Effects of γ -Irradiation on Biosynthesis of Different Types of Ribonucleic Acids in Normal and Regenerating Rat Liver

By GEORGE G. MARKOV, GEORGE N. DESSEV, GEORGE C. RUSSEV and ROUMEN G. TSANEV
Institute of Biochemistry, Bulgarian Academy of Sciences, Sofia 13, Bulgaria

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1. The effect of γ -irradiation (4000rd) on the synthesis of ribosomal (pre-rRNA) and heterogeneous nuclear RNA (pre-mRNA) in normal and in regenerating rat liver was studied by using 40 min labelling with [6- 14 C]orotic acid. 2. Partial hepatectomy caused a sharp transient increase in the specific radioactivity of the endogenous low-molecular-weight RNA precursors in the livers of both normal and irradiated rats. Irradiation of intact animals did not affect the pool. 3. Irradiation enhanced the synthesis of pre-rRNA for at least 12 h. The synthesis of pre-mRNA was also enhanced, but only in the first 3 h after irradiation. 4. Partial hepatectomy strongly stimulated the synthesis of both pre-rRNA and pre-mRNA. 5. The synthesis of pre-rRNA was enhanced also in regenerating liver of animals irradiated before or after the operation. The conclusion can be drawn that the early increase in the synthesis of ribosomal RNA is a non-specific cellular response to different injuring factors. 6. The only case where irradiation caused an early inhibition of RNA synthesis was that of pre-mRNA in regenerating liver. This supports the hypothesis that ionizing radiation does not suppress the transcription *per se* but affects the mechanisms of activation of new genes (cellular programming).

The biosynthesis of RNA in eukaryotic cells is a relatively radioresistant process (for reviews see Kelly, 1961; Ord & Stocken, 1961; Kuzin, 1962; Nygaard, 1962; Markov, 1965; Mitchell, 1966; Shooter, 1967; Tokarskaya, 1972; Komar & Hanson, 1972). In fact, the total amount of RNA per cell in proliferating tissues shows an increase parallel to that of the cell mass even after irradiation with doses of X-rays of several thousand rd, which strongly inhibits the synthesis of DNA and mitosis (Klein & Forssberg, 1954; Whitmore *et al.*, 1961; Markov, 1964). On the other hand, the data concerning incorporation of labelled precursors into RNA are controversial: stimulation, suppression or no effect have been reported (see the reviews quoted).

Recently the problem of the radiosensitivity of RNA biosynthesis has been re-evaluated taking into account the structural, metabolic and functional heterogeneity of RNA (Markov, 1965; Kuzin, 1967; Huystee *et al.*, 1968; Tokarskaya, 1972; Komar & Hanson, 1972). It has been suggested (Nygaard, 1962; Mitchell, 1966; Huystee *et al.*, 1968) that irradiation stimulates the biosynthesis of ribosomal RNA but suppresses that of messenger RNA. However, the synthesis of mRNA was found to be radiosensitive only in cases in which the type of proteins synthesized was changing, such as embryonic development and tissue regeneration (Uchiyama *et al.*, 1966; Kafiani *et al.*, 1966; Kuzin, 1967; Tokarskaya, 1968; Tokarskaya & Umansky,

1968; Umansky, 1968; Umansky & Tokarskaya, 1968; Huystee & Cherry, 1967; Huystee *et al.*, 1968; Fausto & Van Lancker, 1969).

Embryonic development and tissue regeneration possess a common feature: the synthetic programme of the cell changes through the activation of new genes. It has been supposed (Markov, 1965) that the radiosensitivity of these processes depends on whether the cells undergo reprogramming: radiation selectively affects the mechanisms involved in switching the genes on and off. Since the mRNA coding for new proteins may comprise a small part of the total mRNA, it may be difficult to detect radiation-induced changes in mRNA biosynthesis.

To check this hypothesis we have investigated the biosynthesis of different RNA classes in rat liver under different conditions: (1) normal liver of non-irradiated adult rats (group N); (2) intact liver of irradiated adult rats (group I); (3) regenerating liver after partial hepatectomy (group R); (4) regenerating liver in irradiated rats (group IR).

