Expression of the c-*myc* and Ca-ATPase Genes in Cardiac Muscle During Adaptation to Repeated Stress

F. Z. Meerson, V. V. Didenko, Yu. V. Arkhipenko, and V. A. Saltykova

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In the course of adaptation to repeated stress, the expression of the proto-oncogene c-*myc* found to increase much more rapidly than that of the Ca-ATPase gene. It is suggested that an increase in the level of c-*myc* expression may activate the structural Ca-ATPase gene and possibly also the heat-shock proteins.

Key Words: *gene expression; c-myc; Ca-ATPase*

Adaptation of the heart to large loads, which is accompanied by adrenergic effects, consistently induces expression of the proto-oncogene c-*myc* in cardiac cells [8,16,17]. This proto-oncogene is virtually not expressed during periods of physiological rest, but the level of its expression rises rapidly 7- to 10-fold in the first few hours of exposure to an external stimulus [8]. Like other proto-oncogenes, c-*myc* is a trigger gene, i.e., it may be instrumental in increasing the transcription of structural genes in growing and dividing cells [7,10,16]. This has laid the basis for designating proto-oncogenes as adaptation genes, for they play a key structural role in enhancing the function of the heart and other organs when increased demands are made on the organism [5]. In the context of the study reported here it is important that in all such situations an increase in the function of organs and of the organism as a whole is always preceded and accompanied by a stress reaction, and that during an increase in cardiac function the expression of structural genes is very selective. It has been found, for example, that a periodic increase in cardiac function in response to moderate physical exercise results in only a slight increase in heart weight while leading to a selective growth of sarcoplasmic reticulum (SPR) tubules so that the surface area of these, which are the main site of Ca-ATPase, more than doubles [14]. Accordingly, the rate of myocardial relaxation rises [3] and less Ca^{2+} enters the mitochondria, with the result that the heart works more efficiently and oxygen consumption by the myocardium increases [13]. Since the multiple effects exerted by adrenergic hormones and corticosteroids on the heart in the course of adaptation to repeated stress have also been found not to result in an increased mass of that organ, we presumed that activation of c-*myc* may occur early during such adaptation, followed later by augmented expression of the structural gene encoding Ca-ATPase. To examine this possibility we compared, in this study, the expression of c-*myc* and that of the Ca-ATPase gene in C57Bl mice in the course of their adaptation to repeated moderately painful electrostimulations.

MATERIALS AND METHODS

Male C57Bl mice weighing 18-20 g were used, 7 mice in each series of tests. Adaptation to repeated pain stress was carried out in a cage with an elec-

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

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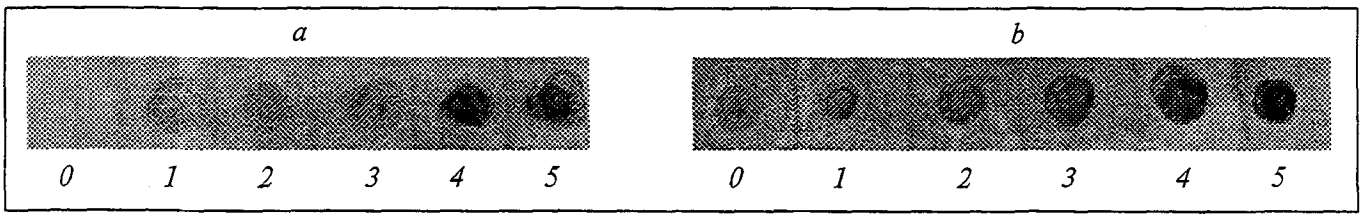

Fig. 1. Blots of c-*myc* (*a*) and Ca-ATPase (*b*) gene RNA in the control (0) and after 1, 2, 3, 5, and 9 days of adaptation.

trified metal floor. Each mouse was subjected to a total of 10 such stressors, once per day at 24-h intervals. The first exposure lasted 5 min (current strength, 0.05 mA), the second 10 min (0.1 mA), and the third and all others 20 min each (0.15 mA). The animals first responded to the pain stimulation by running and squeaking. With subsequent exposures, however, these responses disappeared (only running about the cage was sometimes observed). Exsanguinated hearts from the adapted mice weighed only 5% more than those from controls (an insignificant difference).

