Expression of Collagen Binding Integrins During Cardiac Development and Hypertrophy

Louis Terracio, Kristofer Rubin, Donald Gullberg, Ed Balog, Wayne Carver, Ron Jyring, and Thomas K. Borg

The interaction between components of the extracellular matrix and the cell surface of cardiac myocytes appears to be regulated in part by receptors belonging to the integrin superfamily. The expression of the integrins was investigated at different stages of development of the heart as well as during cardiac hypertrophy. The characterization of the membrane proteins showed that a β1-integrin and associated α-chains were responsible for the interaction with collagen, laminin, and fibronectin. Immunoprecipitation data indicated that the presence of specific α-chains varied with development. These data were correlated with the ability of the isolated myocytes to attach to specific components of the extracellular matrix. The expression of the α1-chain was prominently associated with the recognition of interstitial collagens. The presence of the α1-chain was also associated with stages when collagen synthesis was increased, especially during fetal and neonatal growth and cardiac hypertrophy. Immunohistochemical localization with the antiserum against β1-integrin demonstrated its specific localization near the Z lines of cardiac myocytes. The localization both in vitro and in vivo indicated that the β1-integrin may play a role in myofibrillogenesis during development. The present immunohistochemical, cell adhesion, and biochemical data clearly indicate that integrins play a major role in the regulation of the interaction between cardiac myocytes and the extracellular matrix during development and disease. (Circulation Research 1991;68:734–744)

Numerous investigations have shown that the components of the extracellular matrix (ECM) play an important role in the development of the heart, in the maintenance of normal function, and in disease.1–6 The specific arrangement of the individual components of the ECM, especially the interstitial collagens, plays a significant role in influencing both the form and function of the heart.7–9 The collagens are arranged in a three-dimensional network interconnecting individual myocytes, myocytes and capillaries, and groups of muscle fibers.7–9 At the level of the individual myocytes, these collagens are attached to specific sites on the sarcolemma just lateral to the Z disk.6,10,11 The attachment of the collagen to the sarcolemma appears to be mediated by specific proteins.10–12 Antibodies against proteins that are purported to mediate adhesion of rat myocytes to collagen stain myocytes in a pattern essentially identical to that obtained with anti-vinculin antibodies.11,12 These findings strongly suggest that collagen receptors and vinculin colocalize at the plasma membrane of myocytes near the Z line.11,13

The interaction of ECM components with the surface of cells appears to be regulated in part by specific receptors belonging to the superfamily termed integrins.14–16 This family is composed of dimeric components consisting of a β-subunit and a noncovalently linked α-subunit. The α-subunits (α1–α8), when associated with the β1-subfamily, are also referred to as very late antigens (VLA1–VLA6).17,18 There appear to be at least four distinct groups of β-subunits (β1–β4).17–19 Within each β-subfamily, the β-subunit is associated with a variable set of α-subunits. There is sequence homology between the individual β-subunits (40–50%) and between the α-subunits that have been sequenced (20–60%); however, there appears to be no sequence homology between the α- and β-subunits.14–16 The αβ heterodimers of the β1-subfamily are associated with cells and tissues and are thought to be responsible for cell interactions with the ECM during growth and development. The β2-, β3-, and β4-subfamilies are principally associated with leuko-
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The purification of cardiac membrane proteins was similar to that previously described for hepatocytes. The micromolar fraction (100,000g) was solubilized at 4°C with a buffer containing 1% Triton X-100, 0.05 M Tris, 2 mM phenylmethylsulfonyl fluoride (PMSF), and 1% aprotinin, pH 7.4, and affinity-purified on a lentil lectin-Sepharose column as previously described. The eluate was applied to a differential attachment protocol to separate fibroblasts from myocytes. The myocytes were used immediately for surface labeling or allowed to attach to 100-mm culture plates coated with laminin (10 μg/ml) before labeling. There was no difference observed between attached and unattached cells.

Cardiac hypertrophy was induced in rats by abdominal aortic coarctation as previously described. Briefly, the rats were anesthetized by intraperitoneal injection with acepromazine (0.075 mg/100 g body wt) and sodium pentobarbital (2.8 mg/100 g), and the aorta was banded between the right and left renal arteries. Cardiac hypertrophy was determined by heart weight/body weight ratios, and the myocytes were isolated only from hearts that were clearly hypertrophied. The cells were isolated as described above. The isolated cells were processed for cell surface labeling as described below.