The effect of whole-body irradiation on liver regeneration has been extensively studied and the basic events are well known (see Kelly, 1961; Bucher, 1963; Markov, 1965; Shooter, 1967; Van Lancker, 1970; Bresnick, 1971). Fig. 1 summarizes these data. However, in most papers the radiosensitivity of RNA biosynthesis has been studied in periods 12 h after partial hepatectomy or later, or RNA has not been properly fractionated (Welling & Cohen, 1960). In

Fig. 1. Sequence of the main events in the course of liver regeneration

Irradiation applied at the times indicated by arrows leads to the following effects. (1) Irradiation in S period: mitoses blocked, DNA synthesis continues, S period extended. (2) Irradiation in the late G₁ period: DNA synthesis blocked, the activity of the enzymes involved in DNA replication continues to increase. (3) Irradiation in the first hours after partial hepatectomy (period of reprogramming, early G₁ period): strong inhibition of enzyme activities involved in DNA replication. The same period is actinomycin-sensitive. (4) Irradiation immediately before operation: the effect is the same as in (3).

In the present paper we have studied the period immediately after partial hepatectomy, when cellular reprogramming takes place and the hepatocytes enter the mitotic cycle (Bucher, 1963; Tsanev & Markov, 1964; Grisham, 1969, 1973; Bresnick, 1971; Bucher & Malt, 1971). The irradiation was applied immediately before operation so that the reprogramming proceeded entirely in radiation-damaged liver. In a previous paper (Markov *et al.*, 1973) we have shown that, in accordance with earlier data (Holmes, 1956; Albert & Bucher, 1960), liver regeneration in rats previously irradiated with 4000rd is a special case of disbalanced growth: the synthesis of DNA and mitoses are completely blocked while the synthesis of other cell constituents including RNA and proteins continues.

The results obtained in this paper showed that different types of RNA responded in a different way to irradiation. The synthesis of ribosomal RNA was stimulated independently of the functional state of the liver. An early stimulatory effect was observed also on the synthesis of pre-mRNA, but only in normal liver. In contrast, the irradiation significantly inhibited the synthesis of pre-mRNA in regenerating liver. These data support the hypothesis that irradiation temporarily stimulates the transcription of mRNA on genes operating at the time of irradiation but suppresses the synthesis of new types of mRNA by affecting the mechanisms of activation of new genes.

Materials and Methods

Animals and irradiation

Adult male albino rats, weighing 180–200g, were irradiated with 4000rd of γ -rays by a ^{60}Co unit at a dose rate of 150R/min and under conditions ensuring homogeneity of the field.

Liver regeneration (compensatory hypertrophy) was induced by removing two-thirds of the liver (Higgins & Anderson, 1931).

Labelling of RNA

The rats were injected intraperitoneally with [$6\text{-}^{14}\text{C}$]orotic acid (15mCi/mmol, 15 $\mu\text{Ci}/100\text{g}$ body wt.) 40 min before death. The times of irradiation and partial hepatectomy in different experiments are shown in Table 1.

Determination of the specific radioactivity of the precursor pool

A small piece of liver was chilled in ice-cold 0.14M-NaCl for 5 min and homogenized in 5 vol. of distilled water. Then 1M-HClO₄ was added to a final concentration of 0.2M. The sample was kept in ice for 20 min and neutralized with 1M-KOH to pH 7.0. Samples of the supernatant after removal of KClO₄ were used to determine the specific radioactivity of the total acid-soluble fraction.

The neutralized extract was made 1M in HClO₄ and heated for 30 min in a boiling-water bath. Under these conditions the pyrimidine nucleoside di- and tri-phosphates are converted into monophosphates (Michelson, 1963; Bresnick, 1965). The solution was neutralized with KOH, made 0.05M in HCl and passed through a column (8 mm $\times$ 12 mm) of Dowex 50 (X8; Cl⁻ form). The effluent contained only UMP, since the other bases, nucleosides and nucleotides were retained on the column. This has been checked by t.l.c. on DEAE-cellulose and ion-exchange chromatography of the effluent on Dowex 1 (X8; formate form). The specific radioactivity of the effluent (designated as the UMP fraction) was taken as a measure of the specific radioactivity of the endogenous precursors of RNA.

