Molecular Basis of Cardiac Performance

Plasticity of the Myocardium Generated through Protein Isoform Switches

Bernardo Nadal-Ginard and Vijak Mahdavi
Howard Hughes Medical Institute; Department of Cardiology, Children's Hospital; and Departments of Pediatrics and Cellular and Molecular Physiology, Harvard Medical School, Boston, Massachusetts 02115

Although the physiological properties of the myocardium and their dynamic character have been the focus of intense research during the past three decades, the biochemical and molecular correlates underlying cardiac performance for the most part remain poorly understood. The central role of the myocardium in the maintenance of the cardiac output notwithstanding, until very recently the prevailing view of the heart was that of a biochemically very static organ. Therefore, it did not seem either particularly interesting or suitable to address major questions of cellular and molecular biology affecting normal and pathological states. As a consequence, the impact of the newly developed techniques of recombinant DNA and genetic manipulation in transgenic animals on the understanding of cardiac cellular and molecular biology has been less significant than on other fields of biomedical sciences. With few exceptions, the power of these new techniques and approaches is just beginning to be felt in the field of cardiovascular biology. This state of affairs is more surprising given the biomedical importance of the cardiovascular system in general and the myocardium in particular, as well as the large number of investigators dedicated to its study. This situation is due, at least in part, to the characteristics of the myocardium that, until recently, have made this organ a less than ideal model system for a molecular and genetic approach.

Due to the essential role of the myocardium in the survival of the organism, most of the genetic mutations that significantly affect its development and/or function are likely to be lethal. This feature explains the very small number of mutations described so far affecting the myocardium either in human or animal models. This is in contrast with the large number of mutations affecting blood cells, the endocrine system, and metabolic pathways, among others. The existence of these mutations has provided the port of entry for the molecular dissection of these systems. In addition to the unavailability of mutations, the difficulty in obtaining repeated samples of the myocardium that are suitable for biochemical and molecular analysis from the same animal has also slowed progress. Furthermore, the existence of well-characterized cell lines that can be grown in homogeneous populations and mutated at will are an almost essential requirement for the exploitation of recombinant DNA technology to elucidate regulatory pathways. Given that the cardiac myocyte is a terminally differentiated cell that has lost its ability to replicate in vivo or in vitro shortly after birth (1), no cell lines with well-defined characteristics of cardiac myocytes have been available until now. This combination of characteristics, broadly outlined above, has played an important role in delaying the dissection of the cellular and molecular basis of cardiac performance in physiological and pathological states. Yet it is clear that the application of the modern techniques of cellular and molecular biology holds great promise to elucidate some of the major problems in clinical and experimental cardiovascular medicine. In addition, it is becoming increasingly clear that the cardiovascular system in general and the myocardium in particular are excellent model systems to address not only particular cardiovascular problems but also some broad biological questions that have general significance and are of import to a large number of organs and systems.

It is our objective to review summarily some of the recent advances in the understanding of the biology of the myocardium and its response to physiological and pathological stimuli. Particular emphasis will be placed on the contractile apparatus and on those areas that highlight the extraordinary plasticity of this tissue at the biochemical level and appear particularly suitable for a molecular approach.

The cardiac contractile apparatus

The unit of contraction in the myocardium, as well as in the skeletal muscle, is the sarcomere (2). The contractile properties of the myocardium, both in terms of force generated and velocity of contraction, are dependent on the number as well as the biochemical composition of its sarcomeres. The sarcomere is constituted by seven major proteins and several minor ones organized into thin and thick filaments. The thin filaments, anchored at the Z lines, are formed by a double helix of polymerized sarcomeric actin molecules. In the major groove of this double helix is located a continuous head-to-tail coiled coil of tropomyosin (TM)[1] dimers. Every TM dimer interacts with seven actins and is associated with a troponin (Tn) complex. Each complex is composed of one molecule of each of the three Tns: T, C, and I. This TM-Tn complex is responsible for the calcium sensitivity of the contractile apparatus. It regulates the interaction between the heads of the myosin molecule, located in the thick filament, with actin, the main constituent of the thin filament. Although most is known about this inter-

Address correspondence to Dr. Nadal-Ginard, Howard Hughes Medical Institute, Department of Cardiology, Children's Hospital, 300 Longwood Ave., Boston, MA 02115.
Received for publication 11 September 1989.

J. Clin. Invest.

0021-9738/89/12/1693/08 $2.00
Volume 84, December 1989, 1693–1700

1. *Abbreviations used in this paper:* ANF, atrial natriuretic factor; MHC, myosin heavy chain; MLC, myosin light chain; TM, tropomyosin; Tn, troponin; TRE, thyroid hormone–responsive element.

action, the precise molecular mechanisms responsible for the biochemical-mechanical transduction have not yet been fully elucidated. The thick filament contains the molecular motor of contraction, the myosin heavy chain (MHC). This is a bifunctional molecule that exists in a dimeric form. The two functional domains are constituted by the rod portion and the head. The rod is constituted by the carboxyl-terminal half of the molecule and is a regular coiled coil α-helix responsible for the assembly of myosin into an organized antiparallel thick filament with heads regularly spaced every 14.3 A at both ends with a bare zone in the middle. The rod carries the load during contraction, and due to its antiparallel organization makes possible the shortening of the sarcomere by pulling together two thin filaments pointing in opposite orientation and attached to two neighboring Z lines. During the contraction cycle the head of MHC, constituted by the amino-terminal half of the molecule, interacts directly with the actin molecules in the thin filament and carries the ATPase activity required to produce the physical translocation needed for fiber shortening. The ATPase activity of MHC is modulated by two smaller protein subunits bound to each MHC head, the alkali and essential myosin light chains (MLC). As originally pointed out by Barany (3), there is a direct correlation between the unloaded maximum velocity of shortening of a muscle fiber and the actin-activated ATPase activity of its MHC. Although there are some apparent exceptions to this rule, fibers with a high ATPase activity MHC shorten faster than the ones with a lower enzymatic activity (4, 5). Interestingly, there is an inverse correlation between ATPase activity of the MHC in a fiber and the energetic cost to perform a given workload (4–6). The faster the fiber, the higher the energetic cost to produce the same amount of work. It is clear, therefore, that the type of MHC present in the sarcomeres is physiologically significant and has a profound effect on the contractile properties of the myocardium.

