INFORMATIONAL CONTENT OF POLYTENE CHROMOSOME BANDS AND PUFFS

Authors: **Igor F. Zhimulev**
Elena S. Belyaeva
Valery F. Semeshin
Institute of Cytology and Genetics
Siberian Division of U.S.S.R. Academy of Sciences
Novosibirsk, U.S.S.R.

Referee: M. Ashburner*
Department of Genetics
University of Cambridge
Cambridge, England

I. HISTORICAL PERSPECTIVES

The discussion of the genetic organization of bands and interbands started in the early 1930s. Painter was the first to ask, "Where are the genes? Are they represented by the deeply staining material or by some other part of that region of the chromosome?"[1] The same year, Koltzoff wrote: "It is difficult to decide what structure corresponds to the gene—the chromomere or the piece of genoneme between two chromomeres. The latter assumption seems to me more probable . . . we may, therefore, suppose that the chromatin granules and the "discs" are only joints between the genes and the points where the crossing-over takes place."[2] Contrary to this, Muller and Prokofjeva considered the bands to be the main gene carriers: "The results show that at least two regions of the chromatic nodes . . . contain genes (we leave still open the composition of the nonstaining internodes), and that at least some of these nodes contain *clusters* of genes rather than individual genes."[3] And then we find in Bridges: ". . . each of the faint cross-bands . . . corresponds to *one* locus."[4] Thus, the general lines of the discussion, whether the genes are located in bands or interbands and how many genes there are in a band, were set out as early as 40 years ago. Subsequently, it has been widely assumed that the genes are located in the bands. This appears to have been a result of the stimulating papers by Beermann, Mechelke, Breuer, and Pavan,[5-13] where the puffs of polytene chromosomes were interpreted as morphological manifestations of genetic activity. These puffs were known to be formed from the bands.[5,7,14,15] At first, a band was assumed to contain a single gene: ". . . the chromomeres or bands of the giant interphase chromosomes of Diptera represent operational genetic units, units of transcription—whose activity can be individually controlled . . ."[8] "Chromomeres, the physical equivalents of Mendelian genes . . ."[9] ". . . there is a good reason to believe that genes and bands are related one to one."[16-19]

However, the amount of DNA in a middle-size chromomere, was found by Rudkin[20] to greatly exceed, (by about 30 times), that necessary to code for one polypeptide. In the early 1970s, these findings seem to have given rise to the idea that several genes may be present within a band, and that a band contains complexes of genes with related activities.[21-23] "The 1:1 hypothesis brings us back to the old idea that the number of genes

* The fact that Dr. Ashburner was the referee for this review is not to be taken to mean that he necessarily endorses the conclusions the authors have made.

in the genome is equal to that of the bands which can be seen under a microscope. Thus, taking as a basis the hypothesis "1 gene—1 chromomere," we can think that the number of genes in *Drosophila* and *E. coli* is the same. Long ago when a strict scientific etiquette had not been worked out such a result would be called *reductio ad absurdum*."[23] Edström assumes that ". . . most of the eukaryotic DNA has an exclusive meiotic function. Each gene is associated with meiotic DNA usually in a morphological unit, a chromomere. The gene transcribes in somatic tissues, meiotic DNA in meiotic prophase."[24] Akifjev describes a chromomere as "an evolutionary 'pot', where genetic innovations are constructed on the base of redundant DNA". In his opinion, a chromomere contains a structural gene with all its regulatory sequences and a large amount of silent (and even nonsence) DNA.[25]

All of the ideas mentioned above fail to take into account functions of the interband. Some authors proposed that a band + an interband should be taken as the functional unit.[26-28] All the models considering both the band and the complex (a band + an interband) as possible functional units imply that differential activity of the genome can be expressed only in the formation of puffs. Since there are only about ten large puffs in *Drosophila melanogaster* in the middle third instar, it is hardly believable that these few puffs could provide for a great deal of different processes in living cells. Several models postulate the existence of both continuously functioning genes, i.e., "housekeeping genes" and stage- and tissue-specific ones:[30-33] "The bands contain inactive genes, but some of these become active at various stages of development. On the other hand, active genes are in expanded regions . . . interbands represent genes in a steady state of activity, the genes of puffs become active over a more limited period of time."[32] Finally, we find in Ashburner: "There may be silent bands (with no function), bands with more than one function, or even functions spread over more than one band."[34]

Thus, having discussed the various models, we can summarize that during the last 40 years the theoretician's interest has centered around the problem of the genetic information in bands, interbands, and puffs. However, the existence of numerous hypotheses which are often mutually opposed (e.g., Crick's[26] and Paul's[27] models) seems to indicate the lack of decisive data and the necessity of revising the available ones. Several problems can be pointed out as the key questions of the subject which are still worth discussing:

1. Are there any other chromosome regions except puffs where RNA is synthesized?
2. Is there any difference between the banding patterns in various tissues?
3. How many genes are there in a band (puff)?
4. If there is a single gene in a band, what is the function, if any, of the excess DNA in the band?
5. If there are several genes in a band, to what extent are they functionally related?
6. Is the puff a functional unit?

These problems will be considered in the following sections.

II. LOCALIZATION OF RNA SYNTHESIZING REGIONS IN POLYTENE CHROMOSOMES

RNA synthesis in individual chromosome regions is one of the main indications of their genetic activity. Consequently, studies of the location of transcriptionally active regions are important for the understanding of chromosome organization. In the early cytochemical investigations, the preferential, RNase sensitive, accumulation of RNA-specific dyes (such as toluidine blue, azur B, methylgreen-pyronine) was

demonstrated to be mainly in the nucleoli, Balbiani rings, and puffs.[5,6,14,35–37] Characteristic staining of some diffuse bands, as well as of the smallest puffs (interbands?), has been observed in polytene chromosomes of *Chironomus* and *Drosophila.*[36,38,39] In experiments where the indirect immunofluorescence technique for the detection of RNA-polymerase B was used, these data were confirmed.[40,41] At least a few RNA polymerase molecules are located in practically all interbands. This suggests that "interbands represent either active genes or RNA polymerase storage sites or both."[40]

In 1954 Beermann and Bahr described the ultrastructure of the Balbiani rings in *Chironomus tentans* polytene chromosomes in which elements, like the lampbrushes, "bear characteristic bodies of a maximum diameter of about 30 μm."[42] As a result of further electron microscopical (EM) investigations similar RNP granules were found in polytene chromosome puffs of different species of *Diptera.*[43–46] The sizes of these RNP granules range from 200 to 600 Å and do not depend on the puff sizes or fixation methods. Such RNP granules have been described in unpuffed chromosome regions which display at least a small degree of decondensation: in the vacuoles situated inside large bands, in partly decondensed regions of centromeric and telomeric heterochromatin, and in interbands.[45–49] Skaer[50] has found similar granules in about 33% of the interbands including small ones (0,07μm) in *D. melanogaster* polytene chromosomes. The concentration of RNP granules is different in individual parts of the same chromosome.[45,50] The presence of RNP granules in the interbands suggests that RNA is synthesized in these regions like in the puffs, and that RNP granules are a product of genetic activity of such "micropuffs". RNP granules were found again in interbands of *D. melanogaster.*[269]

The question arises as to whether the RNP granules observed are synthesized *in situ* or whether they can be redistributed from the puffs (or the Balbiani rings) along the chromosomes. It is known that the concentration of RNP granules in decondensed chromosome regions, and particularly in interbands, is higher than in the surrounding nucleoplasm, and lower than in the visible puffs. In some very small interbands the granules are absent. The interbands with a considerable number of granules are often situated close to the interbands which contain only a few granules or do not contain them at all. It means that there is no granule concentration gradient from an interband or a puff to an adjacent interband.

In the experiments on the incubation of salivary glands with a radioactive precursor in vivo or in vitro, it was shown that nucleoli, Balbiani rings, and large puffs are labeled heavily.[36,51–56] The sensitivity of the incorporation to RNase treatment, and the labeling after short periods of incubation with the precursor both in vitro (0.5 to 5 min)[55] and in vivo (1 to 2 min)[36,57] demonstrate that the precursor is incorporated in the RNA, and that this RNA is synthesized *in situ* but is not redistributed from other active regions. The incorporation of the radioactive precursor is almost completely inhibited by actinomycin D (0.1 to 1.0 μg/mℓ),[8,37,58] suggesting that it results from transcription of a DNA template.

It has been shown in most investigations carried out with the use of ^{3}H-uridine that silver grains are situated not only over the puffs but also over other regions.[51–54] In *Drosophila hydei* salivary chromosomes only 35 to 50% of sites with uridine incorporation show morphological characteristics of puffs.[52] About 50% of the total number of silver grains are localized over the typical puffs in *D. melanogaster* polytene chromosomes of 0-hour prepupae.[55,56] If one takes into account that the number of puffs at this stage of development is maximum,[29] then at the other stages the proportion of label over unpuffed regions has to be considerably larger. The question arises: What types of unpuffed regions have transcriptional activity? Unfortunately, the resolution

power of light autoradiography does not permit us to solve in principle the question of interband transcriptional activity, though such an attempt has been made.[51] Investigations on stretched chromosomes[59] also do not resolve this problem (see detailed discussion in Reference 60).

EM autoradiography gives a higher resolution (about 0.2 μm) than light autoradiography. Therefore EM autoradiographical data could have a decisive significance in ascertaining the transcriptional activity of interbands. In one of the first such investigations carried out on *Chironomus thummi* polytene chromosomes,[44] silver grains were shown to be located over loose fibrills near condensed bands, besides the nucleoli and the Balbiani rings.

With the help of EM techniques, we have made a detailed investigation of some regions of *D. melanogaster* polytene chromosomes without obvious puffs.[60,61] These data confirm the notion of the existence of transcriptional activity in completely or partially decondensed structural elements of the chromosomes, and are in good agreement with the above cytochemical, EM, and light microscope autoradiographical data.

All the data confirm, or at least do not contradict, the notion that the interbands are micropuffs. The common objection to such a view is that the DNA amount in interbands is too small to house a gene. At present, there are no sufficiently precise techniques for measuring the DNA content of interbands and the estimates of interband length are rather approximate. Even considering the definition of an interband itself, it is impossible to distinguish between a very small puff and a large interband. Furthermore, in determining the physical sizes of interbands, we come across considerable difficulties; for example, there is a variability in interband width (0.10 to 0.35 μm), and the shape of the border between a band and an interband is irregular, or jagged.[10,62] The estimations of the diameter of DNP fibrills in interbands vary (according to different authors) from 130 to 400 Å.[32,63-65] The packing ratio of DNA in transcriptionally active chromosome regions is about 1.6.[66,67] Taking into consideration these data, there should be at least 1500 base pairs in an interband. Beermann[10] has estimated the mean size of an interband as ~2000 base pairs. According to biochemical investigations the average size of a nuclear poly(A^+) RNA molecule in *D. melanogaster* embryos is about 6000 nucleotides, and the average size of a cytoplasmic poly(A^+) RNA is about 1800 nucleotides.[68,69] If the RNA molecules synthesized in interbands undergo processing to the same extent, then the sizes of coding sequences will be approximately 700 base pairs, which can encode about 240 amino acids. However, we cannot rule out that only a small part of the RNA molecule synthesized in the interband is cleaved during processing. It is also impossible to exclude the hypothesis that the interband RNA does not code for any proteins, but has some regulatory functions in the nucleus.

