MiniReview

Small Guanine Nucleotide-Binding Proteins and Myocardial Hypertrophy

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Abstract—The small (21 kDa) guanine nucleotide-binding protein (small G protein) superfamily comprises 5 subfamilies (Ras, Rho, ADP ribosylation factors [ARFs], Rab, and Ran) that act as molecular switches to regulate numerous cellular responses. Cardiac myocyte hypertrophy is associated with cell growth and changes in the cytoskeleton and myofibrillar apparatus. In other cells, the Ras subfamily regulates cell growth whereas the Rho subfamily (RhoA, Rac1, and Cdc42) regulates cell morphology. Thus, the involvement of small G proteins in hypertrophy has become an area of significant interest. Hearts from transgenic mice expressing activated Ras develop features consistent with hypertrophy, whereas mice overexpressing RhoA develop lethal heart failure. In isolated neonatal rat cardiac myocytes, transfection or infection with activated Ras, RhoA, or Rac1 induces many of the features of hypertrophy. We discuss the mechanisms of activation of the small G proteins and the downstream signaling pathways involved. The latter may include protein kinases, particularly the mitogen-activated or Rho-activated protein kinases. We conclude that although there is significant evidence implicating Ras, RhoA, and Rac1 in hypertrophy, the mechanisms are not fully understood. (Circ Res. 2000;86:1019-1023.)

Key Words: cardiac myocyte hypertrophy ■ Ras ■ Rho ■ signal transduction ■ transgenic mice

Ventricular myocyte hypertrophy is an important adaptive growth response facilitating an increase in myocardial contractility.1 It is associated with changes in myocyte morphology (increased cell size and myofibrillogenesis) and alterations in gene expression (eg, upregulation of genes expressed early in ventricular development, such as atrial natriuretic factor [ANF] and the related B-type natriuretic peptide [BNP]). Reexpression of such genes is a frequently used index of myocyte hypertrophy. Many stimuli induce hypertrophy in cultured cardiac myocytes. The best characterized are those that couple through heterotrimeric G protein–coupled receptors (GPCRs), particularly the G_{q/11}PCR subfamily. These agonists include endothelin-1 (ET-1), α-adrenergic agonists such as phenylephrine (PE), and angiotensin II (Ang II). Because small guanine nucleotide-binding proteins (small G proteins) regulate the growth and morphology of dividing cells, their role in the development of myocyte hypertrophy has become a focus of interest.

The small G protein superfamily comprises a multitude of proteins with relative molecular masses of ~21 kDa, which regulate a wide variety of cellular processes. In their inactive state, they are ligated to GDP and are activated by exchange of GDP for GTP. This is enhanced and regulated by guanine nucleotide exchange factors (GEFs) (Figure 1). The inactive GTPase activity of small G proteins hydrolyzes bound GTP to GDP, returning them to their inactive states. This GTPase activity is stimulated by GTPase-activating proteins (GAPs). In addition, guanine nucleotide dissociation inhibitors restrain small G proteins in their inactive states.

Five subfamilies of small G proteins have been characterized (Ras, Rho, ADP ribosylation factors [ARFs], Rab, and Ran), each consisting of multiple members. All are lipid-modified and associate with membranous structures within the cell. Only the Ras and Rho subfamilies have been studied in the heart. The Ras subfamily includes the 3 classical Ras isoforms (Harvey [HRas], Kirsten [KRas], and NRas [not HRas or KRas]), Rap, and Ral.2 The Rho subfamily includes RhoA, Rac1, and Cdc42.3,4 Mutated forms are used to investigate the involvement of small G proteins in cellular processes. These include constitutively activated forms (eg, [Gly-12→Val]Ras [V12Ras], V12Rac1, and V14RhoA) and dominant inhibitory (dominant-negative) forms (eg, [Ser-17→Asn]Ras [N17Ras], N17Rac1, and N19RhoA).

