Identical Functional Organization of Nonpolytene and Polytene Chromosomes in *Drosophila melanogaster*

Tatyana Yu. Vatolina, Lidiya V. Boldyreva, Olga V. Demakova, Sergey A. Demakov, Elena B. Kokoza, Valeriy F. Semeshin, Vladimir N. Babenko, Fedor P. Goncharov, Elena S. Belyaeva, Igor F. Zhimulev

Institute of Molecular and Cellular Biology of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

**Abstract**

Salivary gland polytene chromosomes demonstrate banding pattern, genetic meaning of which is an enigma for decades. Till now it is not known how to mark the band/interband borders on physical map of DNA and structures of polytene chromosomes are not characterized in molecular and genetic terms. It is not known either similar banding pattern exists in chromosomes of regular diploid mitotically dividing nonpolytene cells. Using the newly developed approach permitting to identify the interband material and localization of interband-specific proteins from modENCODE and other genome-wide projects, we identify physical limits of bands and interbands in small cytological region 9F13-10B3 of the X chromosome in *D. melanogaster*, as well as characterize their general molecular features. Our results suggest that the polytene and interphase cell line chromosomes have practically the same patterns of bands and interbands reflecting, probably, the basic principle of interphase chromosome organization. Two types of bands have been described in chromosomes, early and late-replicating, which differ in many aspects of their protein and genetic content. As appeared, origin recognition complexes are located almost totally in the interbands of chromosomes.

**Introduction**

*Drosophila* salivary gland polytene chromosomes are routinely used as a model for actively functioning interphase euchromatic chromosomes. Aside from their giant size, polytene chromosomes display prominent banding pattern, which is formed by tight alignment of homologous chromomeres, thereby forming a cable of chromatin with stripes of condensed material. Two neighboring chromomeres are separated by an interchromomeric region, which appears as an interband in the context of a polytene chromosome. According to the early estimates, most of the DNA which appears as an interband in the context of a polytene chromosome in *D. melanogaster* is 5% of genomic DNA, i.e. on average one interband corresponds to about 2 kb [1]. Taking into account that nucleosome packaging of chromatin reduces the linear size of these structures, the existing immunostaining methods fail to provide enough resolution in mapping the above-mentioned proteins to faint bands, the existing immunostaining methods fail to provide enough resolution in mapping the above-mentioned proteins to faint bands, interband material further reduces the linear size of these structures, as well as characterize their general molecular features. Our results suggest that the polytene and interphase cell line chromosomes have practically the same patterns of bands and interbands reflecting, probably, the basic principle of interphase chromosome organization. Two types of bands have been described in chromosomes, early and late-replicating, which differ in many aspects of their protein and genetic content. As appeared, origin recognition complexes are located almost totally in the interbands of chromosomes.
chromosomes [44]. This approach took advantage of the fact that when a P-element based transgene integrates into the genome, and if this integration hits an interband region, the transgene forms a new polytene chromosome band that can be clearly visualized at the level of electron-microscopy. Thus, by comparing the physical and cytological maps, one can accurately annotate the sequences adjacent to the integration site of the transpozon as forming an interband. One of the obvious drawbacks of this approach is that it only allows mapping the interband sequences around transgene insertion site, but does not tell us where the band/interband border is; in other words, it fails to provide data that would help characterize the interband as a structure.

More recently, several projects have produced a wealth of information about genome-wide localization patterns of proteins and protein complexes in Drosophila cell lines [45–49]. When we compared these data with 13 chromosome regions that had EM-mapped transgene insertions in interbands, we observed that such regions were associated with interband-specific proteins such as Chriz/CHRO and other “open chromatin” proteins described above. On a physical map, these interband regions typically corresponded to the intergenic regions and 5’-ends of genes, they were associated with ORCs, RNA polymerase II, transcription factors, nucleosome remodeling factor WDS. Additionally, these interbands showed yet another feature of the open chromatin: increased frequency of P-element insertions. Furthermore, these proteins tend to co-localize with each other [50].

In polytene chromosomes, there are several types of bands. First, there are large densely-condensed and late-replicating bands corresponding to the intercalary heterochromatin (IH) [51]. They are bound by SUUR protein, which marks late-replicating regions of the genome and contributes to the phenomenon of under-replication in polytene chromosomes. In these polytene chromosome bands, the DNA strands frequently fail to complete replication and so form chromosome breaks [52,53]. Such IH regions are characterized by lower than the genome-average gene density [54].

Clearly then, differences in gene density, replication timing, associated protein factors observed in interbands, dense IH bands and faint decompacted bands – can serve as convenient marks to precisely map the molecular borders of these structures. Combined with EM analysis, these data allow for the first time to compile an accurate molecular and cytogenetic map of bands and interbands in D. melanogaster salivary gland polytene chromosomes as well as in chromosomes of the cell lines.

Here, we perform analysis of bands and interbands in the region 10A–B (Fig. 1A, B) very well characterized cytology- and genetics-wise before [4,55], in polytene chromosomes from salivary gland cells and interphase chromosomes of mitotically dividing cells. Our results demonstrate high similarity of the banding patterns in both polytene and non-polytene chromosomes.

Results

Electron microscopy analysis of banding pattern in the region 9F13-10B3 of polytene chromosomes

As it is known, Electron Microscopic (EM) sections of polytene chromosomes in which all the known bands in given regions are expected to be seen, occur extremely infrequently. In the majority of sections a complete set of bands is observed only in short chromosome segments, especially in the regions of thin bands. Therefore, several sections of the same chromosome region should be used for analysis of bands (see [2] for more details). The electron microscopy data we obtained for the regions 10A–B (Fig. 1A, B) are consistent with revised map of Bridges [56] (Fig. 1C), if one considers double bands in the Bridges maps as singlets (see [2], for discussion). Figure 1 A, B illustrates three distinct types of domains characteristic of the banding pattern. First, these are two fairly massive bands 10A1–2 and 10B1–2,
encompassing dense chromatin and each flanked from both sides with interbands (with the band 10A2-1 appearing larger than the 10B1-2). In between these two large bands, one can see a “paling” of thin loosely-compacted “grey” bands and interbands, which correspond to the region 10A3-11.

