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Time of recombination in the *Drosophila melanogaster* oocyte: Evidence from a temperature-sensitive recombination-deficient mutant

(DNA replication/meiotic sequence/nondisjunction/premeiotic interphase/synaptonemal complex)

R. F. GRELL

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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ABSTRACT **A temperature-sensitive recombination-deficient mutant, *rec-1*^{26}, has been isolated that permits high frequencies of recombination at the permissive temperature (25°) but greatly decreases recombination at the restrictive temperature (31°). The sensitive period for response of female germ cells carrying this mutant to the restrictive temperature has been defined. Sensitivity begins very close to the time the oocyte enters premeiotic interphase and initiates DNA synthesis; it continues for the duration of premeiotic-S; and it terminates with the completion of S. This time span precisely coincides with the sensitive period for enhancement of recombination by heat in the normal genome and is further characterized by the presence of the synaptonemal complex. These results provide compelling evidence for identifying premeiotic-S as the time of meiotic recombination.**

The time of meiotic recombination in eukaryotes remains unresolved. Traditional concepts continue to place the event at pachytene during meiotic prophase, but persuasive evidence now exists to fix the time much earlier, during premeiotic interphase. Part of this evidence comes from studies of the temporal relationship between recombination and the premeiotic-S phase, arrived at by means of a temperature probe which locates the sensitive period for increasing recombination. Reliable results with this method require that the temperature treatment be applied to a well-synchronized meiocyte population, that response be measured genetically via crossovers, and that the effective treatment period be sufficiently short and the S phase sufficiently long to permit discrimination between response at replication and response at a postreplication prophase stage. Studies meeting these criteria have shown that the heat-sensitive period for induction and enhancement of recombination coincides with premeiotic-S (1–6) or premeiotic interphase (7). The simplest interpretation of these data is that recombination occurs during premeiotic-S. The less likely possibility, that heat acts indirectly and that recombination occurs at a later time when response is absent, has not been rigorously excluded.

An independent approach to the problem appeared possible with a temperature-sensitive recombination-deficient mutant that would permit high frequencies of recombination at the permissive temperature but would decrease or eliminate recombination at the restrictive temperature. The time of recombination would then be revealed by identifying the effective period for reduction of recombination by the restrictive temperature.

A search for such a mutant led to the recovery of a new locus designated *recombination-1* (*rec-1*). The locus is located on the right arm of chromosome 3 within the segment defined by the deficiency, *Df(3R)sbd*^{105}. It is represented by three mutant alleles—namely, *rec-1*^{6}, *rec-1*^{16}, and *rec-1*^{26}. Only *rec-1*^{26} is temperature-sensitive, and this property is dominant to the temperature insensitivity of the two other alleles. None of the alleles displays any evidence of mutator activity as is seen with the mutant *mutator* (8), and all complement fully with mutants at the neighboring *c(3)G* locus. Electron microscopic examination reveals the presence of synaptonemal complexes. The method used to select the mutants and a description of their properties will be published elsewhere.

The present experiments were designed to delineate the period during female germ-cell development when the temperature-sensitive *rec-1*^{26} mutant responds to the restrictive temperature. Identification of the sensitive period as coincident with S now provides an independent criterion for localizing the time of meiotic recombination in a eukaryotic system as occurring during the S phase.

EXPERIMENTAL PROCEDURE

The Pupal System. To study the ability of heat to alter recombination frequency when applied to germ cells of different stages, it is necessary to recover samples that were homogeneous in age at the time of treatment. The pupal system provides such samples. A detailed description of the method (3) and a timetable of oocyte development (5) have been published; a brief outline follows. Parents (P1) of the females to be treated are mass mated in bottles; after several days they are transferred *en masse* to fresh bottles for a restricted egg-laying period and then removed. Developing progeny (G1) are maintained at the control temperature (25° ± 1°) except during temperature treatment. Females developing at 25° begin to produce oocytes between 126 and 132 hr, coinciding with their pupation time at 132 hr; at eclosion, the oldest oocyte in each of the 30–40 ovarioles that make up the two ovaries is immature. Mature stage-14 oocytes ready for fertilization and oviposition are formed ~36 hr later. Upon eclosion, treated females (G1) are mated in vials for 24 hr and transferred to bottles for 24 hr. This procedure limits egg-laying of the G1 female to 8–12 hr, during which she produces 10–15 eggs, corresponding to one-third to one-half of her first set of 30–40 eggs, one from each ovariole. Progeny (G2) are scored for crossing-over and nondisjunction. As judged by reproducibility of heat response, the progeny represent a sample that was well synchronized at the time of treatment (3).

