Environmental Health Perspectives
Vol. 31, pp. 41-43, 1979

Mechanisms of Nondisjunction Induction in Drosophila Oocytes

by B. Leigh*

Quantitative and qualitative studies on the induction of nondisjunction and related phenomena can be carried out using the germ cells of Drosophila. X-Irradiation breaks chromosomes and cold-shock disrupts spindles, these two treatments producing different spectra of nondisjunction in oocytes.

Introduction

Several recent articles elaborate on the usefulness of Drosophila as a test system for the detection of environmental mutagens (*1-3*). In short "In contast to most other assay systems, where only one class of genetic damage can be studied, special test strains permit the simultaneous assessment of the total spectrum of genetic changes, ranging from recessive lethal or visible mutations, small deletions, translocations, chromosome loss, and dominant lethals to nondisjunction and genetic recombination" (*1*).

Nondisjunction is one of the first genetic changes which was shown to be induceable by an external agent, x-irradiation (*4*). A variety of mechanisms have been proposed to account for this phenomenon. These include chromosome stickiness (*5*), damage to the centromeric region of the chromosome (*6*), and damage to the spindle (*7*). The most extensive genetic analysis of the products of induced nondisjunction has been carried out by Parker and coworkers (*8, 9*). Their data are consistent with the model of "heterologous conjunction via chromatid interchanges as the major and perhaps exclusive cause of radiation-induced non-disjunction" (*9*).

The data supporting the model were all obtained from studies on the sex chromosomes and the fourth chromosomes. Puro (*10*), working with the major autosomes, has questioned whether induced chromatid rearrangements direct first division segregation.

*Department of Radiation Genetics and Chemical Mutagenesis, The State University of Leiden and The J. A. Cohen Institute, Wassenaarseweg 72, Leiden, The Netherlands.

In Drosophila there are many genes which function during the regular meiotic process in the oocyte. Mutations of these genes are interesting for two reasons; firstly, many of them cause nondisjunction and secondly, some of them enhance sensitivity to radiation and mutagenic chemicals (*11*). There are several ways in which the meiotic process can be disturbed by such mutations. Often this is associated with a reduction in the amount of recombination. While it has been shown that artificially induced conjunctions probably cause nonhomologous chromosomes to segregate, the converse is not true. Homologous chromosomes which are not conjoined by chiasma usually segregate regularly. To explain this phenomenon, Grell (*12*) has proposed the "distributive pairing" model. According to this model there are two rounds of pairing during meiosis in the oocyte. Firstly the chromosomes pair to permit recombination and thus the formation of regular conjunctions. Then the chromosomes which are nonconjoined form a "distributive pool". Segregation of the chromosomes of this pool is regulated by a specific set of rules. Novitski (*13*) has argued that the observations can be accounted for by assuming a nonhomologous association of all the chromosomes prior to synapsis. Recent cytological studies have confirmed that there is indeed a chromocentral association of all the chromosomes. An example of the complexities which must yet be explained is the behavior of the fourth chromosomes and the compound autosomes (*14*). According to the genetic analysis (*15*), these are two pairs of chromosomes which segregate independently from the "distributive pool". Cytological analysis showed that the orientation of the fourth chromosomes is disturbed in

females carrying compound autosomes.

Compound autosomes segregate regularly in females but at random in males (*16*), this being an expression of the different mechanisms regulating pairing and segregation in the two sexes. The products of autosomal nondisjunction are generally lethal because of aneuploid unbalance. Studies on induced autosomal nondisjunction in oocytes became feasible when it was shown that males carrying compound autosomes regularly produce 25% nullosomic and 25% disomic gametes. Females with either regular metacentric autosomes or with compound autosomes can be exposed to an agent which is expected to induce aneuploidy. Viable zygotes will form with the corresponding nullosomic or disomic male gamete. Bateman (*17*), Savontaus (*18*), and Gibson (*19*) have studied induced nondisjunction in the oocytes of females with regular second chromosomes. With this technique only exceptional progeny are recovered and frequencies must be estimated. This work has shown that autosomal nondisjunction can be induced by radiation and that the ratio of induced losses to gains is about 6:1, a ratio similar to that which has often been found for rod-X chromosomes. Clark and Sobels (*20*) induced nondisjunction by irradiation of females with compound second chromosomes. Theoretically, with this technique there is an uniform viability of regular and exceptional maternal gametes. Therefore, frequencies of induced nondisjunction can be measured directly.

