Localization of RNA polymerase in polytene chromosomes of *Drosophila melanogaster*

(RNA polymerase B and histone H1 antibodies/indirect immunofluorescence/bands and interbands)

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**ABSTRACT** RNA polymerase (RNA nucleotidyltransferase) B (or II) and histone H1 of *Drosophila melanogaster* were localized on salivary gland polytene chromosomes using the indirect immunofluorescence technique. RNA polymerase B is present almost exclusively in puffs and interband regions, whereas histone H1 is found primarily in bands. The puff at region 3C, known to be transcriptionally active in larval salivary glands, gives a bright fluorescence with antibodies against RNA polymerase B. This fluorescence disappears after exposure of the larvae to 37° for 45 min. The heat shock treatment results in a general reduction of fluorescence intensity with the appearance of brightly staining heat shock puffs. Heat-induced removal of RNA polymerase molecules from a puff does not immediately alter its morphology. We propose that an interband represents that fraction of the total number of gene copies in a band that are active, the inactive copies being present in a condensed form in the adjacent band. Large puffs would originate through the decondensation and activation of most or all gene copies in a band.

**Indirect immunofluorescence** has become a widely applied tool for localization of not only surface antigens (1), but also intracellular components such as contractile proteins in nonmuscle cells (2). Recently, this technique has been applied to the study of the distribution of chromosomal proteins in polytene chromosomes of *Drosophila* (3–5). In these studies, chromatin was fractionated and either histones or nonhistone proteins, the latter being of unknown purities and functions, were used as antigens for their intrachromosomal localization. With the availability of antibodies against *Drosophila* RNA polymerase (RNA nucleotidyltransferase) B (6) we possess a probe to study the localization of a chromosomal protein of known function. Upon treatment of squash preparations from *D. melanogaster* salivary glands, Plagens et al. observed a distinct fluorescence pattern with the indirect immunofluorescence technique (7). They observed the α-amanitin-sensitive RNA polymerase B (also called polymerase II) not only in positions of visible puffs, but also at numerous other sites along the polytene chromosomes. Exposure of larvae to 37° resulted in a strong fluorescence at sites corresponding to the most prominent “heat shock” puffs (8). Meanwhile, a systematic study on conditions for preparing chromosomal squashes for immunofluorescence led to the observation that the postfixation with formaldehyde used previously caused shrinking of smaller puffs, yielding the impression of bands in the phase contrast pictures. This led to the original interpretation that RNA polymerase B was located also in bands (7). We studied the distribution of RNA polymerase B and of histone H1 with the improved technique described here and found that RNA polymerase B occupies exclusively uncondensed regions of the chromosomes, whereas histone H1 is found primarily in condensed regions. Heat shock treatment results in a redistribution of RNA polymerase molecules. We assume that interbands represent active genes.

**MATERIALS AND METHODS** Antibodies directed against RNA polymerase B of *D. melanogaster* were obtained as described (6). Antisera against *D. melanogaster* histone H1 were generously provided by Sarah Elgin (Harvard University) and by Hans Will of this laboratory. Both preparations yielded identical results. Sheep anti-rabbit immunoglobulins, coupled with fluorescein isothiocyanate (FITC), were purchased from Wellcome.

Third instar larvae from *D. melanogaster* Oregon wild-type strain, grown at room temperature, were used for squash preparations of salivary glands. In heat shock experiments larvae were kept at 37° for the times indicated in the figure legends.

Salivary glands were dissected in Shield’s medium (9) containing (wt/vol) 0.5% Triton X-100/0.5% Nonidet P-40/0.1% digitonin; this and all subsequent steps were done at room temperature. Within 30 sec the glands were transferred to a fixative solution containing 3.7% acetic acid-formaldehyde in the dissecting medium and were fixed for 1–2 min. Longer fixation times did not increase the amount of histone H1 or RNA polymerase detected on the chromosomes. The glands were then transferred to 45% acetic acid containing 3.7% formaldehyde for 90 sec and covered with a siliconized cover slip; the chromosomes were spread by tapping on the cover slip with the eraser end of a pencil (4). After the slide was frozen in liquid nitrogen, the cover slip was pried off with a razor blade, and the slide was placed in 95% ethanol for 30 min. Prior to treatment with antibodies, the slide was washed in phosphate-buffered saline (0.14 M sodium chloride in 10 mM potassium phosphate, pH 7.5) for at least 30 min.

Chromosome preparations were treated for 30 min with anti-RNA polymerase B immunoglobulins diluted 1:2 with phosphate-buffered saline containing 1 mg of human immunoglobulins (Sigma) per ml, or with antisera against histone H1 diluted 1:50. After a 30-min wash in phosphate-buffered saline, the preparations were reacted with a 1:20 dilution of FITC-coupled sheep anti-rabbit immunoglobulins (30 min), and then washed again in phosphate-buffered saline (at least 30 min).