Heat Treatment. Bottles containing the developing females (G1) of the desired age were placed in an incubator for the treatment period. The restrictive temperature used in all of the tests was 31° ± 0.5°, which is the maximum compatible with viability for the *rec-1* heteroalleles.

***Drosophila* Genotypes.** P1 females were homozygous for the X chromosome markers *yellow*^{2} (*y*^{2}-0.0), *crossveinless* (*cv*-13.7), *vermilion* (*v*-33.0), and *forked* (*f*-56.7). In addition,

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they carried the allele *rec-1*^{16} and the closely linked marker *bithorax*^{34e} (*bx*^{34e}-58.8) on one third chromosome, while the other third chromosome was involved in the reciprocal 2:3 translocation *Xasta* (*Xa*) which served as a balancer. P1 males carried the *rec-1*^{26} allele and *bx*^{34e} on one third chromosome balanced over *Xa*. G1 heat-treated females were heterozygous for *y*^{2}, *cv*, *v*, and *f*; were homozygous for *bx*^{34e}; and carried the heteroalleles *rec-1*^{16} and *rec-1*^{26} in *trans* configuration in their two third chromosomes. G1 females were mated to males carrying on attached-XY chromosome marked with *yellow*^{1} (*y*^{1}) and *Bar* (*B*-57.0) on their X chromosome and carrying in addition a free unmarked Y chromosome ($\overline{XY}$, *yB*/Y). Further descriptions of the mutants may be found in Lindsley and Grell (9).

Procedure. Fifty newly eclosed *y*^{2} *cv v f*/*y*^{2} *cv v f*; *rec-1*^{16} *bx*^{34e}/*Xa* females and 50 *rec-1*^{26} *bx*^{34e}/*Xa* males per bottle (P1) were allowed to mate for 2 days, transferred to fresh bottles for 3 hr of egg laying, and returned to their original bottles to be used again. The developing progeny (G1) were kept at 25° ± 1° except for the period of heat treatment. In the first set of experiments, the period sensitive to the restrictive temperature was roughly defined by treating different groups of progeny with the restrictive temperature (31° ± 0.5°) for 24 hr on sequential days of development beginning with day 2 and ending with day 9. In two cases the restrictive temperature was applied for 48 hr, on days 2 and 3 and on days 6 and 7. The second set of experiments more precisely defined the period sensitive to the restrictive temperature by heat treating at different intervals within the responsive period (120–168 hr). Upon eclosion, the heat-treated G1 females of the proper genotype (*y*^{2} *cv v f*/++++; *rec-1*^{16} *bx*^{34e}/*rec-1*^{26} *bx*^{34e}) were mated, four to a vial, to four $\overline{XY}$, *y B*/Y males for 24 hr, transferred to bottles for 24 hr, and then discarded. G2 progeny were reared at 25° ± 1°, and, following eclosion, the regular males were scored for crossing-over in three regions of the X chromosome—region 1, *y*^{2}-*cv*; region 2, *cv*-*v*; region 3, *v*-*f*. Nondisjunction rates were calculated from the number of matroclinous females (*B*$^+$) and the number of patroclinous males (*y*^{1} and *B*).

