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Proposals to the Problem of Structural and Functional Organization of Polytene Chromosomes

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Summary. The concept of the structural and functional organization of polytene chromosomes is formulated. It is based on the following:

Interbands are transcriptional active chromosome regions; they seem to be responsible for the base metabolism of the cell. The stability of these metabolic processes in the cells of different organs results in constancy of chromosome banding pattern. Thus, each functional state of the cell possesses its own banding pattern which may vary depending on activity level and degree of condensation of interbands.

The band is considered not as a structural or functional unit, but as a region of the chromosome not involved in transcription at a certain point of time; a band may contain one or several genes.

Introduction

Giant polytene chromosomes of *Dipterae* have a typical band pattern produced by the alternation of condensed parts (chromomeres or bands) and decondensed ones (interchromomeres or interbands). The function of these structures is widely discussed at present.

It is assumed that the band is structural unit, information unit and unit of replication and transcription (Beermann, 1965, 1972; Pelling, 1966; Kiknadze, 1967, 1972). Interbands in these models either are not taken into account or are considered as sites delimiting adjacent genes and having no essential genetic function. Some authors consider the complex band plus interband as a complementation unit (Judd *et al.*, 1972; Sorsa *et al.*, 1973). According to Paul (1972), the interband contains promotor and address-loci of the gene situated in the adjacent band. In contrast, Crick (1971) supposed that structural genes for proteins are located generally in interbands and bands are the sites of regulatory elements.

All these models have the common idea that the banding pattern is quite stable and can be distorted only by puffing. If so, the number of functional units in the chromosome is equal to that of bands or interbands.

Study of the functional morphology of *Drosophila melanogaster* salivary gland chromosomes has led us to a quite different idea about their organization. It is based on analysis of three groups of facts.

1. Interband as transcriptionally active Chromosome Region

Autoradiographical study of RNA synthesis in polytene chromosomes has demonstrated that labelled precursors are incorporated not only into typical puffs (Fujita and Takamoto, 1963; Berendes, 1965; Kiknadze, 1965; Mukherjee, 1966; Korge, 1970; Zhimulev and Lychev, 1972) but into other chromosome regions as well, except apparently very compact bands. Even when puffing activity is maximal (0-hour prepupae), about 50% of RNA synthesized in chromosomes of *D. melanogaster* is accounted for in the regions without visible thickening of chromosome diameter (Zhimulev and Belyaeva, 1974). Morphological study of such RNA-synthesizing sites has led us to the conclusion that all the chromosome regions decondense to some extent, namely puffs, diffused bands ("grey" and "dotted" on Bridges' map) and interbands, are active in the transcription. We consider diffused bands and interbands to be small puffs. Diffused bands seem to be the regions where only some part of the DNA is active in RNA synthesis and interbands are apparently small pieces of actively transcribed DNA. In this sense, the interband can be looked upon as a very small puff.

Unfortunately light autoradiography resolution does not allow this conclusion to be properly proved. Electron microscopic data on RNA, RNP-granule localization and ^{3}H-uridine incorporation into interbands (Berendes, 1970; Perov and Chentsov, 1971; Vazquez-Nin and Bernhard, 1971; Gersh and Gersh, 1973) does not provide conclusive evidence since the possible existence of "minibands" was not taken into account by these authors.

The following facts do speak in favour of the transcriptional activity of interbands. Development of prominent puffs is stage-specific and is stimulated by ecdysone (Ashburner, 1972, 1974; Belyaeva *et al.*, 1974; Zhimulev, 1974). Sharp magnification of puffing activity during puparium formation thus runs parallel to drastic changes in the functioning of salivary gland cells and may result in specific

