Evidence for postmeiotic effect of *t* factors causing segregation distortion in mouse

THE *t* complex of the mouse consists of a large series of genetic factors often characterised by embryonic lethality of the homozygotes, reduced male fertility of viable homozygotes, suppression of recombination over a relatively long chromosomal segment and relatively frequent occurrence in natural mouse populations. Most *t* factors also possess a component which interacts with the *Brachyury* (*T*) gene and causes complete absence of the tail in *T*/*t* heterozygotes. One of the most fascinating but least explored characteristics of the *t* factors is their effect on genetic segregation. A male mouse carrying a *t* factor in a heterozygous condition (*t*/—) often transmits the factor to almost 100% of its progeny, in defiance of the Mendelian principle requiring 1:1 segregation.

Table 1 Frequency of metaphase II spreads with one metacentric chromosome in *T7*+/+t^6 males

Animal Number	1M*	OM†	Total	%1M‡	χ^2
199/919	9	12	21	42.9	0.42
199/920	32	37	69	46.4	0.32
199/921	11	17	28	39.3	1.28
199/922	28	36	64	43.8	1.0
199/923	27	32	59	45.8	0.42
199/924	21	27	48	43.8	0.76
199/925	19	30	49	38.8	2.46
200/1151	7	11	18	38.9	0.88
200/1334	29	41	70	41.4	2.06
Total	183	243	426	43.0	8.4

* No. metaphase II spreads with one metacentric (*T7*).
† No. metaphase II spreads without the metacentric.
‡ % spreads with one metacentric.

Although this segregation distortion has been known for almost half a century, it has not been satisfactorily explained. It has been suggested that the distortion may be due to abnormal meiotic divisions (meiotic drive[1]). More specifically, it may be due to a process in which homologous chromosomes distribute themselves unequally during metaphase I of male meiosis with the result that the spermatocytes receive one chromosome of a given pair more frequently than the other. Here we present evidence that in a *t*/+ male, the *t* and the + bearing chromosomes are distributed during metaphase I in a 1:1 ratio, implying that the actual distortion must occur after metaphase I, most likely postmeiotically.

The evidence for the meiotic effect of *t* factors was obtained by cytogenetical means, using a strain of mice recently produced in this laboratory and carrying Robertsonian translocation *T(16;17)7Bn*, abbreviated *T7* (ref. 2). The strain was derived by repeated backcrossing of hybrids between the house mouse (*Mus musculus*) and the tobacco mouse (*M. poschiavinus*) to the inbred strain C57BL/10, and selection for chromosome 17 carrying the *t* complex. During the selection, six of the seven metacentrics present in the tobacco mouse were eliminated and only the one composed in part of chromosome 17 was maintained. The *T7* translocation is easily identifiable both at mitosis (as a single or double metacentric among 39 or 38 telocentrics, depending on the zygosity of the carrier) and meiosis (as a quadrivalent at metaphase I and a metacentric chromosome at metaphase II).

The rationale for the present experiment was as follows: if the segregation distortion is caused by meiotic drive during metaphase I, at metaphase II a *T7*+/+*t* male should show a preponderance of meiotic figures lacking the metacentric marker; if, on the other hand, the distribution of homologous chromosomes at metaphase I is normal, a 1:1 ratio of figures with and without the metacentric can be expected at metaphase II.

The prerequisite of such an experiment is that the *T7* translocation itself does not greatly disturb the segregation of the homologues at meiosis. For this reason, males carrying the *T7* marker but not the *t* factor were also tested.