RESULTS

The effect of the restrictive temperature on germ cells of females treated on successive days of development is shown in Table 1. The frequency of total recombination for the three regions that were measured shows no significant departure from the control level (42.0%) on days 2–5 or on days 8 and 9. A treatment on day 6 (120–144 hr) or on day 7 (144–168 hr) caused a highly significant reduction to 16.3% and 19.3%, respectively. Extension of the treatment period to 48 hr to include both days 6 and 7 (120–168 hr) produced a further reduction to 7.3%, which is significantly greater than that on either day 6 or day 7 ($P < 0.01$). By contrast, a similar extension to include both days 2 and 3 gave a value of 41.9%, demonstrating that lengthening the treatment period does not maximize the effect unless treatment is during the sensitive period. Inspection of regional responses shows significant decreases ($P = 0.01$) with treatments on day 6, day 7, or days 6 and 7 except for region 1 on day 6 for which $P = 0.02$.

The reciprocal relationship that exists between crossing-over and nondisjunction in the X chromosome is shown in Fig. 1. Nondisjunction remained low when crossing-over was high; it increased on days 6 and 7 when crossing-over was decreased; and it reached a maximum (21.2%) with treatment on days 6 and 7, when crossing-over was at its minimum. The greatest reduction in recombination and the greatest increase in nondisjunction coincided with the premeiotic-S period which, for the oocytes under study, occurs between 132 and 162 hr of female development (refs. 3 and 5; unpublished data).

The first set of experiments localized the responsive period for the restrictive temperature to days 6 and 7, corresponding to 120–168 hr. The second set of experiments dissected this 48-hr period and more precisely defined sensitivity as present during the 36-hr interval between 126 and 162 hr. This has been designated the "sensitive" period. Results of the second series of experiments are presented in Table 2. The total frequency of crossing-over for a 36-hr treatment coincident with the sensitive period was 6.8%. A 48-hr treatment, initiated 6 hr earlier (120 hr) and terminating 6 hr later (168 hr), gave a value of 7.3%, which is not significantly different. Response to treatments of 44 and 46 hr duration, administered at times that included the 36-hr sensitive period, gave similar values, 5.4 and 5.9%, respectively.

Elimination of the final 6 hr of the sensitive period caused an increase in crossing-over that becomes significant ($P = 0.10$) when the average for the four runs treated to include the entire period is compared with the average for the two runs exposed for 6 hr less. Further shortening of the treatment time during sensitivity resulted in still higher crossover values.

A negative correlation again is seen between crossing-over and nondisjunction frequencies. Because noncrossover X chromosomes, in the absence of noncrossover heterologues of comparable size, will pair distributively with each other and segregate very regularly (10), the high incidence of X nondisjunction implies the presence of noncrossover major auto-

Table 1. Effect of restrictive temperature given on sequential days of development on X chromosome crossing-over and nondisjunction

Day(s) of treatment	Crossing-over, %				Nondisjunction, %	No. of ♂ progeny
	Region 1	Region 2	Region 3	Total		
Control	7.0 ± 0.5	15.8 ± 0.7	19.2 ± 0.8	42.0 ± 1.0	0.1	2697
2	6.8 ± 0.8	15.1 ± 1.1	15.2 ± 1.1*	37.0 ± 1.5	1.2	1015
2 and 3	8.1 ± 1.0	14.9 ± 1.3	18.9 ± 1.4	41.9 ± 1.8	0.7	793
3	6.1 ± 1.1	12.1 ± 1.5	18.8 ± 1.8	37.0 ± 2.2	1.7	479
4	6.3 ± 1.1	16.6 ± 1.1	17.6 ± 1.1	40.5 ± 1.4	1.0	1189
5	7.1 ± 0.7	14.2 ± 0.9	16.5 ± 1.0	37.8 ± 1.3	0.8	1434
6	3.6 ± 0.9†	6.5 ± 1.2‡	6.2 ± 1.2‡	16.3 ± 1.8‡	12.2	418
6 and 7	1.6 ± 0.5‡	2.7 ± 0.7‡	2.9 ± 0.7‡	7.3 ± 1.1‡	21.2	551
7	3.9 ± 0.04‡	6.6 ± 0.04‡	8.8 ± 0.1‡	19.3 ± 0.8‡	9.4	2510
8	6.8 ± 0.8	15.5 ± 1.2	15.9 ± 1.2	38.3 ± 1.6	0.8	985
9	7.5 ± 0.5	16.1 ± 0.7	18.6 ± 0.8	42.3 ± 1.0	0.4	2671

* Significant at the 5% level.
† Significant at the 2% level.
‡ Significant at the 0.1% level.

