Molecular cytogenetic organization of polytene chromosomes

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The results of the works carried out in the Laboratory of Molecular Cytogenetics (Institute of Cytology and Genetics of Siberian Branch of the RAS, Novosibirsk) devoted to the molecular genetic analysis of main units of polytene chromosomes,*1 bands, interbands, and puffs, as well as intercalary and pericentric heterochromatin,*2 are summarized. The results are discussed in terms of the dynamic model of organization of polytene chromosomes.

Key words: polytene chromosomes, bands, interbands, puffs; genes; heterochromatin; position effect variegation.

Chromosomes are the material carrier of inheritance. They are located in nuclei and consist of deoxynucleoprotein (DNP), which is a DNA molecule bound to various proteins.

The transcription (reading of information from chromosomes) and replication (duplication of the amount of DNA) processes occur during the interval between two cell divisions (interphase). During this period, chromosomes are dispersed and indistinguishable; they become visible only during division, when they are condensed and inactive. In some tissues, the replication process is not followed by cell division, and polytenization takes place, *i.e.*, a considerable multiplication of the number of chromosomes in one nucleus.

Polytene chromosomes are considered to be the best model for studying the organization and functioning of interphase chromosomes. This is primarily associated with the fact that they contain thousands of longitudinally bound individual interphase chromosomes (chromatids) and are easily discernible by an optical microscope. Demonstrating the longitudinal differentiation of DNP packing, chromatids form a unique pattern of dark strips, chromomeres (bands), and light intervals, interchromomeres (interbands).[1] The activation of transcription in individual regions of chromosomes results in the decompactization of the band DNP and formation of a large light intumescence (puff).

The functional significance of the chromomeric organization is widely discussed. We suggested a dynamic model of the chromomeric organization of polytene chromosomes. The model is based on the fact that morphological specific features of the structure of interphase chromosomes are determined by alternating the regions with different degree of DNP packing, which in turn correlates with the transcription activity. Chromomeres are considered as inactive in the transcription of chromosome regions, while interchromomeric regions (interbands) are considered to be permanently transcriptible sequences, whose permanent decompactization creates a relatively unchanged pattern of bands.[2–5]

The analysis of molecular genetic organization of *Drosophila* polytene chromosomes, in other words, the study of the gene content and molecular structure of morphological chromosomal structures (bands, interbands, and puffs) is the main subject of the experimental studies of our laboratory. The most important results obtained in this field are discussed in detail in several monographs.[6–8]

*1 **Chromosomes** are material carriers of inheritance. A simple fission of a cell (mitosis) results in a strong compactization of chromosomes, which become visible to form X-, V-, or dot-like bodies. Such chromosomes are called *mitotic*. In some tissues after the multiple (of the order of 10 cycles) duplication of the amount of DNA, chromosomes do not diverge and are not compactified, and the DNA chains are stretched in length and longitudinally connect with one another to form *polytene* chromosomes, which are several hundred times thicker and longer than mitotic chromosomes. Polytene chromosomes make it possible to visually observe the work of genes: their activation, reading RNA duplicates, and inactivation.

*2 **Heterochromatin** is regions of chromosomes whose properties differ from other (euchromatin) regions by the late and long replication. Heterochromatin is in the condensed state (it is intensely stained) for most of the cell cycle. Heterochromatin is incompletely polytenized compared to nearby sequences (underreplicated), which results in breaks. *Intercalary* (*i.e.*, inserted into euchromatin) heterochromatin is presented by dark compact bands. Heterochromatin surrounding the *centromere* (*i.e.*, the region of the chromosome to which filaments of the fission spindle are attached) is called *pericentric*.

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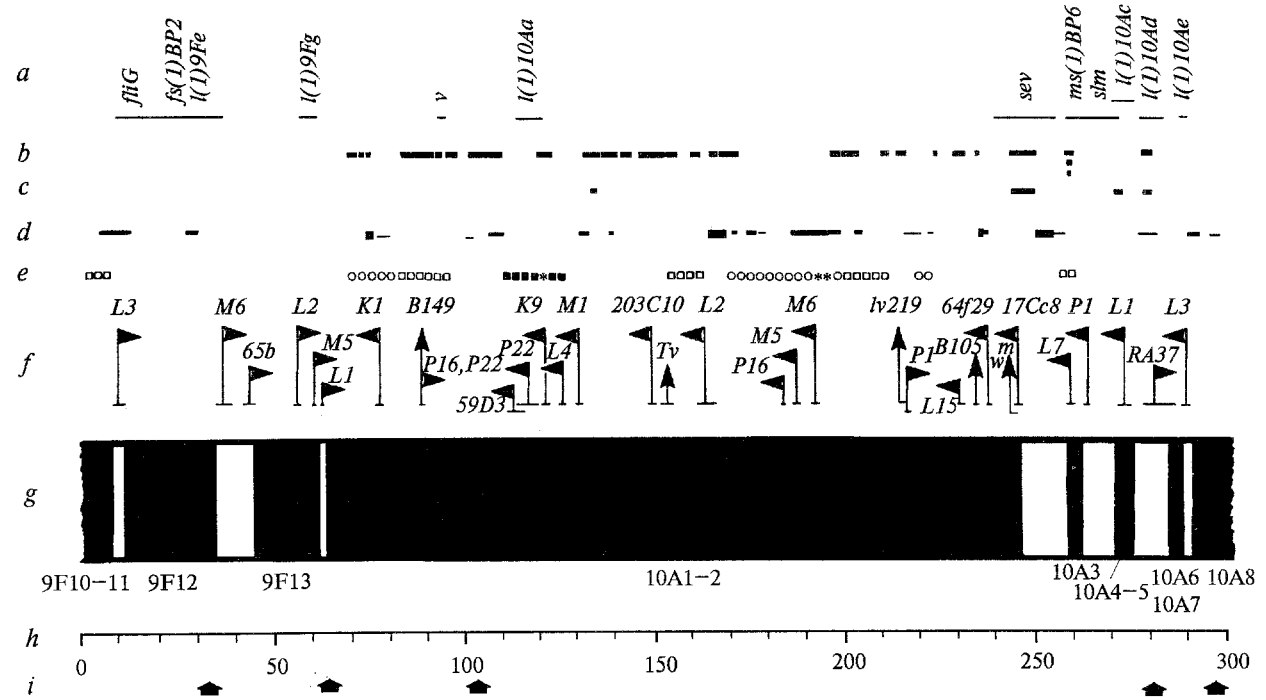

Fig. 1. Molecular genetic organization of the 9F12 — 10A1—2 region of the *X*-chromosome of *Drosophila*: *a*, genes; *b*, transcripts; *c*, clones of cDNA; *d*, fragments containing AC-duplications; *e*, K1 (light squares) and K2 (dark squares) duplications specific for the *X*-chromosome and dispersed (circles) duplications characteristic of the pericentric regions and chromocenter (stars); *f*, breakage points of chromosome rearrangements; *g*, patterns of bands according to EM data; *h*, physical scale of the region (in kb*[8]); *i*, breakage points of "evolutionary rearrangements" (Ref. 9).

Traditional methods for cytogenetic and molecular analysis and an original approach suggested for studying the "artificial" bands, interbands, and puffs appearing in chromosomes due to transposition*[3] in them of DNA fragments with a known molecular-gene content and regulated transcription were used in our works. In all cases, high-quality electron microscopic (EM) analysis, which allows one to observe thin bands and even details of large bands, is necessary.

Analysis of bands

Polytene chromosomes of *Drosophila* are separated into 102 regions (1—102), and each of the regions is subdivided into six sections (A—F) inside which bands are denoted by figures.

The 2B3—7 and 9E—10B regions of the *X*-chromosome of *Drosophila* were analyzed in detail. The saturation of these chromosome regions with mutations*[4] and subsequent relation of genes*[5] to certain bands confirm the hypothesis about the polygenic character of bands, *i.e.*, the possibility of localization of several bound genes within the same band.

The elucidation and localization of genes and chromosome rearrangements in the region of 9F12—10A7 (the region of the *X*-chromosome, removed by $Df(1)v^{L3}$ deletion*[6]) and the complete cloning*[7] of the DNA of this region make it possible to construct its molecular-gene map (Fig. 1). The region contains ~300 kb of DNA[10] located in seven bands and interbands. Twelve genes are observed in this region by genetic methods.[11,12]

Several types of the molecular-gene organization of bands have been revealed.

Complex bands. Three genes (*v, l(1)10Aa,* and *sev*) and two zones of genetically "silent" DNA, in which no genes were revealed, were found in the 10A1—2 band by usual genetic methods of saturation with mutations. One zone of "silent" DNA is localized between the distal*[9] point of the *L1* deletion breakage and *v* gene, while the second zone is localized between the *l(1)10Aa* and *sev* genes and occupies the whole medium part of the band.

*[3] **Transposition** is a transfer of the DNA fragment over chromosomes, *i.e.*, "cutting" from one site and insertion into another site.

*[4] **Mutation** is an inherited change in the gene: it can exert no effect on gene function, change it, or completely inactivate the gene.

*[5] **Gene** is a region of the DNA molecule, a functionally unfissible unit of hereditary information.

*[6] **Deletion** is a loss of the region of a chromosome.

*[7] **Cloning** is the totality of operations on isolation and accumulation of a sufficient amount of a certain DNA sequence in cells of other organisms, *e.g.*, bacteria.

*[8] **kb** (kilo bases) is a thousand pairs of nucleotides, **bp** is a base pair.

*[9] **Distal** means localized farther from the centromere.

According to the recombination estimations, the size of the proximal*[10] zone is ~67 % of the band length, *i.e.*, 120 kb according to the molecular-biological data; the size of the distal zone is 30 kb (17 % of the band); thus, both "silent" zones contain in aggregate ~84 % of the DNA of the band.[10] It can be assumed that "silent" regions of the 10A1—2 band contain genes, which are not revealed by mutagenesis, or these zones are occupied by duplicated DNA sequences, which do not code mRNA*[11] and proteins.