Results and Discussion

Scoring for nondisjunction of two pairs of chromosomes was made possible by adding marked X and Y chromosomes to the stocks with compound second chromosomes. Nondisjunction was induced by irradiating immature oocytes; one-day old females were given an exposure of 1500 R, mated, and then allowed to lay eggs for 4 days. In another set of females nondisjunction was induced by cold treatment of mature oocytes. A summary of the data obtained in these experiments, together with an acomparable set of data on recording nondisjunction (*15*), is given in Table 1. The first two columns show nondisjunction of only the sex chromosomes, the second two columns show nondisjunction of the second chromosomes, and the next four columns show simultaneous nondisjunction of both pairs of chromosomes.

In the control series (a) almost a third of all the exceptions are nullo-X diplo-2. Secondary nondisjunction (b) is typified by high frequencies of diplo-X nullo-2 and nullo-X diplo-2 progeny. Radiation-induced nondisjunction (c) is characterized by a high ratio of X-loss to X-gain. In mature oocytes cold treatment induces high frequencies of all exceptional classes (d).

The most important point to be made from these data is that nondisjunction can be induced in different ways and significant variations between the distribution of the exceptional progeny classes provides a direct indication that different mechanisms are involved.

There is now general agreement that chromosome breakage is one of the primary events in the process of radiation-induced nondisjunction.

The effects of low temperature on mitotic divisions were first demonstrated by Bauer (*21*), who induced triploidy by giving cold shocks to young embryos. Gloor (*22*) aged third instar larvae from two stocks for 10 days at approximately 10°C. Polyploidy was induced in the ganglion cells of one of these stocks. Hildreth and Ulrichs (*23*) cold-aged females for one or two weeks and obtained a significant increase in the frequency of nondisjunction. Tokunaga (*24*) was able to show that mature oocytes

Table 1. Summary of the frequencies of progeny carrying gains and losses of the sex chromosomes (CHR. 1) and compound second chromosomes (CHR. 2).

	Material gametes									
CHR. 1 CHR. 2	0 1	2 1	1 0	1 2	0 0	0 2	2 0	2 0	Exceptions, %	Total no. of gametes
Spontaneous, primary nondisjunction	0.05	0.09	0.12	0.10	0.01	0.19	0.05	0.05	0.67	5808
Secondary nondisjunction in XX/Y females[a]	1.7	1.1	1.5	3.4	0	5.0	3.5	0	16.3	1310
Exposure of immature oocytes to 1500 R x-irradiation	0.80	0.05	0.72	0.65	0.13	0.47	0.08	0.04	2.94	5272
Eggs laid on first day after storage at 10°C	0.9	0.9	2.9	3.8	0.2	0.7	0.7	0.7	10.8	443
Eggs laid on second to fifth days after storage at 10°C	0.25	0.15	0.30	0.13	0.03	0.20	0.08	0	1.12	4003

[a]Data of Grell (*15*).

are the most sensitive stage for this phenomenon. Clark (*25*) was able to induce a small increase in the rate of compound-2 nondisjunction by cold aging. The data in Table 1 were obtained with a stock carrying a *C(2R)RM, vg* chromosome. The distribution of classes is compatible with the interpretation that nonhomologous chromosomes nondisjoin independently. There is no indication that cold aging breaks chromosomes, and Tokunaga found equality of X chromosome losses and gains. The simplest explanation of these observations is that cold aging affects the spindle mechanism (*26*).

Taking into account the theme of this workshop, chemicals which induce only aneuploidies and no other genetic end points, it can be concluded that a simple genetic test for sex chromosome aneuploidy can be used. The studies with complex genetic systems will then be required for an analysis of the mechanism.