The use of the specific radioactivity of the UMP fraction as a measure of the specific radioactivity of the immediate precursors of RNA is correct if UMP, UDP and UTP in the acid-soluble pool are in equilibrium with respect to the radioactivity. The period of 40 min labelling time in our experiments fully satisfies this condition. According to data in the literature a few minutes are sufficient to equalize the specific radioactivities of nucleoside mono-, di- and tri-phosphates (Hurlbert & Potter, 1954; Brum *et al.*, 1956).

Table 1. *Treatments applied to the different experimental groups*

Experimental group (symbol)	No. of experiments	Total no. of animals	Time from the beginning of treatment until death (h)	
			Irradiation	Partial hepatectomy
N	14	20	—	—
I ₃	2	8	3	—
I ₁₂	4	4	12	—
R ₃	6	36	—	3
R ₁₂	11	33	—	12
I ₄ R ₃	6	36	4	3
I ₁₃ R ₁₂	3	9	13	12
I ₃ R ₁₂	4	12	3	12

Isolation of RNA

RNA was isolated by a stepwise thermal fractionation with 0.14M-NaCl-phenol (1:1, v/v), pH 6.2, by the method of Georgiev & Mantieva (1962), modified by including an additional extraction at 85°C (Arion *et al.*, 1967; Markov & Arion, 1973). As shown in detail elsewhere (see Georgiev *et al.*, 1972; Markov & Arion, 1973), this procedure yields as separate fractions: (a) cytoplasmic RNA (0°C RNA fraction, cytoplasmic RNA); (b) nucleolar ribosomal and ribosomal-precursor RNA species (45°C RNA fraction, pre-rRNA); (c) heterogeneous nuclear DNA-like RNA, containing the earlier transcriptional products (85°C RNA fraction, pre-mRNA). Under the conditions of extraction used in the present work, the RNA fractions extracted between 45°C and 63°C represented mixtures of nuclear ribosomal and DNA-like RNA species and therefore were not subjected to further studies.

Purification of RNA

RNA preparations were purified, removing DNA and low-molecular-weight radioactive contaminants as previously described (Markov & Arion, 1973). The cytoplasmic RNA was freed from glycogen by centrifugation at 30000 rev./min for 30 min.

Fractionation of RNA

All RNA preparations were fractionated by electrophoresis in agar gel. The electrophoretograms were analysed for distribution of u.v.-absorbing material and radioactivity as described previously (Tsanev *et al.*, 1966).

Measuring the radioactivity

Portions of the samples were evaporated on aluminium planchets and counted in a windowless gas-flow counter (Vakutronik VA-Z-530).

Results

Specific radioactivity of the endogenous precursor pool of RNA

As shown in Tables 2 and 3, irradiation does not change the specific radioactivity of the endogenous RNA precursors. In contrast, partial hepatectomy strongly affects the endogenous pool. At 3 h after operation an increase of about 80% in the specific radioactivity of both the total acid-soluble fraction and the UMP fraction is observed. A similar increase occurs in regenerating liver of animals irradiated 1 h before operation. At 12 h after operation the specific radioactivity of the pool decreases to the control value (Table 3, Fig. 2).

These data emphasize the importance of knowing the changes in the specific radioactivity of the endogenous RNA precursors. In our study the specific radioactivity of RNA was always expressed relative to the specific radioactivity of the UMP fraction. This ratio multiplied by 100 is designated in the text as the relative specific radioactivity of RNA.

Effect of irradiation and partial hepatectomy on RNA

Electrophoretic profiles. As shown in Figs. 3–5, none of the treatments applied has affected the absorption profiles of the thermal RNA fractions. Both irradiation and partial hepatectomy affect the labelling of these fractions, although the changes are reflected only in the total specific radioactivity (Table 3, see below), since the ratios of the specific radioactivities of the different electrophoretic peaks or zones in a given thermal fraction remain the same (Figs. 3–5). This implies that neither regeneration nor irradiation has an appreciable effect on the post-transcriptional stages in processing of pre-rRNA and pre-mRNA.

Specific radioactivity. The relative specific radioactivities of cytoplasmic RNA, pre-rRNA and pre-mRNA are given in Table 3 and the changes

Table 2. Specific radioactivity of the total acid-soluble fraction and the UMP fraction

For details see the text.

