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Cattanach's Translocation: Cytological Characterization by Quinacrine Mustard Staining

(X chromosome/autosome/translocation/*Mus musculus*/fluorochrome staining)

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ABSTRACT **Metaphase chromosomes of mice carrying Cattanach's translocation, which is the deletion of material from a medium-sized autosome and its insertion into an X chromosome, were stained with quinacrine mustard. Comparison of the fluorescence patterns of these chromosomes with those of the chromosomes of normal mice has allowed the identification of the autosome involved in the translocation and localization of the transposed material within the X chromosome. Since the material inserted into the X chromosome in Cattanach's translocation is known to carry part of linkage group I, we are now able to assign linkage group I to a specific chromosome pair of the normal fluorescent karyotype of the mouse.**

The mitotic metaphase chromosomes of the mouse exhibit virtually no morphological characteristics that would allow them to be individually identified. However, when they are stained with quinacrine mustard and observed under the fluorescence microscope (1), they display prominent characteristic patterns of bright and dark bands. These patterns have permitted the individual identification of every pair in the mouse complement (2, 3). Miller *et al.* (2) have published a fluorescent karyotype for the mouse based on approximate relative length. We (3) measured the chromosomes of many independently arranged fluorescent karyotypes derived from several different inbred and random bred mice, and presented a standard karyotype, where the chromosomes are arranged according to decreasing mean relative length.

Cattanach's translocation (4), *T(1;X)Ct*, involves the transposition of material from an autosome into an X chromosome, so that this X (X^t) becomes the longest chromosome in the complement. This insertional type of translocation must have resulted from at least three breaks. Genetic evidence indicates that the autosomal material involved contains part of the middle region of linkage group I, and that it is inserted into an interstitial location in the X^t chromosome. There is no evidence for a reciprocal translocation of X material to the autosome. Male mice carrying X^t and females heterozygous or homozygous for X^t are viable whether they carry the deleted autosome or a normal autosomal complement. In this study, we have applied the quinacrine mustard staining technique to the chromosomes of mice that carry an X^t chromosome and either A^t or normal autosomes, in an attempt to locate the site of insertion of the transposed material into the X chromosome and to identify the autosome involved.

Abbreviations: X^t, the X chromosome with inserted autosomal material; A^t, the autosome from which material has been deleted.

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MATERIALS AND METHODS

Two adult male mice hemizygous for X^t and two adult heterozygous females were studied, in addition to two 15-day fetuses from the mating of an X^t heterozygote carrying A^t with a normal male. Cell cultures from the adult mice were started by trypsinization of lung fragments. Initiation of fetal cultures, culture conditions, methods of chromosome preparation, quinacrine mustard staining, fluorescence microscopy, and photography have all been described elsewhere (3).

Several karyotypes were prepared for each of the individuals studied; profiles of the distribution of fluorescent intensity along the chromosomes of interest were obtained by the preparation of Polaroid transparencies of the chromosomes and the scanning of these transparencies on a Joyce–Loebl microdensitometer. Length differences between X and X^t chromosomes, and between the A^t autosome and its normal homolog were determined by measurement of these chromosomes on 33 photographs of known enlargement.

RESULTS

One of the fetuses had a fluorescent karyotype indistinguishable from that of a normal male mouse. Cells of the second fetus and the four adults all contained a large chromosome not present in normal mouse cells (Figs. 1, 2). The two adult males and the second fetus did not have a normal X chromosome, but did have the relatively bright, characteristic Y (Fig. 1). The cells of the adult females contained only one normal X (Fig. 2). Comparison of the normal X with the X^t (Fig. 3) shows that some of the inserted material is rather brightly fluorescent and is located between one-half and two-thirds of the way from the centromere end. The breakpoint in the X chromosome appears to be in the region indicated in Fig. 3. The normal X and X^t both have bright distal regions, but the dark band immediately proximal to the bright distal region is wider in X^t than in the normal X. Just proximal to the translocated material is a dark band of the same size as the distal dark band of the normal X chromosome. Thus, the break in the normal X chromosome appears to have occurred distal to the narrow distal dark band.