Thus, the experimental data considered substantiate the view that RNA synthesis is present in interbands. However, at present we can only speculate as to the functional value of this RNA. It is possible that the proposed[33,70] housekeeping genes are located in interbands. The relative stability of the banding pattern is one of the indications of the constant activity of such genes. Progress in this direction will clarify the functional significance of these chromosome elements.

III. STABILITY AND VARIABILITY OF BANDING PATTERN

The stability of the banding pattern of polytene chromosomes seems to be obvious. Nevertheless, the question of banding pattern stability is not yet solved. It is necessary to decide whether the banding patterns are identical in functionally different cells of various tissues, stages of development, and in mutant stocks (with the exception of changes caused by the puffing). It is rather difficult to answer the question, since considerable

controversy surrounds the interpretation of the data. One of its causes is the varying resolution of the bands in various cell types.

The bands are best detected in the light microscope after moderate chromosome stretching.[4,5,10] The different degree of polyteny and the different degree of chromosome extensibility also brings about differences in banding patterns. For example, Beermann found in the third chromosome of *Chironomus tentans* from salivary glands — 380 bands; from Malpighian tubes — 440 bands; from rectum — 380 bands; and from midgut — 490 bands[5] (see also Reference 71). The visualization of bands greatly depends on the fixative used.[62]

With these ideas in mind, we can understand the great diversity in the number of bands in various species (within the *Diptera* order) which Lifshytz[72] and Panitz[73] discuss: in *Glossina morsitans* there are about 600 bands;[74] in *Drosophila lebanonensis* — 1200;[75] in *Chironomus tentans* — about 1600;[36] in *Drosophila hydei* — 2000;[76] and in *Drosophila melanogaster* — 5000.[77] These differences are not likely to have functional significance related to the gene number. However, we cannot exclude various kinds of DNP packing in chromosomes of various species. Thus, there is a great morphological difference between *Chironomus* and *Drosophila* chromosomes, but it disappears after the treatment of *Chironomus* chromosomes with solutions of high ion strength at low pH.[78]

The discussion of the stability of the banding pattern in chromosomes of various organs became most acute in the late 1940s and early 1950s after the appearance of papers by Kosswig and Sengün.[30,71] These authors believed the banding patterns of chromosomes of various organs were nonidentical and dependent on cell function. This point of view, in Beermann's and Clever's[37] opinion, was at variance with the principle of gene linearity in chromosomes.

Having thoroughly studied the banding patterns of chromosomes of four organs,[5,7] Beermann concluded that they were very similar. However, Beermann points out four types of differences which perhaps could not be explained by the differential puffing activity:

1. **Presence-absence** — The band is clearly seen in one tissue and absent in the other.
2. **Single-doublet** — There may be one or two bands in the same chromosome region in various tissues.
3. **Different distance** — Two homologous bands are at a different distance from each other in various tissues.
4. **Tissue-specific intensification of staining interband** — Between two or more adjacent thick bands.[7,10]

Pavan and Breuer[12] found the banding patterns of the A-chromosome from salivary glands and Malpighian tubules to be very similar except for the region 13 (see Figure 1 in Reference 12). But the data presented in the next paper[79] show that the variations are more obvious than the resemblance (see regions 4, 12, 13 of A-chromosome, region 2 of X-chromosome, and others[79]). Enormous differences in the total chromosome structure in various sections of the Malpighian tubules have been demonstrated by Pavan.[80]

We have recently analyzed in detail the patterns of the polytene chromosomes of *D. melanogaster* in various stocks, tissues, and stages of development. We have observed the general stability of patterns and rather numerous variations which correspond to Beermann's types, i.e., interband elongation and band splitting (double-single by Beermann). These variations were characteristic of the cell type (stock, stage, organ), and their visibility did not depend on the degree of polyteny, fixation, or staining technique.[81] Recently, Richards[82] has shown that the banding patterns of fat body polytene chromosomes are basically the same as those of the salivary gland, but again "tissue

differences in relative spacing exist", and then "the region 84AB is characterized by three heavy bands in . . . salivary gland maps, whereas the same region in fat body has four (and sometimes five) heavy bands". More pronounced differences were found in the 93EF region. One can see some cases of variations in the paper by Holden and Ashburner; there are clearly fewer dense bands in the region 98 of the *D. melanogaster* 3R-chromosome from the ring gland as compared to those from the salivary gland (see Figure 2 in Reference 83). But in the mutant l(3)DTS3, the banding patterns of many regions are identical in the two organs. One can find a difference in band numbers and their morphology in the region 121 of *D. hydei* salivary gland and Malpighian tubule chromosomes (see Figure 4 in Reference 84). Large shortened polytene chromosomes with changed banding pattern were found in pseudonurse cells of mutant *fs 231* in *D. melanogaster*.[270]

Small differences in the number of bands between larval salivary chromosomes and adult Malpighian tubule chromosomes can be observed on the photographs of Figure 3 in Reference 85.

The comparison of banding patterns in cells greatly differing in function is of special interest. The nurse cells of oocytes are highly differentiated and provide a growing oocyte with RNA.[86,87] In polyploid nuclei of these cells, typical polytene chromosomes are formed at some stages of development.[88,89] As a result of the comparison of the banding pattern of the nurse cell chromosomes with those of the salivary gland in *Anopheles superpictus*[90] and salivary gland chromosomes with those of the pupal epidermal bristle forming cells in *Calliphora erythrocephala*,[91] it was reported that there is no homology in the patterns. However, careful analysis of the photographs presented in Reference 90 shows that it is possible to find such homology at least in the shortest chromosomes, though the morphology of chromosomes is quite different probably due to different distances between homologous bands and their different thicknesses.

Recently, Redfern[92] described the polytene chromosomes of *Anopheles stephensi* in the nurse cells and in salivary glands. The banding patterns were basically the same in both tissues, though numerous differences are evident mainly by band splitting and apparently differing interband lengths in homologous regions.

The great differences in *Calliphora* nurse cell and epidermal cell chromosomes[91] are still mysterious.

Considerable changes of the banding pattern throughout the year have been observed in *Chironomus plumosus*. In September, before the cold season, the chromosomes are greatly shortened; the band number is minimal, and many adjacent bands identified as separate in summer are fused in chromatin blocks. The number of separate bands visible in autumn is smaller than in summer by a factor of 1.5 to 2.0. In January and February, the chromosomes become longer, and in March, it is possible to see 3 to 4 times more bands than in September.[93,94]

Three karyotypes in *Chironomus Valkanovi* were discovered,[95] each of them being characteristic of the larvae occupying regions of the Black Sea with quite definite salt concentrations. The homologous chromosomes of these three karyotypes differ in general morphology in length and sometimes in the band number (see Figure 4 in Reference 94). Similar differences have been found for *Chironomus plumosus* in populations from different environments.[96]

Two main types of infections are known for *Diptera*: microsporidians and gregarines living in the cytoplasm, and viruses living within the nucleus.[79] The penetration of a microorganism into the cell soon leads to a structural disorganization of the host cytoplasm: reduction of endoplasmic reticulum, Golgi complexes, and myofilaments in muscle cells; the number of mitochondria greatly increases (the parasites do not have their own mitochondria). Infected cells are not lysed during metamorphosis and can exist

as long as 50 days after the infection took place.[79] How do chromosomes respond to such a sharp change in the physiology of the cell? An additional replication of the giant polytene chromosomes is observed simultaneously with the reproduction of the protozoan. The DNA content in the individual chromosomes increases by a factor of 2, 4, 8, 16, or 32 compared with normal ones.[79] Simultaneously, the puffing patterns begin to change; the DNA puffs either disappear or the order of their activity is changed.[79] There remains a considerable incorporation of ^{3}H-uridine.[97] According to Pavan and co-workers, ". . . there is no alteration in the basic banding pattern of the chromosome (Figure 11)."[79] However, in this figure the band number in the second segment from the left in the infected chromosome is smaller than that in the normal chromosome by a factor of 2 or 3. The B-chromosome of *Sciara ocellaris*, being rather long in the normal cells, contracts to half its length at the very beginning of the infection. The number of the observable bands decreases considerably (see Figures 6 to 8 in Reference 98). Both the striking activation of the DNA-synthesizing apparatus during the additional replication cycles of polytene chromosomes, and the considerable increase in the number of mitochondria as the parasites propagate, should result in a constant growth of the mRNA synthesis. Since the majority of the puffs are absent, one may assume that the interbands take part in these typical processes of "housekeeping".

A long-term (5 to 10 days) cultivation of *Rhynchosciara angelae* salivary glands in vitro results in a considerable shortening of the chromosomes. However, there were chromosomes with distinct banding pattern even after 30 days of incubation.[99]

After the transplantation of the larval[100-103] or embryonic[104] salivary glands of *Drosophila* into the imago abdomen and a long-term (for 48 to 50 days) incubation, polytene chromosomes present a range of phenotypes: diffused and weakly stained with orcein, supercontracted with "meanders", or shortened. The shortening of the chromosomes is caused by the fusion of the groups of neighboring bands into chromatin blocks.[103] At the least some of the normal cell reactions remain unchanged after a long-term incubation of the salivary glands. The DNA replication cycles in these transplanted polytene chromosomes are almost normal but somewhat delayed.[101,105,106] Even after 2 to 3 weeks of implantation, heat shock puffs[101] and some of the early ecdysone puffs (there were only 74EF, 75B under analysis)[102] can be induced normally. The ecdysone-stimulated histolysis of the salivary gland takes place.[107] Even after a 40-day implantation, all nuclei were able to incorporate ^{3}H-uridine.[101]

The mutant l(3)tl of *D. melanogaster* is one of the most vivid and best-studied examples of variations in banding pattern.[108-112] A detailed study of the process of chromosome shortening showed that this process is based upon fusion of a few neighboring bands into one new band or block of chromatin.[110,111] In the regions where the condensation has taken place, the intensity of the ^{3}H-uridine incorporation decreases sharply (up to tenfold).[111] In such shortened chromosomes, puffs can still be induced by means of anaerobiosis,[112] heat shock, and at early stages, ecdysone.[111] The pompon-like X-chromosome of males (see below) maintains its dosage compensation.[111]

Sometimes the banding pattern disappears in polytene chromosomes; these shorten, widen, become diffused, "pompon-like". Such pompons (mainly from male X-chromosomes) have been found in the nuclei of infected *Sciaridae* larvae[98] in the salivary glands after prolonged cultivation in vitro[99] or in vivo[102,103] in some lethal mutants of *D. melanogaster*[83,109] and *D. hydei*,[108,112] and during normal development of *Phryna cincta*.[113,114] In the latter case, the clarity of banding depends upon temperature, crowding of culture, and concentration of some salts.[113,114] Such chromosomes were suggested to be "generalized puffs". Perhaps it should be expected from the definition that in this type of chromosomes ^{3}H-uridine incorporation increases. But the papers on infected larvae do not supply such data. The increase of RNA synthesis in *Phryna cincta*

pompons has not been found. The character of DNA replication was similar to that in the autosomes.[114] The level of ^{3}H- and ^{14}C-uridine incorporation in the pompons of l(3)tl larvae was identical to that of normal males, i.e., the X-chromosome was two times more active than the autosomes.[111] Therefore, we have no reason to think that any superactivation of RNA synthesis or puffing of bands occurs in the pompons. It seems more probable that as a result of some physiological changes in the cell, a longitudinal disintegration of DNP threads of polytene chromosomes and their redistribution takes place, and because of this, they become shorter, wider, more diffuse, and lose their banding pattern. It is evident now that chromosome "pomponization" has nothing to do with the problem of variations in the banding pattern under discussion.

