regions

Here we analyze in molecular terms two interband sequences cloned in this study and those of four interbands cloned earlier (Demakov, Semeshin & Zhimulev, 1993; Schwartz, Demakov & Zhimulev, 1998). These data, along with the information available from BDGP databases (Altschul et al., 1997; Adams et al., 2000), permitted to draw up their molecular and genetic maps (Figure 2).

Region 3A4/A6
In strain 148, the P{lArB} transposon is inserted in the 5′ UTR of the *egh* gene adjacent to the 3′ region of the *wds* gene (Figure 2). *egh* encodes a transmembrane protein and is expressed in ovaries (Goode et al., 1996) whereas the *wds* gene product is presumably a transcription factor (Hollmann, 2000). In strain EP(X)0804, another P transposon is inserted at 522 bp proximal to P{lArB}. Flies homozygous for each of these inserts are fertile.

Region 3C5-6/C7
Using literature data (Keppy & Welshons, 1977; Ramos et al., 1989) and BDGP data, we compiled a molecular and genetic map of this region (Figure 2). It contains the 3′ end of *kirre* and the 5′ region of *Notch*. *kirre* encodes a membrane protein with the immunoglobulin-2-type domain, which contributes to cell adhesion upon myocyte fusion. Data on transcriptional activity of this gene in salivary glands are not available. *Notch*, which participates in the control of differentiation, is transcribed in most tissues during the early developmental stages (Ramos et al., 1989), but is not active in salivary glands of third-instar larvae (references in Rykowski et al., 1988). The fa^{swb} deletion does not include the transcribed region of the gene, and the sequence removed by this deletion exhibits strong insulator functions (Vazquez & Schedl, 2000). A substantial part of P element insertions (13 events) in this locus occurred within a small (about 100 bp) region located immediately upstream of *Notch* promoter region (Kelley et al., 1987).

Region 60E8-9/E10
In strain 55, the P{lArB} insertion occurred in the proximal unit (157 bp in length) of a complete tandem repeat. This insertion is located at 1090 bp from the 3′ end of the *RpL19* gene, which encodes for the ribosomal protein L19 (Hart, Klein & Wilcox, 1993) and at 114 bp from the 5′ end of the cDNA of *CG30424* gene with unknown functions. As referenced in FlyBase, seven other sites of P element insertions (*EP(2)2333*, *EP(2)2334*, *EP(2)0755*, *EP(2)2127*, *l(2)k03704*, *EP(2)0835* and *EP(2)0840*) cluster mainly 5′ of the *CG30424* and *RpL19* gene.

Region 61C7/C8
In addition to the pHAP transposon insertion (Bonner et al., 1984; Semeshin et al., 1989; Demakov, Semeshin & Zhimulev, 1993), four other P transposon insertions (*EP(3)3208*, $fs(3)neo^{1}$, *l(3)L1170* and *l(3)05967*) have been found in this region (Figure 2). The latter two insertions are located proximal to the pHAP insertion site and are reported to be recessive lethals. Moreover, *l(3)05967* suppresses the expression of one of the *Krüppel* alleles, $Kr^{If\text{-}1}$, which affects eye development (Carrera et al., 1998). 5′ ends of cDNAs corresponding to several genes with unknown functions also hit to the region. One of them, RE64518, begins 31 bp downstream of the pHAP transposon insertion site (Figure 2). The cDNA has a number of stop codons and should be considered as a 5′ UTR of the uncharacterized gene.

Region 85D9/D10
In strain 12, the P{lArB} insertion occurred in this interband region (Schwartz, Demakov & Zhimulev, 1998). Four other P transposon insertions were also found in this region (*EP(3)3384*, *l(3)L4092*, *EP(3)3690* and *EP(3)0632*, from left to right, Figure 2). Insertion sites for all these transposons are situated upstream of a 20 bp-interval where three different start points for a number of related cDNAs were located (only three cDNAs are shown in Figure 2). cDNAs RE31825, RE70963, and LD02836 begin at 7, 17 and 27 nucleotides from the closest insertion *EP(3)0632*. We sequenced the LD02836 DNA (GenBank accession number AJ421016) and found that its 2112 bp are generated from a putative primary transcript spanning more than 17 kb within the genomic fragment AE003682. At the 5′ end of this cDNA, a number of stop codons were found. The LD02836 (976–1319 bp) appears to be most similar to the part of the *CG16751* gene, which has also been known in FlyBase as *CG33188*. It encodes a product with protein binding function. The last 80 bp at the 3′ end of LD02836 match three uncharacterized cDNA sequences (Figure 2). These results support the suggestion that the 5′ UTR of the *LD02836* gene is located within the 85D9/D10 interband region.

