Polytene chromosomes reflect functional organization of the *Drosophila* genome

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Polytene chromosomes of *Drosophila melanogaster* are a convenient model for studying interphase chromosomes of eukaryotes. They are giant in size in comparison with diploid cell chromosomes and have a pattern of cross stripes resulting from the ordered chromatid arrangement. Each region of polytene chromosomes has a unique banding pattern. Using the model of four chromatin types that reveals domains of varying compaction degrees, we were able to correlate the physical and cytological maps of some polytene chromosome regions and to show the main properties of genetic and molecular organization of bands and interbands, that we describe in this review. On the molecular map of the genome, the interbands correspond to decompacted aquamarine chromatin and 5′ ends of ubiquitously active genes. Gray bands contain lazurite and malachite chromatin, intermediate in the level of compaction, and, mainly, coding parts of genes. Dense black transcriptionally inactive bands are enriched in ruby chromatin. Localization of several dozens of interbands on the genome molecular map allowed us to study in detail their architecture according to the data of whole genome projects. The distribution of proteins and regulatory elements of the genome in the promoter regions of genes localized in the interbands shows that these parts of interbands are probably responsible for the formation of open chromatin that is visualized in polytene chromosomes as interbands. Thus, the permanent genetic activity of interbands and gray bands and the inactivity of genes in black bands are the basis of the universal banding pattern in the chromosomes of all *Drosophila* tissues. The smallest fourth chromosome of *Drosophila* with an atypical protein composition of chromatin is a special case. Using the model of four chromatin states and fluorescent *in situ* hybridization, its cytological map was refined and the genomic coordinates of all bands and interbands were determined. It was shown that, in spite of the peculiarities of this chromosome, its band organization in general corresponds to the rest of the genome. Extremely long genes of different *Drosophila* chromosomes do not fit the common scheme, since they can occupy a series of alternating bands and interbands (up to nine chromosomal structures) formed by parts of these genes.

Key words: *Drosophila melanogaster*; polytene chromosomes; interphase chromosomes; four chromatin state model; fluorescent *in situ* hybridization; genetic organization; bands and interbands of chromosomes.

Политенные хромосомы отражают функциональную организацию генома *Drosophila*

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Политенные хромосомы *Drosophila melanogaster* – удобная модель для изучения интерфазных хромосом эукариот. Им свойственны гигантские размеры в сравнении с хромосомами диплоидных клеток и поперечная исчерченность, возникающая в связи с упорядоченным расположением хроматид. Каждый район политенных хромосом обладает уникальным дисковым рисунком. С использованием модели четырех типов хроматина, которая выявляет домены различной степени компактизации, удалось соотнести физическую и цитологическую карты некоторых районов политенных хромосом и показать основные свойства генетической и молекулярной организации дисков и междисков, описанию которых посвящен данный обзор. Междискам на молекулярной карте генома соответствуют декомпактный aquamarine хроматин и 5′-концы повсеместно активных генов. Серые диски содержат промежуточный по уровню компактизации lazurite и malachite хроматин и в основном кодирующие части генов. Черные плотные транскрипционно неактив-

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ные диски обогащены ruby хроматином. Локализация нескольких десятков междисков на молекулярной карте генома позволила по данным полногеномных проектов подробно исследовать их архитектуру. Распределение белков и регуляторных элементов генома в промоторных районах генов, локализованных в междисках, показывает, что эти части междисков, вероятно, отвечают за формирование хроматина открытого типа, который визуализируется в политенных хромосомах как междиски. Таким образом, постоянная генетическая активность междисков и серых дисков и неактивность генов в черных дисках лежат в основе универсального дискового рисунка в хромосомах всех тканей дрозофилы. Особый случай представляет самая маленькая – четвертая – хромосома дрозофилы с нетипичной белковой композицией хроматина. При помощи модели четырех состояний хроматина и флуоресцентной *in situ* гибридизации была уточнена ее цитологическая карта и определены геномные координаты всех дисков и междисков. Показано, что, несмотря на особенности этой хромосомы, ее дисковая организация в целом соответствует остальному геному. Выбиваются из общей схемы особо длинные гены разных хромосом дрозофилы, которые могут занимать целую серию чередующихся дисков и междисков (до девяти структур), образованных частями этих генов.

