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Synthesis of Ribonuclease-Resistant Ribonucleic Acid by Fowl-Plague-Virus-Induced Ribonucleic Acid Polymerase

By B. W. J. MAHY and P. A. BROMLEY (*Department of Pathology, University of Cambridge*)

An RNA-dependent RNA polymerase is induced in chick-embryo fibroblast cells infected with fowl-plague virus (Skehel & Burke, 1968; Scholtissek & Rott, 1969). The RNA synthesized by this enzyme *in vitro* has now been investigated and compared with RNA formed in infected cells *in vivo*.

Chick-embryo fibroblast cells grown in roller bottles were infected at an input multiplicity of 5–10 plaque-forming units/cell (Borland & Mahy, 1968). The microsomal fraction prepared from cells at 6 hr. after infection was incubated at 28° with a reaction mixture containing (μ moles/ml.): tris-HCl, pH 7.8, 250; 2-mercaptoethanol, 12; $MgCl_2$, 2.5; ATP, CTP and GTP, 0.15 each; [3H]-UTP, 0.005 (5 μ C). Incorporation into acid-insoluble radioactivity increased linearly with time of incubation up to 60 min. When samples of the reaction mixture taken at any time were treated with ribonuclease (1 μ g./ml. for 10 min. in 0.15M-NaCl–0.015M-sodium citrate buffer), only about 50% of the radioactivity was rendered acid-soluble. Labelled RNA extracted from the reaction mixture after 30 min. incubation with phenol–sodium dodecyl sulphate or phenol–cresol (Kirby, 1965) was 37% resistant to ribonuclease treatment. The ribonuclease-resistant fraction showed a sharp thermal transition at about 90° in 0.15M-NaCl–0.015M-sodium citrate buffer.

The polymerase product RNA sedimented in the 7–18s region on sucrose density gradients, and ribonuclease-resistant fractions were found at 12–14s. If the product RNA was treated with ribonuclease before sedimentation, all the acid-insoluble radioactivity banded sharply at 12s. To compare the RNA product obtained *in vitro* with RNA formed *in vivo*, cells were treated with actinomycin D (2 μ g./ml.) at 5.5 hr. after infection then pulse-labelled with [3H]uridine (10 μ C/ml.)

for 15 min. before extraction of RNA from the microsomal fraction. The bulk of the RNA labelled *in vivo* sedimented in the 18s region, i.e. in a similar position to RNA from purified fowl-plague virus (Barry & Davies, 1968), but 5–10% was resistant to ribonuclease and sedimented at 12s.

Hybridization experiments were carried out between 3H -labelled polymerase product RNA and ^{32}P -labelled fowl-plague-virus RNA. The virus RNA was completely susceptible to ribonuclease treatment, but became 37% resistant after mixing with polymerase product RNA under annealing conditions. The results of further annealing studies will be presented.

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The Use of Gradient Centrifugation to Study Events in the Life Cycle of Cultured Cells

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Exponentially growing P815Y neoplastic mast cells (Schindler, Day & Fischer, 1959) were briefly exposed to radioactive precursors, collected by centrifugation, washed and layered on to 40 ml. of a gradient (5–10%, w/v) of Ficoll in growth medium (Morris, Cramer & Reno, 1967). After centrifugation at 80g_{av} for 7 min. the tube was punctured and samples were collected. The number of cells in each sample, and their mean volume, was measured with a Coulter counter. Precursor incorporated into cells was determined by filtering a portion of each sample, washing with trichloroacetic acid and measuring the radioactivity.

The distribution of cells through the gradient showed a broad peak near the centre. The mean volume of cells near the top was about half that at the bottom. When thymidine was used as a precursor, the incorporated radioactivity/cell showed a broad maximum just below the cell peak, in agreement with the results of Morris *et al.* (1967). When proline, uridine or choline was used, the incorporated radioactivity/cell increased from the top to the bottom of the gradient.

