Antibodies to left-handed Z-DNA bind to interband regions of *Drosophila* polytene chromosomes

Alfred Nordheim*, Mary Lou Pardue*, Eileen M. Lafer†, Achim Möller*, B. David Stollar† & Alexander Rich*

* Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
† Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111, USA

Antibodies which are specific to the Z-DNA conformation have been purified and characterized on the basis of their binding to three different DNA polymers which can form this left-handed helix. These antibodies bind specifically to polytene chromosomes of Drosophila melanogaster *as visualized by fluorescent staining. The staining is found in the interband regions and its intensity varies among different interbands in a reproducible manner. This is the first identification of the Z-DNA conformation in material of biological origin.*

LEFT-HANDED double-stranded DNA was first discovered by an atomic resolution X-ray crystallographic analysis of the hexanucleoside pentaphosphate CpGpCpGpCpG (ref. 1). In this conformation the DNA formed a double helix with Watson–Crick base pairs and antiparallel sugar–phosphate chains; the sugar–phosphate backbone followed a zig-zag course and for this reason was named Z-DNA. In Z-DNA the guanine bases have rotated about the glycosydic bond and have assumed a *syn* conformation, in contrast to the *anti* conformation in B-DNA. The early observation by Pohl and Jovin[2] of salt-induced inversion of the circular dichroism spectrum of poly(dG-dC) DNA in 4 M NaCl solution can now be understood as the conformational transition of the right-handed B-DNA to the left-handed Z-DNA[3,4]. Z-DNA has been seen in several crystal structure and fibre analyses[5–8] and generally, DNA with alternating purine–pyrimidine sequences may be expected to have Z-forming potential.

We have recently demonstrated that left-handed Z-DNA is a strong immunogen; in both rabbits and mice antibodies can be raised which are specific for the left-handed Z conformation and will not react with right-handed B-DNA[9]. Because of their specificity, antibodies can be used to determine the distribution

0028–0836/81/490417—06$01.00

of Z-DNA in biological systems. Here we have purified antibodies to Z-DNA from rabbit sera and demonstrate their specificity for the left-handed Z conformation. We have used these antibodies for indirect immunofluorescent staining of *Drosophila melanogaster* polytene chromosomes. Because a large polytene chromosome can consist of a thousand or more individual chromatids in very precise alignment, these chromosomes provide a magnification that cannot be obtained with other types of chromosomes. In addition, polytene chromosomes are interphase chromosomes, active in both transcription and replication, in spite of the relatively condensed state of the chromatin. The alignment of the many chromatids making up a polytene chromosome is so precise that regions of tight coiling in the individual chromatids match up to give the appearance of bands across the width of each chromosome. These bands are separated by interbands in which the chromatid fibres are relatively extended. The banding pattern of each chromosome is essentially constant from nucleus to nucleus and from individual to individual, and must thus reflect basic features of chromatid structure, although the significance of this structure is not understood. Polytene chromosomes therefore provide a unique opportunity for studying the conformational states of specific chromatin regions at times when the chromatin is genetically active.

Indirect immunofluorescence has been widely used to study the distribution of proteins on polytene chromosomes[10], and here we apply this technique to investigate the occurrence and distribution of Z-DNA in these chromosomes. We show that anti-Z-DNA antibodies bind to polytene chromosomes in a very reproducible pattern, exhibiting fluorescence staining exclusively in interband regions in our experimental conditions. This finding may throw some light on the role of Z-DNA in biological systems and may lead to some clues concerning the significance of banding in eukaryotic chromosomes.

Characterization of the antibody

As described earlier[9], the anti-Z-DNA antibodies were raised by injecting rabbits with brominated poly(dG-dC)·poly(dG-dC). This DNA polymer remains in the Z-form even in low salt because most of the bromine atoms are on the C-8 position of guanine, stabilizing guanine in the *syn* conformation. In low salt, non-brominated poly(dG-dC)·poly(dG-dC) is in the B conformation and does not react with the antibody. In 4 M NaCl the unmodified poly(dG-dC)·poly(dG-dC) is converted to the Z form and does react with the anti-Z-DNA antibody.

