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Assignment of Four Linkage Groups to Chromosomes in *Mus musculus* and a Cytogenetic Method for Locating Their Centromeric Ends

(linkage group/centromeres/fluorescent-banding patterns/quinacrine mustard)

D. A. MILLER, R. E. KOURI*, V. G. DEV, M. S. GREWAL, J. J. HUTTON*, AND O. J. MILLER

Departments of Human Genetics and Development, and of Obstetrics and Gynecology, Columbia University, New York, N.Y. 10032; and * The Roche Institute of Molecular Biology, Nutley, N.J. 07110

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ABSTRACT The mitotic chromosomes in primary cultured cells from mouse embryos of the translocation stocks, *T(5;18)26H*, *T(5;11)7Ca*, and *T(14;17)264Ca*, have been identified by their distinctive fluorescent-banding patterns after staining with quinacrine mustard. In this way, linkage group (LG) V has been assigned to chromosome 2, LGXVIII to chromosome 9, and LGXIV and LGXVII to either of chromosomes 3 or 13. The assignment of LGXI to chromosome 6 has been confirmed. The centromeres of chromosomes 1 (LGXIII), 2 (LGV), 9 (LGXVIII), and 16 (LGIX) have been located at the ends nearest the genes fuzzy (*fz*), Danforth's short tail (*Sd*), nervous (*nr*), and *T* of their respective linkage groups by a cytogenetic method. The centromere of the X (LGXX) has been tentatively assigned to the end nearest to the scurfy (*sf*) locus.

Recently, using the technique of Caspersson and associates (1), we have shown that each chromosome in the mitotic complement of the mouse, *Mus musculus*, can be identified by its distinctive fluorescent-banding pattern after staining with quinacrine mustard (2). Using this technique and a series of translocations, we have assigned mouse linkage-group (LG) I to chromosome 8, LGII to 10, LGIII to 12 or 15, LGIX to 16, LGXI to 6, LGXII to 19, and LGXIII to 1, and confirmed the identification of the X chromosome using Cattanach's translocation (2, 3).

We report here four more linkage-group assignments and tentative determination of the centromeric end of five linkage groups by a cytogenetic method.

MATERIALS AND METHODS

Mice of *T(5;11)7Ca* and *T(5;18)26H* homozygous translocation stocks were obtained from the M.R.C. Radiobiological Research Unit, Harwell, through the courtesy of Dr. A. G. Searle. Mice of the *T(14;17)264Ca* heterozygous translocation stock were purchased from the Jackson Laboratory, Bar Harbor, Maine. These will be called *T7*, *T26*, and *T264*, respectively. All three translocations were radiation-induced. The origins (4), genetics (5), and configuration of the pachytene stage of meiosis (6) of the *T7* and *T264* translocations have been described. The genetics of *T26* has been described by Searle (7). The mice carrying the translocation were mated to (C57BL/6J × DBA/2J)F_1 normal mice, and 11- to 14-day embryos were used to establish primary cultures. The breeding of mice, establishment of primary cultures, and preparation of metaphase spreads were done at The Roche Institute of Molecular Biology, while quinacrine mustard staining, fluorescence photomicrography, and karyotyping were done at Columbia University, as described in detail in our earlier report (2).

RESULTS AND DISCUSSION

Nearly 250 quinacrine-stained metaphases were photographed and more than 60 karyotypes were prepared of cells carrying these three translocations. The translocation in *T7* involves chromosomes 2 and 6 (Fig. 1). The breakpoint in chromosome 2 is farther from the distal end than that in chromosome 6. The translocation chromosome with a no. 2 centromere is of the same length as the normal no. 6 chromosome, while the reciprocal translocation chromosome is the same length as the normal no. 2. The translocation in *T26* involves chromosomes 2 and 9 (Fig. 2). The breakpoint in chromosome 2 is close to the distal end, while that in chromosome 9 is close to the centromeric end. The unequal exchange has produced a very long chromosome with the centromere of a no. 2 and a very short chromosome with the centromere of a no. 9. The translocation in *T264* involves chromosomes 3 and 13 (Fig. 3). About equal segments have been exchanged, representing roughly a third of chromosome

