QUINACRINE FLUORESCENT CHROMOSOME ANALYSIS OF THE SNELL TRANSLOCATION IN THE MOUSE

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ABSTRACT

The chromosomes involved in the $T(2;4)Sn$ (formerly designated $T(5;8)Sn$) or Snell translocation in the mouse have been identified as numbers 2 and 4 by analysis of the fluorescent banding patterns of quinacrine mustard-stained chromosomes in primary cultures from heterozygous and homozygous embryos.

IN 1934, SNELL, BODEMANN and HOLLANDER reported the presence of abnormal embryos in female mice which had been mated to sons of X-irradiated males with reduced fertility and they suggested that the reduced fertility was due to radiation-induced translocations. A homozygous translocation stock was developed from one of the mice and is still maintained. These mice have a reciprocal translocation between the two chromosomes carrying Linkage Groups (LG) V and VIII (SNELL 1946), and for some years the line has been designated as $T(5;8)aSn$ or more recently $T(5;8)Sn$. The breakpoint in LG V is about 1 centimorgan (cM) from the non-agouti, *a*, locus and about 19 cM from the pallid, *pa*, locus. The breakpoint in LG VIII is about 21 cM from the misty, *m*, locus and about 29 cM from the brown, *b*, locus in the order breakpoint-*m-b* (SNELL 1946). SNELL observed two brown, pallid mice among 513 progeny of matings of brown females $\times$ pallid males (and vice versa), all heterozygous for the translocation, i.e. matings of the type $+TSn\ b/+ + b \times pa\ TSn\ +/pa\ + +$. Such homozygous progeny could only have arisen by nondisjunction in each parent, giving rise to two unbalanced gametes which complement each other to produce an offspring with a balanced karyotype. From this, SNELL concluded that the pallid and brown loci are on different translocation chromosomes and therefore are on opposite arms in the meiotic pairing configuration. Thus, if the centromere is at the *pa* end of LG V, the centromere must be at the *b* end of LG VIII. Conversely, if *pa* is at the distal end of LG V, *b* is at the distal end of LG VIII.

A further step was possible following meiotic analysis. SLIZYNSKI (1952) reported the presence of typical translocation configurations in 16 of 33 male

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mice heterozygous for the SNELL translocation. FORD, CARTER and HAMERTON (1956) reported in an abstract that such males formed quadrivalents in 8–30% of the primary spermatocytes. The quadrivalents were invariably rings with four (or exceptionally, five) chiasmata at diplotene. Failure of association was said to occur in the distal, or non-centromeric arms, presumably because each break-point is fairly near the end of the chromosome. Since SNELL (1946) reported more than 30% recombination between the *a* locus of LG V and the *b* locus of LG VIII in this translocation, *a* marking the breakpoint, *b* must lie in a centromeric arm of the other chromosome. FORD and his associates (1956) therefore concluded that the centromeres are at the *Sd* (Danforth's short tail) end of LG V and the *b* end of LG VIII. The assignment of the centromere to the *Sd* end of LG V has recently been confirmed by two other methods (MILLER *et al.* 1971a; SEARLE, FORD and BEECHEY 1971).

Cytological identification of the chromosomes involved in the Snell translocation was not possible in mitotic chromosome preparations until quite recently. The development of the quinacrine fluorescent method of chromosome staining (CASPERSSON *et al.* 1968) now permits identification of each chromosome in the mitotic karyotype of the mouse (DEV *et al.* 1971; FRANCKE and NESBITT 1971a). We have applied this method to the study of reciprocal translocations in the mouse, and have used 18 translocation stocks in which two or more translocations involve the same linkage group to assign 14 of the 19 known linkage groups to specific chromosomes (KOURI *et al.* 1971; MILLER *et al.* 1971a,b,c). Similar results have been obtained in four of these translocations by another group (FRANCKE and NESBITT 1971b; NESBITT and FRANCKE 1971a,b). The Committee for Standardized Genetic Nomenclature for Mice (1972) has recently drawn up a suggested standard and unified numbering system for the mouse chromosomes and linkage groups. Since we have assigned LG V to chromosome 2 and LG VIII to chromosome 4 (5 in our previous numbering system, MILLER *et al.* 1971c), $T(5;8)Sn$ becomes $T(2;4)Sn$ in conformity with the new system. Quinacrine fluorescent studies of this translocation are reported here.