Although our initial experiments on polytene chromosomes were carried out with whole sera from rabbits injected with brominated poly(dG-dC)·poly(dG-dC), the more recent experiments have been done with antibodies purified from these sera. The purified antibody was obtained by preparative scale quantitative immunoprecipitation in 4 M NaCl using unmodified poly(dG-dC)·poly(dG-dC), which is in the Z conformation at that salt concentration[2,4]. The specificity of the affinity-purified antibody for the Z conformation was tested on native *Escherichia coli* DNA and three different polymers, poly(dG-dC)·poly(dG-dC), brominated poly(dG-dC)·poly(dG-dC) and the methylated polymer poly(dG-m^{5}dC)·

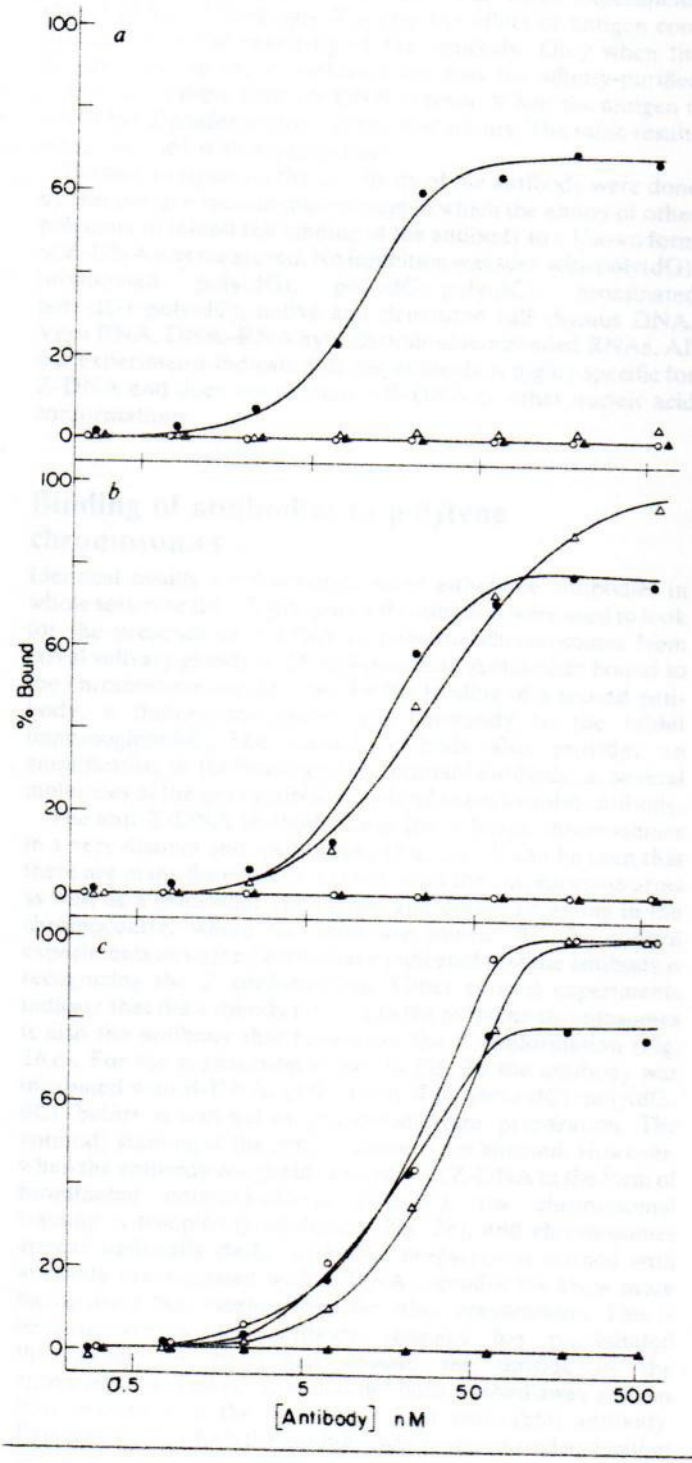

Fig. 1 Specificity of the affinity-purified anti-Z-DNA antibody as measured by direct binding to ^{3}H-brominated poly(dG-dC)·poly(dG-dC) (●), ^{3}H-poly(dG-m^{5}dC)·poly(dG-m^{5}dC) (△), ^{3}H-poly(dG-dC)·poly(dG-dC) (○) and ^{3}H-labelled *E. coli* DNA (▲) in 0.2 M NaCl (*a*), 1.5 M NaCl (*b*) and 4.0 M NaCl (*c*). The affinity-purified antibody was prepared by a modification of the procedure of Kitagawa and Okuhara[22]. A sample of rabbit serum was incubated with an equivalent amount of poly(dG-dC)·poly(dG-dC) (as determined from quantitative precipitation curves) in 60 mM sodium phosphate, 30 mM EDTA, 4 M sodium chloride, *p*H 8.0, for 3 h at 37 °C and overnight at 4 °C. For equivalent proportions of antigen and antibody, 75 A_{260} units of polynucleotide were added to 15 ml of serum. Resulting precipitates were washed three times with cold PBS (10 mM sodium phosphate, 140 mM sodium chloride, *p*H 7.4) and dissolved in 20 mM sodium carbonate, 5% dimethyl sulphoxide (DMSO) *p*H 10.5. The dissolved antibodies and antigen were separated on a DEAE-cellulose column (20 ml) previously equilibrated with 20 mM sodium carbonate, 5% DMSO, *p*H 10.5. The free antibody flowed through on washing with 20 mM sodium carbonate, 5% DMSO, *p*H 10.5. The retained nucleic acid was eluted by 20 mM sodium carbonate, 5% DMSO, 1 M NaCl, *p*H 10.5. The purified antibodies were dialysed against PBS and protein concentration determined by the method of Lowry *et al.*[23]. Radioimmunoassays (RIAs) were performed by incubating 100 μl samples of purified antibody serially diluted into the RIA buffer (60 mM sodium phosphate, 30 mM sodium EDTA, *p*H 8.0) containing 0.2 M NaCl (*a*), 1.5 M NaCl (*b*) or 4.0 M NaCl (*c*) with 100 ng of ^{3}H-labelled nucleic acid in 0.05 ml of the RIA buffer containing the indicated NaCl concentration for 1 h at room temperature. Then 0.05 ml of the γ-globulin fraction of goat anti-rabbit immunoglobulin serum