In addition to the seven major proteins mentioned above, the sarcomere contains a number of proteins, such as α-actinin, C protein, titin, nebulin (7, 8), etc., that are present in lower amounts and are thought to play an important role either in its organization or modulation of function. However, with the exception of α-actinin, which is the main constituent of the Z line and serves to anchor the actin filament (7), the precise physiological function of these minor components of the sarcomere remains to be elucidated.

Although the intrinsic properties of the sarcomere are the main determinants of the contractile state, a number of other molecules such as adrenergic receptors (9), ion channels (10), Na,K ATPase, sarcolemmal and sarcoplasmic calcium pump, sarcoplasmic calcium release channel (11–13), etc. are also involved in its modulation. Most of these molecules exert their effect on contractility by directly or indirectly modulating either the availability or the response to calcium by the contractile proteins.

Each of the contractile proteins is a member of a family of isoforms that exhibit tissue- and developmental stage–specific regulation

The regulated expression of structurally distinct, developmentally regulated, and cell type–specific protein isoforms is a fundamental characteristic of higher organisms. The molecular mechanisms responsible for the generation of this protein diversity can be broadly categorized into two main systems: those that select a particular gene among the members of a multigene family for expression in a particular cell, and those that generate several different isoforms from a single gene. This latter mechanism includes DNA rearrangement and alternative pre-mRNA splicing. Both mechanisms involve the differential use of intragenic sequences that lead to the production of multiple protein isoforms from a single gene. DNA rearrangement appears to be restricted to a very limited set of genes coding for Igs and T cell receptors (14, 15). In contrast, increasing numbers of genes in organisms ranging from insects to human, including their DNA and RNA viruses, are known to be alternatively spliced.

Alternative pre-mRNA splicing is particularly prevalent in striated muscle tissues, including the myocardium. Among the contractile protein genes, this mode of gene regulation has been documented for α- and β-TM, TnT, and the MLCs, in addition to a number of other genes (reviewed in references 16 and 17). Furthermore, the major constituents of the thick filaments (MHCs and MLCs) and thin filaments (actin, TMs, and Tns [C, T, and I]) of mammalian sarcomeres are each encoded by a multigene family of moderate size ranging from four to eight members (2, 8, 18). The expression of each member of these multigene families is regulated at the transcriptional level in a tissue-specific and developmentally regulated manner. The different isoforms of sarcomeric contractile proteins, generated either through the transcription of different genes or from the same gene by alternative pre-mRNA splicing, are able to substitute for each other and to assemble combinatorially when present in the same cell. The restricted combinatorial use of the different members of these multigene families allows for the generation of a moderate number of qualitatively different sarcomere types that, at least in some cases, exhibit significantly different physiological characteristics (8, 9, 18, 19). This potential for the production of different sarcomeres is highly increased by the generation of multiple protein isoforms by individual MLC, TM, and TnT genes. Therefore, to understand the mechanisms involved in generating myocardial protein diversity it is necessary to elucidate both the elements responsible for the selective transcription of a given gene in a particular cell type at a particular time in development or physiological state and the factors that regulate alternative pre-mRNA splicing in the same cell.

During the past six or seven years most of the genes coding for contractile proteins have been identified and cDNA and genomic sequences have been obtained, mapped to the human genome, and characterized for their potential to generate multiple protein isoforms by alternative splicing. Although some of these genes are closely clustered on the same chromosome, as is the case for the cardiac and skeletal MHCs (20–22), most other contractile protein gene families are not linked but scattered on several chromosomes (23, 24). Therefore, although the contractile proteins are assembled in the sarcomere in very precise stoichiometric amounts, their regulation is by necessity complex, since it involved multiple genes that are located in different regions of the genome that, with very few exceptions, do not seem to have common regulatory sequences.

Some of the contractile protein isoforms expressed in the myocardium are shared with the skeletal muscle, while others are expressed exclusively in the myocardium (2, 8, 18; Table I). Moreover, for several of these proteins the atrial and ventricular isoforms are different from each other and both differ from those expressed in the conduction system. Although the physi-

Table I. Expression of Contractile Protein Genes in Striated Skeletal and Cardiac Muscles of Small Mammals