Region 86B4/B6
In strain 2 (Semeshin et al., 1994; Schwartz, Demakov & Zhimulev, 1998), the P{lArB} insertion in this region (Figure 2) occurred 88 nucleotides upstream of the initiation sites for two related cDNAs (GM05287 and GH19011) that correspond

to the *stich1* gene. This gene encodes an RNA polymerase II transcription factor and its mutation affects neuron formation (Prokopenko & Bellen, 2000). This, and three other P element insertions in this region (*EP(3)3120*, *EP(3)3532* and *EP(3)3470*) are homozygous viable and do not show phenotypic effects. No other genes were found in this region. These data suggest that the 5′ non-coding region of *stich1* is situated within the interband region 86B4/B6.

Transcriptional activity of 60E8-9/E10 and 61C7/C8 interbands

As could be seen from the molecular and genetic characterization of the interband regions presented above, most of them are immediately adjacent to or comprise 5′ or 3′ ends of genes, thus opening the possibility that they eventually correspond to gene regulatory regions. A better characterized 3C5-6/C7 region corresponds to the regulatory region of the *Notch* gene, which is not transcribed in salivary glands, i.e. the gene regulatory region acquires a decondensed state without the entire gene being transcribed (Rykowski et al., 1988; Ramos et al., 1989; Vazquez & Schedl, 2000). Does 3C5-6/C7 interband represent an exceptional case or is this situation typical for all interband regions? Could a contrary situation be observed? To answer these questions we examined the transcription activity of 60E8-9/E10 and 61C7/C8 interbands throughout fly development and in third-instar larval salivary glands.

The transcriptional activity of the interband 61C7/C8 was examined with the 61C-3,8HB DNA as a probe (Figure 2). Strong signals corresponding to 3.0 and 3.4 kb transcripts were found at a first-instar larval stage only (Figure 3(A), lane 2). Very weak transcripts if any were detected with this probe in poly $(A)^+$ RNA from other stages (Figure 3(A), lanes 1, 3–5) and from salivary glands (Figure 3(B), lane 6). In fact, the same filters re-hybridized with *actin* DNA probe, gave well-detectable specific signals (Figure 3(A), lanes 1–5 and Figure 3(B), lane 7), indicating that the RNA was undegraded. To verify the RNA detection threshold, hybridization of approximately the same amount of poly $(A)^+$ RNA from third-instar larvae with the *white* DNA probe was carried out under the same conditions, and respective signals were obtained (Figure 3(B), lane 8). As estimated earlier, the abundance of *white* mRNA is very low, constituting about 0.0005% of poly $(A)^+$ RNA (Pirrotta & Bröckl, 1984). Although our experiments do not exclude the possibility that the interband DNA is transcribed and the RNA produced is either extremely unstable or it is present at extremely low level, we think this to be very unlikely. Alternatively one might suggest that the RNA produced is not polyadenylated. However, we suppose that these options are also unlikely, since no signals were detected in hybridization

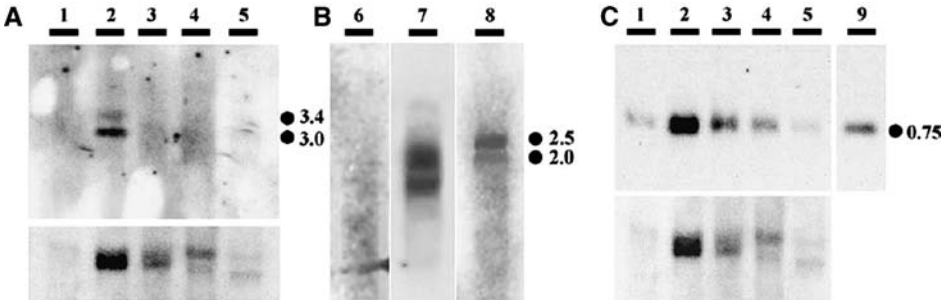

Figure 3. Transcriptional activity of 60E8-9/E10 and 61C7/C8 interband regions as demonstrated by Northern-blotting of Oregon-R RNA. Poly $(A)^+$ RNA was isolated from the following developmental stages: embryos, first-instar larvae, second-instar larvae, early prepupae, adults (1–5 lanes, respectively) (5 μg of RNA per each lane), from salivary glands of third-instar larvae (lanes 6, 7, 9) and from whole third-instar larvae (lane 8) (15 μg of RNA per each lane). RNA samples were blotted and probed with 61C-3.8HB (panels A (lanes 1–5) and B (lane 6)) and with 60E-2.1S (panel C (lanes 1–5, 9)) (see Figure 2 above). The filters were subsequently re-hybridized with *actin 42A* DNA probe as a control (lower panels for A and C, lane 7 for B). The RNA detection threshold was tested by hybridization with the *white* probe (lane 8). Exposure times: 2 days (lanes 1–5, 8, 9), 15 days (lane 6), 4 and 12 h (lane 7 and lower panels, respectively). Lengths of transcripts (in kilobases) are shown on the side of each blot.

experiments of total RNA from salivary glands with the 61C-3.8HB probe (data not shown).