in PBS was added and the mixture kept at room temperature for an additional hour. The resulting precipitate was centrifuged, washed twice with RIA buffer containing the indicated NaCl concentration, dissolved in 1 ml 0.1 N NaOH and counted in 10 ml of Aquasol-2 (NEN). ^{3}H-thymidine-labelled *E. coli* DNA was prepared from a mutant strain B3, as described previously[24], but with a pulse of 0.5 mCi of ^{3}H-thymidine. ^{3}H-labelled poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was synthesized according to Behe and Felsenfeld[11], except that 0.065 mCi of ^{3}H-dGTP (Amersham) was included into the synthesis reaction mixture. The other polymers were radiolabelled by nick translation[25].

poly(dG-m^{5}dC). Behe and Felsenfeld have shown that methylation on the 5 position of cytosine yields a polymer that is converted to Z-DNA at low concentrations of $MgCl_2$, and has a midpoint for conversion by NaCl at 700 mM (ref. 11).

Figure 1 demonstrates the specificity of the purified antibody by showing its direct binding as a function of antibody concentration in three different salt environments using the four polymers. In 0.2 M NaCl, the antibody bound only the brominated poly(dG-dC)·poly(dG-dC) (Fig. 1*a*), the only one which has the Z conformation in 0.2 M NaCl. At 1.5 M NaCl, antibody bound the brominated and methylated polymers, both of which have the Z conformation, but not the two polymers which are in the B conformation at that ionic strength (Fig. 1*b*). In 4 M NaCl the brominated, methylated and unmodified poly(dG-dC)·poly(dG-dC) were bound, but *E. coli* DNA was not (Fig. 1*c*). Poly(dG-dC)·poly(dG-dC) is completely in the Z-DNA conformation in 4 M NaCl, where its Raman spectrum is identical with that of Z-DNA crystals[4]. The three experiments shown in Fig. 1 strikingly illustrate the effect of antigen conformation on the reactivity of the antibody. Only when the polymers are in the Z conformation does the affinity-purified antibody combine with the DNA antigen. When the antigen is not in the Z conformation, no reaction occurs. The same results were obtained with whole serum.

Further analyses on the specificity of the antibody were done by competitive radioimmunoassays in which the ability of other polymers to inhibit the binding of the antibody to a known form of Z-DNA was measured. No inhibition was seen with poly(dG), brominated poly(dG), poly(dG)·poly(dC), brominated poly(dG)·poly(dC), native and denatured calf thymus DNA, Vero RNA, DNA–RNA hybrids or double-stranded RNAs. All our experiments indicate that the antibody is highly specific for Z-DNA and does not recognize B-DNA or other nucleic acid conformations.

