Increased Synthesis of Low-Molecular-Weight Nuclear Ribonucleic Acids of Rat Liver after Gamma Irradiation and Hepatectomy

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Incorporation of [^3H]orotic acid into low-molecular-weight nRNA of rat liver, fractionated on polyacrylamide gels, increased 6–12 h after partial hepatectomy and 6 h after γ -irradiation at 2000 R. The incorporation of orotic acid was particularly increased into the 4.5S, 5S and approx. 10S nRNA fractions. If the irradiation was given after 6 h of regeneration and RNA was isolated from the nucleus 12 h after hepatectomy then the incorporation of orotic acid into these low-molecular-weight nRNA components was greater than after hepatectomy or irradiation alone.

It has been established that liver rRNA synthesis is enhanced after whole-body X-irradiation of animals (Hidvégi *et al.*, 1965, 1966a, 1970; Cammarano *et al.*, 1969; Popov *et al.*, 1971) and RNA polymerase activity is also increased (Omata *et al.*, 1968; Hidvégi *et al.*, 1970; Yatvin, 1970). These were followed by a rise in the rRNA and mRNA contents (Hidvégi *et al.*, 1970). As a consequence, the number of polyribosomes in the liver cytoplasm and protein synthesis increased (Hidvégi *et al.*, 1964, 1966b, 1968; Baeyens & Goutier, 1968). It is not known whether there is a general increase in RNA synthesis due to irradiation, and whether it also involves an increase in low-molecular-weight nRNA.

Similar phenomena are well-known for the regenerating liver. rRNA synthesis (Fujioka *et al.*, 1963; Muramatsu & Busch, 1965) and RNA polymerase activity increased appreciably (Tsukada & Lieberman, 1964) shortly after hepatectomy and, as a consequence, the number of polyribosomes increased in the cytoplasm (Cammarano *et al.*, 1965). New species of RNA, some of them short-lived molecules, were detected only a few hours after hepatectomy (Church & McCarthy, 1967). Low-molecular-weight nRNA synthesis in regenerating liver was studied by Stevely & White (1970), who found a general decrease 6 h after partial hepatectomy, although both rRNA and mRNA syntheses are known to increase at this time.

Our limited knowledge of nRNA synthesis early in liver regeneration has led us to investigate low-molecular-weight nRNA synthesis in liver cells shortly after irradiation or partial hepatectomy, and also to examine the effects of irradiation in the early phase of the regeneration process.

Methods

F₁ hybrids of Wistar R–Amsterdam $\times$ Long-Evans (Chester Beatty hooded) male rats, weighing 180–

200 g, were used. The animals were starved for 24 h before being killed. Partial hepatectomy refers to the removal of about two-thirds of the liver (left lateral and medium lobes) (Higgins & Anderson, 1931). Surgery was done under ether anaesthesia. Whole-body irradiation was performed with γ -rays (2000 R) from ^{60}Co , at an exposure rate of 129 R/min.

At 1 h before being killed the animals were injected intravenously with 60 μCi of [^3H]orotic acid (The Radiochemical Centre, Amersham, Bucks., U.K.; sp. radioactivity 1 Ci/mmol). Either the whole livers of three control animals or eight livers of regenerating animals were pooled. Nuclei were isolated and the whole nRNA was extracted from the nuclei by the sodium dodecyl sulphate–phenol procedure at 60°C (Steele & Busch, 1966; Steele *et al.*, 1965) and fractionated by sucrose-density-gradient centrifugation in the SW27 rotor of the Beckman model L2-65B ultracentrifuge. The gradient was fractionated with an ISCO model D fractionator. Radioactivity was measured in a sample of the fractions (Steele *et al.*, 1965) in a Packard Tri-Carb liquid-scintillation spectrometer. *s* values were assigned by using purified ^{14}C -labelled rat liver 18S and 28S rRNA as markers. The major part of the fractions of low-molecular-weight nRNA (with *s* values of 4–10S) was pooled and precipitated with 2.5 vol. of 95% (v/v) ethanol containing 2% (w/v) potassium acetate. The low-molecular-weight nRNA was fractionated on polyacrylamide gels (Loening, 1967). Two gels were run in parallel from the same samples with the appropriate markers; on one, 30 μg of low-molecular-weight nRNA was loaded on 6 mm-diameter gels, and after electrophoresis the gel was stained with 0.2% Methylene Blue in 0.4 M-acetate buffer (pH 4.7) (Peacock & Dingman, 1967). The stained gel was scanned with a Joyce–Loebl Chromoscan instrument at 620 nm. On the other gel, 150 μg of low-molecular-weight nRNA was loaded on a 10 mm-diameter gel