**Antibodies**

Purified proteins obtained by elution of an immunoaffinity column made with the previously characterized antisera, denoted COLL-CAM II, were used in the initial purification of the collagen-specific integrin from rat heart. These proteins were used to immunize New Zealand White rabbits by previously described procedures. Immunoglobulin G (IgG) was obtained with protein A Sepharose affinity chromatography. A previously described anti-β2 antibody with affinity for collagen from hepatocytes was used to monitor the purification of the β2-subunit from rat cardiac myocytes. Antibodies against the α and β-subunits of the integrin were obtained from Dr. Richard Hynes at the Massachusetts Institute of Technology, Cambridge, and the monoclonal antibody that specifically recognizes the rat α5-subunit (3A3) was obtained from Dr. David Turner, SUNY Health Science Center at Syracuse.

**Affinity Chromatography**

Affinity resins were prepared using cyanogen bro-mide–activated Sepharose 4B (Pharmacia, Uppsala, Sweden). For IgG, fibronectin, and laminin, the purified components were coupled according to the manufacturer’s instructions. Collagen type I affinity resins were prepared by dialyzing type I collagen (Vitrogen, Collagen Corp., Palo Alto, Calif.) against 0.26 M NaCl and 20 mM NaHPO4, pH 8.0 at 4°C, and allowed to react with cyanogen bromide–activated Sepharose 4B.

**Membrane Protein Purification**

The purification of cardiac membrane proteins was similar to that previously described for hepatocytes. The micromolar fraction (100,000g) was solubilized at 4°C with a buffer containing 1% Triton X-100, 0.05 M Tris, 2 mM phenylmethylsulfonyl fluoride (PMSF), and 1% aprotinin, pH 7.4, and affinity-purified on a lentil lectin-Sepharose column as previously described. The eluate was applied to a differential attachment protocol to separate fibroblasts from myocytes. The myocytes were used immediately for surface labeling or allowed to attach to 100-mm culture plates coated with laminin (10 μg/ml) before labeling. There was no difference observed between attached and unattached cells.

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an anti–COLL-CAM II–Sepharose column and washed with 10-bed volumes of a buffer containing 1.0% Triton X-100, 0.10 M Tris, 0.5 M NaCl, and 2 mM PMSF at pH 7.4, followed by 5-bed volumes of the same buffer except with 0.1% Triton X-100. The bound material was then eluted with 0.1 M glycine, pH 3.0, as previously described. The eluted material was immediately neutralized with 1.0 M Tris-HCl, pH 8.0, dialyzed against water, lyophilized, and extracted with 100% ethanol at -20°C. This material was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie blue staining. Protein bands were cut from the gel and electroeluted. This material was used for polyclonal antibody production as described above.

**SDS-PAGE, Electroelution, and Western Blotting**

Electrophoresis was performed on SDS-PAGE gradient gels (5–10%) according to standard procedures. The electroelution of proteins was accomplished by previously published methods. For autoradiograms, the molecular weights were estimated by comparing the blotted proteins relative to the position of 125I-labeled high molecular weight standards (Sigma Chemical Co., St. Louis). Immunoblots of proteins transferred to nitrocellulose were detected using 125I–protein A as previously described.

**Cell-Attachment Assay**

Quantitative analysis of the attachment of cardiac myocytes to specific ECM substrates has been documented in previous publications. The antibodies raised against specific cell surface proteins were used to inhibit the attachment of the myocytes to specific ECM components as described.