In the study, human c-*myc* (1.6 kb fragment) and SPR Ca-ATPase from rat heart (4.1 kb fragment, kindly provided by Dr. Dallman) were used. Human c-*myc* is highly complementary to its rodent counterpart and may be used to synthesize samples for hybridization assays [6]. Highly labeled biotinylated samples were obtained using a USB kit (USA) in accordance with the manufacturer's recommendations.

Total RNA was isolated from mouse hearts using guanidine isothiocyanate and was fractionated by gradient centrifugation in CsCl [9] in a Beckman centrifuge (SW 40Ti, 30,000 rpm, for 24 h at 20°C); it was then applied to prehumidified 20×SSC filters (Zetabind, USA) in an amount of 10 or 3.3 μg per filter, and fixed with ultraviolet at 302 nm.

Hybridization was carried out in 50% formamide by a standard procedure [12] at 42°C over 24 h. The filters were washed in 2×SSC, 0.1% SDS; 0.5×SSC, 0.1% SDS; and 0.1×SSC, 0.1% SDS at room temperature for 15 min in each; the last washing was done in 0.1×SSC, 0.1% SDS at 60°C for 30 min. Sample detection was carried out using a standard kit (Sigma, USA) according to the manufacturer's recommendations.

The filters were subjected to densitometry on a scanning attachment to a Hitachi-557 spectrophotometer in a two-wave mode: at λ_1=596 nm (background) and λ_2=566 nm (spots).

RESULTS

Expression of c-*myc* in the myocardium, virtually nonexistent in control mice, was found to be significant in the test mice after the first day of the adaptation tests (day 1) and continued to increase up to day 9 (Fig. 1, *a*). Expression of the Ca-ATPase gene also increased, but to a much lesser extent than that of c-*myc* (Fig. 1, *b*). After densitometry of the spots obtained, the time course of gene expression was represented in the form of curves (Fig. 2). The level of gene expression (absorbance of the spots on the filters) on day 1 was taken as unity.

Thus, while increases in the expression of both genes were recorded, c-*myc* began to be expressed more actively and the level of its expression on day 9 was 6.5 times higher than on day 1, as compared with only 1.5-1.8 times for the Ca-ATPase gene. These results should be evaluated on the basis of the following considerations.

In a normal myocardium, the number of connective tissue cell nuclei is 3 times higher than that of cardiomyocytes [2]. In the connective tissue cell population under consideration, 84% of the cells are fibroblasts and 16% are histiocytes and endothelial cells. Most of the genetic material in the myocardium is thus contained in fibroblasts rather than in cardiomyocytes. Moreover, fibroblasts are also the main sites of DNA synthesis [2]. For example, it has been found that at a critical stage of cardiac hyperfunction - on day 4 after coarctation of the aorta - ^{3}H-thymidine incorporation into cell nuclei of cardiac connective tissue increases 10-12-fold, whereas its incorporation into cardiomyocytes remains almost unchanged [2].

It is significant that c-*myc* expression precedes cell growth and division. In our study, cardiomyocytes did not grow substantially, and in adult mice these cells divided very rarely. Consideration of these findings in relation to the reported burst of c-*myc* expression occurring in fibroblasts in response to stress mediators such as catecholamines [11] suggests that the increased expression of c-*myc* observed in stress is most likely the result of its enhanced expression in nonmuscle cells of the heart. The situation is different with the gene of the SPR Ca pump, whose main structural unit is Ca-ATPase. SPR, the major function of which is Ca^{2+} transport, is not found in nonmuscle cells, and for this reason an alteration in the level of