Experiments performed to reveal transcriptionally active and repeated DNA sequences also elucidated the molecular-gene structures of the "silent" zones and the band as a whole. The hybridization of clone DNA with labeled cDNA*[12] complementary to different poly(A)$^+$RNA*[13] was chosen as a method for determining the transcription activity of sequences in the band. Poly(A)$^+$RNA was prepared from *Drosophila* embryos and larvae, which were at different stages of development, and from individual larval tissues. The maximum number of the regions homologous to RNA was found in embryos: at least 16 different regions in the 10A1—2 band manifest the transcription activity. The transcription of the other eight fragments of DNA was found in the analysis of the hybridization with cDNA from third instar larvae. As a whole, 24 fragments, which code poly(A)$^+$RNA (see Ref. 13), were found within DNA of the 10A1—2 band. They are sufficiently uniformly distributed in "silent" zones: four fragments are in the distal zone, one fragment probably corresponds to the *vermilion* gene, and other fragments are distributed in the "silent" zone of the medium and proximal parts of the band (see Fig. 1).

No signals exceeding the background hybridization were found for the hybridization with labeled cDNA from cells of salivary glands of third instar larvae, despite prolonged exposures. Thus, it is likely that the sequences of the 10A1—2 band are not transcriptionally active in cells of salivary glands.[13] These data confirm the previous results, which testify that ^{3}H-uridine*[14] is not incorporated into the 10A1—2 band.[14]

The *c901* clone was isolated from the library of cDNA.*[15] It is mapped*[16] at the 135 kb position on the physical map, and its size is 1.3 kb (see Fig. 1, *c*). It was sequenced,*[17] and an open reading frame (ORF),*[18] which contains several AUG codons*[19] coding methionine, was found within this sequence. The first of them is at position 67 from the beginning of the sequenced fragment; the second methionine codon is located at a distance of 22 amino acid residues from the first one toward the C-end of the polypeptide. The sequence, which surrounds the first methionine codon (AAATATG),[13] is in good accordance with the canonical sequence, which surrounds the site*[20] of initiation of translation for *Drosophila* (C/A AA A/C ATG).[15] The second methionine codon is in the context (GGAAATG), which corresponds less to the canonical site of initiation of translation. The absence of the poly(A)-sequence in the sequenced fragment testifies that the *c901* clone is incomplete.[13]

The possible product of the translation of the long reading frame exhibits a substantial homology to protein products, which are coded by the *Delta* and *Serrate* genes of *Drosophila*. The presence of several repeated units 30 to 40 amino acid residues in length is the characteristic feature of these polypeptides. The repeated units are homologous to the amino acid sequence in the protein of the epidermal growth factor (EGF) of mammals. Each of repeated units is characterized by six invariant cysteine residues at a certain distance from one another. Eight repeated units homologous to EGF are localized in the central part of polypeptide, which is coded by the *c901* clone (Fig. 2). It is likely that this sequence is the signal polypeptide, which is usually present in secretory and membrane proteins.[16,17]

In addition to genes and transcriptionally active regions of DNA, repeated sequences of different types are revealed in the composition of the 10A1—2 band. The presence of two variants of repeated K1 and K2 elements[9] is of interest. The K1 repeat has three localization sites within the 10A1—2 band, while the K2 duplication has at least two sites. Both these duplication are

*[10] **Proximal** means localized closer to the centromere.

*[11] **mRNA** are RNA molecules (a portion of the total RNA pool of the cell) that do not contain introns (see Note*[42]); mRNA are translated on ribosomes, *i.e.*, the polypeptide chain is synthesized on them as on matrices.

*[12] **cDNA** is a DNA molecule synthesized on the matrix of poly(A)$^+$RNA.

*[13] **Poly(A)$^+$RNA** are mRNA molecules (a portion of the total RNA pool of the cell) that has a long sequence of residues of adenylic acid at the 3′-end of the chain.

*[14] **Incorporation of ^{3}H-uridine** is the method for revealing transcriptionally active regions of chromosomes, *i.e.*, regions of RNA synthesis. After incubation of the salivary gland in a solution containing ^{3}H-uridine, the gland is used for obtaining a preparation of chromosomes that is covered with the photoemulsion. After **autoradiography** (see Note*[26]) and development of the photoemulsion, aggregates of silver grains are seen only above the regions of chromosomes in which ^{3}H-uridine has been incorporated.

*[15] **cDNA library** is the totality of incorporated, *e.g.*, in plasmids, cDNA molecules synthesized on the total poly(A)$^+$RNA from some tissue, organ, or organism as a whole.

*[16] **Mapping** is the determination of the localization of some DNA sequence or gene on the chromosome map or on the gene map.

*[17] **Sequencing** is the totality of operations for reading the primary sequence of DNA

*[18] **Open reading frame (ORF)** is the region of the DNA molecule that codes amino acids arranged between two stop codons.

*[19] **AUG** is the triplet coding the amino acid methionine, from which the synthesis of any protein begins.

*[20] **Site** is a "place". It is a rather broad term that denotes inclusively some DNA sequence (the site of initiation of transcription), the arrangement of the sequence or gene on the chromosome (the site of localization), and the localization of the incorporation (the site of incorporation) of the transposon (see Note*[25]).

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                                                  C LPGWQGDL C QVPM
C RR----G C DPMN-GY C QRPGE------ C R C RIGYSGEL C DK
C IPLPLPG C QH---GG C TKPFE------ C I C KPGWAGLF C TEPS
C RT----G C -HSTRGY C EAPGE------ C R C RIGYAGRT C SE
C ATM--PG C QH---GT C NKPLE------ C L C LPGYTGLL C QTPI
C D----PD C SKQH-GY C RKPGE------ C R C KVGWTGSQ C DK
C -FVSL-G C AN---GD C EAPWE------ C N C HPGWGGML C DEKLTY
C VEHPDT- C ENG--GK C TSLSRRGWSYQ C Q C RQEFLGKN C EIRD
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Fig. 2. Repeats of amino acids, which are homological to the domain in the protein of the epidermal growth factor of mammals, in the composition of the *c901* clone. One incomplete and seven complete repeats are shown (Ref. 14).

mainly localized in the immediate vicinity of genes and transcriptionally active fragments of DNA. In addition to the 10A1—2 band, they are found in several sites, but only in the *X*-chromosome. These repeats are conservative to some extent, because they are found in the *X*-chromosome of the relative *D. simulans* species and not revealed in genomes of more alienated species (for example, *D. virilis, D. repleta, D. funebris,* and *D. pseudoobscura*). One can believe that the absence of the interlinear polymorphism in the chromosomal localization of *X*-specific repeats testifies in favor of the structural functional role of these elements, for example, in the dose compensation*[21] (see Ref. 18) and primary sex determination[19] and spermatogenesis,[20] because the *X*-chromosome plays a specific role in all processes mentioned.

The M203.5, M193, and M325 DNA fragments from the 10A1—2 band are homologous to several regions of the second, third, and especially, the fourth chromosome, they are *in situ* hybridized*[22] by bases of all chromosome arms.[9] Some other repeats are also found (see Fig. 1). One block of the poly(dA—dC) · poly(dG—dT) homopolymer repeat falls on approximately 15 kb of DNA in the band.[9]

The results of various experiments show that gene units inside the band are not connected functionally to one another and to the material of two adjacent interbands, because, first, the separation of these genes from one another and from the interband material by chromosomal rearrangements does not give the mutant phenotype*[23] (see Ref. 11); second, the experiments on projection of the gene activity on the blastoderm*[24] map show that genes of the same band function independently;[21] third, the experiments on *in situ* hybridization of DNA clones from the 10A1—2 band from *D. melanogaster* to chromosomes of other species reveal that they are present at least in two bands localized in different regions of *X*-chromosomes[22,23] of *Drosophila* of the *virilis, funebris,* and *repleta* groups. The main part of the DNA band (140 kb of 180) exists as a single block in the same order as that of *D. melanogaster* (*cf.* Figs. 1 and 3). Since all DNA sequences from the 10A1—2 band, including those from its "silent" zones, exhibit hybridization signals on chromosomes from *D. virilis*, one may draw a conclusion that they are rather evolutionary conservative and did not undergo elimination as insignificant sequences.

Thus, it can be assumed that the large 10A1—2 band of *D. melanogaster* appeared due to the fusion of two different bands inherent to ancestors and modern species analyzed. It cannot be denied that this band existed in ancestors and remained unchanged in *D. melanogaster*, and its division into two parts occurred in the evolution branch, which then gave other species.

The fact that some species have two individual bands, which enter the composition of the same band in other species, testifies that different DNA sequences of the given band can function independently.[9]

The 9F12 band contains three genes, which are responsible for flying ability (*fliG*), female fertility (*fs(1)BP2*), and viability (*l(1)9Fe*).[12]

The 2B3—5 band contains at least three genes: *sta, BR-C,* and *dor.* The activation of the *BR-C* gene results in the formation of the 2B3—5 puff from the proximal part of the band (see below). The molecular cloning followed by mapping of cDNA and mobile P-element, which causes the mutation of *dor*, shows that this gene is covered (at least partially) by the *P154* deletion, which removes 30 kb of the proximal edge of the 2B3—5 band. Thus, at least three genes are in the 2B3—5 band.

Simple bands. A close correspondence between the numbers of bands and genes is observed in several regions, for example, three genes (*slm, ms(1)BP6,* and *l(1)10Ac*) are mapped in the 10A3—5 region, which contains two bands. The *l(1)9Fg* and *l(1)10Ad* and *l(1)10Ae* genes are found in the regions of the 9F13 and 10A6—7 bands, respectively. The *hfw* gene is localized in the 2B6 band.

"Artificial" bands. An ingenious approach to the study of the band organization is provided by the transformational method. The use of this method makes

*[21] **Dose compensation** is the phenomenon of equalizing activities of the single male *X*-chromosome and two female *X*-chromosomes: for *Drosophila* it is performed due to duplication of the activity of the male chromosome, and for mammals it is performed due to inactivation of one of the female chromosomes.