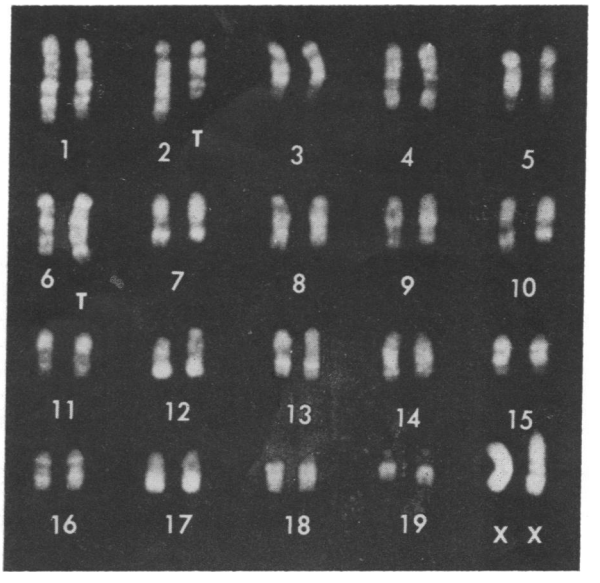

FIG. 1. Karyotype of a cell showing the *T (5;11)7Ca* translocation chromosomes. The distal portions of chromosomes 2 and 6 have been interchanged.

Abbreviation: LG, linkage group.

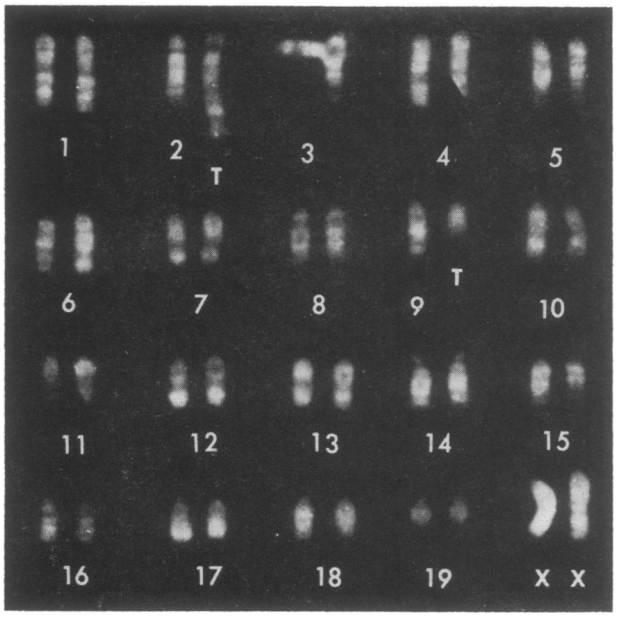

FIG. 2. Karyotype of a cell showing the *T(5;18)26H* translocation chromosomes. The distal portion of chromosome 9 is located at the distal end of chromosome 2.

3 and half of chromosome 13. Chromosomes 10 and 13 are indistinguishable; we previously assigned LGII to chromosome 10 (2). If LGII does not include either LGXIV or XVII, which are present in *T264*, one of these two linkage groups must be on the other chromosome, no. 13.

By correlating these cytologic findings with knowledge of the linkage groups involved in these translocations (Fig. 4), it is possible to assign several of the linkage groups to specific chromosomes. *T7* and *T26* have chromosome 2 and