Unlike the 61C7/C8 and the 3C5-6/C7 interbands, the 60E8-9/E10 region represents a more complicated case. The insertion of P{lArB} transposon there occurred between the 3′ end of the *Rp119* gene and the 5′ end of the unknown *CG30424* gene (Figure 2). Considering the average estimate of interband DNA to be about 2 kb (Beermann, 1972), it is possible that either the 5′ end of *CG30424* or the whole *RpL19* constitute the 60E8-9/E10 interband. The latter option is very intriguing since *RpL19* had been shown to code for ribosomal protein L19 (Hart, Klein & Wilcox, 1993) and thus it is likely to be transcribed in all tissues during development. To test this possibility, the RNA isolated from all stages of fly development and from salivary glands was hybridized with the 60E-2.1S probe (Figure 2). Only one hybridization signal of approximately 750 nucleotides was observed (Figure 3(C), lanes 1–5 and 9), which is comparable with the 769 nucleotides long *RpL19* mRNA (Hart, Klein & Wilcox, 1993). As expected for a 'housekeeping' gene, the transcript was found in all stages and the level of transcription was approximately equal in all samples examined (Figure 3(C)). The data obtained suggest that the decondensed state of the 60E8-9/E10 interband region could depend on the constant activity of *RpL19*.

Discussion

Accuracy of molecular mapping of interband regions

It should be emphasized that even the most precise EM method of P element mapping does not allow determining the distance from the insert to the band/interband borders. As it was reported earlier, new bands can be generated via transposon insertion in either middle part or the edge of the original interbands (Semeshin et al., 1989; Demakov, Semeshin & Zhimulev, 1993). In the latter case, new bands can be detected due to decondensation of the P element 5′ end and the formation of a novel interband separating the inactive transposon material from the adjacent original band. Possible involvement of P element DNA in the formation of interband regions has been shown in case of the native P element insertion into *vermilion* gene (band 10A1-2) resulting in the appearance of a new interband and in the splitting of the band into two unequal parts (Semeshin et al., 1989). However, in either of the two possible variants of P insertions in an interband region, the original interband DNA is located in the immediate vicinity of the transposon, – at one or both sides of it. Thus, we infer that the accuracy of molecular mapping of interband borders in respect to the site of P insertion cannot exceed the interband size, or approximately 2 kb of DNA according to Beermann (1972) and to more recent estimates of *Drosophila* genomic DNA amount (5% DNA present in interbands of the euchromatic DNA (120 Mb) i.e. 6 Mb divided by the number of known interbands (approximately 3000).

P transposon tagged mapping of interband regions

Even though the approach employed is suitable for systematic analysis of many interband regions, one could argue that P elements tend to insert non-randomly and thus only a specific subset of interbands could be investigated in such a way. As we learned from the analysis of genomic DNA sequence, *Drosophila* genes are more or less evenly distributed in the euchromatin (Adams et al., 2000). Assuming that interbands constitute about 5% of euchromatic DNA (Beermann, 1972; Sorsa, 1984) one would anticipate the majority of the genes with their regulatory regions to reside in polytene chromosome bands. Thus, from a set of randomly chosen P transposon insertions, not more than 5% are expected to be mapped to interbands. Nevertheless, of 19 insertions EM-mapped to date 14 ones (or 74%) had been found to occur in the interband regions (Semeshin et al., 1986, 1989, 1994; present work). This finding is in good agreement with the observation that only some loci could be efficiently mutated by P transposon insertions. For example, the analysis of a collection of 3900 P insertion lines associated with recognizable phenotypes showed that 70% of these P transposons were inserted preferentially into about 400 hotspot loci. Extensive comparisons failed to uncover any common biological features such as size, location, or regulation for these loci that would explain why they are so attractive for P elements to integrate (Spradling et al., 1999). It was suggested that the P element transposition

mechanism has a two-fold dyad symmetry and recognizes a structural feature at insertion sites, rather than a specific sequence motif (Liao, Rehm & Rubin, 2000). In addition, about 95% of insertions occur either in 5′ UTRs or within 100–200 bp upstream of the transcriptional initiation site (Spradling et al., 1995).

Here we speculate that the same biochemical processes, which cause the decondensed state of interbands in polytene chromosomes, are also operating in germ line cells, where P elements usually transpose, by somehow changing the chromatin structure and thus facilitating insertions in these regions.

Interbands may correspond to the regulatory regions of genes, which are not active in salivary glands

The decondensed state of interbands makes one expect these regions to contain genes that are actively transcribed in salivary glands, just as it was demonstrated for puffs. However, several lines of evidence indicate that at least some interbands represent a different case. Earlier Rykowski et al. (1988) have shown the 5′ region of the *Notch* locus, which is not transcribed in salivary glands, to be located in the 3C6/C7 interband with its coding part being situated in the 3C7 band. Semeshin et al. (1998) obtained essentially similar results. Using the EM *in situ* hybridization technique they have mapped the 5′ UTR of *muscle-blind* gene into the 54A1-2/B1-2 interband, with its long coding part being in band 54B1-2 and its 3′ UTR residing in the 54B1-2/B4 interband. Genetic organization of other interband regions examined in this study suggests that at least part of interbands may share the same properties. As demonstrated above, the 61C7/C8 interband contains cDNA (RE64518), which is most probably 5′ UTR of a gene with unknown functions, whose transcripts are not detectable in salivary glands. 3A4/A6, 85D9/D10 and 86B4/B6 have all been hit by P transposon insertions more than once. All these regions contain 5′ UTRs. Even though transcription of these genes in salivary glands has never been tested experimentally, the functions and mutant phenotypes of *egghead* and *stich1* located in 3A4/A6 and 86B4/B6 imply that they should not be transcribed there (Goode et al., 1996; Prokopenko & Bellen, 2000).