**Iodination of Myocyte Cell Surface and Immunoprecipitation**

Either freshly isolated or attached cardiac myocytes were surface-labeled with 125I by previously published procedures. Labeled cells were extracted for 30 minutes with a solubilization buffer containing 1% Triton X-100, 1 mM MgCl2, 1 mM CaCl2, and 10 mM Tris, pH 8.0, and a protease inhibitor cocktail (1 mM PMSF, 1% aprotinin, 1 µg/ml pepstatin A, and 2 mM leupeptin). The cells were centrifuged at 15,000 g for 30 minutes, and the supernatant was used for immunoprecipitation. Preimmune IgGs (100 µg/ml) were added to the supernatant and incubated for 4 hours in an end-over-end fashion followed by the addition of 100 µl of protein A–Sepharose 4B (50% slurry in PBS) for 1 hour. This mixture was then centrifuged at 10,000 g for 10 minutes, and the supernatant was incubated with immune IgG (100 µg/ml for polyclonals; 10 µg/ml for monoclonals) for 8 hours and treated as above. The protein A–Sepharose with bound proteins was washed four times with buffer containing 1% Triton X-100, 0.5 M NaCl, 1 mM CaCl2, and 10 mM Tris, pH 7.4, two times with 0.5% Triton X-100, 0.1% deoxycholate, 0.1% SDS, 1 mM MgCl2, 1 mM CaCl2, and 0.1 M NaCl, pH 8.0, and one time in PBS.

SDS-PAGE sample buffer was added to the Sepharose beads with bound proteins; the sample was boiled for 5 minutes and subjected to SDS-PAGE and autoradiography as previously described.

**Microscopic Analysis**

Immunolocalization of the antibodies produced against the heart β1-integrin was visualized by standard immunofluorescence and laser scanning confocal microscopy as previously described. Stereo imaging of the staining pattern was performed using the software developed by the manufacturer (Bio-Rad Laboratories, Cambridge, Mass.). Electron microscopic localization was performed on Lowicryl-embedded (Electron Microscopy Sciences, Fort Washington, Pa.) rat papillary muscle as previously described.

**Results**

**Characterization of Antisera**

The initial purification procedure for integrins from cardiac muscle involved antibody-affinity chromatography with a polyclonal antiserum designated as COLL-CAM II. Immunoblots of lectin-purified heart membranes using this antiserum showed the presence of a distinct band with an Mr of 120,000 and 120,000 (nonreduced) (Figure 1). This antiserum also blocked the attachment of neonatal myocytes to collagen (data not shown). When this antiserum was used to affinity-purify glycoproteins from cardiac membranes, a restricted number of bands were apparent on Coomassie blue–stained SDS-PAGE. These proteins were electroeluted, and the individual fractions were used to raise antisera that were subsequently assayed for their ability to inhibit the attachment of neonatal myocytes to ECM components, especially collagen. Antibodies against the proteins with an Mr of 120,000 (nonreduced) could block the attachment of neonatal myocytes to collagen type I and laminin but not fibronectin or collagen type IV (Figure 2). Immunoblots of lentil lectin–purified cardiac membranes demonstrated that the antiserum recognized a single band with an Mr of 120,000 (Figure 3, lane B). Material eluted from the heart antibody affinity column and immunoblotted with the same antiserum demonstrated two bands similar to the one observed with anti–COLL-CAM II (compare Figure 3, lane C, with Figure 1).

To determine if the antiserum raised against the Mr 120,000 protein from rat heart was related to a known β1-integrin, antibodies against the β1-integrin from hepatocytes were compared with the antibodies made from cardiac membranes. When the proteins eluted from the hepatocyte β1 affinity column were immunoblotted with the antibodies made against heart and hepatocyte integrins, the same or very similar proteins were recognized (Figure 3, lanes D and E). The heart and hepatocyte antibodies were also compared in their ability to immunoprecipitate proteins from neonatal heart cells. The pattern of
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**Figure 1.** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (nonreduced) of rat heart membrane proteins eluted from a lentil lectin affinity column, silver-stained (lane A) and immunoblotted with the COLL-CAM II antisera (lane B). Numbers represent kilodaltons.

proteins immunoprecipitated with each antibody clearly demonstrates that they recognize essentially identical components (Figure 4). These antibodies were similar in their ability to inhibit attachment of neonatal heart cells to collagen and laminin but not fibronectin and collagen type IV (data not shown). From these data, we conclude that the heart antibody recognized a β1-integrin similar to that previously reported from hepatocytes.35