processes of mucoprotein secretion (Poels, 1970, 1972) and preparing for histolysis. It is necessary to emphasize that there are less than 10 prominent puffs in *D. melanogaster* before ecdysone secretion (110 hour stage) (Ashburner, 1972). These puffs obviously can not guarantee all processes of basic cell metabolism. As for small puffs (including interbands), their morphology and RNA synthesis intensity are quite stable in the course of development (Zhimulev and Lychev, 1972; Zhimulev and Belyaeva, 1974: Belyaeva *et al.*, 1974; Zhimulev, 1974). This has led us to suggest that small puffs are the regions with constant transcriptional activity which provide functions common for all cells. The same idea about interband involvement in transcription has been discussed earlier (Fujita, 1965; Speiser, 1974). Probably this stability of basic metabolic processes specifies the relative stability of the chromosome banding pattern in different tissues (Berger, 1940; Beermann, 1950, 1952; Pavan and Breuer, 1952). Though interband sizes are very small (on average about 1500 nucleotide pairs), they are sufficient to code for 30,000 molecular weight protein (Crick, 1971).

Summarising all these considerations, one can argue that though direct evidence in favour of interband transcriptional activity is absent at present, none of the available facts contradicts the assumption that the interband is active in RNA synthesis. This assumption is quite justified from the standpoint of general regularities of chromosome functioning (decondensation of active regions, Koltzoff, 1934).

2. Band is not quite constant Structure

Allowing for interband transcriptional activity, we must assume that in the case of inactivation of the transcriptional processes, the interband material will be condensed and adjacent bands will be united into the new single one.

Actually, the analysis of investigations dealing with chromosome banding pattern in various larval tissues has shown the relativity of its constancy. Only 70% of all bands can be identified comparing chromosome maps of various tissues of *Chironomus tentans* (Beermann, 1952, 1962). There are differences in the band number, (although, the great majority of inconsistencies concerns only the thin bands), width and the distance between them (Beermann, 1962; Berendes, 1966). In some cases it is possible to obverse several bands in the Malpighian tubule chromosome instead of one in the same region in the salivary gland chromosome (e.g. region 13 in AR chromosome of *Rhynchosciara angelae*, Pavan and Breuer, 1952, fig. 1).

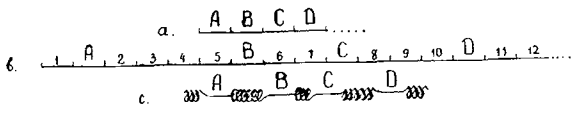

Fig. 1. Probable way of the formation of banded chromosome structure

a. eukaryote ancestor having decondensed chromosome whose assortment of genes provides basic metabolic processes in nondifferentiated cell (A, B, C, D)
b. appearance of new genes in more complicated multicellular organism (1—12), providing tissue- and stage-specificity
c. inactivation of new genes and the formation of bands

Though a detailed description of such tissues was not made, a comparison of photographs of *Calliphora erythrocephala* chromosomes from bristle-forming cells and oocyte nurse cells shows considerable variability both in the chromosome length and apparently the band number (Bier, 1960; Ribbert, 1972).

Contraction and shortening of chromosomes resulting from drastic interband shortening occur in the course of the normal development of *Dasyneura crataegi* larvae in the cells of salivary gland section, whose functions are changed (Henderson, 1967), and in various sections of Malpighian tubules of *Rhynchosciara angelae* too (Pavan, 1965).

Changes in chromosome banding patterns are particularly remarkable when normal cell metabolism is disturbed, for instance, when dipteran larvae are infected with viruses and microsporidia (Diaz and Pavan, 1965; Pavan *et al.*, 1969), at the salivary gland transplantation into adult abdomen (Hadorn *et al.*, 1963; Staub, 1969; Ashburner and Garcia-Bellido, 1973), when salivary glands are incubated for a long time *in vitro* (Simoes and Cestary, 1969) and in the cells of $l_{(3)}tl$ mutant of *Drosophila melanogaster* (Zhimulev *et al.*, 1974).

