Ribonucleic Acid Polymerase Activity in Cardiomyopathic Hamster Heart Nuclei

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Cardiomyopathy in the Syrian hamster is associated with compensatory hypertrophy and heart failure. As yet these processes are not fully understood. Bajusz, Büchner, Onishi & Rickers (1969), by electron-microscope studies, have observed that there is a depression of myofilament formation, which is followed by an increased formation of ribosomes. Lochner, Brink & Brink (1971) have shown that there is an increase in protein synthesis in older age-groups of myopathic Syrian hamsters. As part of a broad programme of investigation into cardiomyopathy of the Syrian hamster, it was decided to study DNA-dependent RNA synthesis in isolated nuclei of myopathic hamsters and control animals.

Cardiomyopathic hamsters (B10 14.6) and control animals imported from the Bio-Research Institute, Boston, Mass., U.S.A., were used in the present experiments. [^{14}C]ATP, [^{14}C]GTP, [^{14}C]CTP and [^{14}C]UTP were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., the disodium salts of unlabelled ATP, GTP, CTP and UTP from Boehringer, Mannheim, Germany, yeast RNA and calf thymus DNA (highly polymerized) from Sigma Chemical Co., St Louis, Mo., U.S.A., and deoxyribonuclease (ribonuclease-free) from Seravac, Cape Town, South Africa.

For the isolation of nuclei, all operations were performed at 0-4°C. Hearts from at least eight animals were pooled and weighed in 0.32M-sucrose containing 3mM-magnesium chloride (homogenizing medium). The procedure of Widnell & Tata (1964) for the isolation of the nuclei was followed, except that after the first low-speed centrifugation the crude nuclear pellet was suspended in a small volume of homogenizing medium and diluted exactly 1:10 with 2.4M-sucrose containing 1mM-magnesium chloride. The suspension was homogenized by ten strokes in a hand-operated ground-glass homogenizer with a loose-fitting pestle and then centrifuged for 2h at 50000g in an SW 25.1 swinging-bucket rotor in a Beckman model L-4 preparative ultracentrifuge. The nuclear pellet was finally resuspended in 2ml of 0.25M-sucrose containing 1mM-magnesium chloride.

The method of Widnell & Tata (1966) for the

assay of RNA polymerase activity in the absence and presence of ammonium sulphate was used, with the exception that the reaction volume was scaled up to 1ml. The specific radioactivity of the [^{14}C]ATP used was 2.5 $\mu\text{Ci}/0.05 \mu\text{mol}$. For the ammonium sulphate-stimulated reaction, no pre-incubation was carried out, but the [^{14}C]ATP was added at zero time. The reactions were terminated by the successive additions of 0.2ml of RNA (yeast, 10mg/ml) and 2ml of 10% (w/v) trichloroacetic acid. The reaction tubes were left in ice for 15min. Zero-time controls were also carried out. The precipitates were collected by centrifugation (2000g for 10min) and washed twice in cold 5% (w/v) trichloroacetic acid (2ml) by low-speed centrifugation, once with ethanol containing 2% (w/v) sodium acetate, and once with ethanol-ether (3:1, v/v). RNA in the washed precipitates was then extracted by means of the Schmidt-Thanhauser procedure (Munro & Fleck, 1966). RNA samples were counted for radioactivity in 15ml of Beller's (1968) solution with a Packard liquid-scintillation counter. DNA was determined by the method of Burton (1956). The specific activity of RNA polymerase was defined as pmol of [^{14}C]ATP incorporated into RNA/mg of DNA in the nuclear suspension.

For the determination of the base composition of synthesized RNA, the method of Marchok & Wolff (1968) was followed, with the exception that ^{14}C -labelled nucleoside triphosphates were used, with a specific radioactivity of 2.5 $\mu\text{Ci}/0.3 \mu\text{mol}$.

In this study, normal hamsters of the same age served as controls. Under both reaction conditions studied, the RNA polymerase activity was significantly higher in the nuclei of the myopathic hamster hearts than in the controls (Table 1). This increase in activity was most marked at an age of 30 days. Thereafter there was a progressive decrease in the difference between the polymerase activities of the two groups of animals.

In both the presence and the absence of ammonium sulphate the reaction was DNA-dependent, since a decrease of [^{14}C]ATP incorporation into RNA was obtained in the presence of 20 μg of deoxyribonuclease. The base composition of the

Table 1. *RNA polymerase activity in the presence and absence of ammonium sulphate in normal and myopathic hamster heart nuclei*

The incubation medium for the assay of RNA polymerase in the absence of $(\text{NH}_4)_2\text{SO}_4$ contained in 1 ml: 50 μmol of tris-HCl buffer, pH 8.5, 2.5 μmol of MgCl_2 , 10 μmol of cysteine, 3 μmol of NaF, 0.3 μmol each of GTP, CTP and UTP, 0.05 μmol of $[^{14}\text{C}]\text{ATP}$ (2.5 μCi) and 0.2 ml of nuclear suspension (150 μg of DNA). Incubation time was 15 min at 37°C. The incubation medium in the presence of $(\text{NH}_4)_2\text{SO}_4$ contained in 1 ml: 50 μmol of tris-HCl buffer, pH 7.5, 2.0 μmol of MnCl_2 , 0.1 ml of a solution of $(\text{NH}_4)_2\text{SO}_4$ saturated at room temperature and adjusted to pH 7.5 with aq. NH_3 , 0.3 μmol each of GTP, CTP and UTP, 0.05 μmol of $[^{14}\text{C}]\text{ATP}$ (2.5 μCi) and 0.2 ml of nuclear suspension. Incubation time was 45 min at 37°C.

Age (days)	Tissue	$[^{14}\text{C}]\text{ATP}$ incorporated into RNA (pmol/mg of DNA)	
		Mg^{2+} - stimulated	Mn^{2+} + $(\text{NH}_4)_2\text{SO}_4$ - stimulated
30	Normal	2900	3600
	Myopathic	5200	4100
60	Normal	2987	3590
	Myopathic	5170	5200
90	Normal	2650	2990
	Myopathic	3480	3600
120-150	Normal	2660	3115
	Myopathic	3190	3700
240	Normal	2400	3010
	Myopathic	2900	3600

RNA product synthesized in the presence of Mg^{2+} resembled that of ribosomal RNA, since a higher content of GTP+CTP (62.6%) than of UTP+ATP (37.2%) was obtained. However, when Mn^{2+} + ammonium sulphate replaced Mg^{2+} the composition of the RNA product resembled that of total nuclear RNA. The RNA had approximately the same content of all four nucleotides, with a higher ATP +

UTP (44.4%) content and a lower GTP+CTP (53.3%) content than ribosomal RNA. It is noteworthy that the reaction in the presence of Mg^{2+} was more sensitive to 10 μg of actinomycin D than when carried out in the presence of Mn^{2+} + ammonium sulphate. Similar base compositions for the RNA synthesized in the presence and absence of ammonium sulphate were obtained by Widnell & Tata (1966) and Blackburn & Klemperer (1967) working with rat liver nuclei, Nair, Rabinowitz & Chen Tu (1967) using rat heart nuclei, and Marchok & Wolff (1968) working with isolated nuclei from developing chick muscle.

The results in the present study suggest that both types of RNA are synthesized by isolated hamster heart nuclei. Further, the radioactivities of both the ribosomal RNA and the DNA-like RNA were greatly increased in the young myopathic hamster hearts, but decreased in the older animals. The reasons for the increased DNA-dependent RNA synthesis in isolated nuclei of myopathic hamster hearts are as yet unknown.

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