When cells that had been separated by gradient centrifugation were resuspended in medium, they grew with a certain degree of synchrony; those from the top of the gradient divided after 10–15 hr. (mean generation time 15 hr.) whereas those from the bottom began division within 5 hr. Hence cells near the top of the gradient appear to be

early in their life cycle (phase G1), and cells near the bottom at the end of the cycle (phase G2). The experiment with labelled thymidine shows that cells near the middle of the gradient are in the period of DNA synthesis (phase S).

These results, in confirmation of other work (Bergeron, Warmley & Pasternak, 1969), indicate that incorporation of proline, uridine and choline into protein, RNA and phospholipid respectively is not limited to a specific portion of the cell cycle (as is the case with thymidine), nor is the rate of incorporation constant throughout the cell cycle. Rather incorporation appears to increase from a low rate in phase G1 to a higher rate in phase G2. Such an increase has been observed by using other techniques in the case of RNA and protein synthesis (e.g. Robbins & Scharff, 1966; Pfeiffer & Tolmach, 1968; Martin, Tomkins & Granner, 1969).

A.M.H.W. is a Medical Research Council Scholar.
J.J.M.B. is a Rhodes Scholar.

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Subcellular Fractionation of *Polytomella caeca* by Zonal Centrifugation

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Lloyd, Evans & Venables (1968) have shown that resting cells of acetate-grown *Polytomella caeca* oxidize propionate only after a lag of 2 hr., and that this adaptation involves the formation of enzymes of β -oxidation. Two of these enzymes, β -hydroxypropionate dehydrogenase and malonic semialdehyde dehydrogenase, were shown by differential centrifugation to be located in a mitochondrial fraction. Present studies were undertaken to confirm this observation and to investigate the distribution of other enzymes in homogenates prepared from suspensions of this organism.

Cells were disrupted in an all-glass hand-operated homogenizer in a medium consisting of 0.32M-sucrose in 24 mM-tris-HCl buffer, pH 7.4, containing

10 mM-EDTA. After the unbroken cells had been centrifuged off, the homogenates were loaded on to a sucrose density gradient (buffered with 10 mM-tris-HCl at pH 7.4) in a B XIV rotor and centrifuged at 35 000 rev./min. for 2.75 hr. (100 000g at the sample zone).

Under these conditions mitochondria (marker enzyme, cytochrome c oxidase) sedimented to their isopycnic density (1.19–1.21 g./ml.). A major peak of catalase activity at density 1.24 (1.22 in an unbuffered gradient) suggests that this organism contains peroxisomes. Three peaks of acid phosphatase activity (substrate, *p*-nitrophenyl phosphate) were located: a 'non-sedimentable' activity and activities associated with particles sedimenting to densities 1.14 and 1.20–1.21 respectively. The distribution of esterase activity (substrate, *p*-nitrophenyl acetate) was similar to that of acid phosphatase. Most of the NADPH-cytochrome c oxidoreductase activity was 'non-sedimentable', whereas NADH-cytochrome c oxidoreductase activity was mainly located in the mitochondrial peak and also at a density of 1.11. A portion of the malate dehydrogenase (40–60%), citrate synthase (70–80%) and NADP-linked isocitrate dehydrogenase (8%) activities was mitochondrial; variation in the sedimentability of these activities may be used as an index of mitochondrial breakage. Isocitrate lyase was nearly all (> 99%) detected as a 'soluble' activity.

β -Hydroxypropionate dehydrogenase was located mainly (> 95%) in the mitochondrial fractions of both propionate-grown cells and acetate-grown cells that had been adapted to propionate.

R.A.C. holds a Medical Research Council Research Studentship.

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Palmitic Acid Uptake and Metabolism by Isolated Rat Liver Cells

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Previous studies on isolated liver cells (Higgins & Green, 1967) raised the possibility that incorporation of fatty acids into plasma-membrane phospholipids was involved in their uptake. The cells used in these earlier studies displayed metabolic deficiencies, but new techniques (Howard, Christensen, Gibbs & Pesch, 1967) give suspensions of cells in much better condition. When such cell suspensions were incubated for up to 2 hr. with a [14 C]palmitic acid-albumin complex in the presence of ATP,