Binding of antibodies to polytene chromosomes

Identical results were obtained when either the antibodies in whole serum or the affinity-purified antibodies were used to look for the presence of Z-DNA in polytene chromosomes from larval salivary glands of *D. melanogaster*. Antibodies bound to the chromosome are detected by the binding of a second antibody, a fluorescein-labelled goat antibody to the rabbit immunoglobulin. The second antibody also provides an amplification of the binding of the primary antibody, as several molecules of the goat antibody can bind to each rabbit antibody.

The anti-Z-DNA antibody stains the polytene chromosomes in a very distinct and specific way (Fig. 2*a*). It can be seen that there are many fluorescent regions on all the chromosome arms as well as a somewhat diminished and diffused staining in the chromocentre, where the arms are joined. All the control experiments described above have indicated that the antibody is recognizing the Z conformation. Other control experiments indicate that the antibody binding to the polytene chromosomes is also the antibody that recognizes the Z conformation (Fig. 2*b*,*c*). For the preparation shown in Fig. 2*b* the antibody was incubated with B-DNA, in the form of poly(dG-dC)·poly(dG-dC), before it was put on the chromosome preparation. The antibody staining of the chromosomes is not affected. However, when the antibody was preincubated with Z-DNA in the form of brominated poly(dG-dC)·poly(dG-dC), the chromosomal staining is completely abolished (Fig. 2*c*), and chromosomes appear uniformly dark. Note that preparations stained with antibody preincubated with Z-DNA reproducibly show more background fluorescence than do other preparations. This is because the antigen–antibody complex has precipitated throughout the field and around the outside of the chromosomes. This complex has not been washed away and has thus reacted with the fluorescent goat anti-rabbit antibody. Experiments in which the antibody was preincubated with either native or denatured DNA from *Drosophila* embryos showed no effect on chromosomal staining.

The same chromosomal staining pattern is produced by whole serum from rabbits injected with brominated poly(dG-dC)·poly(dG-dC), by antibodies affinity purified from this serum by the brominated polymer in low salt, or by antibodies affinity purified from the serum by unmodified poly(dG-dC)·poly(dG-dC) in high salt where this polymer is in the Z conformation. We have also studied an antibody obtained from a rabbit injected with unmodified poly(dG-dC) poly(dG-dC), which exists as B-DNA in physiological salt solution. The antibody produced by this rabbit was of lower titre but was specific for Z-DNA at the dilution used in the experiment. To obtain this antibody, the polymer was injected into the rabbit complexed with methylated bovine serum albumin and it is assumed that the cationic charges on the surface of the methylated bovine serum albumin provided regions which converted local domains of the poly(dG-dC)·poly(dG-dC) into Z-DNA[9]. This last antibody produced without bromine atoms yielded the same chromosomal staining pattern as the other antibody

