DIRECT EVIDENCE FOR THE PRESENCE OF DNA IN INTERBANDS OF DROSOPHILA SALIVARY GLAND CHROMOSOMES

DAVID R. WOLSTENHOLME

Genetical Institute, University of Groningen, Haren (Gr.), The Netherlands, and Max-Planck-Institut für Biologie, Abteilung Beermann, Tübingen, Germany

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FROM the results of experiments involving autoradiography of stretched H\textsuperscript{3} thymidine-labelled salivary gland chromosomes of \textit{Drosophila melanogaster}, and a consideration of previously published evidence, \textsc{Steffensen} (1963) has concluded that DNA (deoxyribonucleic acid) is absent from the interband regions of these chromosomes. In opposition to this conclusion are the results presented in this report of examinations in the fluorescence microscope of acridine orange stained, ribonuclease digested salivary glands of \textit{Drosophila melanogaster}, which provide direct evidence for the presence of DNA in interband regions of polytene chromosomes.

MATERIALS AND METHODS

Salivary glands of living third instar larvae of a laboratory wild-type strain of \textit{Drosophila melanogaster} were removed in Ringer solution (\textsc{Ephrussi} and \textsc{Beadle} 1936) and transferred immediately to a drop of acetic acid-ethanol (1:3) on a 22mm No. 1 coverslip. After 1 minute the fixative was replaced with 45% acetic acid and the glands were squashed by covering with an 18mm No. 1 coverslip and applying pressure. After freezing on solid CO\textsubscript{2}, the coverslips were separated and placed in acetic acid-ethanol for approximately 10 min, rinsed in absolute ethanol and two changes of 95% ethanol for 5 min each and finally air dried.

Ribonuclease digestion of glands was accomplished by incubation of coverslip squash preparations for 4 hr at 38°C in distilled water containing 0.3 mg/ml crystalline ribonuclease (British Drug House) adjusted to pH 7.0 with Na\textsubscript{2}HPO\textsubscript{4}. Deoxyribonuclease digestion involved a 4 hr incubation of squash preparations at 38°C in distilled water containing 10\textsuperscript{-3} M MgSO\textsubscript{4} and 0.5 mg/ml crystalline deoxyribonuclease (British Drug House), adjusted to pH 7.0 with Na\textsubscript{2}HPO\textsubscript{4}. The specificities of the enzyme extractions were controlled by incubating squash preparations in similar solutions with the enzymes omitted. The specificities of the nucleic acid-dye complexes were also controlled by treatment of some salivary gland squash preparations with 10% TCA (trichloracetic acid) at 90°C for 20 min, before staining. The hot TCA treatment was in turn controlled by treatment in parallel of squashes for 20 min with either 10% TCA at 4°C or with distilled water at 90°C. Squash preparations were stained for 30 min at 4°C with acridine orange prepared according to the method described by \textsc{Rabinovitch} and \textsc{Plaut} (1962) and examined in a Zeiss fluorescence microscope using a 100\times fluorospar oil immersion objective, high pressure mercury illumination, a combination of Zeiss BG-12 and BG-38 exciter filters and a combination of Zeiss 53 and 44 barrier filters. Fluorescence micrographs were made on Kodak Plus-X film.

RESULTS AND CONCLUSIONS

In acridine orange stained salivary gland squash preparations, from which the

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RNA (ribonucleic acid) has been removed by ribonuclease digestion, viewed in the fluorescence microscope the chromosomes fluoresce bright yellowish-green in contrast to the faint dull greenish-grey of the cytoplasm. Chromosomes showing various degrees of stretching (which may be compared directly with the stretched chromosomes pictured by Steffensen 1963) have been examined (Figure 1) and the yellowish-green fluorescence appears to be continuous along the lengths

**Figure 1.**—A-F.—Fluorescence micrographs of acridine orange stained, ribonuclease digested salivary gland chromosomes of *Drosophila melanogaster* stretched to various degrees. Fluorescence, shown to be entirely due to the presence of DNA, is continuous along the lengths of all chromosomes. Differences in intensity of fluorescence reflect the banding patterns. In some relatively unstretched chromosomes (D) the brighter fluorescence of the bands obscures the banding pattern. The interband regions indicated by the arrows in E and F seem to comprise a number of fine fluorescent threads (the smallest about 0.3μ in diameter) connecting the bands. All parts of the Figure × 2,100.
of all chromosomes. Differences in intensity of brightness of the yellowish-green fluorescence reflect the well described banding patterns. Details of the banding patterns are sometimes obscured in relatively unstretched chromosomes due to the very bright fluorescence of the bands (see Figure 1D for example) but are clearly visible in stretched chromosomes. In some regions an interband is seen to comprise a number of yellowish-green fluorescent threads connecting the bands and measuring as small as 0.3 μ in diameter (arrows Figure 1E and F). The fluorescence of such threads always appears continuous. The yellowish-green fluorescence is not found in any part of the chromosome if treatment of ribonuclease digested tissues with either deoxyribonuclease or hot TCA (but not deoxyribonuclease buffer, cold TCA, or hot distilled water) precedes acridine orange staining. The greenish-grey background fluorescence is not altered by either deoxyribonuclease or hot TCA treatment. It is concluded, therefore, that the yellowish-green fluorescence is due exclusively to the presence of DNA.

Plaut and Nash (1964) have mentioned that, following staining with fluorescence dyes, no discontinuities in DNA are apparent along the length of salivary gland chromosomes of Drosophila and, contrary to the finding of Steffensen (1963) have shown label resulting from incorporation of H3 thymidine over apparent interband regions of salivary gland chromosomes of Drosophila melanogaster. This latter evidence is subject to criticism owing to the possibility that bands undetectable in the light microscope may occupy apparent interband regions (Beermann and Bahr 1954). The present results, however, provide a direct demonstration of DNA in interband regions of salivary gland chromosomes of Drosophila melanogaster and indicate that, within the limits of resolution of the light microscope system used (about 0.2 μ) DNA is continuous throughout the length of the chromosome (thus confirming the findings of Swift 1962). A similar conclusion has been reached from a study involving acridine orange stained chromosomes of Chironomus tentans (Wolstenholme 1965).

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SUMMARY

Examination in the fluorescence microscope of acridine orange stained, ribonuclease digested salivary glands of Drosophila melanogaster has revealed continuous yellowish-green fluorescence along the lengths of all the chromosomes. The yellowish-green fluorescence is sensitive to both deoxyribonuclease and hot TCA. From these results it is concluded that DNA is present in interband regions and that within the limits of resolution of the light microscope system used (about 0.2 μ) DNA is continuous throughout the length of the chromosome.

LITERATURE CITED