The results discussed have lent support to Kosswig's and Sengün's general ideas of polytene chromosome organization: ". . . it could be imagined that the visible pattern of the chromosome is induced . . . by the differentiated cytoplasm . . ." In other words, we have every reason to think that a definite functional state of the cell and the banding pattern in polytene chromosomes are closely related. In cases when cells have similar types of activity, they have also similar, but perhaps not identical, sets of active (situated, by supposition, in interbands, diffused bands and puffs) and inactive (in condensed bands) genes, and a similar banding pattern in general. Some insignificant fluctuations in the banding pattern were observed in all the cases investigated and these seem to be related with minor differences in gene sets of the cells being compared. Cell specialization, for example RNA synthesis for export (nurse cells) and sharp changes in physiological or ecological conditions, may be accompanied by activation or inactivation of genes which have been inactive or active. Changes of banding pattern in pathological situations when an infection, mutation, or prolonged incubation leads to disorders in a similar way. This idea of a dynamic character of banding pattern which is able to vary according to physiological changes in cells, as we understand it, does not contradict the principle of the linear gene arrangement in chromosomes. The discussion of variations in the banding pattern leads unexpectedly to the conclusion that the bands can be informationally complex. We can imagine that a band contains several independent loci. If all of them are inactive, the material aggregates into one band; if one or two segments of DNA are activated, the bands split into two or three. In this case, the activation of a locus situated at the edge of a band will lead to lengthening of the interband.

IV. INFORMATIONAL CONTENT OF BANDS

The presence of genes in bands follows, first of all, from the fact that they contain 90 to 95% of the whole DNA of polytene chromosomes.[10] This is also evident from the results of *in situ* hybridization of 5S RNA, heat-shocked RNA, cloned poly(A^+) RNA sequences, etc. By means of cytogenetic methods it has so far been impossible to demonstrate the presence of genes within bands, since the only available technique of gene localization by deletions and duplications allows us to map a gene rather exactly only to the chromosome region, but not the individual structure (band or interband) because of a lack of resolution: 1 band ± 1 or 2 adjacent interbands or else 1 interband ± the margins of 1 or 2 adjacent bands (Figure 1). The use of the EM has no significant advantage.[62] It seems that it is this reason that accounts for the fact that the problem of the exact localization of the gene *white* raised at the end of the 1930s[77] still remains controversial.[10,115-117] The only method permitting us to judge the precise localization of a gene in relation to a band (unfortunately, valid only for large bands) seems to require the use of differently directed overlapping deletions, each of which removes only a part of the band (Figure 1). However, by means of this method only one band, 10A1-2 (see below), has been so far studied in *Drosophila*.

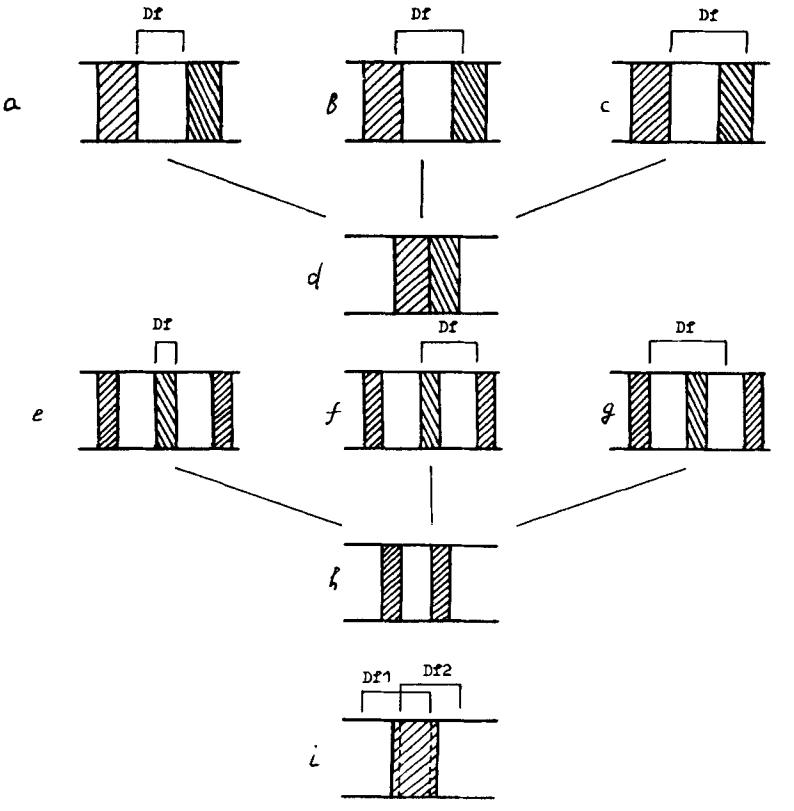

FIGURE 1. Resolution of cytological mapping of deficiencies ends. The banding pattern seems to be similar (d) after removing only interband (a), interband and margins of both neighboring bands (b), interbands and a whole neighboring band (c). A similar banding pattern (h) can be generated by removing only a band (e), band with one (f) or two (g) neighboring interbands. Two overlapping deficiencies (each removes only part of the band) permit a precise mapping of the gene within the band (i).

There are no facts proving the absence of genes in the interbands. And vice versa, it is very difficult to obtain indisputable facts regarding the localization of any genes in the interbands. This means that the results of the experiments on the saturation of some chromosome regions with mutations (Table 1) argue equally well for the correlation of the number of genes with the number of bands, or interbands.[72] In discussing the results presented in Table 1, one has to keep in mind the following circumstances:

1. In most cases there is no agreement between different authors as to the number of bands in the chromosome region which has been saturated, the difference being often larger than twofold (region 2D3 to 2F5, etc.) This leads to great differences in the determination of ratios, which means that it is often impossible to draw any conclusions except that the ratio of the number of essential loci to bands/interbands varies within a considerable range—around 1:1.
2. Saturation has probably not been reached in most regions because in all regions, except for 3A1 to 3B5 and 2B5 to 6, there are numerous "complementation groups", which are represented by only one lethal or semilethal mutation.
3. The "saturation" of the regions was produced mainly with mutations affecting

Table 1
ANALYSIS OF THE DATA ON THE RATIOS OF GENE NUMBER TO BAND NUMBER IN VARIOUS CHROMOSOME REGIONS OF *DROSOPHILA MELANOGASTER*

Chromosome region	Complementation groups	Number of bands (in parenthesis number of "doublets")	Maximal and minimal possible ratios of cistron number to band number	Remarks
1B1-14, X chromosome	14—15 complementation groups of lethal mutations, 4 visible genes *yellow, achaete, scute, silver,* lethal *M(1)Bld*[118]	14(4)[77] 8—9[119] 10[120]	$\frac{19—20}{8—14}$ = 2.5—1.3	Region is not saturated, since there are only 20 mutations giving 14—15 complementation groups; full data have not been published
1E1-2—2A1-2,[a] X chromosome	10 complementation groups of lethal mutations plus 3 separate lethals, plus 2 visible genes *su(w^b)* and *sta*[121,122]	11(4)[77] 7—8[119] 11[120]	$\frac{12—15}{7—11}$ = 2.1—1.1	Three single lethals permit to consider this region as unsaturated; full data have not been published
2B3-4—2B7-8, X chromosome	2 separate complementation groups of visible and lethal mutations *dor* and *swi,* four groups of lethal and visible mutations overlapped by noncomplementing lethals[123]	2—4(2)[77] 2—4[120] 2—4[124]	$\frac{3—6}{2—4}$ = 3.0—0.75	
2D3—2F5, X chromosome	8 complementation groups of lethal mutations, 3 single lethals, visible gene *pn*[125] and female fertility locus *mel(1)R1*[126]	12(2)[77] 7[119] 16[120]	$\frac{12—13}{7—16}$ = 1.9—0.8	Presence of 16 bands[120] was not documented by photographs; allelism of *mel(1)R1* with the rest mutations was not tested
3A1-10, X chromosome	6 loci of lethal mutations, loci *gt, tko, z,* nonlethal break of *T(1;Y)p22*[18,116,117,127]	10(1)[77] 7[119] 8—9[10] 10—13[120]	$\frac{9}{7—13}$ = 1.3—0.7	Presence of 13 bands[120] was not documented by photographs; presence of repetitions in 3A1-4[128] probably prevents revealing other genes
3B1-4, X chromosome	7 cómplementation groups of lethal mutations, *per* locus, 2 female sterility loci[117]	4(1)[77] 5[119] 5[10] 6[120]	$\frac{10}{4—6}$ = 2.5—1.7	Presence of 6 bands[120] was not documented by photographs