	Skeletal muscles			Cardiac ventricle			Cardiac atrium
	Embryonic/neonatal	Adult fast	Adult slow	Embryonic	Adult	Pressure overload adult	Adult
MHC	Embryonic MHC Neonatal MHC	Fast II A MHC Fast II B MHC	Slow I/β-MHC	Slow/β-MHC	α-HMC $+\sim\beta$MHC	Slow/β-MHC $+\alpha$-MHC	α-MHC
MLC	MLC 1e MLC 1f	MLC 1f MLC 3f	MLC 1 slow/cardiac	MLC 1e	MLC 1 slow/cardiac	MLC 1 slow/cardiac + MLCe	MLC 1e MLC 1 slow/cardiac
MLC 2	MLC 2sk	MLC 2sk	MLC 2sk	MLC 2 cardiac	MLC 2 cardiac	MLC 2 cardiac	MLC 2 cardiac
TM	$\beta\beta$	α/β α/α	α/β	β/α	α/α	α/β	α/α
Tns							
T	fast TnT Slow TnT	Fast TNT	Slow TNT	(Emb) cardiac TNT	(Adult) cardiac TNT		Cardiac TNT
C	c TNC(slow/cardiac)	Skeletal TNC	c TNC	c TNC	c TNC	c TNC	c TNC
I	Fast TNI	Fast TNI	Slow/cardiac TNI	Slow/cardiac TNI	Slow/cardiac TNI		Slow/cardiac
Actin	c α-actin Skeletal α-actin	sk α-actin	sk α-actin	sk α-actin c α-actin	c α-actin	c α-actin Skeletal α-actin	c α-actin
Creatine kinase	BB ck BM ck	MM ck	MM ck	BB ck MM ck	MM ck	MM ck BB ck	

MHC, Myosin heavy chain; each member of this gene family is indicated by a prefix indicating the most common nomenclature used to duplicate the gene. MLC, Myosin light chain gene products: LC1e, light chain 1 embryonic; LC1 slow/cardiac, light chain specific for slow and cardiac tissues. LC1f and LC3f are the two products for the myosin light chain ⅓ that is predominantly expressed in fast muscle. LC2sk and LC2 cardiac denote the light chain 2 characteristic of skeletal (sk) and cardiac muscle, respectively. Tropomyosin α and β designate the products of these two genes. α-TM is characteristic of differentiated striated muscle, while β is characteristic of the undifferentiated cells. TNC, TNT, and TNI indicate troponin C, T, and I, respectively. The prefixes cardiac, skeletal, slow, embryonic, etc., indicate the tissue and/or developmental stage where this gene product is predominantly expressed. cα-Actin and skα-actin indicate the isoforms characteristic of adult normal cardiac and skeletal muscle, respectively. Creatine kinase B and M isoform indicate the muscle-specific (M) and nonmuscle (B) isoforms.

ological basis for the selective advantage that has produced this isoform distribution is not apparent from our present understanding of contractility, two main general trends are obvious. In general, the myocardium genes are more likely to be shared with slow than fast skeletal muscle. Embryonic and fetal isoforms are shared more often with striated muscle than their adult counterparts.

Since the MHC isoform switches in the myocardium are physiologically more relevant and are better understood at the molecular level, they will be used to illustrate some of the mechanisms involved. The regulatory processes regulating the expression of these genes appear to be of general significance and, in general terms, are likely to apply to isoform switches in the myocardium.

Isoform switches in response to physiological and pathological stimuli

In the cardiac ventricles of most mammalian species, including the human, three myosin isoforms have been identified based on their electrophoretic mobility: V1, V2, and V3 (24, 25). However, these three myosins are composed of only two distinct types of MHCs, referred to as α and β. V1 and V3 are composed of $\alpha\alpha$ and $\beta\beta$ homodimers, respectively, while V2 is an $\alpha\beta$ heterodimer. These two myosins are produced by two different genes that are closely linked (21, 22) and are located on chromosomes 14 and 3 in mouse and human, respectively (22).

As for all muscle types, the myosin composition of the myocardium is of physiological importance, since the relative distribution of α- and β-MHC is directly correlated with the contractile properties of the heart. The α-MHC, which has high Ca^{2+} and actin-activated ATPase activity (25, 26), is associated with an increased shortening velocity of the cardiac fibers (26, 27). In contrast, the β-MHC, which has lower ATPase activity (25, 26), is associated with slower shortening velocity (26, 27). It is, therefore, interesting that the ratio of these two different cardiac isoforms is developmentally regulated. In the ventricles of all mammalian species studied so far β-MHC is the most abundant isoform in utero until late fetal life (28–30). In small mammals, such as rat and mouse, α-MHC increases immediately before birth and becomes the predominant form throughout perinatal and adult life (28, 29). In contrast, in large mammals such as man α-MHC is only transiently predominant shortly after birth, with β-MHC becoming the most abundant isoform in the adult (28, 30). The situation is different in the atria where α-MHC is the predominant isoform throughout life in both small and large species (2, 29, 30). In addition, in all species studied, including man, the distribution of the cardiac MHC isoforms changes in response to certain pathological and experimental conditions such as work overload (27, 31–34), diabetes (35), gonadectomy (36), and, more importantly, changes in thyroid hormone levels (24, 26, 29, 37–39). These changes are regulated at the level of transcription of the respective genes, since there is a direct correlation between the levels of α- and β-MHC and the corresponding mRNAs (29, 39) and between these and the rate of transcription (40).

It is clear now that thyroid hormone plays a fundamental role in the regulation of the MHC phenotype both in the myocardium and in skeletal muscle (39). At least in mammals, all the genes of the striated MHC multigene family are, without exception, responsive to thyroid hormone. Surprisingly, however, whether the hormone induces or represses the expression of a given MHC depends on the gene itself and the muscle where it is expressed. The same gene can be induced by the hormone in one muscle and repressed in another (39), indicating that the regulation of this gene family by thyroid hormone is likely to be more complex than described so far for a variety of steroid hormones (41). In the ventricular myocardium there is a precise correlation between the levels of circulating thyroid hormone and the relative levels of α- and β-MHC (29). The expression of α-MHC is dependent on the presence of thyroid hormone. In its absence the α-MHC gene is not transcribed. The converse is true for β-MHC. The expression of this gene is repressed by thyroid hormone and it is induced in hypothyroid states (29, 39). The induction of α-MHC at the time of birth is directly correlated with the surge in the circulating thyroid hormone that occurs at this time (29). This effect of thyroid hormone on cardiac MHC expression can be directly demonstrated in experimental animals by manipulating their thyroid state. After surgical or chemical (5-thiouracil) thyroidectomy, the expression of α-MHC is completely suppressed and only β-MHC is expressed in the myocardium. Replacement therapy restores the normal phenotype (29, 39). On the other hand, hyperthyroid states repress the expression of the β-MHC gene at both the mRNA and protein levels and produces a myocardium constituted exclusively by α-MHC (29, 39). That these results are not indirect and are not produced by changes in metabolic state, circulating catecholamines, innervation, etc., due to the general effects of changes in thyroid hormone status, is demonstrated by the fact that they can be reproduced in isolated tissue slices and cells in culture (42).

