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〔Review Article〕

Molecular mechanism of cardiac hypertrophy and cardiac development

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SUMMARY

To understand the heart failure, it is important to elucidate the mechanism of the development of cardiac hypertrophy. Hemodynamic overload, namely mechanical stress, is a major cause for cardiac hypertrophy. To dissect the signaling pathways from mechanical stress to cardiac hypertrophy, we developed the in vitro device by which mechanical stress can be imposed on cardiac myocytes of neonatal rats cultured in the serum-free condition. Passive stretch of cardiac myocytes cultured on silicone membranes induced various hypertrophic responses such as activation of phosphorylation cascades of many protein kinases, expression of specific genes and an increase in protein synthesis. During this process, secretion and production of vasoactive peptides such as angiotensin II and endothelin-1, are increased and they played critical roles in the induction of these hypertrophic responses. Recently candidates for the "mechanoreceptor" which receive mechanical stress and convert it into intracellular biochemical signals have been demonstrated. To treat heart failure, gene therapy and cell transplantation are hopeful strategies. To enable these novel treatments, it is important to understand how normal cardiac myocytes are differentiated. We have isolated a key gene which plays a critical role in cardiac development. A cardiac homeobox-containing gene Csx is expressed in the heart and the heart progenitor cells from the very early developmental stage, and targeted disruption of the murine Csx results in embryonic lethality due to the abnormal looping morphogenesis of the primary heart tube. With a cardiac zinc finger protein GATA4, Csx induces cardiomyocyte differentiation of teratocarcinoma cells as well as upregulation of cardiac genes. Mutations of human Csx cause various congenital heart diseases including atrial septal defect, ventricular septal defect, tricuspid valve abnormalities and atrioventricular block.

Key words: cardiac hypertrophy, mechanical stress, angiotensin II, development, homeobox

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I. Molecular mechanism of cardiac hypertrophy

It is important to understand how cardiac hypertrophy is formed, not only because it shows diastolic dysfunction and often leads to congestive heart failure but also because it is an independent risk factor for many cardiac diseases such as ischemic heart disease, arrhythmia and sudden death[1].

1. MECHANICAL STRESS INDUCES CARDIAC HYPERTROPHY

A growing body of evidence has suggested that humoral factors in the circulating blood may have a marginal effects on the development of cardiac hypertrophy in response to hemodynamic overload and that mechanical stress induces cardiac hypertrophy via increasing the local production of humoral factors. How is the mechanical stress perceived by a cardiomyocyte as a stimulus? With the use of Langendorf preparation, it has been shown that stretch of the ventricular wall as a consequence of increased aortic pressure is the mechanical parameter most closely related to an increase in protein synthesis[8]. This observation has been confirmed by the experiments of cardiocytes cultured in vitro with serum-free media. When cardiocytes cultured on deformable silicone membranes were stretched, an increase in protein synthesis was observed[2]. Stretching cardiocytes also induced expression of specific genes, such as immediate early response genes (IEG) and fetal type genes[3, 4, for a review see ref. 5, 6]. These observations suggest that mechanical stress (hemodynamic overload) induces cardiac hypertrophy by stretching cardiomyocytes.

2. NEUROHUMORAL FACTORS MEDIATE MECHANICAL STRESS-INDUCED CARDIAC HYPERTROPHY

Protein kinase cascade of phosphorylation was also activated by mechanical stress on cardiac myocytes and this event is very similar to that observed when growth factors and cytokines are added to many cell types. It was thus conceivable that cardiac myocytes and non-cardiomyocytes such as fibroblasts, endothelial cells and smooth muscle cells secrete some hypertrophy-promoting factors following stretch and that they induce cardiac hypertrophy by autocrine or paracrine mechanism[7].

(1) Angiotensin II

A growing body of evidence has indicated that the local renin-angiotensin system plays a critical role in the development of cardiac hypertrophy[for a review see ref. 8]. All components of the renin-angiotensin system such as angiotensinogen, renin and angiotensin converting enzyme (ACE) have been identified at both the mRNA and protein levels in the heart. Ang II stimulates protein synthesis in cultured cardiomyocytes[8]. Increases in angiotensinogen and ACE mRNAs have been reported in hypertrophied left ventricle of rats[9]. Subpressor doses of ACE inhibitors can prevent or cause regression of cardiac hypertrophy with no change in systemic systolic blood pressure[10]. An increase in left ventricular mass which was produced by abdominal aortic constriction, without significant increase in plasma renin activity, was completely prevented with an ACE inhibitor without any change in afterload[11]. These results strongly suggest that the local renin-angiotensin system but not the circulating one may play a critical role in pressure overload-induced cardiac hypertrophy.