Under the light microscope, in the context of a normal X chromosome, the band 10A1–2 appears as a “single large dense body even if the chromosome is well-stretched” [57,58], however according to the EM data [4,55] this region in fact harbors five distinct bands. There is one prominent band 10A1–2, and four medium-sized bands, two found distal (9F12 and 9F13, Fig. 1A) and two situated proximal (10A2 and 10A4–5, Fig. 1A, B). Of these four, two bands are located on the sides of the group – 10A2 and 10A4–5, - can sometimes be seen under the light microscope as separate bands, whereas 9F13 and 10A3 are located too close to 10A1–2 and are virtually never seen as separate bodies. The band 10A4–5 appears as the largest among the paling of the 10A3–11 group of bands. The next two thin bands at 10A6 and 10A7 are rarely detected even at the EM level, and even less frequently seen separately from each other. Notably, when these bands do appear separate, the 10A7 band looks larger than the 10A6 (Fig. 1B). The band 10A6–9 is routinely seen even under the light microscope, whereas the band 10A10–11 represents a typical singular faint band.

Mapping of bands and interbands in the 9F13-10B13 region of nonpolytene chromosomes

According to the combined cytogenetic and molecular-genetic analysis, the region of polytene chromosome band 10A1–2 is marked with two genes, vermilion which is located at its distal end [55,57,58], and sevenless, at its proximal side [4,55]. Therefore, we used positions of these two genes on the physical map as starting points for mapping the region in nonpolytene chromosomes.

Figure 2 shows map of the DNA features and protein localization profiles in the region of interest in nonpolytene chromosomes; these data have been generated by fly modENCODE consortium [47–49,59–62] as well as in experiments of genome-wide analysis of chromosome proteins [47].

Much like as was observed with the EM mapping data of polytene chromosomes, one can subdivide this region into three distinct domains. First, there are two zones on both flanks of the region (Fig. 2), which were previously shown to lack any interband-associated proteins. The DNA sequences that map to these zones are very large and are flanked with regions displaying interband-like features (Fig. 2C, D, E, F, G). According to the physical map, the leftmost domain may correspond to the 10A1–2 band, as it comprises vermilion and sevenless genes (Fig. 2A). The other region showing similar properties should correspond to the large band 10B1–2. Mimicking the EM pattern observed in polytene chromosomes, the region in between 10A1–2 and 10B1–2 appears as a paling of alternating interband-like zones and regions lacking interband features (middle of the Fig. 2C, D, E, F, G, H). This region is composed of faint bands intermingled with interbands which corresponds well to the EM mapping data (see Fig. 1).

So, in nonpolytene chromosomes of mitotically dividing cells one can find DNA fragments demonstrating features characteristic to polytene chromosomes, i.e. bands and interbands.

Identical positions of bands and interbands in polytene and nonpolytene chromosomes on genome map

We asked whether interbands have the same borders in polytene and nonpolytene chromosomes. To do so, we first selected two interband fragments on both sides of the 10A1–2 and 10B1–2 bands of nonpolytene chromosomes. The large sizes of both bands allow accurate mapping of said DNA fragments using FISH and immunostaining.

A short chromosome fragment with interband feature which is found immediately distal to the tentative 10A1–2 in nonpolytene chromosomes (arrow 1 on Fig. 2A) has been mapped on polytene chromosomes using three DNA probes (Table 3 in Materials and Methods section): CG1582, ypa 9F and CG15208 (hereafter the names of FISH probes correspond to the gene names that they map to). Given that the 10A1–2 band frequently fuses with the neighboring distal 9F11-12 and 9F13 bands (Fig. 3A), we only analyzed rare polytene chromosome spreads where all the bands appeared separate from each other. The three above-mentioned probes were mapped to the interval between the faint band 9F13 and the distal edge of 10A1–2 (Fig. 3B, data shown for CG15208 only), i.e. in the expected interband 9F13/10A1–2.

The region found on proximal side of the 10A1–2 band of nonpolytene chromosomes, has been mapped using two DNA probes, Vago and CG2076 (arrow 2 on Fig. 2A). The signal maps to the proximal edge of 10A1–2 in polytene chromosomes (Figs. 3B, C show the data for Vago probe only), i.e. to the interband 10A1–2/10A3.

The CG32668 and l(1)10B6 genes (arrows 3 and 4 on Fig. 2A) located distally and proximally to the tentative 10B1–2 band of the nonpolytene chromosomes, map by FISH to the interbands flanking this band in polytene chromosomes (Figs. 3 D, E, F). So, the four probes essentially hybridize to interband regions immediately flanking the two dark bands of the region 10A-B.

To address the question of whether Chriz/CHRO-associated DNA fragments are the same in polytene and nonpolytene chromosomes, we performed simultaneous FISH for these interband sequences and immunodetection of Chriz/CHRO in polytene chromosomes. Figure 4A, B, C shows that there is only one Chriz/ CHRO-positive region between the bands 9F13 and 10A1–2 (marked with an asterisk on Fig. 4B, C, D, E, F), i.e. Chriz/CHRO mapped in the 9F13/10A1–2 interband in nonpolytene chromosomes does map to the 9F13/10A1–2 interband in polytene chromosomes. At the same time, the FISH probe of CG15208 which bind this protein in nonpolytene chromosomes (Fig. 2) displays perfect co-localization with Chriz/CHRO in this region of polytene chromosomes (Fig. 4D, E, F).

We successfully mapped Vago probe on stretched polytene chromosomes which, as accepted [2, for more details], provided greater resolution. Figures 4G, H demonstrate that upon stretching, both the length of large bands and their spacing have dramatically increased, which, in turn, resulted in that Chriz binding pattern in between 10A1–2 and 10B1–2 is now seen as comprising a series of relatively distinct fluorescent bands reminiscent of the EM map for this region (Fig. 4I). We also observed that, first, Vago probe located in interband of the nonpolytene chromosome also completely co-localizes with one of these Chriz/CHRO -positive regions (Fig. 4G, H, I), and this probe itself mapped to the same interband in the proximal edge of the 10A1–2 band of polytene chromosome. Similarly, CG32668 and l(1)10B6 that in nonpolytene chromosomes flanked 10B1–2 from both sides, displayed extensive co-localization with Chriz/ CHRO -bound fragments in the 10B1–2 band in polytene chromosomes (Fig. 5).

Taking into account that Chriz/CHRO is a protein found both in the “open” chromatin of nonpolytene chromosomes and in polytene chromosome interbands tagged with P-element insertions [50], we conclude that DNA sequences associated to
Chriz/CHRO proteins correspond to interbands of polytene chromosomes. This, in turn, indicates that Chriz/CHRO is invariably bound to interbands in chromosomes of various cell types. Thus, we can map all the interband borders in a given region of a physical map, using the localization borders of various interband-specific proteins (Fig. 2C, D, E, F, G).