The molecular mechanism of thyroid hormone action has been elucidated, at least in part, by the demonstration that the c-*erb* protooncogenes are the nuclear receptors for this hormone (43–46). At least two genes with well-defined tissue-specific expression encode this receptor (43–45), and each one can generate several different isoforms by alternative splicing (45, 46). The functional properties of some of these alternatively spliced isoforms are very different and some have lost their ability to bind T3 (46). It has been recently proposed that some of these isoforms that are impaired in their ability to bind ligand might function as antioncogenes and/or anti–receptor molecules (47, 48). This is because of their ability to compete for DNA binding sites, but due to their absence of ligand binding are unable to stimulate transcription (46–48). Yet, whether this is a general phenomenon remains to be demonstrated. The functional T3 receptors are hormone-dependent transcriptional factors that exercise their effect through binding to a thyroid hormone–responsive element (TRE) in the responsive gene (46, 48, 49). The TRE for the human and rat α-MHC genes has been determined by a combination of deletion mapping, site-directed mutagenesis, and in vitro and in vivo binding assays (42, 46, 50). The two genes have a TRE with identical sequence and both are able to confer thyroid hormone sensitivity to heterologous genes (42, 46, 50). Therefore, the sequence containing the TRE is both required and sufficient to confer T3 responsiveness to a gene.

The mechanism of T3 repression of MHC gene expression is less understood. Both the human and rat β-MHC genes have sequences with a high degree of homology to the TRE of the α-MHC genes (42, 50). These sequences do not have an effect on the heterologous gene promoters so far tested, and it is not

clear whether or not they are specifically recognized by the thyroid hormone receptor. Since these putative TRE sequences in the β-MHC genes are overlapping with the CAAT box sequences (42), an essential promoter element in these genes, the possibility that T3 exerts its negative regulatory role by sterically hindering the binding of an essential transcription factor is under investigation.

The results summarized above demonstrate that thyroid hormone plays an important role in regulating cardiac MHC expression and raise the question of whether this hormone is solely responsible for the regulation of these genes. Several lines of evidence indicate that this is not the case. First, it is clear that the α- and β-MHC genes respond to thyroid hormone in a tissue-dependent manner. For example, in the ventricle the α-MHC gene is exquisitely sensitive to T3 and is not expressed at all in the hypothyroid state. However, in the atria of the same heart this gene is practically unresponsive to the hormone. The different behavior in the two tissues is not due to the lack of functional thyroid hormone receptors in the atria, since other genes in this structure are readily responsive to the hormone. A similar phenomenon is apparent for the β-MHC gene. As indicated above, in the ventricle the expression of this gene is repressed by thyroid hormone. Yet, in the same animals its expression continues at an almost normal level in the slow muscle fibers of skeletal muscle. Moreover, the TRE of these genes does not explain their tissue specificity since they act as positive regulators of transcription in the presence of receptor and T3, irrespective of the cell type where they are expressed. In fact, the tissue specificity of these genes is conferred by a combination of positive and negative transcriptional regulatory elements (50). These other regulatory elements are probably responsible for the species-specific differences in the expression of these genes. There are some differences in the organization of the 5′ flanking sequences between rat and human. Although the TRE in the α-MHC gene of the two species is identical in sequence, the human gene is less responsive to the hormone when tested in cotransfection experiments (50). Taken together, these results indicate that in addition to thyroid hormone and its receptors other factors that are specific to different muscles also play an important role. The nature of these factors is not yet known.

The involvement of different regulatory pathways in the expression of the cardiac MHC genes becomes more evident when the changes produced by work overload hypertrophy are analyzed. In small mammals, and particularly in rats, in response to a moderate increase in mean aortic pressure (~ 30 mmHg) produced by aortic coarctation (51), or by other means, there is a rapid induction of β-MHC mRNA followed by the appearance of comparable levels of β-MHC protein, in parallel with an increase in left ventricular weight. A similar change is not detectable in larger mammals, including humans, because β-MHC is the predominant isoform expressed in the normal ventricle. However, in the human atria, which normally expresses α-MHC, a switch to β-MHC is readily apparent in response to increased pressure (52). Therefore, the hypertrophic myocardium induces the expression of β-MHC and represses the expression of the α-gene. Therefore, with respect to the MHC phenotype it resembles the fetal and hypothyroid state. Yet, in these animals the circulating level of thyroid hormone remains normal and their metabolic state argues against hypothyroidism. Other features argue persuasively that this isoform switch produced in response to work overload hypertrophy is not regulated through the thyroid hormone pathway (51).

