CHANGES AT TRANSCRIPTIONAL LEVEL IN THE RAT LIVER FOLLOWING WHOLE-BODY X-IRRADIATION

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1. Introduction

Exposure of animals to a single lethal whole-body dose of X-rays is known to cause enhanced rates of protein synthesis in the liver during 12 to 24 hr post-irradiation. Evidence from various lines indicates that these changes are solely the consequence of a general stimulation in RNA synthesis [1,2]. Previous studies of ours on the mechanism by which radiation exerts stimulatory effect on RNA biosynthesis in the rat liver have revealed that it is the template capacity of chromatin rather than the level or the activity of RNA polymerase which exhibits distinct rise in the interval between 4 and 24 hr post-irradiation [3]. Several workers have recently reported that under conditions which lead to gene activation specific enhancement in synthesis of certain chromosomal acidic proteins can be discerned in animal cells. Thus in rats, selective stimulation in the synthesis of chromosomal acidic proteins has been observed in the liver following administration of cortisol [4] and glucagon [5] and in the liver and the uterus in response to estradiol [6]. In this communication, we present evidence to show that concurrent with amplification in template capacity of liver chromatin, incorporation of labelled amino acid precursors into specific chromosomal acidic proteins in the liver is also selectively stimulated during 4 to 24 hr following whole-body exposure of rats to 1000 R X-rays.

2. Materials and methods

2.1. *Treatment of rats*

Male Wistar strain rats each weighing between 150 and 160 g and fed on a laboratory stock diet were used. They were fasted for 18–20 hr prior to sacrifice.

Rats were given a single whole-body dose of 1000 R X-irradiation by housing them in groups of three in a three-place perspex container located at a distance of 50 cm from 250kV X-ray generator (Siemens, Stabilipan). The latter was operated at 15 mA tube current with an added filter of 2 mm Al. The dose rate was 100 R per min.

2.2. *Assay of template activity*

The template activity of liver chromatin or DNA was determined essentially by the method of Church and McCarthy [7]. The template assay system (0.5 ml) contained besides other components, 0.2 μmoles of [^{14}C] ATP (specific activity 27.2 mCi/mmole), rat liver DNA or chromatin equivalent to 20 μg DNA and rat liver RNA polymerase preparation equivalent to 100 μg protein. Incubation was for 10 min at 37°C.

The chromatin was isolated and purified by the procedure of Marushigae and Bonner [8]. DNA was isolated from rat liver by the procedure of Marmur [9]. RNA polymerase was prepared from rat liver by the method of Roeder and Rutter [10] to the stage of their fraction IV.

2.3. *Measurement in incorporation of labelled leucine into chromosomal acidic proteins*

Irradiated and control rats were pulse-labelled for 1 hr periods by the injections of 600 μCi of DL-[^{3}H] leucine (specific activity, 5 Ci/mmole) and 83 μCi of DL-[^{14}C] leucine (specific activity 0.25 mCi/mmole), respectively. Thereafter, livers from both the irradiated and control rats were pooled and homogenised together in 0.025 M citric acid. The chromosomal acidic proteins (300 μg) isolated from the mixed homogenate

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were co-electrophoresed on a 10% polyacrylamide gel with a running buffer of 0.1 M sodium phosphate (pH 7.2) containing 0.1% sodium dodecyl sulphate according to the procedure described by Shelton and Allfrey [4]. The protein bands were fixed in 20% sulphosalicylic acid and located by staining with Coomassie Brilliant Blue. Radioactivity in fractionated proteins was determined by transverse slicing of the gel in discs of 2 mm thickness and counting in a liquid scintillation spectrometer.

In one set of experiments, administration of radioactive amino acids was reversed-control rats received [^{3}H] leucine and irradiated rats (18 hr post-irradiation) [^{14}C] leucine. The pooled livers from these animals were analysed for labelling patterns of chromosomal acidic proteins by the same procedure as above.

2.4. *Measurement of incorporation of labelled lysine into chromosomal acidic proteins*

Irradiated and control rats were pulse-labelled for 1 hr period by the injections of 400 μCi of L-[^{3}H] lysine (specific activity 1250 mCi/mmole) and 50 μc of L-[^{14}C] lysine (specific activity 30 mCi/mmole). Chromosomal acidic proteins from the mixed liver homogenate were isolated, separated and their labelling pattern analysed by the procedure described in 2.3.

2.5. *Determination of molecular weights of electrophoretically separated proteins*

This was done by the method of Shapiro et al. [11] using proteins of known molecular weights, viz., bovine serum albumin, alcohol dehydrogenase, myoglobin and cytochrome *c*.

3. Results and discussion

Results of an experiment on effect of whole-body exposure of rats to 1000 R X-irradiation on template activity of chromatin in the liver are presented in table 1. It will be seen that the template activity of chromatin, as assessed using exogenous liver RNA polymerase, is only slightly increased at 4 hr following whole-body radiation exposure. It rises to about 25% higher than control at 12 hr post–irradiation and as high as 64% above control at 18 hr post–irradiation. The template capacity exhibits a decline thereafter, being only about 18% higher than control at 24 hr and returning to normal by 36 hr. At these time intervals, the template activity of DNA remains unchanged. This would mean that the radiation-induced alterations in chromatin function are associated, in some way, with non-DNA components of the chromatin.