**Integrin Expression on Developing Cardiac Myocytes**

Both the cardiac and hepatocyte β1-integrin antibodies were used for immunoprecipitation in this study and yielded identical patterns in all cases. The immunoprecipitation data from neonatal myocytes (Figure 4) demonstrated the presence of several high molecular weight protein bands in addition to the
Figure 4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis showing 125I–surface-labeled neonatal cardiac myocytes immunoprecipitated with the cardiac antiserum (lanes A and B) and hepatocyte β1-integrin (lanes C and D). Lanes A and C are nonreduced, and lanes B and D are reduced. The two antisera precipitate essentially identical sets of proteins, indicating that the cardiac antiserum is directed against a β1-integrin. Numbers represent kilodaltons.

band at Mr 120,000. To determine which of these protein bands were known α-chains, serial immunoprecipitations using monospecific antisera against the α5-chain,7 38 α1-chain,37 and αc-chain37 were performed to identify these components (Figure 5). These data indicated that the three α-chains are associated with neonatal heart cells. Furthermore, the immunoprecipitation pattern obtained with a mixture of the three α-chain–specific antibodies was similar to that obtained with the anti-β1-integrin IgG (Figure 5, compare lanes A and E), indicating that several of the high molecular weight bands immunoprecipitated with the anti-β1-integrin antibodies are integrin α-chains.

The expression of the different α-chains at various stages of development was determined by immunoprecipitation with the β1-antisera on 125I-labeled cell surface glycoproteins. Myocytes were isolated from hearts at various stages of development as well as from hypertrophied hearts. These data document that the α-chains appeared to vary with the stage of development and hypertrophy (Figure 6). Fetal cells showed the presence of all three known α-chains as well as other unknown associated proteins (Figure 6, lane A). Neonatal cells possessed a reduced number of bands; however, the three known α-chains were present (Figure 6, lane B). Immunoprecipitation of adult heart membrane proteins revealed the absence of α1- and αc-chains on freshly isolated normal cells (Figure 6, lane C); however, both of these were present on hypertrophied myocytes (Figure 6, lane D). The presence of the α-chains was correlated with the ability of the myocytes to attach to immobilized components of the ECM (Figure 7). Neonatal and fetal myocytes attached with high affinity to fibronectin, laminin, and collagens (Figure 7) and possessed the α-chains associated with these components (Figure 6). Freshly isolated normal adult cells do not attach well to collagen type I and fibronectin (Figure 7) and do not express the α5- and αc-chains (Figure 6, lane C). However, myocytes isolated from hypertrophied hearts showed increased attachment to collagen and fibronectin as well as laminin (Figure 8) and expressed the α1- and αc-chains (Figure 6, lane D).

The specificity of the α-chains associated with the different ECM components was shown by the immunoprecipitation of 125I-labeled myocyte proteins eluted from specific ECM affinity columns (Figure 9). These data showed that on fetal cardiac myocytes (Figure 9, lanes A, B, and C) and neonatal cardiac
myocytes (data not shown) the $\alpha_1$-chain was present in the eluate from collagen (Figure 9, lane A) and laminin (Figure 9, lane B) but not fibronectin (Figure 9, lane C) affinity columns. In normal adults (Figure 9, lanes D, E, and F), where cells have little or no affinity for collagen, there was no $\alpha_1$-chain detectable from the eluate from collagen I-Sepharose (Figure 9, lane D). The $\alpha_1$-chain was present in the eluate from all three ECM affinity columns, whereas

![Image of gel electrophoresis](image)

**Figure 6.** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (nonreduced) showing the expression of different $\alpha$-chains at various stages of development and hypertrophy determined by immunoprecipitation with the cardiac $\beta_1$-integrin on $^{125}$I-labeled myocytes. Lane A: Fetal myocytes. Lane B: Neonatal myocytes. Lane C: Adult myocytes. Lane D: Adult myocytes isolated from hypertrophied hearts.

the $\alpha_5$-chain was present only from the fibronectin affinity column eluate (Figure 9, lane F).

**Inhibition of Cell Attachment**

The inhibition of attachment of isolated fetal and neonatal myocytes to collagen but not fibronectin was demonstrated using antisera against the $\alpha_1$-chain (Figure 10). Attachment of the myocytes to collagen was only inhibited to 50% of control, presumably due to the cells using other $\alpha$-chains to attach to collagen type I.