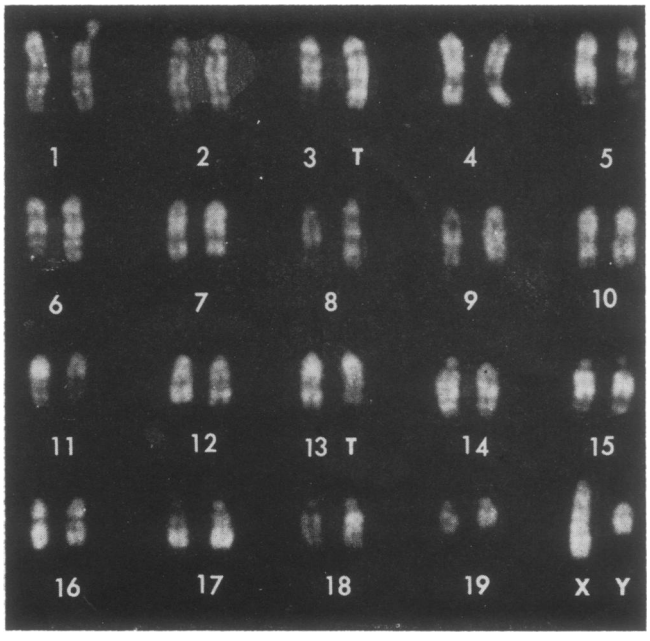

FIG. 3. Karyotype of a cell showing the *T(14;17)264Ca* translocation. The distal ends of chromosomes 3 and 13 have been interchanged.

LGV in common; therefore, LGV is on chromosome 2. The other linkage group involved in *T26*, namely LGXVIII, must be on the remaining chromosome, no. 9. Similarly, in *T7*, LGXI must be on chromosome 6. This assignment of LGXI to chromosome 6 confirms our previous findings with the *T(XI;?)1A1d* translocation (2) (Fig. 4). Gropp and his associates showed, by meiotic studies in F_1 males from a cross of *M. poschiavinus* × AKR/*T1A1d-T1A1d*, that the two chromosomes involved in the *T1A1d* translocation are carried by two different metacentric chromosomes in the tobacco mouse, *M. poschiavinus* (8). Therefore, the two chromosomes involved in *T1A1d*, no. 6 and 15 (2), must each be carried by a different *M. poschiavinus* metacentric chromosome. Recently, Klein has shown that the *H-2* locus, which is part of LGIX (chromosome 16, ref. 2), is carried by one of the *M. poschiavinus* metacentric chromosomes (9), so that 3 of the 14 mouse acrocentric chromosomes that are involved in the Robertsonian translocation chromosomes in *M. poschiavinus* have been identified.

T264 contains chromosomes 3 and 13, and LGXIV and XVII. A more exact assignment of either linkage group involved in *T264* is not yet possible, because there are no overlapping translocations or other cytological markers. Table 1 contains a summary of the linkage-group assignments that we have made using this approach.

In the past, it has been difficult to determine at which end of any linkage group the centromere is located; various approaches to this problem have been developed. Ford *et al.* (10, 11) have obtained information in a particularly favorable case from meiotic studies of translocation heterozygotes. Roderick (12) has suggested the use of paracentric inversions, measuring the relative length of bridges at anaphase of the first meiotic division. Lyon *et al.* (13) have used simple genetic-linkage analysis in mice bearing submetacentric (centric-fusion type) translocations. Searle has developed a powerful genetic method for locating the centromeric end of a linkage group, based on analysis of recombinants among the progeny of translocation intercrosses (matings between mice heterozygous for the same reciprocal translocation) (7).

The quinacrine-fluorescence technique permits a cytogenetic approach to this problem. If the breakpoint in a translocation chromosome is near either the centromeric or distal end, and if the position of the breakpoint in the linkage group is known, it is possible by correlating these findings

TABLE 1. *Chromosome assignment of linkage groups*

Linkage group	Chromosome	Ref.
I	8	3
II	10*	2
III	12 or 15	2
V	2	
IX	16	2
XI	6	2
XII	19	2
XIII	1	2
XIV	3 or 13*	
XVII	3 or 13*	
XVIII	9	
XX	X	3

* 10 and 13 are indistinguishable, and are arbitrarily assigned.

to determine which end of the linkage group is near the centromere. In the case of *T26*, the breakpoint in chromosome 2 is near the distal end (Fig. 5). In the corresponding LGV, the breakpoint is much closer to the end with ragged (*Ra*) as the distal marker than to the end with Danforth's short tail (*Sd*) as the distal marker (7). Therefore, the centromere of LGV can be assigned to the *Sd* end. This is in agreement with the findings of Ford and associates (10) and of Searle (7). *T26* involves chromosome 9, in which the breakpoint is near the centromeric end of the chromosome (Fig. 5). This breakpoint has been mapped close to the quinky (*Q*) locus of LGXVIII (14), closer to the end with the distal marker nervous (*nr*) than the end with the sombre (E^{so}) marker. The centromere can therefore be tentatively assigned to the *nr* end of LGXVIII, confirming the conclusion of Searle and Beechey (14).