Ang II induces activation of protein kinases

including ERKs and expression of immediate early response genes such as *c-fos* and c-jun genes as well as an increase in protein synthesis in neonatal rat cardiocytes through Ang II type 1 receptor (AT1)[12,13]. Activation of ERKs and up-regulation of *c-fos* gene expression by Ang II are blocked by PKC inhibitors, and Ang II actually increases production of inositol phosphates and activates PKC[12,13]. These signals elicited by Ang II in cardiac myocytes are very similar to those evoked by mechanical stress. The involvement of Ang II in the stretch-induced hypertrophic responses was examined using AT1-specific inhibitors. AT1 antagonists partially but significantly suppressed all stretch-induced hypertrophic responses such as an increase in amino acid incorporation, the induction of *c-fos* gene expression and the activation of ERKs[14,15]. It was reported that Ang II is stored in secretory granules in neonatal ventricular myocytes and that mechanical stretch causes a release of Ang II from cultured cardiocytes[14]. Although it remains to be determined how stretch induces release of Ang II from the secretory granules in cardiac myocytes, these results suggest that Ang II is responsible at least in part for hypertrophic responses evoked by mechanical stress on cardiac myocytes.

Intracellular signaling pathways evoked by Ang II are different among cell types. Ang II markedly activated ERKs in cardiac myocytes. Although Ang II activates Fyn, a Src family tyrosine kinase and Ras[16], inhibition of these molecules had no effects on Ang II-induced ERK activation. Instead, down-regulation of PKC or a PKC inhibitor calphostin C abolished Ang II-induced ERK activation[17]. Ang II also activated Raf-1 kinase and MEK, and the activation of these kinases are also suppressed by inhibition of PKC. Since PKC has been reported to directly activate Raf-1 kinase, Ang II activates ERKs possibly through PKC, Raf-1 and MEK. Signaling pathways from AT1 to ERKs are divergent among cell types. In smooth muscle cells, pertussis toxin had no effects but phospholipase C [PLC], Ca^{2+} and Src family are critical for Ang II-induced activation of ERKs. Ang II activates ERKs possibly through Gq-PLCg-Ca^{2+}-Ca^{2+}-dependent tyrosine kinase Pyk2 and Src-Shc-Grb2-Sos-Ras---ERKs[17]. Cardiac fibroblasts produce and secret extracellular matrix proteins such as collagen and fibronectin in response to Ang II, which induces fibrosis of the heart. In cardiac fibroblasts, Ang II activates ERKs through pertussis toxin sensitive G protein, possibly Gi protein. bg subunit of Gi activates Src and then ERKs through Shc-Grb2-Sos-Ras[18]. It remains to be determined why signaling pathways are so divergent among cell types.

(2) Endothelin-1 (ET-1)

ET-1 has been reported to be a strong inducer of cardiac hypertrophy[19]. Stretch-induced activation of ERKs are also suppressed by typeA ET-1 (ETA) receptor antagonist[20], suggesting that ET-1 is also involved in mechanical stress-induced hypertrophic responses. Since ET-1-induced activation of ERKs is also inhibited by down-regulation of PKC, ET-1 activates ERKs possibly through the pathway same as Ang II. Recently it was reported that Ang II does not have direct potent hypertrophy-promoting effects on cardiac myocytes and that Ang II may evoke hypertrophic responses in cardiac myocytes by inducing ET-1 secretion from cardiac fibroblasts[21]. Cross-talk among different cell types and among many molecules might be important not only for maintaining its highly differentiated phenotype but also for inducing cardiac hypertrophy.

The beneficial effects of ET-1 receptor antagonists on myocardial infarction highlighted the importance of ET-1 on cardiovascular remodeling[22]. Recently the importance of Gq protein in the development of pressure overload-induced cardiac hypertrophy was demonstrated using transgenic mice[23]. Class-specific inhibition of Gq-mediated signaling was produced in the heart of transgenic mice by targeted expression of a carboxyl-terminal peptide of the a subunit of Gq. When pressure overload was surgically produced, the transgenic mice developed significantly less ventricular hypertrophy than control mice. Although the prevention of cardiac hypertrophy was not complete in the transgenic mice, this study suggests that Gq-coupled receptors such as AT1, ETA receptor and other seven transmembrane receptors are important in the development of pressure overload-induced cardiac hypertrophy.

