Aust. J. Biol. Sci., 1985, **38**, 1–11

Native Salivary Chromosomes of *Drosophila melanogaster*: Retrospect and Prospect

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Abstract

A method has been developed which, for the first time, allows the preparation of mappable cytological spreads of salivary chromosomes from *D. melanogaster* without exposure to acid fixatives. These isolated native chromosomes show the best preservation of ultrastructure observed to date—ribonucleoprotein particles may be seen to be organized in linear arrays in transcriptionally active puffs and the repeating nucleosome module is present. Native salivary chromosomes are proving useful for the localization of nuclear proteins both at the light microscope and ultrastructural levels. They display only background-level binding of antibodies specific for the Z-DNA conformation. However, Z-DNA immunoreactivity is activated by exposure to acid fixative, first in interbands and then in bands. The Z-conformation in the chromosomes is held in place by elastic torsional strain which appears in the DNA following acid fixation. Native *D. melanogaster* salivary chromosomes offer promise for enabling the probing of the chromatin of known genetic loci for properties dependent on the preservation of macromolecular integrity.

Historical Background

The discovery of giant banded chromatin threads in the nuclei of salivary gland cells of Diptera, such as *Chironomus*, is generally credited to Balbiani in 1881. However, some 50 years elapsed before it was realized that these structures actually represented an enormously amplified form of the interphase chromosomes and their significance to the science of genetics became apparent (Heitz and Bauer 1933; Painter 1933). *Drosophila melanogaster*, the animal whose genetics was then, and still remains, by far the best characterized, has very small mitotic chromosomes. The recognition that giant salivary chromosomes are a faithfully amplified representation of the interphase chromosomes brought this organism from one of the poorest objects for the study of structural details of chromosomes to one of the best. Painter (1934) stated that these giant chromosomes were 'the material of which every geneticist had been dreaming'. Not only was the linear organization of the interphase chromosomes apparent in the light microscope, but genes could be seen at work as local puffed regions along the chromosome.

The salivary gland tissue in Diptera grows by an increase in cell size rather than cell number. During this growth the chromosomes go through some 10 rounds of DNA replication without separation of daughter chromatids so that cable-like structures are built up with approximately 1000 DNA molecules running the length of the 'polytene' chromosomes in register.

0004-9417/85/010001$02.00

Since the 1930's, these chromosomes have generally been isolated for cytological observation by squashing salivary glands in 45% (v/v) acetic acid, a solvent which breaks down the cell and nuclear membranes and generally disrupts the cell contents with the exception of the chromosomes which it toughens and acid-fixes. Whilst this acid fixation does not represent a problem for gross studies of banding patterns, it does have certain disadvantages when more precise molecular questions are to be investigated. For example, acid fixatives can extract chromosomal proteins, change macromolecular conformations, disrupt ultrastructure and destroy biological activity. These problems have been recognized in general terms for decades.

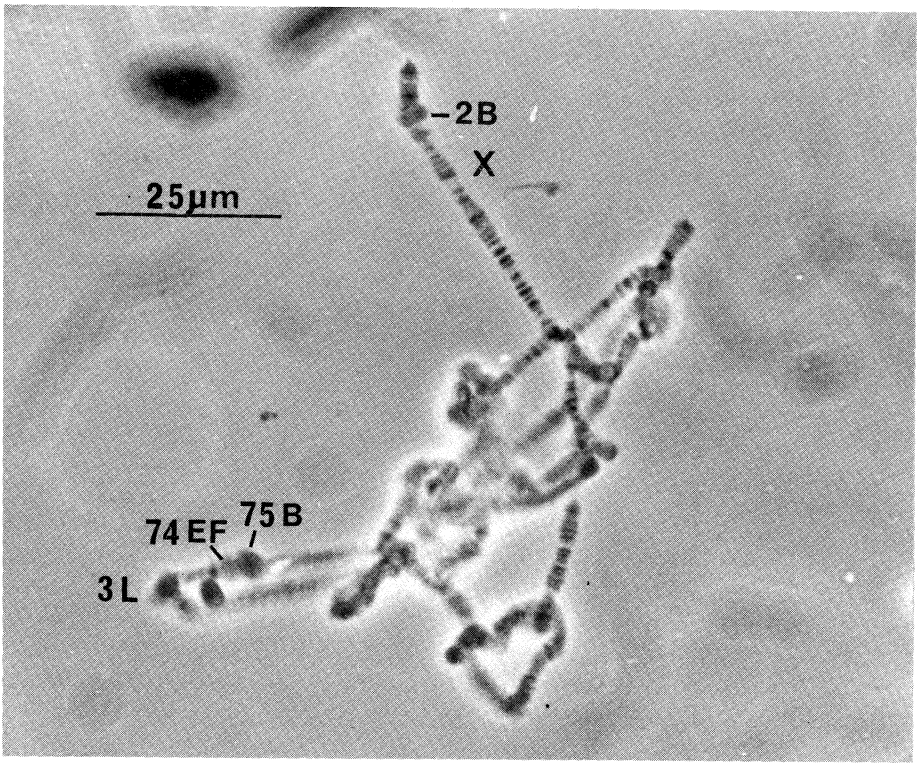

Fig. 1. Phase-contrast micrograph of a *D. melanogaster* chromosome spread isolated by microdissection from a salivary gland nucleus. Identified are: the X chromosome with the large puff at position 2B and chromosome 3L present as a loop with two puffs at positions 74EF and 75B. (Reproduced from Mott *et al.* 1980.)