If the suggestion that part of interbands correspond to the 5′ parts of genes inactive in salivary glands holds true, how can one explain the earlier observations that RNA polymerase II (Pol II) could be detected virtually in all interbands (Jamrich, Greenleaf & Bautz, 1977; Sass & Bautz, 1982)? We think there is no real contradiction. It is well known that several forms of Pol II coexist in eukaryotic nuclei. These forms differ in the phosphorylation state of Pol II largest subunit carboxy-terminal domain (CTD). Several *in vitro* and *in vivo* experiments showed that the enzyme having its CTD unphosphorylated (Pol IIA) is competent exclusively for transcription initiation, while the enzyme isoform with a phosphorylated CTD (Pol IIO) participates in RNA elongation (for review see: Dahmus, 1996). Immunostaining experiments with antibodies against Pol IIA and Pol IIO on polytene chromosomes (Weeks et al., 1993; Kaplan et al., 2000) argue that Pol IIO is located predominantly in puffing regions and in some interbands, whereas only Pol IIA could be detected in most interbands. The staining patterns of these isoforms display only partial overlap. These data make it reasonable to propose that part of interbands harbor promoters and sites nearby that are occupied by transcription activators capable of recruiting Pol IIA there. However, some yet uncovered factors block subsequent CTD phosphorylation of these Pol II molecules, preventing the transcription of adjacent genes. The phenomenon of Pol IIA pausing on the promoter, first demonstrated to be essential for the *hsp70* gene organization (Gilmour & Lis, 1986), was later found to be much more widespread, being intrinsic for the promoters of a number of *Drosophila* genes (Law et al., 1998). We speculate that of many genes evenly distributed along the *Drosophila* euchromatin, only the regulatory regions of some of them have Pol II paused on the promoter and are able to form interbands in polytene chromosomes. In this respect, it is of interest that the genes mapped in the interbands under study and whose exon-intron structure had been resolved, show remarkable similarity in organization of their 5′ ends. Thus, *egghead*, *LD02836* (both depicted in Figure 2), *Notch* (Rykowski et al., 1988), and *muscle-blind* (Semeshin et al., 1998) all have their 5′ untranslated first exon separated from the coding part by a long intron. It is tempting to suggest that such organization is necessary for the genes

whose promoter is permanently occupied by Pol II, perhaps, to reduce the level of undesirable transcription to be produced by rare polymerase molecules that escaped pausing. However, the connection between localization of paused Pol IIA in interband regions and the decondensed state of interband chromatin is not very clear. It is worth noting that pausing of polymerase is definitely not sufficient to produce an interband since the very *hsp70* promoter, known to be bound by Pol IIA, is situated inside of 87A7 band (Udvardy, Maine & Schedl, 1985). Probably other biochemical activities that may be associated with Pol IIA arrested on promoters, are involved in building a special state of interband chromatin. One of such activities could be phosphorylation of the histone H3 N-terminal tail. Thus, a recently discovered tandem kinase JIL-1 capable of phosphorylating H3 *in vitro* was shown to be localized to most of interband regions (Jin et al., 1999). More important is that the reduced level of JIL-1 in mutant flies results in severe reduction of H3 Ser10 phosphorylation and breaks the polytene chromosome banding pattern with the interband regions being completely condensed (Wang et al., 2001).

Model of polytene chromosome organization

Summarizing the arguments mentioned above, the following rough picture of polytene chromosome organization can be drawn (Figure 4). On a cytological level, the euchromatic part of polytene chromosome is represented as a set of alternating condensed elements (bands) and decondensed ones (interbands and puffs). With respect to their genetic organization, most puffs correspond to the genes temporarily active in salivary glands, while interbands could presumably be divided into two classes. Interbands of the first type (I) correspond to the regulatory regions of genes inactive in salivary glands. However, correlation of the regulatory regions of genes with this type of interbands is not obligatory. For instance, at least 24 genes were found in the 10A1-2 band (Kozlova, Zhimulev & Kafatos, 1997), but only two of them, situated on the edges of the band, are able to have regulatory regions located within the neighboring interbands. Interbands of second type (II) correspond to the genes constantly active in this tissue (housekeeping genes).

