Bands & Condensed Chromatin as Sites of Transcription in Polyene Chromosomes of Drosophila

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The question of heterogeneous DNA of bands and other condensed chromatin masses in transcription in polyene chromosomes of salivary glands of Drosophila has been analyzed by electron microscopy and autoradiography (in light and electron microscope level) following a 4 hr in situ pulse labeling with H-uridine. Ultrastructural studies show that many of the thick bands have regions of high electron density within their 3-dimensional structure. Tightly packed chromatin granules are seen to be specifically associated with the less electron dense regions (grainlessness) of several thick and thin bands and also with the polytenization around the condensed masses in beta heterochromatin of chromosomes. Autoradiographic studies show that certain bands and the condensed masses of beta heterochromatin incorporate into significant levels. Majority of the interbands are not labeled with H-uridine. It is suggested that in polyene chromosomes, bands and other condensed chromatin masses (like the beta heterochromatin) may become transcriptionally active without apparent decondensation. Most of the interbands probably were not transcribed.

The distinct band-interband organization of polyene chromosomes has evolved interest in their genetic correlates. The one to one relationship of the number of bands (and interbands) with the number of genetically determined complementation groups in a given chromosome segment has been implied to suggest that a polyene chromosome band with its associated interband constitutes a unit of function. One question which has repeatedly been discussed in this context is the localization of the informational part of a genetic unit in the band or the interband. Various conditions have led to the belief that the coding sequences of a genetic unit are located within the band DNA 2-4. However, on the basis of the amount of DNA present in bands and interbands, respectively, and on other considerations, Cuck 5 suggested that while the coding sequences are located within interbands, the band DNA has a regulatory role. Similarly, on the basis of light microscope (LM) autoradiographic data on H-uridine incorporation in polyene chromosomes it has been suggested that in addition to pulses nearly all interbands synthesize RNA while the bands do not. It has also been suggested that in polyene nuclei, RNA polymerase H is present in all interbands and pulses but not in bands 6. On the basis of ultrastructural studies, Shriver et al. concluded that the transcriptionally inactive regions are within the interbands and pulses. Implications of these apparently conclusive evidences for occurrence of transcription only on pulse and interband regions of polyene chromatin, a few recent observations have questioned these generalizations and have raised the possibility of RNA synthesis at certain condensed regions of polyene chromosomes. Lathrop et al. showed a high rate of RNA synthesis in the beta heterochromatin of the chromosome of polyene nuclei of D. melanogaster. Studies on in situ hybridization of cytoplasmic or nuclear DNA to polyene chromosomes have shown a specific hybridization of RNA to certain bands. More recently, Anazawa and Barka 7 have shown a high degree of H-uridine incorporation over a significant number of condensed bands of D. melanogaster polyene chromosomes. The present paper provides additional evidence for RNA synthesis by condensed chromatin regions in polyene nuclei of Drosophila. For this purpose, ultrastructural and autoradiographic (light as well as electron microscopic) studies have been done.

Materials and Methods

Salivary glands from late 3rd instar larvae of wild types of D. melanogaster and D. simulans were used for these studies. For ultrastructural observations, salivary glands from D. melanogaster larvae were fixed with glutaraldehyde and postfixed with osmium tetroxide and embedded in Epon-Araldit mixture as described earlier. Ultrathin (~100 nm) sections of glands, double stained with uranyl acetate and lead citrate, were examined under transmission electron microscope. For localization of sites of RNA synthesis, Hm (LM) and electron microscope (EM) autoradiographic studies have been done. For EM autoradiographic observations, salivary glands from late third instar larvae of D. melanogaster were pulse labeled in vivo with H-uridine (1Mc/Cml; sp. act. 12.6C/mCml, BARC, Trombay) for 5 min following which the glands were fixed in acetone-methanol (1:3) and squashed in 50% acetic acid. After removal of coverslips, the preparations were stained with carbol-fuchsin and coated with Bhof Land emulsion for autoradiography. Autoradiographic exposure was between 21 and 28 days at 4°C.

