55. Cytogenetic Evidence for and Frequency of Adjacent-2 Disjunction in Murine T(1;13)70H Male Heterozygotes

By Mitsuo OSHIMURA, Shin-ichi SONTA, and Avery A. SANDBERG

Roswell Park Memorial Institute, Buffalo, New York 14263

(Comm. by Sajiro MAKINO, M. J. A., April 12, 1976)

The frequency of adjacent-2 disjunctions (13%) in mouse translocation heterozygotes, T26H, T70H and T264Ca, was reported on by Searle *et al.* (1971). We attempted to estimate the frequency of adjacent-2 disjunctions by direct cytogenetic analyses in meiosis-II.

Materials and methods. Mice with the T(1;13)70H homozygous translocation (hereafter referred to as T70) were obtained through the courtesy of Dr. A. G. Searle, M. R. C. Radiobiological Research Unit, Harwell, United Kingdom. This translocation is due to an unequal reciprocal translocation leading to a large marker chromosome consisting of most of #13, including its centromere, and a preponderant part of #1; and also leading to a small marker containing the centromere of #1 and a small amount of translocated material from #13 (Miller *et al.* 1971; Boer and Gijzen 1974, Standardized Genetic Nomenclature for Mice 1972). The former marker will be designated as M1 and the latter as M2. The T70 translocation heterozygotes were derived from a T70/T70 φ $\times$ C3Hf/HeHa σ cross. Testes from five male T70 heterozygotes, injected intraperitoneally with colcemide (2 mg/Kg b.w.) 4 hrs before sacrifice, were minced in 0.075 M KCl and fixed with 3:1 methanol/acetic acid. Air-dried slides were subjected to a C-banding technique (Sumner 1972).

Results. Examinations of metaphase-I in 450 cells indicated 18 rings of IV; 394 chains of IV; and 38 chains of III+I. In 657 metaphase-II cells (Fig. 1) the chromosome counts varied from 19 to 21. On the basis of the C-banding analysis of the metaphase-II the cells could be classified regarding 1) the total chromosome number, and 2) the number and morphology of the M1 and M2 markers, which were difficult to determine by means of conventional techniques.

Of the 657 cells studied, 194 had a chromosome consisting of unequal chromatids (M1') in which its longer chromatid was similar to an M1 and an M2 marker, indicating the crossing-over between M1 and #13 in the interstitial segment (M1', M2 class) (Fig. 2); 216 of M-II cells had only an M1' and no M2 (M1', - class); and 31 cells had an M2 with unequal chromatids (M2') in addition to the M1', suggesting crossing-over between M1 and #13 and between M2 and #1 in their

interstitial segments ($M1'$, $M2'$ class) (Fig. 3). The marker chromosome combinations and nearly equal numbers of cells of the ($M1'$, $M2$) and ($M1'$, -) class suggest that these classes are in fact complementary; cells of the ($M1'$, -) class probably possess a normal #1. Thus, most of the cells of these three classes are considered to have resulted from alternate or adjacent-1 disjunction. The above three classes of cells,

Disjunction type	Chromosome no. and markers		No. of cells	Disjunction type	Chromosome no. and markers		No. of cells
Alternate & Adjacent-1		$20, M1', M2$	182	3:1		$21, M1', M2$	10
	$20, M1', M2$	29			$21, 2M1', M2$	1
	$20, M1', -$	199			$21, 2M1', -$	1
Adjacent-2		$20, -, 2M2$	2	Equational		$21, M1', M2''$	1
	$20, 2M1', -$	103			$20, M1', M2$	2
	$20, -, M2$	90			$20, -, M2''$	1
		19	12				
		19	2				
		19	17				
			3				
			2				

Fig. 1. The frequency of various classes of 2nd spermatocytes following alternate, adjacent-1, adjacent-2, 3:1 and equational disjunctions. Black chromatids and centromeres originated from chromosomes #13 and the white ones from #1. The asterisk indicates karyotypes in which a normal chromosome #1 was probably present.