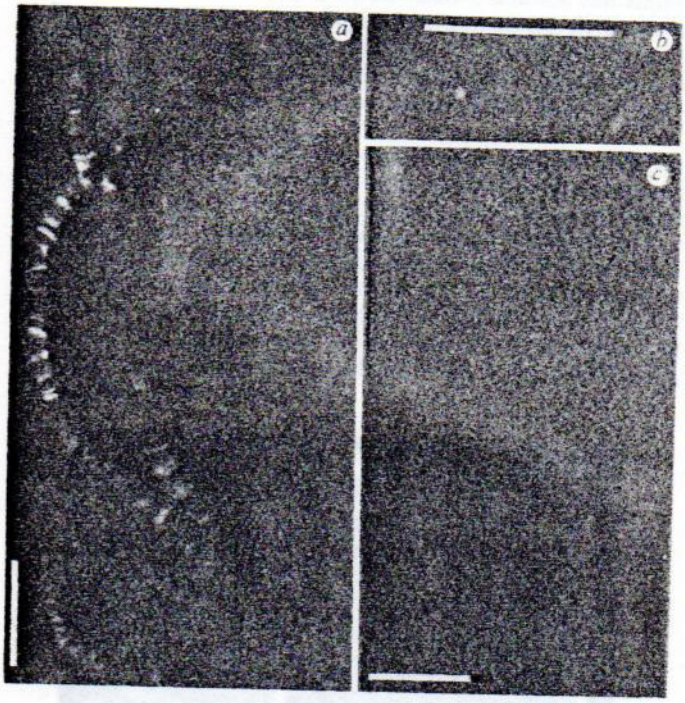

Fig. 2 Fluorescence micrographs of polytene chromosomes demonstrating the specificity of the reaction between anti-Z-DNA antibody and fixed chromosomes. *a*, Chromosomes stained with anti-Z-DNA antibody by our standard procedure. *b*, Chromosomes stained with anti-Z-DNA antibody, which had been preincubated with poly(dG-dC)·poly(dG-dC) in the B conformation. The pattern of fluorescence staining is identical to that produced by the antibody without B-DNA competitor. *c*, Chromosomes stained with anti-Z-DNA antibody, which had been preincubated with the Z-form brominated poly(dG-dC)·poly(dG-dC). No chromosomal fluorescence staining is detectable although the precipitated antigen–antibody complex produces an increased general background of fluorescence. Salivary glands were dissected out of third instar Oregon R larvae (gt-1 stock) into a droplet of 45% acetic acid in Ringer's buffer on a coverslip. After 2–5 min a microscope slide was touched to the drop on the coverslip, the gland was squashed mechanically and the slides were placed on the flat surface of a block of dry ice. After 20 min freezing on dry ice the coverslips were pried off and the slides were briefly immersed horizontally in PBS. Excess PBS was removed from the slide without completely drying the surface; 0.02 ml antibody-containing PBS solution was then added and incubated for 15 min at 37 °C. The slides were then gently washed in PBS and fluorescein isothiocyanate (FITC) conjugated IgG fraction of goat anti-rabbit serum (Cappell) was added at a 1:250 dilution in PBS, followed by another 37 °C incubation for 15 min. Unbound FITC-conjugated secondary antibody was gently washed out and a clean coverslip was mounted with a drop of PBS. The slides were viewed and photographed in a Zeiss microscope using both incident UV illumination and phase contrast optics. Photographs were taken using Kodak Ektachrome 400 film. For the blocking experiments of *b* and *c*, the antibody solutions contained the respective competitor DNAs at concentrations of 0.5 mg ml^{-1} and were preincubated for 30–45 min at 37 °C before staining of the polytene chromosomes. Scale bar, 10 μm.

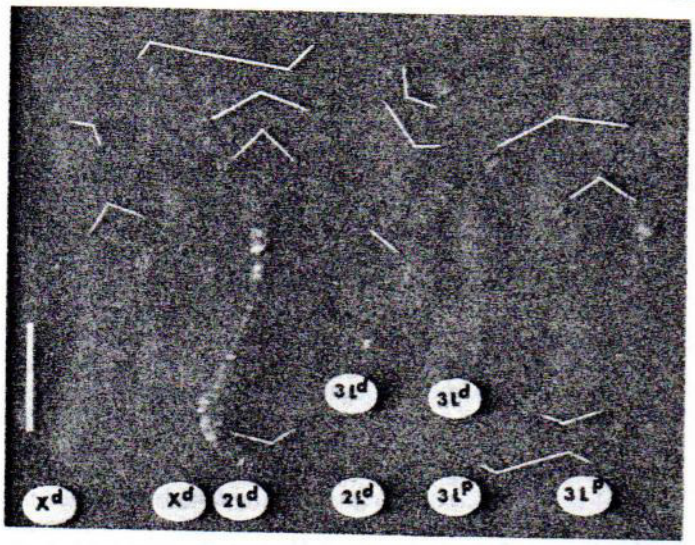

Fig. 3 Fluorescence micrographs of chromosomes stained with the anti-Z-DNA antibody demonstrating the constancy of the fluorescence pattern from nucleus to nucleus. Sections of chromosomes from different nuclei are aligned and selected fluorescent regions are marked to orient the viewer. d marks the most distal sections of chromosome arms X, 2L and 3L; p designates the proximal part. Fluorescence staining and visualization was done as described in Fig. 2. Scale bar, 10 μm.

preparations. None of the normal rabbit sera which we have used nor incubation with fluorescent labelled goat anti-rabbit antibody alone has produced staining of the polytene chromosomes. Thus, all our control experiments indicate that the antibody is recognizing the Z-conformation in the polytene chromosomes, as it does in the immunoprecipitation experiments.

The anti-Z-DNA antibodies produce a distinctive pattern of staining on the polytene chromosomes; the fluorescent segments seen on the chromosome vary both in size and intensity, but the size and staining intensity of any particular region is reproducible and appears to reflect the nature of the chromatin. Figure 3 shows several examples of chromosomal segments from different nuclei. The reproducibility of the staining pattern is most conveniently seen when the two homologues that make up a chromosome have become unpaired along a part of their length. Figure 4 shows two examples that demonstrate the similarity of the staining pattern in the asynapsed chromosomes.