For EM autoradiography, the glands from late third instar larvae of D. melanogaster were pulse labeled with H-uridine (1Mc/Cml; sp. act. 13.6C/mCml, 239
Radiochemical Centre, Ameriose) for 5 min. Following the pulse, the glands were washed, fixed and processed for EM autoradiography as described earlier. Autoradiography exposure in this case was up to 20 days.

Results

Localization of perichromatin granules — Transcription products are identifiable as ribonucleoprotein perichromatin granules (peg) and, in general, their distribution is a good indication of active gene sites. Peg of various size ranges are generally distributed in puffs and a few interbands. The distribution of peg in relation to condensed chromatin has been specifically examined in this study and the observations on this aspect are presented below and in Figs 1-3.

Figure 1 shows electron micrographs of 2 adjacent
sections through the same unidentified chromosome segment. There is no major puff in this segment. Nevertheless, a large number of peg is seen in many of the regions and their distribution is essentially similar in the two sections. It may be noted that a number of bands have an irregular structure and the organization of such bands is not identical in two adjacent sections—in one section, the band may appear as a homogeneously condensed mass of fibrils while the same band in another section could have lateral disruptions or may contain regions of low electron density within its thickness. It is significant that specifically associated with most such bands are a large number of typical peg. As seen in Fig. 1, the interbands adjacent to bands marked with arrows are free of peg while in close proximity to these bands are clusters of peg. These granules are particularly abundant in regions where the bands appear laterally broken or in areas which appear as varicosities within the band structure.

or band regions specifically associated with perichromatin granules are indicated by arrows. Very few interband regions are seen to contain perichromatin granules. 250 = binding membrane, 5g = secondary granules. The bar represents 0.5 μm.
Figure 2 shows part of two large puffs. In both the puffs, a large number of peg (20-25 nm) is seen arranged in definite arrays. In the lower puff in Fig. 2, several small blocks of condensed chromatin are present which appear to be remnants of bands which decondensed concomitantly with puff development. The point of interest here is that the small condensed chromatin masses are surrounded by a narrow zone of perichromatin with which rows of perichromatin granules are closely associated. This suggests that these chromatin masses are involved in the formation of the associated peg. It is noteworthy that the interbands between the two puffs are almost free of peg. Shih and Blandau had suggested that "perichromatin granules spread along the chromosomes away from their original site of synthesis and pass between the bands where they are ejected, until the granules are contained in an unbroken band above and below the puff." However, in the present study, no evidence for migration of peg to interbands outside the puff area through the lateral discondensation in the bordering bands could be obtained. As can be seen in Fig. 2, the two interbands (double arrows) immediately next to the two puffs are separated from the respective puff region by laterally broken bands and yet both these interbands are nearly free of peg. A similar distribution of peg was seen in a few other puff regions also.

The presence of two types of peg (20-25 nm and 40-45 nm) and RNA synthesis in the beta heterochromatin of polytene nuclei of Drosophila has been reported earlier. The beta heterochromatin has an irregular organization with clumps of condensed chromatin masses and large intervening electron...
A narrow zone of perichromatin surrounds each of the condensed chromatid masses. As can be seen in Fig. 3a, the smaller peg (present all over the beta heterochromatin region) is always clustered in rows or loops near perichromatin of the condensed masses. The large interwinding spaces are totally devoid of these RNP granules. Figure 3b shows the arrangement of the larger sized peg which are restricted to the beta heterochromatin at the base of one chromosome arm. As discussed earlier, these pegs are generally seen in large clusters in one or more electron dense regions, however, in addition to such larger clusters, smaller number of these granules are also present in rows or singly, attached to the perichromatin at and around condensed masses in the region (Fig. 3b). It is possible that in this region of beta heterochromatin, the larger peg are synthesized over a wider area in close association with the perichromatin of condensed masses and subsequent to synthesis, these particles are stored or accumulated in clusters until their release to the nuclear sap.