In the case of *Chironomus*, it has long been possible to isolate microsurgically the salivary chromosomes without exposure to acid fixatives (D'Angelo 1946; Lezzi and Robert 1972). However, despite considerable efforts in the United States involving at least three laboratories over the past two decades, the problem of preparing cytologically mappable spreads of *D. melanogaster* salivary chromosomes without acid fixation proved more refractory (Hewson Swift, personal communication). It emerges that there are a number of technical and biological reasons for this.

Isolation of Salivary Chromosomes of *D. melanogaster* without Exposure to Acid Fixatives

On attempting microsurgical isolation of the chromosomes from *D. melanogaster* salivary glands in physiological saline, we ran into two major problems vis-à-vis the situation in *Chironomus*. Firstly, the chromosomes, known to contain about an order of magnitude fewer DNA molecules, have considerably less tensile strength. Care with micromanipulation and stabilization using trace amounts of formaldehyde (0·05% w/v) or physiological concentrations of polyamines have been successfully employed to combat this. The second problem was more frustrating. For most of the time the chromosomes are multiply attached to the internal surface of the nuclear membrane (Hill and Watt 1978). These attachments, the physical reality of which became apparent to us during micromanipulative attempts to remove chromosomes from the confines of the nucleus, have recently been independently characterized by Agard and Sedat (1983) using computer-assisted tomography to reconstruct the three-dimensional architecture of the *D. melanogaster* polytene nucleus.

We have found a narrow window towards the end of the third-larval instar where the attachments between the chromosomes and the nuclear membrane weaken. At this time it is possible to tear open cells to release nuclei; then an incision is made in the nuclear membrane and the chromosomes are withdrawn with fine glass needles. The freed chromosomes from one nucleus are shown in Fig. 1. Most striking in this phase-contrast micrograph are the X chromosome with transcriptionally active ecdysterone-induced puffs in section 2B and chromosome arm 3L present as a loop with two ecdysterone-induced puffs at loci 74EF and 75B. At the light-microscope level the preservation of structure is quite comparable to that of acid-fixed chromosomes. We shall refer to chromosomes prepared in this way without exposure to acid fixatives as 'native'.

Ultrastructure of Native Salivary Chromosomes of *D. melanogaster*

The distal region of the X chromosome is depicted at higher magnification in the electron micrograph in Fig. 2. Ultrastructure is seen to be excellently preserved despite the absence of acid fixation during the isolation procedure. It is at least comparable to, and in some respects better than, that in classical acid-squash preparations (see, for example, Sorsa and Sorsa 1967; Berendes 1970); moreover, there is no confusing background due to the network of nucleoplasmic fibrils which extends right through the chromosomes in whole glutaraldehyde-fixed nuclei (Skaer and Whytock 1977). The puff at 2B and some, but not all, interbands contain putative ribonucleoprotein (RNP) granules of diameter 30–45 nm, consistent with transcription at these sites (Skaer 1977) and according with the view of Crick (1971) that transcription occurs in interbands and puffs.

The RNP particles in the puff can be seen, in more open regions, to be arranged in linear arrays. Towards the periphery of the puff these structures display looped configurations (insert in Fig. 2). This is of interest since such loops have long been seen in the Balbiani rings of *Chironomus* (Beerman and Bahr 1954) but have never previously been observed in the puffs of *D. melanogaster* chromosomes. The particles appear to be clustered about an axial filament of diameter approximately 10 nm. By analogy with the interpretation of such structures in *Chironomus* (Stevens and Swift 1966; Lamb

and Daneholt 1979), we provisionally interpret the arrays of particles in puff 2B as nascent RNP attached to axes of transcriptionally active deoxyribonucleoprotein.