In all these cases chromosomes are strongly shortened due to the fusion of many bands and interbands into massive blocks of compact material. It is conceivable that this change of banding pattern is accounted for by changing the number and activity of RNA-synthesizing regions: inactivation of the transcription processes leads to DNP condensation in the earlier active regions (puffs, interbands) resulting in fusion of adjacent bands. Shortened chromosomes thus represent the alternation of transcriptionally active regions and large inactive blocks. The latter seem to contain a lot of genes.

All the cases mentioned above, thus, do not allow us to consider the band as a quite stable morphological structure of polytene chromosome. Banding pattern seems to be created by alternation of the regions both active (interbands, puffs) and inactive (bands) in transcription. Since the assortment of active loci in different tissues is different to some extent, banding patterns of their chromosomes are adequately changed; in other words we can speculate that banding pattern represents functional activity of chromosomes in a given tissue.

3. There can be more than one Gene in one Band

If the thesis about transcriptional activity of interbands and probable fusion of bands at interband inactivation is accepted, we can conclude that there

will be more than one gene in a band resulting from fusion of several. Apparently the number of genes in one band in a "normal" chromosome can vary and is dependent upon chromosome formation in the course of evolution, i.e. upon the distribution of "constantly active" and "stage specific" genes along chromosome length. We may think of the eukaryote ancestor having an assortment of genes providing basic metabolic processes in the nondifferentiated cell (A, B, C, D, fig. 1a). When new genes (1—12 in fig. 1 b) providing organ- and stage-specificity appeared the system of their inactivation has formed. This system in some way or other had been associated with condensation of chromosome material i.e. with chromomere formation (figs. 1b, c). If the distribution of new genes among constantly active ones was not regular then the number of genes in the chromomere might vary. Concepts about genetical complication of the band and transcriptional activity of the interband allow us to interpret a number of facts: formation of puff from the part of band (fig. 2a—b), moving of puff maximum (figs. 2a, c, d, e), elongation of interband (fig. 3), formation of one band from several (figs. 4a, c, d) and the reverse process (figs. 4a, b).

This interpretation of structural chromosome organization conflicts with the widespread opinion that the chromomere (band) is the unit of genetic information. Analysis of data in this area is of particular importance. The experiments on saturation of X-chromosome regions (Judd *et al.*, 1972) and the whole 4th chromosome of *D. melanogaster* (Hochman *et al.*, 1964; Hochman, 1971, 1973) with lethals and visible morphological mutations, followed by complementation tests have shown that the number of complementation groups is correlated with band number. Discussing these facts, however, we have to take into account the following.

1. The technique of recognition of lethals and morphological mutations permits to detect only vital genes or those for morphological features. "Meanwhile, in *Drosophila* over 30 genes have a known gene product, of which there are 14 at which "null" alleles eliminate the protein, its activity, or the RNA product entirely, and of these only the *bobbed* locus has lethal alleles and 5 other loci exhibit morphological phenotype" (O'Brien, 1973). Obviously the study of lethal and visible mutations does not permit the recovery of all genes.

2. The study mentioned above (Judd *et al.*, 1972) was carried out on the chromosome part (3AB) having mostly very thin bands, in which the relationship 1 gene: 1 band is more probable than in thick ones. According to Lefevre (1971) there may be but one genetic function ("vermilionness" and lethality) associated with the thick band 10A1—2; this band is not actually double.

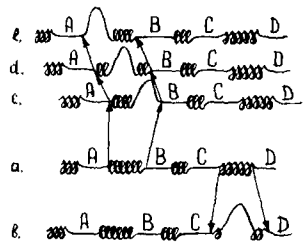

Fig. 2

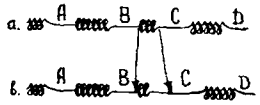

Fig. 3

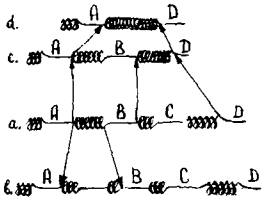

Fig. 4

Figs. 2—4. Interpretation of some known phenomena from the point of view possibility of transcription in interband and presence of more than one gene in band
2a, 3a, 4a — the part of chromosome from differentiated cell with the definite banding pattern (see fig. 1)

