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Activation of myosin heavy chain genes during cardiac hypertrophy

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Abstract. Nick translation of intact rat heart nuclei has shown that the incorporation of [^{3}H]–dATP is greater in hypertrophic heart nuclei than in normal heart nuclei suggesting that hypertrophic heart nuclei have more DNase I sensitive regions than normal heart nuclei. DNase I sensitivity analysis has shown that the rate and extent of digestion of myosin heavy chain genes are greater in hypertrophic than in normal heart nuclei. Dot blot hybridization analysis of myosin heavy chain transcripts from hypertrophic heart nuclei using myosin heavy chain cDNA as probe has shown that the sensitivity of myosin heavy chain genes to DNase I in hypertrophic heart nuclei correlates with myosin heavy chain gene activation and increased number of transcripts.

Keywords. Cardiac hypertrophy; nick translation of intact nuclei; DNase I digestion pattern; myosin heavy chain genes.

Introduction

Cardiac hypertrophy is a basic adaptive response of the heart to any increased functional demand. The development of cardiac hypertrophy is associated with enhanced transcription and translation, and a consequent increase in the ribosomal RNA, poly (A)-containing RNA, and myofibrillar and cytoplasmic protein contents of cardiac muscle cells (Zak and Rabinowitz, 1979). The increase in RNA synthesis reflects an altered conformation of chromatin (Limas, 1982). The regions of chromatin that are engaged in transcription and those that are not, generally show a differential sensitivity to DNase I digestion (Weintraub and Groudine, 1976). Although it is clear that the transcriptionally active regions of the chromatin are maintained in a conformation distinguishable from that of the transcriptionally inert chromatin, the nature of the changes which accompany gene activation remains obscure.

Exploiting the sensitivity of transcriptionally active chromatin to DNase I, we carried out the nick-translation of intact nuclei obtained from both normal and hypertrophic hearts at low concentrations of DNase I, so that the active genes are nicked rather than cleaved (Levitt *et al.,* 1979). In DNase I sensitivity experiments, nuclei from normal and hypertrophic heart were compared in terms of the rate and extent of digestion of the chromatin. At the more specific gene level we have attempted to study the activation of myosin heavy chain genes which is responsible for major changes in the Ca-dependent ATPase activity of myosin (Lompre *et al.,* 1984) during cardiac hypertrophy.

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Materials and methods

Induction of cardiac hypertrophy

Cardiac hypertrophy in female albino Wistar rats was induced by following the method of Rakusan and Poupa (1966) with minor modifications. A tantalum hemoclip (Edward Week and Co., Cat. No. 523135) was placed around the proximal ascending aorta just distal to the coronary ostis. A sham operation was performed on control animals and no band was placed around the ascending aorta. The development of hypertrophy was calculated as per cent increase in the ratio of heart weight (wet wt.) to body weight with respect to sham-operated controls (Meenakshi *et al.,* 1983).

Isolation and nick translation of nuclei

The isolation of nuclei was carried out by the method of Jackowski and Liew (1980). The nuclear pellet was purified by pelleting through a step gradient of 2·4 and 1·6 M sucrose at 100,000 *g* for 1 h at 4°C in a Hitachi SCP 85H centrifuge. The DNA content of the nuclei was determined by Burton's (1956) procedure. Nuclei were pelleted and rinsed in nick translation buffer (50 mM Tris pH 7·9, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 10 μg/ml BSA) and then used for nick translation as described by Levitt *et al.* (1979).

DNase I sensitivity studies

Nuclei from sham-operated and hypertrophic hearts were suspended in 50% w/v glycerol, 10 mM Tris pH 7·4, 10 mM NaCl and 3 mM $MgCl_2$, and aliquots digested with various concentrations of pancreatic DNase I (Sigma) at 37°C for 5 min (Dimitriadis and Tata, 1980). DNA extracted after DNase I treatment was run on a 1% agarose gel. The pattern of digestion was analysed by electrophoresis on a 12% denaturing polyacrylamide gel (Noll, 1974). The gel was stained in 0·005% Stains-all in 50% formamide, destained in water, and scanned in an LKB Ultrascan.

Dot hybridization analysis

Plasmid pcMHC 5, a pBR322 derivative containing myosin heavy chain cDNA from rat heart (Mahdavi *et al.,* 1982), and plasmid pPC-P450–91, a pUC9 derivative containing cytochrome P450e cDNA from rat liver (Ravisankar and Padmanaban, 1985) were nick-translated as described by Rigby *et al.* (1977). Total nuclear RNA was isolated from sham-operated and hypertrophic hearts as described by Penmann (1966). Spotting of nucleic acids on nylon filters and hybridization with nick-translated probes (specific activity 1×10^8 cpm/μg DNA) were done according to the protocols given by the manufacturer. Quantitative analysis of dot blots was done by cutting out the radioactive, spots on the nylon filter and counting in a liquid scintillation counter.

