THE IDENTIFICATION OF THE CHROMOSOME BEARING LINKAGE GROUP XII IN THE MOUSE*

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Received June 21, 1971

TO date, it has been impossible to assign specific linkage groups of the mouse to specific chromosomes because of the lack of distinguishing morphological characteristics among the chromosomes. The 40 acrocentric chromosomes (2n=40) all blend into a continuous array when arranged by size. Several workers have reported that a few chromosomes show subtle morphological differences (FORD *et al.* 1956; STICH and HSU 1960; CHU and MONESI 1960; LEVAN, HSU and STICH 1962; FORD and WOOLLAM 1963; CRIPPA 1964; STEVENS and BUNKER 1964; BENNETT 1965; FORD 1966a and b; and GRIFFEN 1966). C. E. FORD (1966b) has characterized three pairs of autosomes, 14, 18, and 19, in mitotic chromosomes from CBA/H mice; FORD reports that each of these three pairs, and occasionally pair 15 (personal communication), has a proximal secondary constriction near its centromere. It is unknown whether these regions are true secondary constrictions (sites of ribosomal RNA synthesis). We have called these regions negative-staining heteropycnotic regions (NHR). For a detailed description of pairs 18 and 19, see FORD (1966a).

In this study we have used two translocations, both known to involve a common linkage group and both having one of the easily identifiable chromosomes, to identify a specific chromosome with a specific linkage group. In addition, one other translocation was analyzed to strengthen our conclusions.

Translocation *T(2;12)163H* (hereafter *T163*) was first cytologically described by EVANS, LYON and DAGLISH (1967) as the result of centric fusion of a medium-sized and the shortest autosome (19). Mice homozygous for *T163* have 38 chromosomes (2 submetacentrics and 36 acrocentrics). EVANS *et al.* showed that the Linkage Group (L.G.) II gene *se* was linked to *T163*. A report by LYON, BUTLER and KEMP (1968), suggested that the long arm of the *T163* submetacentric chromosome carried L.G. II. Later LYON (1969) reported that one of the arms of the *T163* submetacentric chromosome, probably the short arm, carried L.G. XII.

Translocation *T(1;12)145H* (hereafter *T145*) was first cytologically described by LYON and MEREDITH (1966) as a reciprocal translocation. *T145*/+ mice have one morphologically distinguishable chromosome which is slightly smaller than

* Supported in part by a U. S. Atomic Energy Commission Contract AT(30-1)-2902, Public Health Service Training Grant 5 T1 GM 658, and an allocation from NIH General Research Support Grant RR-05545 from the Division of Research Resources to The Jackson Laboratory.

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the Y chromosome. The involvement of L.G. I in *T145* was given in the list of translocations from HARWELL in 1967 (MNL 1967). Later, LYON (1969) reported that L.G. XII was the other linkage group involved. Since *T145*/+ males are sterile, the translocation is maintained in females in the heterozygous state.

Translocation *T(1;X)Ct* (hereafter *TCt*), commonly called Cattanach's or the *flecked* translocation, is a nonreciprocal translocation between the chromosome bearing L.G. I and the X chromosome (see EICHER 1970, for a more detailed description). In *TCt*, a region from the middle of L.G. I has been inserted into the X chromosome (X^{fd} chromosome). The resulting deficient autosome I is called autosome I^{Df}.

In this study we analyzed mitotic chromosome preparations from bone marrow cells of mice carrying the translocations *T163*, *T145*, and *TCt*. Evidence is presented to show that chromosome 19 carries L.G. XII.

In addition, we present data on the time of DNA replication of chromosomes 14, 18, and 19, showing that chromosome 19 is the first chromosome to complete its DNA replication.

MATERIALS AND METHODS

Chromosome preparations from bone marrow cells of males *T163/T163*, *T163*/+, X^{fd}/Y I/I *T163*/+, X^{fd}/Y I/I, and *T145*/+ and their normal +/+ sibs were prepared using hypotonic treatment followed by fixation in 1:3 glacial acetic acid:absolute methanol. Cells were air-dried on slides and stained with Giemsa or carbol-fuchsin.