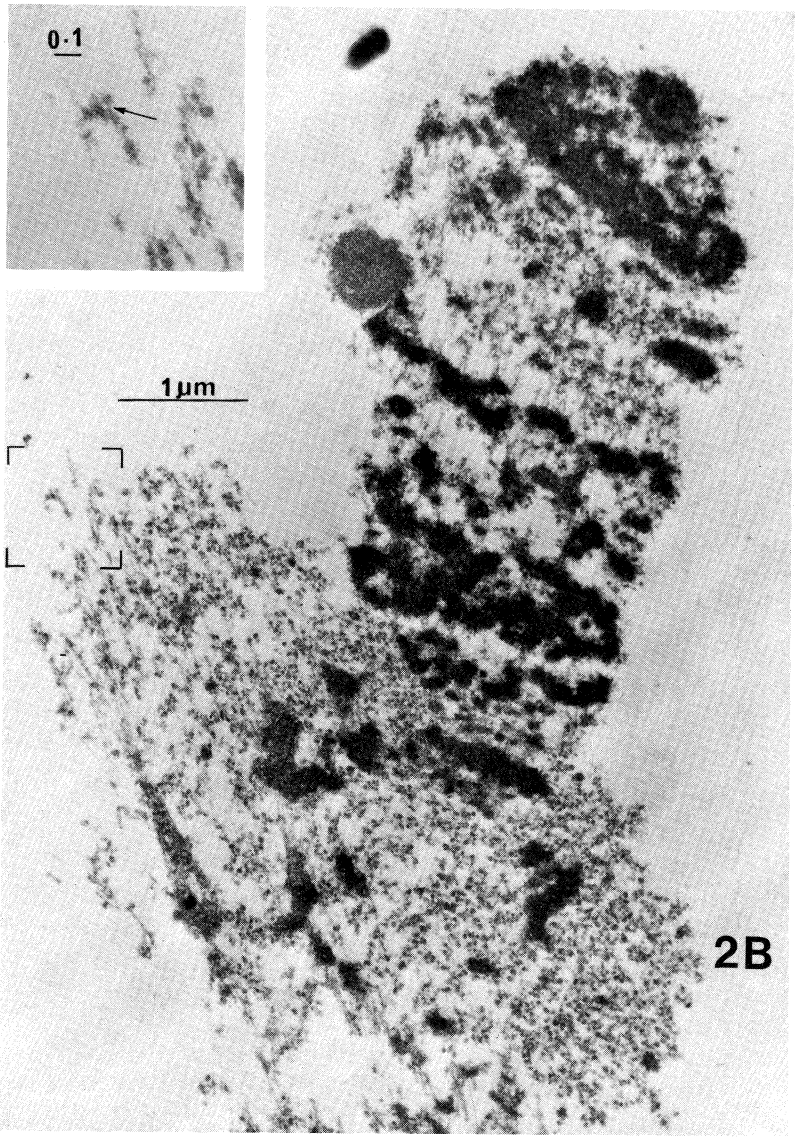

Fig. 2. Electron micrograph of the distal region of the X chromosome of *D. melanogaster*. Inset is a higher magnification of a peripheral region of puff 2B showing a 'looped' chromatin fibre bearing nascent RNP particles. (Reproduced from Mott *et al.* 1980.)

Occasionally a stalk-like structure can be seen apparently attaching a particle to the axial filament (see arrow in the insert).

Nucleosome Module Present in Spreads of Native Salivary Chromosomes of *D. melanogaster*

It has not been possible to demonstrate the presence of nucleosomes in *D. melanogaster* salivary chromosomes isolated as classical acetic acid squashes. There is, in fact, a paucity of demonstrations of the basic nucleosome repeat in these, the most studied of all chromosomes. However, it is possible to isolate microsurgically unfixed salivary chromosomes in buffer A of Hewish and Burgoyne (1973), the solvent in which early experiments with nuclease gave the first evidence for a 200 base pair repeating module in chromatin. Salivary chromosomes in buffer A may be placed directly onto the glow discharged surface of a carbon-coated electron microscope grid and then spread by a slightly modified form of the technique of Miller and Beatty (1969). Individual nucleoprotein fibres with a characteristic repeating nucleosome organization are resolved emanating from the chromosomal material as shown in Fig. 3 (see also Hill *et al.* 1982).

