Low-Molecular-Weight Nuclear Ribonucleic Acid Synthesis after Partial Hepatectomy

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Low-molecular-weight RNA from rat liver nuclei has been resolved into a number of distinct species by polyacrylamide-gel electrophoresis (Peacock & Dingman, 1967; Dingman & Peacock, 1968). We have examined the synthesis of these species after partial hepatectomy.

Partially hepatectomized male rats (180–200g) were injected intramuscularly with 100 μ Ci of 32 P_i/100g body wt. 30 min before death. Six hepatectomized and two control animals were used at each time-interval and tissue phosphate soluble in 20% (w/v) trichloroacetic acid was used as a reference standard for determining relative specific radioactivities. RNA was prepared from nuclei by using a hot-phenol-detergent technique (Burdon & Clason, 1969) and subjected it to electrophoresis through 7.5% (w/v) polyacrylamide gels. These were stained with Toluidine Blue and scanned with a microdensitometer and then sliced and assayed for radioactivity.

The staining pattern of normal liver showed two major peaks in the region of 5S and 7S (assessed by comparison with tRNA standard). The 5S RNA/7S RNA ratio was 1.10:1 in control animals, 1.19:1 at 2h after hepatectomy, 1.34:1 at 6h and 0.96:1 at 20h.

The radioactivity profile gave a large number of counts in the first two slices, due to RNA of sedimentation coefficient 18S and above, and in the region 4–18S 12 peaks were resolved. The ratio of counts due to high-molecular-weight RNA in gels from hepatectomized animals to that of controls rose after operation from 1.3:1 at 2h to 2.9:1 at 20h.

At various times after hepatectomy changes could be observed in the incorporation of radioactive isotope into specific peaks in the 4–18S region. At 6h there appeared to be a fairly general depression of all low-molecular-weight nRNA synthesis.

The results obtained indicate that the change in genetic expression required for hyperplasia after partial hepatectomy is accompanied by changes in the pattern of synthesis of low-molecular-weight nRNA. This lends support to the suggestion that these species may be involved in control mechanisms (Prestayko & Busch, 1968).

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The Isolation and Properties of Large Amounts of Messenger Ribonucleic Acid for Mouse Globin

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A considerable body of evidence now exists indicating that the 9S RNA component found in reticulocyte polyribosomes in the mRNA for globin. This component is of approximately the correct size and is sensitive to nuclease degradation and polyribosome sonication (Chantrenne, Burny & Marbaix, 1967; Williamson, Lanyon & Paul, 1969), and it appears to be able to direct protein synthesis in mixed systems with *Escherichia coli* components (Laycock & Hunt, 1969), in mixed mouse-rabbit systems (Lockard & Lingrel, 1969) and in a mixed system utilizing a genetically determined amino acid substitution in the α -chain of rabbit globin (Schapira, Dreyfus & Maleknia, 1968).

For a detailed study of the properties of this mRNA, a large-scale preparative procedure minimizing contamination of 9S material by breakdown products of rRNA and other RNA species is essential. We have therefore developed techniques to isolate large amounts of pure globin mRNA.

Mouse reticulocytes were prepared (80% reticulocytosis) after phenylhydrazine injection, and total RNA was prepared from the postmitochondrial supernatant or from isolated polyribosomes. Approx. 30mg of total RNA was centrifuged in a 5–20% (w/v) sucrose gradient in the B-XIV Ti zonal ultracentrifuge rotor at 47000 rev./min for 3h. The addition of diethylpyrocarbonate (Baycovin) to all buffers greatly decreased ribonuclease-mediated breakdown, and was essential to obtain reproducible results. A 9S-containing fraction was taken, which also included the 12S RNA found in these preparations (Labrie, 1969; Lanyon *et al.* 1968; Laycock & Hunt, 1969) and small amounts of 5S and 18S RNA.

The pooled fractions from several zonal runs, amounting to 3–5mg in all, were finally purified by preparative polyacrylamide-gel electrophoresis (Lanyon, Paul & Williamson, 1968) with a 3% gel. Milligram quantities of 9S RNA, apparently uncontaminated by other RNA species, were obtained by these procedures.

Although the 9S RNA migrates as a single component on 2.4% polyacrylamide gels, it splits into two major components on 6% gels. The equal

size of these components, the extent of their separation and their equal labelling *in vivo* is consistent with the interpretation that they represent the mRNA species for the α - and β -globin chains. The 12S RNA also has a unique labelling pattern with [32 P]P_i *in vivo*, distinct from both 9S RNA and rRNA, and thus is unlikely to be an rRNA breakdown product.

The molecular weight of the 9S mRNA, as calculated from its sedimentation coefficient and its migration in polyacrylamide gels, is slightly greater than expected from the known size of the globin amino acid chain. DNA-RNA hybridization results with the mRNA, described in the following communication (Williamson, Morrison & Paul, 1970), may indicate a non-messenger function for a portion of the mRNA polynucleotide chain.

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Hybridization with Deoxyribonucleic Acid of Messenger Ribonucleic Acid for Mouse Globin

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The isolation of large amounts of purified mRNA for globin (Morrison, Williamson, Lanyon & Paul, 1970) has made the study of its hybridization to DNA possible. Because of the difficulty of obtaining sufficient amounts of high-specific-radioactivity RNA after labelling *in vivo*, the RNA was isolated unlabelled and then tritiated with [3 H]dimethyl sulphate (Smith, Armstrong & McCarthy, 1967). Such RNA methylated *in vitro* shows the same specificity in DNA-RNA hybridization as RNA labelled *in vivo*. Mouse embryo DNA used in the hybridizations was purified by centrifugation through caesium chloride.

Hybridization was in formamide at 37°C, by the Gillespie-Spiegelman technique as modified by McConaughy, Laird & McCarthy (1969). The conditions are calculated to be equivalent to hybridization at 67°C in 4×SSC. A saturation value of 0.5% of the genome was found, corresponding to approx. 50000 gene copies for the mRNA, many more than anticipated. The hybridization reaction is competed out to 100% with unlabelled 9S mRNA, but only slightly competed by a mixture of 18S and 28S rRNA. Saturation occurs only at fairly high RNA/DNA ratios, but is very fast, being substantially complete in the first 10 min of reaction.

Crude thermal-denaturation profiles were obtained by heating the filters after hybridization in 1ml of 1×SSC at increasing temperatures. A low T_m for the hybrid was obtained, indicating a very extensive mismatching of base pairs.

The simplest explanation of these results is that inherent similarities between stretches of the amino acid sequences of proteins allow cross-hybridization to occur with extensive mismatching between one mRNA chain and the DNA genes coding for other polypeptide chains. This would certainly be expected for similar proteins such as the globins of haemoglobin and myoglobin, but the extent of hybridization seen here seems rather too large. It is also possible that the large extent of hybridization is due to the pairing, not only of the coding portion of the messenger RNA, but also to hybridization of the portion 'extra' to the expected messenger length (see Morrison *et al.* 1970). Such an extra sequence might be involved in initiation (Labrie, 1969) or in control of translation by 'ticketing' (Sussman, 1970), or be a transcribed portion of a control sequence for transcription (Britten & Kohne, 1968; Georgiev, 1969). In any of these cases such a sequence might be expected to be repeated at many points through the genome, not only at the site of globin mRNA.

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