Table 2 Comparison of results with different *t* factors

Genotype	No. of males tested	Frequency of metaphase II spreads with one metacentric chromosome in males of indicated genotype: 1M/T*	%1M†	χ^2‡	χ^2§	Segregation of the metacentric chromosome to offspring of males tested for the presence of this chromosome in metaphase II: 1M/T‖	% progeny with 1M	χ^2¶	χ^2**	Segregation of *t* factor in the absence of the metacentric chromosome: Ot/T††	%Ot‡‡
+/+/+/+	1	4/69§§	5.8	54.0	46.4						
T7+/+/+	1	57/122	46.7	0.52							
T7+/+/*T*	5	70/155	45.2	1.46	0.15	73/137	53.3	0.60			
T7+/+/+t^{w2}	1	21/47	44.7	0.54	0.08	1/18	5.6	14.2	0.47	15/137	10.5
T7+/+/+t^{w5}	5	85/116	42.3	4.8	1.57	2/67	3.0	59.2	3.03	4/44	9.1
T7+/+/+t^6	9	183/426	43.0	8.4	2.38	10/177	5.6	139.2	65.92	25/72	34.7
T7+/+/+t^{12}	4	78/170	45.9	1.16	0.04	2/92	2.2	84.2	23.88	49/205	23.9

*Number of metaphase II spreads with one metacentric (*T7*)/total number of scored spreads.
†% spreads with one metacentric.
‡Observed frequencies of 1M spreads were compared to expected frequencies (that is 50%).
§Observed frequencies of 1M spreads were compared to observed frequencies of 1M spreads in *T7*+/+ + male.
‖Number of progeny with one metacentric/total number of progeny. Progeny arose from matings of mice with the genotype indicated in the first column to +/+/+ + or +/+/+*T* mice. Presence or absence of the metacentric chromosome (*T7*) was determined in mitotic metaphase spreads.
¶Observed frequency of progeny with *T7* from the matings of *T7*+/+/+*T* or *T7*+/+/+*t* × +/+/+ + or +/+/+*T* was compared to expected frequency (that is 50%).
**Observed frequency of progeny with *T7* from the mating of *T7*+/+/+*t* × +/+/+ + or +/+/+*T* was compared to observed frequency of progeny from the mating +/+/+*t* × +/+/+ + or +*T*/+ + (see column 12).
††Number of progeny without *t*/total number of progeny. Progeny arose from matings of +*T*/+*t* or +/+/+*t* to +/+/+ + mice. Segregation of + chromosome was detected by H-2 typing.
‡‡% progeny without *t* from matings in the absence of *T7*.
§§As B10.A lacks the metacentric chromosome, the four spreads scored as 1M must be reading errors. Two telocentric chromosomes which happen to be positioned in the spreads close to each other with their centromeres almost in contact and their arms pointing in opposite directions resemble and are confused with a metacentric chromosome. Such a reading error, however, is so small that it does not influence the conclusions drawn from the data.

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In the experiment, $T7+/T7+$ females were mated to $+t/+T$ males and two types of progeny were produced: $T7+/+t$ (normal tail) and $T7+/+T$ (short tail). Both the normal tail and the short males were further mated to $++/++$ or $++/+T$ females; the progeny of these matings were killed, mitotic chromosome preparations were prepared from bone marrow[3] and the segregation of the $T7$ chromosome was determined. This progeny test provided the necessary control for demonstrating that the t factor distorted segregation whereas the $T7$ translocation did not. If this were the case, one would expect the progeny of the $T7+/+T \times ++/++$ mating to segregate in a 1:1 ratio of mice with and without the metacentric, and the progeny of the $T7+/+t \times ++/++$ mating to have preponderance of mice without the metacentric. After a sufficient number of progeny were obtained from the $T7+/+T$ and $T7+/+t$ males, the animals were killed meiotic chromosome preparations were made from their testes[4], and the metaphase II spreads evaluated for the presence or absence of the metacentric chromosome. Twenty to twenty-five slides were made from each male and only metaphase II spreads of 19 telocentrics and one metacentric, or 20 telocentrics were counted. Four different t factors were tested: t^6, t^{12}, t^{w2}, and t^{w5}.

