CYTOLOGICAL IDENTIFICATION OF THE CHROMOSOMES INVOLVED IN SEARLE'S TRANSLOCATION AND THE LOCATION OF THE CENTROMERE IN THE X CHROMOSOME OF THE MOUSE*

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ABSTRACT

The autosome in Searle's X-autosome translocation has been shown to be chromosome 16. The breakpoint in chromosome 16 is slightly proximal to the middle and in the X is slightly distal to the middle.——Available evidence indicates that either Linkage Group XV or Linkage Group XIX is carried on chromosome 16.——The centromere of the X chromosome is at the *spf* end of the linkage group.

THE *T(X;?)16H* translocation in the mouse, commonly known as Searle's translocation, involves a reciprocal exchange between the X chromosome and a small autosome (SEARLE 1962; LYON *et al.* 1964; EICHER 1970). Genetically, *T(X;?)16H* (hereafter *T16*) is unique among mouse X-autosomal translocations in that it gives the appearance of interfering with the normal process of X-inactivation. In *T16*/+ females the sex-linked allele on the translocated X appears completely dominant over its allele located on the intact X chromosome; it is as if the X involved in the translocation is the active X in most or all cells. This dominance effect has been noted for the four X-linked loci *Ta* (tabby), *Bn* (bent-tail), *Blo* (blotchy), and *spf* (sparse-fur) (LYON 1963, 1966a; LYON *et al.* 1964). Since the order of genes in the X chromosome is *spf—Bn—T16* breakpoint *—Ta—Blo* (LYON *et al.* 1964; LYON 1966b), the effect occurs in regions both distal and proximal to the breakpoint.

The autosomal linkage group involved in *T16* has not yet been identified. We report here the cytological identification of the autosome involved in *T16* (chromosome 16). Since chromosome 16 is one of the six chromosomes not yet assigned a linkage group (COMMITTEE ON STANDARD GENETIC NOMENCLATURE FOR MICE 1972), the autosomal linkage group remains unknown.

MATERIALS AND METHODS

Chromosome preparations were made from bone marrow cells of non-tabby (*T16* +/Y) males

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from the cross of *T16* +/+ *Ta* ♀ × + *Ta*/Y ♂ using a 30 min colchicine treatment followed by hypotonic treatment and fixation in 1:3 glacial acetic acid:absolute methanol. The cells were flame or air-dried onto slides and stained with quinacrine mustard as described by FRANCKE and NESBITT (1971a). In all, 11 metaphase plates were photographed and karyotyped. Chromosome images from polaroid transparencies of enlargements of metaphase plates were scanned on a Joyce Loebl microdensitometer to produce curves representing the distribution of fluorescent intensity along each chromosome. Maxima on the curves correspond to those regions with the most fluorescence.

As recommended by the COMMITTEE ON STANDARDIZED GENETIC NOMENCLATURE FOR MICE (1972), italicized bold-faced numbers within the parentheses of translocation symbols refer to chromosome numbers; i.e., *T(**14**;**15**)6Ca* is a translocation involving chromosomes 14 and 15. When the numbers within the parentheses are only italicized, they refer to linkage group numbers; i.e., *T(3;?)6Ca* is the same translocation as that given above but 3 refers to Linkage Group III carried on chromosome 14 whereas the other linkage group, here unknown, is carried on chromosome 15.

RESULTS AND DISCUSSION

None of the cells examined contained a normal X chromosome. Instead, there was a chromosome resembling an X but with its distal region shortened and uniformly brightly fluorescent rather than showing the distally decreasing fluorescence of the normal X (Figure 1). The distal region of this chromosome resembled the distal region of a normal chromosome 16.