*[22] ***In situ* hybridization** (hybridization "in the site") is the method that makes it possible to determine the exact localization of the DNA fragment on the chromosome, either the tissue or organ where RNA or the protein function.

*[23] **Phenotype** is the totality of external properties of an organism.

*[24] **Blastoderm** is the one layer of cells that forms the blastula, the embryo at the earliest stage of development.

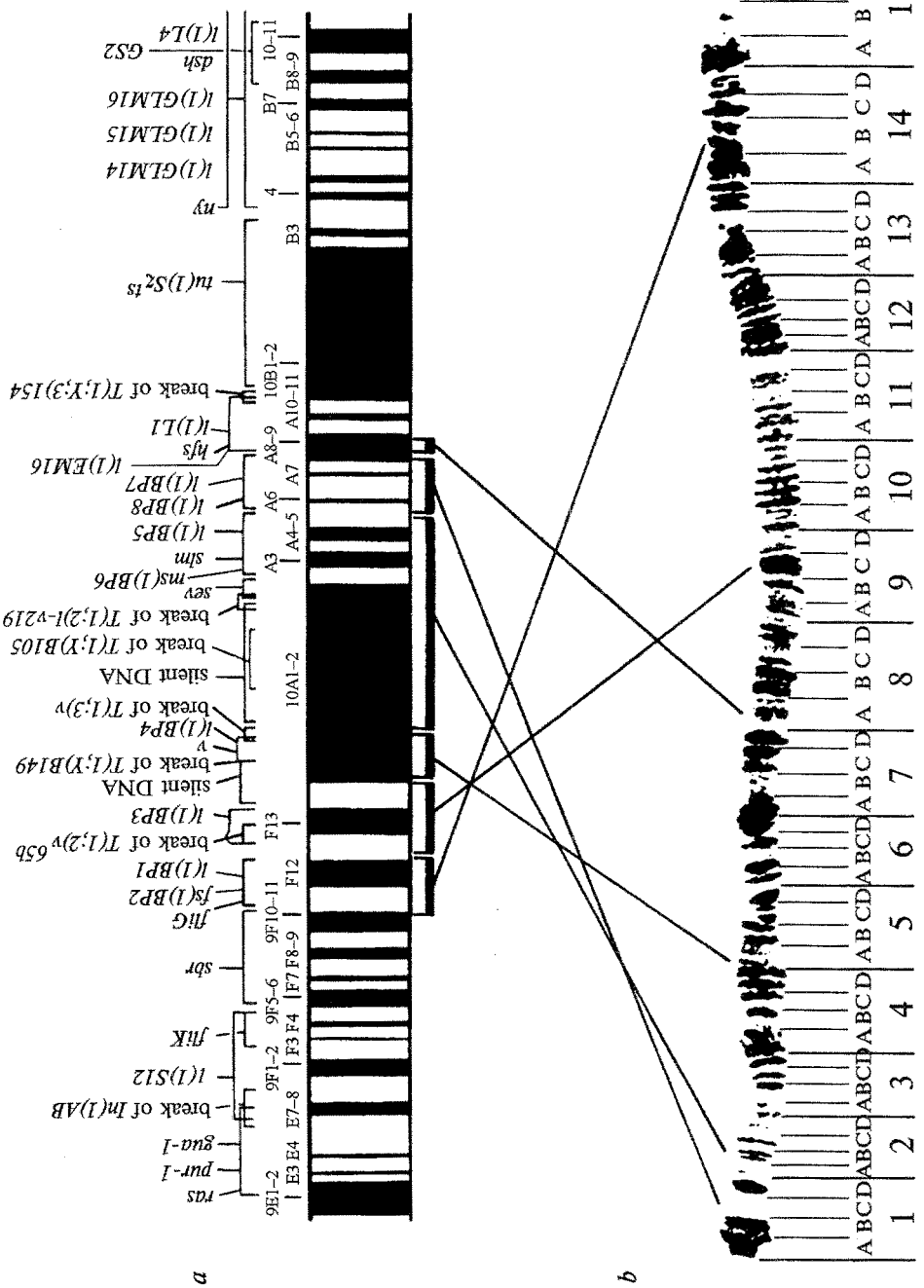

Fig. 3. Localization of DNA sequences from the 9F12—10A7 region of *D. melanogaster* in polytene chromosomes of *D. virilis*: *a*, scheme of the 9F—10B region of *D. melanogaster*; *b*, map of the *X*-chromosome of *D. virilis* (Ref. 9).

it possible to insert DNA sequences with known molecular-gene characteristics into the genome. Insertion sites of the structure studied can be revealed by *in situ* hybridization, and then electron microscopy allows one to understand what morphological structure (bands or interbands) has been formed. Thus, bands or interbands that appeared due to gene manipulations, which are not found in native polytene chromosomes, can be called artificial bands.[24,25]

Different DNA fragments are in composition of transforming structures (transposons*[25]): the mobile P-element, which provides insertion; marker genes (*rosy, Adh, etc.*), which allow one to judge whether insertion has occurred or not; DNA of genes, whose expression is under study; genes of heat shock proteins, which are immediately activated at high temperature or under other stress factors.

According to the data of EM analysis, one new band formed from DNA of transposon is formed in 10 of 13 transformed regions studied.[26] DNA sequences of these "artificial" bands retain functional activity: marker genes exhibit normal functioning, and heat shock genes are easily activated to form a puff.

The transcription of DNA of one of the genes from the transposon (for example, for induction of the heat shock gene or transcription of the P-element) results in the division of the band and the formation of the puff and interband from the corresponding part of the band.[24–27]

These facts allow us to draw the following conclusions:

1. Since a single band is formed from heterogeneous (in a functional sense) DNA, it is evident that all DNA undergo continuous compactization without the formation of the band-interband pattern. This means that the arrangement of the gene in the neighboring interband and band is not necessary, as has been assumed for the *Notch* gene,[28] *i.e.,* bands are not related functionally to interbands, and there is no basis to speak about the cytogenetic unit "band + interband".

2. All genes, which are taken from different bands and present in the transposon, are compacted in one band. Therefore, there are no special mechanisms of compactization of different genes or specific sites for each gene, which restrict this process.

3. All these facts testify that bands are neither units of gene function nor compactization units.

Analysis of interbands

It is established by the autoradiographic*[26] studying the transcription that ^{3}H-uridine is incorporated into all regions of polytene chromosomes, which exhibit decompactization of chromatin, including puffs, duffuse bands, and probably interbands.[14] These data are confirmed by the results of EM autoradiography.[29]

The high resolution achieved when antibodies to molecular DNA/RNA hybrids are used allowed one to observe distinctly the transcription activity in some interbands that is visible with an electron microscope: 1E1—2/1E3—4, 21D1—2/21D3, 21D3/21D4, 21D4/21E1—2, and 100B3/100B4—5. These interbands are transcripted at all studied stages of development: from the third instar larvae to four- to six-hour pre-pupa.[30]

The activation of small genes, which are in composition of transposons based on the P-element, can serve as a transcriptional model for interbands, because the activation of bands ~1 kb in length (*Sgs7, hsp18.5*) results in chromosomes, whose structures are morphologically indiscernible from interbands. The activation of longer fragments (>3 kb) results in the formation of a puff. After the insertion*[27] of the P-element into the 10A1—2 band, the latter is decomposed into two parts with an interband between them, which is probably caused by the transcription of DNA of the P-element.[25]

Electron microscopy reveals the tetramer of the *pry-1* plasmid*[28] (54 kb), in which each monomer containing DNA of the *ry* and *w* genes is restricted by DNA of the P-element and bound to the next monomer by DNA of the *pBR322* plasmid, as the diffuse chromatin complex consisting of four loosened bands and interbands. It is likely that this structure is caused by alternating of compacted DNA of the inactive *ry* gene and decondensed DNA of the transcriptionally active P-element.[25]

The sequence of DNA from the transposon, which is inserted into the interband, can be used as a probe in screening*[29] genome libraries prepared from transformed lines, which allows one to clone DNA of the interband. DNA of the interband has primarily been isolated and sequenced using this approach.[31] The analysis of the sequence of 1289 bp in length shows two overlapped ORF 354 and 555 bp long. The longer frame contains the insertion site of the P-element localized, according to EM analysis, in the middle of the interband.[32]

The analysis of the sequences of nucleotides, which compose these two reading frames, testifies that none of them begins from the ATG-codon. The comparison between the frequencies of using codons in these reading frames and mean frequencies of codons, which have experimentally been determined for proteins of *D. melanogaster*,[33] shows that they differ substantially.[32] All this can mean that both reading frames in interbanded DNA are not functional, although the possibility

*[25] **Transposon** is a fragment of a DNA molecule that can move in the genome, *i.e.,* be cut from one site and incorporated into another site.

*[26] **Autoradiography** is the reduction of silver grains under the action of β-irradiation of tritium.

*[27] **Insertion** means inclusion or incorporation.

*[28] **Plasmid** is a small ring DNA molecule containing genes of antibiotic sensitivity and capable of autonomic replication in cells of bacteria.

*[29] **Screening** is the totality of operations for isolating the clone containing a certain DNA sequence from the library.