**Immunolocalization**

The $\beta_1$-integrin from the heart was localized by immunofluorescence and confocal microscopy to the surface of the myocytes (Figures 11–13). Images of freshly isolated fetal and neonatal myocytes demonstrated that the $\beta_1$-integrin was localized in a random pattern on the surface of the cells (Figure 11). As the cells attached to the substrate and spread in culture, the $\beta_1$-integrin immunoreactivity clustered at the sites of attachment (Figure 11d). Once spread and adapted to culture, the $\beta_1$-integrin was localized in the focal adhesions at the periphery of the cells and in a sarcomeric pattern on the cell surface (Figure 12). Stereo imaging demonstrated that the $\beta_1$-integrin localization was restricted to the cell substrate interface (Figure 12c). Freshly isolated adult cardiac myocytes exhibited $\beta_1$-integrin that was localized at the cell surface and distributed in a precise, repeating pattern that appeared to be near or at the Z bands (Figure 13). High resolution immunoelectron microscopy of rat papillary muscle (data not shown) demonstrated that the $\beta_1$-integrin was localized at the Z line. Although not every Z line exhibited...
clusters of immunogold staining, the only site to possess multiple gold particles was the Z line.

Discussion

The intimate association of the ECM with cardiac myocytes at various times of development appears to be regulated in part by specific receptors on the cell surface. The data presented here indicate that the interaction with interstitial collagens, fibronectin, and laminin involves a β2-integrin and associated α-chains. The cells of the liver and heart are both known to form stable contacts with interstitial collagen. Data from these investigations as well as hepatocyte and myocyte adhesion data support the concept that they may have a common β2-integrin. The immunoprecipitation profiles from surface-labeled myocytes using the β2-antiserum from heart and liver were virtually identical (Figure 4). Proteins affinity-purified on β1 affinity columns from heart and liver resulted in the elution of proteins of similar Mr, under reducing and nonreducing conditions on SDS-PAGE. The purified IgGs from the antisera against the cardiac β1-integrin inhibited the attachment of neonatal cardiac myocytes to collagen and partially to laminin in a manner similar to that of the β1-antiserum from liver. Thus, immunological, biochemical, and cell attachment data demonstrate that the β1-integrin from cardiac muscle is very similar, if not identical, to the β1-integrin from hepatocytes.

The association of α-chains with the β1-chain and the specific binding of some α/β integrin heterodimers to ECM components were shown by immunoprecipitation and affinity chromatography on immobilized ECM components followed by immunoprecipitation. Previous investigations have demonstrated that the αc-chain was associated with cell attachment to collagen and laminin. Whereas other α-chains, such as the α5- and α6-chains, have also been demonstrated to regulate binding to collagen, the αc-chain did not appear to be present on the surface of cardiac myocytes at various stages of development. However, specific anti-αc antisera that reacts with rat cells was not available to perform the immunoprecipitation studies necessary to preclude its presence on myocytes. Previous studies have proposed that the αc-chain is present on cardiac fibroblasts; however, there were few fibroblasts in the isolated myocyte cell preparations. The presence of the multiple α-chains for attachment to the same component (i.e., α1 and α5 for collagen) may merely represent duplicity or may indicate a specialization in the function for the separate α-chains. The attachment of fibroblasts to collagen in vivo is different from that of the myocytes. Since fibroblasts are migratory cells, they make transient attachments tangential to the collagen fibril, whereas collagen attaches to myocytes principally in a perpendicular fashion at specific sites near the Z line. Thus, it is possible that the presence of the αc- and α5-chains on cardiac myocytes

Figure 10. Graph showing antibody inhibition of cell attachment to immobilized extracellular matrix components (fibronec- tin [FN], laminin [LN], and collagen types I and III [CI&III]) using the anti-α5 antiserum. This antiserum inhibited the attachment of neonatal myocytes to interstitial collagen and laminin by 50%.
myocytes and the α1- and α2-chains on fibroblasts may reflect a difference in how cells interact with collagen in vivo.