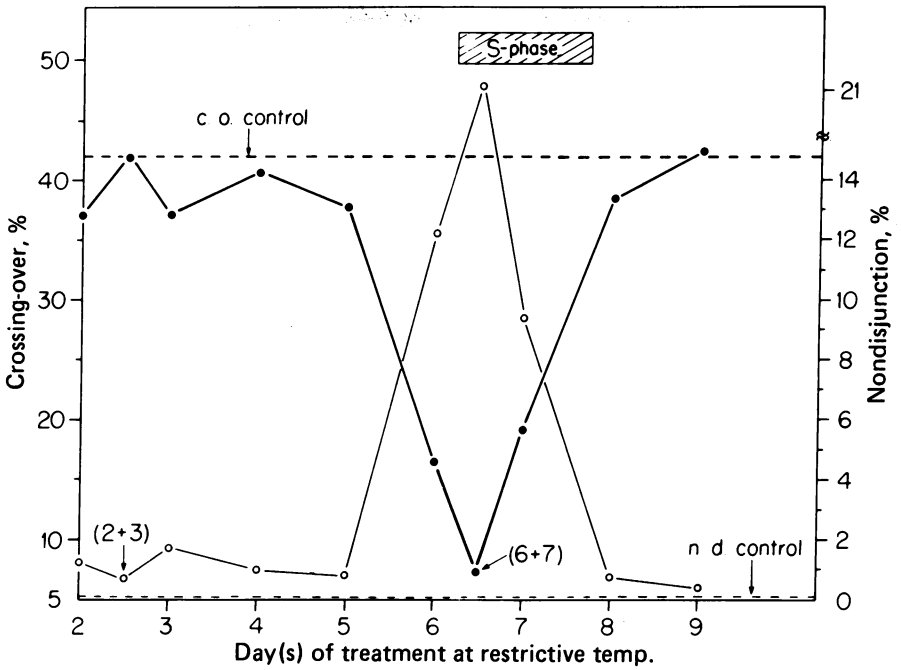

FIG. 1. Effect of restrictive temperature (31°) on crossing-over and nondisjunction on different groups of *rec-1*^{26}/*rec-1*^{16} females heat-treated for 24 hr on sequential days of development from day 2 to day 9 or heat-treated for 48 hr on days 2 and 3 or on days 6 and 7. ●, Crossing-over; o, nondisjunction; - - - -, control; S-phase is premeiotic S. c.o. control, crossing-over control; n.d. control, nondisjunction control.

somes in the distributive pool. Nonhomologous pairing between noncrossover Xs and autosomes produces the X nondisjunction that is observed and must be accompanied by autosomal nondisjunction leading to lethal aneuploid products. An indication of autosomal aneuploidy comes from the great reduction in the number of progeny per female from ~16 in the control to ~4 when heat treatment covers the "sensitive" period.

DISCUSSION

Studies have repeatedly shown that heat-induced increases in recombination in the *Drosophila* oocyte are limited to the premeiotic-S phase (1, 3–5). The coincidence has been demonstrated for more than 20 regions of the genome where significant increases have been measured. In no case has the increase been found to persist beyond S. A recent study (6) provides evidence for narrowing the time of coincidence for specific regions. The heterochromatin spanning the centromere of chromosome 2, which is late labeling in embryonic and cultured cells of *Drosophila* (11, 12), shows a dramatic increase in recombination during the final one-third of S, and the contiguous region, carrying the sequences coding for histones, where there is reason to suspect late replication, peaks during the final one-sixth of S (6). Finally, the dot-like fourth chromosomes, which normally do not recombine in the diploid female, can be induced to do so with heat treatment but only if the treatment is given during S (2).