3C1—3C7, X chromosome	3 visible genes *white, roughest, verticals* and locus of lethal mutations *Notch;* no other lethals[118,129]	7(2)[77] 4[119] 5—7[120] 4[129]	? (See remarks)	According to standard map[77] in the *white-split* interval there are 14 more loci; the mutations were obtained by various authors and were not tested on allelism each other
3C8—3D3, X chromosome	Absence of loci of visible or lethal mutations,[118] locus *Sgs-4,* coding for salivary gland secretion polypeptide[130]	8(2)[77] 7[120]	$\frac{1\ (?)}{6^a—8} = ?$	
10A1-2—10A4-5 X chromosome	3 complementation groups of lethal and semilethal (slm), mutations, nonessential genes *v, sev, ms(1)BP-6*[131]	7[62]	$\frac{6}{3} = 2.0$	Chromosome region was mapped with EM
Band 15A1, (cytological localization of *r* locus,[122,132] X chromosome	The first 3 enzymic activities of pyrimidine biosynthesis[116,132]	1[77]	3:1	Localization of *r* precisely in the band 15A1 was not documented by photographs; these three activities may be activities of a single polypeptide
18F4-5—19D3, X chromosome	5 complementation groups of lethal mutations, 4 visible genes[133]	20(8)[77]	$\frac{9}{12^a 20} = 0.8—0.5$	Three single mutations permit to consider this region as unsaturated
19E1-2—20A1-2, X chromosome	14 complementation groups of lethal mutations, 2 visible genes, 3 loci of male fertility[133,134]	16(7)[77]	$\frac{12—19}{9^a—16} = 2.1—0.7$	12—number of loci according to Shalet and Lefevre;[133] 19—total number of loci according to both research groups; allelism of some mutations used by them was not tested
34D1-2—35B10, 2L chromosome	21 complementation groups of lethal mutations, 9 visible genes[135]	33(9)[77]	$\frac{30}{24^a—33} = 1.3—1.0$	Region seems to be not saturated since 6 loci consisted from the only mutation; presence of intercalary heterocromatin in 34EF—35A (see [4]) probably prevents revealing another genes
37B10—37C4,[c] 2L chromosome	11 complementation groups of lethal mutations plus visible mutation *hook*[136]	8(2)[77]	$\frac{12}{6^a—8} = 2.0—1.5$	Full data have not been published

Table 1 (continued)
ANALYSIS OF THE DATA ON THE RATIOS OF GENE NUMBER TO BAND NUMBER IN VARIOUS CHROMOSOME REGIONS OF *DROSOPHILA MELANOGASTER*

Chromosome region	Complementation groups	Number of bands (in parenthesis number of "doublets")	Maximal and minimal possible ratios of cistron number to band number	Remarks
87C1-9, 3R chromosome	4 complementation groups of lethal mutations plus *kar* locus were located only in 87C4-5—87C9[137]	6[77]	$\frac{5}{6} \sim 1.0$	Complementation groups have not been isolated in 87C1-3 region, which contains repetitions (see discussion in Section VI)
87D2-4—87E12-F1, 3R chromosome	21 complementation groups of lethal and visible mutations[138]	24—28(2-4)[77]	$\frac{21}{22^a—28} \sim 1.0$	Three single lethals permit to consider the region as unsaturated
[139]Chromosome 4	37 complementation groups of lethal mutations, 6—8 visible genes, minor genes (number was not determined) influencing number of sternopleural bristles, ether resistance, the penetrance of *witty*[139]	50(17)137[77]	$\frac{43\text{-}?}{33^a—137} = ?$	Number of bands 137 was not documented; chromosome seems to be not saturated since 10 loci consisted from the only mutation; calculated number of loci is 67—75;[137] chromosome contains 3 or 4 bands containing A-T sequences which probably do not code for proteins (see discussion in Section V)

[a] Cytological limits of *Df(1)sta* used for saturation of the region 1E1-2—2A1-2[121] are broader: 1E1-2—2B3-4.[271] In independent experiments using other sets of deficiencies in the region 1E1-2—2A1-2, 10 complementation groups were detected. Locus *sta* was found to be located in the 2A1-2—2B3-4 region.[271,272]

[b] Number of bands considering Bridges' doublets as singlets. Artifact nature of doublets was discussed widely; see recent discussion in Reference 62.

[c] 12 (possibly 13) genes are not evenly distributed within the region. Dopa decarboxylase locus plus 5 lethal genes are in a maximum of 3 bands, probably part of the 37C1-2 singlet plus bands 37C3 and 4.[273]

viability, i.e., lethals and semilethals. Genetic variation is not restricted to the essential loci.[140] As the selection criteria change, it becomes possible to detect new loci. Enzyme null alleles were recovered at 13 of 25 loci in *D. melanogaster.*[274] Thus, specialized selection experiments have recently detected in the X-chromosome no less than 30 loci which affected the ability to fly,[141] and about 50 loci concerned with the fertility of females.[142] Also, loci controlling the circadian rhythms, behavior, sight, mutagen susceptibility, etc. are known. One may think that the range of possible mutations is not restricted to this list. Recently, an attempt was made to detect sequences whose breakage by means of chromosome aberrations is not manifested in the phenotype and has no lethal effect.[117] An unequivocal interpretation of these data is rather difficult, since it is not clear whether this breakage took place in a really nonessential locus, in a small repetition, or in a spacer between genes.

The presence of gene duplication in a region makes it difficult or even impossible to detect mutations. The absence of lethals in the region 3C1 to 3C7 is possibly due to the presence of a duplication, as has already been discussed.[129] The same is true for the 3A, 34EF, 87C regions and for the fourth chromosome. Some other difficulties were discussed earlier.[117] Taking into account what has just been told, one may conclude that the data given in Table 1 concern only the most easily detectable loci.

In spite of these limitations, at least in four regions of the *Drosophila* genome, it has been established that the number of loci exceeds that of bands or interbands. In the region 1B1 to 14, the number, even of essential loci, is larger than that of bands (interbands); in addition, there are four nonessential genes known in this region. In the region 3B1 to 4, seven essential and three nonessential loci correspond to 5-6 bands (interbands). In the region 10A1-2 to 10A4-5, there are three loci of lethal mutations and three nonessential loci, and only three bands (interbands) (see also 37B10 to C4, Table 1). In chromosome 4, there are 33 to 50 bands, and there have been found 37 essential and 6 to 8 nonessential loci and also some modifier loci; besides, there are A-T—rich repetitions in four bands. In all these cases while one can see a close correspondence between the number of bands (interbands) and the number of essential loci, there is an excess when nonessential loci are considered. From the point of view that a band is a "lair" built especially for a single Mendelian gene,[9] it is difficult to account even for a slight deviation from a 1:1 ratio.

The question of the genetic content of a band as a morphological structure of the polytene chromosomes can be discussed at present only by considering the band 10A1-2 of the X-chromosome of *Drosophila.* Morphologically, this band is single and uniform.[62] A series of differently directed overlapping deletions which removed different parts of the band[16] were mapped by means of the EM with maximum possible accuracy.[62] As a result of complementation analysis, using a series of newly induced mutations[131] and a series of previously known mutations,[16,143,144] a cytogenetic map of 10A1-2 has been built. At the left edge of the 10A1-2 band there are silent DNA zone and two loci: *v* and *l(1)BP4,* which occupy a region of 0.08 map units. Then there comes a huge silent zone (0.38 map units) and a nonessential locus *sev* which has been mapped by means of EM[131] to the very right edge of the 10A1-2 band. It is possible that recently revealed locus *ms(1)BP6* is also located within the right part of the band (Table 1). Quite plausible considerations are given to account for the contradiction between the amount of DNA (the recombination size of the band is about 200 kb) and the number of DNA sequences found (5). Perhaps, not all the genes have been detected in the band. Indeed, three nonessential loci (*csk, wgv, osh*) affecting mainly the wing position in flies are known. These loci are recombinationally inseparable from *vermilion.*[77] It is possible that by changing the criteria for the selection of mutations, one can expect detection of new loci in this band.

Are the five above-mentioned sequences (three loci and two silent zones) within the band 10A1-2 functionally related, i.e., are they in one transcript under a common control or can they be transcribed independently? The translocation *T(1,3)v* breaks the band 10A1-2 approximately in the middle, and so separates its two parts spatially; nevertheless, the heterozygotes *T(1,3)v/ Df(1)v*^{L3} have a normal phenotype and fertility, which argues for the absence of any regulatory functions of the right half of the band 10A1-2 (and the adjacent right hand interband) for the transcription of the loci situated in the left part. The left part of the band and the left interband are not necessary for transcription of the *sev* locus, since *T(1,3)v/sev* heterozygotes have a *sev*$^{+}$ phenotype. These facts are evidence for the independent functions of DNA sequences localized in the band. At least this band is not related fuctionally to these two neighboring interbands. In addition, these data allow us to predict that the band 10A1-2 will split if the genes *v* or *l(1)BP4* are activated and that the marginal interband will be elongated if the locus *sev* is activated. The possibility of the construction of such compound bands has recently been shown experimentally.[145] Full data about genetic anatomy of the 10A1-2 band are published in References 275 and 276.

The analysis of the data presented in this section is evidence that even in such a large band as 10A1-2 there are few essential loci. A homozygous deficiency of the distal part of the medium-size band 3C2-3 gives only the *white* phenotype.[10,117] It is possible that in small bands there may be no essential loci at all. On the other hand, as a whole, a ratio rather close to 1:1 for essential loci and a certain excess of nonessential ones have been found.

For further analysis it is first necessary to consider certain other facts characterizing the lethal mutations. It has been shown[146,147] that 40 to 50% of X-chromosome mutations taken at random did not form any viable gynandromorphs in adult flies; i.e., these lethals are most probably cell lethals. This is supported by the fact that there is preferential embryonic or early larval mortality of individuals homozygous for these lethals. Between 16.3 and 40% of lethals do not give rise to any viable cell clones obtainable by means of somatic crossing over.[147] Experiments on the production of cell clones having a hemizygous deletion of a fragment of chromosome from 2 to 30 bands long have demonstrated that cell lethals are distributed in the genome rather uniformly, there being cell lethals in 5 out of the 9 smallest deletions removing 2 to 4 bands/interbands. As the size of the chromosome fragment removed increases, so does the proportion of deletions including a cell lethal.[148] The normal alleles of cell lethals must work in most cells. Permanent work probably means permanent transcription, and therefore decondensation of the chromosome material. Interbands are permanently decondensed in most cells. This is why it is logical to consider that it is in the interbands that some of the essential loci are localized, while the nonessential ones are situated mainly in the bands. (This conclusion was made by A. V. Bgatov in our laboratory.) This would clearly explain three facts:

1. The ratio between the number of essential genes in the region and the number of interbands is close to 1:1
2. The absence (band 3C2) or the small number (band 10A1-2) of essential loci in the bands
3. The rather high proportion of cell lethals among the essential loci

V. "INTERCALARY" HETEROCHROMATIN

Some regions have been found in the polytene chromosomes of *Drosophila* which have a number of properties in common with those of the centromeric heterochromatin, such

as ectopic pairing and an increased frequency of chromosome rearrangement breakpoints. These regions, by analogy with the centromeric heterochromatin, were called intercalary heterochromatin. Some regions of the intercalary heterochromatin in salivary gland chromosomes appeared to occupy whole subdivisions composed of many bands and interbands.[149] More recently, other properties have been found such as the late DNA replication in these regions,[150] high breakability,[151] increased concentration of asynapsis ends, i.e., strong somatic conjugation,[152] and the presence of repeated sequences.[128]

The regions having all these characters have a similar localization.[151,153,154] One can be sure that the carriers of the properties of intercalary heterochromatin are single compact bands, rather than whole regions, since it is in individual bands that late replication has been found.[15,150,151] By using chromosome rearrangements, it was demonstrated that in the 3C region it is not the whole region of compact bands 3C1-7 that replicates late, but only the interval 3C3 to 3C5, corresponding to the segment between *white* and *roughest* on the genetic map.[150] Single bands participate in ectopic conjugation; topographically, the breakages in the weak spots occur always in definite places, namely just on either the left or right margin of the band.[151] In addition, cloned DNA sequences, hybridize quite distinctly to a single band.[153] In some chromosome regions there are series of such bands lying close together due to which one gets an impression of the existence of whole heterochromatin regions. The finding of similar or identical DNA sequences in the regions of ectopic contacts[153,155,156] witness for the fact that the ectopic conjugation is made on the "similar-to-similar" principle. This, in turn, demonstrates that in the genome of *D. melanogaster* there are no less than 300 bands (the number of ectopically conjugated bands according to Kaufmann and Iddles[157]) which at least in combinations by the pair show the presence of homologous and most probably repeated sequences.