Some of the fluorescence photographs in Figs 2–4 were exposed for long periods to give a stronger fluorescent signal. However, photographs were also taken with a shorter exposure so that the image would be in the linear range of the film response, and here, the darker non-fluorescent segments were as dark as the general background. From analyses of negatives of these photographs using a densitometer tracing to look at the variations in the amplitude, we estimate that the intensity of different fluorescent segments roughly varies by a factor of 5–10 in brightness.

To determine which segments of the *Drosophila* chromosome are fluorescing, we compared the pattern of bands seen in phase microscopic photographs with the fluorescence pattern (Fig. 5). Photographs of segments from various parts of the *Drosophila* chromosome were cut along the chromosomal axis and the phase photographs were compared with the fluorescence photographs. Figure 5 shows that the fluorescent segments are the interbands and not the bands. The bright areas in the fluorescence photograph are found in the position of the light interband regions in the phase photographs. Note that there is not necessarily a correlation between the width of the interband segment and its fluorescent intensity.

Thus, the antibody binding results shown in Fig. 1, together with the negative controls, clearly demonstrate that we are using an antibody that is specific for the Z-DNA helix. It reacted with three polymers only in distinct conditions required for each to occur in the Z conformation, and did not react with *E. coli* DNA in any of these conditions, nor with single-stranded DNA and a variety of polynucleotides, including brominated polymers. The bromination was not required either for induction of Z-specific antibody or for its serological reactivity. The antibody that binds to polytene chromosomes shows the same properties as those measured in the binding experiments; chromosomal staining is blocked only by molecules that have a Z conformation and yield an immune precipitate with the antibody.

Tests for effects of experimental procedures

As the chromosomes in almost all cytological preparations have been subjected to treatments that remove or alter cellular components, this raises the question of whether sample preparation induces formation of Z-DNA in the polytene chromosomes. At present, the only way we can approach this question is by testing the effects of various preparative procedures, and we have therefore tested preparations from salivary glands that were incubated for 2–5 min at room temperature in 45% acetic acid, squashed in the same solution, frozen on dry ice and treated in various ways before incubation with antibody. After freezing and removal of the cover slips, the slides were: (1) placed directly in phosphate-buffered saline (PBS), or (2) immersed in ethanol, air dried and then placed in PBS, or (3) placed directly in a post-fixative solution containing 3.7% formaldehyde and then in PBS. Alternatively, glands were fixed by the formaldehyde technique of Silver and Elgin[12], squashed in 45% acetic acid with 10 mM $MgCl_2$ and frozen on dry ice, then immersed in ethanol and air dried before incubation with antibody in PBS.

These treatments might be expected to produce different types of artefact, yet all result in qualitatively the same staining pattern. The first procedure produces by far the most intense staining pattern and has been used as our standard preparative technique. Less intense staining occurred when formaldehyde was used for fixation and so further study will be required to determine whether bright staining was due to selective removal of proteins during exposure to 45% acetic acid. In the post-fixed preparations it is likely that the formaldehyde is itself blocking the antibody binding site.

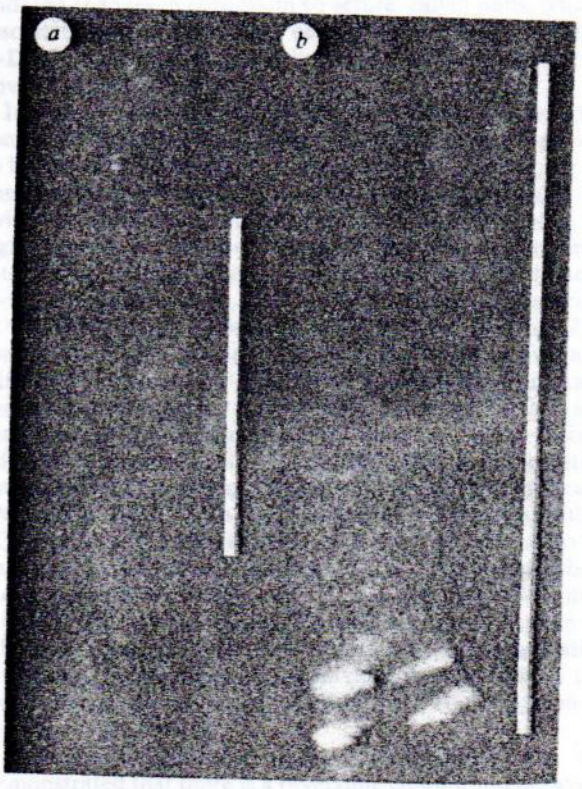

Fig. 4 Reproducibility of the anti-Z-DNA antibody staining pattern as seen in two examples of asynapsis (*a*, *b*). The unpaired homologues of a polytene chromosomal arm show very similar fluorescence staining in the homologous regions. Experimental protocol was as described in Fig. 2.

