# THE EFFECT OF X-RAYS ON THE FUNCTIONAL STRUCTURES OF THE Y CHROMOSOME IN SPERMATOCYTES OF *DROSOPHILA HYDEI*

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The paired loop-like formations that are developed by the Y chromosome in the nuclei of primary spermatocytes of *Drosophila hydei* have been shown to be similar in nature to the loops of amphibian lampbrush chromosomes (8, 6). Each pair of loops has its own specific organizer. The sites of these organizers on the Y chromosome have been mapped (5). The loops have been found to collapse after a treatment with actinomycin (9). As this agent is known to block the DNA-dependent RNA synthesis, this result suggests that the loops are modifications of the chromosome structure at sites of active genes.

The present study of the loops of the Y chromosome in *D. hydei* spermatocytes shows that the morphological effects produced by actinomycin can be simulated by x-irradiation with doses ranging from 1,000 to 10,000 r. These changes are most conspicuous in the pair of loops designated as the "threads" (Fig. 1 *a*). The compact proximal sections of this loop pair become shorter and thicker. Moreover, the material puffs and forms small cavities. At five to 7 hours after irradiation, the threads have the appearance of a string of hollow beads (Fig. 1 *b*). The number of these beads or spheres decreases gradually. At 15 to 20 hours after irradiation, one usually finds only one pair of rather big spheres located near the nucleolus. The threads have completely disappeared at 24 to 30 hours after irradiation (Fig. 1 *c*). The fate of the diffuse distal segments of the threads could not be followed because they become invisible soon after irradiation. However, alterations occurring in this segment could be followed in *tube-distal*, a mutation which changes the distal segments of the threads into two knots of narrow tubes (3, 4). Whereas the behavior of the unchanged proximal segments in the mutated type is similar to that in the normal type, the distal tubes swell after irradiation. In later stages they are shortened, and finally they disappear.

The other pairs of loops show also striking alterations after x-irradiation. The matrix of a second pair of loops, designated "clubs," swells, and the normally attached granules are spread gradually over the whole nuclear area. Around a third loop pair, the so called "tubular ribbons," many new granules are formed which are never found in non-irradiated nuclei. The material of the tubular ribbons which normally appears in phase contrast as a large gray field (Fig. 1 *a*, *T*) is clotted together. The "pseudonucleolus" seems



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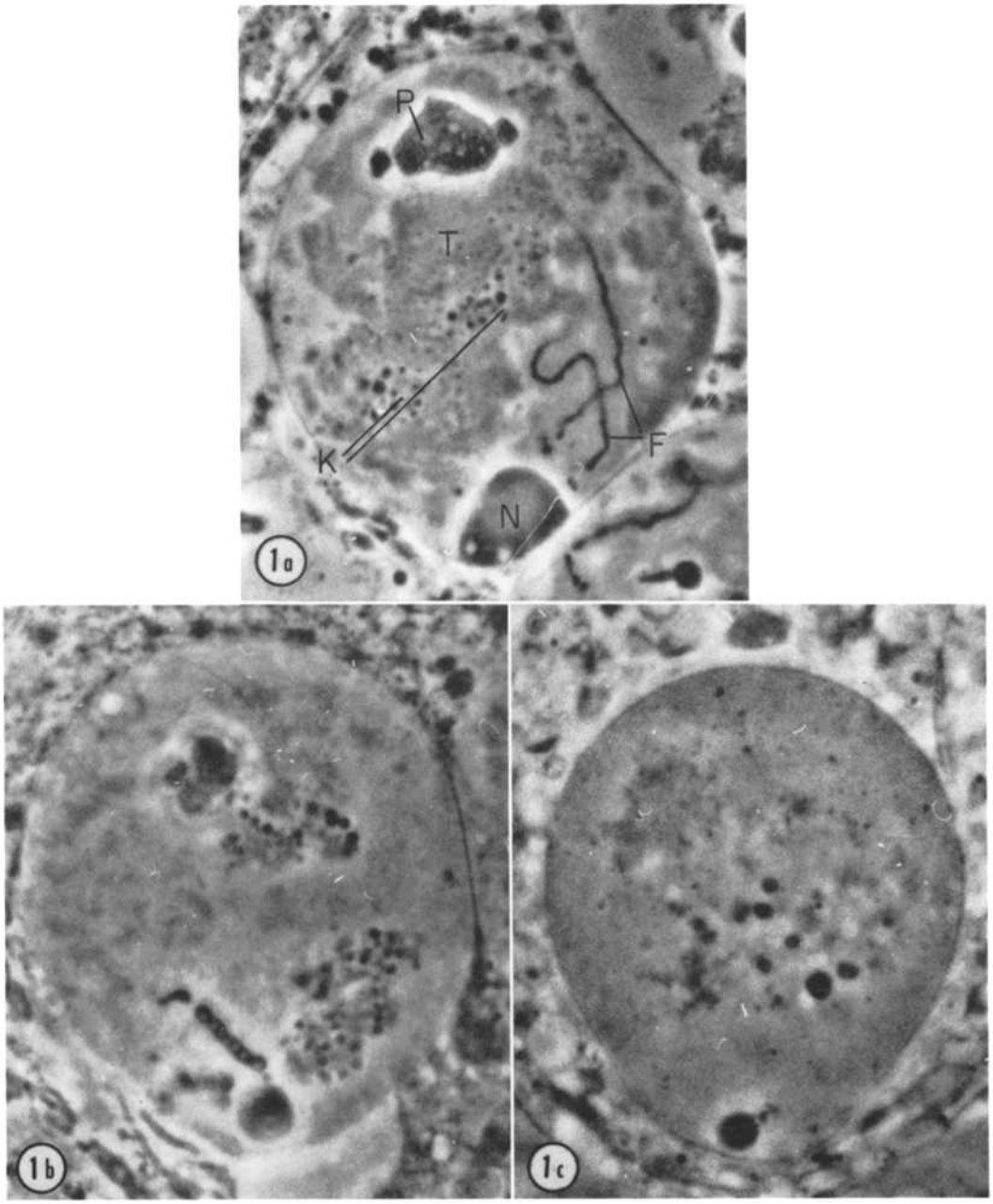