Considering the structural organization of polytene chromosomes, two different, but not mutually exclusive, concepts could be proposed. According to the first one (Figure 4(A)), each chromatid of a polytene chromosome independently undergoes its differential compaction with bands being composed of a nucleosome fiber further folded in some sort of high-order structure. As it concerns the decondensed chromatin of puffs and interbands, it is not clear whether it has the same molecular organization. On the one hand, both the DNA of heat-shock puff 87A (Levy & Nöll, 1981) and the chromatin of interband regions (Sorsa, 1984) were considered to be devoid of nucleosomes. However, another set of experimental data available indicates that at least for some interbands this may not be the case. Using high-resolution fluorescent *in situ* hybridization Rykowski et al. (1988) have estimated the 3C6/C7 interband DNA to be folded in a 10 nm fiber, which corresponded to the nucleosomal level of chromatin packaging. The organization of 61C7/C8 interband DNA into nucleosomes was later directly demonstrated (Schwartz, Demakov & Zhimulev, 2001). We suppose that puffs and type II interbands containing 'housekeeping' genes are represented by the chromatin with altered nucleosomal organization that results from the transcriptional machinery passing through. Interbands of the type I are made up of the nucleosome fiber, which is unsuitable for further folding as a result of histone modification by activities presumably associated with the paused Pol II. These may include histone acetylation, phosphorylation or ubiquitination (Jenuwein & Allis, 2001).

The second concept (Figure 4(B)) assumes that while the decondensed state of puffs and type II interbands is caused by disturbance of their nucleosome organization, the difference in compaction of bands and type I interbands resides in the high levels of chromatin folding. Such interbands and bands contain the same folded nucleosome fiber, but bands further form some sort of chromatin loops, while type I interbands are prevented from doing so by tight contacts either with so-called chromosome scaffold or with a homologous sister chromatid. Even though currently the phenomenon of nuclear matrix/chromosome scaffold (Laemmli et al., 1992; Razin, Gromova & Iarovaia, 1995) is strongly criticized (Hancock, 2000), it is worth mentioning that the DNA of

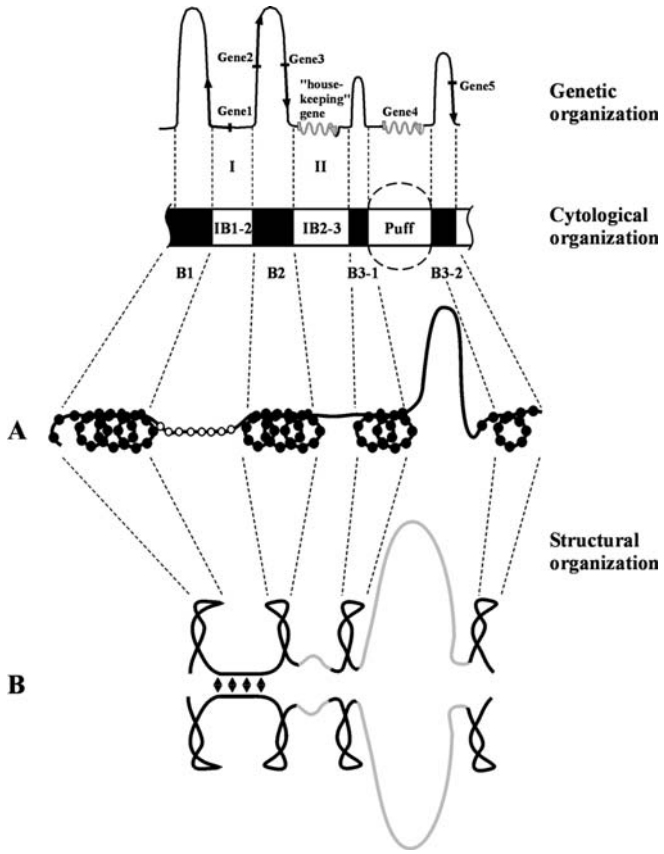

Figure 4. Schematic representation of structural and functional organization of polytene chromosome. Cytologically similar bands (or interbands) are heterogeneous from a genetic point of view. Band 1(B1) contains a coding part of Gene1 (shown as a thick gray line with the transcription direction marked by an arrowhead) whose regulatory region is located in a neighboring type I interband (IB1-2). Gene1 is silent in salivary glands. Band 2 is polygenic and contains, for illustration, two inactive genes. Interband IB2-3 of type II contains short constantly active 'housekeeping' gene. Bands B3-1 and B3-2 originate from a polygenic band B3, which is splitted by a puff as a result of temporary Gene 4 activation. Two concepts of structural organization of the same polytene chromosome segments are presented. (A) A single chromatid in band regions has nucleosome organization (filled circles) and is folded in a super-nucleosome fiber. DNA of IB1-2 is also packed into nucleosomes (open circles), but their histones are covalently modified preventing the chromatin fiber from further folding. As a result of transcription activity, the chromatin of IB2-3 and the puff loose normal nucleosome organization and subsequent folding. (B) A schematic picture of a path that chromatin fibers of two sister chromatids undergo inside a polytene chromosome. The part of the chromatids organized into nucleosomes is shown in black. The parts having their nucleosomal organization disturbed are depicted in gray. Intense contacts between two sister chromatids or between each individual chromatid and a chromosome scaffold in interbands of the type I represented by IB1-2 are shown by double arrows.

some interbands specifically interacts with a protein fraction proposed to be involved in the maintenance of nuclear architecture (Schwartz et al., 1999). In addition, the possibility of intense contacts between regulatory regions of homologous chromosomes is illustrated in transvection experiments (Pirrotta, 1999).