MATERIALS AND METHODS

Homozygous $T(2;4)Sn$ mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. Some matings of TSn mice were set up to obtain homozygous embryos; in other cases TSn males were mated with (C57BL/6J $\times$ DBA/2J) F_1 females to obtain heterozygous offspring. Primary cultures were set up from 11–14 day old embryos. The breeding of animals, establishment of primary cultures, and preparation of metaphase spreads were carried out at The Roche Institute of Molecular Biology, whereas quinacrine mustard staining, fluorescence photomicrography, and karyotyping were done at Columbia University, as described in detail in an earlier report (MILLER *et al.* 1971b).

Meiotic chromosome preparations were prepared for $TSn/+$ males by the method of EVANS *et al.* (1964).

RESULTS AND DISCUSSION

The presence of a translocation in the TSn was tested for by carrying out meiotic studies on two male mice which were obligate heterozygotes. A quad-

rivalent was observed in 64 of the 97 first meiotic division cells examined, indicating that a translocation was present.

About 100 mitotic metaphase spreads were analyzed and 28 karyotypes were prepared from primary embryonic cultures of heterozygous embryos. A partial karyotype and an idiogram of the translocation chromosomes are shown in Figure 1. The distal segments of chromosomes 2 and 4 have been exchanged; the abnormal number 2 is slightly shorter than a normal 2 and has a bright end, and the abnormal 4 is slightly longer than a normal 4 and has a dull end. Similar results were found in the seven cells karyotyped from homozygous embryos.

Although the Snell translocation is difficult to demonstrate because of the small amount of chromosomal material which has been exchanged, the results conform to our expectations on the basis of other translocations. Figure 1 illustrates the findings in two translocations which also involve chromosomes 2 and 4. The

FIGURE 1.—Partial karyotypes showing quinacrine mustard stained translocation chromosomes and their normal homologues from the $T(2;4)Sn$, $T(2;8)26H$ and $T(2;4)13Ca$ translocation stocks. The left member of each pair is a normal chromosome, which is numbered. The right member of each pair is the translocation chromosome with the same centromere. Arrows indicate the cytologic breakpoints. The Sn breakpoint in chromosome 2 is near the distal end and its position is cytologically indistinguishable from that of $T26$. The TSn breakpoint in chromosome 4 is closer to the distal end than is the corresponding $T13$ breakpoint. An idiogram of the chromosomes involved in the TSn translocation has been included for comparison.

T(2;8)26H (previously *T(5;18)26H*) translocation involves chromosome 2 (LG V) and chromosome 8 (LG XVIII) (MILLER *et al.* 1971a). The genetic breakpoint in chromosome 2 has been mapped very close to the *a* locus (SEARLE, FORD and BEECHEY 1971), i.e., in the same region as the breakpoint in the *TSn* translocation. As indicated by the arrows, the cytologic breakpoint in chromosome 2 is at approximately the same place in both the *T26* and the *TSn*, near the distal end of the chromosome.

The *T(2;4)13Ca* (previously *T(5;18)13Ca*) translocation involves the same chromosomes as the *TSn* translocation, chromosome 2 (LG V) and chromosome 4 (LG VIII) (MILLER *et al.* 1971c). In the *T13* translocation the genetic breakpoint in chromosome 2 is closer to the centromere than is that in the *TSn* translocation (BEECHEY and SEARLE, personal communication). The genetic breakpoint in chromosome 4 in the *T13* translocation has been mapped between the *b* and *m* loci (BEECHEY and SEARLE, personal communication), i.e. 20–30 cM closer to the centromere of the chromosome than is the breakpoint in the *TSn* translocation. Since the cytological breakpoint in *T13* is about two thirds of the distance from the chromosome 4 centromere, the breakpoint in *TSn* should be located in the distal third of chromosome 4, and that is what we found (Figure 1). The order centromere-breakpoint *T13*-breakpoint *TSn* provides additional confirmation of the assignment of the centromere to the *wd* (waddler), i.e., the *b* end, of chromosome 4, using what we have called the two-breakpoint method (MILLER *et al.* 1971a).

SLIZYNSKI (1952) presented pachytene maps purporting to show the breakpoints in the mid portion of each chromosome involved in the Snell translocation. A similar pachytene map was presented by GRIFFEN (1955), but a consideration of the relative lengths of the chromosomes casts doubt on the reliability of this system. The longest chromosomes in the pachytene map, in order of decreasing length, are LG V, VIII, III, I, IX, XX, XI, XIII and II (GRIFFEN 1960). The corresponding chromosome numbers would be 2, 4, 14, 7, 17, X, 6, 1 and 9. Since the mitotic chromosomes were numbered in order of decreasing length, either there is differential contraction or the pachytene analysis is incorrect.

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