Although there was a considerable male-to-male variation in the individual experiments (Table 1), the overall results with the different t factors were comparable (Table 2). In the meiotic test (Table 2), the observed frequency of 1M spreads (that is metaphase II spreads with one metacentric chromosome) in $T7+/++$ mice was not significantly different from the observed frequency of 1M spreads in $T7+/+t$ mice. When the observed frequency of 1M spreads in $T7+/+t$ mice, however, was compared to the expected frequency (50%), significant difference was found for some t factors (t^6 and t^{w5}). The departure from the expected value was most likely caused by the presence of the metacentric chromosome. (The frequency of 1M spreads in $T7+/++$ animals was lower than the expected 50% value, but for the number of animals used, the difference was not statistically significant.)

In the progeny test (Table 2), two t factors (t^{w2} and t^{w5}) were transmitted in almost exactly the same ratios in $T7+/+t \times ++/++$ matings as in $+T/+t \times ++/++$ or $++/+t \times ++/++$ matings. The other two factors tested (t^6 and t^{12}), on the other hand, were transmitted in significantly lower ratios in $T7+/+t \times ++/++$ matings than in $+T/+t \times ++/++$ or $++/+t \times ++/++$ matings. This latter observation suggests that $T7$ interacts with t factors postmeiotically, resulting in an increased transmission ratio of t. This interaction, however, can be detected only with t factors that, in the absence of $T7$, have low (t^6) or moderate (t^{12}) transmission ratios. The nature of the interaction is not known.

We conclude that two types of segregation distortion operate in the experiments we describe; one a very minor type occurring during the first meiotic division and another which has a very strong effect postmeiotically. The meiotic distortion can most likely be attributed to the presence of the $T7$ translocation, whereas the strong distortion is caused by the t factors and it may be enhanced by the $T7$ chromosome. Thus, the distortion resulting from t factors does not occur in metaphase I as a result of a structurally abnormal chromosome but rather postmeiotically. This conclusion is in agreement with the data of the effect of late mating[5,7], which shows a normalisation of the transmission ratios as a result of the shorter period of time between insemination and fertilisation. Further evidence for the postmeiotic effect of t factors is provided by recent work[8] in which epididymal sperm was incubated *in vitro* and the number of surviving sperm were estimated at intervals by a cytotoxic test using antisera directed against a specific t factor. They demonstrated that the number of t bearing sperm increases with time as the number of $+$ sperm decreases.

The data we present, however, do not rule out the possibility of a biochemical activity related to the t factors that may occur before or early in meiosis but have a postmeiotic effect as has been suggested by Erickson[7].

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Factor which affects the mode of genetic recombination in *E. coli*

THE normal recombination process in *Escherichia coli* requires a pathway involving a functional *recB* and *recC* gene product (henceforth designated the RecBC pathway).[1] The *recB* and *recC* genes map as closely linked cistrons near *thy* on the *E. coli* chromosome map (54 min)[2,3]. ATP-dependent DNase (*recBC* DNase) has been identified as the product encoded by these genes[4–6]. Another gene essential for recombination proficiency, *recA*[7], is located between *cysC* and *pheA* (51 min)[8]. Genetic transformation studies[9–12] showed that the most efficiently transformable *E. coli* strain (genotype: $recB^-$ $recC^-$ $sbcB^-$) lacks the DNase but retains recombination proficiency because of the $sbcB^-$ allele which acts as an indirect suppressor of the $recB^-$ $recC^-$ mutations[13]. This strain uses a recombination pathway (designated as the RecF pathway)[1] which does not involve the ATP-dependent DNase but consists of products of several recombination genes[13]. Restoration of the ATP-dependent DNase to this strain by introduction of the $recB^+$ $recC^+$ alleles reduced the transformation frequency by a factor of approximately five[9]. *In vitro* studies demonstrated that this DNase degrades linear DNA molecules extensively, while it has no effect on circular DNA[4]. The adverse effect of the DNase on transformation may therefore be due to destruction of linear donor DNA molecules before they reach the recombination machinery. These results raise the possibility that in normal cells the DNase affects the mode of recombination by selecting or destroying DNA molecules of a particular structure.

The transfer of genetic material can result in the formation of two possible types of recombinant clones: (*a*) substitution type resulting from replacement of recipient genetic material by a homologous segment of donor material, and (*b*) addition type

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