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Technical Progress Report

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X-RAY INDUCED SYNTHESIS OF DNA IN POLYTENE CHROMOSOMES*

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The study was undertaken to ascertain whether DNA synthesis could be induced in the polytene chromosomes of Drosophila melanogaster by X-irradiation, whether the production of new breaks in chromosomal DNA would lead to detectably new sites of DNA synthesis. It was hoped that positive results, if obtained, would not only permit an experimental approach to the question of control mechanisms of normal DNA replication but also provide a good basis for exploring the nature of chromosomal breakage repair in cytologically favorable material. As is detailed below, we have observed X-ray induced synthesis of DNA in our material; moreover, this synthesis can be distinguished from that associated with normal DNA replication. Our initial objectives have thus been achieved and we can now undertake a more detailed analysis of repair versus replicative chromosomal DNA synthesis.

Methods:

Salivary glands from Drosophila melanogaster (Oregon R) were excised from third instar larvae grown at 18° C in standard corn meal-agar medium, enriched with extra yeast which was added after egg hatching. The larvae were selected among those which had first emerged from the food. We have also used glands which were transplanted (from synchronously grown third instar larvae) into the abdominal cavity of adults and cultured for nine to nineteen days.

The two lobes from each gland were separated and one used for treatment, the other for control. They were placed individually in the depressions of a disposable microtiter plate containing a small

amount of Ephrussi-Beadle medium.

The experimentals were irradiated and high specific activity tritiated thymidine was subsequently added to both experimentals and controls. A solution of ten microcuries per milliliter of tritiated thymidine was employed and the pulse lasted 15-20 minutes.

Several doses of X-rays (1000r, 5000r, 10,000r, 30,000r, and 100,000r) were used in the first experiments. Later, 30,000r was usually used. After incubation in thymidine, the glands were smeared on gelatinized slides in a drop of 45% acetic acid for fixation. After removal of the coverslip, they were post-fixed in 1:3 acetic-alcohol, stained in a solution of 2% orcein in 65% acetic acid for 15-20 minutes, dehydrated, passed through a solution of 1% parlodion in amyl acetate, run down to distilled water, and finally covered with Kodak A. R. 10 autoradiographic stripping film. The slides thus prepared were placed in light-proof boxes and stored at 4° C for two weeks, after which they were developed in D19b Kodak developer and examined.

Analytical Procedure

Several hundred experimental and control nuclei were analyzed following the procedure outlined in the original proposal. The parameters taken into consideration were as follows:

1. Frequency of labeled nuclei.
2. Proportion of "discontinuous" and "continuous" labeling.
3. Frequency of labeling at specific loci and frequency of joint labeling at normally correlated loci.
4. Iso-labeling at homologous loci in unpaired regions of homologous chromosomes.

5. Distribution of autoradiographic grains across versus along the chromosome axis.

It had previously been observed in this laboratory that during normal chromosomal DNA replication, labeling is always observed in the chromocenter. Chromocentral labeling was therefore used as the criterion for determining, especially in doubtful cases, whether or not a nucleus was undergoing normal DNA replication.

A nucleus was considered continuously labeled when the label was distributed along the chromosomes without obvious interruption. In cases of light spot labeling along the chromosomes a cluster of at least 4 grains referable to a band or small chromosome region was taken as indication of localized DNA synthesis.

When considering the pattern of labeling at individual and correlated loci only those chromosome segments where the patterns of incorporation have already been established (Howard & Plaut, J. Cell Biol., in press, 1968) were observed. These segments are (according to Bridges' salivary chromosome map) regions 1, 2, 3, and part of 4 of the X chromosome; regions 58, 59, and 60 of chromosome II R and 61, 62, 63, and part of 64 of chromosome III L.

Results

A number of observations which we consider of interest and permit some preliminary conclusions have been made to date.