Ключевые слова: *Drosophila melanogaster*; политенные хромосомы; интерфазные хромосомы; модель состояний хроматина; флуоресцентная *in situ* гибридизация; генетическая организация; диски и междиски хромосом.

Introduction

The *Drosophila melanogaster* polytene chromosomes have been used as a model for studying interphase chromosomes for many years, since this is the only type of chromosomes that can be visualized along their entire length in the interphase nucleus. These giant chromosomes are formed in specialized cells (e. g. salivary gland cells and nurse cells) due to numerous replication cycles without subsequent segregation of daughter chromatids, which remain closely connected to each other. The chromomeric pattern of interphase chromosomes, therefore, becomes contrastingly expressed in the form of alternating dark and light transverse stripes. Densely packed chromatin in polytene chromosomes forms black bands, moderately condensed regions form loose gray bands, and light decondensed regions are interbands.

The model of four chromatin states

In recent years, a large amount of whole genome data on the distribution of various histone modifications, chromatin proteins, and regulatory elements of the *Drosophila* genome has been accumulated (modENCODE Consortium..., 2010). These data have a marking corresponding to the physical genomic coordinates. To analyze the interrelation of the functional domains formed by these characteristics, with the structural, morphological, and genetic organization of *Drosophila* chromosomes, it is necessary to know the correspondence of polytene chromosome cytological structures (bands and interbands) to their genomic coordinates. To achieve this goal, we developed a computer model of four chromatin states, which has a good correspondence to the cytological structures of polytene chromosomes (Zhimulev et al., 2014).

Earlier, about 30 interbands have been localized on the physical map of the *Drosophila* genome using different methods. Using modENCODE data, a set of proteins associated with DNA sequences, corresponding to these interbands in cell cultures, was determined (Demakov et al., 1993, 2011; Semeshin et al., 2008; Vatolina et al., 2011a, b; Zhimulev et al., 2014). Based on the distribution of these proteins, using a computer algorithm, our model of four chromatin states, conventionally named as colors, was developed (Zhimulev et al., 2014). Further, in order to avoid intersections with other works, the colors of chromatin states were renamed according to the names of the minerals (Boldyreva et al., 2017).

Aquamarine chromatin (former cyan) contains maximum amount of open chromatin proteins, that are the basis of the model, and occupies about 13 % of the genome. This chromatin state corresponds to 5′ UTRs of ubiquitously active genes (Zhimulev et al., 2014; Zykova et al., 2018). All the interbands with determined genomic coordinates contain this chromatin type (Zhimulev et al., 2014). Ruby chromatin (former magenta) occupies about 50 % of the genome and it lacks proteins characteristic of open chromatin (Zhimulev et al., 2014). It is localized in black transcriptionally inactive bands, mainly containing tissue- and stage-specific genes, including the largest bands of intercalary heterochromatin (Khoroshko et al., 2016; Kolesnikova et al., 2018). Lazurite (former blue) and malachite (former green) chromatin states have an intermediate degree of compaction and mainly correspond to gray bands and transition zones between the interbands and black bands (Zhimulev et al., 2014; Boldyreva et al., 2017; Khoroshko et al., 2018). Lazurite chromatin is enriched with coding parts of genes, and malachite – with introns and intergenic spacers (Zykova et al., 2018). Thus, the model of four chromatin types confirms the identity of the organization of polytene chromosomes and chromosomes of diploid cells. This review shows examples of successful application of this model in determining the localization of bands and interbands on the physical map of the *Drosophila* genome.