Especially interesting are the results of the localization in polytene chromosomes of two DNA fragments, Dm 225 and Dm 234B, which are complementary to poly(A^+)RNA of *Drosophila* cell culture. The total number of hybridization sites in *Drosophila* strains was 67 for Dm 225 and 41 for Dm 234B. These clones are repeated in the *Drosophila* genome hundreds of times, that is why it is believed that in each "nest" of localization of clones there are about 10 copies.[153] The localization of hybridization sites largely coincided with the heterochromatin regions. This has allowed us to consider that in the bands of the intercalar heterochromatin there are DNA sequences on which mRNA is transcribed.[153] The plausibility of such a conclusion is evidenced by the results of mapping of the total poly(A^+)RNA whose sites[128,158] also coincide with those of intercalary heterochromatin. When analyzing the photographs,[153] one can notice that the accumulation of label is found over very short pieces of chromosomes which can reasonably be considered as separate bands, e.g., in 39E1-2 (the authors call it 39CD), 42A1-2 (42A), 56F1-9 (56EF), 57A1-4 (57A), 89E1-4 (89EF), and 94A1-4 (93E and 93EF). The two clones are hybridized with the regions 42A1-2, 57A1-4, 89E1-4, 94A1-4, the region 39E1-2 with the clone Dm 225 and with histone mRNA,[159] the region 56F1-9 with the two clones, 5S RNA, and $tRNA^{Glu}$.[160,161] Loci of three tRNAs ($tRNA_3^{Gly}$, $tRNA_2^{Lys}$, $tRNA_3^{Met}$) were found to be located in 56EF region.[277] If the label accumulations in all these cases are really connected with the same bands (there are no accurate data on this point), then at least two or even four (56F1-9) "nests" of repetitions coding for different RNA are localized in each of them. The absence of unequivocal data on the cytological localization of DNA sequences does not allow us to consider still other examples. Clones Dm 225, Dm 234,[162] and Dm 702[163] have been mapped in the region 84DE; $tRNA_{3b}^{Val}$ in the band 84D3-4.[164] In nine other regions — 21F-22B, 25D, 49F, 51CD, 57B, 59AB, 87A, 98A, and 98CD — the clones Dm 225, Dm 234B,[162] and Dm 702[163] have been localized.

The molecular heterogeneity of the bands seems to be witnessed for by the fact that

many bands containing simultaneously highly and moderately repeated sequences have been found in the polytene chromosomes of *Rhynchosciara americana*.[165] In the paper by Ananiev and co-workers,[153] there are other data which give additional evidence for the informational complexity of the intercalar heterochromatin bands: in homologous regions of unpaired chromosomes only one of the homologues contained label after the hybridization of DNA clones although the banding pattern in these homologues seems to be the same. Evidently, such bands must consist not only of DNA complementary to Dm 225, but also of some other sequences. The same was found for the localization of satellite 1H in hybrids of *D. virilis* x *D. a. americana*[166] and that of *copia* sequence in *D. melanogaster*.[167] The asynchronous completion of replication in two unpaired homologues of the 3R-chromosome of *Drosophila*[168] also seems to be accounted for by the unequal amount of late-replicating material in the weak point 95A.

In polytene chromosomes of *D. melanogaster* and *D. simulans* stained with quinacrine, bright fluorescent bands are found mainly in six regions (Table 2). However, in only one of them (101F) was the fluorescence seen in all of the strains studied of the two species. In the other regions, the manifestation of the character varies greatly, and with only one exception (102F in *D. simulans*),[155] the banding pattern appears the same both when there is fluorescence and when there is none.

There are reasons to believe that Q-bands are visualized in chromosome regions enriched with A-T-sequences[174-178] and which do not seem to code for any protein. Thus, one is led to think that such bands contain some poly A-T sequences (repeats) whose number changes considerably (giving an appearance ranging from a bright fluorescence to its complete absence), and some other DNA.

The facts considered in this section allow us to draw a conclusion regarding the existence in the genome of *D. melanogaster* of a large number of bands having a complicated structure due to repetitions, i.e., such bands which contain homologous sequences involved in ectopic conjugation and clusters of sequences for several different poly(A^+)RNA. One may suppose that there are two kinds of complexity in the information content of the intercalary heterochromatin bands: the one due to repeats of one sequence, and the one due to the localization of "nests" of repetitions of different sequences. The variation in the localization of cloned fragments of Dm 225 or A-T sequences in the chromosomes of larvae of different strains whose banding pattern is the same suggest that in such bands there are, besides the repeated genes or A-T sequences which are not always detected, still some other sequences which are always present. A scheme of such a complex band can be built after considering some peculiarities of reiterated DNA replication. Such a phenomenon as underreplication of the centromeric heterochromatin in the process of polytenization is well known.[179] Probably the underreplication of the repeated regions in the intercalar heterochromatin results in the formation of weak points.[151] There exist considerable differences in the DNA amount of various satellites in different tissues of *D. virilis*[180] and *Calliphora erythrocephala*[181] whereby in *D. virilis* a 30% excess of satellites with respect to nuclei of brain cells was found in the ovarium of pupae, which may result from an excessive replication of satellite sequences. There are also considerable interspecific differences in the number of different satellites in the chromocenters in *Drosophila* of the *virilis* group[182] and between *D. melanogaster* and *D. simulans*.[183] The amounts of simple sequences corresponding to satellite DNA in each location in the euchromatin may vary between species of *D. virilis* group, between different stocks of the same species, and even between individuals of the same stock.[166] A significant increase in the amount of A-T material was found in chromosomes of *D. lummei* as a consequence of a decrease of the environmental temperature,[184] in *D. melanogaster* due to the position effect,[185] and during development.[171] Differences in polyteny of certain bands were found in salivary gland

Table 2
VARIATIONS OF LOCATIONS OF Q-BANDS IN THE POLYTENE CHROMOSOMES OF *DROSOPHILA MELANOGASTER* AND *DROSOPHILA SIMULANS*

Species, stocks	Dye	Chromosome regions					
		101F	102B	102D	102F	81F	83E
D. melanogaster, wild type[169]	Quinacrine	+	+	−	+	+[a]	−
D. melanogaster[170]	Sarcolysine-acridine	+	−	+	−	+	−
D. melanogaster, Swb-9[171]	Quinacrine mustard	+	−	+	+	+	−
D. melanogaster, Oregon-R[172]	Quinacrine dihydrochloride	+	−	+	+	+	+[b]
D. melanogaster[173]	Quinacrine *dihydrochloride*	+	+	+	−	—[c]	—[c]
D. simulans[171]	Quinacrine mustard	+	−	+	−	+	−
D. simulans, yw[172]	Quinacrine dihydrochloride	+	−	−	−	−	−
D. simulans, wild-type stocks from Honduras, from Madison, *v* stock[155]	Quinacrine dihydrochloride	+	−	−	+	—[c]	—[c]

[a] Named by the author as 20D.
[b] Only in some individuals of this stock.
[c] No data.

and fat body chromosomes of *D. gibberosa*.[278] Variations in the Q-bands fluorescence have been found as a result of chromosome aberration[171,186] and cell culture.[187] These facts demonstrate the variation of the number of copies within a cluster of repeated sequences both at the haploid level and at the level of polytene chromosomes, i.e., the number of copies in each chromonema of a polytene chromosome may be larger or smaller than in a haploid one. If this property is common to all the repetitions, then one can imagine two homologous bands differing in the number of repetitions (of poly A-T sequences or reiterated sequences) and having some other (stable) sequences. The variation in the reiteration degree of a part of the band might lead to inconsistency of manifestation of such above mentioned properties as *in situ* hybridization, fluorescence, ectopic conjugation, and late replication, while the banding pattern would remain constant.

The facts considered in this section show the existence of a class of informationally complex bands, and besides, they may account for the results of the cytogenetic "saturation" of some regions. Thus, it is not ruled out that a relatively small number of genes discovered at least in the regions 3AB and 3C of the X-chromosome and in chromosome 4 is connected with the localization of some repetitions in these bands (Table 1).

VI. INFORMATIONAL CONTENT OF PUFFS

Beermann was the first to suppose that puffs are sites of local decondensation in the

chromosomes, being a morphological manifestation of genetic activity. It took almost 25 years to prove this hypothesis. The first step was the finding of an enhanced transcription in puffs (review[188]) which perfectly suited the "DNA-RNA-protein" scheme. An important stage was the study of behavior of the puffs in development, which has demonstrated their being regulated by ecdysone.[45,188,189] Correlations have been found between puffing activity and the behavior of some biochemical and physiological characters,[188] and yet, there remained doubts since there were no direct proofs of template RNA synthesis in puffs. These doubts were the basis of some critical statements as to the role of puffs in the control of protein synthesis.[190,191] The breakthrough took place in studies of the so-called "heat-shock" puffs of *Drosophila* which were found to contain genes coding for a special class of heat shock-induced proteins.[192]

At present, the information content of puffs is studied intensely by means of direct genetic and biochemical methods. However, many aspects of the genetic organization of puffs are still obscure. How complicated is this organization? Is the puff a morphological equivalent of the activity of one gene — a transcription unit? It is just these aspects of the problem of the informational content of puffs that will be dealt with in this section.