The functions of specific small G proteins are not fully understood. Classical Ras isoforms regulate cell survival, growth, and division, effects that are probably mediated through the extracellular signal–regulated protein kinase (ERK) subfamily of the mitogen-activated protein kinases (MAPKs) or phosphatidylinositol 3'-kinase (PI3K) (Figure 2). RhoA, Rac1, and Cdc42 are involved in the regulation of the actin/myosin cytoskeleton in many cells.4 The effects of RhoA on cellular architecture may be mediated through...
Rho-dependent Ser/Thr protein kinases. Rac1 and Cdc42 stimulate p21-activated kinases (PAKs), which may regulate disclosures of p21-activated kinases (PAKs), which may regulate discations that the biological functions of specific proteins in vivo will be resolved readily. However, experience so far has shown that some of these studies are equivocal. Although developing methodologies that allow inducible tissue-specific activations of transgenes or tissue-specific knockouts may be more revealing, mouse models may never fully simulate the situation in humans. To study small G proteins, transgenic mice that cardiосpecifically (predominantly postnataⅽally) overexpress HRas or RhoA have been developed. Hunter et al produced mice that were homozygous for a cardiac-targeted V12Hras transgene. V12Hras mRNA was expressed predominantly in the ventricles, although it was also expressed at a lower level in the atria. These animals displayed hallmarks of left ventricular (LV) hypertrophy (increased LV to body weight ratio, myocyte cross-sectional area, and abundance of ANF transcripts) in the absence of systemic hypertension or changes in heart rate. Although the age at which the mice were examined was not specified, the studies were conducted at a stage when the mice seemed to be progressing from compensated hypertrophy into diastolic heart failure and showed no chamber dilatation. The phenotype was qualitatively similar to human compensated (left-sided) hypertensive heart disease, but there was also evidence of diastolic dysfunction as seen in human familial hypertrophic cardiomyopathy. The overall interpretation is that HRas promotes cardiac hypertrophy in this context and reproduces features of hypertrophic cardiomyopathy rather than dilated cardiomyopathy. The involvement of HRas was not entirely unambiguous because there was no significant hypertrophy in the right ventricle, although expression of the transgene was similar to that in the LV. Furthermore, heterozygous littermates showed no LV hypertrophy despite significant expression of V12Hras mRNA. Additional analysis of transgenic mice derived from V12Hras homozygote parents that were echocardiographically selected and demonstrably hypertrophic suggested considerable variability of phenotype. Approximately 25% of the mice died within 3 weeks. Survivors showed evidence of myofibrillar disarray, but other changes (eg, fibrosis) were variable in extent. In addition, expression of hypertrophic index genes suggested that the transcriptional changes differed from those seen in experimental in vivo pressure overload. Thus, although Ras promotes hypertrophy in transgenic animals, its precise role in the pathophysiological hypertrophic response in healthy animals remains unclear. Ras-dependent signaling probably represents one of the many pathways activated during a pleiotropic response to biomechanical stress. The precise overall phenotype reflects the balance of these pathways in the overall context of the genetic and environmental background.

Transgenic mice overexpressing wild-type RhoA or a constitutively activated RhoA mutant in the atrial and ventricular compartments have been generated. Heterozygotes died prematurely with signs of ventricular dilatation (but not increased mass) and dysfunction, accompanied by changes in hypertrophic index gene expression, increases in atrial mass, marked conduction abnormalities, and other signs of heart failure. Although these mice are of interest, the phenotype is so dissimilar from compensated hypertrophy that firm conclusions about the role of RhoA in hypertrophy cannot be drawn. Given the role of RhoA in regulating cytoskeletal organization in other cells, and allowing for possible leakage of transgene expression during development, the effects of RhoA could represent developmental modulation of cellular architecture.
Small G Proteins and Hypertrophy in the Cultured Cardiac Myocyte Model

Ras Subfamily

Several Ras family proteins are readily detected in primary cultures of neonatal rat ventricular myocytes, including HRas, NRas, KRas, and Rap111 (and unpublished data, A.C. and P.H.S., 1999). Transient transfection with a V12Ras expression vector or microinjection of the protein induces hypertrophic gene expression and increased cell profile.12–14 Although microinjection of V12Ras protein or adenoviral infection of the V12ras gene may induce myofibrillogenesis,12,13 this is not observed in transfection studies.12,14 The reasons for this are unclear but may reflect levels of V12Ras expression. Inhibitory N17Ras prevents PE-induced hypertrophic changes.12 However, its effects are mediated through interaction with Ras.GEFs, which are promiscuous with respect to the activation of small G proteins.16 Thus, some responses inhibited by N17Ras may be mediated by non-Ras small G proteins.