Expt no.	Animals ...	Specific radioactivity											
		Controls (N)			Irradiation (I ₃)			Partial hepatectomy (R ₃)			Irradiation + partial hepatectomy (I ₄ R ₃)		
		Total acid-soluble fraction	UMP fraction		Total acid-soluble fraction	UMP fraction		Total acid-soluble fraction	UMP fraction		Total acid-soluble fraction	UMP fraction	
1	c.p.m./E ₂₆₀ unit % of control	13220	79200		13830	80300		26000	159000		31800	160000	
		100	100		105	102		197	202		241	202	
2	c.p.m./E ₂₆₀ unit % of control	19210	94800		21100	91000		33200	156300		36500	152300	
		100	100		110	96		173	165		190	161	
Mean	%	100	100		108	99		185	184		216	182

Fig. 2. Effect of different treatments on the specific radioactivity of the UMP fraction
Experimental groups are as in Table 1.

relative to a normal or regenerating liver are shown in Figs. 6–8. The data reveal that irradiation affects RNA biosynthesis in a different way depending on the type of RNA and the functional state of the liver.

In intact animals 3h after irradiation (Fig. 6) a considerable increase in the specific radioactivity of all RNA fractions is observed. The increase is particularly high for pre-rRNA and cytoplasmic RNA, whereas the relative specific radioactivity of the nuclear pre-mRNA is only 22% higher than that of the control. The enhanced synthesis of ribosomal RNA persists at least as long as 12h after irradiation. At that time, however, the synthesis of pre-mRNA has already decreased to that of the controls.

Partial hepatectomy also stimulates the synthesis of all types of RNA (Fig. 7, Table 3). At 3h after operation the increase in the labelling of all fractions is similar to that observed in intact liver 3h after irradiation (cf. Fig. 6). At the 12th hour of regeneration, however, the synthesis of pre-rRNA continues to increase and the relative specific radioactivity reaches values several times higher than that of the controls. The relative specific radioactivity of the nuclear pre-mRNA 3h after operation, however, is only 40% higher than that of the normal liver and there is no further increase as regeneration proceeds (Fig. 7).

The synthesis of RNA in regenerating liver of irradiated animals shows some interesting features (Table 3, Fig. 8). At 3h after partial hepatectomy the synthesis of pre-rRNA in pre-irradiated animals is stimulated to the same extent as in intact irradiated

Fig. 3. Electrophoretic profiles of rat liver cytoplasmic RNA (0°C RNA fraction)

RNA was isolated from animals treated (experimental groups) as indicated in Table 1. —, E_{260} ; ----, radioactivity measured by the blackening of the radioautographs scanned at 550nm. Arrows indicate the position of 28S, 18S and 4S rat liver cytoplasmic RNA run in parallel slots as markers.

or in regenerating liver (Table 3) and even a small additive effect is observed (Fig. 8).

In 12h regenerating liver an increase in the labelling of pre-rRNA is observed also in the pre-irradiated rats ($I_{13}R_{12}$): the relative specific radioactivity of pre-rRNA is higher than that in normal liver and in liver 12h after irradiation (Table 3). However, in pre-irradiated animals the synthesis of pre-rRNA at this later period of regeneration is not so great as in regenerating non-irradiated liver (Fig. 8). This means that the continuous increase of the synthesis of ribosomal RNA which normally occurs after partial hepatectomy is suppressed in pre-irradiated animals at later periods after irradiation. It should be pointed out that in intact animals at this time after irradiation (12h) the synthesis of pre-rRNA still remains enhanced (Fig. 6).

The finding that irradiation provokes an early increase in biosynthesis of ribosomal RNA in resting hepatocytes raises the question whether cells of

regenerating liver will respond to irradiation in the same way. To answer this question rats were irradiated 9h after partial hepatectomy and killed 3h later. The results of this experiment (Table 3, Fig. 8, group I_3R_{12}) show that irradiation of the regenerating liver additionally stimulates the synthesis of pre-rRNA which has been already enhanced by the partial hepatectomy. It can be concluded that irradiation provokes an early increase in the synthesis of ribosomal RNA independently of the functional state of the liver.