A. Number of Bands Involved in Puff Formation

The opinions on this question are extremely contradictory, in part a reflection of the diversity of puff morphology.[7,14,34,39,193-200] We shall try to point out several ways of puff formation:

1. A puff is formed from one band, e.g., the puffing in III-$b_3$11 region in *Chironomus thummi thummi* begins with the separation and decondensation of the distal (but sometimes proximal) part of a thick band, the decondensation spreading subsequently to the whole band.[201] Keyl considers that this band represents a sequence of tandem duplications, each of which may be activated independently. In *D. melanogaster,* an example of such a puff formation may be the puff 2B11.[124] For some puffs developing from one band mutant variants have been described.[202]
2. The majority of morphologically well-studied puffs begin their development by the decondensation of one band, but then spread two or three adjacent ones. Examples of this are the Balbiani rings in *Chironomidae*[7,39] and 63E in *D. melanogaster.*[81]
3. Examples of despiralization of a series of neighboring bands during puff forming can be found in many papers.[7,50,194-200] In these cases, electron microscopic studies are especially important as they which permit us to rule out the presence of compact bands separating neighboring puffs. For example, according to Sorsa[64] the puff 85D1-2 — 85D3 in *D. melanogaster* consists at least of two bands, and the puff 85F occupies all the bands between 85E15 and 85F10. Large puffs occupying whole lettered subdivisions in the chromosome map of *D. melanogaster* are formed in 82EF, and 71DE. Beermann[7] has introduced, in this connection, a concept of "the place of origination" ("Entstehungsort") of a puff, which points to the band where the decondensation starts, and of an "activity zone" ("Aktivitätszone") which includes also the bands "secondarily involved" in the puffing.

   One could think that the bands adjacent to the "main" one are decondensed passively due to purely mechanical causes.[50] In that case, it is difficult to understand why some large puffs do not go beyond one band while others, e.g., the giant Balbiani rings, involve only the closest neighbors, and yet others (as indicated above) consist of dozens of bands decondensed to about the same de-

gree. Evidence against passive despiralization also comes from the incorporation of ^{3}H-uridine over the whole region of the many-band puff, even after a 1-min incubation of cells with the precursor.[57] This fact, however, should be interpreted with caution. Thus, in the region of the 2B1-10 puff of *D. melanogaster,* decondensation and label incorporation are found in all bands. However, a cytogenetic analysis has demonstrated that it is only the central bands 2B5-6 that participate in transcription, since a removal of the adjacent thick "doublets" 2B1-2, 2B7-8, 2B3-4, and 2B8-9 by rearrangements does not influence the ^{3}H-uridine incorporation and the puff size.[124] Moreover, the bands of other regions shifted towards 2B5-6 are not involved in puffing. The 2B1-10 region is evidently a reversed repeat,[203] and many ectopic threads arise between the marginal "doublets" within 2B1-10. The ectopic contacts are preserved also at the time of puff activation which leads to the mixing of transcriptionally active and inactive regions, and to a partial decondensation of the latter.[124] Such a passive decondensation, however, can hardly take place in regions of a different organization where the position of bands involved in puffing is not affected by any ectopic contacts.

The decondensation of many bands in the region of the puff and the label over them could be accounted for by the accumulation of the products of puff activity.[50,204] We could not, however, find any redistribution of labeled RNA in the zone of a few large puffs in *D. melanogaster.*[57] This contradiction may be connected with the fact that Berendes' data were obtained using an Actinomycin block, which is known to disturb both transcription and RNA transport.

Cases of the simultaneous decondensation of a few neighboring bands are known. For example, the puff 63BC in *D. melanogaster* reaches its maximum within 5 min after an exposure of cells to 37°C. During this short interval, the puffing of a series of 4-5 bands between 63B1-2 and 63C1-2 takes place. All this region is labeled with ^{3}H-uridine during the first minute of induction when the band borders in the puff are still distinct. The pattern of labeling does not change as the incubation time increases. Most actively labeled is the central part of the puff.[57,60] It is remarkable that in *in situ* hybridization with RNA isolated from the cytoplasm of cells subjected to a heat shock, the distribution of silver grains shows the same pattern[159] although the authors believe that only one or two central bands participate in the hybridization.

The existence of complex multiband puffs is confirmed also by cytogenetic data. Very demonstrative is the independent functioning of two parts of the puff 71DE in *D. melanogaster* which, being separated by a translocation, puffed simultaneously at the corresponding stage of development when the puff 71DE normally is increased.[29] The data on inactivation of one of the bands in the complex puffs II/O and I/D-9 in *Acricotopus lucidus* are also well known.[193] This does not, however, prevent the other bands from being active. On the other hand, in some cases a series of neighboring bands can behave as an integral system controlled by one genetic locus. It has been established that the mutation *sal* in *D. pseudoobscura* switches a group of bands off from active puffing; however, although these are localized in the same chromosome, they are not in the immediate vicinity of the *sal* locus.[205] Similar to this is the behavior of the puff 64C which is active in the *vg-6* strain of *D. melanogaster.* In this case the localization of the mutation inactivating the puff does coincide with the position of the latter.[202] On the basis of photographs, it appears that the puff 64C develops from two thick "doublets" — 64C9-10 and 64C11-12 — although the multilocality of puffing in this case is not certain.

4. Among the multiband puffs, a special place is occupied by regions with a movement of the puff maximum, a classical example of which is the BR4 in *Acricotopus lucidus,* where about 20 bands are involved successively in puffing.[194] Here, evidently a successive activation of independent loci takes place: thus, as salivary glands are transplanted from larvae into pupae, the regression of puffing in the distal zone of BR4 is accompanied by only a partial activation of the proximal bands, which results in a series of nonactive bands appearing between the active zones.[195] However, as a whole, the phenomenon of the movement of the puff maximum is rather rare. Many cases of "movement" of the active zone are better described by the term "puff extension" which can take place in both directions from the puff center.
5. Finally, sometimes a puff is formed by a fusion of two different active zones separated by a nonactive region. For example, the BR1 of *Chironomus tentans* is composed of two parts, BR1a and BR1b; both parts have normal activity, when separated by a translocation.[7]

Thus, already at the morphological level there is evidence of the informational complexity of many active chromosome regions. A confirmation of this may be obtained at genetical and biochemical levels. Below, we are going to consider the available, at present rather fragmentary, data on certain puffs.

B. Molecular Genetic Aspects of Active Regions Organization

The data on the BR-RNA synthesis and metabolism are summed up in a series reviews.[206-208] From the point of view discussed here, the following facts are of principal importance: In BR1 and BR2 of *C. tentans,* giant RNA molecules with a molecular weight of 12×10^6 Daltons (about 37 kb) are synthesized.[209] The 75S RNAs of BR2 and BR1 have different nucleotide sequences.[207,210] These molecules pass, without any obvious processing, to the cytoplasm.[209,211,212] The BR1 in *C. tentans* is composed of two different transcriptional zones. Only one of them participates in hybridization with poly(A^+) RNA from salivary glands.[213]

The question of the number of copies for 75S RNA in BR2-DNA, and the number of bands containing these copies remains obscure.[7,213-216] In any case, irrespective of whether or not the BR2-DNA contains one or a few coding sequences, it must be repeated internally.[207] This may be evidence in favor of (although it does not prove) the polycistronic nature of 75S RNA. According to EM data, each chromatid of both BR1 and BR2 in *C. tentans* contains one active transcription unit about 7.7 μm long.[67,209]

In BR1 and BR2 of *C. thummi* beside the 75S RNA, smaller RNA molecules have been found.[217] The number of bands in these BRs have not been studied in detail; however, it is possible that BR1 in *C. thummi* is as complex as that in *C. tentans.*

Two types of RNA molecules are synthesized in BR1 of *Acricotopus lucidus:* fraction I in the range of 10 to 20 $\times$ 10^6 Daltons, and fraction II — of considerably smaller molecules.[218] It is very impressive that the additional fraction is not synthesized in individuals with an inversion, which includes the BR1 site, and is accompanied with a diminishment of the BR1 size. It is probably a consequence of the inactivation of one of two independent loci within BR1.

The most probable function of BRs, i.e., the control of the synthesis of the salivary gland secretion protein,[214,219,220] has still not been proved. A big progress was achieved in the study of Balbiani Rings: formation of BR2 in *C. tentans* originates from band IV-3B10, the amount of DNA in which is about 470 kb. "Since there are only between one and four 75S RNA genes, 37 kb in size, in BR2 chromomere, most of the BR2 DNA must consist of DNA not coding for 75S RNA."[279] The structure of BR loops, active units

of transcription, was studied in detail.[280] Presence of periodicities and tandem repeats in a BR gene was found.[281] New data support that "75S RNA with an origin in the BR1 and BR2 codes for the giant secretory protein component I".[282] It is a fact that even if in some BRs there is only one transcriptional unit, its size is an order of magnitude larger than that of an average single gene. This circumstance, together with the high intensity of transcription,[14,67] provides for the giant size of BRs.

3C11-12 is the first region in the X-chromosome of *D. melanogaster* for which a direct evidence of the connection of puff development with the synthesis of a definite protein, i.e., the fourth fraction of the salivary gland secretion coded by the locus *Sgs-4* has been obtained. The interval for the *Sgs-4* was restricted by means of deletion mapping to a region between the 3C9-10 and 3D1-2 containing, according to Bridges,[77] two thin bands. In Korge's opinion, there is only one band in this interval, from which the puff is formed.[130,221] Its behavior correlates developmentally with the secretion protein synthesis. Any more precise determination of puff limits is possible only with EM. It is not known whether there are any other sequences in the puff. In any case, there are no genes influencing viability there.[118]

The gene for the third fraction of salivary gland secretion was localized between the 68C8-11 and 68A8-9, i.e., in the region where the puff is formed.[221,222] The hybridization of the RNA from the cytoplasm of the salivary glands labeled at the period of activity of the puff 68C with the region of the puff is clearly multilocal.[223] These data suggest that in this puff there may be other transcribed sequences besides the gene for the fraction 3 of the secretion.

A detailed review[192] is dedicated to this class of puffs discovered by Ritossa.[224] They will be considered here in brief only with respect to their genetic organization.

From the data of deletion mapping,[225] these puffs develop from the bands 87A7 and 87C1, respectively. One has to note, however, that this region, with poorly identifiable thin bands, is rather difficult for deletion mapping, especially at the level of light microscopy.[64] The puffs 87A and 87C contain sequences 2.4 kb long, which code for a heat shock protein of 70,000 Daltons.[226,227] There are at least two 2.4 kb sequences at 87A and three somewhat different ones at 87C.[226,228,229] The puff 87C, besides, contains a repeated sequence of 1.5 kb[226] (the $\alpha\beta$ segment).[163] The transcripts of these latter segments (fraction A-4 of hs RNA) are present in the cytoplasm, but no corresponding protein was found.[226] In both 87A and 87C puffs there is still another transcribable sequence of an obscure significance, i.e., 0.8 kb segment.[226] Finally, a spacer of about 7.4 kb which hybridizes to 87A, the chromocenter, and to 30 other chromosome regions unrelated to the hs puffs has been found in a clone containing $\alpha\beta$ segments.[163] The presence in puffs of such a sequence, widely represented, in the genome has also been found by Schedl and co-workers.[230] There are no less than three such spacers in 87C. The puff 87C seems to be complex in the informational sense; it is so, at least with respect to the multiplicity of its sequences. Probably, it was just this circumstance that has prevented the isolation of any mutations in the regions of 87A and 87C.[135]

The morphology of this puff was described above as an example of a puff formed as a result of simultaneous decondensation (as can be evaluated visually) of several bands. The puff is hybridized with the heaviest hs RNA fraction A-1,[231] which agrees well with the data on the hybridization of this region with an RNA that, transcribed in a cell-free system, gives an 83,000 Daltons protein, i.e., the largest of hs proteins.[232,233]

In this puff in *D. melanogaster,* the A-3 fraction of hs RNA corresponding to a message for the 68,000 Daltons hs protein is synthesized.[226,232,233] The DNA complementary to this RNA is present in the bands 95D10, D11 (or even 95E1), which are certainly situated more proximally than the puff maximum, virtually at its base.[159] It seems that bands are active in the puff which contains other sequences not involved in the hybridization with the cytoplasmic RNA of heat-shocked cells.

