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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

# *Drosophila* Polytene Chromosome Bands Formed by Gene Introns[1]

Academician **I. F. Zhimulev[a, b], L. V. Boldyreva[a], O. V. Demakova[a], G. V. Poholkova[a], V. A. Khoroshko[a], T. Yu. Zykova[a], S. A. Lavrov[c], and E. S. Belyaeva[a]**

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**Abstract**—Genetic organization of bands and interbands in polytene chromosomes has long remained a puzzle for geneticists. It has been recently demonstrated that interbands typically correspond to the 5'-ends of house-keeping genes, whereas adjacent loose bands tend to be composed of coding sequences of the genes. In the present work, we made one important step further and mapped two large introns of ubiquitously active genes on the polytene chromosome map. We show that alternative promoter regions of these genes map to interbands, whereas introns and coding sequences found between those promoters correspond to loose grey bands. Thus, a gene having its long intron "sandwiched" between to alternative promoters and a common coding sequence may occupy two interbands and one band in the context of polytene chromosomes. Loose, partially decompacted bands appear to host large introns.

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Polytene chromosomes were rediscovered in 1934. They were described as peculiar giant chromosomes that are formed via non-disjunction of individual chromosomes, which have undergone multiple rounds of replication.

For several decades, polytene chromosomes have served as the best model of eukaryotic interphase chromosomes even though the very organization of polytene chromosomes has never been formally proven to be identical to that of regular chromosomes from mitotically dividing diploid cells. In polytene chromosomes, two major features stand out: they are very large (about 1000 times longer than the chromosomes from drosophila mitotic cells) and they show characteristic banding pattern. The nature of this pattern lies in varying degree of chromatin compaction along the chromosome body: densely packed regions form transverse dark stripes (bands) alternating with decompacted light regions referred to as interbands. Each chromosomal fragment, not to mention each chromosome, has a highly specific, unique, and reproducible pattern of bands (alternation of bands having distinct morphology, staining intensity, width, as well as degree of condensation); furthermore, every band in the chromosome can be recognized, described, and labeled on the chromosome map.

Genetic organization of polytene chromosomes (i.e., correspondence between genes and their structural parts vs bands and interbands) has long been and still is an unresolved question, much as the functional organization of morphological elements in polytene chromosomes. This was mainly due to the lack of appropriate methods that would allow accurate mapping of band and interband borders in the genome.

Several hypotheses were put forward, which assigned various parts of the genes to specific structures found in polytene chromosomes. These ideas are reviewed in [1, 2], so we will only briefly touch upon them in the present work. Three hypotheses are of special interest. First, regulatory parts of genes were suggested to map to interbands, whereas structural parts of genes are found in adjacent bands [3]. The other two hypotheses [4, 5] set interbands as encompassing house-keeping genes, with neighboring bands hosting one [4] or more [5] tissue-specific genes.

Transposons were used to tag interbands and so to move from cytology analysis to their molecular mapping in the genome [6]. This, in turn, has helped mapping the binding sites of chromatin proteins as well as many functional elements in interbands (modEncode project, [7]). The list of proteins highly enriched in the cytologically mapped "reference" interbands has been obtained, and this information allowed to infer the positions of interbands across the on a genome-wide scale. Thus, interbands turned out to correspond to the promoters of constitutively active genes that harbored the binding sites for RNA polymerase II, nucleosome remodeling complexes, and pre-replica-

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[a] *Institute of Molecular and Cellular Biology, SB RAS, Novosibirsk 630090, Russia*
[b] *Novosibirsk State University, Novosibirsk, 630090 Russia*
[c] *Institute of Molecular Genetics RAS, Moscow, 123182 Russia*
*e-mail: zhimulev@mcb.nsc.ru*

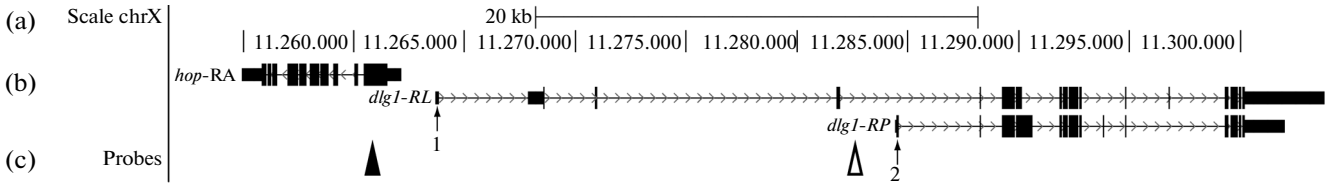


**Fig. 1.** Molecular and genetic map of the chromosome region encompassing *dlg1*. (a) Genomic scale (bp). (b) Structure of long transcripts of *dlg1* and *hop* genes. Exons are shown as black rectangles. Introns are depicted as thin lines with arrows indicating the direction of transcription. The first and second promoters of *dlg1* are marked (1 and 2). (c) Positions of the FISH probes: black triangle indicates the probe from the upstream promoter, with empty triangle showing the position of the probe from a second downstream promoter region.