FIGURE 1 Loops of the Y chromosome in the nuclei of primary spermatocytes of *Drosophila hydei* after x-irradiation with 7,500 r at 100 kv. *a*, Control nucleus before irradition. *b*, 7 hours after irradiation. *c*, 30 hours after irradiation. *F*, "threads"; *K*, "clubs"; *N*, nucleolus; *P*, "pseudonucleolus"; *T*, "tubular ribbons." Phase contrast photographs of living nuclei. × 1,600



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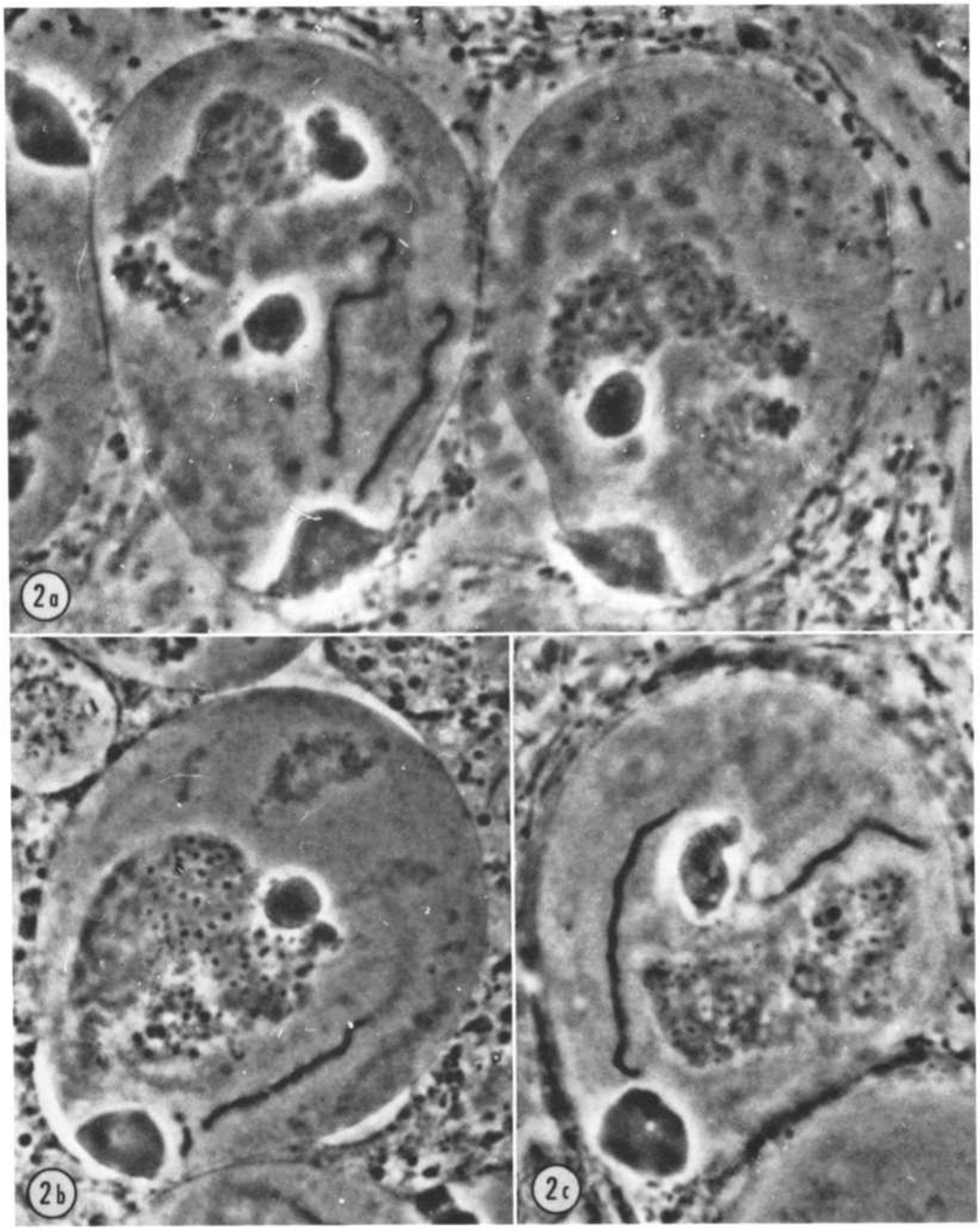