The isoform switches produced in response to increased afterload are not limited to MHC. In fact, a general myocardial response to work overload occurs rapidly and affects a multitude of cellular compartments (53). This response is characterized by the reexpression of the protein isoforms normally expressed in fetal life, but that are normally suppressed in adulthood. To the best of our knowledge this phenomenon has been demonstrated for all the gene phenotypes analyzed so far, including other contractile proteins such as skeletal α-actin (53, 54), MLC 1 (55), and β-TM (53); membrane proteins like the Na,K ATPase (the cardiac glycoside receptor) (56); secreted molecules such as atrial natriuretic factor (ANF) (53); and those involved in ATP regeneration such as creatine kinase (57). With the exception of ANF, all the above examples represent the reexpression of an isoform normally expressed only during fetal and early postnatal life that is later replaced by the corresponding adult isoform. ANF expression in the ventricles is normally suppressed after birth and is not replaced by another isoform. Its expression, however, is rapidly reinduced in response to the hypertrophic stimulus.

From the above observations it is clear, therefore, that myocardial hypertrophy is not only a quantitative phenomenon that results in an increase in cardiac mass, but more importantly, results in a significant qualitative change in important constituents of the myocardium. In general, these changes produce a muscle that has many of the biochemical characteristics of the fetal myocardium.

What is the stimulus for this dramatic and concerted change in myocardial gene expression in response to work overload? A potential candidate is thyroid hormone itself. However, as already indicated above, no changes in thyroid hormone levels are detected in these animals. Furthermore, if thyroid hormone were responsible it should be possible to reestablish the normal phenotype in response to thyroid hormone therapy. Yet this is not the case. Thyroid hormone can overcome the effect of pressure overload on MHC gene expression, but has no effect on the other phenotypic changes (51, 53). Administration of high doses of T3 in the hypertrophic animals produces a rapid deinduction of the β-MHC gene with the concomitant induction of the α-gene, despite the fact that these animals have a higher degree of hypertrophy than those with simple hemodynamic overload (51, 53). None of the changes in the expression of other genes are affected by the hormone. These results give further support to the contention that the changes induced by hemodynamic overload are not secondary to thyroid hormone changes. However, in the case of the MHC genes T3 has a dominant effect and can overcome the regulatory mechanisms induced by the hypotrophic stimulus. This behavior highlights the complex interplay that exists between hemodynamic and hormonal stimuli in myocardial gene expression.

It is noteworthy that in the aortic coarctation animal model system, increased afterload is not the only consequence of the manipulation. It might produce a rise in circulating catecholamine levels and/or activation of the renin-angiotensin system secondary to decreased renal blood flow. NE (58, 59) and possibly angiotensin II could, in principle, directly stimulate myocardial cell hypertrophy independently of the hemodynamic effects. The effect of NE on cardiac cell growth in culture has been shown to be mediated by stimulation of the

α1-adrenergic receptor (58), which couples the hydrolysis of membrane phosphatidylinositol followed by the release of IP3 (60) and activation of protein kinase C (61). Furthermore, phorbol esters, direct activators of protein kinase C, can produce hypertrophy and isoform switches when administered to cultured neonatal cardiac cells (62). However, the fact that the atria and right ventricles of animals with aortic coarctation do not exhibit the isoform transitions described above strongly suggests that these humoral mechanisms do not play an important role, if they are involved at all, in the processes described here.

Work overload and stretch produce the rapid expression of a set of protooncogenes involved in normal cell growth

The cardiac response to normal growth requirements, as well as to work overload, is dependent on the developmental state of the organ. During fetal and early postnatal life the demand for an increased cardiac mass are fulfilled mainly by an increase in the number of myocytes (hyperplasia). However, soon after birth cardiac myocytes lose their ability to divide (1). Later in life, demand for increased mass is fulfilled exclusively through an increase in the size of a fixed number of preexisting myocytes. The molecular mechanisms responsible for the loss of replicative ability (terminal differentiation) remain completely unknown. Genes involved in determining the myogenic lineage and terminal differentiation in skeletal muscle, such as MyoD (63), myogenin (64), and Mif5 (65), that function as tissue-specific transcriptional factors, are not involved in the determination and differentiation of the cardiac myocytes, since they are not expressed in these cells. It is likely that a family of genes with functional similarities but with significant sequence divergence from the ones identified in skeletal muscle is responsible for the cardiac phenotype.

What is the mechanism involved in inducing cell growth and isoform switches in response to work overload? The observed reexpression of fetal isoforms in cardiac hypertrophy is reminiscent of the mitogenic response of many differentiated cell types, such as hepatocytes, which often involves the suppression of the adult phenotype and reexpression of the fetal pattern, such as the inhibition of albumin and induction of α-protein expression during liver regeneration (66). In a general biological context, cardiac hypertrophy could be considered the equivalent of the growth response exhibited by most cell types in response to mitogens. In this particular case the growth response is carried out by terminally differentiated cells (myocytes) that are unable to undergo cell division and have only the hypertrophic response open to them. If this hypothesis were correct, it would be expected that the initial response to the hypertrophic stimuli would mimic early events of cell division induced by growth factors in a large variety of cell types.

One of the early responses of stationary cells to growth stimuli is the induction of a series of protooncogenes, such as c-*fos* and c-*myc*, among others, that directly or indirectly turn on the cascade of events that lead to cell division. Recently it has been demonstrated that these protooncogenes are bona fide transcriptional factors (67). Furthermore, c-*myc* is able to induce a family of heat shock or stress proteins that are involved in protecting the viability of cells under adverse conditions by mechanisms that are not fully elucidated, but might affect proper protein folding (68) and/or modulation of gene transcription (67).