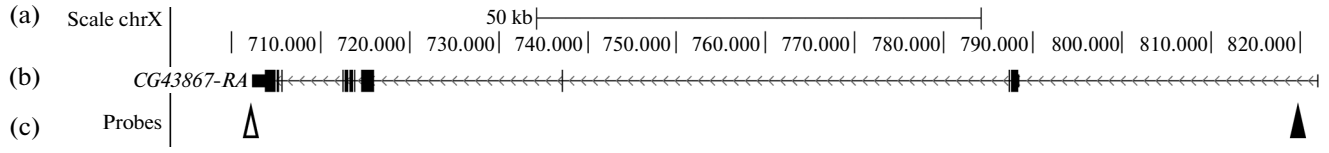


**Fig. 2.** Molecular and genetic map of the region around *CG43867*. (a) Genomic scale (bp). (b) Structure of *CG43867* gene. Black bars denote gene exons. Introns are shown as thin lines with arrows indicating the direction of transcription. (c) Positions of the FISH probes used. Black triangle corresponds to the position of the "promoter" probe, with empty triangle indicating the position of the "3'-end" probe.

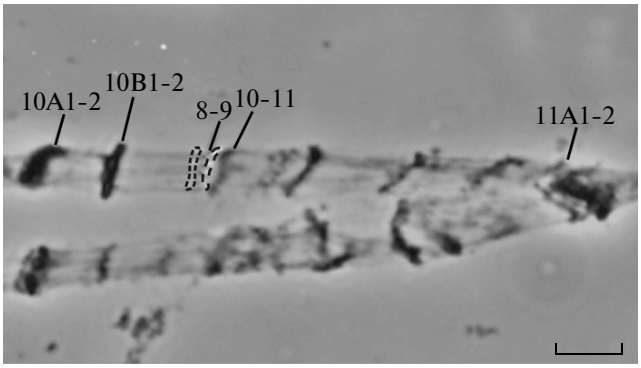


**Fig. 3.** FISH analysis of *dlg1* probes in the region 10B of drosophila X chromosome. Fluorescence signals are labeled as dashed (Flu signal) and broken (TAMRA signal) lines. Two short lines and one long line point to the two fluorescence signals and the loose band in between. Here and in Fig. 4, scale is 2 μm.

tion machinery. As a result, the borders of bands and interbands could be unambiguously and accurately established on the physical map. House-keeping genes were demonstrated to occupy two structures in the context of interphase chromosomes. Namely, interbands were shown to map to active gene promoters, whereas adjacent loose bands corresponded to the structural parts of house-keeping genes [2].

Our group has recently established that banding pattern is a universal organization principle, which is common to chromosomes from both polytene and regular non-polytene diploid cells. Positions, span, as well as ensembles of associated proteins turned out to be identical in interbands from both types of chromosomes [8]. This observation makes it even more attractive to use polytene chromosomes as a model for eukaryotic interphase chromosomes.

In the present work, active gene promoters were localized in the genome and on polytene maps, and so we for the first time established that polytene chromosome bands may be formed by the long introns of active genes. This, in turn, has an important implication that different parts of the same gene may show distinct degrees of chromatin condensation/decondensation.

Specifically, we used publicly available data (FlyBase, release 5.50) on genomic localization of two X-chromosomal genes, *dlg1* and *CG43867* (Figs. 1 and 2). Both genes are ubiquitously expressed across different developmental stages with low or moderate intensity [9, 10] and map to the regions 10B8-9–10B10-11, and 1C1-3–1D1-2, respectively. *Dlg1* spans some 40 kb, of which about 18 kb are found within the first long intron of the gene, right between two alternative transcription start sites (arrows 1—transcript variant L and 2—transcript variant P in Fig. 1). This long intron encompasses several very short exons (indicated as short transverse stripes in the intron, Fig. 1b). We prepared TAMRA-dUTP-labeled DNA probe from the fragment of *hop* gene, which is found next to the first promoter of *dlg1* and maps to an interband (fragment size is 669 bp, genomic coordinates 11260452–11261121). Appropriately, the DNA from the vicinity of the downstream promoter of *dlg1* (genomic coordi-

nates 11282249–11282987) was PCR-amplified and labeled with Flu-dUTP (Fig. 1b). Next, we performed high-resolution FISH according to the manual [11] and showed that these two promoter fragments can be detected as two separate signals, both mapping to the interbands of polytene chromosomes, with a diffuse grey band 1B8-9 found in between (Fig. 3, signals are circled with dashed and broken lines) Thus, taking into account that two FISH probes are separated by 18 kb (harboring the intron of *dlg1*) on the genomic map and by a diffuse band 10B8-9 on the cytology map, we conclude that this band is composed of the intronic material found between two alternative promoters of *dlg1* (see Fig. 1).