For the DNA replication study, four X^{fd}/+ I/I^{Df} and three X^{fd}/0 I/I females were injected with 50 μC ^{3}H-thymidine (6C/mM, Schwartz BioResearch), injected one hour later with 0.5 ml of a 1% colchicine solution, and killed by cervical dislocation one hour after colchicine treatment. Slides of bone marrow cells were processed as given above. Well-spread metaphases were located and photographed. The slides were dipped in NTB-2 liquid emulsion (Kodak), exposed for 28 days, and developed. Karyotypes of labeled metaphases were prepared and analyzed. (Detailed methods and results of this study can be found in EICHER 1967.)

RESULTS AND DISCUSSION

In chromosome preparations from bone marrow cells of *T163* mice we found that: (1) the short arm of the submetacentric chromosome displayed an NHR near the centromere; (2) cells from *T163/T163* mice lacked chromosome 19; and (3) cells from *T163*/+ mice had one chromosome 19 (Figure 1a).

In *T145* mice we found that: (1) cells from *T145*/+ mice had one chromosome 19, one larger than normal chromosome with an NHR near its centromere (larger than chromosome 14), and one extra small chromosome about the size of the Y chromosome (Figure 1c and 1d); and (2) cells from the normal sibs had two normal chromosomes 19, and no unusually large chromosome with an NHR nor an extra small chromosome (Figure 1b).

In mitotic metaphases from X^{fd}/Y I/I *T163*/+ we found that: (1) there was one normal appearing very large chromosome, the X^{fd} chromosome; (2) there was one submetacentric chromosome (*T163*) which had an NHR on its short arm; and (3) there was one normal chromosome 19 (Figure 1a). Mitotic meta-

phases from X^{fd}/Y I/I males showed that two normal chromosomes 19 and the large X^{fd} chromosome were present.

Karyotypes from X^{fd}/X^{fd} I/I^{Df} and $X^{fd}/0$ I/I females showed that: (1) both kinds of females had two normal chromosomes 19; (2) females X^{fd}/X^{fd} had two normal appearing very large chromosomes (X^{fd}) while females $X^{fd}/0$ had one normal appearing very large chromosome; and (3) females X^{fd}/X^{fd} had an extra small chromosome present which never displayed an NHR and which never was seen in the $X^{fd}/0$ females. This small chromosome was assumed to be I^{Df}. Chromosome I could not be identified.

Since the X^{fd} and I^{Df} chromosomes in *TCt* do not involve chromosome 19, whereas both *T163* and *T145* involve chromosome 19 and share a common linkage group (L.G. XII), we conclude that L.G. XII is carried on chromosome 19, the smallest autosome observed in mitotic cells. Furthermore, we conclude that chromosome 19 is the short arm of the *T163* submetacentric chromosome, and that it has contributed its centromere and NHR to the large chromosome with the NHR seen in *T145*/+ cells. The extra small chromosome seen in *T145*/+ cells contains the centromere region of L.G. I.

A total of 56 tritiated thymidine labeled metaphases from X^{fd}/X^{fd} I/I^{Df} females and 62 labeled metaphases from $X^{fd}/0$ I/I females were analyzed. Data from both kinds of females were combined. Seventy-three of the 118 labeled cells were sufficiently labeled to be analyzed with respect to the labeling patterns in chromosomes 14, 18, and 19.

Of these 73 labeled metaphases, 25 had chromosomes 14, 18, and 19 labeled, 32 had chromosomes 14 and 18 labeled, and 16 had chromosomes 14 labeled.

We conclude that, of the three chromosomes which normally display an NHR, chromosome 19 finishes its DNA replication first and chromosome 14 finishes its DNA replication last. Furthermore, it was noted that chromosome 19 was the first chromosome of all the chromosomes to finish its DNA replication; in metaphases which had only one pair of chromosomes unlabeled, this pair was always number 19.

