MASTER

Technical Progress Report

COO-1747-2X-RAY INDUCED SYNTHESIS OF DNA IN POLYTENE CHROMOSOMES¹

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For introduction, objectives, and general experimental procedures followed, see our previous report, August 15, 1968.

The experiments reported here further support and extend our initial studies on the synthesis of DNA in the polytene chromosomes subjected to irradiation and give evidence for apparently hitherto unknown characteristics.

Studies have been made: 1. On the effect of a number of chemicals which are known to inhibit normal DNA synthesis. a) Fluorodeoxyuridine (FUdR) which acts by blocking the synthesis of thymidylate synthetase. Salivary gland nuclei incubated in FUdR (10^{-6} M) for 25-30 minutes stop replicating DNA, as revealed by the subsequent addition of tritiated deoxycytidine or deoxyadenosine; these are not incorporated. b) Hydroxurea, (OHU) with a more generalized effect, blocking the nucleotide monophosphate reductase. It was used at 10^{-3} M during 30 minutes. c) Mercapto-1-(B-4 pyridethyl) benzimidazole (M.P.B.) which blocks also RNA and protein synthesis and which was used at concentrations of 25-30 $\mu\text{g/ml}$ during 20-30 minutes. Not much is known as to the precise site at which M.P.B. acts on DNA or RNA. It probably interferes with purine and pyrimidine metabolism. 2. RNA inhibitors, namely Actinomycin D (AMD) which binds to DNA, interfering with normal template activity. It was used at concentrations of 2 and 4 $\mu\text{g/ml}$ during 20 minutes. 3. Protein inhibitors. So far only cycloheximide has been used in some preliminary experiments, at concentrations of 10 $\mu\text{g/ml}$ during 25-30 minutes, and no conclusive results have been obtained.

These compounds were tested, singly or combined in some cases, in the salivary glands of Drosophila melanogaster and D. hydei. The results obtained are abstracted in the following table.

Inhibitors	DNA		RNA synthesis
	Normal synthesis	unscheduled synthesis	
FUdR 10^{-6} M	-		+ (to -)
FUdR 10^{-6} M + X-Rays 30Kr	-	+	
OHU 10^{-3} M	-		
OHU 10^{-3} M + X-Rays 30Kr	-	+	
AMD 2-4 μ g/ml	+ (to -)		-
AMD 2-4 μ g/ml + FUdR 10^{-6} M	-		-
AMD 2-4 μ g/ml + FUdR 10^{-6} M + X-Ray 30Kr	-	+	-
AMD 2-4 μ g/ml + OHU 10^{-3} M + X-Rays 30Kr	-	+	-
MPB 25 μ g/ml	-		-
MPB 25 μ g/ml + X-Rays 30Kr	-	+	-

Normal DNA synthesis in the salivary gland chromosomes is immediately inhibited, as judged autoradiographically, by FUDR, OHU, and MPB, that is to say no matter where in the synthetic pathway the blockage occurs. This may reflect the presence of an extremely critical level of available nucleosides at any time during normal DNA synthesis. On the other hand, unscheduled (X-Ray induced) synthesis is not affected by the presence of these inhibitors. Moreover, it takes place in nuclei which have ceased to replicate normally as well as in mutants where DNA synthesis is genetically impaired (see below).

Actinomycin D, at the doses used, completely suppressed all detectable RNA synthesis after 30 minutes; when such treatment was followed by the addition of OHU or FUDR, there was, as expected, no label incorporation into DNA or RNA. When cells thus treated were irradiated, however, unequivocal DNA precursor incorporation were observed in a pattern identical to that of repair replication. The incorporation sites were randomly distributed but more numerous when AMD treatment preceded treatment with FUDR or OHU than when the latter two inhibitors were used alone. From this one could infer some kind of interaction between radiation and AMD which results in a larger number of single strand breaks. It is also possible that AMD modifies the DNA template at binding points in such a way as to delay the repair process and thus allows for a higher level of accumulation of introduced precursor. Under this hypothesis, the increased labeling would be explained by a higher specific activity of labeled precursor in the internal pool.

The experiments in which FUDR and OHU were used in various combinations were repeated with the addition of a 30 minute 37°C temperature shock 15-20 minutes after inhibition had been induced. This

added treatment had no detectable effect on repair synthesis.

Thus the system responsible for the repair of damaged DNA in polytene chromosomes operates in the absence of normal DNA synthesis, RNA synthesis, and probably protein synthesis, and in the presence of reagents which inhibit these processes. It seems highly probable that it is quite different as well as independent from that responsible for normal, replicative DNA synthesis. Whether the enzymatic machinery used for repair exists in the cell per se before irradiation or whether the enzymes are freshly synthesized against pre-existing messenger RNA molecules can be established through more extensive experimentation with inhibitors of protein synthesis. Our present tendency is to favor the first-named alternative.

Since the phenomenon of repair replication is presumably under genetic control the study of certain variations may afford important information. With this idea in mind we are studying and selecting mutants of D. melanogaster whose phenotypes lead us to suspect variations in DNA and RNA synthesis at various times during development.

We have selected so far some obvious ones such as lethal giant larvae, the crossover suppressor of Gowen and others. We have prepared or are preparing suitable stocks.

Lethal giant larvae, 1(2)gl, is a mutant which is located in the second chromosome at 0.0. It was selected because it shows a number of developmental abnormalities correlated with a reduced amount of DNA in the nuclei of the salivary gland; also, the amino acids, peptides and proteins, as well as the hormone level, are affected.