Figure 2. Localization of proteins and DNA elements in 9F13 – 10B3 region of nonpolytene chromosomes (according to data of modENCODE). The positions of proteins were located as described in Material and Methods. A - physical map of DNA; positions of v and sev genes are taken from FlyBase, arrows 1–4 indicate position of probes for FISH on physical map. B - P-elements density in the region calculated as number of insertions per 1 kb in 10 kb interval (data on insertions are taken from FlyBase). C - interband specific and active chromatin specific proteins in S2 cells [48]. D - DNase I hypersensitivity sites (DHS) in S2, BG3 and Kc cells [48]. E - ORC2-binding sites in S2, BG3 and Kc cultural and salivary gland cells [59]. F - histone H1.dips localization in Kc cells [45]. G - histone H3.3 localization in S2 cells (modENCODE, Henikoff group). H - 30 chromatin states in BG3 and S2 cells [48]. I - nucleosome turnover dynamics in S2 cells [60]. J - D1 localization in Kc cells [47]. K - SUUR localization in Kc cells [47]. L - Lamin localization in Kc cells [47]. M - early (up) and late (down) replication in S2, Kc and BG3 cells [61]. N - gene density (number of genes per 10 kb of DNA) [54].

doi:10.1371/journal.pone.0025960.g002
In order to determine the span of DNA sequences in each interband in this region, we first plotted the localization profiles for proteins on nonpolytene chromosomes and those we previously selected as markers for the transposon-tagged interbands of polytene chromosomes: this list included Chriz/CHRO, WDS, ORC, BEAF as well as DNase I hypersensitive sites and histone H1 density dips (see Fig. 2). Using these data, we identified the limits of interbands (Table 1). Within the region of interest, there are nine such interband regions, which is exactly the number of interbands on the Bridges [56] and EM (see Fig. 1) maps. Table 1 shows the coordinates of interband borders and the length of interband DNAs. Distances between interband borders, thus, correspond to the lengths of bands, totaling eight (in agreement with both Bridges and EM maps. Two out of these bands can be classified as late replicating bands, while the rest six are regular early-replicating (Table 1). The chromosome region from 9F13 to 10B3 encompasses 428,307 bp, nine interbands account for 20,100 bp, i.e. 4.69% of DNA length. Interbands range from 1,336 to 4,181 bp in size, with average size about 2,233 bp. Lengths of DNA sequences mapping within bands vary in much broader range. Eight bands concerned here account for 408,207 bp DNA, i.e. average length of DNA per band is roughly 51 kb. The largest IH bands 10A1–2 and 10B1–2, span 189 kb and 170 kb, respectively, whereas the smallest bands from around the 10A6 region are as short as 2761 bp, which is about 69 times less than found in the largest band 10A1–2. The region of faint bands at 10A3–10A11 encompasses about 52 kb DNA, and these bands show much more constrained variation, from 2761 to 14634 bp, being 8725 bp on average.

Accurate mapping of band and interband borders on the physical map and proper identification of the band/interband material, allows us to describe and characterize two types of polytene chromosome bands: late- and early-replicating bands, and give new insight into the structure of interbands.

Late-replicating IH bands 10A1–2 and 10B1–2 composed of compacted material, as is seen by cytology [51]. Degree of DNA compaction in them, i.e. the ratio of visual length of the bands to their actual DNA length, is highest (158- and 204-fold compaction, respectively) (Table 2). These bands are late replicated in both polytene [51] and nonpolytene [61] chromosomes (Fig. 2N).

The DNA in such bands displays several common features, such as noticeably decreased level of ORC2 localization (Fig. 2E), complete absence of any of the “open chromatin” ensemble of proteins which are normally found in interbands (Chriz/CHRO, BEAF-32, RNA polymerase II, BRE-1, WDS, NURF, TRX) (Fig. 2C, D, E, F, G), and low frequency of integration of P-element based transgenes, insertions of which are also characteristic for open chromatin (Fig. 2B).

Recently, by integrative analysis of genome-wide binding maps 53 broadly selected chromatin components in Drosophila cells it was shown that the genome is segmented into five principal chromatin types that are defined by unique combinations of proteins and
form specific domains. Each of these chromatin were condition-
ally labeled with a color: BLUE and BLACK – repressive
chromatins, RED and YELLOW – transcriptionally active
chromatins, GREEN – heterochromatic domain (see Filion
et al., 2010 [47] for details and protein compositions of each
domains). In other work the genome-wide chromatin landscape
based mainly on eighteen histone modifications and several non-
histone chromatin proteins was summarized by 30 combinatorial
patterns or states [48].

Bands like 10A1–2 and 10B1–2 can be categorized as having
the chromatin state 30, which is described as lacking any active
chromatin marks in the fly modENCODE [48]. In both 10A1–2
and 10B1–2 bands, strong enrichment for SUUR, D1 and lamin B
is observed [47,63] (Fig. 2L, M) which is characteristic for BLACK
chromatin, with depletion for H3.3 (Fig. 2G) and low level of
newly synthesized histone subunits (Fig. 2J). These regions show
low gene density, which is characteristic of late replicating regions
of the genome [54,64] (Fig. 2O), they show no depletion for
histone H1, which is necessary for higher-order nucleosome
packaging (Fig. 2F).

Yet, these bands display several distinct features. The band
10A1–2 is virtually homogeneous in terms of its principal
chromatin “color” - it is BLACK [47] throughout, showing
pronounced enrichment in SUUR, lamin B, and D1 proteins.

Figure 4. Colocalization of DNA probes limiting the 10A1–2
band with the Chriz/CHRO protein. Localization of the Chriz
protein in the 10A–B region (A–C); the bands in the region under phase
contrast (A), the Chriz/CHRO protein, asterisk points to the interband
9F13/10A1-2 B), match of Chriz/CHRO location and phase contrast (c),
colocalization of the DNA probe CG15208, limiting distal side of the
band 10A1–2 and Chriz/CHRO protein (D–F); colocalization of the DNA

Figure 5. Colocalization of DNA probes limiting the band
10B1–2, and Chriz/CHRO protein. Banding pattern in the region
10A–B (DAPI) (A), immunostaining of Chriz/CHRO and FISH of the DNA
probe (A), immunostaining of Chriz/CHRO, FISH of the DNA probe and
DAPI (C). Bar represents 5 μm.
doi:10.1371/journal.pone.0025960.g004
Only its proximal-most part shows some contribution of BLUE and YELLOW chromatins (Fig. 2I).