Table 1
Effect of whole-body X-irradiation on template activity of purified chromatin in vitro

	AMP incorporated* pmoles/10 min	
	DNA	Chromatin
Un-irradiated	141.3	37.5
Irradiated (1000 R) hr		
4	140.7	40.9
12	139.0	46.8
18	143.2	61.5
24	141.0	44.2
36	-	36.0

* RNA synthesis by enzyme alone (9.6 pmoles) and chromatin alone (1.0 pmole) is substracted. Each value is an average of two independent determinations.

To determine whether specific alterations in synthesis of chromosomal acidic proteins are associated with these modulations in chromatin functions, a procedure similar to that described by Shelton and Allfrey [4] was adopted. At various times following whole-body X-irradiation, rats were injected with [^{3}H] leucine and sacrificed 1 hr later. The livers from irradiated rats were pooled with those from unirradiated control animals which had been injected with [^{14}C] leucine 1 hr prior to sacrifice. Chromosomal acidic proteins isolated from the mixed homogenates were subjected to gel electrophoresis as described under Materials and methods.

Radioactivity for each of the transversely cut 2 mm gel slices was determined and the results are illustrated in fig. 1. The upper curves represent ^{14}C counts and ^{3}H counts in electrophoretically migrated proteins derived from unirradiated and irradiated (18 hr post-irradiation) rats, respectively. The ^{3}H/^{14}C ratios (lower part of the figure) reflect relative rates of labelling of individual protein components from irradiated and control animals. These ratios are more or less uniform along the gel excepting for the proteins located at slice numbers 12–13 and 19–20 where the ratios are higher than the rest. This would indicate that the synthesis

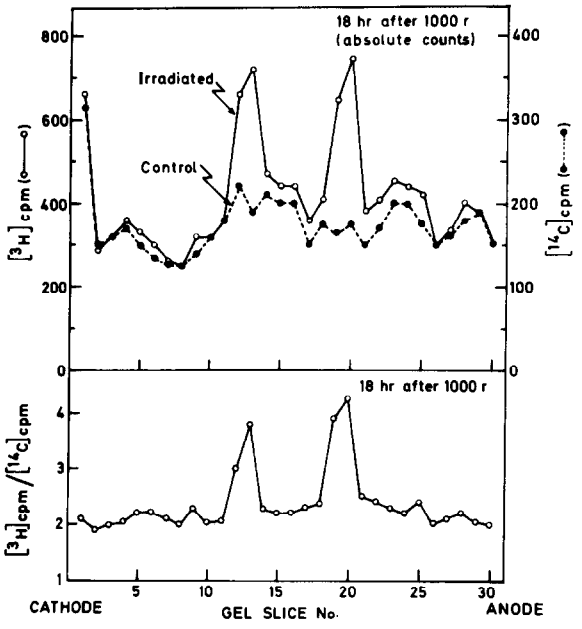

Fig. 1. Labelling patterns of electrophoretically separated chromosomal acidic proteins from livers of irradiated rats (injected with [^{3}H] leucine) and un irradiated controls (injected with [^{14}C] leucine).

of these components is selectively stimulated in irradiated animals. Alternatively, rates of degradation of these components could have been specifically suppressed resulting in relative enhancement in their labelling.

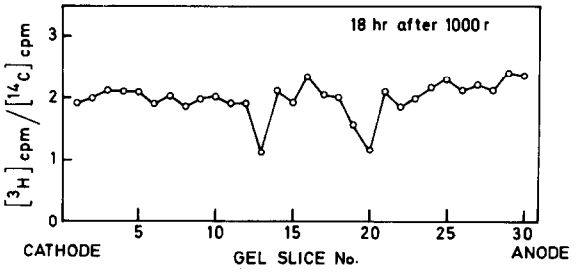

Fig. 2. Labelling patterns of electrophoretically separated chromosomal acidic proteins from livers of irradiated rats (injected with [^{14}C] leucine and un-irradiated control (injected with [^{3}H leucine). Radioactivities of protein components (absolute counts) are as follows. Total counts placed on the gel: 10 300 cpm of ^{3}H and 5 340 ppm of ^{14}C; slice No. 13: 310 cpm of ^{3}H and 360 cpm of ^{14}C; slice No. 20: 310 cpm of ^{3}H and 350 cpm of ^{14}C.

A reverse experiment in which control rats received [^{3}H] leucine and irradiated rats (18 hr post-irradiation) [^{14}C] leucine was also carried out. As seen in fig. 2, there was a decrease in the ratios of ^{3}H/^{14}C of the protein components belonging to the same regions, viz., slice numbers 12–13 and 19–20. Thus the observed effect is not an artefact arising from counting anomalies. In another set of experiments labelled lysine instead of leucine was used, and the results are illustrated in fig. 3. Again, the proteins migrated to positions 12–13 and 19–20 of the gel have shown specific enhancement in the relative rates of labelling as a result of whole-body radiation exposure. This may eliminate the possibility that the observed effects could arise from differences in specific radioactivities of the amino acids in liver soluble pools of control and irradiated rats.