Slices of the ultraviolet incorporation — Light microscope autoradiography—LM autoradiographs of squashed preparations of D. nanus polytene chromosomes following a 5 min pulse of 3H-uridine have been studied. In the present study, the right arm of the chromosome 2 (2R) has been examined in some detail. In order to obtain a greater degree of resolution of labelled sites, some preparations were made with more than the usual degree of chromosome stretching. In such stretched chromosomes, the interband regions get extended so that the bands appear more widely spaced. Two sets of LM autoradiographs of 2R of D. nanus are shown in Fig. 4: in one set (a, c, e) one complete 2R is shown in moderately stretched condition and in the other set (b, d, f), some parts of 2R are shown in a relatively more stretched condition. As expected, the puff sites usually incorporate a much higher level of 3H-uridine. In puffs in sections 4b, 4a (Fig. 4a, b and 40B (Fig. 4e, f), the 3H-uridine incorporation during the pulse of 5 min is limited to a narrow zone of the enlarged puff and most of the label in such puffs appear to be directly overlying the small darkly stained chromatin blocks. Very few interbands appear to be labelled distinctly with 3H-uridine. As seen in Fig. 4, only about 12 interband regions can be considered to be labelled with 3H-uridine along the entire length of 2R of D. nanus. However, it may also be noted that in some of the labelled interband regions (e.g., the interbands preceding bands 50Cl, 50C2, 52B5 in Fig. 4d) the silver grains appear

Fig. 1-3 b. Electron micrographs of two different regions of beta heterochromatin showing the relationship of perichromatin to the condensed chromatid masses. (a) The clusters of densely packed perichromatin granules surrounding the condensed masses are free of these granules. (b) A feature of this region is the presence of large number of perichromatin granules, which at many places, are clustered in groups of similar size and in rows (arrow) rather than in the interband regions (double arrow). The interband region of the chromosome 2 (2R) may be seen around all

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in a definite row and at the margin, the possibility of the presence of a thin band under the label can not be ruled out. Furthermore, some regions which appear as long interbands in stretched chromosomes, are seen as small gaps in recently spread chromosomes from the same or different dina (compare section 46C in Fig. 4a and b and section 53A in Fig. 4e and f).

In no case, a major part of any interband was seen to be labelled with H-uridine; the silver grains over the few interbands which were considered to be labelled, were always restricted to a narrow zone close to the band.

A considerable number of bands on chromosome 2A (and on other chromosome arm) of D. melanogaster have been labeled with H-uridine. Some of the well labelled thick bands seen in Example 1, Fig. 4a and b, and section 53A in Fig. 4e and f are 53A-5, 53C-7 and 53B-5. Additional examples of labelled thick bands are 43B-3, 43A, 43C, 49B1, 49C, 50A, 50C, etc. Examination of stretched chromosomes (Fig. 4b, d and f) establishes beyond doubt that the labelling of bands in these cases is not due to scatter from radioactivity in the adjacent interband regions (e.g. compare the labelling in section 56A in Fig. 4a and 4b. In a few regions of 2R, the silver grains are either on the band as well as on a narrow adjacent region of interband (e.g. 49C-4, 50C) or only at the interface of band and interband (e.g. 53A-5). In other cases, a major part of the interband, however, remains unlabelled.

Electron microsche autoradiography - More than 50 EM autoradiographs of unsphaled salivary glands of D. melanogaster have been examined to ascertain the site of H-uridine incorporation in polytene nuclei. This analysis confirms the results obtained from LM autoradiographs described above. No significant labelling of unsphaled bands is seen. It is not generally possible to identify a given chromosome segment, and, therefore, the present observation refers to the study only of the nucleolus and surrounding puff or band etc.

The autoradiographs are exceptionally labelled and very often the silver grains over the nucleolus are arranged in definite rows (Fig. 5a), reflecting some order of the arrangement of intercalary DNA. The interband regions remain unlabelled in most cases (Fig. 5b), although a few are low labelled. In each of the autoradiographs, several bands are always labelled. Figures 5b, c, and d show autoradiographs of some unidentified chromosome segments. The labelling of a major number of bands in these segments is very significant.