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   1 GCCCCGCAACATTTTTATTGTTCCGTAGATTACAACACATAGGAAAACGCGAGAAGAGCT
  61 GAAAAATTTTCGTTGTCGAGGAGGAGAGTGCGCTTCACACCGAAATATCAGTATACTGAT
 121 GTGACAAAATGCAAAAGTAGCACAGATACAAATGCAGATAGGGATACTCTTCTCGCAGTC
 181 TTCGAAAAAGAAGGCGTCTGGAAGGGGATCGACTGGAAGGGGCAGTGTCGGTTTGTTTGT
 241 GGAATGCCGTTTTGTAAGTTTCTTATGCATGCGACTTCAAACATAGTTCGGCATCGAAAC
 301 TTTCTAGCACACCGACACACATACGAACGCGATCCAGCCGACACACACACACACACGCAC
 361 GCAGCCACACACTTAAGCGACTTTCGAAAGGTACAACTTTTTACGAAGTCGCTGCCTCGG
 421 CCGCTGTGCAGCCGACGCCACTGCCGCTGCCGCTGTCGCTGCCTCTGTCGACTTCGAATT
 481 CCAACGCCAAGATGAAAGATCGGCGCAAAAGAAAAGAAATATTCATTCAGTAAAATTTCA
 541 TAGCTGCAGCCGCATGGTTGTGCCGCTCTCGCTGCTCTTGCTTTTCGCGCAACAAACCGG
 601 AACGAGAAACACATAAATATAAAAGTGTGAACATTGGCGCACATATAAAAACTTAAAACT
 661 TAACTTAACTTGAGCAACATGAACAATAACACCGGAAGCGGTTCCAGCGAAGAGGTTCCA
 721 AGGAGAGCAGACACAACCGCATTCCAGAAAGTTTAAATAACGCTGGAAGGAGGGGGAAAG
 781 TGGAAAACTAAACTCGAACTCGAACTCAGTGTGCCAGTGTATGTGTGTGGAATGCAGAAG
 841 AGGAAGAAGCAGCAGCAGCAGAATAAGCAGCGAATAGAAAATATGTCTTCAAACTGGTTT
 901 TTCGGTTTTTAGACTGTCGTCGTCGGCCAATCGGGTTCCATTGACATCCGAACGAAAAAA
 961 ATAATGCCTAACCTTCGATGGGAACACTCGTAAGTCGGAATTCGAAGTCCGCCATGCACA
1021 CACAACGCCGTTTATTGGCTGAAATTGGATGCTGTGTGTATGTGCGGTTATTTAGAAATT
1081 CGAAATTGAATTTCAGCGTATGCGCGGCCACCCAGAAATTCAAATTAAAATTTTCAAGCG
1141 CGAGCCATACGACCGAGCGTAAATTTTTGAGACCCGTTTCATTCTTCCCGACTCGACGAT
1201 CCTAACCTTCACTGAGAACAGGAGTTAGCCAGCCAGAACGCGATTGGAACGATCGGACGG
1261 ACACTAGTCGCCCGCCAATACAAGCGCAC
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Fig. 4. DNA nucleotide sequence from the region of the interband of the 61C7/C8 line of flies *Oregon-R*. Open reading frames are designated by points *1* and *2*. The insertion site of the P-transposon in the *Adh*^{hs61C} line (triangles) and the octamer that is repeated during this process (wavy line) are indicated. Restriction*[31] sites: P, PstI; R, EcoRI; S, SalGI (Ref. 34).

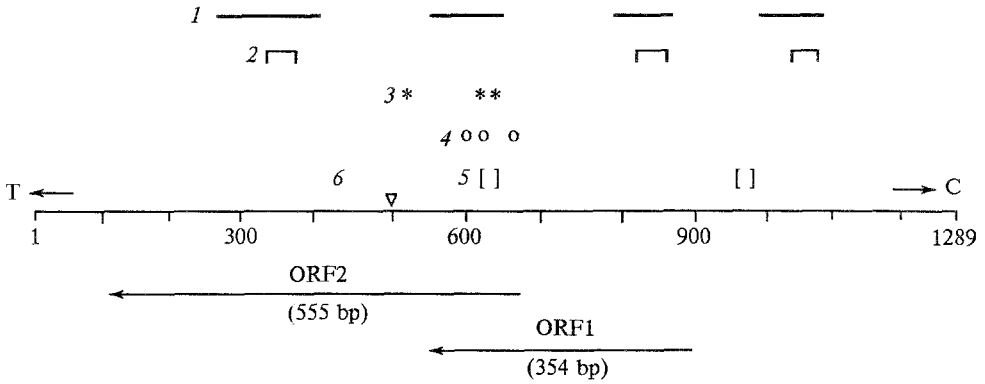

Fig. 5. Structure of the DNA sequence from the 61C7/C8 interband: *1*, regions containing direct and inverted repeats; *2*, regions of possible formation of *Z*DNA; *3*, regions of probable attachment of topoisomerase II; *4*, possible sites of autonomic DNA replication (ARS); *5*, regions of probable attachment to the nuclear matrix (T-SAR-boxes); *6*, insertion site of transposon in the *Adh*^{hs61C} line. ORF1 and ORF2 are taken from Ref. 34.

that they belong to exons*[30] of some gene cannot be excluded.

Some peculiarities can be mentioned in addition to other specific features of the fragment of interbanded DNA (Fig. 4).

1. The sequence is not found anywhere else in the genome, *i.e.*, it is unique.

2. An enrichment in AT-pairs is observed: although for the whole sequence the mean content of AT-pairs is 53.4 %, *i.e.*, it is close to that found for *D. melanogaster* (52.2 %),[33] regions of DNA 12 bp long and longer are also found, in which the content of AT-pairs is more than 80 %.

3. No substantial homology between sequences of nucleotides from the interband and no other homology known in the database are revealed.

*[30] **Exons** are regions of the coding region of a gene that remains when **introns** (see Note*[42]) are cut from mRNA after transcription.

*[31] **Restriction** is the enzymatic reaction of recognition of specific DNA sequences (4 to 7 bp) and the two-strand breakage of the DNA filament inside this sequence or in its vicinity.

4. Repeats from 8 to 50 bp long with a degree of homology between repeated units of 40 % and higher are found (Fig. 5).

5. The homopurine/homopyrimidine tracts, whose existence assumes the formation of the *Z*-form of DNA and some possibly functional (regulatory) sequences (see Fig. 5), are found.

6. DNA of the interband is homologous to the sequences that bind proteins of the nuclear matrix*[32] (MAR) in cells of the reinoculated culture of Kc cells of *Drosophila*, and the interband can be the matrix-bound region of the chromosome.[34]

The method of the P-element-mediated enhancer trapping (regions that enhance gene transcription) was used to elucidate the possible functional role of interbands. The removal of the transposon containing terminal fragments of the P-element between which the *lacZ* genes from *E. coli* and *Adh* and *ry* from *D. melanogaster* are inserted was activated in the *Drosophila* genome. When the transposon is inserted into the enhancer region, it is expected that it enhances the action of the weak promoter*[33] at the 5'-end of the P-element, and this switches on the expression*[34] of the *lacZ* gene localized below. The activity of the latter is revealed by the staining reaction between β-galactosidase (the enzyme coded by this gene) and a substrate.[35] In two lines (12 and 41), the *lacZ* gene functioned in the majority of tissues of embryos, larvae (including salivary glands), and imago (adult flies); and it functioned only in embryos in two other lines (2 and 3). After revealing the region of the transposon insertion by *in situ* hybridization and EM-mapping of chromosome regions, it turned out that there are insertions in interbands in the 85D9—10 and 86B4—6 regions in lines 2 and 12, respectively. The correlation found between the constancy of the transcription activity of the *lacZ* gene in line 12 and decompactization of DNP of the interband can testify that the permanently functioning enhancer exists in this interband.[27]

Analysis of puffs

The chromosome regions from which puffs are formed should be localized exactly for better understanding mechanisms of activation of banded DNA in the induction of the transcription leading to the formation of local decompactization of DNP, *i.e.*, puffing. The EM analysis made it possible to map 37 regions of polytene chromosomes of *Drosophila melanogaster* in which ~50 puffs are localized. The further experiments on studying different stages of the puff formation allowed one to divide all puffs into four types according to the number of bands (1, 2, 3, or 4) involved in the process of DNA decompactization in the puff formation. The number of puffs of each type was 19, 23, 7, and 2, respectively. The cases when only a part of the band is activated is observed.[36,37] All these data testify that many large puffs are morphologically and genetically complex formations.

It is known that the activity of puffs of *Drosophila* at the end of the third instar larval age and of pre-pupa changes subsequently, *i.e.*, each puff is formed exactly at a certain time and in a certain sequence, and the whole cascade is induced by the steroid hormone ecdysterone.[38] Thus, it is easy to imagine that mutations in genes localized in one of the puffs and induced during the first minutes of contact of cells with the hormone can stop or considerably change the development of the whole cascade.

The 2B1—10 region of the *X*-chromosome of *Drosophila* containing the early, *i.e.*, one of the first to appear in the cascade, ecdysterone 2B3—5 puff was saturated with mutations to find three genes: *BR-C, hfw,* and *dor.* The mutation distortions of these genes result in the changes or complete halt of the development of the cascade of puffs induced by the hormone.[39—44]

The whole region of the genome ~300 kb in size containing these three genes was cloned.[45—47] The analysis of localization of chromosome rearrangements, which restrict the *BR-C* gene on the genetic, cytogenetic, and molecular maps, shows that the molecular boundaries of the *BR-C* gene correspond exactly to the cytological boundaries of the chromosome region from which the early ecdysterone-induced 2B3—5 puff is developed.[46]

Multiple distortions of larva and adult organs related to loosing the sensitivity of cells to the hormone are developed in homozygotes*[35] with respect to mutations of the *BR-C* gene.

1. Cuticular cells (cuticle is the external shell) of gynandromorphous*[36] larvae, in which mutations of the *BR-C* gene are in the hemizygotic*[37] state, do not under go sclerotization (hardening), while other cells react normally to the hormone and are sclerotized.[48] Cells of mutants with respect to the *BR-C* gene are not histolized (do not disappear) *in vitro* in response to ecdysterone.[49] Larval salivary glands of homozygotes with respect to the *rbp*-allels*[38] of the *Br-C* gene are not histolized after

*[32] **Nuclear matrix** is the "framework" of the nucleus, specific protein fibrils, which maintain the spatial organization of the nucleus and, hence, allow its normal functioning.