3. MECHANISMS BY WHICH MECHANICAL STRESS IS CONVERTED INTO BIOCHEMICAL SIGNALS

(1) Stretch-Sensitive Ion Channels

Many cells respond to a variety of environmental stimuli by ion channels in the plasma membrane. Mechanosensitive ion channels have been observed with single-channel recordings in more than 30 cell types of prokaryotes, plants, fungi and all animals so far examined [for a review see ref. 24]. The activation of stretch-sensitive channels has been proposed as the transduction mechanism between load and protein synthesis in cardiac hypertrophy [25]. The stretch-sensitive channels allow the passage of the major monovalent physiological cations, Na^+ and K^+, and the divalent cation, Ca^{2+}. With the use of a Ca^{2+}-binding fluorescent dye (fluo3) and the patch clamp technique, mechanically induced Ca^{2+} influx through stretch channels was shown to lead to waves of calcium-induced calcium release[26]. Therefore, stretch-sensitive ion channels are one of good candidates of initial responder for mechanical stress.

The importance of Ca^{2+} in the development of cardiac hypertrophy was recently highlighted [27]. Overexpression of constitutively active mutants of a Ca^{2+}-dependent phosphatase calcineurin and of its downstream transcription factor NFAT3 induced marked cardiac hypertrophy in transgenic mice and phenylephrine- and Ang II-induced cardiomyocyte hypertrophy in vitro was inhibited by calcineurin inhibitors[27]. These observations suggest that an increase in intracellular Ca^{2+} levels could induce cardiac hypertrophy by activating calcineurin. We examined whether calcineurin is also involved in load-induced cardiac hypertrophy. Pressure overload-induced cardiac hypertrophy was significantly suppressed by calcineurin inhibitors such as cyclosporine A and FK506[28,29]. We examined this possibility using the in vitro stretch device. When cultured cardiac myocytes were stretched by 20% for 30 min, brain natriuretic peptide (BNP) gene was upregulated. The upregulation of BNP gene was suppressed by Gadolinium (an inhibitor of stretch-sensitive ion channels) and attenuated by FK506 (a calcineurin inhibitor). These results suggest that Ca^{2+}, which enters cardiomyocytes through stretch-sensitive ion channels, plays a critical role in stretch-induced gene expression.

(2) Extracellular Matrix and Cytoskeleton

Many results have suggested that the mechanical stress is transduced into the cell from the sites at which cells attach to extracellular matrix (ECM)[for a review see ref. 30]. Therefore, transmembrane ECM receptors, such as the integrin family, are good candidates for mechanoreceptors. A large extra-

cellular domain of integrin receptor complex binds various ECM proteins, while a short cytoplasmic domain has been shown to interact with the cytoskeleton in the cell [for a review see ref. 31]. Since cytoskeleton proteins can potentially regulate plasma membrane proteins such as enzymes, ion channels and antiporters, mechanical stress could modulate these membrane associated proteins and stimulate second messenger systems through the cytoskeleton. Integrins can transmit signals not only by organizing the cytoskeleton but also by altering biochemical properties such as the extent of tyrosine phosphorylation of a complex of proteins including $pp125^{fak}$ [30]. Stretch of cultured mesangial cells actually enhanced phosphorylation of $pp125^{fak}$ [32]. We have recently obtained the results indicating that integrin plays a critical role in stretch-induced hypertrophic signals (manuscript in preparation). When outside-in signal of integrin was inhibited, stretch-induced activation of p38MAPK was strongly suppressed. Stretching induced tyrosine phosphorylation of both FAK and Src. Stretch-induced activation of p38MAPK was abolished by overexpression of FAT and CSK,which are inhibitors of FAK and the Src family, respectively. These results suggest that mechanical stress activates p38MAPK through the integrin-FAK-Src pathway in cardiac myocytes.