The interbands are decompacted structures containing promoters of ubiquitously active genes

As mentioned above, aquamarine chromatin mainly corresponds to the interbands of polytene chromosomes containing promoters of ubiquitously active genes (Zhimulev et al., 2014). In the recent study by T.Yu. Zykova and co-authors (2019), the molecular and genetic organization of 33 interband sequences located on the physical map of the *Drosophila* genome was studied in detail. It turned out that the interbands have diverse genetic structure. The majority of the interbands studied contain one gene with one transcription start site, and the remaining interbands contain one gene with several alternative promoters, two or more unidirectional genes and genes with "head-to-head" orientation. In addition, there are complexly organized interbands with three or more genes having unidirectional or bidirectional orientation (Zykova et al., 2019).

Interbands containing unidirectional and bidirectional genes were studied in detail. The distribution of various factors that may influence the formation of open chromatin in the interbands is of particular interest. In this work, using modENCODE data, it was shown that in the interband sequences containing a single gene, the peak of CHRIZ and BEAF-32 insulator protein localization (Vogelmann et al., 2014) is located at the transcription start site, and the peak of RNA polymerase II lies 200 bp upstream of this site. In the interbands containing promoters localized "head-to-head", these insulator proteins lie between the transcription initiation sites, and the peaks of RNA polymerase II correspond to these two gene promoters (Zykova et al., 2019). Short noncoding RNAs, the products of the paused RNA polymerase II (Nechaev et al., 2010), replication complex protein ORC2 (Eaton et al., 2011), DNAse I hypersensitive sites (Kharchenko et al., 2011), *P*-element insertions (FlyBase r.5.57), and "broad" promoters (Hoskins et al., 2011) in the interbands under study are located in the regions of transcription start sites. Thus, they have one peak in the interbands with one gene, and two peaks in the interbands containing two opposite directed genes (Zykova et al., 2019).

Despite the fact that the average interband size is about 3.5 kb, it was shown that various open chromatin elements, characteristic of interbands, do not occupy the entire sequence of aquamarine chromatin fragments corresponding to these structures. They have narrow localization in regulatory regions of genes beginning in the interbands (Zykova et al., 2019). In addition, the location of 5′ gene ends with ubiquitous expression in aquamarine chromatin and its high enrichment in ORC2 protein (Zhimulev et al., 2014; Zykova et al., 2018) confirms the fact that the interbands combine the processes of transcription initiation and replication. The authors conclude that regulatory region of an active gene is apparently critical for the formation of an open chromatin domain, which is visualized as a light interband in polytene chromosomes (Zykova et al., 2019).

Atypical cases of gene localization in the structures of polytene chromosomes

To sum up, it has been shown that the banding pattern is a universal principle of both polytene chromosome and diploid cell chromosome organization, and housekeeping genes are embedded in two interphase chromosome structures: their promoters are localized in the interbands, and the structural parts lie in adjacent loose bands (Zhimulev et al., 2014). In addition, it has recently been discovered that long *D. melanogaster* genes can occupy several morphological structures of polytene chromosomes (Zhimulev et al., 2016; Khoroshko et al., 2019). For example, using fluorescent *in situ* hybridization (FISH), it was shown that long introns of the ubiquitous active genes *dlg1* (~18 kb) and *CG43867* (~106 kb), interrupted by short rare exons, form loose gray bands 10B8-9 and 1D1-2, respectively, and their 5′ and 3′ regulatory regions lie in the decompacted interbands of the X chromosome (Zhimulev et al., 2016). Thus, it has been found that in some cases polytene chromosome bands can be formed from the material of only one part of a gene, namely, long non-coding introns of actively transcribed genes, which allowed the authors to conclude that there is a differential pattern of chromatin condensation of

gene parts during its activation (Zhimulev et al., 2016). A special case in studying gene localization in the morphological structures of polytene chromosomes is the *dunce* (*dnc*) gene, the size of which (167.3 kb) is 25-fold longer than the average length of a *D. melanogaster* gene (Khoroshko et al., 2019). The *dnc* gene has 17 transcripts responsible for various biological functions. About 94 % of the *dnc* transcript is occupied by introns, in which eight short genes are localized. The gene is mostly represented by inactive ruby and malachite chromatin states (93 %), which is consistent with the total size of introns. The majority of gene transcript 5′ ends corresponds to open aquamarine chromatin. To determine the localization of the *dnc* gene on polytene chromosome preparations, the authors performed FISH analysis using three probes from aquamarine chromatin corresponding to the 5′ and 3′ UTRs of the longest transcript of this gene and an alternative promoter of a group of transcripts. According to the hybridization results, the *dnc* gene is localized within nine cytological structures, namely, five interbands and four bands (3C7/C8-3D1 – 2/D3-4). The results do not correspond with the previously dominated ideas about the localization of genes in only one structure (Khoroshko et al., 2019). The material of introns, which constitute the largest part of the *dnc* gene length, forms not only loose gray and dense dark bands, but also interbands, in which chromatin is open for replication (Khoroshko et al., 2019).

