SHORT COMMUNICATION

SEXUAL DIFFERENCES IN MEIOTIC DISJUNCTION OF MURINE T(1;13)70H TRANSLOCATION HETEROZYGOTES

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Studies on murine meiotic T(14;15)6Ca translocation heterozygotes of both sexes indicated that there is no meiotic, gametic or zygotic selection attributable to genome imbalance, at least by the late blastocyst stage (Oshimura and Takagi 1975). The higher yield of translocation trisomic offspring from the female than the male T(14;15)6Ca heterozygotes could be satisfactorily explained by the higher incidence of non-disjunction, involving the minute marker chromosomes, in the primary oocyte (22.2%) than in the spermatocyte (4.4%) (Oshimura and Takagi 1975). Recently, we reported the cytogenetic evidence for the adjacent-2 disjunction and estimated the incidence of various classes of M-II cells following alternate/adjacent-1, adjacent-2, 3:1 and equational disjunction at the M-I division of T(1;13)70H translocation (hereafter T70) heterozygotes (Oshimura *et al.* 1976). In the present study, we attempted to ascertain whether there are any significant differences between the sexes in the disjunctive behaviour at the M-I division of the T70 heterozygotes.

The T(1;13)70H 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 a small marker containing the centromere of #1 and a small amount of materials from #13 (Miller *et al.* 1971; Committee on Standardized Genetic Nomenclature for Mice 1972). The former marker will be designated as M_1 and the latter as M_2 . Since there is chiasma formation in at least one of the interstitial segments which lies between the centromere and the point of exchange, alternate and adjacent-1 disjunction are genetically equivalent. Furthermore, since the "unequal" reciprocal translocation T70 was used, we can expect to observe (1) one chromosome with chromatids of unequal length ($M_1/13$, hereafter M_1') at the second meiotic metaphase, following alternate/adjacent-1 disjunctions of chain quadrivalents because of chiasma formation in one of the interstitial segments, or (2) two different chromosomes with chromatids of unequal length (M_1' and $M_2/1$, hereafter M_2') following alternate/adjacent-1 disjunction of ring quadrivalents because of chiasma formation in two of the interstitial segments. On the other hand, if adjacent-2 disjunction occurs in chain

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Disjunction type	Chromosome no. and markers		No. of cells ♂ (%) ♀		Disjunction type	Chromosome no. and markers		No. of cells ♂ (%) ♀	
Alternate & Adjacent-1		20, M_1, M_2 19, —	225 (28.1)	25 (23.4)	3 : 1		21, M_1, M_2	12 (1.5)	10 (9.8)
	20, M_1, M_2' 19, —	13 (1.6)	1 (0.9)			21, $2M_1, M_2$	1 (0.1)	1 (0.9)
	20, $M_1, -$ 19, —	241 (30.0)	21 (19.4)			21, $2M_1, -$	1 (0.1)	—
Adjacent-2		20, — $2M_2$	4 (0.5)	—	Equational		21, M_1, M_2'	1 (0.1)	2 (1.9)
	20, $2M_1, -$ 19, —	123 (15.3)	18 (16.7)			20, M_1, M_2	3 (0.4)	1 (0.9)
	20, —, M_2 19, —	111 (13.8)	16 (14.8)			20, —, M_2'	1 (0.1)	1 (0.9)

Fig. 1. The frequency of various classes of 2nd spermatocytes and oocytes 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.

quadrivalents, we can expect an equal number of cells with $2M_1'$ and cells with only M_2 at the second meiotic metaphase. The adjacent-2 disjunction in ring quadrivalents is expected to give rise to an equal number of cells with $2M_1'$ and $2M_2'$. T70 translocation heterozygotes of 10 females and 7 males, derived from a cross with 3Hf/HeHa(ϕ), were utilized in the present study. The slides for 2nd meiotic metaphases of the both sexes were prepared according to the technique of Oshimura and Takagi (1975). The estimation of various classes of 2nd meiotic metaphases was made on the basis of the chromosome number and the morphology of these marker chromosomes. In order to ascertain the position of the centromeres of markers, the C-banding technique (Sumner 1972) was applied to the slides of the male heterozygotes.