Rho small G protein family consisting of Rho, Rac and Cdc42 are important for the function of integrins through regulating actin cytoskeleton[33]. When functions of Rho family were inhibited by C3 exoenzyme or by overexpression of Rho GDP dissociation inhibitor and dominant negative mutants, stretch-induced activation of ERKs and expression of IEG were strongly suppressed[34]. Recently it has been reported that C3 exoenzyme inhibits MEKK- and PHE-induced ANP expression but not actin organization, indicating that Rho regulates ANP gene expression but not sarcomere assembly[35]. Sah et al. also reported that Rho is involved in PHE- and Gaq-induced ANP expression but not in Ras- induced ANP expression[36]. Activated RhoA and Ras produced a synergistic effects on ANP gene expression, suggesting that Rho functions in a pathway separate from but complementary to Ras. Treatment of myocytes with Ang II caused a formation of nonstriated actin fiber (premyofibrils), which was abolished by the C3 treatment[37]. Expression of constitutively active RhoA caused the formation of premyofibrils. These results suggest that RhoA is involved in premyofibril formation but not in mature myofibril formation.

4. FUTURE DIRECTIONS

Once mechanical stress is received and converted into biochemical signals, the signal transduction pathway leading to an increase in protein synthesis is similar to the pathway which is activated by various humoral factors such as growth factors, hormones and cytokines in many other cells. Recently, yeast genes encoding members of MAP kinase have been isolated by complementation of yeast mutations as an essential protein for restoring the osmotic gradient across the cell membrane in response to increased external osmorality[38]. This result suggests that mechanical stress-induced intracellular signal transduction pathways are highly conserved in evolusion.

Although many biochemical events which occur in cardiac myocytes subsequent to mechanical stretch have been clarified, a main intriguing question remains unanswered. How is mechanical stress converted into biochemical signals? In other words, what is the mechanoreceptor or the transducer for

mechanical stress in cardiac myocytes? It is conceivable that by stretching the plasma membrane, mechanical stress directly changes conformations of the functional proteins in the membrane such as ion channels, receptors and G-proteins, or directly activates enzymes such as phospholipases by physically placing the enzymes close to their phospholipid substances in the plasma membrane. As mentioned above, the integrin-cytoskeleton complex seems to be an alternate candidate structure for a mechanoreceptor and a transducer. Integrin-cytoskeleton proteins not only play a "passive" role such as maintaining the cell structure, but also may play "active" roles in regulation of cellular functions such as protein phosphorylation and activation of an antiporter. The cytoskeleton has been also shown to play an important role in secretion. Mechanical stress may stimulate secretion of some cytokines, which generate multiple intracellular signals as a secondary event. Further studies are necessary to identify specific signaling molecules, including mecha-noreceptors and mechanotransducers, and characterization of these molecules may pave the way to understand the definit mechanism of mechanical stress-induced cardiac hyper-rophy and to finally clarify the mechanisms by which adaptive cardiac hypertrophy deteriorates into congestive heart failure.

II. Molecular mechanism of cardiac hypertrophy

Murine Csx / Nkx-2.5 is a homeobox-containing gene originally identified as a potential vertebrate homolog of Drosophila gene tinman[39,40]. Loss-of-function mutant of Drosophila tinman gene exhibits complete loss of heart formation, indicating that tinman is essential for the Drosophila heart formation[41,42]. The expression of Csx / Nkx-2.5 is also highly restricted to the heart and the heart progenitor cells from the very early developmental stage when the two heart primordia are symmetrically situated in the anterior lateral mesoderm[39,40], and targeted disruption of the murine Csx/Nkx-2.5 results in embryonic lethality due to the abnormal looping morphogenesis of the primary heart tube[43]. Furthermore, Csx/Nkx-2.5-family genes are also identified in various vertebrate species from zebrafish to humans [44-47]. These results indicate that Csx/Nkx-2.5 is also essential for the normal heart development and morphogenesis in vertebrates like tinman gene in Drosophila, and suggest that the regulatory mechanism of heart development controlled by Csx / Nkx-2.5 is highly conserved in evolution. In order to clarify the molecular framework of vertebrate cardiogenesis, the identification of upstream regulatory factors that control the expression of Csx/Nkx-2.5 gene and the downstream targets of Csx / Nkx-2.5 protein is necessary[48-50].