Not surprisingly, therefore, c-*fos* and c-*myc* mRNAs begin to accumulate within 1 h after the increase in afterload, reach high levels within 3 h, and return to the basal levels in < 24 h. Similarly, the mRNA for one of the major stress proteins, HSP 70, is also increased within 30 min of increasing aortic pressure (53). Thus, similar to the mitogenic response of a variety of cell types, induction of the cellular protooncogenes and major stress protein genes reflect early changes occurring in the nuclei of myocardial cells in response to acute pressure overload and appear to play an important role in mediating the hypertrophic response. Recently it has been demonstrated that several growth factors, including transforming growth factors β and basic fibroblast growth factor, applied to cardiocytes in culture induce a pattern of contractile protein and protooncogene expression that is very similar to the one produced by work overload in the intact heart (69). These results demonstrate that the lack of mitogenic response by the cardiac myocytes is not due to a loss of receptors for growth factors. They also give further support to the hypothesis that work overload affects gene expression through mechanisms similar to or shared by the growth factor receptors. The inability of the cardiocytes to mount a full mitogenic response in response to work overload or growth factors remains an important challenge that also applies to all other terminally differentiated cells, such as neuron and certain epithelial cells. On one hand, these cells could have irreversibly lost the expression of some of the genes required to traverse the cell cycle. In that case it should be impossible for them to reenter the cell cycle in response to any stimulus. On the other hand, as part of the terminally differentiated program they could induce an inhibitor of the cell cycle. In that case, repression or neutralization of the inhibitor should enable the cells to cycle again. Recently, recessive cellular oncogenes with many of the properties required for this role have been described. One of them, the product of the retinoblastoma gene, has been shown to belong to this class. The activity of this gene product is neutralized by certain viral oncogenes, SV40 T antigen (70) and adenovirus E1A (71). Based on the finding that SV40 T antigen is able to reinduce the ability to cycle to terminally differentiated myotubes (72), it has been possible to reinduce the cell cycle in terminally differentiated cardiocytes and to create cell lines that express many of the differentiated characteristics (Thompson, R., B. Nadal-Ginard, and V. Mahdavi, unpublished observations). These results suggest the presence of inhibitors in the differentiated cardiocytes. Identification of the molecule(s) involved could provide the tool required to induce cardiac muscle regeneration.

In various models of cardiac hypertrophy, systolic and diastolic wall stress have both been implicated as major determinants of the degree and pattern of hypertrophy during pressure and volume overload (73). In addition, studies using isolated heart preparations have demonstrated that increased wall tension alone can directly stimulate protein synthesis (1, 2). Although the precise molecular mechanisms by which wall stress is communicated to the myocyte nucleus remain to be elucidated, the recently discovered stretch-sensitive ion channels (74) provide a likely candidate for the sensor mechanism. These channels could provide a very sensitive measure of wall stress. The ionic changes produced by their opening or closing could trigger a second messenger cascade (perhaps involving IP3) that results in the changes in gene expression described above. The recent demonstration that stretch of isolated car-

diocytes in culture induces the changes of contractile gene and protooncogene expression described for work overload and growth factors is in agreement with this hypothesis.

In physiological terms, the reexpression of the fetal isogenes might be a beneficial adaptation to hemodynamic overload. As a consequence of the changes induced in the thin and thick filaments during cardiac hypertrophy, sarcomeres with significantly different functional properties are produced. For the myocardium, the fetal isoform of MHC has been shown to be bioenergetically more efficient than that of the adult. Moreover, because ANF has potent natriuretic, diuretic, and vasodilatory effects, the marked induction of this molecule in the ventricle in response to increased blood pressure might be interpreted as an adaptational response to reduce hemodynamic load imposed on the ventricle.

In conclusion, the results summarized here demonstrate that the myocardium is a biochemically very plastic tissue that is amenable to cellular and molecular dissection and can serve as a good experimental model to address some important questions that are relevant to the cardiovascular system but are also of general biological significance. In addition, it is clear that cardiac hypertrophy is not a simple quantitative increase in ventricular mass, but a qualitatively different and heterogeneous process that is highly influenced by the nature of the hypertrophic stimulus and the developmental stage of the myocardium. Induction of cellular protooncogenes that play a role in cell growth in the very early stages of work overload hypertrophy mimics the mitogenic response to growth factors by a variety of cells. The quantitative and qualitative changes in the expression of contractile and regulatory genes that occurs later, most likely represent only a small sample of the changes produced in the myocardium in response to the hypertrophic stimuli. The fact that each fetal gene examined so far is reexpressed in response to pressure overload hypertrophy suggests that reinduction of the fetal program might be a general adaptive process to hemodynamic stress. Further work is needed, however, to elucidate the precise mechanisms by which the hemodynamic and/or mechanic stimuli are converted into biochemical signals that lead to quantitative as well as qualitative changes in gene expression. A better understanding of the genes involved in converting precursor mesenchymal cells into the cardiogenic pathway, the cell-specific transcriptional factors responsible for the expression of the cardiac specific genes, and the genes involved in blocking these cells in the terminally differentiated phenotype is also required. This information is essential to be able to manipulate the process of cardiac hypertrophy and changes of contractile state to physiological advantage.