As far as pre-mRNA in 3h and 12h regenerating liver is concerned, irradiation leads to a suppression of its synthesis independently of the time of irradiation (Table 3, Fig. 8, group I_4R_3 , $I_{13}R_{12}$ and I_3R_{12}).

The labelling of cytoplasmic RNA is strongly stimulated by both irradiation and partial hepatectomy (Table 3, Figs. 3, 6 and 7). As seen in Fig. 3, the cytoplasmic RNA is slightly contaminated with newly synthesized pre-rRNA (mainly 32S), which

Fig. 4. Electrophoretic profiles of nuclear pre-rRNA (45°C RNA fraction)

Experimental groups and explanations are as in Fig. 3.

confirms our previous finding (Markov & Arion, 1973). The combined application of irradiation and partial hepatectomy causes an early stimulation and a late inhibition of the labelling of cytoplasmic RNA (Fig. 8). The latter effect is most probably due to the decreased inflow of mRNA into the cytoplasm owing to inhibited synthesis of pre-mRNA.

Discussion

Methodological considerations

The results presented in this paper clearly demonstrate that to reveal and interpret correctly the

effect of radiation on RNA biosynthesis, three important methodological requirements should be met. (a) The different types of RNA should be separated and differentially studied. (b) The functional state of the cellular population should be considered. (c) The changes in the radioactivity of RNA should be related to that of the endogenous RNA precursors.

In this work a clear separation of ribosomal from non-ribosomal nuclear RNA of messenger type was achieved by using the method of stepwise extraction of RNA with 0.14M-NaCl-phenol at different temperatures (see Georgiev *et al.*, 1972; Markov & Arion, 1973). The results obtained in the present study confirmed the efficiency of this method for

Fig. 5. Electrophoretic profiles of nuclear pre-mRNA (85°C RNA fraction)

Experimental groups and explanations are as in Fig. 3.

Table 3. Effect of irradiation and partial hepatectomy on the specific radioactivity of different RNA fractions

The specific radioactivities of the UMP and RNA fractions are directly comparable within an experimental series; to facilitate the comparison between different series the specific radioactivities are also presented as a percentage of the specific radioactivity of either group N or group R. For further details see the text.

Expt. series	Expt. group	No. of expts.*	Specific radioactivity of UMP (c.p.m./ E_{260} unit)	Relative specific radioactivity of RNA		
				Cytoplasmic	Pre-rRNA	Pre-mRNA
I	N	2 (4)	87000 (100%)	0.10 (100%)	3.5 (100%)	22.4 (100%)
	I ₃	2 (4)	85650 (98%)	0.22 (220%)	9.4 (269%)	27.4 (122%)
	R ₃	2 (12)	158050 (182%)	0.16 (160%)	8.4 (240%)	31.9 (142%)
	I ₄ R ₃	2 (12)	156150 (179%)	0.21 (210%)	9.5 (271%)	20.3 (91%)
II	R ₃	4 (3)	83675 (100%)	0.18 (100%)	2.9 (100%)	37.3 (100%)
	I ₄ R ₃	4 (3)	85125 (102%)	0.23 (128%)	3.5 (123%)	19.2 (51%)
III	N	4 (1)	56350 (100%)	0.12 (100%)	5.3 (100%)	42.2 (100%)
	I ₁₂	4 (1)	61250 (108%)	0.33 (295%)	7.2 (136%)	41.6 (99%)
IV	N	8 (1)	56080 (100%)	0.12 (100%)	4.7 (100%)	29.6 (100%)
	R ₁₂	7 (3)	57900 (103%)	0.89 (795%)	17.1 (363%)	42.3 (143%)
V	R ₁₂	4 (3)	53000 (100%)	0.85 (100%)	15.4 (100%)	52.6 (100%)
	I ₃ R ₁₂	4 (3)	48750 (92%)	0.62 (73%)	19.5 (127%)	34.1 (65%)
VI	R ₁₂	3 (3)	77500 (100%)	0.92 (100%)	18.7 (100%)	32.1 (100%)
	I ₁₃ R ₁₂	3 (3)	85800 (110%)	0.59 (64%)	9.8 (52%)	18.5 (58%)

* Numbers in parentheses indicate the number of animals in each experiment.