1. DOWNSTREAM TARGET GENES OF CSX[51]

To search for the direct downstream target genes of Csx/Nkx-2.5 protein, we co-transfected luciferase reporter constructs containing various cardiac-specific gene promoters and the Csx/Nkx-2.5 expression vector into COS-7 cells, and examined whether the transcription of native promoters was activated by the overexpression of Csx/Nkx-2.5. Among the reporter constructs tested, Csx/Nkx-2.5 most strongly transactivated the ANP-luciferase reporter containing 600bp 5' flanking region of rat ANP gene, suggesting that ANP gene is a downstream target of Csx/Nkx-2.5. Although deletion of the ANP 5' flanking region from -600 to -390 did not significantly change the fold activation of the ANP promoter by Csx/Nkx-2.5, the deletion between -390 and -160 markedly reduced

the fold induction of the promoter activity, indicating that the Csx / Nkx-2.5-responsive element is situated between -390 and -160 of ANP 5' flanking region. The consensus Csx/ Nkx-2.5-binding element (TGAAGTG) was located at -250 of ANP promoter. The deletion or the mutation of the Csx / Nkx-2.5-binding element located at -250 of the ANP promoter markedly reduced the fold induction of transactivation by Csx/Nkx-2.5.

2. CAS/NKX-2.5 AND GATA-4 INTERACT AND SYNERGISTICALLY TRANSACTIVATE ANP PROMOTER

Because GATA site was located at -280 just distal from the Csx/Nkx-2.5 binding site, we examined whether Csx/Nkx-2.5 and GATA-4 cooperatively regulate the transcription of ANP gene. When ANP(300)-luc construct containing 300bp 5' flanking region of ANP promoter was co-transfected with Csx/Nkx-2.5 expression plasmid alone, modest transactivation of ANP promoter was observed. When ANP(300)-luc was co-transfected with GATA-4 expression plasmid alone, no significant increase in the promoter activity was observed, suggesting that GATA-4 is a weak transactivator of ANP promoter in our assay condition. However, overexpression of both GATA-4 and Csx / Nkx-2.5 induced much stronger activity of ANP promoter than that induced by the expression of Csx / Nkx-2.5 alone, suggesting that Csx / Nkx-2.5 and GATA-4 synergistically transactivate ANP promoter.

Removal of the region between -300 and -270 of the ANP promoter, which resulted in the deletion of the GATA site at -280, had little effect on the Csx / Nkx-2.5-GATA-4 synergism as well as Csx / Nkx-2.5-induced activation. However, deletion of the promoter region between -270 and -240, in which the Csx/Nkx-2.5 binding site is located, abolished not only Csx/Nkx-2.5-induced activation of ANP promoter but also the cooperativity between Csx/Nkx-2.5 and GATA-4, suggesting that the Csx/Nkx-2.5 binding site, but not the GATA site at -280, is required for the Csx / Nkx-2.5-GATA-4 transcriptional synergism. Co-immunoprecipitation experiments revealed that Csx/Nkx-2.5 and GATA-4 interact in vivo.

GST pull-down assay showed that the homeodomain of Csx/Nkx-2.5 and zinc finger domain of GATA-4 are important for their association.

In *Drosophila*, the homeobox-containing gene *tinman* is expressed in the developing dorsal vessel, and its expression is regulated by *decapentaplegic* (*dpp*), a member of the transforming factor-β (TGF-β) superfamily [52].

3. BMP, A MEMBER OF TGFb SUPERFAMILY PLAYS A CRITICAL ROLE IN CARDIOMYOCYTE DIFFERENTIATION [53]

To determine whether BMP, a mammalian dpp homolog, is necessary for cardiomyocyte differentiation, we used a clonal derivative named P19CL6, which efficiently differentiates into beating cardiac muscle in adherent culture with 1% DMSO. In order to confirm that BMP signaling pathways are involved in cardiomyocyte differentiation in P19CL6 cells, expression vectors carrying mutants of TAK1, which is a member of MAPKKK family and is thought to be one of the mediating molecules that transduct BMP signaling [54], were transfected into P19CL6noggin cells. Two days after the initiation of the DMSO treatment (day 2), expression plasmids containing wild-type mouse TAK1 cDNA (TAK1WT), constitutively active form of TAK1 (TAK1 Δ N), and dominant negative

form of TAK1 (TAK1KN) were transiently transfected by the lipofection method. We designated these cells +TAK1 Δ N and +TAK1KN, respectively. The culture was maintained continuously in differentiation medium, until immunofluorescense study or RNA extraction was performed on day 14. Unlike the control P19CL6noggin cells, P19CL6-noggin+TAK1WT and +TAK1ΔN cells partially differentiated into mononucleate, spontaneously beating cardiomyocytes, suggesting that overexpression of TAK1 can rescue the differentiation defect of P19CL6noggin