Results and discussion

Nick translation of intact nuclei

In an earlier report, we had shown that template activation in heart nuclei occurs in the early stage of the development of cardiac hypertrophy (Kamala *et al.,* 1986). Extending this initial observation, we have studied gene activation in hypertrophic heart in general as well as at a specific gene level (myosin heavy chain) during cardiac hypertrophy. The advantage of nick translation studies of intact nuclei is that an active transcriptional state of chromatin is indicated by increased incorporation of label in the nick translation reaction because of the higher DNase I sensitivity of "active" chromatin. This was shown by hybridizing the nick-translated DNA with total cellular RNA (Levitt *et al.,* 1979). Nick translation studies have also been useful in localizing DNase I sensitive regions of chromatin in interphase nuclei and in dividing cells (Hutchison and Weintraub, 1985). The increased incorporation of [^{3}H]–dATP in hypertrophic heart nuclei reflects the activation of regions of chromatin for transcription (table 1). This could be due to changes in the conformation of transcribed regions of chromatin, which then become more accessible to regulatory proteins, nucleases and polymerases.

Table 1. Nick translation of nuclei.

Source of nuclei	Incorporation of [^{3}H]–dATP (cpm/μg DNA)
Sham-operated heart	$7{\cdot}7 \pm 0{\cdot}3 \times 10^4$
Hypertrophic heart	$10{\cdot}0 \pm 0{\cdot}4 \times 10^4$

All values are mean ± SD of mean of 3 experiments.

D Nase I digestion pattern

Increasing the DNase I concentration in a DNase I digestion reaction results in active nuclear DNA sequences being rapidly digested and solubilized. When nuclei from sham-operated and hypertrophic hearts were treated with various concentrations of DNase I (0, 2, 10 and 50 U/mg DNA) for a constant incubation time (5 min) and the extracted DNA electrophoresed on neutral 1 % agarose gel and stained with ethidium bromide, different patterns were observed (figure 1). At various DNase I concentrations, DNA of hypertrophic heart nuclei was found to be digested to a greater extent than DNA of control sham-operated heart nuclei. Further, the rate of production of smaller fragments was faster in hypertrophic heart nuclei (data not shown). When the DNA fragments obtained after DNase I treatment (150 U/mg DNA for 5 min) of sham-operated and hypertrophic heart nuclei were analysed by denaturing PAGE, the usual ladder-like pattern of bands differing in chain length by 10 nucleotides was observed (figures 2 and 3). An intense band at 80 nucleotides reflects the periodicity of the DNA superhelix in the nucleosome. The 10-bp-interval cleavage pattern with DNase I was obtained for both sham-operated and hypertrophic heart nuclei but the production of smaller fragments was more in hypertrophic heart nuclei. This reflects a greater accessibility of the DNA in the

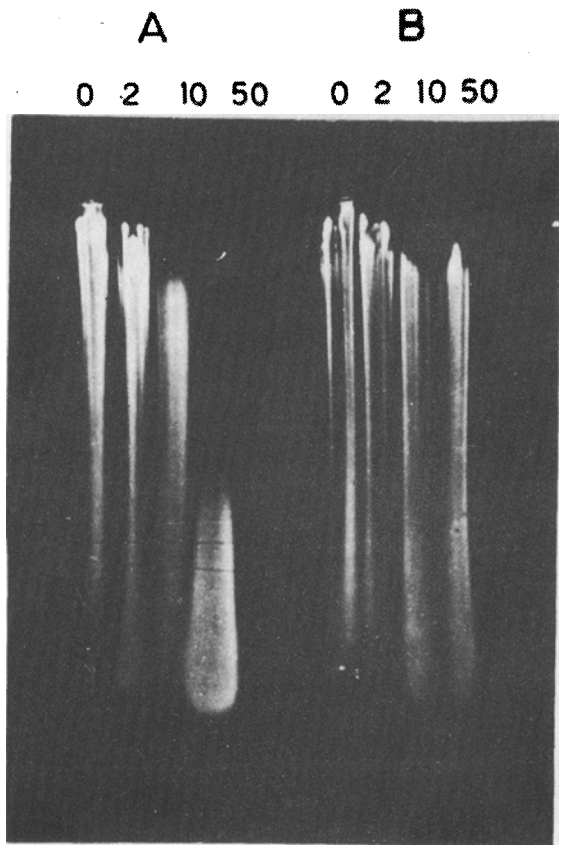


Figure 1. Agarose gel electrophoresis of the DNA released after DNase I digestion. Nuclei from (A) hypertrophic heart and (B) sham-operated heart were incubated with increasing concentrations (U/mg DNA) of DNase I and DNA was extracted after 5 min.

chromatin of hypertrophic heart nuclei to DNase I. Fragments smaller than 50 nucleotides are not seen in the photograph (figure 2) probably because of the low efficiency of precipitation of the fragments by ethanol (Sollner-Webb and Felsenfeld, 1977).