Fig. 6. Effects of irradiation on the relative specific radioactivity of different RNA fractions of intact rat liver

All data are expressed as a percentage of the values for the control group (N). Experimental groups are as in Table 1.

Fig. 7. Effect of partial hepatectomy on the relative specific radioactivity of different RNA fractions of non-irradiated rat liver

All data are expressed as a percentage of the values for the control group (N). Experimental groups are as in Table 1.

studying differences in the metabolism of the different nuclear RNA species.

According to our results partial hepatectomy causes an early temporary increase in the specific radioactivity of the endogenous low-molecular-weight precursors of RNA in the liver. Such an effect has been observed also by Bucher & Swaffield (1969). On the contrary, irradiation does not affect the specific radioactivity of the metabolic pool either in intact or in regenerating liver. These findings emphasize the condition that for a correct interpretation of the labelling data the changes in the endogenous pool should be taken into consideration. Theoretically one should determine the specific radioactivity of the immediate precursor, i.e. UTP in the case of pulse-labelling with [¹⁴C]orotic acid. However, in our experiments a labelling time of 40 min was used, which was long enough to equalize the specific radioactivities of UMP, UDP and UTP (Hurlbert & Potter, 1954; Brum *et al.*, 1956). For this reason the use of a simpler technique was justified in which all phosphorylated uridine derivatives were hydrolysed

Fig. 8. Effect of irradiation on the relative specific radioactivity of different RNA fractions of regenerating rat liver

All data are expressed as a percentage of the corresponding values for non-irradiated regenerating livers (group R). Experimental groups are as in Table 1.

to UMP and the specific radioactivity of the latter was determined.

According to the equations of Zilversmit (Zilversmit *et al.*, 1943; Zilversmit, 1960) the ratio between the specific radioactivity of RNA and its precursor(s) would be proportional to the real synthetic rate if the following conditions are met: (1) the specific radioactivity of RNA is negligible as compared with that of the precursor; (2) the steady-state concentration of the RNA fractions does not change during the period studied; (3) the specific radioactivity of RNA increases linearly with time. In our experiments these conditions are far from being fulfilled. They may be valid only for the cytoplasmic RNA in normal liver. However, this is certainly not the case in regenerating and in irradiated liver, where the concentration of RNA changes. For this reason the relative specific radioactivity should be regarded as a value showing only a relative increase or decrease in the synthetic rate of a given RNA fraction under different conditions. The relative specific radioactivity of different RNA fractions should not be used for estimating differences in their synthetic rate.

Effect of irradiation on RNA synthesis in normal liver

Our results show that the total body γ -irradiation of rats with a dose of 4000rd enhances the incorporation of [^{14}C]orotic acid in all thermal RNA fractions. A similar stimulation lasting for 6h was found by Hidvégi *et al.* (1970) for total liver nuclear RNA after irradiation with 2000rd. Mantieva *et al.* (1966) have also found a 1.5–3.5-fold increase of ^{32}P incorporation into the total rat liver nuclear RNA extracted with 0.14M-NaCl-phenol-sodium dodecyl sulphate at 60°C as well as into fractions extracted at 40°C and 60°C separately. The differential study of the radiation effect on the synthesis of pre-rRNA and pre-mRNA in the present work permits a better understanding of this effect. Our results show that the synthesis of both pre-rRNA and pre-mRNA is stimulated but with ribosomal RNA the effect persists for a longer time, at least for 12h. It is important to note that in normal liver the synthesis of pre-mRNA is also stimulated. Thus our data do not support the view that irradiation suppresses the synthesis of mRNA as a whole. On the contrary, it seems to exert an early stimulatory effect on the transcription.

Three possible mechanisms of the radiation-induced stimulation of RNA synthesis in normal liver may be discussed. (1) It could be mediated through the adrenals. However, irradiation has been found to stimulate the biosynthesis of rat liver nuclear RNA in adrenalectomized animals (Cammarano *et al.*, 1969; Popov *et al.*, 1971). (2) It is possible that other humoral factors are involved in the radiation-induced stimulation of RNA synthesis in the liver,

since this effect persists after shielding the organ during irradiation (Várterész *et al.*, 1967). (3) A direct stimulatory effect should also be taken into consideration. It has been shown that the synthesis of ribosomal RNA is stimulated in irradiated plant tissues, where the effect cannot be explained by humoral factors (Huystee & Cherry, 1967; Romani & Ku, 1970).