The fourth chromosome is a specific domain of *Drosophila* chromatin

Mapping of the fourth chromosome, the smallest chromosome in the *D. melanogaster* genome, is of particular interest due to its unusual organization. The euchromatic arm of this chromosome, which looks like a dot on metaphase spreads, becomes visible after numerous endocycles in salivary gland cells. On the map by C.B. Bridges (1935), it corresponds to cytological sections 101 and 102, but there is no detailed cytological map. This *Drosophila* chromatin domain is unusual since it has high gene density and, at the same time, possesses heterochromatin characteristics (Riddle et al., 2009). Absence of recombination under normal conditions in the fourth chromosome of *D. melanogaster* leads to the accumulation of repeated sequences and mobile genome elements (Slawson et al., 2006). Chromatin in this domain shows high H3K9me enrichment (Riddle, Elgin, 2006), and this modification is mainly catalyzed by dSETDB1 histone methyltransferase specific for this chromosome (Seum et al., 2007; Figueiredo et al., 2012). The protein HP1a, which recognizes this mark and binds to typical heterochromatin regions, is also associated with the polytenised part of the fourth chromosome (James et al., 1989). A unique property of the *D. melanogaster* fourth chromosome is the protein POF (painting of fourth) that is localized mainly in the interbands (Larsson et al., 2001). Here we show the localization of POF on the fourth chromosome of the *SuUR Su(var)3-9* double mutant *D. melanogaster* larval salivary glands (Figure).

It was shown that POF and HP1a bind to the fourth chromosome interdependently and there is a mechanism balancing the action of POF and HP1 proteins, which provides fine-tuning of gene expression on this chromosome (Johansson et al., 2007a, b). It has been hypothesized that HP1a initially binds active gene promoters with high affinity regardless of

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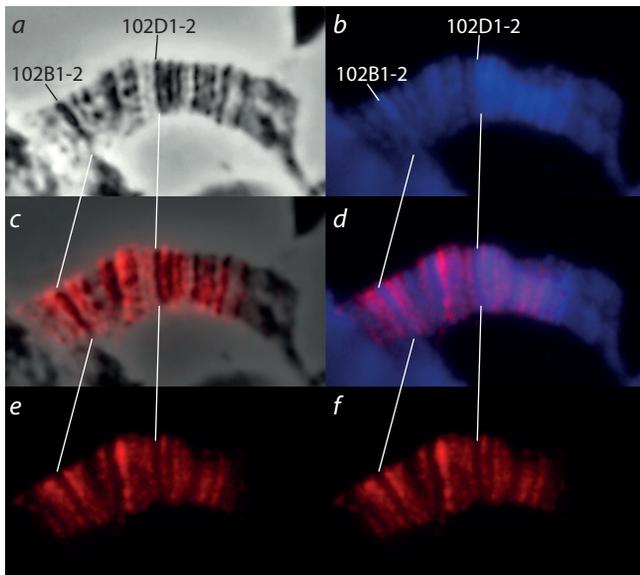
POF localization on the polytene fourth chromosome of *D. melanogaster*.
We used *SuUR^ES Su(var)3-9^06* double mutant flies because of suppressed underreplication for better spreading of the *Drosophila* fourth polytene chromosome on preparations (Andreyeva et al., 2007; Demakova et al., 2007). The preparations were made according to B. Czermin et al. (2002).
a – phase contrast image of the fourth chromosome; *b* – DAPI staining; *c* – merged phase contrast and POF staining; *d* – merged DAPI and POF staining; *e, f* – POF staining. Antibodies are the same as in J. Larsson et al. (2004). POF decorates predominantly the interbands and gray bands of the fourth chromosome.