The specific cutting of DNA in chromatin is an intrinsic property of the DNA double helix and the frequency of cutting is dependent on the exposure of the potential sites to nuclease attack (Lutter, 1978). It has been shown that the rate and extent of digestion by DNase I are greater in chromatin obtained from young (18–20 weeks) rat brain than in that obtained from adult (90–97 weeks) brain (Chaturvedi and Kanungo, 1985). DNase I sensitive sites have been found to be stabilized by high mobility group proteins 14 and 17 (Nicolas *et al.,* 1983). In an earlier report we had shown that 0·35 M NaCl extractable proteins contribute to the enhanced DNase I sensitivity of hypertrophic heart nuclei (Kamala *et al.,* 1986).

Activation of myosin heavy chain genes

Results of dot blot hybridization experiments using the myosin heavy chain cDNA

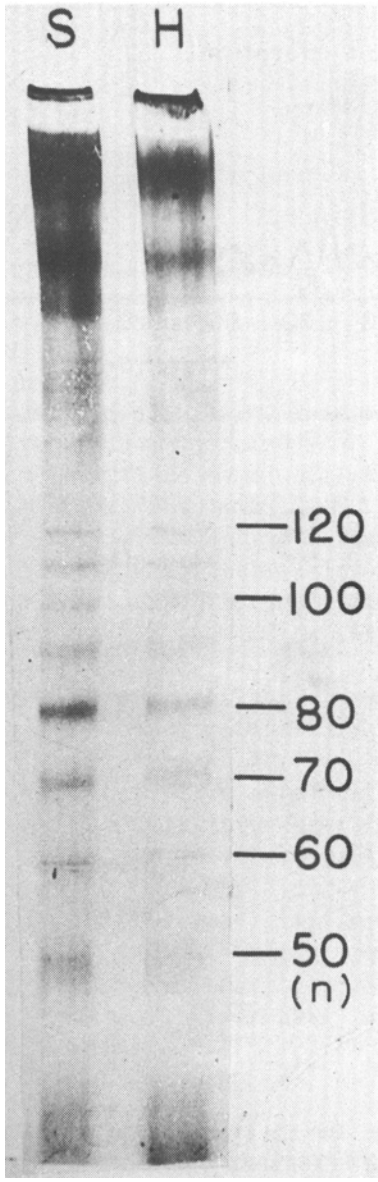


Figure 2. DNA fragments obtained after DNase I treatment of nuclei of sham-operated (S) and hypertrophic (H) hearts. Numbers are fragment sizes (n, nucleotides).

probe (pcMHC 5) clearly indicate that the extent of hybridization of the probe with DNA from DNase I treated hypertrophic heart nuclei was very much reduced compared to that with total undigested DNA from hypertrophic heart nuclei (figure 4, C and D). In contrast, hybridization with DNA from sham-operated heart nuclei was virtually unaffected by DNase I treatment (figure 4, A and B). When the same samples were analysed with the cytochrome P450e cDNA probe (pPC-P450–91), the hybridization pattern was only slightly affected by DNase I treatment in both sham-operated and hypertrophic heart nuclei (figure 5). Cytochrome P450e is induced by