The present study has demonstrated that some interbands correspond to the regulatory regions of genes, which are inactive in salivary glands. This observation led us revise the currently accepted view that any decondensed state of a polytene chromosome region should be associated with transcriptional activity of genes. Further experiments revealing the chromatin modifications of different interbands and the characterization of the proteins that catalyze these modifications are necessary to understand the mechanisms driving differential condensation of eukaryotic interphase chromatin.

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References

Adams, M.D., S.E. Celniker, R.A. Holt, C.A. Evans et al., 2000. The genome sequence of *Drosophila melanogaster*. Science 287: 2185–2195.

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Zh. Zhang, W. Miller & D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. 25: 3389–3402.

Beermann, W., 1972. Chromomeres and genes, pp. 1–33 in Results and Problems in Cell Differentiation, Vol. 4, edited by W. Beermann. Springer, Berlin, Heidelberg, New York.

Bonner, J.J., C. Parks, J. Parker-Thornburg, M.A. Mortin & H.R.B. Pelham, 1984. The use of promoter fusions in *Drosophila* genetics: isolation of mutations affecting the heat shock response. Cell 37: 979–991.

Bridges, C.B., 1938. A revised map of the salivary gland X-chromosome of *Drosophila melanogaster*. J. Hered. 29: 11–13.

Carrera, P., S. Abrell, B. Kerber, U. Walldorf, A. Preiss, M. Hoch & H. Jackle, 1998. A modifier screen in the eye reveals control genes for Kruppel activity in the *Drosophila* embryo. Proc. Natl. Acad. Sci. USA 9: 10779–10784.

Crick, F., 1971. General model for the chromosomes of higher organisms. Nature 234: 25–27.

Cuvier, O., C.M. Hart & U.K. Laemmli, 1998. Identification of a class of chromatin boundary elements. Mol. Cell Biol. 18: 7478–7486.

Dahmus, M.E., 1996. Reversible phosphorylation of the C-terminal domain of RNA polymerase II. J. Biol. Chem. 271: 19009–19012.

Demakov, S.A., V.F. Semeshin & I.F. Zhimulev, 1993. Cloning and molecular genetic analysis of *Drosophila melanogaster* interband DNA. Mol. Gen. Genet. 238: 437–443.

Frisch, M., K. Frech, A. Klingenhoff, K. Quandt, I. Liebich & T. Werner, 2000. A new tool for the *in silico* prediction of matrix attachment regions in large genomic sequences, pp. 27–34 in Proceedings of the German Conference on Bioinformatics, edited by E. Bornberg-Bauer, U. Rost, J. Stoye & M. Vingron. Logos Verlag, Berlin.

Gersh, E.S., 1975. Sites of gene activity and of inactive genes in polytene chromosomes of Diptera. J. Theor. Biol. 50: 413–428.

Gilmour, D.S. & J.T. Lis, 1986. RNA polymerase II interacts with the promoter region of the noninduced *hsp-70* gene in *Drosophila melanogaster* cells. Mol. Cell Biol. 6: 3984–3989.

Goode, S., M. Melnick, T.B. Chou & N. Perrimon, 1996. The neurogenic genes *egghead* and *brainiac* define a novel signaling pathway essential for epithelial morphogenesis during *Drosophila* oogenesis. Development 122: 3863–3879.

Hancock, R., 2000. A new look at the nuclear matrix. Chromosoma 109: 219–225.

Hart, K., T. Klein & M. Wilcox, 1993. A *Minute* encoding a ribosomal protein enhances wing morphogenesis mutants. Mech. Dev. 43: 101–110.

Hollmann, M., 2000. *Drosophila melanogaster WDS* (*wds*) and *egghead* (*egh*) genes, complete cds. GenBank/EMBL/DDBJ 2000.2.9. AF233288.

Jamrich, M., A.L. Greenleaf & E.K.F. Bautz, 1977. Localization of RNA-polymerase in *Drosophila melanogaster* polytene chromosomes. Proc. Nat. Acad. Sci. USA 74: 2079–2083.

Jenuwein, T. & C.D. Allis, 2001. Translating the histone code. Science 293: 1074–1080.

Jin, Y., Y. Wang, D.L. Walker, H. Dong, C. Conley, J. Johansen & K.M. Johansen, 1999. JIL-1: a novel chromosomal tandem kinase implicated in transcriptional regulation in *Drosophila*. Mol. Cell 4: 129—135.

Kaplan, C.D., J.R. Morris, C.-T. Wu & F. Winston, 2000. Spt5 and Spt6 are associated with active transcription and have characteristics of general elongation factors in *D. melanogaster*. Genes Dev. 14: 2623–2634.