The band 10B1–2 appears as a more complex body. In both polytene and nonpolytene chromosomes, this band always appears as a single unit, flanked by interbands from both sides. However, depending on the differentiation stage, the chromatin state within 10B1–2 can change. For instance, in diploid cells, the RED chromatin typical of interbands is present, or some features of chromatin state become apparent (Fig. 2H). Despite the fact that overall gene density throughout 10B1–2 is decreased, the corresponding late completion of replication and SUUR binding in Kc cells is only observed in the distal 40% of this band (Fig. 2N, O). In Kc cells, Lamin B is found associated with both a fraction of late-replicating sequences and all of the early-replicating sequences of 10B1–2 (Fig. 2K, L, M, N). Notably, one of the bands - 10A1–2 - has all its genes replicating late, whereas only a fraction of genes from within 10B1–2 appears late-replicating (Fig. 2N). In this latter case, one can view the 10B1–2 band as only partially composed of late-replicating material.

And, finally, the band 10B1–2 is mosaic in terms of “colored” chromatin types [47]. Even though it is mostly composed of BLACK and BLUE chromatins, it also encompasses YELLOW chromatin at its distal edge, which correlates with localization of histone H1 dips (Fig. 2I).

Very thin (ca 9 kb/band on average, see above) early-replicating bands are harbored in the region 10A3–10A11 (Figs. 1 and 2). These bands are distinct from IH bands in many ways. They complete replication early, do not contain BLACK or BLUE chromatin, however, and much like the IH bands, they show poor enrichment (if any) for ORC2 (Fig. 2D, E). Morphology-wise, they appear less dense, the degree of DNA compaction in them was found to be 16–38 fold, which is much higher than in interbands, but lower than in IH bands. Notably,

### Table 1. Coordinates and sizes of bands and interbands on physical map of the 9F13 – 10B3 region.

| Cytological region | Position in genome (in nucleotides) | Length (in nucleotides) |
|--------------------|-------------------------------------|-------------------------|
| 9F13/10A1–2Mb      | 10792800..10793400                  | 600                     |
| 10A1–2b            | 10793401..10984199                  | 190798                  |
| 10A1–2/10A3Mb      | 10984200..10985600                  | 1400                    |
| 10A3b              | 10985601..11002399                  | 16798                   |
| 10A3/10A4–5Mb      | 11002400..11004400                  | 2000                    |
| 10A4–5b            | 11004401..11017399                  | 12998                   |
| 10A4–5/10A6Mb      | 11017400..11020400                  | 3000                    |
| 10A6b              | 11020401..11022399                  | 1998                    |
| 10A6/10A7b         | 11022400..11024000                  | 1600                    |
| 10A7b              | 11024001..11029999                  | 5998                    |
| 10A7/10A8–9b       | 11030000..11033000                  | 3000                    |
| 10A6–9b            | 11033001..11041199                  | 8198                    |
| 10A8–9/10A10Mb     | 11041200..11044600                  | 2800                    |
| 10A10–11b          | 11044601..11048599                  | 4598                    |
| 10A10–11/10B1–2b   | 11048600..11050000                  | 1400                    |
| 10B1–2b            | 11050001..11217799                  | 167798                  |
| 10B1–2/10B3Mb      | 11217800..11220400                  | 2600                    |

Notes: IB-interband; B-band.

Data of release FB2011_03 were used.

doi:10.1371/journal.pone.0025960.t001

### Table 2. DNA compaction ratio of the bands in the 9F13 -10B3 region.

| Bands and interbands (IB) | Length along chromosome axis (mcM) (α) | Physical sizes (bp) (β) | Length of DNA (μm) (c = β×0.34) | Compaction ratio (c/α) |
|---------------------------|----------------------------------------|------------------------|----------------------------------|------------------------|
| 9F13/10A1–2Mb             | 0.043±0.009                            | 600                    | 0.20                             | 4.65                   |
| 10A1–2b                   | 0.410±0.046                            | 190798                 | 64.87                            | 158.22                 |
| 10A1–2/10A3Mb             | 0.095±0.019                            | 1400                   | 0.48                             | 5.05                   |
| 10A3b                     | 0.078±0.006                            | 16798                  | 5.71                             | 73.21                  |
| 10A3/10A4–5Mb             | 0.065±0.008                            | 2000                   | 0.68                             | 10.46                  |
| 10A4–5b                   | 0.092±0.007                            | 12998                  | 4.42                             | 48.04                  |
| 10A4–5/10A6Mb             | 0.144±0.023                            | 3000                   | 1.02                             | 7.08                   |
| 10A6b                     | 0.057±0.006                            | 1998                   | 0.68                             | 11.93                  |
| 10A6/10A7b                | 0.045±0.008                            | 1600                   | 0.54                             | 12.00                  |
| 10A7b                     | 0.064±0.005                            | 5998                   | 2.04                             | 31.87                  |
| 10A7/10A8–9b              | 0.144±0.006                            | 3000                   | 1.02                             | 7.08                   |
| 10A8–9b                   | 0.087±0.003                            | 8198                   | 2.79                             | 32.07                  |
| 10A8–9/10A10b             | 0.117±0.006                            | 2800                   | 0.95                             | 8.12                   |
| 10A10–11b                 | 0.073±0.003                            | 4598                   | 1.56                             | 21.37                  |
| 10A10–11/10B1–2b          | 0.0975±0.0055                          | 1400                   | 0.48                             | 4.92                   |
| 10B1–2b                   | 0.279±0.011                            | 167798                 | 57.05                            | 204.48                 |
| 10B1–2/10B3Mb             | 0.086±0.006                            | 2600                   | 0.88                             | 10.23                  |

α – estimated on electron microscope sections of 50 polytene chromosomes.

Notes: IB-interband; B-band.

Data of release FB2011_03 were used.

doi:10.1371/journal.pone.0025960.t002
Interphase Chromosomes in Drosophila

two bands, 10A3 and 10A4–5, that are adjacent to 10A1–2 display the level of compaction somewhat higher, around 54–63 fold (Table 2). These bands do not associate with RNA polymerase II, interband-specific proteins, although they are composed of YELLOW, i.e. transcriptionally-active, chromatin [47]; according to [48] they contain chromatin states 22–24 and 30. It must be emphasized that these bands lack DNase I hypersensitive sites.