FIGURE 2 Spermatocyte nuclei 78 hours after irradiation with 7,500 r (*a*), 5,000 r (*c*), and 2,000 r (*b*), showing irreversible damage of the Y chromosome loops. *a*, Nucleus with normally regenerated threads (left), and nucleus with complete suppression of thread regeneration (right). *b*, Nucleus showing partial suppression of thread regeneration. *c*, Nucleus with breakage in thread-like loop. Phase contrast photographs of living nuclei. × 1,600.



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to lose material. Its structure appears much lighter than under normal conditions. Sometimes, it becomes nearly invisible (Fig. 1 *c*). These changes occur all at the same time and reach a maximum at about 24 to 30 hours after irradiation. The nucleolus is also changed. It becomes spherical in shape and often detaches itself from the nuclear membrane (Fig. 1 *b* and 1 *c*) (where it is normally located).

The alterations show only a weak dependence on the irradiation dose. With doses between 2,500 and 10,000 r, the maximum effect attained and the speed with which it develops are the same. With lower doses, *e.g.* 1,000 r, only some of the spermatocyte nuclei are affected. Many nuclei do not show any alterations.

In accordance with the experiments using actinomycin, we find that those structural alterations caused by x-irradiation are also reversible. At 80 to 100 hours after irradiation, a gradual but complete regeneration of the loops occurs. Again, these processes can most easily be followed in the thread-shaped pair of loops. At first, two very short pieces of compact threads which are connected by a diffuse distal section appear near the nucleolus. These extend gradually until they attain the dimension and appearance of the original threads in untreated nuclei (Fig. 2 *a*, nucleus on the left).

Unlike actinomycin, x-irradiation may cause also some irreversible changes in the Y chromosome loops. In some nuclei, for example, the regeneration of the threads is blocked either on both sides (Fig. 2 *a*, nucleus on the right), or on only one side (Fig. 2 *b*). Breaks within a loop can also be seen occasionally (Fig. 2 *c*). In contrast to the lampbrush chromosomes in amphibian oocytes in which breaks of the loops have been observed immediately after the irradiation (10), so far no such direct breaks have been detected in the loops in spermatocytes of *Drosophila hydei*.

In view of our findings, one may raise the question concerning whether the primary effects of both actinomycin and x-irradiation are on the same target; *i.e.* the priming activity of the DNA in RNA synthesis. Whereas the synthesis of DNA is well known to be radiosensitive (see, however, references 11, 12), there has been no evidence so far that x-rays inhibit the RNA polymerase system *in vivo*. On the contrary, Chambon *et al.* (1) reported that whole body irradiation with 1,500 r had no effect on the DNA-dependent RNA synthesis in regenerating rat liver cells. However, it was recently shown that the *in vitro* priming ability of the DNA of *Escherichia coli* (2) and of calf thymus (13) is severely depressed by x-irradiation. The doses used in these experiments were the same as in our experiments. Stronger support for the assumption of such direct radiation effects would be the demonstration of inhibition of messenger RNA formation in the loops by either actinomycin or x-rays. Experiments using $H^3$-uridine are in progress.

An alternative explanation for the observed effects would be the assumption that proteolytic enzymes are activated by x-irradiation (see 7 for references) which might dissolve the coat of ribonucleoproteins attached to the DNA axis in the loops.

## SUMMARY

At least five sites of the Y chromosome of *Drosophila hydei* form loops of specific morphology which seem to occur in correlation with a phase of activation of certain genes in spermatocyte nuclei. X-irradiation in doses of as low as 1,000 r causes characteristic morphological alterations of the loops. Exactly the same alterations have been observed earlier after treatment with actinomycin. The alterations are reversible. As expected, and unlike actinomycin, x-irradiation causes also some irreversible damage; *i.e.*, complete or partial suppression of loop regeneration, as well as breaks within the loops. The results are not inconsistent with the suggestion that in *Drosophila* spermatocytes the DNA-directed synthesis of RNA can be reversibly inhibited by x-irradiation.

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