and after electrophoresis the gel was cut into 1 mm slices and radioactivity measured (Loening, 1967) after digestion with 1 ml of 25% (v/v) NH_3 .

Results

First, the incorporation of orotic acid into whole nRNA was studied after γ -irradiation and hepatectomy. The labelled whole nRNA was fractionated by sucrose-density-gradient centrifugation.

In the non-hepatectomized and unirradiated control animals (Fig. 1a) most of the radioactivity was found in the fractions sedimenting between 28 and 35S, and less in fractions sedimenting between 10 and 18S. Radioactivity is rather low in the low-molecular-weight nRNA fractions sedimenting between 4 and 10S. At 6 and 12h after irradiation incorporation into each fraction of the whole nRNA increased by 30–60% (Figs. 1b and 1c). At 6h after partial hepatectomy incorporation increased 2–3-fold (Fig. 1d) and at 12h 3–4-fold (Fig. 1e) in all the fractions, not specifically in any single RNA fraction. Provided that nRNA was isolated 12h after hepatectomy and irradiation was given after 6h (Fig. 1f), then a rather high incorporation of orotic acid was found in the various fractions. Under these conditions (Fig. 1f) the incorporation was higher in nRNA

fractions sedimenting more slowly than 28S than in the same fractions if the nRNA was obtained from animals killed either 6h after irradiation (compare Figs. 1f and 1b) or 12h after partial hepatectomy (compare Figs. 1f and 1e).

The fractions of low-molecular-weight nRNA were pooled (hatched areas in Fig. 1), precipitated and further fractionated on polyacrylamide gels. The low-molecular-weight nRNA fractions described previously were found (Knight & Darnell, 1967; Nakamura *et al.*, 1968; Weinberg & Penman, 1968, 1969; Dingman & Peacock, 1968; Prestayko *et al.*, 1970). An RNA of approx. 10S can also be observed, located on the gel between 9 and 10cm. The 1h incorporation time was insufficient for significant labelling of the various low-molecular-weight nRNA species in the control animals (Fig. 2a). At 6 and 12h after irradiation, however (Figs. 2b and 2c), incorporation in fractions 4.5S and 5S had greatly increased. The incorporation was rather high in the 10S species, particularly 12h after irradiation (Fig. 2c). At 6h after partial hepatectomy (Fig. 2d) incorporation was greatly increased in the 4.5S and 5S species, significantly increased in the approx. 10S fractions and only slightly increased in the so-called U_1 RNA (sedimenting at approx. 7S) (Prestayko *et al.*,

Fig. 1. Sucrose-density-gradient profiles of the labelled whole nRNA

Rats were injected with [^3H]orotic acid intravenously 1h before being killed. Whole nRNA (2mg) was applied to a linear 10–45% (w/w) sucrose gradient, containing 0.1 M-NaCl–0.01 M-sodium acetate–1 mM-EDTA (pH 5.1) buffer, and centrifuged at 0°C at 27000 rev./min in an SW 27 rotor for 16h. —, E_{254} ; ●—●, radioactivity of 0.3 ml samples of 1 ml fractions. The low-molecular-weight nRNA fractions (hatched area) were pooled and precipitated from two or three identical gradients. (a) Non-hepatectomized and unirradiated control; (b) 6h after irradiation; (c) 12h after irradiation; (d) 6h after partial hepatectomy; (e) 12h after partial hepatectomy; (f) 12h after partial hepatectomy, with γ -irradiation at 6h.