Interbands. appear decondensed in terms of their morphology. The minimal DNA compaction according to data of Table 2, is observed in interbands (3- to 15-fold. Additionally, they display major features of “open” chromatin, such as DNase I hypersensitive sites (DHS), interband-specific proteins Chriz/CHRO, BEAF, PolII, various transcription factors, histone variant H3.3, nucleosome-remodelling factors such as WDS and BRE-1, histone H1 dips. In all interbands, the chromatin is YELLOW or RED (see Fig. 2), which is indicative of their participation in transcriptional activity [47].

According to the 30 chromatin state model [48], interbands fall into states 1–6. These are exactly the same properties that we previously found specific for 13 interbands mapped throughout the analysis of P-transgene insertions [50]. In that work we showed, that 11 of the 13 transgene-tagged interbands corresponded to either intergenic regions or 5′-ends of genes. Moreover, chromatin state 1 is enriched in TSSes, 5′ UTRs and start codons [48]. As the Figure 6 demonstrates the comparison of gene localization from FlyBase with chromatin state 1 [48], interbands/bands, enrichment profiles for Chriz/CHRO, WDS, ORC2 and a nucleosome density plot. The dashed lines that mark the 9F13/10A1–2 interband, maps to the intergenic region between the 5′-ends of CG1528 and CG15268. The interband 10A1–2/10A3 comprises the 5′-half of the CG2076 gene (see Flybase). The interband 10A3/10A4–5 maps to the intergenic region between CG42249 and Hsp60. Likewise, interband 10A4–5/10A6 is found between divergently transcribed CG11122 and Rpl3. The central part of the 10A6/10A67 interband corresponds to the 5′-end of Gip-hp, center of 10A7/10A8–9 interband maps to the 5′-end of Kbp104a and CG18292, the interband 10A8–9/10A10–11 corresponds to the common upstream regulatory regions for ran and CG1906. The 5′-end of Dic2 makes up the 10A10–11/10B1–2 interband, whereas 10B1–2/10B3 interband occupies the 5′-ends of l(1)10Bb and CG1637 (Fig. 6). Thus, of the nine interbands studied in this work, eight map to the 5′-ends of genes, intergenic regions or first exons of genes.

DNA sequences that bind ORC2 in the nonpolytene chromosomes are unevenly distributed along this region [49]. For example, the band 10A1–2 lacks any ORC2 binding (Fig. 2E), whereas 10B1–2 shows four regions or ORC2 enrichment in Kc cells, but not in other cell types (Fig. 2E). ORC2 appeared virtually uniformly present throughout the region 10A3–10A11, with all origin recognition complexes being invariably found in interbands (Figs. 2 and 6). For instance, this 68 kb-long region (between 10A1–2 and 10B1–2 bands) encompasses 5–7 ORC2 binding sites (13.6 – 9.7 kb per ORC2), whereas 10A1–2 region shows no ORC2 binding over 190 kb, in the 10B1–2 band the ORC2 density varies from 42.5 kb/ORC2 to complete absence in 168 kb in chromosomes of some cell lines.

Discussion

The major conclusion that can be drawn from the present work is that both polytene and non-polytene chromosomes of mitotically active cells display band/interband organization. Furthermore, localization and protein content of interband chromatin in these types of interphase chromosomes are identical. This conclusion is based on the following two groups of facts.

Previously, we studied organization of interbands in diploid cells by accurately mapping P-element-tagged interbands in the genome [50]. Analysis of thirteen interband regions thus mapped demonstrated their identical organization in polytene and non-polytene chromosomes. More detailed analysis of two of these interbands firmly established that “interband as a stretch open chromatin is conserved in structure … between cell lines” [63]. In the present work, we used a reverse approach. We first mapped the interbands in the region 9F13-10B3 based on the localization of interband-enriched chromatin features. Then, using FISH with DNA probes from interband regions, we observed that interband positions in diploid cells matched those found in polytene chromosomes. This conclusion is also supported by perfect co-localization of interband DNA and interband-specific protein CHRIZ.

![Figure 6. Relation of genetic map, and band/interband pattern in the region 9F13 – 10B3.](https://example.com/figure6.png)

A - predicted bands  B - FlyBase genes  C – 30 chromatin states in BG3 and S2 cells [48]  D – DNase I hypersensitivity sites (DHS) in S2, BG3 and Kc cells [48]  E – ORC-binding sites in S2, BG3 and Kc cells [59]  F – Nucleosome Density (modENCODE, Henikoff group)  G – active chromatin specific [48] and - interbands specific proteins. Predicted interbands (dotted vertical lines are according to peaks in distribution of corresponding elements, solid lines reflect the edges of distributions of different characteristics).

doi:10.1371/journal.pone.0025960.g006
Based on these data, we hypothesized that interbands could represent a basic unit of interphase chromosome organization across different cell types. This conclusion is consistent with earlier observations of conserved banding pattern in different larval and adult cells in many dipteran species (see [1] and [66] for more details and references). Importantly, it must be noted that those cytological observations were indeed limited to tissues with polytene chromosomes. We generalize this conclusion as applied to the chromosomes from diploid non-polytene cells. Conserved localization and organization of interbands thus argues in favor of their important functions in the chromosome.

Presently, interbands' functions remain enigmatic. In this respect, it would be of utmost interest to explore the mechanisms of binding, roles and interplay between interband-specific proteins, such as CHRIZ, Z4 and JIL1, which were previously shown to contribute to the maintenance of open chromatin structure in interbands [6,38]. It has recently been demonstrated that CHRIZ and Z4 form a complex required to recruit JIL1 kinase thereby enabling H3S10 phosphorylation of histones in interband nucleosomes. This might in turn result in chromatin decondensation in interbands [65].

Open chromatin is required for binding of pre-replication machinery components [49]. So, given that interbands are particularly rich in ORC2, they might have a special role in replication initiation.