Fig. 2a. The formation of puff from part of band — only some of genes in band are activated; Fig. 2c, d, e. Movement of "puff maximum" inside one band: successive activation of various genes in band; Fig. 3b. Elongation of interband — activation of gene, situated on the edge of band; Fig. 4b. The formation of two bands from the one during the activation of gene in the middle of inactive gene complex; Fig. 4c—d. The formation of "new" band when genes in interbands are inactivated

Data on the complementation groups of the 4th chromosome are difficult to discuss since the number of bands has not been precisely distinguished: 50 bands according to Bridges (1935) (in Lindsley, Grell, 1968) and 137 bands according to Slizynski (1944).

The DNA amount per haploid genome of *D. melanogaster* is known to be 0.18 pg (Rasch *et al.*, 1971; Rudkin, 1972) or about 1.8×10^8 pairs. The size of the structural gene is assumed to be 1000 nucleotides, then 0.18 pg of DNA can possess information about the structure of 1.8×10^5 protein molecules. If even 90% of DNA possesses no genetic information, the remaining 10% is sufficient for 18,000 genes, or 14,000 bearing in mind that unique DNA accounts for no more than 85% (Wu *et al.*, 1972) of the haploid genome. Similar estimations for genome size in *D. melanogaster* were obtained by other authors (Laird and McCarthy, 1969; Golubovsky, 1972). These estimations, however, seem to be minimal. It is known that the recombination size of the *D. melanogaster* genome is about 280 map units (Golubovsky, 1972). The map unit thus accounts for $\frac{1.8 \times 10^8}{280} = 6.4 \times 10^5$ nucleotide pairs. Recombination sizes of genes with known end product, such as *v* (*vermilion*) and *ry* (*rosy*), were determined as 5×10^{-3} and 9×10^{-3} map units, respectively (Green, 1954; Schalet *et al.*, 1964), that is equal to 3.2×10^3 and

5.8×10^3 nucleotide pairs. Recombination analysis seems to take into account both structural and regulatory locus parts. One can conclude from this that the haploid genome of *Drosophila melanogaster* may contain 30—50 thousands of genes of such size. Recently it was shown that more than 30% of unique-sequence *D. melanogaster* genome (24% of the total genome) is represented in RNA transcripts at the pupal stage (Turner and Laird, 1973). It means that only in pupae there are 23,000 transcripts with a mean size of 1000 nucleotides. Estimation of the gene number in *Drosophila* is difficult nevertheless because of the lack of data on the size of m-RNA precursor. More concrete calculations can be made for *Dictyostelium discoideum*. It is known that unique sequences in its genome are represented by 3×10^7 nucleotide pairs (Firtel and Bonner, 1972) and the unique part of the m-RNA precursor consists of 1000—1100 nucleotides (Firtel and Lodish, 1973). The number of genes in *Dictyostelium* can thus equal 30,000. It is necessary to emphasize that 56% of unique DNA in *D. discoideum* is transcribed during the 26-hour development cycle. This means that, in this period, 16—17,000 transcripts of unique sequences of 1000 nucleotides (Firtel, 1972) are functioning in the cell. It is of interest that about 20% of unique DNA (or about 5,700 genes) is represented in RNA existing at all developmental stages. Many of these genes are suggested to relate to "housekeeping" functions (Firtel, 1972). The number of such „house-keeping" genes in *D. discoideum* coincides with the number of interbands in *D. melanogaster*.

There are sufficient grounds for believing that the number of genes in *Drosophila* is not less than 20,000—30,000. This value is one order of magnitude more than the number of bands in *Drosophila melanogaster* polytene chromosomes: according to light and electron microscopic data there are not more than, 5000 of them (Bridges, cited in Lindsley and Grell 1968; Sorsa, 1969; Berendes, 1970; Sorsa and Sorsa, 1973).

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