To make further elucidation of how BMP signaling is involved in the induction of cardiac-specific transcription factors, expression vectors containing Csx / Nkx-2.5 and GATA-4 were transfected into P19CL6noggin cells and their cardiogenic activity was examined. Expression plasmids containing human CSX1a cDNA, mouse GATA-4 cDNA and both of them were transfected into P19CL6noggin cells. We designated these cells P19CL6-noggin+CSX, +GATA-4 and +CSX+GATA-4, respectively. Interestingly, although P19CL6-noggin+CSX and P19CL6noggin+GATA-4 did not differentiate into beating cardiomyocytes, only P19CL6noggin+CSX+GATA-4 did differentiate in part into rhythmically contracting cardiomyocytes. These results suggest that both Csx/Nkx-2.5 and GATA-4, but not either of them, are required for cardiomyocyte differentiation in P19CL6noggin cells and that both of them are regulated by BMP function.

4. SPECULATION ON REGULATORY NETWORK CONTROLLING HEART DEVELOPMENT

In the avian embryo, recent excellent work by Lassar and colleagues has revealed that BMP-2 and BMP-4 administration can induce full cardiac differentiation in the anterior medial mesoderm, a tissue that is not normally cardiogenic, but not in the posterior mesoderm, and also has demonstrated that administering noggin to explanted precardiac mesoendoderm inhibits cardiac differentiation of this tissue[55]. Taken together, it has been considered that BMP is necessary for avian cardiogenesis, but not sufficient on its own and that the anterior endoderm is likely to produce a factor that interacts with BMP to induce cardiogenesis specifically in anterior mesoderm[55]. Our current study utilizing P19CL6, a newly-established useful in vitro model system, has demonstrated for the first time that BMP is indispensable for the induction of cardiomyocyte differentiation in murine cell line. On the other hand, when cultured continuously without the DMSO treatment, even parental P19CL6 cells do not differentiate into cardiomyocytes (data not shown). This finding indicates that BMP function on its own does not seem to be sufficient for cardiogenesis and that another crucial factors for cardiomyocyte differentiation in this cell line are probably some signals induced by the DMSO treatment. The precise roles for DMSO in the differentiation of P19 cells are currently unknown. We consider that DMSO, when administered at appropriate concentration, is likely to transactivate some unknown factors which are involved in the cardiac specification in this cell line, and both of BMP and the unknown factors seem to cooperatively function to determine the cardiogenic cell fates. In P19 cells, untreated aggregates contain a few extra-embryonic endodermal cells as well as undifferentiated embryonal carcinoma cells, and the similar endoderm-like cells are also observed in P19CL6 cells (data not shown). It may be possible that factors produced by these endoderm cells after the DMSO treatment function on other

undifferentiated cells to become cardiac muscle just like the model presented previously regarding the avian cardiogenesis, in which signals from anterior endoderm interact with BMP to induce cardiogenesis specifically in anterior mesoderm[55]. Identification of the unknown factors induced by the DMSO treatment in this P19 cell derivative can be the great breakthrough for the elucidation of the mechanisms of cardiac development.

要　旨

心不全発症の機序を知るには，心肥大発生の機序を理解することが重要である。一種の機械的刺激である血行力学的負荷が心肥大形成の主要な刺激である。機械的刺激により心肥大が形成される機序を明らかにするために，我々は伸縮自在のシリコンゴムで培養皿を作り，その上に培養したラットの新生仔心筋細胞を伸展した。心筋細胞を伸展したところ，種々のリン酸化酵素が活性化され，特異的遺伝子の発現が誘導され，蛋白質の合成が亢進した。この過程においてアンジオテンシンⅡやエンドセリンなどの血管作動物質が重要な役割をしていた。最近，機械的刺激を受容し，生化学的シグナルに変換する“機械的刺激受容体”の実体もわかってきた。

心不全の治療法として遺伝子治療や細胞移植治療は有望である。これらの新しい治療法を可能とするには，心臓の発生，心筋細胞分化に関する理解が必要である。我々は心臓の発生に必須な転写因子としてホメオボックス遺伝子 Csx を単離した。Csx は胎生早期より心臓の予定中胚葉領域に発現する。Csx を欠失したマウスの心臓は発生が途中で止まり，胎生9.5日で致死となる。ジンクフィンガーモチーフを持つ転写因子 GATA-4 と会合することにより，Csx は心筋細胞分化を強力に推進する。Csx の変異は心房中隔欠損症，心室中隔欠損症，エプスタイン奇形，房室ブロックなど多くの心疾患の原因となることも明らかとなってきた。

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