

in buffer. The chromosomes were located by phase contrast microscopy and then the fluorescent reaction was observed in a beam of ultraviolet light using the appropriate exciter and barrier filters.

Antiserum produced against the extract of nonbasic proteins reacted specifically with the chromosomes and more intensely with the loops than with the chromomeres. The antiserum produced against histone reacted primarily with the chromomeres (Fig. 3d). Control preparations using normal rabbit serum reacted nonspecifically with all structures, nucleoli as well as chromosomes, but only at a relatively high serum concentration. The greatest serum dilution at which a fluorescent reaction could be obtained (see Table 1) was routinely used to avoid cross reaction and nonspecific effects. There was some reaction with the loops with anti-histone serum but this is most likely to result from contamination of histone extract with some nonbasic protein. In fact a few minor bands of high molecular weight protein could be seen on the electropherogram (Fig. 2). Low concentrations of histones may well be present in the loops but the technique used here is probably not sensitive enough to detect these.

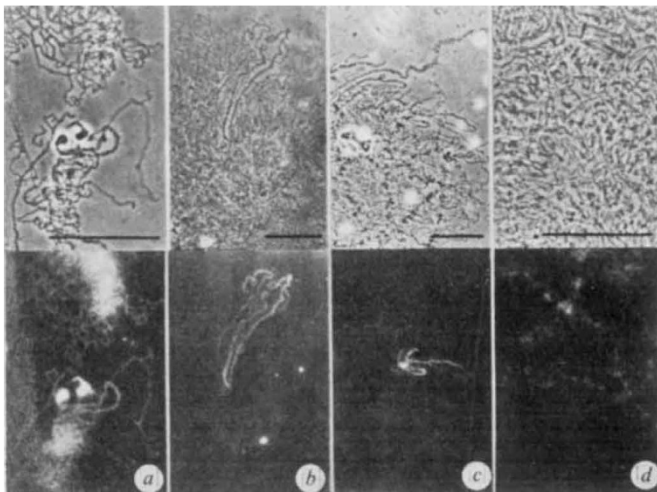


Fig. 3 Phase contrast and fluorescence photomicrographs showing location and specificity of reaction of fluorescein-conjugated antibodies with lampbrush chromosomes. *a*, Antiserum produced against RNP-protein fraction 2 at a concentration of 1:1,000; *b* and *c*, antiserum against fraction 3 at a concentration of 1:1,250; *d*, antiserum against histone at a concentration of 1:1,000. The photomicrographs were taken on Ilford FP4 (1 s exposure for phase; 2.5 min exposure for fluorescence) and developed in Paterson's Acutol. The bars represent 10 μ m.

Of the protein fractions derived from solubilised RNP-fractions 1, 2 and 4 stimulated antibodies which reacted specifically with all the chromosome loops (Fig. 3a), that is, one or more of the proteins present in these fractions were common to the RNP matrix of all loops. As a result of the distribution of different polypeptides between these fractions (see Fig. 1 and Table 1) at least two different proteins are common to all loops. Antibodies produced against fraction 4 showed some cross reaction with the chromomeres. Furthermore, antibodies against fraction 5 reacted specifically with the chromomeric axis. Fraction 5 gave a very similar banding pattern on SDS-acrylamide gels to that of histone (Fig. 2). Because this fraction was not present in all RNP protein extracts (Fig. 1) fraction 5 was judged to be contaminating histone probably as a result of excessive homogenisation of the oocytes.

The most interesting and unexpected result was given by antiserum produced against fraction 3. This antiserum reacted with the proteins associated with only certain loops—approximately 10 loop pairs in each chromosome complement. These loops did not have any peculiar morphology and could not be

identified before the fluorescent reaction (Fig. 3b and c). As far as can be discerned the same loops give this specific reaction in all chromosome preparations and are randomly distributed. Because the whole length of spatially isolated loops gives a specific reaction this is conclusive evidence of the integrity of the loop structure, that is, the lampbrush loop is a functional unit. Evidence that the loop is a unit of transcription will be reported later (J.S., unpublished work). The protein which is the main component of fraction 3 and which probably elicits the specific loop reaction has a molecular weight of approximately 35,000.

Thus histones are present primarily in the regions of condensed DNA (chromomeres) in lampbrush chromosomes (previously shown to contain basic protein by staining¹⁰ while RNP-derived nonbasic proteins are restricted to the loops. The implications of the high protein content of nuclear RNP remains unknown. Preliminary evidence suggests that various types of enzymic activity are present, specifically the ability to polymerise ribonucleotide triphosphates and to cleave large nuclear RNA into smaller fragments. Homoribonucleotide polymerases¹¹ and endonuclease¹² have already been reported to be present in mammalian RNP particles. Obviously enzymes concerned with the processing of primary transcript RNA can only account for a very small amount of protein which may even not be visible in gel electropherograms. A secondary RNP formed during oocyte maturation has been isolated and has been shown to have a similar protein constitution to the RNP described here. Results pertaining to the protein content of the different RNP products at various stages in the processing of RNA transcripts will be reported later.

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Erratum

In the article "Deterioration of high school students' attitudes to physics" by P. L. Gardner (*Nature*, **250**, 465; 1974) the following corrections should be made to the 3rd paragraph: line 5, for 4 yr read 2 yr; lines 10-11 should read "in coeducational State high schools in moderately affluent suburban areas of Melbourne."