It is also important to consider particular properties of interband DNA organization. At the level of DNA sequence, interbands are poorly conserved; they typically correspond to intergenic, 5'–regulatory and 5'–untranslated regions of genes. Notably, not all interband material is functionally equivalent. For instance, DNaseI hypersensitive sites (DHSs) and histone H1 dips were particularly enriched in interbands [50]. Several DHSs were discovered in an interband 3C6/C7, which maps to the 5'–regulatory region of the Notch gene. The faswb deletion which

![Image](https://example.com/image.png)

**Figure 7. Comparison of extents of band/interbands in polytene chromosomes according to Flybase r5.25 (A, B) and to the data of this study (C).** Physical DNA map is situated between 10792800 and 11220400 positions of the map of Flybase.

doi:10.1371/journal.pone.0025960.g007

**Table 3. Coordinates and descriptions of probes, selected for FISH mapping on polytene chromosomes in the region 9F13 – 10B3 (coordinates correspond to the version dm3 (r5.24), FlyBase).**

| Probes                  | Localization of probes on cytological map (See Results) | Description of probes | Primers                                      | Coordinates of probe |
|-------------------------|--------------------------------------------------------|-----------------------|----------------------------------------------|---------------------|
| CG1582                  | DNA of the interband 9F13/10A1–2 (arrow 1 on Fig. 2)   | The fourth coding exon of the gene CG1582 | 5'-GCTTTTCCCTCGCCCAAGCGC-3'; 5'-AAGAGGGCGGCGTTGAGCGT-3' | 10791234..10791994  |
| spas_9F_10A             | DNA of the interband 9F13/10A1–2 (arrow 1 on Fig. 2)   | Intergenic fragment between genes CG1582 and CG15208 | 5'-GGGCCCAAGTGTGAAGCGC-3'; 5'-GCTGCCAACCCGTTGAGCGT-3' | 10793577..10794176  |
| CG15208                 | DNA of the interband 9F13/10A1–2 (arrow 1 on Fig. 2)   | Coding exon of the gene CG15208 | 5'-GCTCTATGCGCTGAACTCC-3'; 5'-ACAGATGTCGCGGCTGAACTCC-3' | 10794863..10795492  |
| Vago                    | DNA of the interband 10A1–2/10A3 (arrow 2 on Fig. 2)   | The part of coding first exon, intron and parts of the second exon of the vago gene | 5'-GGTGCCAGCAAGGACTCC-3'; 5'-AATCTGGGCGGGCTGAACTCC-3' | 10983458..10983987  |
| CG2076                  | The fragment of the interband 10A1–2/10A3 (arrow 2 on Fig. 2) | The second, third, and fourth coding exons, as well as the second, third and fourth introns of the CG2076 gene | 5'-TGCGAAGCAGCGAAGCGAAGGAGGAGG-3' | 10984947..10985771  |
| CG32668                 | Fragments of the interband 10A6–9/10B1–2 (arrow 3 on Fig. 2) | First coding exon of the GG2668 gene | 5'-CGGAGATTCGCGCGGCTCTGC-3'; 5'-CAAGGAGATTCGCGCGGCTCTGC-3' | 11050925..11051486  |
| H(1)10Bb                | Fragments of the interband 10B1–2/10B3 (arrow 4 on Fig. 2) | Coding fragments of first and second exons and intron of the H(1)10Bb gene | 5'-CGTGGAGAAGCAAGCGGAGCGGAGCGG-3'; 5'-TCTACGCGGAGCGGAGCGGAGCGG-3' | 11218702..11219201  |

doi:10.1371/journal.pone.0025960.t003
removes 900 b.p. including these DHSs, leads to the disappearance of this interband [41–43]. Removal of just 246 b.p. closest to the transcription initiation site from within these 900 b.p. and then fixed in a 3:1 mixture of ethanol and acetic acid for 30 minutes at 2°C, squashed in 45% acetic acid, snap-frozen in liquid nitrogen and stored in 70% ethanol at −20°C. For fluorescence in situ hybridization (FISH) on polytene chromosomes DNA probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP (Roche) in random-primed polymerase reaction with 30 minutes at 2°C, squashed in 45% acetic acid, snap-frozen in liquid nitrogen and stored in 70% ethanol at −20°C. For fluorescence in situ hybridization (FISH) on polytene chromosomes DNA probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP (Roche) in random-primed polymerase reaction with Klenow fragment [70].

Materials and Methods

Cytological analysis of polytene chromosomes

Salivary gland polytene chromosome squashes were prepared for cytological electron microscopy analysis and examined as described earlier [69]. The 120–150 nm sections were cut using an LKB-IV (Sweden) ultratome and examined under a JEM-100C (JEOL, Japan) electron microscope at 80 kV.

Fluorescence in situ hybridization (FISH)

Larvae were grown at 22°C in uncrowded vials on standard flyfood. Salivary glands were dissected in Ephrussi-Beadle solution, and then fixed in a 3:1 mixture of ethanol and acetic acid for 30 minutes at −20°C, squashed in 45% acetic acid, snap-frozen in liquid nitrogen and stored in 70% ethanol at −20°C. For fluorescence in situ hybridization (FISH) on polytene chromosomes DNA probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP (Roche) in random-primed polymerase reaction with Klenov fragment [70]. Table 3 summarizes all the probes used in this study.

Chromosomes were examined using epifluorescence optics (Olympus BX50 microscope) and photographed with CCD Olympus DP50.

**GEO data used (http://www.ncbi.nlm.nih.gov/gds/).

**modENCODE data used (http://www.modencode.org/Genomes.shtml).

doi:10.1371/journal.pone.0025960.t004

*modENCODE data used (http://www.modencode.org/Genomes.shtml).