RNA synthesis in regenerating rat liver

As shown in the present work, in regenerating liver the synthesis of both pre-rRNA and pre-mRNA is enhanced. The stimulation reaches a maximum 12h after operation, when a massive production of ribosomes takes place. Even at that time of highest synthetic activity, the percentage increase of the incorporation into the nuclear pre-mRNA remains low compared with that of the incorporation into pre-rRNA. However, if we take into account the high turnover rate and the small amount of the 85°C RNA fraction containing the pre-mRNA, it seems likely that the increase of 30–40% in the relative specific radioactivity of this fraction approaches the maximum possible incorporation, i.e. the value at which an equilibrium of the radioactivity between the endogenous precursors and the pre-mRNA is established.

Effect of radiation on RNA biosynthesis in regenerating liver

In regenerating liver irradiation causes a pronounced imbalance between the synthesis of pre-rRNA (stimulation) and that of pre-mRNA (inhibition). In pre-irradiated rats, 3h after partial hepatectomy the synthesis of ribosomal RNA is stimulated at least to the same extent as in operated non-irradiated animals. It is noteworthy that irradiation stimulates the synthesis of ribosomal RNA also if animals are irradiated 9h after partial hepatectomy, i.e. at a time when the hepatocytes have already entered the mitotic cycle and are intensively producing ribosomal RNA. Similar data with rat fetuses have been reported by Simić *et al.* (1967). These results demonstrate that both resting and proliferating hepatocytes respond to irradiation by increasing the synthesis of ribosomal RNA.

It is striking that the synthesis of ribosomal RNA may be stimulated by such different factors as irradiation (which blocks cell proliferation) and partial hepatectomy (which stimulates cell proliferation). It is worth noting also that we have found a stimulation of the synthesis of ribosomal RNA after such a non-specific treatment as sham operation (R. G. Tsanev, G. G. Markov & G. N. Dessev, unpublished work). It might be that increased production of ribosomes is needed for cell recovery after damage. This suggestion is supported by the data

of Djordjevic *et al.* (1968) who have found that blocking the synthesis of rRNA obstructs the recovery of the cells from radiation damage. In any case the results obtained firmly show that enhanced synthesis of ribosomal RNA is not always related to cellular proliferation.

One of the most important facts established in the present work is that the synthesis of pre-mRNA in normal and in regenerating rat liver is affected by irradiation in quite an opposite way. In normal liver radiation causes an early stimulatory effect, whereas in regenerating liver it is inhibited. The explanation of this difference should be sought in the different nature of pre-mRNA synthesized in normal and in regenerating liver. In normal liver only those types of mRNA are synthesized which are necessary for production of proteins connected with the specialized functions of the hepatocytes. The stimulation of the synthesis of these mRNA species implies that irradiation stimulates the transcription of cistrons already operating at the time of irradiation. In regenerating liver a sequential synthesis of new types of mRNA occurs (Church & McCarthy, 1967), which is needed for growth and division of the hepatocytes. Therefore it can be concluded that irradiation suppresses the production of only those types of mRNA whose synthesis sets in as a result of reprogramming. This would mean that the radiosensitive phase of mRNA biosynthesis is not the process of transcription *per se* but the switching on of new genes transcribing new types of mRNA. This conclusion is supported by recent studies of the effect of irradiation on the biosynthesis of RNA in plants (Umansky & Tokarskaya, 1968; Huystee *et al.*, 1968; Tokarskaya & Umansky, 1968) and in animal embryos (Umansky, 1968). It is also in agreement with the finding that the incorporation of amino acids into proteins in a microsomal cell-free system prepared from 3h regenerating liver was suppressed when the rats were irradiated with 4000rd before the partial hepatectomy. The same dose had a stimulatory effect on a cell-free system from intact rat liver (Tasheva *et al.*, 1973).

The molecular mechanisms determining the radiation damage of the process of reprogramming are not clear. This may be a consequence of direct structural damage to the chromatin, or alternatively, some control mechanisms regulating gene expression may be affected. Insufficient experimental evidence is available to justify any further speculation.

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