References

1. Zak, R. 1974. *Circ. Res.* 35(Suppl 2):17–26.
2. Swynghedauw, B. 1986. *Physiol. Rev.* 66:710–771.
3. Barany, M. 1967. *J. Gen. Physiol.* 50:197–218.
4. Sheuer, J., and A. K. Bahn. 1979. *Circ. Res.* 45:1–12.
5. Schwartz, K., Y. Lecarpentier, J. L. Martin, A.-M. Lompre, J. J. Mercadier, and B. Swynghedauw. 1981. *J. Mol. Cell. Cardiol.* 13:1071–1075.
6. Alpert, N. R., and L. A. Mulieri. 1982. *Circ. Res.* 50:491–500.
7. Obinata, T., K. Maruyama, H. Sugito, K. Kohouma, and S. Ebashi. 1981. *Muscle & Nerve.* 4:456–488.
8. 1986. Molecular biology of muscle development. *UCLA (Univ. Calif. Los Angel.) Symp. Mol. Cell. Biol. New Ser.*
9. Stiles, G. L., M. G. Caron, and R. J. Lefkowitz. 1984. *Physiol. Rev.* 64:661–743.
10. Catteral, W. A. 1986. *Annu. Rev. Biochem.* 55:953–986.
11. Herrera, V. L. M., J. R. Emanuel, N. Ruiz-Opazo, R. Levenson, and B. Nadal-Ginard. 1987. *J. Cell Biol.* 105:1855–1865.
12. MacLennan, D. H., C. J. Brandl, B. Korczak, and N. M. Green. 1985. *Nature (Lond.).* 316:696–700.
13. Brandl, C. J., N. M. Green, B. Korczak, and D. H. MacLennan. 1986. *Cell.* 44:595–607.
14. Siu, G., M. Kronenberg, E. Strauss, R. Haars, T. W. Mak, and L. Hood. 1984. *Nature (Lond.).* 311:344–350.
15. Honjo, T., and S. Habu. 1985. *Annu. Rev. Biochem.* 54:803–830.
16. Breitbart, R. E., A. Andreadis, and B. Nadal-Ginard. 1987. *Annu. Rev. Biochem.* 56:467–495.
17. Smith, C. W. J., D. Knaack, and B. Nadal-Ginard. 1988. *In* Intervening Sequences in Evolution and Development. E. Stone, editor. Oxford University Press, Oxford, UK. 97–118.
18. 1988. Cellular and molecular biology of muscle development. *UCLA (Univ. Calif. Los Angel.) Symp. Mol. Cell. Biol.*
19. Pette, D., and G. Vrbova. 1985. *Muscle & Nerve.* 8:676–689.
20. Leinwand, L. A., E. K. Fournier, B. Nadal-Ginard, and T. Shows. 1983. *Science (Wash. DC).* 221:766–769.
21. Mahdavi, V., A. P. Chambers, and B. Nadal-Ginard. 1984. *Proc. Natl. Acad. Sci. USA.* 81:2626–2630.
22. Saez, L. J., K. M. Gianola, E. M. McNally, R. Feghali, R. Eddy, T. B. Shows, and L. A. Leinwand. 1987. *Nucleic Acids Res.* 15:5443–5459.
23. Czosnek, H., U. Nudel, Y. Mayer, M. Shani, P. F. Barker, D. D. Pravtcheva, F. H. Ruddle, and D. Yaffe. 1982. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1299–1305.
24. Hoh, J. F. Y., P. A. McGrath, and H. T. Hale. 1978. *J. Mol. Cell. Cardiol.* 10:1053–1076.
25. Pope, B., J. F. Y. Hoh, and A. Weeds. 1980. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 118:205–208.
26. Schwartz, K., Y. Lecarpentier, J. L. Martin, A.-M. Lompre, J. J. Mercadier, and B. Swynghedauw. 1981. *J. Mol. Cell. Cardiol.* 13:1071–1075.
27. Lompre, A. M., K. Schwartz, A. D'Albis, G. Lacombe, N. V. Thiem, and B. Swynghedauw. 1979. *Nature (Lond.).* 282:105–107.
28. Lompre, A. M., J. G. Mercadier, C. Wisnewsky, P. Bouveret, D. Pantaloni, A. D'Albis, and K. Schwartz. 1981. *Dev. Biol.* 84:286–291.
29. Lompre, A.-M., B. Nadal-Ginard, and V. Mahdavi. 1984. *J. Biol. Chem.* 259:6437–6446.
30. Chizzonite, R. A., and R. Zak. 1984. *J. Biol. Chem.* 259:12626–12632.
31. Mercadier, J.-J., A.-M. Lompre, C. Wisnewsky, J.-L. Samuel, J. Bercovici, B. Swynghedauw, and K. Schwartz. 1981. *Circ. Res.* 49:525–532.
32. Gorzq, L., P. Pauletto, A. C. Pessina, S. Sartore, and S. Shiaffino. 1981. *Circ. Res.* 49:1003–1009.
33. Litten, R. Z., B. J. Martin, R. B. Low, and N. R. Alpert. 1982. *Circ. Res.* 50:856–864.
34. Scheuer, J., A. Malhotra, C. Hirsch, J. Capasso, and T. F. Schaible. 1982. *J. Clin. Invest.* 70:1300–1305.
35. Dillman, W. 1980. *Diabetes.* 29:579–582.
36. Malhotra, A., S. Penpargkul, F. S. Fein, E. H. Sonnenblick, and J. Scheuer. 1981. *Circ. Res.* 49:1243–1250.
37. Everett, A. W., W. A. Clark, R. A. Chizzonite, and R. Zak. 1983. *J. Biol. Chem.* 258:2421–2425.
38. Chizzonite, R. A., A. W. Everett, W. A. Clark, S. Jackovcic, M. Rabinowitz, and R. Zak. 1982. *J. Biol. Chem.* 257:2056–2065.
39. Izumo, S., B. Nadal-Ginard, and V. Mahdavi. 1986. *Science (Wash. DC).* 231:597–600.
40. Umeda, P. K., J. E. Levin, A. M. Shinha, L. L. Cribbs, D. S. Dawling, D. J. Ende, H.-J. Hsu, E. Dizon, and S. Jakovcic. 1986. Molecular Biology of Muscle Development. C. Emerson, D. Fischman, B. Nadal-Ginard, and M. A. W. Siddiqui, editors. Alan R. Liss, Inc., New York. 809–823.