*[33] **Promoter** is the region of the DNA molecule (~40 bp) at the 5'-boundary of the gene specifically recognized by the RNA-polymerase, enzyme that performs transcription.

*[34] **Gene expression** is the functioning, manifestation of the gene, *i.e.*, the existence of some external property determined by one or another gene.

*[35] **Homozygote** is a cell or organism containing two similar allels (see Note *[38]) in the given locus of homological chromosomes.

*[36] **Gynandromorph** is an individuum containing groups of cells, tissues, or organs with the chromosomal set of different sexes.

*[37] **Hemizygotic gene** is a gene presented in the genotype as the only specimen.

*[38] **Allel** is an alternative form of a gene characterized by a nucleotide sequence that is inherent only in this allel.

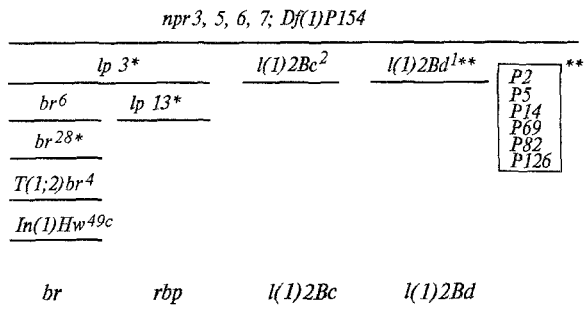

Fig. 6. Complementation map for some mutations and chromosome rearrangements that damage the *BR-C* gene. Comments are given in the text.

the pupa ecdysis and can be found even in very late pupae.[50]

2. No ecdysterone-induced puffs, including the 2B3—5 puff, are developed in homozygotes with respect to the strongest alleles of the *BR-C* gene (*npr5* and *npr6*). However, the 2B3—5 puff is not active at the proper time. The pattern of puff induction is unchanged when ecdysterone is added *in vitro* to the system.[40]

3. It is shown at the molecular level that the product of the *BR-C* gene controls the activity of genes localized in other puffs, in particular, in intermoulting, ecdysterone-repressed,[51—54] and early[55] and later ecdysterone-induced puffs.[53,54,56] A trans-regulatory effect of the *BR-C* gene on the expression of the *LSP2* and *FBP1* genes induced by ecdysterone in adipose bodies of larvae is found.[57]

These data testify that the *BR-C* gene is the key in the development of the cascade of the gene activity induced by ecdysterone.

Various mutations induced by ethylmethanesulfonate, X-ray irradiation, and insertions of the P-element were obtained in the region of the 2B3—5 puff. These genetic experiments revealed complicated complementation interrelations.[39,42,58] The complementation map*[39] of some mutations (mainly of mutations induced by insertions or related to chromosome rearrangements) is shown in Fig. 6. Mutations induced in the system of the P—M hybrid disgenesis are denoted by stars. Two stars indicate hypomorphous mutations, which decrease the viability only in the heterozygote*[40] with deletions or "long" non-complementing allels. Therefore, their position in the complementation map is arbitrary (the detailed map has previously been described in Refs. 42, 58, and 59). Some mutations are mapped in the physical map[46,47,53,60—64] (Fig. 7).

Mutations, which reveal the *br* phenotype, are localized in the interval between the point of the $In(1)Hw^{49c}$ inversion break and the *br28* mutation (Fig. 7, *e*), *i.e.*, they are at the position of 110 to 200 kb in the map. Mutation damages, which result in lethality, have the same wide localization within the gene (Fig. 7, *g*). $In(1)Hw^{49c}$ breaks DNA from the left side of the first exon. This break is not lethal. All other chromosome rearrangements that distort the integrity of DNA between the first exon and the last domain Zn-finger *3* (*z3*-domain) are lethal in the homozygote. However, they reveal a peculiar behavior in the heterozygote with $In(1)Hw^{49c}$. Heterozygotes with respect to this inversion and rearrangements, which break the gene between the first and second exons (from br^{26} to br^{6}), are almost completely viable (which is shown by hatching in Fig. 7, *g*). Heterozygotes between *P3* and *P13* mutations (see hatching in Fig. 7, *g*) induced by insertions of the P-element into the exon, which is localized above the crusted exon (*5*, see Fig. 7, *b*), and the $In(1)Hw^{49c}$ inversion are also viable. Heterozygotes with respect to the inversion and rearrangements, which damage the crusted exon and exons with *z*-domains (*npr7* and *Df(1)P154*), are completely lethal (dark rectangles in Fig. 7, *g*). This probably means that the lethality appears due only to the distortion of the crusted exon and *z*-exons at one chromosome and some sequences at the 5′-end of the gene in another chromosome.

The nonlethal phenotype *rbp* (Fig. 7, *f*), the absence of visible phenotypes for hypomorphs*[41] (Fig. 7, *h*), and the *br* phenotype (see Fig. 7, *e*), which is caused by $In(1)Hw^{49c}$, are related to insertions of mobile elements to the fourth exon, which is more distal than the medullar exon, to the intron*[42] (rbp^{in}), or to the region above its transcripted part ($In(1)Hw^{49c}$) with damages of the gene. Thus, one can draw a conclusion that there is no correlation between the localization of mutations and their belonging to one or another complementation group.

Mutations, which result in sterility, are localized at both ends of the physical map. For example, *Tp(1;3)sta* is sterile in the homozygote. Females that are heterozygous with respect to this transposition or *Df(1)St490* deletion and any mutations localized at the right (Fig. 7, *d*) are also sterile, except *Tp(1;3)sta/npr7* and *Df(1)St490/npr7*, which are partially fertile. $Df(1)St490/T(1;2)br^{4}$ females are also partially fertile. It is likely that eggs in ovaries of sterile females are developed normally, but the females do not lay them.[59] Thus, the fertility function is localized at the 95—210 kb position on the physical map of DNA. Since the breaks of DNA by the *T(1;3)sta* or *Df(1)St490* transpositions also exert an effect on fertility, some portion of the

*[39] **Complementation map** is the scheme of interaction between different allels of one gene in the heterozygote (see Note*[40]).

*[40] **Heterozygote** is a cell or organism containing two different allels in a given locus of homological chromosomes.

*[41] **Hypomorph** is a mutation that is manifested only in the heterozygote with deletion, *i.e.*, when one copy of the mutant gene is present in the genome.

*[42] **Introns** are the regions of the coding part of the gene that do not code information and are cut from mRNA after transcription; and the remaining regions are called **exons** (see Note*[30]).

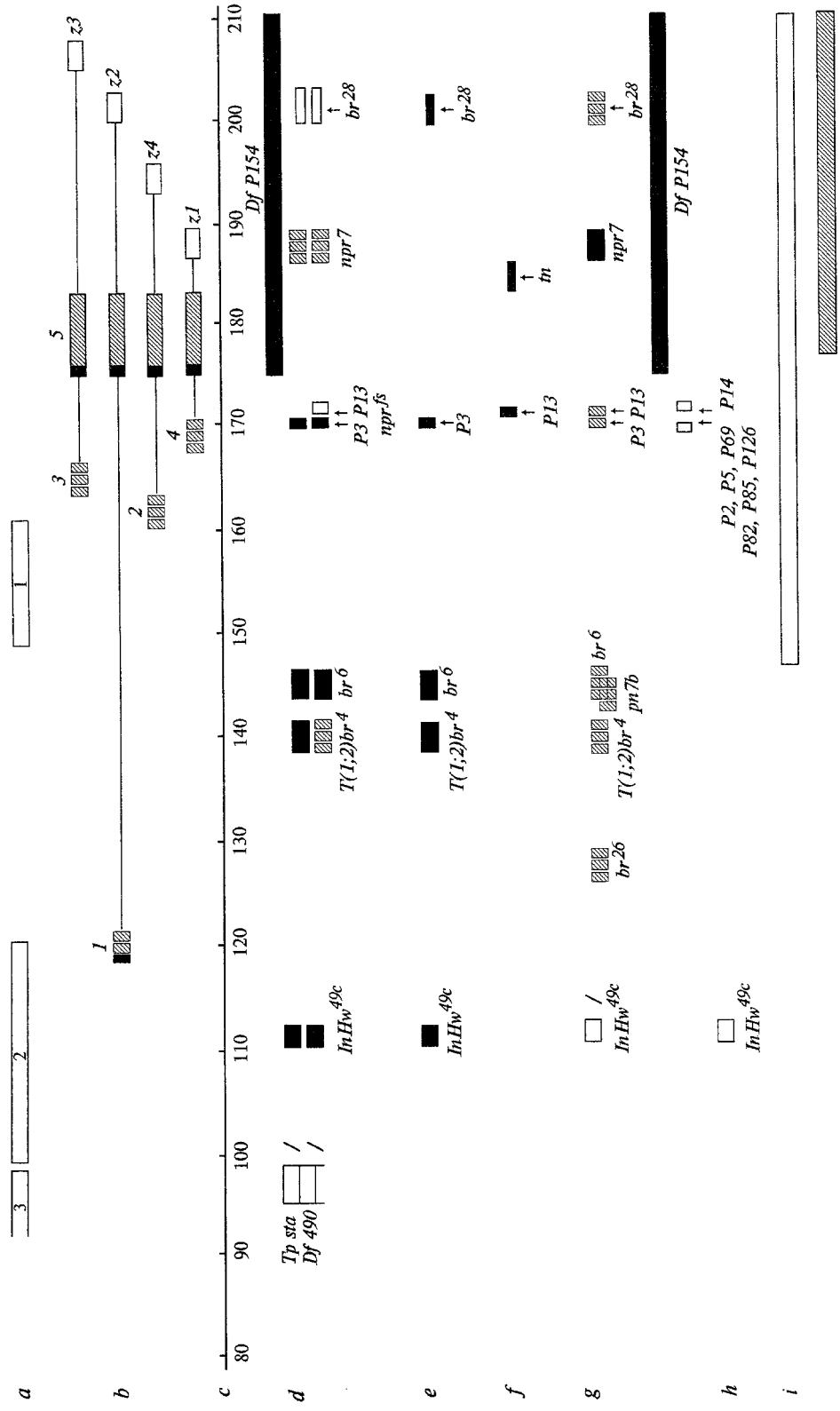

Fig. 7. Molecular map of the *BR-C* gene of *D. melanogaster*: *a*, suggested regulatory elements; *b*, intron-exon transcriptional map, z1—z4 are the domains of "zinc fingers" (see Ref. 65); *c*, physical map of DNA;[45—47] *d*, localization on the DNA map of damages that cause female sterility for homozygotes with respect to *Tp(1;3)sta* or *Df(1)St490* and to any of the mutations mapped at the right: mutations that give complete sterility are indicated by dark rectangles, and mutations that give partial[59] sterility are shown by hatched rectangles (the data on the localization of the *gypsy* element (npr^{5s}) are taken from Refs. 62 and 66); *e* and *f* are the *br* and *rbp* phenotypes, respectively; *g*, localization of breaks that result in a decrease in the viability (for comments see in the text); *h*, hypomorphs;[64] *i*, 2B3—5 ecdysterone-induced puff: a light rectangle indicates a puff that is induced normally from the DNA fragment in the chromosome with br^6 inversion, and a hatched rectangle shows a puff that has reduced sizes in the chromosome with *Df(1)P154* inversion.