The cells of one of the females and one of the adult males contained completely normal autosomal complements (Fig. 2). The cells of the second fetus and the other adults contained an extra small chromosome, which resembles pair no. 17 of the

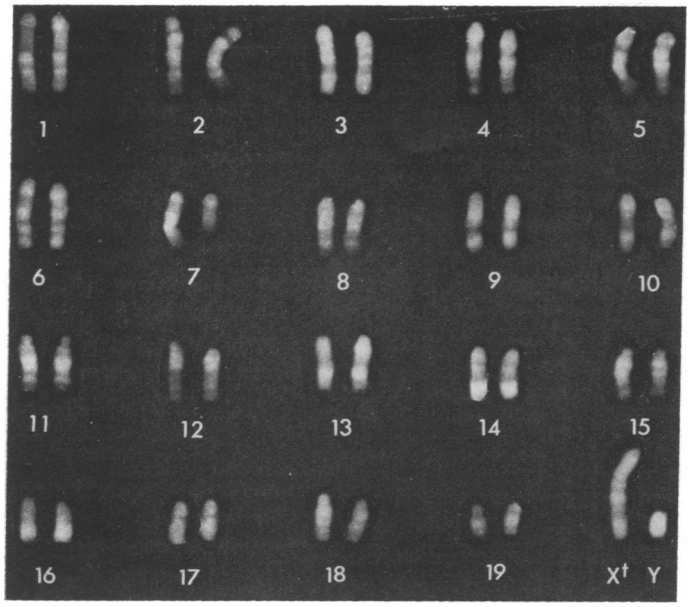

FIG. 1. Fluorescent karyotype of male mouse carrying Cattanach's translocation. Note deletion of bright material from middle of one member of pair no. 7. Karyotype is arranged in the order that we proposed (3).

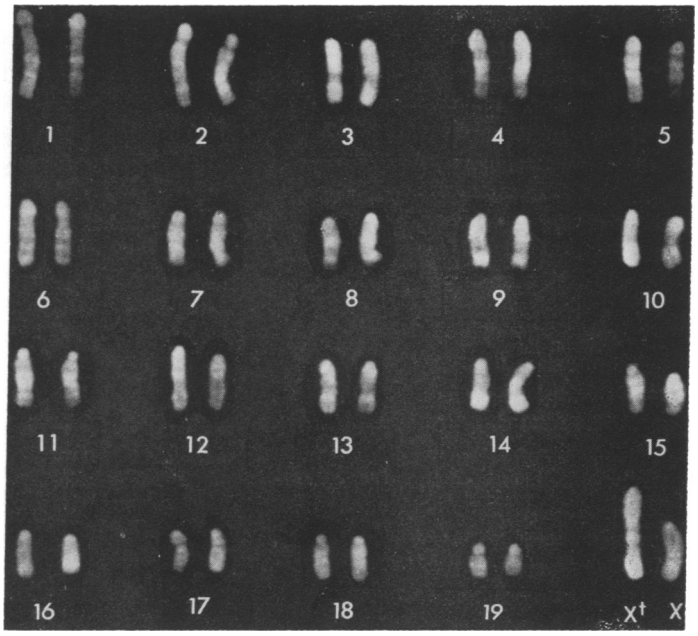

FIG. 2. Fluorescent karyotype of female translocation heterozygote. Note normal pair no. 7. Karyotype is arranged in the order that we proposed (3).

normal fluorescent karyotype (3), and lacked one member of pair no. 7 (Fig. 1). The extra small chromosome appears to be a no. 7, with most of the brighter material deleted from the middle region. There is commonly a dark band near the centromere in pair no. 7 that is apparently fully retained in the extra small chromosome (Figs. 1 and 3). The material inserted into the X chromosome being relatively bright is compatible with it being the middle region of a no. 7 chromosome. From our examination of X^t chromosomes, it appears that one of the breaks in the no. 7 must be in a dark region and, since a small amount of bright material remains in the deleted no. 7, the second break must have occurred in a bright region (assuming the translocation is indeed nonreciprocal). Since the proximal dark band does not seem to be reduced in the deleted no. 7, we conclude that the dark transposed material originates from the distal region. Proposed locations for the breakpoints are shown in Fig. 3. If our interpretation of the breakpoints is correct, it predicts that the transposed autosomal material has not been inverted during translocation, that is, it has the same orientation relative to the centromere of X^t that it had to the centromere of no. 7.

Fluorescent intensity distribution profiles for the normal X, normal no. 7, X^t, and 7^t chromosomes are shown in Fig. 4. The shapes of these curves support the interpretation that material from the middle region of a chromosome no. 7 has been inserted into an X chromosome in Cattanach's translocation.

Measurements of the chromosomes of interest at ×1800 enlargement showed that the difference between the mean lengths of chromosomes X and X^t is about 2.8 mm, and between 7^t and the normal 7 is about 3.1 mm (Table 1).

TABLE 1. *Chromosome lengths**

Chromosome	Mean length (mm)
X^t	16.5 ± 0.96 (33)†
X	13.7 ± 0.58 (12)
No. 7	9.5 ± 0.47 (50)
7^t	6.4 ± 0.41 (14)

* Measured at ×1800 magnification; † Number of chromosomes measured.