For microscopy, a preparation was covered with a drop of phosphate-buffered saline, a cover slip was applied, and the specimen was photographed using incident ultraviolet illumination in a Zeiss Standard microscope with an incident UV attachment and fluorescence optics. Phase contrast or bright field photographs were taken after the preparations were stained with lacto-acetic orcein (10).

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*Abbreviation: FITC, fluorescein isothiocyanate.*

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RESULTS

The distribution of RNA polymerase B on polytene chromosomes of *D. melanogaster*, as observed by the immunofluorescence technique, is shown in Fig. 1. At this magnification we can discern a distinct banding pattern with brightly staining regions, some of which can be identified as puffs in the corresponding phase contrast picture (not shown here since a similar comparison was published elsewhere (7)). Not all of the typical landmark puffs described by Ashburner (8), however, are intensely decorated by the fluorescence dye, indicating that not all puffed regions contain an equal concentration of RNA polymerase B. Furthermore, the fluorescence intensity within a puff does not necessarily correlate with its size. Some regions that could be considered either as interbands or as small puffs fluoresce brightly, such as the site in region 3C, which was identified by deletion analysis as representing a gene coding for a secretory protein (11). A comparatively low level of fluorescence is observed in the heterochromatic chromocenter. Because there are three types of RNA polymerase of similar structures in eukaryotes, it is possible that our antibodies stain polymerases A and C as well. High concentrations of polymerase A should be found in nucleolar regions and, since little fluorescence is observed in these areas, it seems that, at least for polymerase A, the crossreaction is minimal under our conditions. Antiserum against calf thymus RNA polymerase A (kindly provided by Dr. C. Kedinger) gave considerable fluorescence at the nucleolus but not in chromosomes (M. Jamrich, unpublished work). Although it is known that transcription at most chromosomal sites is inhibited by concentrations of α-amanitin that do not affect polymerase C (12), fluorescence at some sites could be due to this enzyme.

Because of the low magnifications required to accommodate an entire chromosome set in a single picture such as in Fig. 1, such pictures lack sufficient structural details to establish a conclusive correlation between the banding patterns observed by fluorescence and by phase contrast microscopy. In the following figures we therefore show short segments of polytene chromosomes at much higher magnifications, which allow such correlations to be made. The segment shown in Fig. 2 represents regions 21D through 24D of Bridges' map. The fluorescence along this segment (Fig. 2 upper) is interrupted by 15 conspicuous gaps, which correspond to the 15 dark staining "bands" appearing after orcein staining (Fig. 2 lower). According to Bridges's map, not only would these 15 landmarks actually consist of two or more closely adjacent subbands, but there would also be at least one or two additional faint bands in each "interband," thus raising the actual number of bands in this segment to approximately 40. While it is generally difficult to demonstrate this much detail under the conditions used, it is interesting to find the indications of substructure using im-
mofluorescence. For example, the “interband” between 21E and 22A shows a brighter left half, while the less bright other half appears faintly striated. For 24B, immunofluorescence demonstrates the double-band nature of this structure by exhibiting a thin, but clearly visible, interband. It is possible that some of the striking differences of fluorescence intensity observed in different “interbands” are caused by the presence of puffed faint bands in some intervals. However, the clear-cut delimitation of fluorescence at the “band/interband” boundaries argues against the possibility that all “interband” fluorescence is derived from puffing. The conclusion seems justified that polymerase B is located in all interbands in polytene chromosomes. In contrast, the immunofluorescence obtained with antibodies against H1 gave a banding pattern that was practically identical to that seen by phase contrast (Fig. 3), indicating a strict proportionality between condensation of chromosomal material and presence of histone H1.

The terminal segment of the X chromosome containing region 3C is shown in Fig. 4. A comparison of the fluorescence intensities of this with its neighboring regions confirms the presence of a high concentration of RNA polymerase molecules at the site of Korge’s gene (11), which is transcriptionally active in salivary glands. The same site appears as a weakly staining interband or puff in the corresponding phase contrast picture (Fig. 4 right).

In Drosophila, as well as in many other dipteran species, a small number of new puffs are induced in the salivary glands after a heat shock treatment (8, 13). The induction of these puffs is paralleled by the appearance of a similar number of new polypeptides (14, 15), with the majority of newly synthesized RNA species hybridizing in sttu to the heat shock puffs, while the expression of originally active genes appears to be turned off (16, 17). Exposure of the larvae to 37°C for various lengths of time resulted in a gradual reduction of fluorescence intensity at most sites, with the appearance of intense fluorescence at sites identified as heat shock puffs (for the latter observation see also refs. 7 and 18). After 45 min at 37°C a barely detectable fluorescence remains in region 3C, suggesting that nearly all RNA polymerase molecules had left the originally active site. On the other hand, a new band of fluorescence appears near the tip of the X chromosome at position 1A (Fig. 5). This region has so far not been described as becoming activated through a heat shock treatment. It is the only one on the X chromosome found to be labeled by the fluorescent dye after a 45-min heat shock.