REFERENCES

1. Sobels, F. H., and Vogel, E. Assaying potential carcinogens with Drosophila. In: Proceedings of the meeting of the Scientific Committee, 12 December 1975, Carlo Erba Foundation, Occupational and Environmental Health Section, p. 117.
2. Sobels, F. H., and Vogel, E. The capacity of Drosophila for detecting relevant genetic damage. Mutat. Res. 41: 95 (1976).
3. Würgler, F. E., Sobels, F. H., and Vogel, E. Drosophila as an assay system for detecting genetic change. In: Handbook of Mutagenicity Test Procedures, B. J. Kilby, M. Legator, W. Nichols, and C. Ramel, Eds., Elsevier/North Holland Biomedical Press, Amsterdam, 1977.
4. Mavor, J. W., The production of nondisjunction by x-rays. J. Exptl. Zool. 39: 381 (1924).
5. Rapoport, J. A. The effect of x-rays on nondisjunction of the fourth and X-chromosomes in *Drosophila melanogaster*. Biol. Zh. 7: 661 (1938).
6. Grell, R. F., Munoz, E. R. and Kirschenbaum, W. F. Radiation-induced nondisjunction and loss of chromosomes in *Drosophila melanogaster* females. Mutat. Res. 3: 494 (1966).
7. Traut, H. Nondisjunction induced by x-rays in oocytes of *Drosophila melanogaster*. Mutat. Res. 10: 125 (1970).
8. Parker, D. R. Radiation induced nondisjunction and Robertsonian translocation in Drosophila. Mutat. Res. 24: 149 (1974).
9. Parker, D. R., Williamson, J. H., and Gavin, J. The nature and time of occurrence of radiation-induced nondisjunction of the acrocentric x and fourth chromosomes in *Drosophila melanogaster* females. Mutat. Res. 24: 135 (1974).
10. Puro, J. Recovery of radiation-induced autosomal chromatid interchanges in oocytes of *Drosophila melanogaster*. Hereditas 88: 203 (1978).
11. Boyd, J. B., Golino, M. D., Nguyen, T. D., and Green, M. M. Isolation and characterization of x-linked mutants of *Drosophila melanogaster* which are sensitive to mutagens. Genetics 84: 485 (1976).
12. Grell, R. F., A new hypothesis on the nature and sequence of meiotic events in the female of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. (U.S.) 48: 165 (1962).
13. Novitski, E. An alternative to the distributive pairing hypothesis in Drosophila. Genetics. 50: 1149 (1964).
14. Puro, J., and Nokkola, S. Meiotic segregation of chromosomes in *Drosophila melanogaster* oocytes. Chromosoma. 63: 273 (1977).
15. Grell, E. H. Distributive pairing: mechanism for segregation of compound autosomal chromosomes in oocytes of *Drosophila melanogaster*. Genetics 65: 65 (1970).
16. Holm, D. G., Deland, M., and Chovnich, A. Meiotic segregation of C(3L) and C(3R) chromosomes in *Drosophila melanogaster*. Genetics 56: 565 (1967).
17. Bateman, A. J. Nondisjunction and isochromosomes from irradiation of chromosome-2 in Drosophila. In: Effects of Radiation on Meiotic Systems. IAEA, Vienna, 1965, p. 63.
18. Savontaus, M.-L. x-ray induced autosomal nondisjunction in relation to crossing over in *Drosophila melanogaster*. Hereditas 86: 115 (1977).
19. Gibson, W. G. Autosomal products of meiosis arising from radiation-induced interchange in female *Drosophila melanogaster*. Ph.D. Thesis, University of British Columbia, Canada, 1977.
20. Clark, A. M., and Sobels, F. H. Studies on nondisjunction of the major autosomes in *Drosophila melanogaster*. I. Methodology and rate of induction by x-rays for the compound second chromosome. Mutat. Res. 18: 47 (1973).
21. Bauer, H. Auslösung von Polyploidie durch Kälte bei *Drosophila melanogaster*. Z. Naturforschg. 1: 35 (1946).
22. Gloor, H. Polyploidy in Drosophila larvae by cold treatment. Dros. Inf. Service. 24: 82 (1950).
23. Hildreth, P. E., and Ulrichs, P. C. A temperature effect on nondisjunction of the X-chromosome among eggs from aged Drosophila females. Genetics 40: 191 (1969).
24. Tokunaga, C. Aspects of low-temperature-induced meiotic nondisjunction in Drosophila females. Genetics 66: 653 (1970).
25. Clark, A. M. Low temperature shock and the segregation of iso-second chromosomes in females. Dros. Inf. Service 51: 26 (1974).
26. Inové, S., Borisy, G. G., and Kiehart, D. P. Growth and lability of Chaetopterus oocyte mitotic spindles isolated in the presence of porcine brain tubulin. J. Cell. Biol. 62: 175 (1974).