DNA SYNTHESIS AND DNA POLYMERASE ACTIVITY IN DIFFERENTIATING CARDIAC MUSCLE[1]

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SUMMARY: DNA synthesis in cardiac muscle of the rat declines rapidly following birth and is essentially "turned off" by the 17th day of postnatal development. Soluble DNA polymerase activity also decreases progressively with age, reaching adult levels of almost zero by the 17th day of development. These results indicate that cessation of DNA synthesis in differentiating cardiac muscle may be attributed to the loss or inactivation of DNA polymerase.

INTRODUCTION

The fully differentiated cardiac muscle cell of the adult is a stable entity which has apparently lost the ability to replicate its DNA and undergo mitosis (1-6). Little is known about the mechanisms responsible for this loss of synthetic activity or precisely when it occurs during differentiation. Autoradiographic studies using tritiated thymidine indicate that in the rat, DNA synthesis in cardiac muscle occurs during the early stages of postnatal development but not in the terminally differentiated adult cells (1-3,6). Histologic studies have also shown that mitotic activity ceases during early postnatal development (1-5). The studies reported in this communication were undertaken to determine the point during development that cardiac muscle cells lose the ability to replicate DNA and to explore possible factors responsible for the cessation of synthetic activity.

METHODS

Pregnant rats, carefully timed, were obtained from Holtzman (Madison, Wisconsin). Neonatal rats were injected subcutaneously with [^{3}H]thymidine, 2 μCi/g body weight (specific activity 20 Ci/mM, New England Nuclear), and sacrificed one hour later by decapitation. Hearts were rapidly removed, the atria and large vessels trimmed away and the ventricles homogenized in 0.154 M NaCl with a glass homogenizer fitted with a Teflon pestle. An equal volume of cold 10% perchloric acid was added to the homogenate and the acid-insoluble

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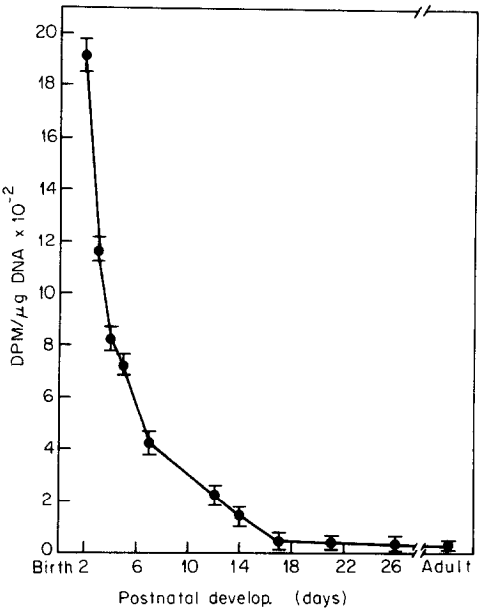

Figure 1: Incorporation of [^{3}H]thymidine into DNA of cardiac muscle at various times during development. Each value represents the mean ± SE of the mean of six individual determinations. In younger animals, 3 hearts were pooled for each determination.

material was washed twice with ice-cold 5% perchloric acid, twice with ethanol: ether (3:1), resuspended in 5% perchloric acid and heated to 70°C for 30 minutes. The amount of DNA in the acid-soluble fraction was determined using the Burton modification of the diphenylamine reaction (6) with calf thymus DNA as the standard. Incorporation of [^{3}H]thymidine into DNA was measured by counting an aliquot of the acid-soluble fraction in a Packard Tri-Carb liquid scintillation spectrometer.

Soluble DNA polymerase activity was assayed by measuring the initial rate of incorporation of [^{3}H] dTTP into an acid-insoluble product using heat-denatured DNA as the primer-template. The reaction mixture contained the following in a total volume of 0.5 ml: 20 μmoles Tris-HCl, pH 7.5; 2 μmoles $MgCl_2$; 0.25 μmoles β-mecaptoethanol; 30 nmoles each of dATP, dGTP, and dCTP; 100 μg of calf thymus DNA; 81 pmoles [^{3}H] dTTP (specific activity 54 Ci/mM, Amersham/Searle); and 0.1 ml of 105,000 x g supernatant as the polymerase source. The reaction was carried out at 37°C for 15 minutes and terminated

TABLE I

CHARACTERIZATION OF THE DNA POLYMERASE REACTION

REACTION MIXTURE	% OF COMPLETE REACTION MIXTURE
Complete[a]	100
-DNA	1.6
-dCTP, dGTP, dATP	3.5
-$MgCl_2$	2.5
+DNase[b]	0.4
+RNase[b]	96.7

[a]Reaction carried out with enzyme prepared from 1-day old neonatal rats.