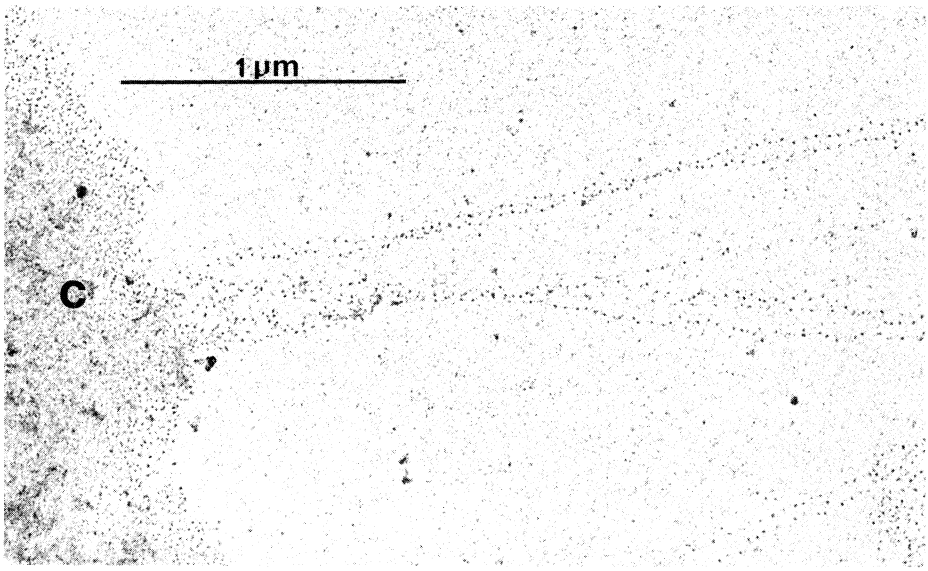

Fig. 3. Electron micrograph of a native *D. melanogaster* salivary chromosome spread by a modification of the Miller technique showing nucleosome-bearing fibres emanating from the bulk of the chromatin, designated *C*. (Reproduced by permission of The Rockefeller University Press from Hill *et al.* 1982.)

Immunolocalization of Chromosomal Proteins of *D. melanogaster*

In addition to DNA, the histone and non-histone chromosomal proteins constitute major components of the chromatin complex. In salivary chromosomes, the chromatin of known genetic loci is arrayed in linear order. It is possible to map the distribution of particular proteins along these chromatin structures by employing immunofluorescence techniques. Much work has been done in mapping chromosomal proteins on 45% (v/v) acetic acid squash preparations of *D. melanogaster* salivary chromosomes (see, for example, Elgin *et al.* 1977; Will and Bautz 1980).

However, many chromosomal proteins are acid-extractable, e.g. the histones, high mobility group proteins (Goodwin *et al.* 1977) and a subset of other non-histones (see e.g. Hill *et al.* 1984). One may combat the extraction effect of acetic acid by cross-linking the chromatin in the salivary gland to varying extents with formaldehyde prior to, or during, acid-squashing.

A more rigorous approach may be to employ native salivary chromosomes that have never been exposed to acid (Hill and Watt 1977). Monoclonal antibodies against an acidic chromosomal protein of molecular weight 74 000 display the distribution along the distal region of the native X chromosome shown in Fig. 4*b*. Acidic chromosomal proteins are generally found to be associated with regions of gene activity. This form of analysis is currently being extended to the ultrastructural level using electron-dense labelling of antibodies (Mott *et al.* 1982). Because native salivary chromosomes isolated by microsurgery display better preservation of ultrastructure than hitherto possible, this approach should take the localization of chromosomal proteins to a new level of molecular precision.

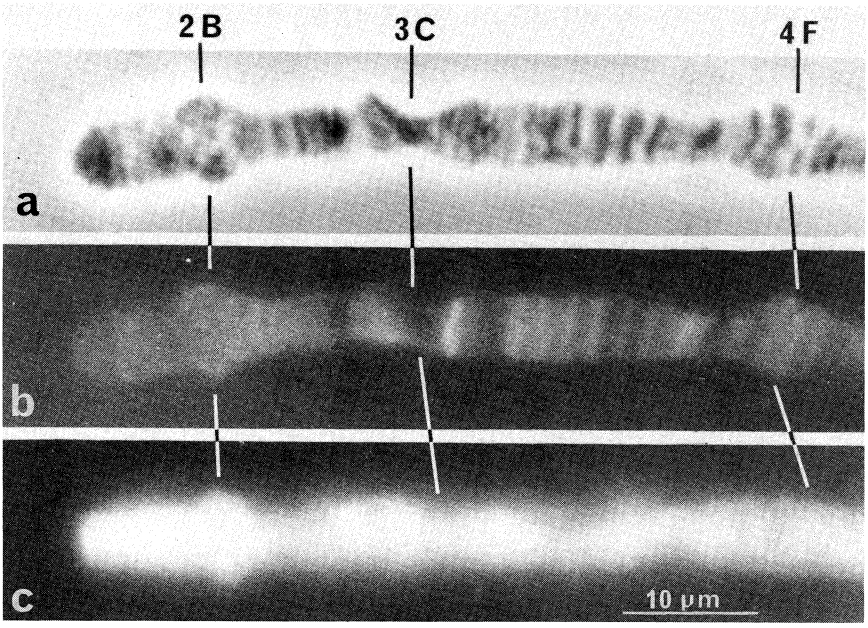

Fig. 4. Immunolocalization of chromosomal proteins on the distal region of the native X chromosome of *D. melanogaster*. Phase-contrast micrograph (*a*), fluorescence micrograph depicting the binding of a monoclonal antibody against an acidic chromosomal protein of molecular weight 74 000 (*b*), and fluorescence micrograph showing the binding of a monoclonal antibody against histone H1 (*c*). (From R. J. Hill, F. Watt and A. Underwood, unpublished results.)