**GEO data used (http://www.ncbi.nlm.nih.gov/gds/).

doi:10.1371/journal.pone.0025960.t004

| Table 4. Accession numbers of chromosome proteins. |
|-----------------------------------------------|
| No. | Proteins and antibodies | Accession numbers (id)* |
|-----|-------------------------|-------------------------|
| 1   | BEAF-32; BEAF-HLB.S2    | modENCODE_274           |
| 2   | BEAF-32; BEAF-70.S2     | modENCODE_922           |
| 3   | Chr1 (or Chromator); Chro/Chr1.WR.S2 | modENCODE_279 |
| 4   | Chr1 (or Chromator); Chro/Chr1.BRS.S2 | modENCODE_278 |
| 5   | RNApol II; RNA pol II(ALG).S2 | modENCODE_329 |
| 6   | Trx Trx.C.S2             | modENCODE_332           |
| 7   | GAF; GAF.S2              | modENCODE_285           |
| 8   | BRE1; BRE1_Q2539.S2     | modENCODE_923           |
| 9   | NURF301; NURF301_Q2602.S2 | modENCODE_947 |
| 10  | WDS; WDS_Q2691.S2       | modENCODE_953           |
| 11  | H3K9acS10P (new lot); H3K9acS10P_(new_lot).S2 | modENCODE_2660 |
| 12  | ORC2; ORC2.C.S2         | modENCODE_2753          |
| 13  | ORC2; ORC2.BG3          | modENCODE_2754          |
| 14  | ORC2; ORC2.Kc           | modENCODE_2755          |
| 15  | Nucleosome_Density.S2   | modENCODE_2506          |
| 16  | 30-State_Chromatin.S2   | modENCODE_3365          |
| 17  | 30-State_Chromatin.BG3  | modENCODE_3364          |
| 18  | H3.3                    | GSE4091**               |
| 19  | H1 dips                 | GSE16885                |
| 20  | Five chromatin types    | GSE22069                |
| 21  | Lamin                   | GSM509085               |
| 22  | D1                      | GSM55042                |
| 23  | SUUR                    | GSM550486               |
| 24  | Gene density            | GSE16531                |
| 25  | CATCH-IT                | GSM494308               |
| 26  | replication timing of S2 cells | GSM336376 |
| 27  | replication timing of Kc cells | GSM336362 |
| 28  | replication timing of CB cells | GSM336363 |
| 29  | DH5s in S2, Kc, BG3 cells | http://compbio.med.harvard.edu/flychromatin/data.html |

*modENCODE data used (http://www.modencode.org/Genomes.shtml).

**GEO data used (http://www.ncbi.nlm.nih.gov/gds/).

doi:10.1371/journal.pone.0025960.t004
them either on corresponding page in GEO (http://www.ncbi.nlm.nih.gov/geo/), or in supplementary to the original papers (Table 4). We used two types of data from modEncode: Smoothed M-value enrichment profiles represented as histograms and Regions of significant enrichment. Protocols of data processing are described in the corresponding section of modMine (http://intermine.modencode.org). Track for density of P-element insertion site was based on FlyBase, r5.32. We treated the data using the sliding window of 1 kb size with the step 500 b.p.

For visualization of data we used UCSC Genome browser (http://genome.ucsc.edu). Custom scripts were used to adjust data in accordance with UCSC format.

References

1. Beermann W (1972) Chromomeres and genes. In: Beermann W, ed. Results and problems in cell differentiation. V. 4. Berlin, Heidelberg/New York: Springer, pp 1–33.
2. Zhimulev IF (1996) Morphology and structure of polytene chromosomes. Adv Genet 34: 1–47.
3. Zhimulev IF, Belyaeva ES, Semeshin VF, Koryakov DE, Demakov SA, et al. (2004) Polytene Chromosomes: 70 Years of Genetic Research. Int Rev Cytol 241: 203–275.
4. Kozlova TYu, Semeshin VF, Tretjakova IV, Kokoza EB, Pirrorta V, et al. (1994) Molecular and cytogenetical characterization of the 10A1–2 band and adjoining region in the Drosophila melanogaster polytene X chromosome. Genetics 136: 1063–1073.
5. Andreyenkova NG, Kokoza EB, Semeshin VF, Belyaeva ES, Demakov SA, et al. (2009) Localization and characteristics of DNA underreplication zone in the 75 C region of intercalary heterochromatin in Drosophila melanogaster polytene chromosomes. Chromosoma 118: 741–751.
6. Eggert H, Grotchak, A. Saumweber H (2004) Identification of the Drosophila interband-specific protein Zt as a DNA-binding zinc-finger protein determining chromosomal structure. Cell Sci 117: 4253–4264.
7. Rith U, Wang D, Ding Y, Xu Y-Z, Qi H, et al. (2004) Chromator, a novel and essential condensomin protein interacts directly with the putative spindle matrix protein skeleton. J Cell Biochem 93: 1033–1047.
8. Grotchak AA, Eggert H, Gan M, Mato J, Zhimulev IF, et al. (2005) Cirr3, a chromom-domain protein specific for the interbands of Drosophila melanogaster polytene chromosomes. Chromosoma 114: 54–66.
9. Zhao K, Hart CM, Laemmli UK (1995) Visualization of chromosomal domains with boundary element-associated factor BEAF-32. Cell 81: 879–89.
10. Jiang N, Emberly E, Cuvier O, Hart CM (2009) Genome-wide mapping of boundary element-associated factor (BEAF) binding sites in Drosophila melanogaster links BEAF to transcription. Mol Cell Biol 29: 3536–3568.
11. Elgin SCR, Amore SA, Eisenberg JC, Fleischmann G, Gillmour DS, et al. (1988) Distribution patterns of nonhistone chromosomal proteins on Drosophila chromosomes: Functional correlations. In: Gustafson JP, Appel R, ed. Chromosome structure and function: impact of new concepts. New York: Plenum, pp 145–156.
12. Jamrich M, Greenleaf AL, Bautz EK (1977) Localization of RNA polymerase in polytene chromosomes of Drosophila melanogaster. Proc Natl Acad Sci USA 84: 789–793.
13. Sass H (1982) RNA polymerase B in polytene chromosomes: immunofluorescent and autoradiographic analysis during stimulated and repressed RNA synthesis. Cell 26: 269–278.
14. Sass H, Bautz EK (1982a) Immunoelectron microscopic localization of RNA polymerase B on isolated polytene chromosomes of Chironomus tentans. Cell 28: 269–278.
15. Sass H, Bautz EK (1982b) Interbands of polytene chromosomes: binding sites and start points for RNA polymerase. Chromosoma 86: 77–93.
16. Weeks JR, Hardin SE, Shen J, Lee JM, Greenleaf AL (1994) Locus-specific variation in phosphorylation state of RNA polymerase II in vivo: correlation with gene activity and transcript processing. Genes Dev 7: 2329–2344.
17. Gillmour DS, Lo JT (1986) RNA polymerase II interacts with the promoter region of the non-induced hsp70 gene in Drosophila melanogaster cells. Mol Cell Biol 6: 3984–3989.
18. Law A, Hirayoshi K, O’Brien T, Lis JT (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.
19. Kaplan CD, Morris JR, Wu C, Winston F (2000) Stn5 and stn6 are associated with active transcription and have characteristics of general elongation factors in D. melanogaster. Genes Dev 14: 2623–2634.
20. Liu JT, Mason P, Peng J, Price DH, Werner J (2000) Poly-TFIIA kinase recruitment and function at heat shock loci. Genes Dev 14: 792–803.
21. Schwartz BE, Lalorache S, Suter B, Lo JT (2003) Cdk7 is required for full activation of Drosophila heat shock genes and RNA polymerase II phosphorylation in vivo. Mol Cell Biol 23(19): 6876–6886.