The present experiments strongly support the interpretation that response to heat identifies the time of recombination. In these studies, exposure of the *rec-1*^{26} allele to the restrictive temperature caused a drastic reduction in recombination. The sensitive period for reduction was coincident with S. Thus, acting in diametrically opposite ways, heat treatment identifies the period of sensitivity for a reduction in exchange by the temperature-sensitive allele and the period of sensitivity for the induction or enhancement of exchange by the normal genome; the heat-sensitive period is identical for both responses and coincides, in both cases, with premeiotic S.

Table 2. Effect of restrictive temperature at different intervals between 120 and 168 hr on X chromosome crossing-over and nondisjunction

Time of treatment,*	Total treatment time, hr	Treatment time during sensitive period, hr	Crossing-over, %				Nondisjunction, %	No. of ♂ progeny
			Region 1	Region 2	Region 3	Total		
Control	–	–	7.0	15.8	19.2	42.0 ± 1.0	0.1	2697
120–144	24	18	3.6	6.5	6.2	16.3 ± 1.8	12.2	418
144–168	24	18	3.9	6.6	8.8	19.3 ± 0.8	9.4	2510
120–148	28	22	2.8	4.4	3.8	11.1 ± 2.3	14.4	180
137–165	28	25	3.4	5.8	6.1	15.3 ± 1.7	5.6	411
120–156	36	30	1.3	3.8	3.2	8.3 ± 1.3	14.0	472
126–156	30	30	1.5	3.1	5.7	10.2 ± 1.3	8.6	547
120–168	48	36	1.6	2.7	2.9	7.3 ± 1.1	21.2	551
120–164	44	36	1.0	1.6	2.9	5.4 ± 1.2	18.4	315
122–168	46	36	1.3	2.9	1.6	5.9 ± 1.3	23.2	306
126–162	36	36	0.8	2.3	3.7	6.8 ± 1.1	15.4	488

* Sensitive period is between 126 and 162 hr.

Recombination requires an intimate association of homologues. It has been widely assumed that homologues are unpaired during premeiotic interphase when DNA replication occurs and that homologous pairing is initiated after DNA synthesis ceases at the zygotene stage of prophase (13). On the basis of these assumptions, recombination could not occur before zygotene. Nevertheless, the cytological literature is replete with observations of associations between homologues during premeiotic anaphase, telophase, and interphase (14). A diagnostic feature of homologous pairing is the synaptonemal complex. Serial reconstructions of electron micrographs (15–17) and idiograms constructed from electron micrograph whole-mount spreads (18) convincingly show that the synaptonemal complexes lie between paired homologues and that they correspond in number to the haploid set of bivalents.

Thus, according to traditional precepts, the synaptonemal complex should not be present between 132 and 162 hr, when DNA replication is in progress, in our oocyte sample. Yet electron micrographs of carefully timed oocytes taken at 6-hr intervals between 132 and 162 hr have revealed long stretches of synaptonemal complex as early as 138 hr (5, 19). Furthermore, electron micrograph autoradiographs of oocytes labeled with [^{3}H]thymidine demonstrate the presence of label (indicating DNA synthesis) and synaptonemal complexes (indicating homologous pairing) in the same oocyte nuclei at 144 hr (5, 19) and between 132 and 162 hr (unpublished data). Because the first oocytes in the present sample are formed at about 132 hr, it is evident that homologues are in the synapsed condition required for recombination from the beginning of premeiotic interphase and throughout premeiotic S.

The presence of synaptonemal complexes during S has recently been found in stage 3 (DNA synthetic period) of pollen mother cells of *Triticum aestivum* (20). Simchen (21) reported that, in *Saccharomyces cerevisiae*, irreversible commitment to meiotic recombination occurs during premeiotic S, probably at its very early stages, and that synaptonemal complexes are present at this time. In view of the nonconformance of three of the best characterized genetic organisms to the traditional view, a thorough reexamination of current concepts concerning the time of recombination in eukaryotes is plainly in order.

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