When acid-squashed chromosomes are employed to map the distribution of histone proteins, the results obtained are dependent on details of the chromosome isolation and fixation procedures. For example, there is a marked but variable lowering of anti-H1-induced immunofluorescence especially over puffs (Bustin *et al.* 1978). However, when chromosomes isolated at neutral pH are treated with monoclonal antibodies against histone H1, the marked tendency to lowered fluorescence over puffs is not apparent (Fig. 4*c*). It is likely that acetic acid extracts histone H1 from salivary chromosomes

and that this extraction may be only partially prevented by formaldehyde fixation (Bustin *et al.* 1978). Thus it would appear that the more rigorous approach of completely avoiding exposure of the chromosomes to acid offers definite advantages for *in situ* protein localization analyses.

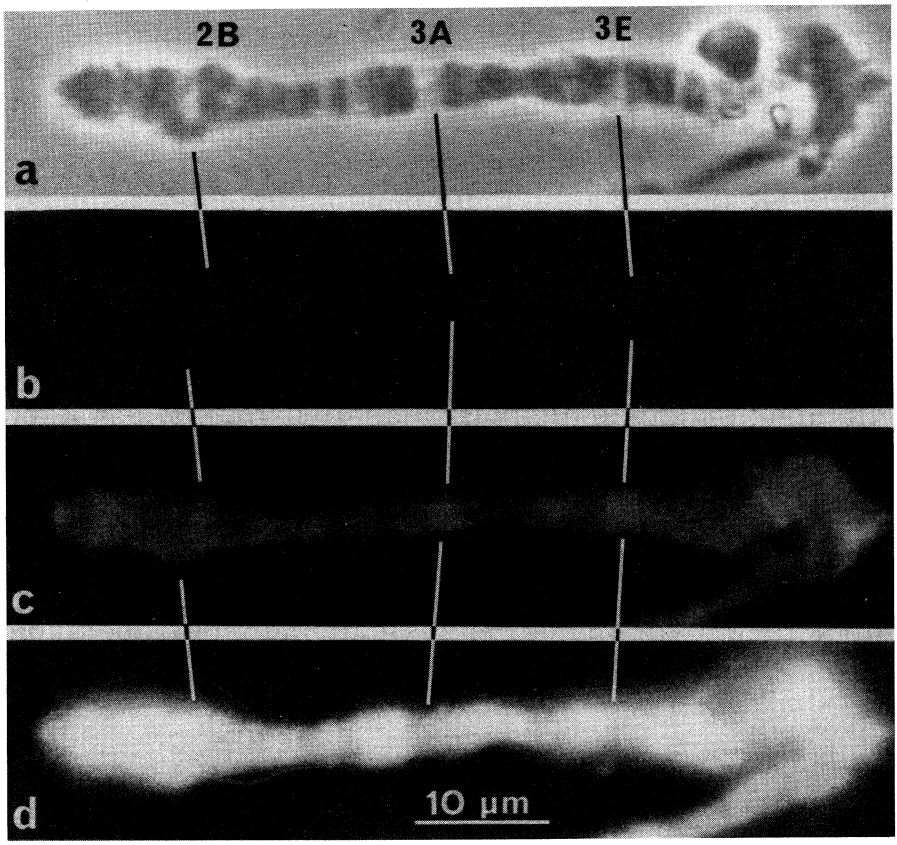

Fig. 5. Phase contrast (*a*) and Z-DNA antibody-stained fluorescence micrographs (*b–d*) of the distal region of the X polytene chromosome of *D. melanogaster*. The chromosome was isolated without exposure to acid. In (*b*) the chromosome was treated directly with Z-DNA antiserum. In (*c*) it was treated with the antiserum after exposure for 5 s to 45% (v/v) acetic acid and return to neutral pH—fluorescence is predominantly over interbands and puffs. In (*d*) it was treated with antiserum after exposure for 30 s to acid. Intense fluorescence now follows the mass distribution of the chromosome. (Reproduced by permission from *Nature*, Vol. 305, pp. 338–40. Copyright © 1983 Macmillan Journals Limited.)

Observation of Macromolecular Conformation *in situ*—Example Z-DNA

The isolation of the salivary chromosomes at physiological pH and ionic strength should leave the conformation of macromolecules *in vivo* essentially intact; this can then be observed *in situ* on the chromosome, by probing with ligands such as steroid hormones or antibodies specific for conformational determinants.

There is currently considerable interest in the conformation that DNA itself adopts in chromosomes. Evidence is growing from X-ray diffraction studies for variations in the conformation of DNA in response to base sequence and changes in microscopic

environment. These variations range from minor changes in the right-handed B-structure, originally modelled by Watson and Crick in 1953, to a radically different left-handed structure termed Z-DNA, exhibited by crystals of alternating dG–dC copolymers (Dickerson *et al.* 1982). An important question now is to what extent do these potential variations in DNA conformation occur in chromosomes *in vivo*?