Rho Subfamily

RhoA and Rac1 are implicated in cardiac myocyte hypertrophy. Transfection or injection of activated RhoA stimulates ANF expression15,17,18 and myofibrillogenesis.15,19 Dominant inhibitory mutants of RhoA prevent PE-stimulated,17,20 Gq-stimulated,20 or Ras-induced20 hypertrophy. Furthermore, C3 exoenzyme, a clostridial toxin that selectively inhibits Rho, inhibits ANF expression induced by hypertrophic stimulation.17–20 Because Rho is implicated in the regulation of the actin-myosin cytoskeleton,4 studies have focused on its role in myofibrillogenesis. V14RhoA promotes formation of myofibrillar structures,15,19 and in some studies C3 exoenzyme inhibits this process.15,19 However, others have failed to detect an effect of C3 exoenzyme on actin myofilaments.18 These discrepancies may reflect the extent of exposure to the toxin or route of administration. The effects of RhoA on the myofibrillar apparatus may be secondary to modification of the nonmyofibrillar cytoskeleton. Thus, electroporation of C3 exoenzyme into myocytes causes disassembly of focal adhesions and loss of β-actin nonstriated fibrils.21

As with V12Ras or V14RhoA, transfection of cardiac myocytes with V12Rac1 increases expression of ANF and BNP.22 Adenoviral infection of myocytes with V12Rac1 also increases ANF expression and promotes morphological changes associated with myocyte hypertrophy, and infection with N17Rac1 inhibits PE-induced hypertrophy.23 These results implicate both RhoA and Rac1 in cardiac myocyte hypertrophy.

Signaling Through Small G Proteins

Mechanisms of Activation

The classical Ras isoforms are rapidly activated in myocytes exposed to Ang II, ET-1, and PE.11,24 These agonists stimulate protein kinase C (PKC) through Gq/11,PCRs, the Gq/11 heterotrimeric G proteins, and phospholipase Cβ.3 Phorbol esters, which activate PKC directly and induce myocyte hypertrophy,1 also stimulate Ras.GTP loading.11 Conversely, inhibition of PKC reduces Ras.GTP loading by GPCR agonists.11 These findings suggest that the activation of Ras by Ang II, ET-1, or PE is PKC dependent. However, other PKC-independent signaling molecules (eg, the pertussis toxin-sensitive Gq, heterotrimeric G proteins) may also be involved.11 It is not clear how GPCR-mediated signaling couples to Ras. As with receptor protein tyrosine kinase stimulation of Ras, adapter proteins (GRB2, She) may be involved, which activate the Ras.GEF Sos.2

Activation of Rho subfamily proteins in cardiac myocytes is not well characterized. Ang II induces RhoA translocation to the particulate fraction, consistent with its activation.19 ET-1 and PE stimulate GTP loading of RhoA and Rac1 (A.C. and P.H.S., unpublished data, 1999). The mechanisms involved are not clear, but in other cells, Rac1 activation is dependent on PI3K,3 which is itself a Ras effector (see below). Additionally, Ras.GEFs are promiscuous and may stimulate GTP loading of several small G proteins.16