As illustrated in fig.4, the effects are apparent even at earlier times of 4 and 8 hr post-irradiation though not as much pronounced as at 18 hr post-irradiation. At 18 hr, labelling of the proteins is about 130% higher than that in non-irradiated controls (fig.1). Similar analysis at 24 hr post-irradiation revealed that

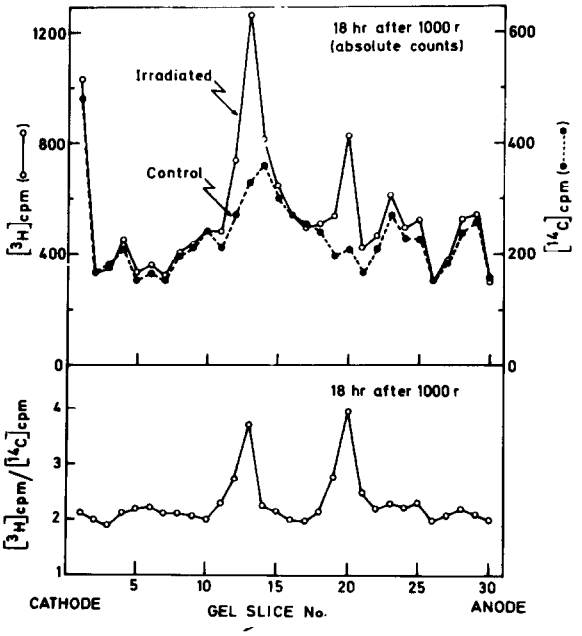

Fig. 3. Labelling patterns of electrophoretically separated chromosomal acidic proteins form livers of irradiated rats (injected with [^{3}H] lysine) and un-irradiated controls (injected with [^{14}C] lysine).

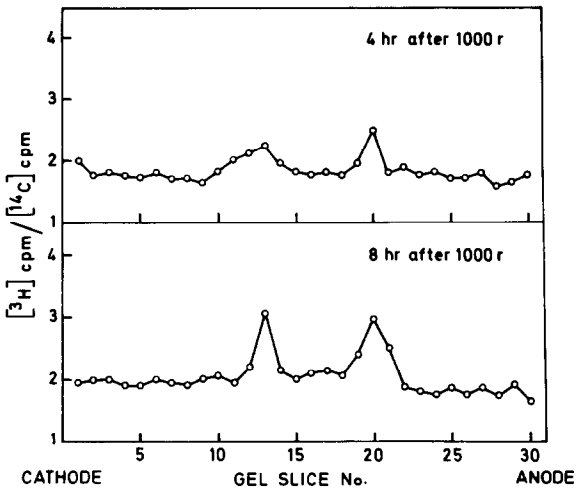

Fig. 4. Relative rates of labelling of electrophoretically separated liver chromosomal acidic proteins at different periods post-irradiation. Irradiated rats received [^{3}H] leucine and un-irradiated controls [^{14}C] leucine. Radioactivities of protein components (absolute counts) are as follows: For 4 hr after 1000 R - total counts placed on the gel: 5975 cpm of ^{3}H and 4970 cpm of ^{14}C; slice No. 13: 365 cpm of ^{3}H and 165 cpm of ^{14}C; slice No. 20: 435 cpm of ^{3}H and 175 cpm of ^{14}C. For 8 hr after 1000 R - total counts placed on the gel: 6295 cpm of ^{3}H and 4885 cpm of ^{14}C; slice No. 13: 555 cpm of ^{3}H and 185 cpm of ^{14}C; slice No. 20: 505 cpm of ^{3}H and 170 cpm of ^{14}C.

the magnitude of increase is much smaller being only 22% higher than normal. Thus, the degrees of stimulation in the labelling of the proteins belonging to slice numbers 12–13 and 19–20 at various times following radiation exposure seem to correspond well with the increments in template efficiency of chromatin observed at these times (table 1). It is quite conceivable therefore that the proteins may have some role in radiation-induced modification in chromatin function. From the relationship of electrophoretic mobilities versus molecular weights of standard proteins, the mol wt. of the proteins for slice numbers 12–13 can be approximately estimated in the range of 57 000–53 000 and for slice numbers 19–20 in the range 34 000–31 000.

The observed changes at the transcription level in the liver may not be the consequence of radiation effects being exerted directly on the liver itself but are probably mediated by some factors elaborated by other organs in response to radiation. This may be surmised from the fact that protection of the liver by shielding during total-body irradiation does not suppress the stimulus in the RNA synthetic rate in this organ [2.12]. It is known that irradiation of animals leads to increased output of adrenal steroids [13] and there are reports which indicate that the alterations brought about in the liver at transcriptional level could be prevented if adrenals are removed prior to radiation exposure [14,15]. It is possible therefore that radiation-induced release of adrenal steroids may lead to enhanced synthesis (or increased accumulation due to decreased rates of degradation) of the chromosomal proteins situated at the gel regions corresponding to slice numbers 12–13 and 19–20.

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