There has been a recent realization that the giant salivary chromosomes, used for over a decade to probe the nature of DNA sequences and associated proteins at specific loci, also offer the possibility of studying DNA conformation *in situ* in chromosomes. In 1981 there was considerable excitement at the Massachusetts Institute of Technology (MIT) when it was found that antibodies specific for the Z-conformation of DNA bound to classical preparations of salivary chromosomes (Nordheim *et al.* 1981). Subsequently two other laboratories reported the binding of Z-DNA antibodies to salivary chromosomes (Lemeunier *et al.* 1982; Jovin *et al.* 1983). However, there was a paradox: some workers observed Z-DNA predominantly in interbands, others in bands and still others in a specific subset of interbands and bands.

The polytene chromosomes used for these studies were isolated by variations on the classical acid-squashing technique in general use since the 1930s. When chromosomes isolated by microsurgery at neutral pH were tested for Z-DNA antibody binding, a different result was obtained (Hill and Stollar 1983). The level of fluorescence observed was virtually background (Fig. 5*b*). However, when native chromosomes were exposed to 45% (v/v) acetic acid fixative for 5 s and subsequently treated with Z-DNA antibodies at neutral pH, fluorescence appeared predominantly over more open regions of the chromosomes—interbands and puffs (Fig. 5*c*). On exposure to 45% acetic acid for 30 s there was a massive enhancement of Z-immunoreactivity and now fluorescence followed the mass distribution of the chromosome, i.e. it occurs predominantly over bands (Fig. 5*d*). This suggests that the different interband and band fluorescence patterns obtained at MIT and also at the Max Planck Institut may arise from differential solvent effects during the fixation procedures used in the two laboratories.

What could be the molecular basis for the massive enhancement of Z-DNA immunoreactivity on acid fixation? A detailed discussion of this question has been presented elsewhere (Hill *et al.* 1984; Hill 1984). However, a possible major contributory mechanism, for which there is now some experimental support, may be of general interest. As the pH decreases through the range 3 to 2 there is a marked disruption of nucleosome structure followed, at slightly lower pH, by effective displacement of the four core histones (Cole and Lawson 1979). This process releases the negative superhelical turns of DNA which were stabilized on the surface of the nucleosome. Model experiments in which synthetic negative superhelical turns have been introduced into bacterial plasmids containing alternating purine–pyrimidine tracts have shown that superhelical densities of the order of those released on nucleosome disruption are sufficient to induce B to Z transitions (Haniford and Pulleyblank 1983).

If torsional stress released into DNA molecules on nucleosome disruption were responsible for activating Z-DNA immunoreactivity, the antibody binding might be sensitive to relaxation of supercoiling. The effect of topoisomerase I on antibody binding is shown in Figs 6*a*–6*d*. Figs 6*a* and 6*b* show the level of Z-DNA antibody binding to a chromosome that has been exposed to topoisomerase I and then to 45% acetic acid. Figs 6*c* and 6*d* depict a similar experiment, except that the chromosome was treated with 45% acetic acid and then topoisomerase I before antibody treatment. Topoisomerase I treatment, after acid exposure but not before, lowers antibody binding

to background (Hill and Stollar 1983). In other words, the Z-conformation detected in acid-treated chromosomes *is* associated with torsional stress in the DNA, torsional stress that is itself only free in the DNA following acid exposure.