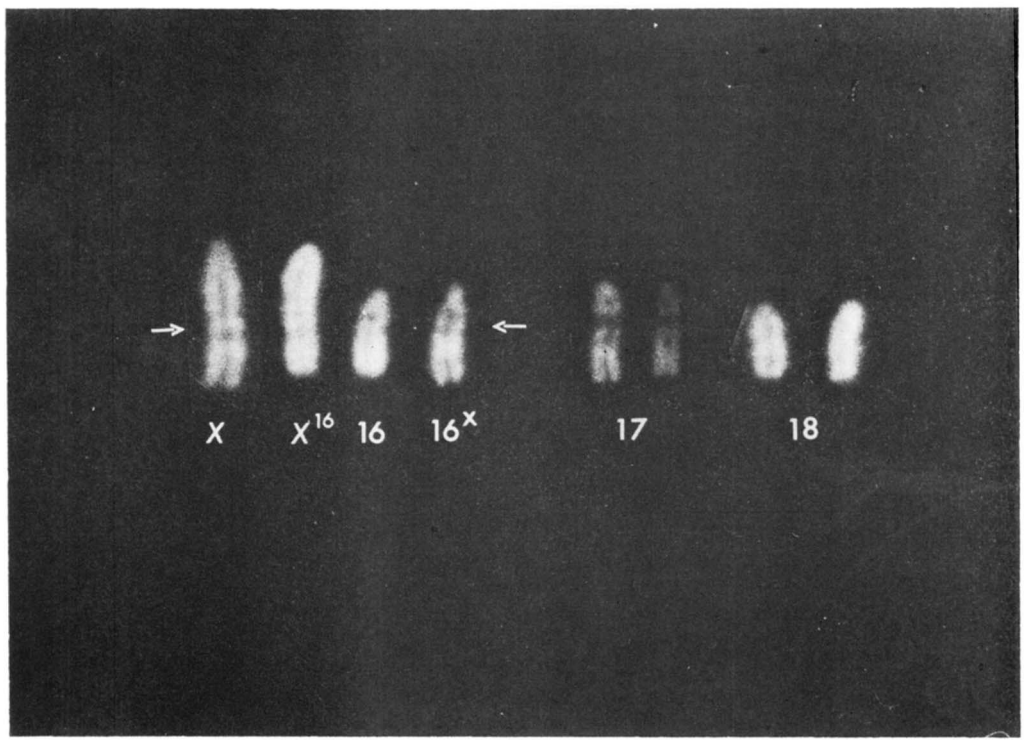

FIGURE 1.—Chromosomes X^{16}, 16, 16^{X}, 17 and 18 from the cell of a *T16*/Y male. A typical normal X chromosome from a CBA/J female is included for comparison. The normal chromosome pairs 17 and 18 are included because, by size, they could be confused with chromosome 16. The arrows indicate the breakpoint regions in the X and 16^{X}. Magnification: 2600×.

Only one normal chromosome 16 could be found in each cell; the other autosomes 17 and 18, which might have been involved in *T16* as expected because of their size, were each represented by two apparently normal chromosomes. In addition, there was a small abnormal chromosome with the proximal region of 16 and a lengthened bright distal region characteristic of the distal region of the normal X (Figure 1).

We therefore interpret *T16* as being a reciprocal translocation between the X chromosome and chromosome 16. To comply with the suggestions of the COMMITTEE (1972), SEARLE's translocation can now be designated as ***T(X;16)16H***.

Figure 2 shows fluorescence intensity profiles of the normal X chromosome, the *T16* X with the distal region of a 16 (hereafter X^{16}), the normal 16, and the *T16* 16 with the distal region of an X (hereafter 16^{X}). Comparison of X, X^{16}, 16, and 16^{X}, as shown in Figure 1, with the intensity profiles suggests the breakpoints are as indicated by the arrows in Figure 1.

FORD and EVANS (1964) described the breakpoints in *T16* as near the middle of the autosome and slightly distal to the middle in the X chromosome. OHNO and LYON (1965) described the breakpoints in *T16* as distal in the autosome and in the middle of the X chromosome (see EICHER 1970 for a discussion of this subject). Our data tend to support the observations of FORD and EVANS as to the location of the breakpoint in the X but disagree with both groups as to the location of the breakpoint in the autosome, since the breakpoint in 16 is proximal to the middle. Because both groups agreed that the translocated chromosome that paired with the Y chromosome was smaller than the reciprocal product, and we now know that the Y chromosome pairs at its distal (non-centromere) end with

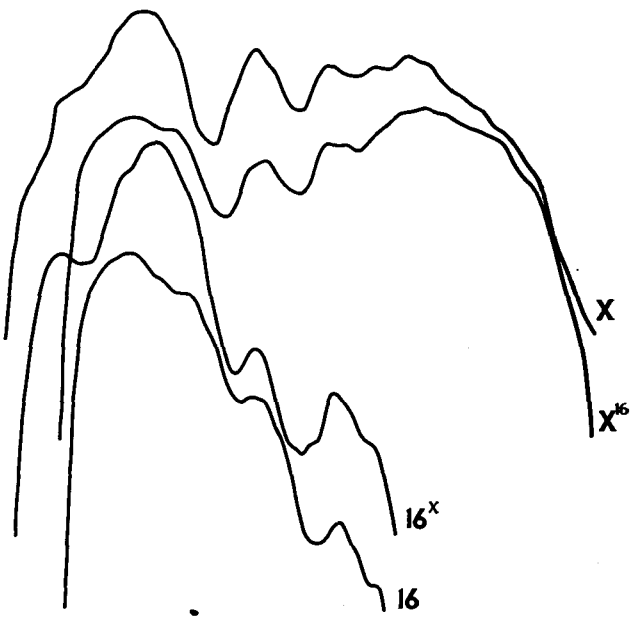

FIGURE 2.—Intensity profile of chromosomes X, X^{16}, 16^{X}, and 16 from Figure 1. Centromeres are at the right. Homologous regions are aligned.

the Y chromosome (HSU *et al.* 1971; EICHER and ZWERLING, unpublished data), we would expect the smallest translocated chromosome to be chromosome 16^{x}. This is in fact what we observed.