Not only small puffs like that in region 3C but also some of the large ones appear depleted of RNA polymerase B molecules after heat shock treatment. This is seen in Fig. 6, which shows that two large puffs observed in the phase contrast picture do not fluoresce after they are stained for RNA polymerase. Heat shock induced puffs, in contrast, fluoresce brightly. Thus, the heat shock treatment appears to result in the removal of RNA polymerase molecules from preexisting puffs without immediately altering their morphology.

**DISCUSSION**

By the immunofluorescence technique we find RNA polymerase B, the enzyme implicated in the synthesis of precursor mRNA, almost exclusively in interbands and puffs but not in
bands. The fact that we observe at least some RNA polymerase molecules in practically all interbands suggests that interbands represent either active genes or RNA polymerase storage sites, or both. Studies on incorporation in vivo and in vitro of [3H] uridine into Drosophila salivary glands after detection of the nascent RNA by in situ radioautography indicated that RNA is synthesized in puffs and in interbands but not in bands (19, 20). The most detailed studies made so far suggest that practically all interbands may be sites of low level RNA synthesis (19). Thus, our results on RNA polymerase localization and the incorporation data complement each other, and we are tempted to conclude that in addition to those sites that are puffed, all interbands may represent active genes. Such a situation would appear to support the idea, proposed by Crick in 1971, that structural gene sites in an uncondensed state (interchromomeres) alternate with condensed regulator regions (chromomeres) in eukaryotic interphase chromosomes (21). An apparent difficulty with this model results from the fact that there is hardly enough DNA in the interbands as a whole [by most estimates not more than 5% (22)] to account for the finding that in Drosophila larvae as much as 24% of the unique sequence DNA, i.e., about 20% of the total DNA, becomes transcribed (23). In other words, while the average amount of DNA of an interband would suffice to produce just enough RNA to code for a protein of 30,000-40,000 daltons, the synthesis of any additional, e.g., "regulatory," RNA sequences would have to be assigned to bands. Transcribing bands do, of course, occur and are classified as puffs. The fraction of the total DNA represented by bands that are puffed is not precisely known but should amount to at least 10% (24). The data reported in ref. 23 are therefore not in conflict with the finding that transcription is confined to interbands and puffs. However, there remains another problem. Assuming that the presence of polymerase B in all interbands means ongoing transcription of all structural genes, the question arises of how differential gene activation is achieved during cell differentiation. One might imagine that for transcription to become developmentally effective in an interband the adjacent band must be opened up for cotranscription. In principle, such a situation would not be different from the already mentioned alternative one that interbands are storage sites for polymerase B. In that case, however, it is hard to see why structural genes should not be assigned to the bands rather than interbands in the first place.

Notwithstanding these considerations, we should like to mention a further possible way of viewing the functional organization of chromosomes that is limited to the polytene state. Like Crick (21), we suggest that the cytogenetic unit represents a band plus adjacent interband, but only if the gene is transcriptionally active. Of the 2n homologous copies of a single cytogenetic unit, only a fraction may be located in an interband and would be active, with the remainder of the copies (usually the larger fraction) being located in the band in an inactive, i.e.,

**Fig. 4.** (Left) Indirect immunofluorescence visualization of RNA polymerase in distal region of the X chromosome. (Right) Phase contrast photograph of the same specimen after orcein staining. (Approximately X2500.)

**Fig. 5.** Effect of heat shock (45 min) on RNA polymerase distribution in the distal region of the X chromosome. (Approximately X2300.) (Left) Indirect immunofluorescent visualization of RNA polymerase. (Right) Phase contrast view of the same specimen after orcein staining.
condensed form. For totally inactive genes, the cytogenetic unit would consist of a band only and, having no interband, it would be fused with the condensed fraction of its neighboring chromosome. Further activation of an already active gene results in the decondensation of further gene copies, i.e., transfer of DNA from the band to the interband. If more gene copies are decondensed than have space in the interband region, a puff appears. Decondensation of all homologous gene copies results in a large puff and the disappearance of the band.

Since each chromatid consists of a continuous DNA strand transversing the entire length of a giant chromosome, it is clear that every chromomere of one band must be connected with a chromomere of the next band, i.e., a small segment of each of the inactive gene copies must go through the interband as connector DNA which, since interbands have an average width of 0.12 μm (25), need to represent little more than 1% of the total DNA. The decondensed active genes can occupy the space between the bands which is limited by the length of the connectors. Our conclusions are not at variance with the generally accepted view based on many detailed observations by both light and electron microscopy that the cytogenetic unit is identical with a band or a part of a band plus its adjacent interband. The new aspect originating from our data and from those cited (19) would be the suggestion that a major portion of the genome is expressed in differentiated cells but that, in polytene chromosomes, not all of the 2n DNA strands of a band need to be present in a decondensed, i.e., transcriptionally active, form.

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