We have determined that late 3rd instar larvae do not incorporate H^3 -thymidine in at least 80% of their salivary gland nuclei, the rest showing a continuous but light pattern of incorporation.

The labeling with H^3 -uridine is also affected, appearing light and spotty in comparison with the wild type. When these nuclei were examined for unscheduled synthesis after X-irradiation, we observed a distinct but light incorporation of the label in 50-60%.

There seems to exist a decrease in the amount of H^3 -thymidine incorporation with approaching death at pupation. Thus one might expect an increase in the frequency of spontaneous chromosome breaks and/or aberrations. The examination of the ganglion cells failed to confirm these expectations, but further tests should and will be made.

Concerning Gowen's cross-over suppressor, c(3)G, located in the 3rd chromosome at 57.4, some preliminary experiments failed to show incorporation of label after X-irradiation and inhibition with hydroxyurea when applied to nuclei from homozygous females. If confirmed, these results would be of great interest, in light of the hypothesized relationship between repair replication and recombination.

Some of our time has been devoted to the work summarized below. Its inclusion within the scope of this project appears to us justified in that it contributes to our understanding of normal DNA replication patterns against which X-ray induced DNA synthesis is studied.

On temporal autonomy of DNA replication in chromosome rearrangements and a developmental mutant.

Previous studies by Plaut and collaborators (1) on the replication of DNA in the polytene chromosomes of D. melanogaster led to the conclusion that replication is specific for small chromosomal regions and could be regarded as a property of such regions. Subsequently it was shown that this property persists after a given region has been translocated to another chromosome (2).

We are presently testing further these results by studying other types of rearrangements including a Y:3 reciprocal translocation, two duplications Dp(1:f) 1337 and Dp(1:4) w^{m5} , and some "normal" X-chromosome "repeats". In all cases salivary glands from third instar larvae were incubated with H^3 -thymidine and analysed autoradiographically.

The pattern of labeling in the translocated 3R segment of the T(Y;3) and in the two duplicated segments was synchronous with that of their respective normal homologs. (All three of these segments are attached to different heterochromatic regions involving the Y, X or 4th chromosomes.) The very short segment of the short arm of the Y, which is attached to the proximal euchromatic 3R at 99A, showed a very low frequency of label which may be interpreted as due to its heterochromatic nature. In a few cases we clearly observed label in the chromocentral region and in the Y segment, but none was present in the euchromatin.

It has also been possible to observe the pattern of replication in certain bands which are normally present in the X-chromosome, in the form of "repeats". First we examined the so called Kossikov's duplication involving bands 11A - 11B and 12D - 12E. In most of the cases when one part of the repeat was labeled the other was also labeled. Later we extended our observations to 3C2 (white) and 3A3 (zeste). It appears that in about 70-80% of the cases both loci label synchronously, which is very interesting considering the known genetic interactions between these two genes.

In addition to the above chromosome rearrangements, we have studied a mutant, lethal giant larvae (l(2)gl), known to affect the normal rate of growth and DNA synthesis in the salivary glands and

other organs. The analysis of the pattern of incorporation in this mutant gave evidence of a continuous pattern in those nuclei which were still replicating (5-20%) of the 3rd instar.

These observations show that the time of replication of DNA in a given chromosome segment remains the same even when the segment is placed into a new position in the genome. The results with "repeats" show further that the pattern of replication persists for a long time during evolution, even when the genes involved have changed their function. The pattern can, however, probably be changed by gene mutations which affect synthesis.

Below is an abstract of a paper to be given at the November 1969 meeting of the American Society for Cell Biology.

X-ray induced DNA synthesis in polytene chromosomes.¹

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This study was undertaken to ascertain whether DNA synthesis can be induced by X-irradiation in polytene chromosomes and whether the presumptive production of breaks in chromosomal DNA will lead to detectable new sites of DNA synthesis.

Excised salivary glands from 3rd instar larvae of Drosophila melanogaster (Oregon R), the number 1(2)gl, and a wild type strain of D. hydei were used.

Tests were made on freshly excised glands where DNA and RNA synthesis had been inhibited (by pretreatment with 10^{-6} M FUdr and 5µg/ml of Actinomycin D or with a 10^{-3} M soln. of hydroxyurea and 5µg/ml of Actinomycin D), on glands transplanted and cultured in the abdominal cavity of adult flies (such glands show little or no thymidine incorporation after 8 days of implantation), and on glands from 1(2)gl, where thymidine incorporation is restricted to 10% or less of the nuclei. The two lobes from each gland were separated, one was used for treatment and the other as control, 30,000r of X-rays was the usual dose of irradiation; the glands were subsequently incubated with tritiated thymidine and studied autoradiographically.

The results obtained show that X-rays induce thymidine incorporation in salivary gland chromosomes which appears to be related to the repair synthesis observed in other cell types. In contrast to normal replicative synthesis, the sites of incorporation are randomly distributed and fewer grains are observed over the chromocentral region. Moreover, labeling never extends across the entire chromosome at any one point, suggesting the involvement of less than all the DNA helices at any one site. These differences as well as the fact that the induced synthesis is observable in nuclei which no longer replicate DNA and in nuclei where normal DNA and RNA syntheses have been inhibited suggest that this presumptive repair synthesis is a separate process from normal chromosomal DNA replication.

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