BR-C gene should be localized to the left of their break points.

Action foci*[43] of the *lt76* mutation of the allel of the *l(1)2Bc* complementation group were localized on the map of blastodermal primordia, using the gynandromorph method and fate mapping*[44] (see Ref. 67). Two foci are found: one is in the front part of the blastoderm in the zone from which the front section of the nervous system and the head are developed; and the second focus is revealed in the blastodermal primordium from which the abdomen and the genital imaginal disk are developed.[68]

To elucidate the reasons for the sterility related to mutations of the *BR-C* gene, ovaries from *y npr*^{3} *w/y*$^+$ *In(1)Hw*^{49c}*w* larvae were transplanted to larvae of the same age of *Fs(1)K1237* females heterozygotus in sterility mutation. Own ovaries of females of the latter type are underdeveloped, because the development was ceased at the stage of vitellogenesis.*[45] If transplanted ovaries were adnated to oviducts of recipients, female-recipients became fertile, and their progeny had genotypes*[46] of the donors. Mutants of *BR-C* normally synthesize vitelline proteins, which enter composition of eggs. Thus, the function of donor ovaries remain normal after grafting to a recipient. This means that mutations of the *BR-C* gene exert no direct effect on ovaries.[69]

The complex organization of the transcription of the *BR-C* gene means that there is a complex organization controlling this process. In fact, it turned out that ecdysterone induces normal development of the puff in the 2B3—5 region of chromosomes of salivary glands with *Df(1)pn7b*, *Df(1)br*^{26}, and *T(1;2)br*^{4} rearrangements or in females homozygous with respect to *br*^{6} and *Hw*^{49c} inversions (see Refs. 39 and 46). Break points of these rearrangements are mapped at positions between 113.7 and 148 kb (see Fig. 7, *g*). Thus, despite the fact that chromosome rearrangements remove the 5′-region of a gene, including the first exon, all sequences necessary for reception of the signal of the ecdysterone-receptor complex and forming the puff remain in the residual part of the gene. Therefore, we assumed[46] that the element that controls the transcription and induction of the puff by the ecdysterone in cells of salivary glands should be between the *br*^{6} break point and the second exon (148—160 kb). From the viewpoint of the authors of Ref. 65, two promotors localized at the 165 and 167 kb positions should exist. However, to provide viability and normal development, the whole gene should be transcribed, and hence, the regulatory zone should be above the first exon. This region can be localized between *Tp(1;2)sta* and *Df(1)St490* break points from the distal side of the gene and the first exon, *i.e.*, between 99 and 190 kb.[46] Viewpoints about localization of the promoter at the 102—115 kb[53,63] or 120 kb[65] positions are known.

Finally, one can postulate the existence of the third regulatory element (3, see Fig. 7, *a*), which is necessary for the complete gene functioning. The breakage of the DNA region by the *Tp(1;2)sta* and *Df(1)St490* chromosome rearrangements results in the female fertility. The proximal boundary of this regulatory zone coincides with break points of these rearrangements, and the distal boundary is at the left, but it is yet not mapped.

It has been mentioned above that mutations of the *BR-C* locus exert a strong effect on the development of puffing in cells of salivary glands.

It has reecently been shown that salivary glands of homozygotes with respect to the *rbp*-allel of the *BR-C* gene are not histolized during metamorphosis.[50] Ecdysterone-induced puffs are absent for 0-hour prepupa homozygous with respect to this allel. No ecdysterone-induced puffs are found in salivary glands of prepupa including 24-hour pre-pupa.[70]

It is likely that the tight localization of three genes, *BR-C*, *hfw* (*swi*), and *dor*, involved in processes of ecdysterone induction is evolutionary conservative. The *in situ* hybridization of chromosomes of eight distant species was performed using labeled DNA clones of *D. melanogaster* containing fragments of these genes. For *D. funebris*, *hydei*, *repleta*, *mercatorum*, *paranaensis*, and *D. virilis*, all clones were hybridized with the region of the same puff at the very end of the *X*-chromosome, the labeled region of *D. kanekoi* is also localized in the puff but further from the telomere,*[47] and in *D. pseudoobscura* this puff is mapped in the proximal region of the *X*-chromosome. For all species, the morphology of the puff, in which *BR-C*, *dor*, and *hfw* genes are arranged, strongly resembles the morphology of the 2B3—5 in *D. melanogaster*.[71]

Intercalary heterochromatin

It is likely that the compacted material of polytene chromosomes, which form bands, is not functionally homogeneous. It can decompacted to form puffs at various stages of ontogenesis. At the same time, in chromosomes of salivary glands the regions of the intercalary heterochromatin (IHC)[72—74] are observed, which resemble in properties the regions of pericentric heterochromatin found in mitotic and polytene chromo-

*[43] **Action focus** is the tissue or organ in which the gene functions.

*[44] **Fate map** is the scheme that shows the tissue or organ that should be developed from one or another region of the blastoderm.

*[45] **Vitellogenesis** is the stage of development of the egg at which its fast growth and accumulation of nutrients in the form of a vitellus occur.

*[46] **Genotype** is the totality of genetic information about the organism; the genetic structure of the species according to the genes studied.

*[47] **Telomere** is the terminal region of the chromosome.

somes (see below). The regions of IHC are presented by dark compact bands in which DNA is lately replicated and incompletely polytenized compared to adjacent sequences. Breaks (so-called weak points) appear in them due to the incomplete replication. These regions undergo ectopic (nonhomological) pairing between one another and regions of the pericentric heterochromatin.[75]

It has previously been assumed that the specific features of IHC listed above exist due to a large accumulation of DNA in bands of this type. However, study of the distribution of the amount of DNA in the regions of polytene chromosomes followed by analysis of specific frequencies of appearance of one or another property per DNA mass unit show that the properties of IHC are determined by the specific character of its structure.[76]

It could also be expected that the manifestation of similar properties by different IHC bands is caused by the resemblance in the primary DNA structure in these bands. However, no satellite DNA*[48] typical of the regions of the pericentric heterochromatin was found in the IHC regions. Moreover, in some bands the DNA sequences, whose functions are known, are localized, and these sequences differ. The cluster of histone genes is localized in one of the IHC regions, the cluster of the ribosome 5S RNA is in the second region, and the gene of the early embryo development, *bithorax*, is found in the third region. The data presented allow one to assume that the resemblance in behavior of IHC regions can be related to the presence of similar proteins in them. These can be proteins involved in the supercompactization of some regions of chromosomes, whose functioning is not necessary during the most period of the development.[7]

The change in the frequency of appearance of IHC properties affected by modificators of the position effect variegation (see below), in particular, when the hetrochromatinic *Y*-chromosome is removed from the genome, can favor this assumption. Frequencies of breaks in IHC regions increase considerably for *X0* males, which indicates an increase in the degree of underreplication.[77] It can be assumed by analogy to phenomena of the position effect variegation that special proteins localized in the regions of the pericentric and intercalary heterochromatin are involved in processes of compactization of chromatin. When the *Y*-chromosome is removed from the genome, a considerable portion of a substrate for binding these proteins disappears, while the other proteins are redistributed between IHC regions. This results in an increase in the level of compactization, and, hence, the degree of underreplication in IHC increases as well.

*[48] **Satellite DNA** is the non-coding region of the DNA molecule, which consists of the long (several kb) tandem duplication of short (5 to 10 bp) nucleotide sequences.

Pericentric heterochromatin

A chromosome can be divided into two parts, whose properties differ distinctly: euchromatin and heterochromatin. The latter is replicated later and longer, exists in a compact state for most of the cell cycle, is stained more intensely, and is exclusively depleted in genes and enriched in repeats, mainly, in satellites.

Heterochromatin, which surrounds the centromere (see Note*[2]), is called pericentric heterochromatin and comprises from 1/4 to 1/2 of the lengths of different arms. For example, the whole *Y*-chromosome consists of heterochromatin.

Two types of pericentric heterochromatin are distinguished in polytene chromosomes: the tightly compacted α-heterochromatin and more loosened diffuse β-heterochromatin. α-Heterochromatin is enriched in satellite sequences, and β-heterochromatin is enriched in mobile elements, which are often immobilized (*cf.* Ref. 7).