In fact, majority of silver grains in these and other

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**Fig. 4** - LM autoradiographs of chromosome arms 2R of D. melanogaster, to show sites of H-uridine incorporation. Moderately stretched chromosome 2R is shown in a 3 parts (a, c), while the full-length chromosome 2R is shown in b. Sections of chromosome are shown in b, d and f. The numbering of different regions is in the basis of our original photomontage (published) of D. melanogaster polytene chromosomes. The 2R is divided into 36 regions (from 4A to 4G; from 4H to 56A, and 8A to 8C) as well as interbands (Fig. 4a, b, c and d). Since each section starts with an interband and ends with a band, a section is usually divided into two regions: (a) a region in which the bands are labelled and (b) a region in which the interbands are labelled. This section has been divided into 2 regions, 2450

**Fig. 5** - EM autoradiographs of chromosome arms 2Rs of D. melanogaster, to show sites of H-uridine incorporation. The specimen is of D. melanogaster larva fed on H-uridine for 1 hour. The autoradiographs are of unstained chromosome spread on paraffin. The labelling of the nucleolus is observed. The labelling of the interbands is not observed. This section has been divided into 3 regions: (a) a region in which the bands are labelled and (b) a region in which the interbands are labelled. This section has been divided into 2 regions, 2450
their original sites of synthesis. Unlike Silver's contends, it is suggested that there may indeed be small regions of limited decondensation within bands and RNA synthesis proceeds in such band regions. The view is supported by several observations. In some cases, the bands containing or associated with

PEG are bordered on either side by interbands which are almost devoid of PEG—this makes it unlikely that the PEG in these bands are the result of transcriptional

activity of adjoining interbands. The localization of

PEG in beta heterochromatin also supports the view that transcription products can arise in close association with condensed chromatin.

The brief duration of the uridine pulse (3 min) and the measured resolution offered by autoradiography of stretched chromosomes and by EM autoradiography ensure that the location of silver grains in the autoradiograms represent the original sites of transcrip-

Fig. 5— Autoradiographic labeling of silver-stained nuclei of Drosophila heterozygous for two chromosomes. The set of chromosomes on the right were labeled by the silver grains over the chromosomes are not from adjacent daughter cells. All autoradiographs are labeled. (A) Autoradiographs show labeling of bands. (B) Autoradiographs show labeling of bands. (C) Autoradiographs show labeling of bands. (D) Autoradiographs show labeling of bands. (E) Autoradiographs show labeling of bands. (F) Autoradiographs show labeling of bands. (G) Autoradiographs show labeling of bands. (H) Autoradiographs show labeling of bands. (I) Autoradiographs show labeling of bands. (J) Autoradiographs show labeling of bands. (K) Autoradiographs show labeling of bands. (L) Autoradiographs show labeling of bands. (M) Autoradiographs show labeling of bands. (N) Autoradiographs show labeling of bands. (O) Autoradiographs show labeling of bands. (P) Autoradiographs show labeling of bands. (Q) Autoradiographs show labeling of bands. (R) Autoradiographs show labeling of bands. (S) Autoradiographs show labeling of bands. (T) Autoradiographs show labeling of bands. (U) Autoradiographs show labeling of bands. (V) Autoradiographs show labeling of bands. (W) Autoradiographs show labeling of bands. (X) Autoradiographs show labeling of bands. (Y) Autoradiographs show labeling of bands. (Z) Autoradiographs show labeling of bands.
An essentially similar model of protein chromatin organization has been suggested by Janknecht et al. However, the constancy of banding pattern in polytene chromosomes appears to be fairly well documented. Furthermore, the present data and those of Anasagasti and Barka show that almost none of the interbands may be active in transcription at any significant level, while certain unspalled band regions do transcribe. It is, therefore, suggested that the interbands represent permanent structural features of polytene chromosome organization and they may contain sites with which RNA polymerase II is associated without necessarily transcribing. This view would explain the immunofluorescent localization of RNA polymerase II on almost all interbands. The informational DNA of a given cytogenetic unit is visualized to be located mainly in bands, presumably close to the junction of band and interband. Depending upon the regulatory events, a part or all of the multiple strands of informational DNA in a given polytene band becomes transcriptionally active. Just a few of the strands are activated, there is only a partial decondensation of a band and these decondensed strands may give rise to "uns毫不" or lateral disarrangements within a band structure, as seen in the electron micrographs of presumably active bands. Such partially decondensed bands will also show $Hi$-or $Hi$-like satellite. If most or all of the 2 strands of a polytene band are activated, a small or big puff may result, depending upon the size of the transcription unit, the rate of synthesis and turnover of the transcription product.

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