DISCUSSION

The autosome from which the material transposed to the X chromosome in Cattanach's translocation has been derived is no. 7 of our normal fluorescent karyotype (3). Since the autosomal material involved in this translocation carries part of linkage group I, it follows that chromosome 7 carries linkage

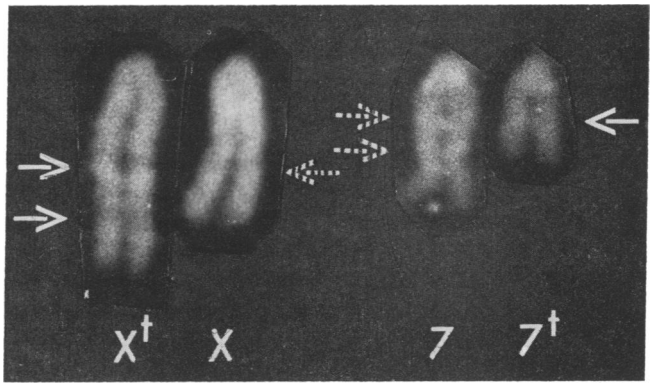

FIG. 3. Enlarged view of X and X^t chromosomes, and the normal and deleted no. 7 chromosomes from a cell from a translocation heterozygote. *Dotted arrows* point to normal chromosomes, and indicate regions where breaks must have originally occurred to give rise to Cattanach's translocation. *Solid arrows* point to the rearranged chromosomes. In the case of 7^t, the *arrow* points to the region of healing of broken ends after the deletion of material. In the case of the X^t chromosome, the *arrows* designate the limits of the transposed material.

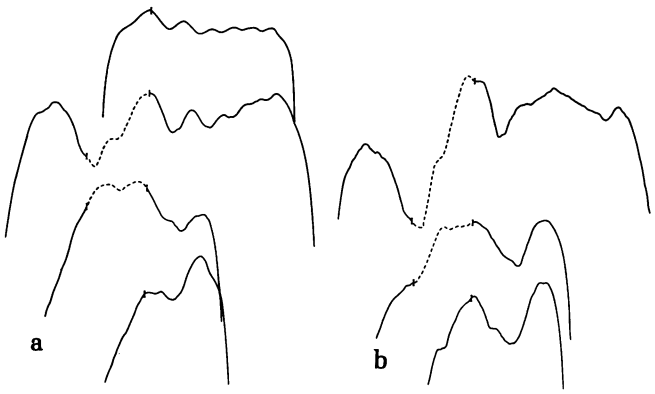

FIG. 4. Fluorescent intensity distributions. All curves are presented with the region representing the centromere area of the chromosome to the right. Regions of the curves thought to represent the material involved in the transposition are shown with *broken lines*. The curves in group *a* represent, from the top, X, X^t, 7, 7^t chromosomes from a cell from a female translocation heterozygote. The group in part *b* contains, from the top, X^t, 7, and 7^t chromosomes from a cell of a male translocation carrier.

group I. This corresponds to chromosome 8 in the arrangement of Miller *et al.* (2). One of the breakpoints in linkage group I is known to lie in the two map-unit interval between the shaker-1 locus and the hemoglobin β chain locus, *sh-1* being included in the transposed material (5), and *Hbb* not included (6). The other breakpoint lies somewhere between the ruby eye-2 locus, which is included in the transposition, and the quivering locus, which is not included (7). The transposed autosomal material is, thus, about 20–30 map-units long. Measurement of the length differences between 7 and 7^t and between X and X^t chromosomes on the photographs, and then correction for the enlargement factor, leads us to estimate that the transposed fragment is on the average about 1.6 μm long. This amounts to about 12–18 map units/μm in chromosomes in an average state of contraction, or between 50–80 nm (500–800 Å) per map unit. Somewhat shorter estimates of the lengths of X (13 mm) and X^t (15 mm) chromosomes at $\times$1800 enlargement were obtained by Nesbitt and Gartler (8). After fluorescent staining, moderately elongated chromosomes generally show clearer banding patterns than do contracted chromosomes, and our selection of cells for the study may have caused us to overestimate average chromosome lengths to some extent.

The breakpoint in the X chromosome has been shown to be very close to the jimpy (*jp*) locus (9), though it is not known whether this locus is proximal or distal to the translocated material in X^t. This locus must then be located near the proximal edge of the bright distal region in the X chromosome, as indicated in Fig. 3. Linkage maps of linkage groups I and XX (the X chromosome) that show the loci whose locations have been discussed in this paper, can be found in (10).

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