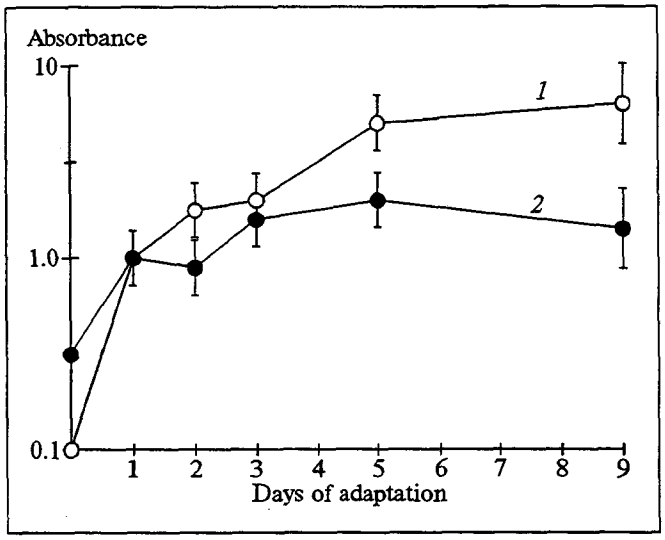

Fig. 2. Variations in levels of c-*myc* (*1*) and Ca-ATPase (*2*) RNA in the myocardium of mice in the course of adaptation (RNA level on day 1 of adaptation was taken as unity).

expression of this gene reflects a process occurring in cardiomyocytes.

That the increase in c-*myc* expression occurs mainly, if not exclusively, in nonmuscle cells and is much larger than the increase in Ca-ATPase gene activity can probably be explained on the basis of the following analogies. When the functional activity of brain neurons rises, the surrounding neuroglial cells transmit to these neurons ready-made respiratory enzymes and nucleotides required for RNA synthesis, thereby acting as donors of plastic resources [2]. As regards the heart, it has been shown that during hyperfunction of this organ, DNA synthesis in fibroblasts (as assessed by ^{3}H-thymidine incorporation) proceeds at the highest rates in areas where the cardiomyocytes are particularly hypertrophic [2]. It has been suggested that accessory cells can perform a donor function not only in the brain but also in the heart [14]. If so, then intensified functioning of the genetic apparatus in cardiac nonmuscle cells will be necessary in order that the latter cells may fully perform their donor functions for cardiomyocytes. It is also important to note that comparison of c-*myc* and Ca-ATPase gene activities in the heart can indicate how the state of its muscle and nonmuscle cells has changed, thereby providing a more discriminative approach to evaluating the effects exerted by particular factors on the myocardium.

Since the expression of c-*myc*, as assessed from the elevation in the concentration of its mRNA, was found to be much higher than Ca-ATPase gene expression assessed in the same way, the following two points, of key importance in this study, should be emphasized.

1. Activation of the proto-oncogene c-*myc* may be thought to play the role of a trigger for Ca-ATPase gene expression. This phenomenon is biologically important because a selective increase in the power of the Ca pump in myocardial cells is essential not only for adapting the heart to major loads but also for increasing the resistance of this organ to ischemia, reperfusion, high Ca concentrations, and other injurious factors that act by raising Ca levels in the sarcoplasm.

2. Previously, with special reference to the heart, we provided evidence substantiating the existence of a phenomenon of adaptive stabilization of structures (PASS) [4,15]. It has been found that PASS is responsible for a direct increase in the resistance to autolysis of myocyte organelles such as SPR, mitochondria, and nuclei [15], and that, moreover, it is coincident with the accumulation of HSP70-family proteins; thus, HSP70 accumulation and PASS both reach their maximum only by days 6-8 of adaptation [1]. The results of the present study merit testing the hypothesis that the early activation of the c-*myc* proto-oncogene demonstrated in this study triggers, during adaptation to stress, HSP70 accumulation and the development of PASS.

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