In a previous report (2), we showed that *T(9;13)190Ca* involves chromosomes 1 and 16. We found that the breakpoint in chromosome 1 is located far from the centromere (Fig. 5). It is also far from the fuzzy (*fz*) locus on LGXIII (5), indicating that the centromere of LGXIII is at the *fz* end. This confirms the findings of Searle and Beechey (15). The Cattanach translocation, *T(1;X)Ct*, involves the cytologically identifiable chromosomes 8 and X (3). The breakpoint in the X chromosome falls rather far from the centromere in the distal portion of the arm; the breakpoint has been mapped (16) quite far from the scurfy (*sf*) distal marker, and quite near the distal marker at the other end, gyro (*Gy*). We therefore conclude that the centromere lies at the *sf* end of this chromosome.

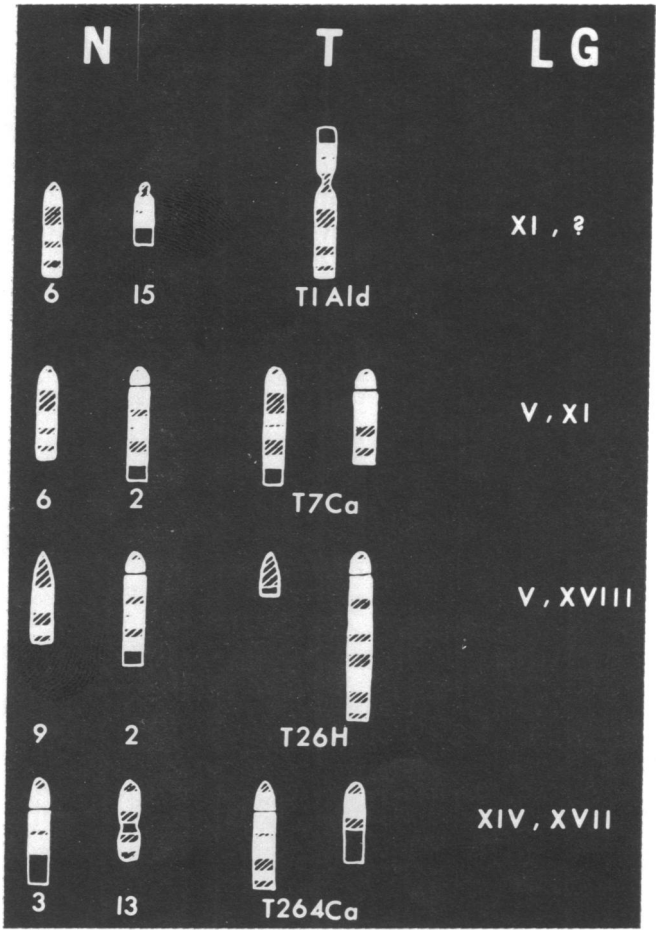

FIG. 4. Diagram of the translocations. *N* represents the normal chromosomes. *T* represents the translocation chromosomes. *LG* indicates the linkage groups involved in the translocation. A karyotype of a *T1Ald* cell was published previously (2).

