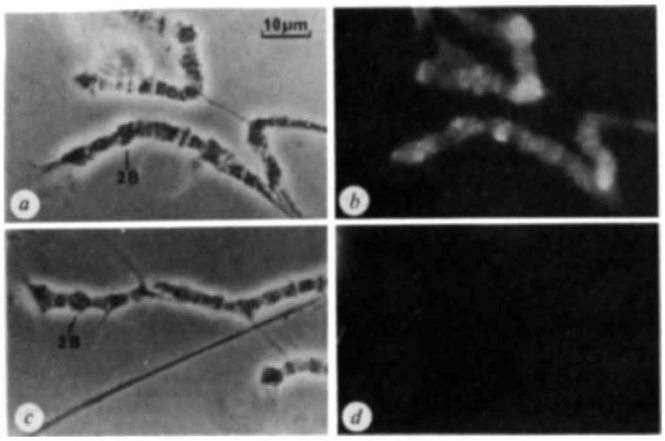

Fig. 3 The effect of topoisomerase I on Z-DNA antibody-binding. *a*, Phase contrast, and *b*, Z-DNA antibody-treated X chromosome that had been exposed to topoisomerase I (0.2 U μl^{-1} calf thymus enzyme for 30 min at 37 °C) and then 45% acetic acid (30 s). *c*, Phase contrast, and *d*, antibody-treated X chromosome that had been exposed to 45% acetic acid (30 s) followed by topoisomerase I. In *b*, the level of fluorescence is typical of an acid-treated chromosome. It is reduced to background in *d*.

from closed circular DNA extracted from SV40 chromatin[19]). It is of interest that Hamada *et al.*[23] have reported the equivalent of 2×10^3 copies of alternating dT-dG sequences, 50 bp long, with Z-DNA forming potential in the *Drosophila* genome. It has recently been shown[24,25] that such (dT-dG)–sequences can be driven from the B- to the Z-conformation by a superhelical density of 0.047.

It is quite possible that 45% acetic acid (apparent *p*H 1.6) may facilitate the transition from B to Z by a number of mechanisms in addition to providing torsional free energy. (1) In Z-DNA, the base pairs are rotated 180° away from the position they have in B-DNA[1]. Strand separation at lower *p*H may well facilitate this rotation. (2) In Z-DNA, guanosine is in the *syn* conformation[1]. There is optical evidence consistent with a change from the *anti* to the *syn* conformation for guanosine on protonation of the base[26]. (3) Protonation of the primary phosphates in DNA, which has begun by *p*H 1.6 (ref. 27), might favour the Z-conformation in which phosphate groups are closer than in the B[1]. (4) The incorporation of DNA into nucleosome core particles stabilizes the B-conformation relative to the Z[28]; conversely, release from a nucleosomal organization should intrinsically favour a B to Z transition.

In conclusion, we have demonstrated that: (1) Z-DNA antibodies display only background-level binding to native *Drosophila* polytene chromosomes; (2) the binding is massively enhanced by prior exposure to 45% acetic acid; (3) depending on the extent of acid exposure, binding can be localized primarily to interbands or bands; and (4) the binding is dependent on torsional strain of supercoiling that, like the binding itself, can only be detected subsequent to acid treatment. This is the first demonstration that the Z-conformation in a cytological preparation of eukaryotic chromosomes is dependent on torsional strain. We have also shown that antibodies reacting with B-DNA have access to DNA in the native chromosomes.

These findings indicate that a signficant proportion of DNA, in both bands and interbands, at least has a propensity to adopt a Z-conformation. However, the possibility that DNA, in chromatin, may be driven towards the Z-conformation by the liberation of torsional stress on the disruption of nucleosomes is a very real one, especially at low *p*H in acid fixatives. The fact that the torsional stress that may be generated on core histone extraction is in the range that can drive B to Z transitions, necessitates great care in extrapolating to *in vivo* conformations from the properties of chromosomes prepared in 45% acetic acid. Furthermore, the demonstration, described above, that variation in the conditions of acid fixation can lead to qualitative variation in the pattern of anti-Z antibody binding, offers a likely explanation for the paradox presented by different interband and band binding patterns observed in different laboratories to date[6–8]. We have also recently observed that exposure of native chromosomes to 95% ethanol also leads to Z-DNA antigenicity, although to a lesser extent than 45% acetic acid (unpublished data).