The polytene chromosome is segmented into bands and interbands. The bands vary in size, yielding a banding pattern that is invariant unless chromosomal rearrangements occur. These banding patterns are useful for genetic mapping studies and for studies of evolutionary relationships between species; however, their role in chromosome structure is unknown. Banded polytene chromosomes have also been reported in several other eukaryotic organisms including protozoa and plants[13]. It has been suggested that this banding might be a general property of the organization of the eukaryotic chromosome[13]. *Drosophila* has ~5,000 bands as well as interbands which have been identified and mapped. On average, a band plus interband contains some 30 kilobases of DNA[13]. The DNA in the interband has been estimated as ranging from 5% (ref. 13) to 30% (ref. 14) of the total DNA, depending on the method of measurement. Electron micrographs of *Drosophila* chromosomes show bands as containing darkly staining, densely clumped units while the interbands are visualized as elongated fibrils 50–60 Å in diameter[13]. Indirect immunofluorescence has revealed a number of proteins in the bands[15–17] and interbands[16–18].

An important question in any immunofluorescence experiment is whether the antigen is present but not accessible for reaction. This question is especially relevant to our experiments because staining is limited to the least compact regions of the chromosomes. Although anti-histone antibodies can cause brighter staining in the more compact regions than in interbands[15] it is possible that the DNA, or a particular portion of it, is not as available as the histones. Although we cannot rule out the presence of Z-DNA in the band regions, our experiments do show that it is either more abundant or more accessible in the interbands. Either of the alternatives is interesting and a decision between the alternatives may help explain the significance of chromosomal banding. This question is under further study with antibodies that react with other forms of DNA.

The reproducibility and uniformity of the staining pattern throughout the entire length of the polytene chromosome are remarkable. We have compared the staining pattern among different giant polytene chromosomes within an individual and between individuals. The staining pattern of Z-DNA antibodies appears to be a constant feature of the chromosomes in much the same way that the banding in the phase contrast microscope appears constant. This suggests that the pattern is an intrinsic property of the chromosome rather than, for example, a property induced through the mechanical action of squashing the chromosome, for then one would expect significant differences in the staining pattern in various parts of the chromosome, depending on the degree of mechanical stress to which each segment was subjected.

The intensity of staining in different interbands varies considerably, and seems to be unrelated to the width of the interband; the origin of the variability is not known. The possibility that the brighter segments contain a larger number of closely packed and unresolved band–interband units is unlikely, because we have occasionally seen extremely stretched regions in which the interbands are considerably extended and still do not break up into smaller fluorescent segments.

It would be interesting to know the proportion of the interband DNA that needs to be Z-DNA to produce the observed fluorescent pattern. This is difficult to estimate because we have no absolute standard which will allow us to determine accurately the amount of primary antibody bound from the amount of fluorescence detected. As a lower limit it is possible that only a small stretch of some 6–8 base pairs out of the 3 kilobase pairs in the average interband might be in the Z conformation. In a polytene chromosome, where 1–2,000 copies of such a sequence are lying in register, one would have enormous numbers of antibodies arrayed transversely across the interband. Because of the limited optical resolution in the light microscope and the broadening of the fluorescent image, one site would appear to occupy the entire 0.1 μm of an average interband. As an upper limit, the entire interband could be in the Z-DNA conformation. Although this seems implausible, further work will be required before we can make a more accurate estimate.