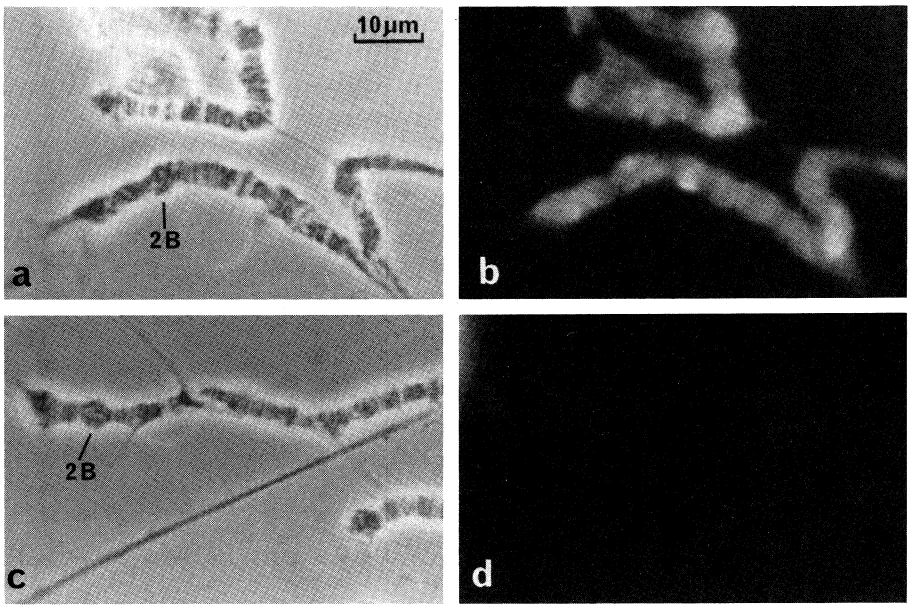

Fig. 6. The effect of topoisomerase I on Z-DNA antibody binding. (*a*) Phase-contrast and (*b*) Z-DNA antibody-treated X chromosome of *D. melanogaster* that has been exposed to topoisomerase I and then 45% (v/v) acetic acid. (*c*) Phase-contrast, and (*d*) antibody-treated X chromosome of *D. melanogaster* that had been exposed to 45% (v/v) acetic acid followed by topoisomerase I. In (*b*) the level of fluorescence is typical of an acid-treated chromosome. It is reduced to background in (*d*). (Reproduced by permission from *Nature*, Vol. 305, pp. 338–40. Copyright © 1983 Macmillan Journals Limited.)

Future Directions

The availability of *D. melanogaster* salivary chromosomes that have not been subjected to acid fixation should prove useful for a number of applications including the following:

(1) The immunofluorescence localization of nuclear proteins at a level of resolution approaching individual genetic loci. Avoidance of exposure to acid fixatives during chromosome isolation avoids complications such as acid extraction of proteins and denaturation of three-dimensional antigenic determinants.

(2) Because the native chromosomes display the best preservation of ultrastructure available to date, they are uniquely suited to the localization of proteins within active loci at a macromolecular level using electron-dense-labelled antibodies in the electron microscope.

(3) Refinements of micromanipulative procedure are beginning to allow the placement of known chromosomal segments at positions of defined coordinates on electron-microscope grids. With this innovation and Miller spreading of the unfixed chromosomal material, it should be possible to examine directly in the electron microscope nucleosomal organization and transcription patterns within known genetic loci.

(4) Investigations of the molecular conformation of chromatin and chromatin components, e.g. the DNA itself, *in situ* at known genetic loci can be undertaken without complications resulting from perturbations occurring at low pH.
(5) The localization of biological activities such as hormone binding sites or enzymatic functions, which is only possible when higher orders of protein structure are not irreversibly destroyed, should be attainable.
(6) Finally, in the future it may be possible to observe and even control the activation *in vitro* of specific genetic loci on the native chromosomes.

Acknowledgments

My thanks to colleagues who have made important contributions during the course of this project: in particular Fuji Watt, Margaret Mott, Anne Underwood, Theodora Fifis and Mary Alice Yund. I would like to acknowledge the support of Dr G. W. Grigg.

This article is dedicated to Dr J. M. Rendel who introduced me to *Drosophila melanogaster*, and to Professor H. G. Callan who first showed me salivary chromosomes.

References

Agard, D. A., and Sedat, J. W. (1983). Three-dimensional architecture of a polytene nucleus. *Nature (London)* **302**, 676–81.

Beerman, W., and Bahr, G. F. (1954). The submicroscopic structure of the Balbiani ring. *Expl. Cell Res.* **6**, 195–201.

Berendes, H. D. (1970). Polytene chromosome structure at the submicroscopic level. I. A map of region X, 1-4E of *Drosophila melanogaster*. *Chromosoma* **20**, 118–30.

Bustin, M., Kurth, P. D., Moudrianakis, E. N., Goldblatt, D., Sperling, R., and Rizzo, W. B. (1978). Immunological probes for chromatin structure. *Cold Spring Harb. Symp. Quant. Biol.* **XLII**, 379–88.

Cole, R. D., and Lawson, G. M. (1979). Selective displacement of histone H1 from whole HeLa nuclei: effect on chromatin structure *in situ* as probed by micrococcal nuclease. *Biochemistry* **18**, 2160–6.

Crick, F. H. C. (1971). General model for the chromosomes of higher organisms. *Nature (London)* **234**, 25–27.

D'Angelo, E. G. (1946). Microsurgical studies on *Chironomus* salivary gland chromosomes. *Biol. Bull.* **90**, 71–87.

Dickerson, R. E., Drew, H. R., Conner, B. N., Wing, R. M., Fratini, A. V., and Kopka, M. L. (1982). The anatomy of A-, B-, and Z-DNA. *Science (Wash., D.C.)* **216**, 475–85.

Elgin, S. C. R., Silver, L. M., and Wu, C. E. C. (1977). The *in situ* distribution of *Drosophila* nonhistone chromosomal proteins. In 'The Molecular Biology of the Mammalian Genetic Apparatus I'. (Ed. P. O. P. Ts'o.) pp. 127–41. (North-Holland: Amsterdam.)

Goodwin, G. H., Rabbani, A., Nicolas, R. H., and Johns, E. W. (1977). The isolation of the high mobility group non-histone chromosomal proteins HMG14. *FEBS Lett.* **50**, 413–16.