Cytologically, the breakpoint in the X chromosome in *T16* is more proximal to the centromere than that for Cattanach's translocation *T(7;X)Ct* (hereafter *TCt*; formerly *T(1;X)Ct*) as described by FRANCKE and NESBITT (1971b). Since the order of genes in the X is *spf—Bn—T16—Ta—(TCt, Gy)* (CATTANACH 1966), the cytological evidence combined with the genetic evidence confirms that the centromere of the *X* is near the *spf* rather than *Gy* (Gyro) end of the linkage group (EICHER 1970; SEARLE and BEECHEY 1971; MILLER *et al.* 1971).

OHNO and LYON (1965) calculated that the autosome involved in *T16* should be 1.33 times the length of the Y chromosome. Their measurements were made on meiotic chromosomes from *T16* males. Measurements of mitotic chromosomes showed that chromosome 16 is 1.39 (3.82/2.75) times the length of the Y (COMMITTEE 1972). As determined by quinacrine fluorescent staining, the conclusion that *T16* involves chromosome 16 agrees very well with the relative size for the autosome as determined by OHNO and LYON.

The only mouse linkage groups (LG) still unassigned to chromosomes are IV, VI, VII, XV, XVI, and XIX. No genes are known for LG XIX. The chromosomes still unassigned to linkage groups are 3, 11, 12, 15, 16 and 18. Using the data obtained by FRANCKE and NESBITT (1971b) and KOURI *et al.* (1971) on the *TCt* translocation, it is possible to estimate the expected genetic length of chromosome 16 and thus eliminate some linkage groups for chromosome 16.

TCt involves an insertion from the middle region of chromosome 7 (LG I) into the X chromosome (CATTANACH 1961; EICHER 1971). The insertion includes the genetic region in chromosome 7 from *sh-1* (shaker-1) to *ru-2* (ruby-2) (EICHER 1967, 1970) representing from 20 to 30 cM. The present known genetic length of chromosome 7 is approximately 60 cM. FRANCKE and NESBITT (1971b) and KOURI *et al.* (1971) concluded that approximately one-third of chromosome 7 was involved in the translocation. One-third of chromosome 7 contains 1.81 (5.43/3) percent of the mitotic haploid complement (MHC) (COMMITTEE 1972). Assuming that the known genes in chromosome 7 are evenly dispersed along its length, one percent MHC would contain from 11.1 to 16.6 cM (20/1.81 or 30/1.81). Since chromosome 16 represents 3.86% MHC, it is expected to contain from 43 to 64 cM.

It is unlikely that either Linkage Groups IV or VII are carried on chromosome 16: *T16* was checked for linkage to steel (*Sl*, LG IV) and rex (*Re*, LG VII) (LYON *et al.* 1964) both of which occupy central locations, and would therefore be expected to show linkage to the central *T16* breakpoint. The gene *Va* (varitint-waddler) in LG XVI was tested for linkage to *T16* with negative results (LYON, personal communication). LG XVI contains 31 cM if *Va is* located near the end of LG XVI. LG XVI contains 60 cM if *Va* is located near the middle of LG XVI (GREEN 1971). Whichever is the case, if LG XVI is carried on chromosome 16, linkage to *Va* would be expected. With regards to LG VI, the gene *Ca* (caracul), located near one end (GREEN 1971), showed no linkage to *T16* (SEARLE, personal

communication). Since the physical breakpoint in chromosome 16 is slightly proximal and LG VI contains 54 cM (GREEN 1971), even if *Ca* is at one extreme end of the chromosome, linkage of it to *T16* would have been expected.

GREEN (1963), using the chiasmata counts of SLIZYNSKI (see CARTER 1954) and CREW and KOLLER (1932), estimated that the total autosomal map length was approximately 2000 cM. The total autosomal percent MHC equals 94.82 (100—MHC of X). Thus, one percent MHC equals 21.3 cM. On this basis, chromosome 16 is expected to contain 82.3 cM (3.86 × 21.3). Linkage Groups IV and VII are still most likely not carried on chromosome 16 because of the failure of linkage of *T16* with *Sl* and *Re*. Linkage Group VI could be carried on chromosome 16 only if its now distal gene *uw* (underwhite) is actually central (GREEN 1971). The argument regarding independence of *T16* and LG XVI is the same as given in the previous paragraph.

We believe the evidence favors the likelihood that LG XV or XIX is carried on chromosome 16.

One other translocation involves chromosome 16. This is *T7Bnr*, one of the seven metacentric chromosomes of the tobacco mouse (*Mus poschiavinus*) (ZECH *et al.* 1972). *T7Bnr* is the translocation involving LG IX (chromosome 17) that KLEIN isolated (1971) and called *T1*. This metacentric chromosome has been shown to involve chromosomes 16 and 17 (EICHER and KLEIN unpublished data; ZECH *et al.* 1972). Thus, if our conclusions are correct, *T16* and *T7Bnr* have a chromosome (linkage group) in common.

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