Coupling of Ras to Hypertrophy

Ras binds to and activates several signaling proteins, including c-Raf (MAPK kinase kinase of the ERK cascade), PI3K, and Rap.GDS (GEF for the Rap-like protein Rap) (Figure 2).2 The ERK cascade is implicated in hypertrophy, as reviewed recently.1 Thus, potent hypertrophic stimuli (eg, ET-1, PE, and phorbol esters) activate the ERK cascade, activated components of the cascade stimulate ANF expression and increase myocyte cell profile, and dominant inhibitory components attenuate agonist-stimulated responses. However, not all agonists that activate ERKs induce hypertrophy, and there is little evidence that ERKs promote myofibrillogenesis. Adenoviral infection of signaling molecules has generally been more successful in promoting myofibrillogenesis, but such experiments have not been reported for ERK cascade components. It is probable that additional signaling pathways are necessary for myofibrillogenesis. Studies with V12Ras double mutants that couple selectively to c-Raf, PI3K, or Rap.GDS indicate that c-Raf and Rap.GDS specifically promote hypertrophic gene expression, whereas PI3K has a more global effect.14 PI3K may act at the level of protein synthesis (reporter genes are transcribed and must be translated for detection), because signaling from PI3K promotes protein synthesis in cardiac myocytes.1,25 Indeed, increased protein synthesis is a hallmark of hypertrophy, and PI3K inhibitors suppress protein synthesis in cardiac myocytes.1

Some researchers propose that Ras-mediated activation of the JNK cascade is more relevant to hypertrophy.24 One line of evidence is that JNK activity is increased, albeit minimally (50%), in hypertrophied hearts of the V12Hras transgenic mouse with no increase in ERK activity. Because these animals were examined at 6 weeks (a considerable period of time subsequent to the initiating hypertrophic signals), JNK activation may reflect the stress of progression from compensated hypertrophy into heart failure. Indeed, there is evidence from patients with heart failure subsequent to ischemic heart disease that this is the case.26 We have previously reviewed the evidence implicating JNKs in hypertrophy,6 and our view is that this pathway is more probably associated with apoptosis.
One function of MAPKs is to modulate gene transcription by phosphorylation of transcription factors (eg, Elk1, c-Jun, and ATF2), altering their transactivating activities. Consistent with this, ERKs and JNKs are active in the nuclei of myocytes after stimulation, and c-Jun and ATF2 are both phosphorylated in myocytes subjected to cellular stress or exposed to hypertrophic stimuli.

**Coupling of RhoA and Rac1 to Hypertrophy**

The signaling pathways activated by RhoA to promote hypertrophy are not understood. In other cells, RhoA regulates cell morphology and contractile activity, and, because myocyte hypertrophy involves changes in cell shape, cytoskeletal modifications are likely to occur. RhoA activates several protein kinases, two groups of which have been identified: protein kinase N/PKC-related kinases and Rho kinases. The Rho kinases (\(\approx 160\) kDa) include Rho kinase itself (ROK\(_{\alpha}\) or ROCK2) and the related p160ROCK (ROK\(_{\beta}\) or ROCK1, also known as Rho kinase). ROK\(_{\alpha}\) may stimulate LIM kinase to phosphorylate and inactivate cofilin. Because cofilin promotes actin depolymerization, the net effect of RhoA through this pathway is to promote formation of actin fibrils. RhoA activation of Rho kinase promotes phosphorylation of myosin light chains (MLCs) and inhibition of MLC phosphatase. This increase in MLC phosphorylation regulates cytoskeletal organization.

ET-1–induced hypertrophy is inhibited by the Rho kinase selective inhibitor Y27632, and some dominant-negative mutants of Rho kinase inhibit PE-, ET-1–, or RhoA-induced hypertrophy, suggesting that Rho kinase is involved in the hypertrophic response. However, interpretation of some of these experiments may not be simple. For example, the Rho-binding domain of Rho kinase inhibits V14RhoA- or PE-induced myofibrillogenesis, but this may reflect general sequestration of activated RhoA rather than Rho kinase acting as a signaling intermediate. The kinase-dead form of Rho kinase should be a more specific inhibitor of Rho kinase signaling, but this did not inhibit PE-induced myofibrillogenesis and was less effective than the Rho kinase–binding domain in inhibiting myofibrillogenesis or ANF expression induced by activated RhoA. However, others showed that the kinase-dead Rho kinase inhibited ET-1–induced BNP expression. Equally contradictory are the data indicating that N19RhoA, but not the Rho kinase–binding domain, inhibits V12Ras-induced ANF expression. Thus, the role of Rho kinase in hypertrophy requires further clarification.