even though containing preponderantly 20 chromosomes, had 31 cells (7%) with 19 chromosomes. Except for the cells with 19 chromosomes in the ($M1'$, -) class (to be described later), all other cells with 19 chromosomes were considered to have arisen technically from the cells with 20 chromosomes. Of all the cells examined, 106 metaphase-II cells had 2 $M1'$ and no $M2$ ($2M1'$, - class), which could be expected from adjacent-2 disjunction at anaphase-I (Fig. 4). The combined frequency of spermatocytes (94 cells) characterized by either (-, $M2$) (Fig. 5) or (-, $2M2'$), which is complementary to ($2M1'$, -), further substantiates that the quadrivalent frequently undergoes adjacent-2 disjunction. The two classes of cells, ($2M1'$, -) and (-, $M2$), had cells with 19 chromosomes and are regarded as artificially derived from their respective cells with 20 chromosomes. Thus, the cytogenetic analyses made it possible to estimate the frequency of adjacent-2 disjunction at about 30%.

Numerical non-disjunction at anaphase-I produces an equal number of M-II cells (2nd spermatocytes) with 19 and 21 chromosomes.

Since most of the M-II cells with 21 chromosomes included an M2, the assumption is possible that the M2 is responsible for the 3:1 disjunction in male T70 heterozygotes. If this is the case, we can expect an equal number of cells with $[21(M1', M2)]$ and $[19(M1', -)]$. Hence, about 10 cells out of 17 of the $[19(M1', -)]$ class must have been derived through 3:1 disjunction. Actually, there were 4 cells with an M2 consisting of a single chromatid (M2'') which might have resulted from equational separation of the M2 univalents at anaphase-I.

Fig. 2. Metaphase with 20, M1', M2 karyotype in 2nd meiotic metaphase. Arrows show M1' (large) and M2 (small) markers. Fig. 3. Metaphase with 20, M1', M2' karyotype. Arrows point to M1' and M2' markers. Fig. 4. Metaphase with 20, 2M1', - karyotype. An M2 marker was not observed. Arrows point to two M1' markers. Fig. 5. Metaphase with 20, -, M2 karyotype. An M1' marker was not observed. Arrow points to an M2 marker.

Discussion. The use of male mice with an unequal reciprocal translocation, T(1;13)70H, and the application of a C-banding technique made it possible to estimate the incidence of various classes of M-II cells following alternate, adjacent-1, adjacent-2, 3:1 and equational disjunctions at the M-I division. The data presented indicated that the frequency of adjacent-2 disjunction is nearly 30%, which is twice of that presented by Searle *et al.* (1971) in an extensive genetical analysis in F1 from a crossing of T70 heterozygotes. It is apparent that the different genetic backgrounds of mice used and variation in the approaches used may be responsible for the difference between the frequency of adjacent-2 disjunctions estimated in the present study and that genetically arrived at by Searle *et al.* (1971).

In the present study of T70 translocation we also observed the presence of non-disjunction and equational separation of M2 markers, similar to data obtained in the T6 heterozygotes by Oshimura and Takagi (1975). Since the incidence of non-disjunction in the primary oocytes is significantly higher than in spermatocytes of the T6 translocatin males (Oshimura and Takagi, 1975), it will be important

to see whether there exists a significant difference in the incidence of non-disjunction between the sexes with the T70 translocation.

Summary. A C-banding technique applied to the second meiotic spermatocyte metaphases affords an estimate of the frequency of adjacent-2 and 3:1 disjunctions at the first meiotic division in male T(1;13)70H heterozygotes. The frequency of adjacent-2 disjunction is about 30% and that of 3:1 disjunction 4%.

Acknowledgements. We are very grateful to Dr. A. G. Searle for the T70H strain used, and to Emeritus Professor Dr. Sajiyo Makino and Dr. Nabuo Takagi for valuable advice and revision of the MS.

References

- Boer, P. D., and Gijzen, M. V.: *Can. J. Genet. Cytol.*, **16**, 783-788 (1974).
Committee on Standardized Genetic Nomenclature for Mice: *J. Hered.*, **63**, 69-72 (1972).
Miller, O. J., Miller, D. A., Kouri, R. E., Dev. V. G., Grewal, M. S., and Hutton, J. J.: *Cytogenetics*, **10**, 452-464 (1971).
Oshimura, M., and Takagi, N.: *Cytogenet. Cell Genet.*, **15**, 1-16 (1975).
Searle, A. G., Ford, C. F., and Beechey, C. V.: *Genet. Res.*, **18**, 215-235 (1971).
Sumner, A. T.: *Exptl. Cell Res.*, **75**, 304-306 (1972).