H3K9me, and then it propagates through H3K9 methylation and loop contacts with chromatin containing this mark (Figueiredo et al., 2012). Recently, an optimal autonomous POF target has been identified. It consists of a gene and a block of X chromosome-specific 1.688 satellites downstream of the gene. The fourth chromosome seems to interact with POF due to the cooperative effect of a large number of suboptimal combinations of genes with repeats (Kim et al., 2018).

The study of the fourth chromosome at the cytological level is complicated by its small size, a large number of gray bands with an intermediate level of compaction, and frequent ectopic contacts of its distal end with the chromocenter. In our recent work, a fine mapping of all bands and interbands of this chromosome on the physical map of the *Drosophila* genome was carried out using the four chromatin state model (Zhimulev et al., 2014) and FISH (Sidorenko et al., 2018). In this study, to obtain preparations with good morphology of the fourth chromosome, we used a fly stock with suppressed underreplication, containing mutations in the *SuUR* and *Su(var)3-9* genes and linked X and Y chromosomes. Due to the matching of the fourth chromosome cytological map to the genomic coordinates, the distribution of various chromatin properties in bands and interbands was investigated using data from whole genome projects. It was shown that the interbands contain aquamarine chromatin and 5′ UTRs of ubiquitously active genes, gray bands contain lazurite chromatin and coding parts of these genes, and black bands contain ruby chromatin and are polygenic, or also contain coding parts of genes (Sidorenko et al., 2018). The distribution of various characteristics in the domains of active and repressed chromatin of the fourth

chromosome was studied. POF (modENCODE data; Lundberg et al., 2013) and HP1a (modENCODE data) proteins, providing a special organization of this chromosome, are predominantly localized in aquamarine (interbands) and lazurite (gray bands) chromatin states (Sidorenko et al., 2018). Only ruby chromatin is enriched in the modified histone H3K27me3 (Sher et al., 2012), which corresponds to the data on other chromosomes (Sidorenko et al., 2018). DNase I hypersensitive sites, ORC2 protein and *P*-elements (modENCODE data) are predominantly located in open aquamarine chromatin, while the fourth chromosome-specific element *1360* (Kholodilov et al., 1988) occupies the band chromatin types (Sidorenko et al., 2018). Compared to the rest of the genome, the fourth chromosome contains half the size of compact ruby chromatin, which is associated with a large number of loose gray bands and the absence of intercalary heterochromatin bands. Notably, despite the peculiarities of the fourth chromosome, its band organization corresponds to the organization of the rest of the *D. melanogaster* genome (Sidorenko et al., 2018).

Conclusion

Thus, *D. melanogaster* polytene chromosomes together with the four chromatin state model (Zhimulev et al., 2014; Boldyreva et al., 2017; Zykova et al., 2018) provide a good model for studying the organization of interphase chromosomes. Using this system, we confirmed that the banding pattern is the general principle of interphase chromosome organization (Zhimulev et al., 2014). The model of four chromatin states in combination with FISH allows matching the morphological chromosome structures to the genomic coordinates to investigate them further using the data of genome-wide projects. The general regularity of molecular and genetic organization of interphase chromosome cytological structures is that the interbands contain mainly open aquamarine chromatin and 5′ UTRs of housekeeping genes, gray bands are enriched in lazurite and malachite chromatin states and coding parts of genes, black bands and intercalary heterochromatin bands contain compact ruby chromatin and tissue- and stage-specific genes (Zhimulev et al., 2014; Khoroshko et al., 2016; Zykova et al., 2018). This rule holds true for the organization of the fourth chromosome of *Drosophila* (Sidorenko et al., 2018). In addition, there are some atypical cases of band and interband genetic organization, with one long gene occupying up to nine cytological structures (Zhimulev et al., 2016; Khoroshko et al., 2019).

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