The gene *CG43867* has a very large span of about 120 kb, with an intron separating the promoter and the part of CDS common to all transcript isoforms (about 106 kb) (Fig. 2). Much as above, several short exons (Fig. 2b), and seven nested genes (FlyBase) are found within the intron. To perform FISH analysis, we labeled DNA probes with Flu (X:818796-820307, fragment size 1512 bp) and TAMRA-dUTP (X:701877-702715, fragment size 839 bp). These probes map to the promoter and the 3'-end of the gene, respectively (Fig. 2). We observed that these fragments are visualized as two separate signals in the region 1CD of the polytene X chromosome. Both of the signals map to interband regions and are separated by a diffuse grey band 1D1-2 (Fig. 4, pseudo-colored signals are overlaid on phase contrast image and are circled with a dashed and a broken line). Similarly, given that the two probes are found 116 kb apart on the one hand and are separated by a grey diffuse band 1D1-2 on the other, we conclude that this band is composed of the intron joining the promoter and protein-coding regions of *CG43867*. Notably, the gene's 3'-end maps to the next adjacent interband (Fig. 2).

Thus, we provide evidence that in polytene chromosomes, a single band may form from a defined part of the gene. This part may show certain degree of chromatin condensation, whereas the rest of the gene body (for instance, promoter(s)) remains highly decompacted. Until recently, gene activity was believed to be strongly correlated with local chromatin decondensation, a position that dominated the field of cytogenetics for several decades. Two most notable examples that support this view are as follows. First, chromosomes are known to undergo repeated rounds of compaction/decompaction throughout the cell cycle as they shuttle between the tightly condensed and transcriptionally inactive mitotic state and the interphase state, wherein the chromosomes are decondensed and transcription is at its maximum. Second, polytene chromosome bands hosting inactive genes may become locally decondensed and form puffs as a consequence of gene activation [12].

About one year ago, our group reported on the exceptions to this generalization. Namely, we showed that ubiquitously active //housekeeping// genes may occupy two structures in the context of polytene chromosomes, with their 5'-ends mapping to the highly decondensed chromatin of interbands, where nucleosome remodeling factors, ORCs, and transcription start sites co-localize. Thus interbands in interphase chromosomes serve as a hub where all the genetic functions replication, transcription, nucleosome remodeling act at a time. This is likely due to invariably high degree of decondensation of this chromatin region in polytene chromosomes. We show that the remainder of the gene (i.e., its exon/intron part) resides in the nearby loose band. Even though the band appears somewhat decompacted, it still appears as a band morphology-wise [2]. Thus, in polytene chromosomes two distinct structures having contrasting levels of chromatin compactization may encompass two parts of the same gene. We speculate that both of them contribute equally to the gene function, despite localization to distinct morphological structures, an interband and a grey loose band.

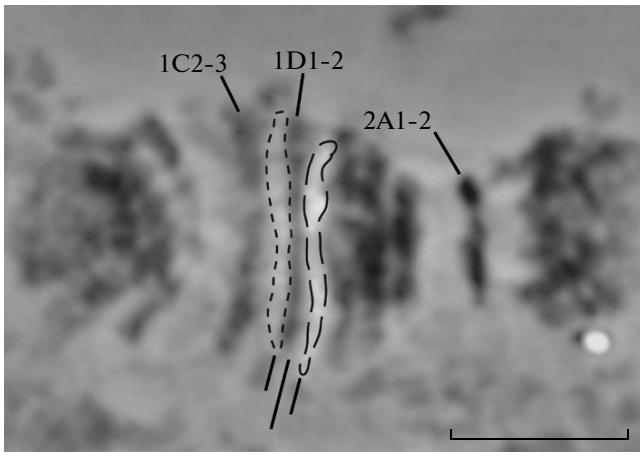


**Fig. 4.** FISH analysis of probes around the *CG43867* gene in the region 1C-D of the X chromosome. Fluorescence signals are labeled as dashed (Flu signal) and broken (TAMRA signal) lines. Two short lines and one long line point to the two fluorescence signals and the loose band in between.

Our work provides important insight into differential decondensation of chromatin template (chromosome-wide) of a non-coding part of a gene. Specifically, our data support the idea that long introns of active genes remain partially condensed despite their localization between two highly decondensed promoter regions (interbands). Furthermore, tighter packaging of material composed by long introns of active genes has been recently demonstrated at the genome-wide level [13].

## ACKNOWLEDGMENTS

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