Experiments using freshly excised salivary glands:

It has been possible to establish that X-irradiation leads to synthesis of DNA at a large number of sites on the chromosomes as manifested by the incorporation of H^3 -thymidine. The sites of incorporation appear randomly scattered over the euchromatin; only a few silver grains have been detected over the chromocentral region. We suggest that this process represents the repair of X-ray damage to

chromosomal DNA and that it resembles the phenomenon of repair replication described in bacteria and other cells. We have observed such induced DNA synthesis with all X-ray doses used except the lowest, 1000r. In the latter case the results are ambiguous and more data will have to be obtained.

The identification of labeling as induced was possible due to the following considerations: In untreated glands, about 50% of the nuclei show label incorporation. Only a few of these nuclei have continuously labeled chromosomes; the majority are spot labeled with recognizable and predictable patterns. In glands irradiated with doses above 1000r, the frequency of labeled nuclei greatly exceeds 50% (90% with a dose of 100,000r). Moreover, a large proportion of the labeled nuclei in irradiated glands had unlabeled chromocenters and were thus not undergoing normal replicative DNA synthesis. These nuclei showed random labeling over the euchromatic chromosome regions and no tendency toward spot labeling patterns. Since this particular distribution of incorporation sites is never seen in unirradiated nuclei its identification as induced is definite.

Experiments with glands cultured as implants:

At the time the above mentioned observations were being made Dr. and Mrs. Cordeiro, in our laboratory, had discovered that 95% or more of salivary gland nuclei (from synchronized mid-third instar larvae) cultured as implants in the abdominal cavity of adult females would stop incorporating tritiated thymidine on about the 7th or 8th day after implantation. (These nuclei, even when aged for almost thirty days in implants, retained the ability to incorporate tritiated uridine and are thus viable.) The fact that normal chromosome replication was suppressed (or terminated) in almost all of these nuclei led us to inquire whether cultured nuclei were respons-

ive to the action of radiation and whether it might therefore be possible to study induced synthesis in the absence of normal replication.

It was found that implanted glands do indeed show an increase in labeling following irradiation in a manner quite comparable to freshly excised glands. In a typical experiment where implanted nuclei 8, 15, and 19 days old were irradiated with 30,000r and immediately incubated with tritiated thymidine an average of 50% showed induced incorporation of the label. About 2% of the experimental and control nuclei evidenced normal replication in the form of continuous or spot labeling. Experiments using different doses such as 5000r and 15,000r gave similar general results.

Concerning the duration of this effect, we can only say at this moment that it lasts for at least 20 minutes after irradiation and probably begins immediately after. Experiments are in progress to determine the duration more precisely.

Experiments have also been designed to determine the relationship between different radiation doses and the amount of label incorporated. Preliminary results indicate a direct relationship for doses ranging between 5000r and 30,000r.

Discussion

The results thus far obtained show clearly that a wide range of doses induce a kind of DNA synthesis in the salivary gland nuclei which can be detected by the incorporation of tritiated thymidine and which appears to be closely related to, if not identical with, the phenomenon of repair replication known in bacteria and reported for various other types of cells.

In our system this effect has been induced and observed in the extended interphase polytene chromosomes at highly localized single

spots, which are randomly distributed throughout the euchromatic chromosome region.

No autoradiographic grain clusters were observed at any points along the chromosomes and there was no evidence of isolabeling in unpaired regions, indicating that the events must be highly localized, probably not affecting more than part of a single chromosome (or single DNA helix) in the closely packed bundle. It would appear that this generalized and yet local effect of radiations is not equivalent to the stimulus responsible for normal chromosomal DNA replication because if it were, one should observe clusters characteristic of the temporal patterned replication. Repair replication thus is probably not a partial reinitiation of normal synthesis, but a separate process which can operate independently. This conclusion is also supported by the fact that in the transplanted glands induced synthesis occurs in nuclei which were no longer engaged in normal replicative DNA synthesis. In replicating nuclei, both processes may occur simultaneously.