The results of the molecular analysis of one of clones of 2846 bp in size isolated from the long arm of the *Y*-chromosome of *Drosophila* are presented below. The determination of the nucleotide sequence and the computational simulation of the data show that the clone contains the DNA fragment 1176 bp long restricted by terminal inverted repeats 37 bp long, which are flanked by direct repeats 6 bp long. This fragment, which we call the "element 1360", is enriched in AT and saturated with short direct and inverted repeats with different degrees of homology, regulatory sequences necessary for transcription, and sequences with potentials for forming the *Z*-form of DNA and autonomic replication. The *in situ* hybridization method shows that this element exhibits the localization in euchromatic arms of chromosomes, which varies in various lines. This makes it possible to consider this element to be a new, unusually short labile element. In addition, it is localized over the whole length of the fourth chromosome and in all pericentric regions: in the β-heterochromatinic and nearby euchromatinic parts of chromosomes. Thus, β-heterochromatin of almost all chromosomes is enriched in DNA of the "element 1360".[78]

It is known that pericentric heterochromatin in polytene chromosomes is underreplicated. The level of its underreplication can vary in various tissues.

It is normal for *Drosophila* that nurse cells of imago oocytes are endopolyploid*[49] and do not contain polytene chromosomes of the classical type. The formation of polytene chromosomes in pseudonurse cells of oocytes[79] is one of manifestations of the *otu* mutation, which results in the female sterility. Thus, there is a possibility to study the structure and functioning of interphase

*[49] **Endopolyploid** is a tissue or organism with several copies of chromosomal sets (more than two) in nuclei.

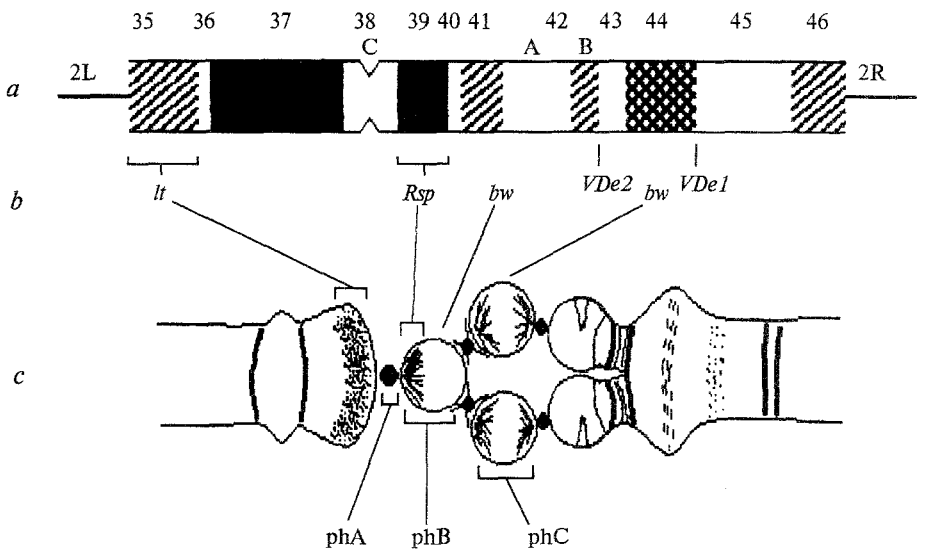

Fig. 8. Molecular cytogenetic map of the region of pericentric heterochromatin of the second chromosome of *Drosophila*: *a*, map of heterochromatinic blocks in mitotic chromosomes; *b*, chromosome rearrangements and names of clones; *c*, blocks of heterochromatin (phA—phC) in polytene chromosomes of pseudonurse cells of otu^{11} mutants.

chromosomes of germ line cells. It turned out that in polytene chromosomes of this type the manifestation of properties typical of heterochromatin of salivary glands (both pericentric and intercalary chromatins) is decreased. A considerably larger amount of heterochromatin is found in pseudonurse cells than in somatic cells of larval salivary glands, which testifies that the degree of underreplication is weakened in these regions.[80] In addition, in IHC regions breakage frequencies and, hence, the level of underreplication and ectopic conjugation are considerably lower than in polytene chromosomes of salivary glands.

The assignment of heterochromatinic blocks in chromosomes of pseudonurse cells to particular arms and their correspondence to segments of mitotic heterochromatin were determined for the second chromosome by rearrangements with breakage points, which are exactly mapped relative to heterochromatinic segments in mitotic chromosomes (Fig. 8, *a*), and by *in situ* hybridization of DNA clones from heterochromatin.

It turned out that many heterochromatinic fragments of mitotic chromosomes can be found in polytene chromosomes of pseudonurse cells (Fig. 8, *c*).

Position effect variegation

Studying euchromatin regions when they are transferred to regions of pericentric heterochromatin due to rearrangements is of special interest. In some cases, this results in the change in the character of functioning of relocated genes: they are inactivated, *i.e.*, endure the position effect variegation, because inactivation does not occur in all cells. We used rearrangements in which the *BR-C* gene is inactivated, which is localized in the 2B3—5 puff and initiates the cascade of puff changes in response to ecdysterone. The manifestation of the mutant *BR-C* phenotype in cells of salivary glands is in the absence of hormone-induced puffs (see above). Therefore, it is possible to compare the genetic activity and the state of a chromosome in the same cell.

This approach allowed us to obtain direct proofs in favor of the fact that the genetic inactivation occurs in parallel to the cytological compactization of the inactivating region.[81,82] The compactization usually starts from the region of contact with heterochromatin and subsequently extends further.[83,84]

The observation of the discontinuous compactization was unexpected; in this case, compact (heterochromatinized) regions of chromosomes alternate with regions with the typical pattern of bands and normal genetic activity.[85]

The cytological and molecular genetic analysis shows that genetically inactivated compact regions of chromosomes acquire features typical of heterochromatin. They become lately replicated, and a portion of DNA is underreplicated, which results in the formation of weak points (breaks) and ectopic contacts.[85,86] The HP1 protein specific for heterochromatin is revealed in compact regions of euchromatin and heterochromatin.[87]

Like the general manifestation of properties of heterochromatin, the position effect variegation in cells of the embryonic path is considerably weakened. The absence of the suppression of the 6-PgD gene expression for the position effect variegation in ovaries of imago is shown biochemically.[88] The compactization of regions of polytene chromosomes in pseudonurse cells of the otu^{11} mutant is also very much weakened. This is expressed in a drastic decrease in the frequency of abundance of rearranged chromosomes with compacted re-

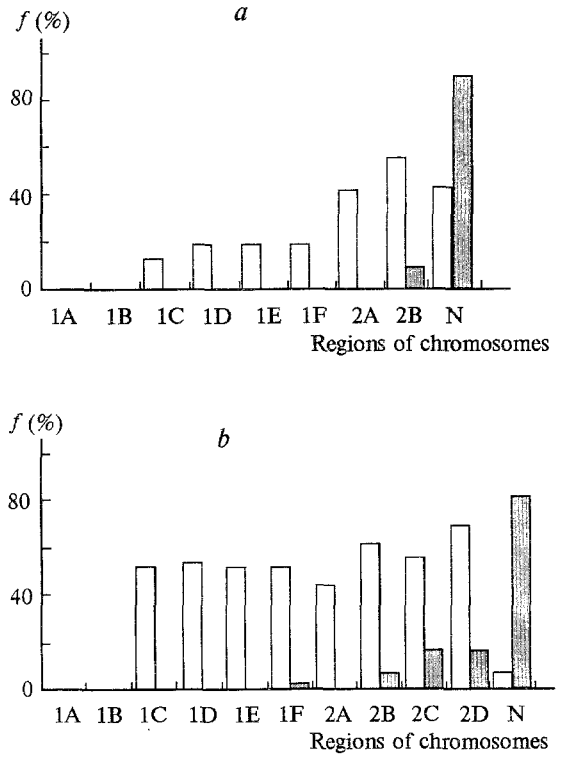

Fig. 9. Frequency (*f*) of the compact state of the region and length of the compactization of euchromatin in *Dp(1;f)1337* (*a*) and *Dp(1;1)pn2b* (*b*) chromosome rearrangements for the position effect variegation in chromosomes of salivary glands (light columns) and pseudonurse cells of oocytes (dark columns) of *otu*^{11} mutants of *D. melanogaster* (N indicates chromosomes with normal morphology).

gions and in a considerable decrease in the length of compactization compared to those in cells of salivary glands of the same genotype (Fig. 9).

At least three questions appear in considering any models of gene inactivation related to the position effect variegation. 1. Why is the position effect variegation mosaic, *i.e.*, a certain region of a chromosome is inactivated in one cell and is not inactivated in another cell? 2. What properties of pericentric heterochromatin are responsible for its ability for supercompactization, and, in addition, why can it involve euchromatic regions transferred to its vicinity in supercompactization? 3. Why do regions that are normally euchromatic have the ability for heterochromatization?

Let us consider the problems listed above in detail.

1. When two chromosome rearrangements (identical or different), each of which undergoes the position effect variegation, are introduced into the genome, inactivation processes in them are independent. The frequency of the simultaneous compactization of two rearrangements in one nucleus is equal to the product of the individual compactization frequencies of each of them. The compactization length of euchromatin in one rearrangement is independent of the degree of compactization of another rearrangement in the same nucleus. The results obtained testify that the statistical variation of the concentration of compactifying factors at the euchromatin—heterochromatin boundary in each nucleus is the basis for initiating compactization. These data also show that there are no substantial differences in the concentrations of compactifying factors between individual nuclei.[87]

2. It becomes clear due to many studies of the position effect variegation that genetic inactivation is far from occurring in each chromosome rearrangement that transfers euchromatin to the vicinity of heterochromatin.[7] It is probably related to the fact that heterochromatin is heterogeneous in its ability to cause compactization. For example, it has been reported[89] that three revertants were obtained in the chromosome with *In(1)w*^{m4} inversion. These revertants exhibit the position effect variegation despite the fact that the DNA fragment from heterochromatin at least 3 kb in length was transferred along with the w^+ gene to a new position.

