LETTERS TO NATURE

DNA Loss during Robertsonian Fusion in Studies of the Tobacco Mouse

THE centric fusion of two telocentric chromosomes to form a metacentric chromosome, described by Robertson¹, is one of the basic mechanisms for altering the karyotype of eukaryotes. In conjunction with other processes, such as geographical isolation, it is frequently one of the initial steps in the formation of new species. Electron microscopy of mammalian chromosomes has suggested that both of the centromere regions may be retained during the fusion process². A similar conclusion can be drawn on the basis of staining of the centromeric heterochromatin³. In spite of the retention of these regions it is likely that the fusion process is basically a reciprocal translocation event resulting from a crossover within the chromatin fibres that go to make up the centromeric region. The reciprocal product is presumably lost as it is too small to possess a kinetochore.

Until recently, there has been no way to measure the quantity of DNA lost during this process, but two observations now allow this to be assessed. The first is the observation that the DNA of centromeric heterochromatin of the mouse is predominantly satellite DNA^{4,5}. This normally constitutes approximately 10% of the mouse genome. The second is the report of a new species of mouse, the tobacco mouse, or *Mus poschiavinus*. This species was found in a geographical isolate in Switzerland and is cytologically different from *Mus musculus* in that there are seven metacentric chromosomes resulting from the Robertsonian fusion of fourteen telocentric chromosomes⁶. If significant amounts of DNA are lost during the fusion process there should be a detectable decrease in the concentration of the centromeric satellite DNA.

To test this, DNA was isolated from the livers of *Mus musculus* and *Mus poschiavinus* and the amount of satellite DNA was determined by analytical ultracentrifugation (Fig. 1). Enlarged tracings of the photographs were made with a Joyce-Loebl microdensitometer, and the amount of satellite DNA measured using a DuPont 310 curve resolver. These techniques would readily allow the detection of a 10% decrease in satellite DNA (that is, 9.5 to 8.5%) and on repeated samples could detect a 5% decrease. From 6 to 9 μ g of both types of DNA was simultaneously centrifuged utilizing a double cell rotor with mask and timer. Three different runs were made and multiple tracings analysed from each run. From thirty such tracings (fifteen each) *Mus musculus* showed 9.46% (s.d. 0.35) satellite DNA and *Mus poschiavinus* showed 9.58% (s.d. 0.17). These values were not significantly different.

This suggests that less than 0.5% of the genome is lost following the occurrence of seven Robertsonian fusions or less than 1.5×10^7 nucleotide pairs per fusion. This would be equivalent to 5,100 μ m of DNA, or 100 μ m of chromatin, assuming a packing ratio of 50 lengths of DNA to 1 length of chromatin⁷. This would be equivalent to less than the size of the smallest of avian microchromosomes and would probably be invisible to light microscopy (unpublished results of D. E. C. and T. A. Okada).

Fig. 1 Analytical ultracentrifugation of DNA from *Mus musculus* (N) and the tobacco mouse, *Mus poschiavinus* (T). The methods of DNA isolation, ultracentrifugation and analysis by curve resolving have been described elsewhere⁸.

The occasional loss of detectable amounts of centromeric DNA during Robertsonian fusion has been shown by Chen and Ruddle³ utilizing heterochromatic staining of chromosomes of some mouse cell lines (LMTK-). In one metacentric chromosome most of the centromeric heterochromatin was missing. Since this would represent a loss of less than 4% of the satellite DNA it would not have been detected by the technique that we used.

Even though relatively small amounts of DNA seem to be deleted during the process of centric fusion, the loss of as little as 1 μ m of DNA would be harmful if that DNA contained essential genes. It is not unlikely that the positioning of non-essential repetitive satellite DNA of the centromere plays a role in protecting the organism from deleterious effects of even a small loss of DNA⁸.

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