There is evidence that Rac1 participates in the hypertrophic response. In some cells, Rac1 activates PAKs, which may promote activation of ERKs or stimulate JNKs and p38-MAPKs. In cardiac myocytes, Rac1 cooperates with c-Raf to promote ERK activation and ANF expression (A.C. and P.H.S., unpublished data, 1999). Although Rho family proteins may promote JNK activation in cardiac myocytes, we have no evidence to suggest that they activate p38-MAPKs (A.C. and P.H.S., unpublished data).

As mentioned above, myocyte hypertrophy involves changes in cell architecture and cell-cell interactions. Activation of PAKs by Rac1 and Cdc42 regulates cell-cell interactions and cell architecture in other cells by modulating the actomyosin cytoskeleton and cell motility and migration. PAKs affect the phosphorylation state of cytoskeletal MLCs, although whether they promote or diminish phosphorylation is controversial. Thus, PAKs may phosphorylate and inactivate MLC kinase and diminish MLC phosphorylation (which should antagonize the effects of RhoA), but they have also been reported to promote MLC phosphorylation, potentially reinforcing the effects of RhoA. Whether the increase in MLC phosphorylation is mediated through MLC kinase is not clear, although PAKs may directly phosphorylate MLC. Additionally, PAKs may also phosphorylate LIM kinase, which may be expected to synergize with RhoA to promote cytoskeletal organization. To our knowledge, there is no direct evidence in cardiac myocytes to link activation of Rac1 to any of the downstream molecular-signaling events described here.

**Conclusions**

The overwhelming body of evidence from studies in cultured cardiac myocytes strongly implicates Ras, RhoA, and Rac1 in the development of hypertrophy, but the signaling pathways involved are only partially understood. Although it is clear, in our opinion, that the ERKs are likely to play a significant role in the \(G_{q11}\),PCR/PKC-mediated hypertrophy induced by ET-1 and phorbol esters, other pathways obviously contribute to PE-induced hypertrophy. It is feasible that the overall balance of signals promoting hypertrophy determines the response of the cell. For example, for phorbol esters, the powerful activation of the Ras \(\rightarrow\) ERK cascade alone is sufficient to induce hypertrophy. In contrast, an agonist such as PE may modestly activate several pathways (perhaps stimulating Ras, RhoA, and Rac1) over a longer time period to produce the same overall effect. Such complex interactions between signaling pathways may explain why transgenic models have been less than illuminating with the phenotype rarely simulating a compensated hypertrophy (as opposed to a pathological decompensated hypertrophy). The principal conclusion from such models is that constitutive activation of Ras or Rho signaling produces pathological changes in the heart that may not necessarily be related to myocyte growth.

**Note Added in Proof**

The generation of a mouse cardiospecifically expressing a \(V12Rac1\) transgene has been reported recently (Sussman MA, Welch S, Walker A, Klevitsky R, Hewett TE, Price RL, Schaefer E, Yager K. Altered focal adhesion regulation correlates with cardiomyopathy in mice expressing constitutively active rac1. J Clin Invest. 2000;105:875–886). Two distinct phenotypes (a lethal neonatal dilated cardiomyopathy and a resolving transient cardiac hypertrophy in juveniles) were detected. Because PAK translocated from the cytoplasm to the cytoskeleton, the authors suggest that activation of Rac1 may induce reorganization of focal adhesions and the cytoskeleton, and this is an event common to both phenotypes.

**Acknowledgments**

We thank Dr. Ken Chien (University of California, San Diego) for his helpful comments. Owing to space limitations, we have been unable to include many original references and apologize to the authors involved.
References

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*Circ Res.* 2000;86:1019-1023
doi: 10.1161/01.RES.86.10.1019

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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