Acknowledgments

The authors are grateful to Drs. Yu.Ya. Sheveljov and A.A. Gortchakov for invaluable discussion and advice.

Author Contributions

Performed the experiments: OVD LVB VFS. Wrote the paper: IFZ TYV ESB. Conceived the experiments and analyses: IFZ TYV ESB. Performed bioinformatics analyses: TYV VNB EBK FPG SAD.
43. Ramos RGP, Grimwade BG, Wharton KA, Scottigale TN, Artavàns-Tsakonas S (1989) Physical and functional definition of the Drosophila Nch locus by P element transformation. Genetics 123: 337–348.
44. Demakov SX, Semeshin VF, Zhimulev IF (1993) Cloning and molecular genetic analysis of Drosophila melanogaster interband DNA. Mol Gen Genet 238: 437–445.
45. Braunschweig U, Hogan GJ, Page L, van Steensel B (2009) Histone H1 binding is inhibited by histone variant H3.3. The EMBO J 28: 3635–3645.
46. van Steensel B, Braunschweig U, Filion GJ, Chen M, van Bennet JG, et al. (2010) Bayesian network analysis of targeting interactions in chromatin. Genome Res 20(2): 190–200.
47. Filion GJ, van Bennet JG, Braunschweig U, Talbout W, Kind J, et al. (2010) Systematic protein location mapping reveals five principal chromatin types in Drosophila cells. Cell 143: 212–224.
48. Kharchenko PV, Alekseyenko AA, Schwartz YB, Minoda A, Riddle NC, et al. (2010) Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. Nature 471(7339): 480–485.
49. MacAlpine HK, Gortázar R, Powell SK, Hartemink AJ, MacAlpine DM (2010) Drosophila ORC localizes to open chromatin and marks sites of cohesin complex loading. Genome Res 20: 201–211.
50. Vatolina T Yu, Demakov SA, Semeshin VF, Makunin IV, Babenko VN, et al. (2011) Identification and molecular genetic characterization of the polytene chromosome interbands in Drosophila melanogaster. Russian Journal of Genetics 47(5): 15–26.
51. Zhimulev IF, Semeshin VF, Kulichkov VA, Belyaeva ES (1982) Intercalary heterochromatin in Drosophila. I. Localization and general characteristics. Chromosoma 87: 197–228.
52. Belyaeva ES, Zhimulev IF, Volkova EI, Alekseyenko AA, Moshkin YuM, et al. (1998) Su(UR)ES: a gene suppressing DNA underreplication in intercalary and pericentric heterochromatin of Drosophila melanogaster polytene chromosomes. Proc Natl Acad Sci USA 95(13): 7532–7537.
53. Belyaeva ES, Zhimulev IF, Volkova EI, Alekseyenko AA, Kriventseva EV, Belyaeva ES, et al. (2005) Genomic analysis of Drosophila chromosome underreplication reveals a link between replication control and transcriptional territories. Proc Natl Acad Sci U S A 102: 8269–8274.
54. Belyaeva ES, Babenko VN, Makximov DA, Shloma VV, Kvon EZ, et al. (2010) SUUR joins separate subsets of PAG, HP1 and B-type lamin targets in Drosophila. J Cell Sci 120: 2344–2351.
55. Zhimulev IF, Pokhol'kova GV, Bgatov AV, Semeshin VF, Belyaeva ES (1981) Fine cytogenetical analysis of the band 10A1–2 and the adjoining regions in the Drosophila melanogaster X chromosome. II Genetical analysis. Chromosoma 82: 25–40.
56. Bridges CB (1938) A revised map of the salivary gland X-chromosome of Drosophila melanogaster. J Hered 29: 11–13.
57. Lefèvre G, Jr. (1969) The eccentricity of vermilion deficiencies in Drosophila melanogaster. Genetics 63: 589–600.
58. Lefèvre G, Jr. (1971) Salivary chromosome bands and the frequency of crossing over in Drosophila melanogaster. Genetics 67: 497–513.
59. Eaton ML, Prinz JA, MacAlpine HK, Tret'yakov G, Kharchenko PV, et al. (2011) Chromatin signatures of the Drosophila replication program. Genome Res 21(2): 164–174.
60. Deal RB, Henikoff JG, Henikoff S (2010) Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. Science 328: 1161–1164.
61. Schwaiger M, Stadler MB, Bell O, Kohler H, Oakeley EJ, et al. (2009) Chromatin state marks cell-type- and gender-specific replication of the Drosophila genome. Genes Dev 23(3): 289–301.
62. Celinker SE, Dillon LA, Gerstein MB, Gussalsh KG, Henikoff S, et al. (2009) Unlocking the secrets of the genome. Nature 459: 927–930.
63. Pindyurin AV, Moorman C, de Wit E, Belyakin SN, Belyaeva ES, et al. (2007) SUUR recruits JIL-1 to polytene chromosomes, a requirement for interband-specific phosphorylation of H3S10. J Biosci 36: 425–430.
64. Babenko VN, Pokhol'kova GV, Kokoza EB, Andreyenkov NG, Belyakin SN, et al. (2009) Characteristics of molecular-genetic organization of intercalary heterochromatin band 10A1–2 in X chromosome of Drosophila melanogaster. Doklady Biochemistry and Biophysics 424: 27–30.
65. Gan M, Moebus S, Eggert H, Saumweber H (2011) The Chriz-Z4 complex over in Drosophila melanogaster, a requirement for interband-specific phosphorylation of H3S10. J Biosci 36: 425–430.
66. Zhimulev IF (1999) Genetic organization of polytene chromosomes. Adv in Genetics 40: 1–123.
67. Andreyenkov OV, Volkova EI, Demakov SA, Semeshin VF, Zhimulev IF (2010) The decompact state of interchromomeric chromatin from the 3C6/C7 region of Drosophila melanogaster is determined by short DNA sequence. Doklady Biochemistry and Biophysics 431: 57–59.
68. Sorsa V (1988) Chromosome maps of Drosophila. Vol. 2. Boca Raton: CRC Press, Inc. pp 147.
69. Semeshin VF, Belyaeva ES, Shloma VV, Zhimulev IF (2004) Combined immunostaining and FISH analysis of polytene chromosomes. Methods Mol Biol 247: 289–303.