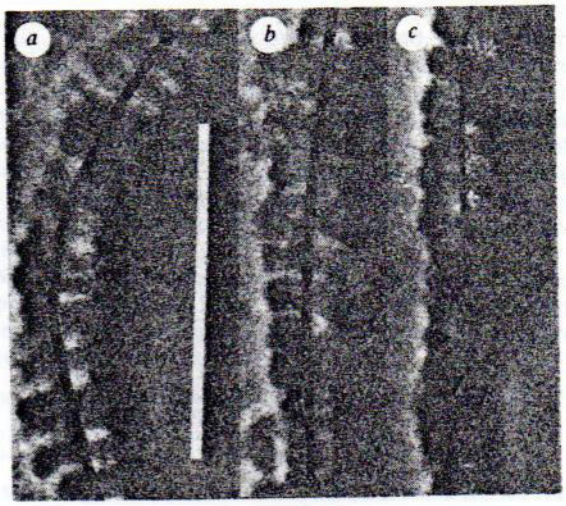

Fig. 5 Comparison of phase contrast and anti-Z-DNA antibody-stained fluorescent photographs of polytene chromosomes. Chromosome sections from three different nuclei (*a–c*) are shown. Analogous sections of the same chromosome are compared by cutting the photographs down the chromosomal axis and comparing the two halves. The light areas of the left phase contrast photographs correspond to the interbands while the darker sections are the bands. The white sections of the right darkfield fluorescent photographs show the position of the anti-Z-DNA fluorescent stained antibodies. It can be seen that the interbands are fluorescing. Note that the intensity of fluorescence is not necessarily related to the width of the interband. Fluorescence staining was done as described in Fig. 2 legend. Scale bar, 5 μm.

Possible functional significance of Z-DNA

Because the Z conformation is a reversible structural form of DNA it is an attractive candidate for having a regulatory role in genetic activity. Many regions of the polytene chromosomes undergo changes in chromatin structure, called puffs, which are associated with transcription of certain genes. The patterns of Z-DNA staining during such structural changes will be of obvious interest.

In physiological salt conditions the Z-DNA conformation is somewhat less stable than the B conformation and therefore has to be stabilized. There may be four ways of maintaining the Z conformation in biological systems. (1) Supercoiling. Z-DNA twists the double helix in a left-handed mode, opposite to the right-handed B-DNA conformation. Because of this one can interchange strongly negative supercoiled DNA for segments of Z-DNA (L. Peck, A. N., A. R. and J. C. Wang, in preparation). (2) Binding to proteins which are specific for the Z conformation. These probably involve electrostatic interactions with basic residues. (3) Binding to specific ions. For example, spermine or spermidine can stabilize crystals of Z-DNA so that they form with a regularity which yields an atomic resolution diffraction pattern[1,7]. (4) Modification such as methylation of cytosine in the 5 position (although not necessarily applicable to *Drosophila* DNA[19]). In the absence of methylation poly(dG-dC)·poly(dG-dC) requires 700 mM Mg^{2+} to convert to the Z conformation. Behe and Felsenfeld[11] have shown that when the methyl group is present, the Z conformation is formed in 0.6 mM Mg^{2+}. It is interesting that spermine is even more effective, forming the Z conformation at a concentration of 0.002 mM. Further work must be done before we fully understand the parameters which stabilize the Z conformation in the polytene chromosome.

Work with synthetic deoxypolynucleotides has amply demonstrated that there is a reversible equilibrium between the right-handed B conformation and the left-handed Z conformation in molecules in which there is an appropriate sequence. Recent work with cloned segments of poly(dG-dC)·poly(dG-dC) in plasmids have demonstrated that it is possible for these segments to undergo a B to Z transformation even though they

are enclosed in plasmid segments containing B-DNA (ref. 20 and L. Peck, A. N., A. R. and J. C. Wang, in preparation). It is thus reasonable to imagine that appropriate segments in chromatin might undergo similar changes in conformation. Z-DNA segments may have the property of not only changing the local environment near a particular gene, but through interaction with supercoiling these segments could modify the transcribability of DNA regions far removed from the site at which the Z-DNA segment is found.

The striking observation here is that an antibody specific to left-handed Z-DNA binds in a regular fashion to the interband regions of the *D. melanogaster* polytene chromosomes. Our control experiments strengthen the conclusion that this binding is due to a Z-DNA conformation found in the interbands. Band–interband units seem to be basic units of gene activity[13] replication[21] and perhaps chromosomal synapsis. It is tempting to suppose that Z-DNA might be a conformational switch which is involved in the control of transcription or of other of these activities. As Z-DNA appears to be a fundamental component of the polytene chromosome, these chromosomes may provide a unique opportunity for further exploration of the role of Z-DNA.

We thank Dr Roberta Farrel for assistance. This work was supported by grants from the NIH, NSF and the American Cancer Society. A.N. and A.M. are supported by grants from the Deutsche Forschungsgemeinschaft.

Received 3 August; accepted 22 October 1981.

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