I wish to thank Dr. MARY F. LYON for making *T163* and *T145* available. Dr. MARGARET C. GREEN and Dr. THOMAS H. RODERICK gave helpful comments regarding the manuscript.

SUMMARY

In the mouse, chromosome 19 has been shown to carry L.G. XII. It is the smallest autosome seen in mitotic metaphase plates and usually has a negative-staining heteropycnotic region near its centromere. Chromosome 19 is the first autosome to finish its DNA replication.

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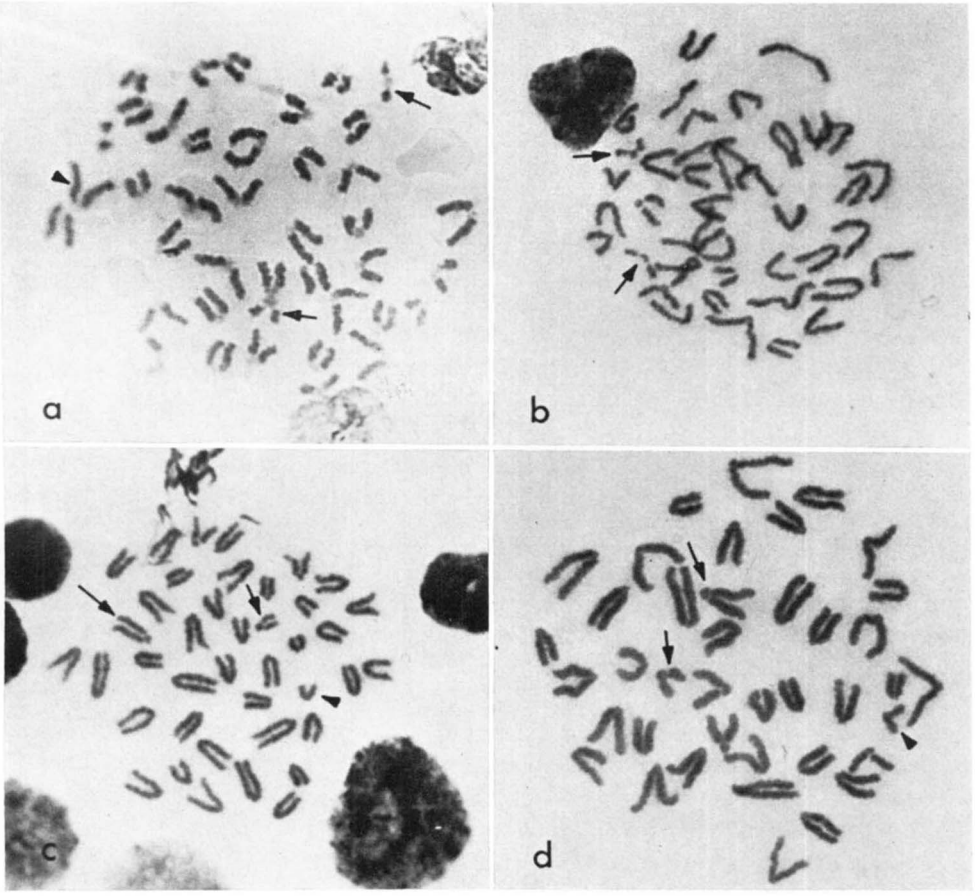

FIGURE 1.—Mitotic metaphases from bone marrow cells. a. X^{fd}/Y I/I *T163*/+. Arrows designate chromosome 19 involved in *T163* submetacentric chromosome and normal chromosome 19. The arrow head indicates the X^{fd} chromosome. b. Normal Male. Arrows designate normal chromosomes 19. c. and d. +/Y *T145*/+. Arrows designate the large chromosome with NHR and normal chromosome 19. The arrow head points to the small marker chromosome. Magnification: approximately 1600 ×.

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