Kellum, R. & P. Schedl, 1992. A group of scs elements function as domain boundaries in enhancer-blocking assay. Mol. Cell Biol. 12: 2424–2431.

Kelley, M.R., S. Kidd, R.L. Berg & M.W. Young, 1987. Restriction of P element insertions at the *Notch* locus of *Drosophila melanogaster*. Mol. Cell Biol. 7: 1545–1548.

Keppy, D.O. & W.J. Welshons, 1977. The cytogenetics of a recessive visible mutant associated with a deficiency adjacent to the *Notch* locus in *Drosophila melanogaster*. Genetics 85: 497–506.

Korge, G., I. Heide, M. Sehnert & A. Hofmann, 1980. Promoter is a important determinant of developmentally regulated puffing at the *Sgs-4* locus of *Drosophila melanogaster*. Develop. Biol. 138: 324–337.

Kozlova, T., I.F. Zhimulev & F.C. Kafatos, 1997. Molecular organization of an individual *Drosophila* polytene chromosome chromomere: transcribed sequences in the 10A1-2 band. Mol. Gen. Genet. 257: 55–61.

Laemmli, U.K., E. Kas, L. Poljak & Y. Adachi, 1992. Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. Curr. Opin. Genet. Dev. 2: 275–285.

Law, A., K. Hirayoshi, T. O'Brien & J.T. Lis, 1998. Direct cloning of DNA that interacts *in vivo* with a specific protein:

application to RNA polymerase II and sites of pausing in *Drosophila*. Nucl. Acids Res. 26: 919–924.

Levy, A. & M. Nöll, 1981. Chromatin fine structure of active and repressed genes. Nature 289: 198–203.

Liao, G., E.J. Rehm & G.M. Rubin, 2000. Insertion site preferences of the P transposable element in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 97: 3347–3351.

Mott, M.R. & R.J. Hill, 1986. The ultrastructural morphology of native salivary gland chromosomes of *Drosophila melanogaster*: the band-interband question. Chromosoma 94: 403–411.

O'Kane, C.J. & W.C. Gehring, 1987. Detection *in situ* of genomic regulatory elements in *Drosophila*. Proc. Natl. Acad. Sci. USA 84: 9123–9127.

Paul, J., 1972. General theory of chromosomes structure and gene activation in eukaryotes. Nature 238: 444–446.

Pirrotta, V., 1999. Transvection and chromosomal trans-interaction effects. Biochim. Biophys. Acta 1424: M1–M8.

Pirrotta, V. & Ch. Bröckl, 1984. Transcription of the *Drosophila white* locus and some of its mutants. EMBO J. 3: 563–568.

Prokopenko, S.N. & H.J. Bellen, 2000. From phenotype to gene function: a molecular screen for novel genes required for the development of the peripheral nervous system. Dros. Res. Conf. 41: 727C.

Ramos, R.G.P., B.G. Grimwade, K.A. Wharton, T.N. Scottgale & S. Artavanis-Tsakonas, 1989. Physical and functional definition of the *Drosophila Notch* locus by P element transformation. Genetics 123: 337–348.

Razin, S.V., I.I. Gromova & O.V. Iarovaia, 1995. Specificity and functional significance of DNA interactions with the nuclear matrix: new approaches to clarify the old questions. Int. Rev. Cytol. 162B: 405–448.

Rykowski, M.C., S.J. Parmelee, D.A. Agard & J.W. Sedat, 1988. Precise determination of the molecular limits of a polytene chromosome band: regulatory sequences for the *Notch* gene are in the interband. Cell 54: 461–472.

Sambrook, J., E.F. Fritsch & T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sass, H. & E.K.F. Bautz, 1982. Interbands of polytene chromosomes: binding sites and start points for RNA polymerase. Chromosoma 86: 77–93.

Schwartz, Yu.B., S.A. Demakov & I.F. Zhimulev, 1998. Cloning and analysis of DNA from 85D9/D10 and 86B4/B6 interband region. Genetika 34: 905-913 (in Russian).

Schwartz, Yu.B., E.S. Ioudinkova, S.A. Demakov, S.V. Razin & I.F. Zhimulev, 1999. Interbands of *Drosophila melanogaster* polytene chromosomes contain matrix association regions. J. Cell Biochem. 72: 368–372.

Schwartz, Yu.B., S.A. Demakov & I.F. Zhimulev, 2001. Polytene chromosome interband DNA is organized into nucleosomes. Mol. Genet. Genom. 265: 311–315.

Semeshin, V.F., I.F. Zhimulev & E.S. Belyaeva, 1979. Electron microscope autoradiographic study of transcriptional activity of *Drosophila melanogaster* polytene chromosomes. Chromosoma 73: 163–177.

Semeshin, V.F., E.M. Baricheva, E.S. Belyaeva & I.F. Zhimulev, 1982. Electron microscopical analysis of *Drosophila* polytene chromosomes. I. Mapping of the 87A and 87C heat shock puffs in development. Chromosoma 87: 229–237.