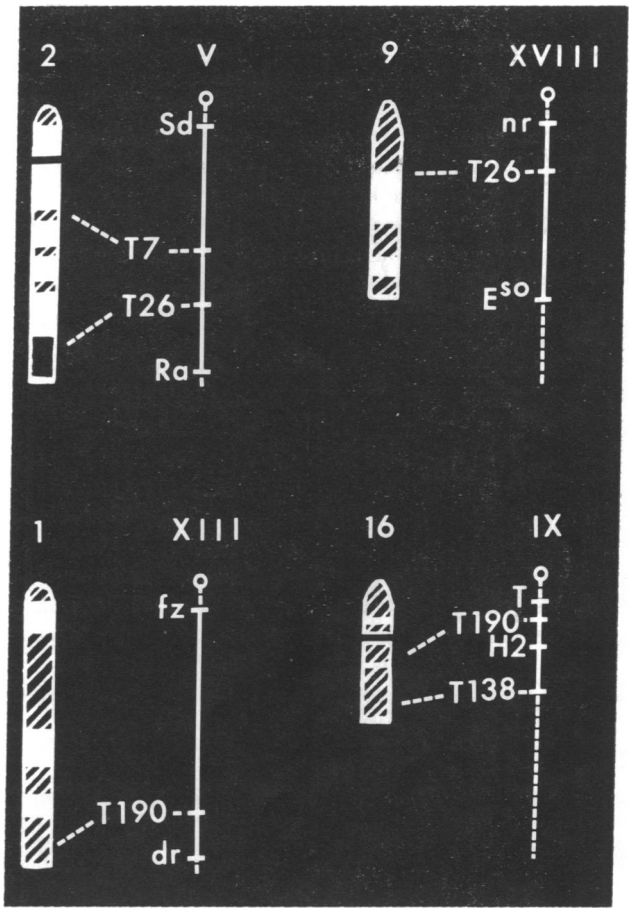

FIG. 5. Diagram showing the approximate locations of the breakpoints of the translocations. The figure to the left of each pair shows the location of the breakpoint in the chromosome. The four chromosomes have been drawn to the same relative length, based on our measurements. The figure to the right of each pair shows the breakpoint in relation to the known distal genetic markers of the linkage group. The linkage groups have been drawn to the same relative scale, based on published map distances. See text for method of determining the centromeric end of the linkage group, indicated by a small circle.

This one-breakpoint method of identifying the centromeric end of a linkage group has several drawbacks. Cytological assessment of the position of a breakpoint is inexact and only translocations in which the breakpoint is located close to one or the other end of the chromosome give unambiguous information. The accuracy of the method also depends on the completeness of the linkage map and the accuracy of assignment of the breakpoint within it.

An alternative method, which does not have these drawbacks, can be used if two translocations involving the same chromosome and having breakpoints sufficiently far apart to be recognized cytologically are available. It is possible to correlate the position of these breakpoints, relative to the centromere, with the position of the breakpoints in the linkage group to indicate which end of the linkage group is the centromeric end. *T7* and *T26* both involve chromosome 2, with

the breakpoint in the *T7* being closer to the centromere than that in *T26*. *T7* and *T26* both involve LGV, with the breakpoint in *T7* (5) closer to the *Sd* end of the linkage group than that in *T26*. Therefore, the *Sd* end of the linkage group must be the centromeric end (Fig. 5). This is in agreement with the findings of the first method described above. The *T(9;13)-190Ca* and *T(2;9)138Ca* have chromosome 16 and LGIX in common (2). The breakpoint of *T190* is closer to the centromere of chromosome 16 than is that of *T138*, and the breakpoint of *T190* is closer to the *T* locus of LGIX than is that of *T138* (5) (Fig. 5). Therefore, the centromere is at the *T* end of LGIX. This is in accord with the conclusion of Lyon and her associates (13), based on linkage studies in the *T(2;12)163H* and *T(2;9)138Ca* translocation stocks.

This two-breakpoint method for identifying the centromeric end of a linkage group should be generally useful. All that is required is that two translocations involving the same chromosome have breakpoints that are sufficiently far apart to permit cytological recognition of their positions relative to the centromere, and that the breakpoints be mapped with sufficient accuracy to determine their order along the linkage group. The utility of translocations in assigning linkage groups to specific chromosomes is now well established, whether one examines meiotic configurations in double translocation heterozygotes (13), or mitotic chromosomes by the methods used in the present study.

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