In our work, the revertants were obtained by irradiation of the *T(1;2)dor*^{var7} chromosome rearrangement, in which the 1A—2B fragment of the *X*-chromosome was transferred to pericentric heterochromatin of the second chromosome and was attached to it in the 2B7—8 region, which results in the appearance of the position effect variegation of genes localized more distal than 2B7—8. Of revertants obtained, the *rev45* line is of special interest. In this line, the portion of 2Lh—2Rh heterochromatin, which has previously been in the contact with the 1A—2B fragment, removed to the vicinity of the 2B7—8 — 7A region. In the *rev45* chromosome in the region of the *dor*, *BR-C*, and *sta* genes (1A—2B7), no inactivation appears even under the action of modifying agents, which enhance the position effect variegation. The inactivation in the 2B7—8 — 7A fragment appeared simultaneously (Fig. 10). The lengths of compactization is extremely long: it covers up to 170 bands (the longest compactization described in the literature). Nevertheless, it turned out that closest 17 kb of DNA from the heterochromatinic region found in the initial *T(1;2)dor*^{var7} line are retained in the *rev45* line at the point of the eu-heterochromatin contact as in other revertants obtained.

Thus, simple neighboring of euchromatin with any regions of heterochromatin does not result in the compactization. It is also evident that there are some centers of compactization, which cause the position effect variegation. They can be arranged rather far from the breakage point of the chromosome rearrangement (in the given case, >17 kb). Their movement due to new rearrangements can drastically change the direction and intensity of the compactization.[90,91]

The 1A—2B7 region from the *T(1;2)dor*^{var7} rearrangement of another revertant, *rev60*, obtained in the same experiments relocates to the pericentric region of the *X*-chromosome along with the fragment of heterochromatin of the *2R*-chromosome. The articulation oc-

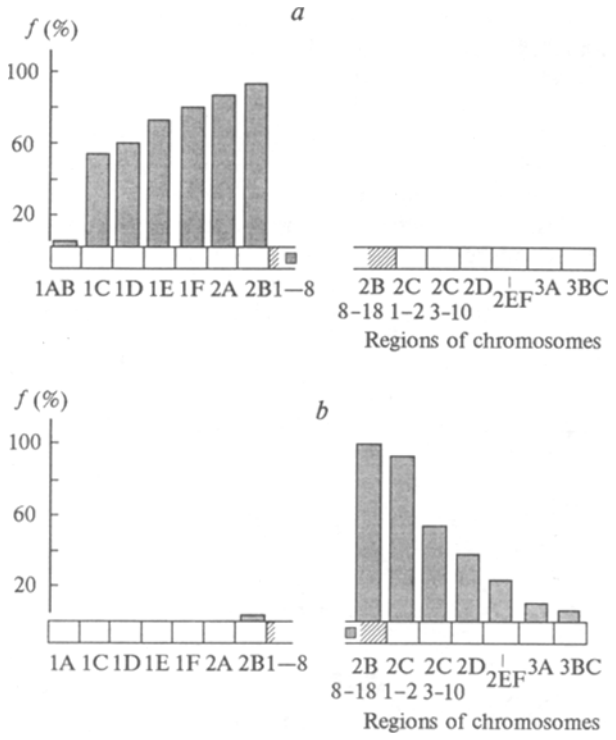

Fig. 10. Frequency (*f*) of the compactization of euchromatin[91] (1AB — 2B1—8 and 2B—3BC regions) in the initial *T(1;2)dor*^{var7} line (*a*) and for changes in the composition of neighboring heterochromatin in the *rev45* line (*b*).

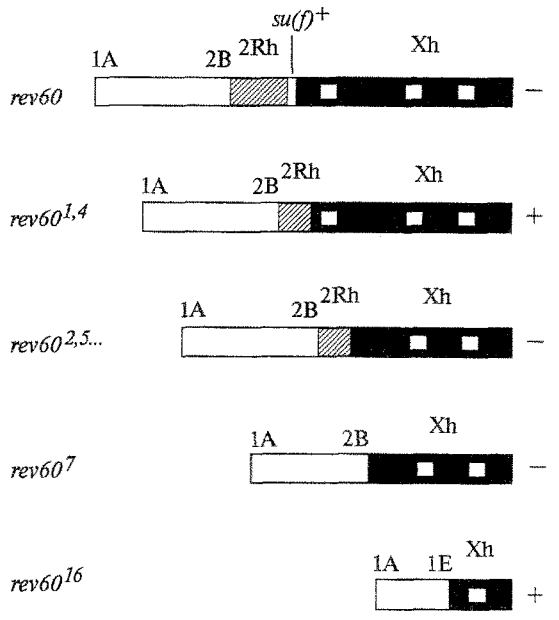

Fig. 11. Variation of the induction of the position effect variegation in the euchromatinic region of the *X*-chromosome (1A—2B) as a function of the structure of neighboring heterochromatin (Xh) in the (*rev60—rev60*^{16}) lines of *Drosophila*. Light squares designate suggested compactization centers; "+" and "−" denote the presence or absence of genetic inactivation with the position effect variegation, respectively.

curs in such a way that the *su(f)* gene is retained between *2R*- and *X*-heterochromatin, and the position effect variegation in genes in the 1A — 2B7—8 region is not manifested itself.[91] Irradiation of the *rev60* chromosome results in the formation of two lines in which the position effect variegation appears again after removing the region of the chromosome that contains the *su(f)* gene. It can be assumed that the induction of the position effect variegation occurs due to approach of the 1A—2B7 fragment to the inactivation centers localized in heterochromatin of the *X*-chromosome (Fig. 11).

Researchers of our laboratory have proposed a model of the compactization of euchromatin for the position effect variegation based on the concept about the statistical distribution of molecules of protein-compactifiers around centers of the initiation of compactization. The model is based on two statements: 1. Centers of the initiation of compactization are present both in euchromatin and heterochromatin (Fig. 12). Compactifying proteins are accumulated in them. The compactization is revealed only in pericentric heterochromatin (see Fig. 12, *I*), if the centers are separated by a region of euchromatin (it is limited by points) without such centers. In the case of the direct contact of the centers when euchromatin (*II*) is removed, compactifying proteins from euchromatin form multilamellar complexes with proteins of heterochromatin. If the compactifying ability of such a complex is weak, the compactization is revealed only in heterochromatin (see Fig. 12, *II*, *a*), and if the ability is strong, there is continuous compactization (see Fig. 12, *II*, *b*) (Ref. 92). 2. Molecules of protein-compactifiers interact not only with DNA sites in chromatin, but also with one another to form a multimeric complex. The nonuniform center distribution in euchromatin can result in long and discrete compactization with the position effect variegation.[92]

Dynamic character of the organization of polytene chromosomes

A polytene chromosome as a whole can be considered as a dynamic formation. Regions of chromosomes 1 to 3 kb in size with short open reading frames are always in the decompactization state (interbands) and arranged in a chromosome at the intervals of 5 to 160 kb (on the average, 30 kb). Regions of chromosomes between them (bands) are in the state of reversible compactization and be completely decompactified when genes, which are contained in them, are activated (the development of the puff). When only individual regions inside the band are activated, the band is fissured into several thinner bands to form decompactified intervals between them.[1,93]

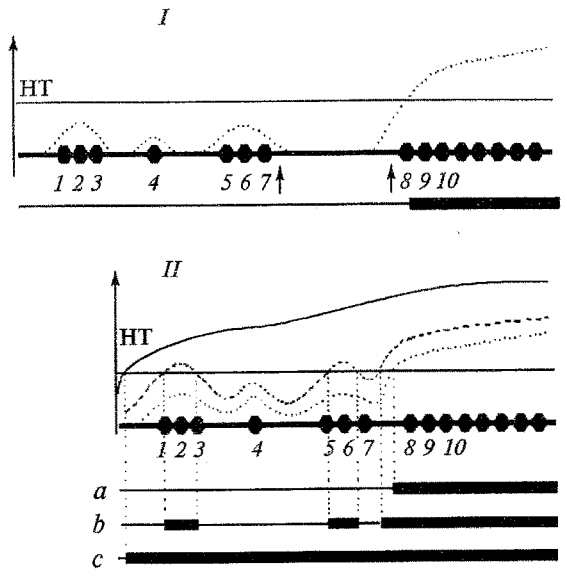

Fig. 12. Scheme illustrating the appearance of compactization with a position effect variegation of the mosaic type: *1—7,* compactization centers in euchromatin; *8—10,* compactization centers in heterochromatin (HT is the heterochromatinization threshold).

The results of experiments with transformants*[50] show that genes entering the composition of the same transposon, which form an entire band, function (and are decompactified) independently both of one another and nearby interbands.

The data on the evolutionary separation of regions of bands and on their independent existence in the other type also testify that their functioning is not adherent.

It is likely that there is the system of strongly inactivated regions in the genome in addition to regions of chromosomes with the regular pattern of bands capable of reversible and irreversible compactization. This is first pericentric heterochromatin, nearby regions of euchromatin inactivated at the position effect variegation, and intercalary heterochromatin. The genetic compositions of these regions differ: the regions of pericentric heterochromatin are depleted in genes and enriched in repeats. Euchromatin contains the usual set of genes. As for the IHC regions, they contain genes (for example, blocks of repeats of 5S rRNA, genes of histones, and genes of the early embryonic development, such as *Antp* or *BX-C*). It is assumed that all these genes as well as the whole regions of pericentric heterochromatin are deeply repressed.[5] It is likely that the repression is performed by special compactifying proteins of the HP1 type[94].

The state of the deep repression probably appears at the early development. For example, pericentric heterochromatin is not revealed by standard C-staining at initial stages of the embryonic development. The staining is observed only at the end of fission divisions.[95] It turned out that the temperature-sensitive period of the genetic inactivation and compactization at the position effect variegation coincides in time with the initial period of revealing heterochromatin.[84,96]

Thus, the variety of morphological elements in polytene interphase chromosomes is determined by their genetic content and the state of their activity, which correlates with the degree of compactization-decompactization of chromatin.

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*[50] **Transformant** is an individuum containing an alien DNA fragment incorporated in its chromosomes by chemical, physical, or biological manipulations.

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