DNA FRAGMENTATION AS A DEVELOPMENTAL PROGRAM FOR CELLULAR AGING IN CARDIAC MUSCLE

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Several years ago we determined that semiconservative DNA replication in the intact rat heart and in isolated individual cardiac muscle cells essentially ceases by the third week of postnatal development (1-3). Temporally correlated with this loss of DNA replicative ability was the disappearance from these cells of the putative replicative DNA polymerase, DNA polymerase α (1,3). An obvious question to ask of this data was: does DNA synthesis cease in these cells because the activity of this enzyme is lost, or is the activity of this enzyme lost because DNA synthesis ceases? Which is cause and which is effect? In an attempt to answer this question, DNA template availability (capacity to prime for DNA replication) and the number of 3'-OH termini in the DNA (origins of DNA replication) were measured in cardiac muscle nuclei and chromatin prepared at various times during the developmental period when DNA replication is being and has been restricted. These parameters were assessed using several different exogenous DNA polymerases (4). It was observed that DNA template and 3'-OH termini available to exogenously added DNA polymerases do not change as cardiac muscle differentiates and the rate of DNA synthesis ceases (4) (Fig. 1). It was concluded from these experiments that DNA replication ceases in these cells because the activity of a replicative DNA polymerase is lost, rather than the activity of this enzyme being lost because DNA synthesis ceases.

3'-OH TERMINI IN ADULT RAT CARDIAC MUSCLE

The surprising observation to come from these studies was that the number of 3'-OH termini was substantially increased in nuclei and chromatin of cardiac muscle of the adult rat (Fig. 1). It was shown that this increase was not due to elevated endonuclease or exonuclease activity in nuclei or chromatin of the adult (4).

Further data to show that DNA is more fragmented in cardiac muscle from adult animals compared to cardiac muscle from neonatal animals are given in
Figure 1. Determination of DNA template availability and 3'-hydroxyl termini in nuclei isolated from cardiac muscle during postnatal differentiation and from the adult. Nuclei were isolated from animals of the indicated age (adults were females that weighed approximately 250 g) and incubated in a reaction mixture designed to measure template availability (open symbols) or 3'-hydroxyl termini (closed symbols). Nuclei (approximately 5 μg of DNA) were incubated for 30 min at 37°C with 4 units of E. coli DNA polymerase I at pH 8.5 (○, ●), 4 units of M. luteus DNA polymerase at pH 7.0 (Δ, △) or 4 units of DNA polymerase α at pH 6.5 (□, ■). Further details are given in the text and Claycomb (4).

Figure 2. In these studies nuclei were isolated from cardiac muscle of 1-day old and adult rats. These nuclei were incubated in reaction mixtures containing dCTP, dGTP, 3H-dATP, BrdUTP and E. coli DNA polymerase I. BrdUTP was used in this assay to measure any density heterogeneity if it existed in the DNA. E. coli DNA polymerase I will catalyze the incorporation of BrdUTP into DNA at sites in the DNA where there are free 3'-OH groups. The more free 3'-OH sites in the DNA, the more BrdUTP will be incorporated and hence this DNA will be more heterogeneous (depending on the length of the DNA between 3'-OH sites) than DNA that had fewer 3'-OH sites. Following this incubation, the DNA was extracted and analyzed in alkaline CsCl equilibrium density gradients. DNA from neonatal rats shows a homogeneous pattern in these gradients. DNA from the adult is very heterogeneous both by optical density and radioactive profiles. This indicates that DNA from cardiac muscle of the adult is much more fragmentated than DNA from cardiac muscle of neonatal animals. A report by Jackowski (5) confirms this finding. This fragmentation or increased number of 3'-OH sites in the DNA of the adult is quantitated in Figure 1. The data