Figure 1 shows the results obtained. A total of 802 2nd spermatocytes and 108 2nd oocytes were counted and analyzed. The marker chromosome combinations and the nearly equal number of cells of the (M_1', M_2) and $(M_1', -)$ classes in both sexes suggest that these classes are, in fact, complementary. Of all the cells examined, 123 (15.3%) were second meiotic metaphase cells in the males and 18 (16.7%) in the females had $2M_1'$ and no M_2 , $(2M_2', -)$ (Fig. 2a, b), which would be expected from adjacent-2 disjunction. The combined frequency, characterized either by $(-, M_2)$ or $(-, 2M_2')$, is similar to that of the $(2M_1, -)$ class. Thus, the cytogenetic analysis made it possible to estimate the incidence of adjacent-2 disjunction and revealed no frequency difference between the sexes (30.1% in the male and 31.5% in the female). Numerical non-dis-

Fig. 2. Metaphases with 20, 2M₁' — karyotype in 2nd meiotic metaphase of male (a) and female (b) mice. An M₂ marker was not observed. 2M₁' are closely contiguous with each other. Arrows point to two M₁' markers.

junctions at anaphase-I produce an equal number of second metaphase cells with 19 and 21 chromosomes. Since most of the M-II cells with 21 chromosomes included an M₂, it is possible that M₂ is responsible for the 3:1 disjunction. If this is the case, we can expect an equal number of cells with 21 chromosomes (M₁', M₂) and 19 chromosomes (M₁', —); a total of 13 cells with (19, M₁', M₂) might be attributed to the loss of a chromosome from (20, M₁', M₂) in the male and 1 cell with (19, M₁', M₂) from (20, M₁', M₂) in the female. This implies that a comparable number of (19, M₁', —) also had technically arisen from (20, M₁', —). Thus, reasonably good estimates for the frequency of non-disjunction would be twice the percentage frequency of (21, M₁', M₂) cells (3.0% in the male and 19.6% in the female). Hence, the frequency of non-disjunction involving the minute marker chromosomes was significantly different between the sexes ($P < 0.0001$; $\chi^2 = 49.9$).

The tendency for female heterozygotes to contribute to a higher yield of chromosomally unbalanced progeny than the heterozygous males, has been reported for Robertsonian-type translocations (Baranov and Dyban 1971; Gropp 1973) and in a reciprocal translocation (Oshimura and Takagi 1975). In contrast to these translocations, White *et al.* (1974) found more trisomic embryos associated with male rather female mice doubly heterozygous for Rb1Wh and 163H. Thus, the present study supports the notion that each structural change is unique and that the different translocations behave differently at meiosis (Oshimura and Takagi 1975). Moreover, the evidence suggests that the non-disjunction involving the minute marker and the adjacent-2 disjunction in T70 heterozygotes seems to be controlled by different factor(s); this is

pointed to by the presence of sexual differences in the non-disjunction including the M_2 marker and no sexual differences in adjacent-2 disjunction.

Another class of M_2 , consisting of a single chromatid (M_2''), was present in a small proportion of the cells (equational segregation) of both sexes. Moreover, in about 80% of the cells with $2M_1'$ (cells derived from adjacent-2 disjunction), the latter were contiguous with each other in metaphase-II (Fig. 2a, b). This suggests that $2M_1'$ move to the same pole without them becoming separated at anaphase-I. Alternatively, non-random distribution of chromosomes in the 2nd metaphase plate could also have occurred.

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LITERATURE CITED

- Baranov, V. S., and A. P. Dyban, 1971 Embryogenesis and peculiarities of karyotype in mouse embryos with centric fusion of chromosomes (Robertsonian translocation). *Ontogenez*. **2**: 164-176.
- Committee on Standardized Genetic Nomenclature for Mice, 1972 Standard karyotype of the mouse, *Mus musculus*. *J. Hered.* **63**: 69-72.
- Gropp, A., 1973 Reproductive failure due to fetal aneuploidy in mice. *Proc. VII Wld Cong. Fertil. Steril.*, 1971, pp. 326-330 (Excerpta Medica, Amsterdam 1973).
- Miller, O. J., D. A. Miller, R. E. Kouri, V. G. Dev, M. S. Grewal, and J. J. Hutton, 1971 Assignment of linkage groups VIII and X to chromosomes in *Mus musculus* and identification of the centromeric end of linkage group I¹. *Cytogenet.* **10**: 452-461.
- Oshimura, M., and N. Takagi, 1975 Meiotic disjunction in T(14;15)6Ca heterozygotes and fate of chromosomally unbalanced gametes in embryonic development. *Cytogenet. Cell Genet.* **15**: 1-16.
- Oshimura, M., S. Sonta, and A. A. Sandberg, 1976 Cytogenetic evidence for and frequency of adjacent-2 disjunction in murine T(1;13)70H male heterozygotes. *Proc. Japan Acad.* **52**: 199-202.
- Sumner, A. T., 1972 A simple technique for demonstrating centromeric heterochromatin. *Exptl. Cell Res.* **75**: 304-306.
- White, B. J., J. H. Tjio, L. C. Van de Water, and C. Crandall, 1974 Trisomy 19 in the laboratory mouse. I. Frequency in different crosses at specific developmental stages and relationship of trisomy to cleft palate. *Cytogenet. Cell Genet.* **13**: 217-231.