Errata

Co-transfection of normal NIH/3T3 DNA and retroviral LTR sequences: a novel strategy for the detection of potential c-*onc* genes

by **R.Müller** and **D.Müller**

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An error appeared in the title of the original manuscript. The correct title is given above.

Immunological detection of left-handed Z DNA in isolated polytene chromosomes. Effects of ionic strength, pH, temperature and topological stress

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On page 722 of this article, the text in the left-hand column was inadvertently transposed. The correct text is presented opposite.

the following advantages: (i) the signals obtained by immunofluorescence are amplified due to the high degree of polytenization; (ii) there exists detailed knowledge about the effects of ionic strength and pH on the structural state of the chromosomes (Robert, 1971; Lezzi and Robert, 1972); (iii) conditions are known for optimal transcriptional activity (Hameister, 1977; Mähr *et al.*, 1979; Sass, 1980); (iv) the ionic concentrations prevalent *in vivo* in the salivary gland nuclei have been measured (Kroeger *et al.*, 1973; Palmer and Civan, 1975); (v) the conditions necessary to deplete specific types of chromosomal proteins have been determined (Bastian, 1983); and (vi) numerous studies on the binding of anti-Z DNA antibodies using fixed preparations of the same chromosomes have been carried out (Jovin *et al.*, 1983a, 1983c; Robert-Nicoud *et al.*, 1983; Zarling *et al.*, 1984a, 1984b) including quantitative immunofluorescence microscopy (Arndt-Jovin *et al.*, 1983; Jovin *et al.*, 1983b). The latter studies have demonstrated that at saturating antibody concentrations, one antibody binds per 3000 – 15 000 bp in the bands and that the immunofluorescence pattern reflects the sequence specificity of the anti-Z DNA antibody. We have exploited the unfixed polytene chromosomes to investigate the influence on anti-Z DNA immunofluorescence of conditions known to effect the B→Z transition, such as ionic strength and temperature, protein extraction and DNA supercoiling. In parallel, we have studied the effect of acid pH on the B→Z transition of polynucleotides in solution and documented the consequences of acid treatment both on protein extraction from, and anti-Z DNA immunofluorescence in, the chromosomes so as to determine the role fixation plays in the frequency and distribution of Z DNA in natural sequences.

Results

Antibody binding to isolated polytene chromosomes at physiological ionic strength and pH

Isolated unfixed polytene chromosomes from insect larvae provide a unique system for studying the distribution of Z DNA sequences under conditions as close as possible to the physiological. However, in carrying out immunological experiments on these chromosomes, we found that the physical exposure of DNA determinants for antibody binding may change, depending on a variety of environmental factors. We have used polyclonal antibodies to histone H3 and a monoclonal antibody to histone H1 to assess the general degree of chromatin accessibility under given experimental conditions.

The immunofluorescence pattern obtained with anti-histone H3 antibodies in isolated polytene chromosomes at physiological pH and ionic strength is shown in Figure 1A. By serial focusing through the chromosome structure, we observe the fluorescence to be distributed at the periphery in a sheath-like fashion. Similar results are obtained with a monoclonal anti-histone H1 antibody.

A weak peripheral binding of anti-Z DNA antibodies is also seen after indirect staining of isolated polytene chromosomes in the same medium (Figure 1B,C). Use of direct fluorescein-labeled anti-Z DNA antibodies shows the same fluorescence distribution suggesting that a limited penetrability of the first antibody may be the primary cause of the observed outside binding. It follows that this pattern is unlikely to reflect the actual distribution of Z DNA tracts in the chromosomes. Therefore, we tested a variety of factors which might enhance the accessibility of determinants in chromosomal substructures.