The *93D* puff is not hybridized specifically with any of the hs-poly(A^+) RNA but to poly(A^-) RNA and the total RNA of imaginal discs labeled during the shock are clearly complementary to the DNA of two adjacent bands, and it is just these (93D3 and 93D5) that form the puff.[159,234] The protein coded by this region is not known. The *67B* puff is hybridized with the A-5 fraction of hs RNA corresponding to the 23,000 and 26,000 Daltons hs proteins.[232] Recently, electrophoretic variants of 23,000, 27,000, and 28,000 hs proteins have been mapped. "They all map to the region of the chromosome which contains only one hs puff" — 67B.[235,236] The puff 67B seems to arise from band 67B1-2; the participation of neighboring bands is very probable, but not evident.

Concluding the description of the hs-puffs of *D. melanogaster,* one should point to the fact that all these regions (except the 63BC) are considerably activated at different stages of development.[29] As for 63BC, it shows significant incorporation of ^{3}H-uridine without the typical swelling.[54-56] It still remains obscure, what functions they perform at this time.

New data on the *hs* puffs follow. The 87A locus has two genes for *hsp* 70000 in a divergent orientation. The 87C locus contains three *hsp* 70000 genes: two on the distal end and one on the proximal end with opposite orientation. The DNA fragment 38 kb long is located between them. It consists of repeated sequences; their transcription is activated by heat shock. The 87C locus extends for approximately 55 kb, about twice an "average band". These data demonstrate the presence of several independently transcribed sequences inside a band.[283]

Complex informational content of a puff was strictly shown for *hs* puff 67B where genes for four smaller *hsp* were found.[284-286] It is rather evident that all four *hsp* genes should occupy the same band since they are located within a 12-kb region at chromosome; they are not transcribed in the same direction.

The *2-48BC* puff of *D. hydei* is induced by high temperature, vitamin B6, etc.[196] During the first 5 min of shock, the bands B4, B5, B6 and to a smaller extent C1 and C2, are decondensed. In 10 min from the beginning of induction, in the region of all these bands, granules of 300 to 400 Å appear, and in the region of B4 and B5, the formation of large aggregates of granules begins which are a specific product of this puff. In 30 min from the beginning of the shock, these aggregates are found over the whole region of the puff. Thus, "all attempts to define the origin of a puff in terms of the decondensation of the particular band have so far failed in *D. hydei*."[45] After 1 min pulse, the incorporation of ^{3}H-uridine gave at first a pattern known as a "single transverse array of grains".[204] This pattern was a reason to think that the transcription in a complex puff is limited to DNA of one band. However, repeated experiments on puffs with vitamin B6 have demonstrated "two or even three transverse arrays or label distributed over a broad area", i.e., over the whole puff.[196] On the basis of this, Berendes and colleagues hypothesized that in 2-48BC different RNAs both with respect to their location and "their information content" can be synthesized.[196] This hypothesis has been confirmed to a certain extent: in the 2-48BC puff isolated by means of a microdissection, two fractions, 40S and 16S RNA, are found which are also found in the nuclear sap.[237,238] In addition, a small 3-4S RNA from salivary glands is complementary to the same sites within the puff;[196] these sequences seeming thereby to be repeated. It is not ruled out, however, that the 3-4S RNA is a product of RNA degradation.[238]

Apparently homologous to 2-48BC, *D. virilis* is the puff 20CD whose interesting peculiarity, distinguishing it from the rest of hs puffs, is the absence of regression in a long-term temperature shock.[239]

The *2B5-6* puff of *D. melanogaster* is so far the only one whose information content is being studied by the method of "saturation" with visible and lethal mutations.[123,240] The puff 2B5-6 is a typical early 20-OH ecdysone puff in the sense that its activity is sharply increased immediately after the contact of cells with 20-OH ecdysone.[29] It was possible to

localize, in the region where this puff develops, a series of ethyl methanesulfonate-induced and earlier known mutations (*br, dor*) which are divided into six complementation groups. Two of them are independent, and the rest overlap with a series of noncomplementary lethals. The complicated complementation pattern of these last groups can be accounted for by the existence of interallelic complementation within one locus, or of series of cistrons united by a common regulatory zone in an operon-like system.

Considering the organization of active chromosome regions, one should not leave out such regions as the histone locus, the 5S rRNA, and tRNA genes.

The ^{3}H-9S RNA of sea urchin which contains information about all or most histone types hybridized only with region 39DE of polytene chromosomes of *D. melanogaster*.[159] Complexity of the transcriptional organization of chromomere is supported by new data about histone genes,[287,288] tRNA genes,[289-293] and genes for rRNA.[294] The polylocality of labeling, i.e., participation of many neighboring bands in hybridization, is beyond any doubt. There is nothing surprising about it, since the total length of the histone genes amounts to about 500 kb (100 gene sets with the tandem length of about 5 kb).[241] In *Drosophila,* unlike in sea urchin, the five histone genes do not function as a single transcriptional unit. Histone gene repeat units are interrupted by sequences, which are not homologous to histone DNA. In the region 39DE, there is a small permanent puff both in the larvae when DNA replication and histone synthesis takes place, and in prepupae when these processes do not occur. Thus, the activity of histone genes is not manifested distinctly in puffing, and one cannot rule out that the zones of their active transcription are interbands. On the other hand, histone genes demonstrate another important feature of organization: in this case the band is neither a genetic nor a transcription unit. These peculiarities of organization of the histone region are also characteristic of other regions with repeated genes, 5S ribosomal and tRNA.

The *5S* rRNA genes in *D. melanogaster* are represented by a single block of total length of about 60 kb, consisting of about 160 identical units of about 370 nucleotides each.[242-244] This gene cluster has been localized genetically[245,246] and by means of *in situ* hybridization[244,247] in the 56EF region. The labeling region after hybridization consists of four compact bands 56F1-9 and possibly the thin 56E bands adjacent to it. Judging by the cluster length, it must occupy no less than two bands. The thick 56F1-9 bands do not show any signs of transcription,[56] however, it is possible that the transcriptionally active part of the genes is adjacent to them in the thin decondensed bands of 56E region. Single genes or gene groups within a cluster are transcribed independently; a deletion of a part of a cluster does not abolish the activity of the rest.[246] A single repeated unit of 5S DNA is transcribed into 5S RNA in oocyte system.[248]

In *C. thummi* the ^{125}I-5S RNA hybridizes with the B3c-e region consisting of two thick and two thin bands.[249]

In *C. tentans* using ^{3}H-labeled electrophoretically isolated RNA from salivary glands, Wieslander[250] established that the region of localization of 5S genes, 2A, is composed of two distinctly discretely labeled bands. The localization of the 5S genes in other *Dipteran* species can be found in References 251 to 255.

In a haploid genome of *D. melanogaster* there are 600 to 800 t-RNA genes; therefore, there is an average of about 10 genes for each t-RNA sequence.[255] It is only the use of highly purified individual t-RNA species that has allowed the localization of some of the genes in *D. melanogaster*.[256-259] It is interesting that isoacceptor types of t-RNA genes can be situated in different regions, and vice versa, different types in one region. Unfortunately, the localization is not always determined accurately with respect to individual bands. Nevertheless, there are examples of one class of t-RNA genes being located in a number of adjacent bands.

A common property of the regions of histone, 5S− and t-RNA genes, is the frequent

distribution of these genes in a series of adjacent bands. These bands may be compact, with a very low level of ^{3}H-uridine incorporation, or they may show some degree of puffing, the puffs being either permanent or varying during development. All these facts together suggest that the activity of these classes of genes is not necessarily manifested at the morphological level in the form of puffing.

What is the cause of the absence of a manifest connection between the functioning of such genes and puffing? Possibly, this is accounted for by the small number of active copies within single gene clusters, and this situation is aggravated by the small sizes of the synthesized RNA; it is not excluded that their activity is controlled also at the level of individual chromomeres within the band (not all homologous chromomeres are active). The latter circumstance would involve a specific level of regulation which is possible only in polytene chromosomes.

The analysis of the organization of active sites of chromosomes is still far from complete. The available evidence in this field underlines the complexity of the problem rather than solving it. However, it is becoming more evident that the "1 band (1 gene) — 1 puff — 1 transcript" scheme is too simple; in any case, it is not applicable to all the puffs. The existence of multigene bands with independent transcription of single genes within them is demonstrated by the regions of the 5S RNA genes and the histone genes. The same conclusion has followed already from the data on the multiplicity of transcription units in nucleolus organizers[260] (see also Panitz[73]).

On the other hand, we have the problem of the existence of puffs which are complex at the morphological level, and formed by a series of bands. At present we do not know of any cases in which it has been unequivocally established that the formation of a large puff is caused by the activity of a single gene of normal size. No doubt, there exist active regions in the chromosomes where RNA is transcribed from a single sequence; but the question arises as to whether this activity does find its expression at the level of formation of large typical puffs. Judging by the size of the BR2 transcript (37 kb, 7.7 μm) one can conclude that the length of an average transcribable sequence in *D. melanogaster* (6 kb according to Lengyel and Penman[69]) is equal to about 1 μm at the same degree of decondensation as in the loops of BR2; in other words, the loop of an average unique gene is by an order of magnitude. However, one has to bear in mind that in the puffs, the degree of decondensation is never as high as in a BR;[45,64] the same is true concerning the density of RNA polymerases.[14,67] It is possible that in puffs some of the homologous chromomers are not active at all.[64] This suggests that an average unique gene may be active although there is only a slight decondensation of the band in which it is contained or, it may have the appearance of an interband. One gets an impression that large puffs of the genome are complexes of active genes with many transcriptionally active sequences:

1. These may be numerous copies of one gene situated in one or a few neighboring bands.
2. The puffing bands may contain different sequences transcribed polycistronically or independently, but participating in one metabolic pathway and activated by a common inducer.
3. A puff may arise due to the activity of several different genes controlling unrelated processes, but situated close to each other and activated by a common signal. In both latter cases the absence of the inducer due to a single mutation may switch off a series of genes in neighboring bands.
4. Finally, a common puff can be formed by some simultaneously but independently activated genes. Such a common active zone may arise from two different active regions separated by inactive material, as in the case of BR1 in *C. tentans* or of a combination of BR7 and nucleolus in *Acricotopus lucidus*.[261] A successive

activation of neighboring bands leads to the phenomenon of "puff maximum movement" or to the extension of the puffing to adjacent regions.