[b]The trichloroacetic acid precipitates of these reactions were suspended in 1.0 ml of 10 mM Tris-HCl (pH 7.5) containing 10mM $MgCl_2$ and incubated with either 100 μg of DNase or RNase (Worthington) for 30 minutes at 37°C. The incubation was terminated with 2 ml cold 10% trichloroacetic acid and the acid-insoluble radioactivity counted on filters as described in the text.

by adding 2 ml of ice-cold 10% trichloroacetic acid containing 2% sodium pyrophosphate. The acid-insoluble material was collected on Whatman GF/C glass fiber filters and washed with ice-cold 5% trichloroacetic acid containing 2% sodium pyrophosphate. The filters were dried and counted in toluene-PPO-POPOP in a liquid scintillation spectrometer. Zero time controls were subtracted when expressing the activity. Protein was estimated by the procedure of Lowry et al. (7) using crystalline bovine serum albumin as the standard.

RESULTS

The rate of DNA synthesis in cardiac muscle of the rat during postnatal development is shown in Fig. 1. The incorporation of [^{3}H]thymidine into DNA declines rapidly following birth and is essentially "turned off" by the 17th day of development. This cessation of DNA synthesis is not due to decreased uptake of [^{3}H]thymidine, alterations in the radioactive pool size or in-

creased catabolism of the isotope during development.

Characterization of the DNA polymerase reaction is given in Table 1. Incorporation of [^{3}H]dTTP into the acid-precipitable product is proportional to the amount of protein added to the reaction mixture and is linear for 45 minutes of incubation. DNA, dNTPs, Mg^{++} and β-mecaptoethanol are required for full enzymatic activity. The radioactive product of the reaction is almost completely hydrolyzed by DNase but is insensitive to RNase. The activity of soluble cardiac muscle DNA polymerase declines progressively during early postnatal development reaching adult levels of almost zero by the 17th day (Table 2). This represents approximately an 80-fold decrease in activity. The decline in enzymatic activity is not due to increased endogenous DNase or phosphatase activity during this period of development. Recombination experiments (assay of neonatal enzyme extracts with adult enzyme extracts) gave additive results, indicating that differences in enzyme activity are not due to the presence or absence of activators or inhibitors at different times during development.

DISCUSSION

The data presented in this communication demonstrate that DNA biosynthesis as measured by [^{3}H]thymidine incorporation in cardiac muscle of the rat has essentially stopped by the 17th day of postnatal development. This cessation in DNA synthesis is correlated temporally with an almost total loss of soluble DNA polymerase activity. Soluble DNA polymerase activity has also been reported to decline greatly in skeletal muscle cells in culture (9, 10) and *in vivo* (11) when the cells change from a proliferative to a fused, nonproliferative population. In regenerating rat liver and in mouse L cells, Chang *et al.* (12,13) have recently demonstrated quite conclusively that the 6 to 8S soluble DNA polymerase activity varies with the rate of DNA synthesis and the proliferative activity of the cells. Other studies (14-16) also indicate a relationship between DNA synthesis and the activity of DNA polymerase.

TABLE 2

SOLUBLE DNA POLYMERASE ACTIVITY IN CARDIAC MUSCLE DURING DEVELOPMENT

DAYS OF POSTNATAL DEVELOPMENT	SPECIFIC ACTIVITY [^{3}H] dTTP INCORPORATED (pmoles/mg protein/15 min.)
1	10.02
10	3.10
17	0.19
Adult	0.14

Reaction mixtures contained between 600 and 800 μg of protein.

It is now generally held that a cell which is actively synthesizing DNA has already made the decision to divide. Therefore, the control of cell division and proliferation may ultimately reside with the factors regulating and needed for the synthesis of DNA. Results of the studies reported here suggest that cessation of DNA synthesis and cell division in developing cardiac muscle cells may be attributed to the loss or inactivation of DNA polymerase. The mechanism by which DNA polymerase activity or availability is regulated in cardiac muscle is presently under investigation.

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