The expression of the α1-chain correlated with periods of increased collagen synthesis and deposition. Initially, during early heart development, expression of the interstitial collagens I and III is apparent but remains stable throughout the later stages of fetal growth. During neonatal development, especially the first 4–5 days post partum, collagen synthesis is elevated, presumably due to the increased pressure and volume changes associated with birth and the development of the left ventricle. These changes are associated with the formation of the elastic, stress-tolerant network of three-dimensional connective tissue. During cardiac hypertrophy, there is also an associated increase in collagen. The elevation of the α1-chain on the surface of myocytes isolated from hypertrophied hearts as well as from neonatal animals correlates the expression of the α1-chain with collagen synthesis and implicates it in collagen attachment to the myocytes.
Since the $\alpha_\text{I}$-chain also has affinity for laminin, its expression also appears to correlate with the expression of laminin on myocytes that are undergoing hypertrophy. Myocytes from adult hearts show an increase in the laminin associated with the basement membrane both in vivo and in vitro.\textsuperscript{4,52}

The role of the $\alpha_\text{I}$-$\alpha_\text{II}$-chains are more difficult to explain in relation to development and hypertrophy, although their presence correlates very well with the ability of myocytes to attach to ECM components, especially fibronectin. Immunofluorescent staining data indicate that fibronectin is associated with times when collagen synthesis is also high.\textsuperscript{53} However, these data may be more coincidental than functional, since the attachment of collagen to the cell surface of myocytes has been shown to be independent of fibronectin.\textsuperscript{12,45} More investigations are necessary to resolve these complex interactions; however, it is clear that the $\alpha$-chains may interact with one or more ECM components.\textsuperscript{14,22,37,38,54} These investigations have demonstrated that adhesion and interaction are dependent on cell type and origin of the cell. Clearly, freshly isolated cardiac myocytes are different from established cell lines from tumors.

The reason for the presence of the $\alpha_\text{II}$-chain in extracts of adult myocyte membranes after affinity chromatography but its absence from freshly isolated whole membranes is not clear. It is possibly due to concentration of the $\alpha_\text{II}$-chain during affinity chromatography coupled with an increased efficiency of the $\alpha_\text{II}$-chain binding to fibronectin in the presence of Mn\textsuperscript{2+} ions.\textsuperscript{55} These two factors could account for the increased signal after affinity purification on fibronectin.

The data on adhesion at different stages of development of the heart indicate that the $\alpha$-chains might be associated with the expression of various ECM components. During fetal development, when the ECM is undergoing rapid changes in components, there are multiple $\alpha$-chains available to react with ECM components that could be important in the differentiation of the various regions of the heart. In response to physiological stimulation of the adult heart, the increased expression of the $\alpha$-chains could be associated with the increase of specific ECM components such as the interstitial collagens.\textsuperscript{4} The data presented in this report document the expression of the $\alpha_\text{I}$-chain, which is associated with collagen and laminin, during periods of fetal and neonatal growth and myocardial hypertrophy.

The localization of the $\beta_\text{I}$-integrin from cardiac muscle to specific regions on the myocytes is significant in the formation of the stress-tolerant network of the ECM.\textsuperscript{11} The colocalization of the $\beta_\text{I}$-integrin with cytoskeletal components at or lateral to the Z disk could be intimately associated with cardiac mechanics.\textsuperscript{11,56} Although it is still hypothetical that this region represents a site of transmission of mechanical stress across the sarcolemma, the localization of integral membrane proteins with affinity for the ECM and the cytoskeleton at this site supports this concept.\textsuperscript{16,57,58} In early fetal development, when mechanical stress is low, the ECM connections are not as prevalent as the cell-to-cell connections; however, in later stages, especially neonatal development and hypertrophy, ECM connections are essential.\textsuperscript{4,5,59} If synthesis of the collagenous components of the ECM are blocked or inhibited, aneurysms are apparent and eventually lead to rupture.\textsuperscript{5,59}

The studies presented here indicate that expression of the $\beta_\text{I}$-integrin and the associated specific $\alpha$-chains on cardiac myocytes may be correlated with physiological factors, such as changes in pressure and volume, associated with growth as well as disease. This expression may be the result of a complex set of both mechanical and chemical signals that also influence the secretion of specific ECM components. Further investigations will be necessary to examine the regulatory components of this complex interaction. Both specific chemical factors, such as growth factors, and mechanical stimulation may be required for the formation of the elastic, stress-tolerant network that is essential for the adaptation of the heart to the increased work load.
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References

41. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets:
Procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350–4354


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