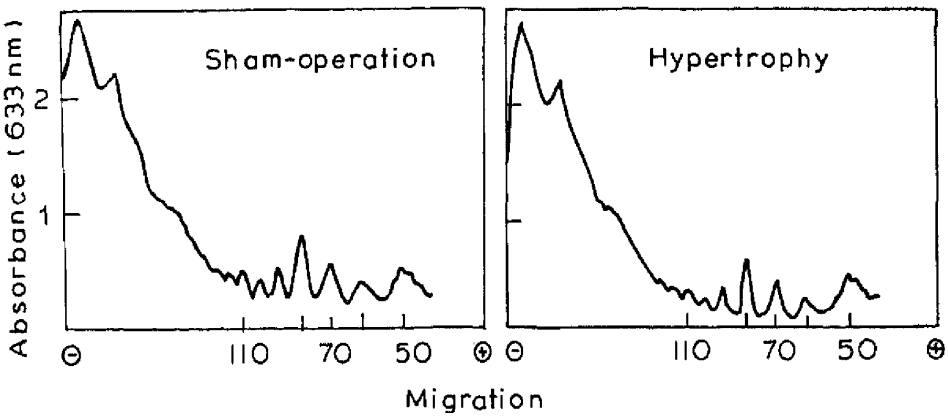
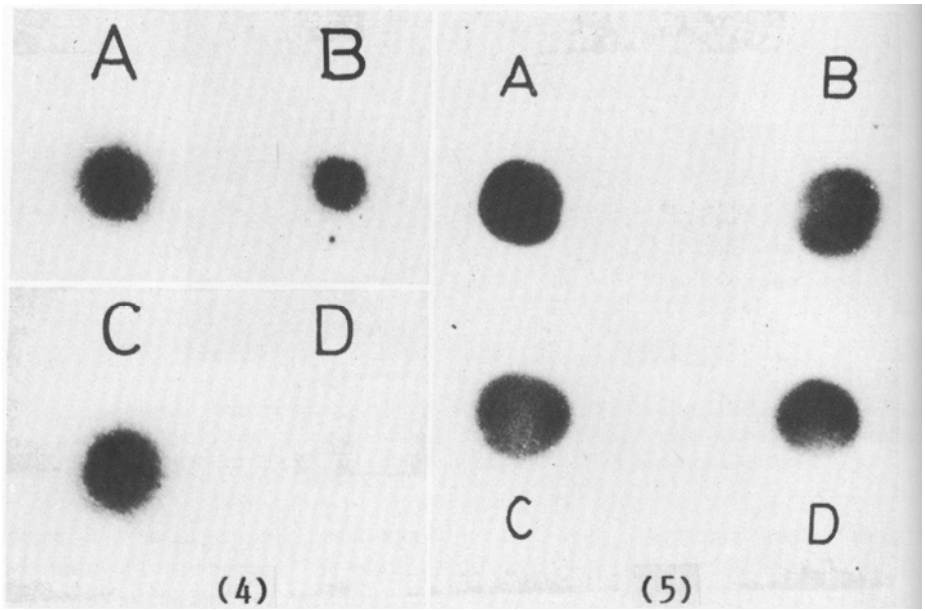


Figure 3. Densitometric scans of DNA fragments produced by DNase I.



Figures 4 and 5. Dot blot hybridization of DNA from untreated and DNase I treated sham-operated and hypertrophic heart nuclei with nick-translated (4) myosin heavy chain cDNA probe (pcMHC5) and (5) cytochrome P450e cDNA probe (pPC-P450–1). (A), Sham-operated heart nuclei; (B), sham-operated heart nuclei+DNase I (10% acid solubility); (C), hypertrophic heart nuclei; (D), hypertrophic heart nuclei+DNase I (10% acid solubility).

phenobarbitone in rat liver but the gene is not active in heart. The results therefore indicate the specific activation of myosin heavy chain genes in hypertrophic heart nuclei.

Increased nuclease sensitivity appears to be an important criterion for confirming the transcriptionally open conformation of chromatin. We also checked whether the increased DNase I sensitivity of myosin heavy chain genes in hypertrophic heart

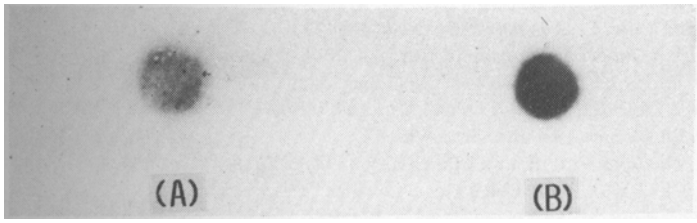


Figure 6. Dot blot hybridization of nuclear RNA from sham-operated and hypertrophic heart nuclei with nick-translated myosin heavy chain cDNA probe (pcMHC 5). (A), RNA from sham-operated heart nuclei; (B), RNA from hypertrophic heart nuclei.

nuclei can be correlated with increase in myosin heavy chain transcripts in the nuclei. Figure 6 shows the dot blot hybridization of nuclear RNA extracted from sham-operated and hypertrophic heart nuclei with the myosin heavy chain cDNA probe. The hybridization signal observed in the RNA sample from hypertrophic heart nuclei was stronger than that in the RNA from sham-operated heart nuclei. This result and the results shown in figure 4 establish a correlation between DNase I sensitivity of myosin heavy chain genes in hypertrophic heart nuclei and their transcriptionally active state. There are, however, instances where DNase I sensitivity is not correlated with active transcription of genes (Stalder *et al.,* 1980). In the case of vitellogenin genes also, DNase I sensitivity parallels transcriptional activation (Folger *et al.,* 1983). DNase I sensitivity is not restricted to the coding regions of the genes but extends upstream and downstream (Strobe *et al.,* 1981). Koropatnick and Duerksen (1987) showed that protein-encoding DNA sequences that are available for transcription are more sensitive to DNase I than those which are unavailable for transcription and that the increase in nuclease sensitivity is detected in actively transcribed metallothionenin-I and α-fetoprotein genes in embryonic liver. Our results show that the activation of myosin heavy chain genes detected by DNase I sensitivity can be correlated with increase in myosin heavy chain transcripts. It appears that the change in chromatin structure of specific genes during cardiac hypertrophy is associated with the transcriptional state.

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