Semeshin, V.F., E.S. Belyaeva, I.F. Zhimulev, J.T. Lis, G. Richards & M. Bourouis, 1986. Electron microscopical analysis of *Drosophila* polytene chromosomes. IV. Mapping of morphological structures appearing as a result of transformation of DNA sequences into chromosomes. Chromosoma 93: 461–468.

Semeshin, V.F., S.A. Demakov, M. Perez Alonso, E.S. Belyaeva, J.J. Bonner & I.F. Zhimulev, 1989. Electron microscopical analysis of *Drosophila* polytene chromosomes. V. Characteristics of structures formed by transposed DNA segments of mobile elements. Chromosoma 97: 396–412.

Semeshin, V.F., V.A. Chernukhin, I.V. Shabel'nikov, L.V. Omel'yanchuk, E.S. Belyaeva & I.F. Zhimulev, 1994. Cytogenetic analysis of insertions belong to interbands of *Drosophila* polytene chromosomes. Genetika 30: 927-933 (in Russian).

Semeshin, V.F., R. Artero, M. Perez Alonso & V.V. Shloma, 1998. Electron microscopic *in situ* hybridization of digoxigenin-dUTP-labelled DNA probes with *Drosophila* polytene chromosomes. Chromosome Res. 6: 405–410.

Siden-Kiamos, I., R.D. Saunders, L. Spanos, T. Majerus, J. Treanear, C. Savakis, C. Louis, D.M. Glover, M. Ashburner & F.C. Kafatos, 1990. Towards a physical map of the *Drosophila melanogaster* genome: mapping of cosmid clones within defined genomic divisions. Nucl. Acids Res. 18: 6261–6270.

Singh, G.A., J.A. Kramer & S.A. Krawetz, 1997. Mathematical model to predict regions of chromatin attachment to the nuclear matrix. Nucl. Acids Res. 25: 1419–1425 (http: // www.futuresoft.org/MAR-Wiz).

Skaer, R.J., 1977. Interband transcription in *Drosophila*. J. Cell Sci. 26: 251–266.

Sorsa, V., 1984. Electron microscopic mapping and ultrastructure of *Drosophila* polytene chromosomes, 2, pp. 75–107 in Insect Ultratructure, edited by King, R.C. & H. Akai. Plenum Press, New York.

Spradling, A.C., D.M. Stern, I. Kiss, J. Roote, T. Laverty & G.M. Rubin, 1995. Gene disruptions using P transposable elements: an integral component of *Drosophila* genome project. Proc. Natl. Acad. Sci. USA 92: 10824–10830.

Spradling, A.C., D. Stern, A. Beaton, E.J. Rehm, T. Laverty, N. Mozden, S. Misra & G.M. Rubin, 1999. The Berkeley *Drosophila* genome project gene disruptions project: single P element insertions mutating 25% of vital *Drosophila* genes. Genetics 153: 135–177.

Udvardy, A., E. Maine & P. Schedl, 1985. The 87A chromomere. Identification of novel chromatin structures flanking the heat shock locus that may define the boundaries of higher order domains. J. Mol. Biol. 185: 341–358.

Vazquez, J. & P. Schedl, 1994. Sequences required for enhancer blocking activity of scs are located within two nuclease-hypersensitive regions. EMBO J. 13: 5984–5993.

Vazquez, J. & P. Schedl, 2000. Deletion of an insulator element by the mutation facet-strawberry in *Drosophila melanogaster*. Genetics 155: 1297–1311.

Vlassova, I.E., G.H. Umbetova, V.H. Zimmermann, C. Alonso, E.S. Belyaeva & I.F. Zhimulev, 1985. Immunofluorescence localization of DNA: RNA hybrids in

Drosophila melanogaster polytene chromosomes. Chromosoma 91: 251–258.

Wang, Y., W. Zheng, Y. Jin, J. Johansen & K.M. Johansen, 2001. The JIL-1 tandem kinase mediates histone H3 phosphorylation and is required for maintenance of chromatin structure in *Drosophila*. Cell 105: 433–443.

Weeks, J.R., S.E. Hardin, J. Shen, J.M. Lee & A.L. Greenleaf, 1993. Locus-specific variation in phosphorylation state of RNA polymerase II *in vivo*: correlations with gene activity and transcript processing. Genes Dev. 7: 2329–2344.

Zhao, K., C.M. Hart & U.K. Laemmli, 1995. Visualization of chromosomal domains with Boundary Element Associated Factor BEAF-32. Cell 81: 879–889.

Zhimulev, I.F., 1996. Morphology and structure of polytene chromosomes. Adv. Genet. 34: 1–497.

Zhimulev, I.F., 1999. Genetic organization of polytene chromosomes. Adv. Genet. 39: 1–589.

Zhimulev, I.F. & E.S. Belyaeva, 1975. ^{3}H-uridine labelling patterns in the *Drosophila melanogaster* salivary gland chromosomes X, 2R and 3L. Chromosoma 49: 219–231.