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1. Wang, A. H.-J. *et al. Nature* **282,** 680–686 (1979).
2. Wells, R. D. *et al. Prog. Nucleic Acid Res. molec. Biol.* **24,** 167–267 (1980).
3. Cantor, C. R. *Cell* **25,** 293–295 (1981).
4. Dickerson, R. E. *et al. Science* **216,** 475–485 (1982).
5. Watson, J. & Crick, F. H. C. *Nature* **171,** 737 (1953).
6. Nordheim, A. *et al. Nature* **294,** 417–422 (1981).
7. Arndt-Jovin, D. J. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
8. Lemeunier, F., Derbin, C., Malfoy, B., Leng, M. & Taillandier, E. *Expl Cell Res.* **141,** 508–513 (1982).
9. Painter, T. S. *Genetics* **19,** 175–188 (1934).
10. Hill, R. J. & Watt, F. *Cold Spring Harb. Symp. quant. Biol.* **42,** 859–865 (1978).
11. D'Angelo, E. G. *Biol. Bull.* **90,** 71–87 (1946).
12. Silver, L. M. & Elgin, S. C. R. in *The Cell Nucleus* Vol. 5 (ed. Busch, H.) 215–262 (Academic, New York, 1978).
13. Hewish, D. R. & Burgoyne, L. A. *Biochem. biophys. Res. Commun.* **52,** 504–510 (1973).
14. Mott, M. R., Burnett, E. J. & Hill, R. J. *J. Cell Sci.* **45,** 15–30 (1980).
15. Hill, R. J. *et al. J. Cell Biol.* **95,** 262–266 (1982).
16. Lafer, E. M., Moller, A., Nordheim, A., Stollar, B. D. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **78,** 3546–3550 (1981).
17. Nordheim, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7729–7733 (1982).
18. Dick, C. & Johns, E. W. *Expl Cell Res.* **51,** 626–632 (1968).
19. Germond, J. E., Hint, B., Oudet, P., Gross-Bellard, M. & Chambon, P. *Proc. natn. Acad. Sci. U.S.A.* **72,** 1843–1847 (1975).
20. Singleton, C. K., Klysik, J., Stirdivant, S. M. & Wells, R. D. *Nature* **299,** 312–316 (1982).
21. Peck, L. J., Nordheim, A., Rich, A. & Wang, J. C. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4560–4564 (1982).
22. Nordheim, A. *et al. Cell* **31,** 309–318 (1982).
23. Hamada, H., Petrino, M. G. & Kakunaga, T. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6465–6469 (1982).
24. Haniford, D. B. & Pulleyblank, D. E. *Nature* **302,** 632–634 (1983).
25. Nordheim, A. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1821–1825 (1983).
26. Guschlbaner, W. & Courtois, Y. *FEBS Lett.* **1,** 183–186 (1968).
27. Jordan, D. O. *The Chemistry of Nucleic Acids,* 206–216 (Butterworths, London, 1960).
28. Nickol, J., Behe, M. & Felsenfeld, G. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1771–1775 (1982).

Common site of integration of HTLV in cells of three patients with mature T-cell leukaemia–lymphoma: a retraction

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WE recently reported in this journal (Hahn *et al.* **303,** 253–256; 1983) that DNA from one fresh cell sample and two cell lines that are positive for HTLV contained provirus integration at the same site while 10 other HTLV-positive DNA samples did not. The conclusion was based on finding a rearranged DNA band that hybridized to a unique flanking cellular sequence called S2. In a note added in proof, we stated that an additional patient contained HTLV at the same locus. We have since carried out an extensive survey of HTLV-positive cells and have not seen a recurrence of a rearranged S2 band. In re-evaluating the early data, we found that the DNA samples that contained rearranged S2 sequences were contaminated by CR1, a recombinant phage containing HTLV and S2 sequences. This was not found in any of the other HTLV provirus-positive fresh cells or cultured lines.

Therefore, although we have evidence for HTLV provirus in many fresh leukaemic cell samples and cell lines, Wong-Staal *et al.*, *Nature* **302,** 626–628; 1983; and unpublished data), so far there is no evidence for a common integration site. Furthermore, the cell line HUT78 is provirus-negative.