41. Evans, R. M. 1988. *Science (Wash. DC).* 240:889–895.

42. Mahdavi, V., G. Koren, S. Michaud, C. Pinset, and S. Izumo. 1989. Cellular and Molecular Biology of Muscle Development. L. Kedes and F. Stockdale, editors. Alan R. Liss Inc., New York. 369–379.

43. Sap, J., A. Munoz, K. Damm, Y. Goldberg, J. Ghysdael, A. Leutz, H. Beug, and B. Vennstroem. 1986. *Nature (Lond.).* 324:635–640.

44. Weinberger, C., C. C. Thompson, E. S. Ong, R. Lebo, D. J. Gruo, and R. M. Evans. 1986. *Nature (Lond.).* 324:641–646.

45. Thompson, C. C., C. Weinberger, R. Lebo and R. M. Evans. 1987. *Science (Wash. DC).* 237:1610–1614.

46. Izumo, S., and V. Mahdavi. 1988. *Nature (Lond.).* 334:593–595.

47. Damm, K., C. C. Tompson, and R. M. Evans. 1988. *Nature (Lond.).* 339:593–597.

48. Koenig, R. J., M. A. Lazar, R. A. Hodin, G. A. Brent, P. R. Larsen, W. W. Chin, and D. D. Moore. 1989. *Nature (Lond.).* 337:659–661.

49. Glass, C. K., R. Franco, C. Weinberger, V. R. Albert, R. M. Evans, and M. G. Rosenfeld. 1987. *Nature (Lond.).* 329:738–741.

50. Thompson, W. R., G. Koren, S. Izumo, V. Mahdavi, and B. Nadal-Ginard. 1989. The Proceedings of the Symposium on Ethiology and Morphogenesis of Congenital Heart Diseases. E. Clarck, editor. *In press.*

51. Izumo, S., A. M. Lompre, R. Matsuoka, G. Koren, K. Schwartz, B. Nadal-Ginard, and V. Mahdavi. 1987. *J. Clin. Invest.* 79:970–977.

52. Mercadier, J. J., P. Bouveret, L. Gorza, S. Schiaffino, W. A. Clar, R. Zak, B. Swynghdavw, and K. Schwartz. 1983. *Circ. Res.* 53:52–62.

53. Izumo, S., B. Nadal-Ginard, and V. Mahdavi. 1988. *Proc. Natl. Acad. Sci. USA* 85:339–343.

54. Schwartz, K., A. M. Lompre, P. Bouveret, C. Wishewshky, P. Oliviero, S. Alonso, and M. Buckingham. 1985. *J. Mol. Cell. Cardiol.* 17(Suppl 3):22. (Abstr.)

55. Hirzel, H. O., C. R. Tuchschmid, J. Schneider, H. P. Krayenbuehl, and M. Schub. 1985. *Circ. Res.* 57:729–740.

56. Cantley, L. C. 1981. *Curr. Top. Bioenerg.* 11:201–237.

57. Ingwall, J. S., M. F. Kramer, M. A. Fifer, W. Grossman, and P. Allen. 1985. *N. Engl. J. Med.* 313:1050–1054.

58. Simpson, P. C., and A. McGrath. 1983. *J. Clin. Invest.* 22:732–735.

59. Lacks, M. M. 1987. *Am. Heart J.* 94:349–399.

60. Berridge, M. J., and R. I. Irvine. 1984. *Nature (Lond.).* 312:315–321.

61. Nishizuka, Y. 1984. *Nature (Lond.).* 308:693–698.

62. Simpson, P. C. 1985. *Circ. Res.* 56:884–894.

63. Lassar, A. B., B. M. Paterson, and H. Weintraub. 1986. *Cell.* 47:649–656.

64. Wright, W. E., D. A. Sasson, and V. K. Lin. 1989. *Cell.* 56:607–617.

65. Braun, T., G. Buschhausen-Denker, E. Bober, E. Tannich, and H. H. Arnold. 1989. *EMBO (Eur. Mol. Biol. Organ) J.* 8:701–709.

66. Ruoslanhti, E., H. Pihko, and M. Seppala. 1974. *Transplant. Rev.* 20:38–48.

67. Johnson, P. F., S. L. McKnight. 1989. *Annu. Rev. Biochem.* 58:799–839.

68. Rothman, J. E. 1989. *Nature (Lond.).* 340:433–434.

69. Schneider, M. D., H. T. Shih, and T. G. Parker. 1989. *J. Mol. Cell. Cardiol.* 21(Suppl III):A67. (Abstr.)

70. DeCaprio, J. A., J. W. Lulow, J. Figge, J.-Y. Shew, C.-M. Huang, W.-H. Lee, E. Marsilio, E. Paucha, and D. M. Livingston. 1988. *Cell.* 54:275–283.

71. Whyte, P., K. J. Buchkovich, J. M. Horowitz, S. H. Friend, M. Raybuck, R. A. Weinberg, and E. Harlow. 1988. *Nature (Lond.).* 334:124–129.

72. Endo, T., and B. Nadal-Ginard. 1989. *UCLA (Univ. Calif. Los Angel.) Symp. Mol. Cell. Biol. New Ser.* 93:95–104.

73. Grossman, W. 1980. *Am. J. Med.* 69:576–584.

74. Guharay, F., and F. Sachs. 1984. *J. Physiol.* 352:685–701.