Stressing the complexity of the genetic organization of prominent puffs, we are, however, far from the extreme view that the whole decondensed puff material is transcriptionally active, and we believe that in some cases a passive despiralization takes place.

The concepts regarding the genetic complexity (or multiplicity) of prominent puffs developed here are, in many aspects, similar to the views of Burnet and Hartmann-Goldstein[198] and Ashburner.[262]

VII. A MODEL OF DYNAMIC ORGANIZATION OF POLYTENE CHROMOSOMES

The data presented in Sections II to VI are evidence for the importance of taking into account the following facts and ideas, while discussing the problems of organization of polytene chromosomes.

1. At all levels of chromosome organization, the process of chromatin decondensation correlates, in one way or another, with transcriptional activity. In the interphase nucleus, ^{3}H-uridine incorporation mainly takes place in the diffuse chromatin regions.[263] If a chromosome fragment is transferred to heterochromatin, the genes localized in the former are repressed, and the chromosome fragment is transformed into a compact, condensed chromatin block with a low level of ^{3}H-uridine incorporation.[264,265] In a similar way, the two alternative states, in the sense of DNP condensation (a compact band, and the puff formed from it), reflect the minimum and the maximum transcriptional levels respectively. The interbands whose material seems to be decondensed in the same degree as the puff material should also be considered as minor puffs. An intermediate position is occupied by diffused grey bands.
2. The banding patterns are only relatively constant, i.e., in normally functioning cells with a background of considerable stability, one can find some cases of variation: elongation of the interbands and the splitting of bands. The range of variation increases as the cell physiology varies both in normal development (different ecological conditions, cell specialization) and in some pathological conditions (prolonged incubations, mutations, infections). The main trend of alterations in these cases is the condensation of interband material, and, as a consequence, band fusion and shortening of the chromosome. This point contradicts the majority of models described in Section I.
3. A band does not seem to be a "lair" of one gene. By means of cytogenetic data it has been proved that in the band 10A1-2 there are a few functionally unrelated cistrons and DNA sequences. The bands of the intercalary heterochromatin are functionally complex since they can include different families of cistrons which are repeated several times. It is logical to consider that an activation of transcription of such a family of repetitions will result in the formation of a large puff while the activate a single locus in the band 10A1-2 will result in the splitting of this band and the formation of a new interband (micropuff).
4. So far, it has not been demonstrated that in the activation of a band (puff formation) only one transcript is synthesized on its template. Moreover, the examples of the 5S RNA genes and of the histone genes witness for the absence of a 1 band:1 transcript correspondence.

5. The number of essential loci in those chromosome regions studied extensively corresponds to (or is somewhat larger than) the number of bands/interbands in the region. The fact that a considerable part of the lethals are cell lethals implies that these loci are active in many cells, i.e., the respective chromosome regions must be decondensed in many cells. Both facts suggest that at least some of the essential loci are situated in the interbands.
6. It seems reasonable to distinguish two types of genes according to their involvement in differentiation: those which function permanently and provide for the general cellular functions (respiration, oxidative phosphorylation, replication, transcription, translation etc.) and the tissue- and stage-specific genes which do not function in all cell types. Recently, a new hypothesis for the possible functional value of "constitutive transcription units" was proposed by Davidson and Britten.[266]

We think that these statements clearly describe the consideration contained in the model of dynamic organization of polytene chromosome proposed by Zhimulev and Belyaeva.[70] We hypothesize that in the chromosomes there are two types of regions, i.e., those permanently transcribed which provide for the housekeeping of the cell functions, and those transcribed only at certain stages of development in certain tissues, i.e., those providing for differentiation, or else not transcribed at all (as, probably, the "silent" zone in the band 10A1-2 (Figure 2a). It is evident that if regions are permanently active, they must be permanently decondensed. These characteristics coincide with those of interbands. One may expect that mutations of such genes must display a lethal effect. Temporarily active regions are not functioning, in most cells; they are condensed and form the bands (Figure 2a). The mutations of such genes either are not manifested in the phenotype due to the repetition of their respective cistrons, or display a more limited effect as compared to the lethals, since these genes are manifested only in certain tissues. Depending on the number of genes (or more exactly, on the amount of inactive DNA between two active interbands) the band size varies. A band in this model is considered not as a structural and functional unit of the polytene chromosome, but as a fragment of temporarily or constantly inactive DNA. Thus, the pattern of the bands of a polytene chromosome, i.e., the pattern of alternation of condensed and decondensed and, consequently, transcriptionally differently active regions, corresponds to a definite functional state of the cell. The stability of the main metabolic processes in cells is reflected in the stability of the banding pattern. If the type of cell activity changes, one of the following events can take place: activation of the whole (or of the majority) band material which brings about the decondensation of the band and the formation of a puff; or the activation of a part of the material which leads to the splitting of the band (formation of a micropuff) or the elongation of the interband (Figure 2b). Considerable changes in the functional state can produce considerable alterations of the banding pattern by means of the same universal mechanism — condensation of the chromosome material with simultaneous inactivation of transcription (Figure 2c). Note that we are speaking only about the correlation between decondensation and transcription without considering all the possible levels of control of RNA synthesis, and we do not suppose an absolutely strict quantitative correspondence between the degree of transcription and the decondensation level. One should also keep in mind that while the hypothesis of the transcriptional activity of the interbands is sufficiently documented with facts, the hypothesis of the localization of essential genes in them is but an alluring speculation, and even at this level it requires a reserve; it is evident that not all interbands have essential functions, and not all bands are deprived of them.

This model has a number of features in common with the models which Kosswig and

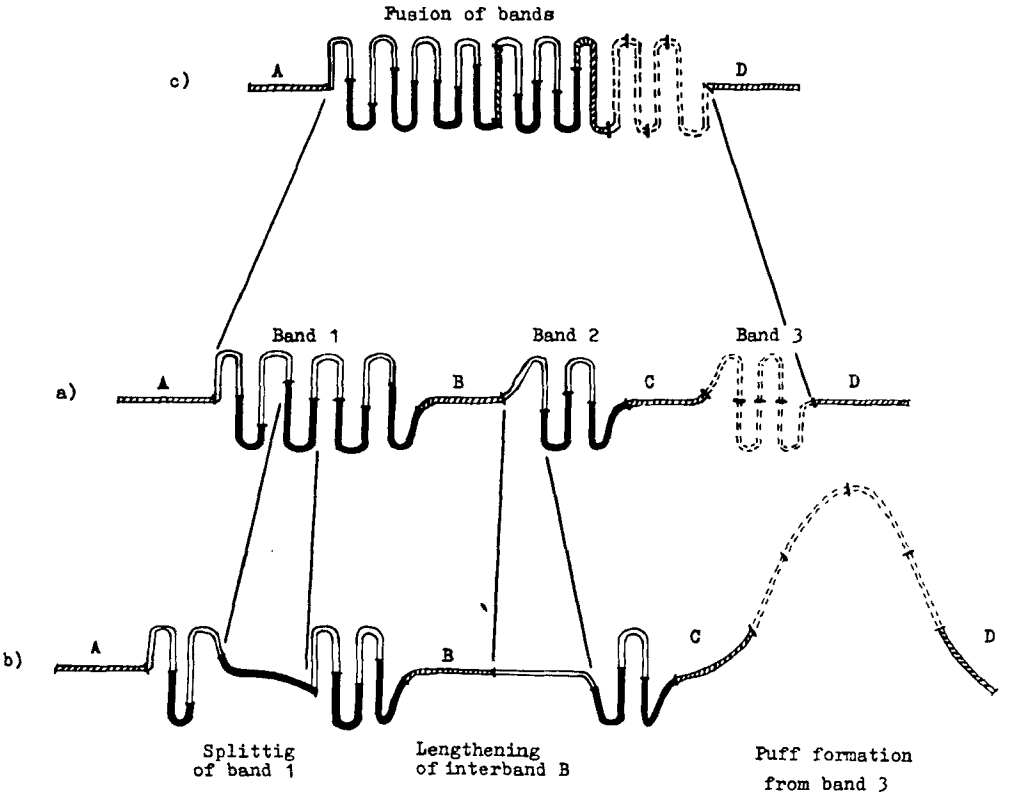

FIGURE 2. Schematic representation of the model of dynamic organization of polytene chromosomes. (a) The initial banding pattern. A, B, C, D — constantly transcribed sequences — interbands. Bands 1, 2, and 3 — series of inactive stage- or tissue-specific genes. Sequences are activated independently (in the bands 1 and 2) or under common control (band 3). (b) Changes of banding pattern caused by the activation of the sequences: splitting of the band, lengthening of the interband, and puff formation. (c) Inactivation of the interbands results in fusion of bands and interbands into a new block of chromatin.

Sengün,[30] Fujita,[31] Akifyev,[25] Speicer,[33] and Gersh[32] proposed for polytene chromosomes. At present, the organization of other chromosome types is also considered from this point of view. The idea of the dynamic nature of chromomeres, especially in mitotic and meiotic chromosomes, is discussed at length in the paper of Lima de Faria.[267] Quite recently, Callan came to the conclusion that "the chromomeres of lampbrush chromosomes far from being themselves functional units, are passively generated by the transcriptional activity of interspersed functional units".[268]

In conclusion, it is necessary to say that in this review we are not considering the aspects of the structural organization of a band, the molecular organization of the genome, or genetic fine structure, etc. In spite of recent advances, there are no new data that allow us to better interpret the functional significance of the banding organization of the chromosome. The role and the distribution of different types of sequences (unique and repeated) with respect to the structural subunits of the chromosome are obscure, although according to a witty observation of Lima de Faria,[267] "it is obvious that there is a 95% chance that any property found in the chromosome will be located in a chromomere". In our review which is rather limited, and naturally, largely subjective, we have given ourselves one aim, i.e., to shatter the widespread idea of a chromomere and a puff as a structural, genetic, and molecular unit of the polytene chromosome.

ACKNOWLEDGMENTS

We thank Drs. M. Ashburner, V. A. Berdnikov, M. D. Golubovsky, I. Kiss, and G. Richards for reading and criticizing the manuscript; Prof. Dr. A Chovnick, Drs. J. Gausz C. P. F. Redfern, and D. Ribbert for supplying us with unpublished data; and V. E. Grafodatskaya for help in this work.

We also thank G. Richards for help in preparing the English version of this manuscript.

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