Haniford, D. B., and Pulleyblank, E. D. (1983). Facile transition of poly[d(TG) . d(CA)] into a left-handed helix in physiological conditions. *Nature (London)* **302**, 632–4.

Heitz, E., and Bauer, H. (1933). Beweise fur die chromosomen-natur der Kernschleifen in den knauelkernen von Bibio bortulanus I. *Z. Zellforsch.* **17**, 68–82.

Hewish, D. R., and Burgoyne, L. A. (1973). Chromatin sub-structure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. *Biochem. Biophys. Res. Commun.* **52**, 504–10.

Hill, R. J. (1984). On the status of the Z-DNA question for animal chromosomes. *Bio-Essays* **1**, 244–9.

Hill, R. J., Mott, M. R., Burnett, E. J., Lowenhaupt, K., and Elgin, S. C. R. (1982). Nucleosome repeat structure is present in native salivary chromosomes of *Drosophila melanogaster*. *J. Cell Biol.* **95**, 262–6.

Hill, R. J., and Stollar, B. D. (1983). Dependence of Z-DNA antibody binding to polytene chromosomes on acid fixation and DNA torsional strain. *Nature (London)* **305**, 338–40.

Hill, R. J., and Watt, F. (1977). Chromosomal proteins of *Drosophila melanogaster* and an approach for their localization on polytene chromosomes. *Chromosoma* **63**, 57–78.

Hill, R. J., and Watt, F. (1978). Native salivary chromosomes of *Drosophila melanogaster*. *Cold Spring Harb. Symp. Quant. Biol.* **XLII**, 859–65.

Hill, R. J., Watt, F., and Stollar, B. D. (1984). Z-DNA immunoreactivity of *Drosophila* polytene chromosomes. Effects of 45% acetic acid, 95% ethanol and DNAase I nicking. *Exp. Cell Res.* **153**, 469.

Jovin, T. M., *et al.* (1983). Generation of left-handed Z-DNA in solution and visualization in polytene chromosomes by immunofluorescence. *Cold Spring Harb. Symp. Quant. Biol.* **XLVII**, 143–54.

Lamb, M. M., and Daneholt, B. (1979). Characterisation of active transcription units in Balbiani rings of *Chironomus tentans*. *Cell* **17**, 835–48.

Lemeunier, F., Derbin, C., Malfoy, B., Leng, M., and Taillandier, E. (1982). Identification of left-handed Z-DNA by indirect immunofluorescence in polytene chromosomes of *Chironomus thummi*: *thummi*. *Exp. Cell Res.* **141**, 508–13.

Lezzi, M., and Robert, M. (1972). Chromosomes from unfixed salivary chromosomes of *Chironomus*. In 'Developmental Studies on Giant Chromosomes'. (Ed. W. Beerman.) pp. 35–57. (Springer-Verlag: Berlin.)

Miller, O. L., Jr, and Beatty, B. R. (1969). Visualization of nucleolar genes. *Science (Wash., D.C.)* **164**, 955–7.

Mott, M. R., Burnett, E. J., and Hill, R. J. (1980). Ultrastructure of polytene chromosomes of *Drosophila* isolated by microdissection. *J. Cell Sci.* **45**, 15–30.

Mott, M. R., Hill, R. J., and Burnett, E. J. (1982). Immunolocalization of chromosomal proteins on polytene chromosomes of *Drosophila*. *Micron* **13**, 301–2.

Nordheim, A., Pardue, M. L., Lafer, E. M., Moller, A., Stollar, B. D., and Rich, A. (1981). Antibodies to left-handed Z-DNA bind to interband regions of *Drosophila* polytene chromosomes. *Nature (London)* **294**, 417–22.

Painter, T. S. (1933). A new method for the study of chromosome rearrangements and the plotting of chromosome maps. *Science (Wash., D.C.)* **78**, 585–6.

Painter, T. S. (1934). Salivary chromosomes and the attack on the gene. *J. Heredity* **25**, 465–76.

Skaer, R. J. (1977). Interband transcription in *Drosophila*. *J. Cell Sci.* **26**, 251–66.

Skaer, R. J., and Whytock, S. (1977). The fixation of nuclei in glutaraldehyde. *J. Cell Sci.* **27**, 13–21.

Sorsa, M., and Sorsa, V. (1967). Electron microscopic observations on interband fibrils in *Drosophila* salivary chromosomes. *Chromosoma* **22**, 32–41.

Stevens, B. J., and Swift, H. (1966). RNA transport from nucleus to cytoplasm in *Chironomus* salivary glands. *J. Cell Biol.* **31**, 55–77.

Will, H., and Bautz, E. K. F. (1980). Immunological identification of a chromocenter-associated protein in polytene chromosomes of *Drosophila*. *Exp. Cell Res.* **125**, 401–10.

Manuscript received 23 October 1984, accepted 31 October 1984