Fig. 2. Polyacrylamide-gel profiles of the labelled low-molecular-weight nRNA

Rats were injected with [^3H]orotic acid intravenously 1 h before being killed. Fractionation was performed on 10% polyacrylamide gels (12 cm long) in 0.04 M-Tris-0.04 M- NaH_2PO_4 -0.001 M-EDTA (pH 7.6) buffer, at 4°C, at 4 mA/gel for 12 h. —, Curve scanned by the Chromoscan densitometer at 620 nm; ●—●, radioactivity of 1 mm slices. (a)–(f) are as defined in Fig. 1.

1970). At 12 h after partial hepatectomy the increase in incorporation was similar to that after 6 h (Fig. 2e), except that the incorporation was substantially lower in the 4.5S fraction (compare Figs. 2e and 2d). Provided that the experimental animals were irradiated 6 h after hepatectomy and killed after 12 h (Fig. 2f) the radioactivity profile resembled the profile obtained 6 h after hepatectomy alone (compare Figs. 2f and 2d), but the increase in incorporation was even greater in 5S and 10S fractions. It should be noted that neither the appearance nor the disappearance of some RNA fraction was observed in the experiments reported here, nor was any significant shift found in the extinction profile.

Discussion

The incorporation of labelled orotic acid was increased in the low-molecular-weight nRNA species during 6–12 h of liver regeneration and 6 h after irradiation, particularly in the 4.5S, 5S and approx. 10S RNA fractions. It might be noteworthy that both irradiation and partial hepatectomy increase the synthesis of the same low-molecular-weight nRNA

species. This may suggest that the treatments have affected control mechanisms that are common for these low-molecular-weight nRNA species.

The biological role of low-molecular-weight nRNA is still uncertain. The rapid increase in labelling of the 5S RNA is probably connected with the increase in rRNA which is observed 12 h after hepatectomy (Fujioka *et al.*, 1963; Muramatsu & Busch, 1965) and 6 h after X-irradiation (Hidvégi *et al.*, 1965, 1966a, 1970; Cammarano *et al.*, 1969).

The large increase in labelling of the approx. 10S RNA may be related to histone mRNA production. Histone synthesis is known to increase in the liver after partial hepatectomy (Holbrook *et al.*, 1962).

The 4.5S nRNA is very rapidly labelled compared with the other low-molecular-weight nRNA species (Moriyama *et al.*, 1969; Weinberg & Penman, 1969), especially 6 h after hepatectomy in our experiments. However, 12 h after hepatectomy the labelling of this RNA decreased. The rapid increase and subsequent decrease in labelling of the 4.5S RNA during the first 12 h after hepatectomy may be related to specific functions with respect to regulation of transcription during the first hours of the regeneration process.

The possibility exists that the 4.5S RNA, either alone or in a complex with some protein, may provide a recognition site for RNA polymerase. It is relevant now to study the function of 4.5S RNA, since it has been isolated in pure form and the nucleotide sequence of the 4.5S₁ RNA has been determined (Ro-Choi *et al.*, 1972).

The time of low-molecular-weight nRNA synthesis in the cell cycle has been studied. The studies on HeLa cells indicate that the low-molecular-weight nRNA species is synthesized throughout the cell cycle and its synthesis is not co-ordinated with DNA synthesis (Weinberg & Penman, 1969). Studies with synchronized baby-hamster kidney cells suggest that the low-molecular-weight nRNA species are synthesized before or immediately after DNA synthesis (Clason & Burdon, 1969). The data presented here suggest that in regenerating liver the synthesis of 4.5S, 5S and approx. 10S low-molecular-weight nRNA species precedes the DNA synthesis which starts only 12–15h after hepatectomy (Grisham, 1962; Lieberman & Kane, 1965).

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