Increased Myocardial Rab GTPase Expression
A Consequence and Cause of Cardiomyopathy

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Abstract—The Ras-like Rab GTPases regulate vesicle transport in endocytosis and exocytosis. We found that cardiac
Rabs1, 4, and 6 are upregulated in a dilated cardiomyopathy model overexpressing β2-adrenergic receptors. To
determine if increased Rab GTPase expression can contribute to cardiomyopathy, we transgenically overexpressed in
mouse hearts prototypical Rab1a, the small G protein that regulates vesicle transport from endoplasmic reticulum to and
through Golgi. In multiple independent mouse lines, Rab1a overexpression caused cardiac hypertrophy that progressed
in a time- and transgene dose–dependent manner to heart failure. Isolated cardiac myocytes were hypertrophied and
exhibited contractile depression with impaired calcium reuptake. Ultrastructural analysis revealed enlarged Golgi stacks
and increased transitional vesicles in ventricular myocytes, with increased secretory atrial natriuretic peptide granules
and degenerative myelin figures in atrial myocytes; immunogold studies localized Rab1a to these abnormal vesicular
structures. A survey of hypertrophy signaling molecules revealed increased protein kinase C (PKC) α and δ, and
confocal microscopy showed abnormal subcellular distribution of PKCα in Rab1a transgenics. These results indicate
that increased expression of Rab1 GTPase in myocardium distorts subcellular localization of proteins and is sufficient
to cause cardiac hypertrophy and failure. (Circ Res. 2001;89:1130-1137.)

Key Words: Rab1 GTPase ■ transgenic mouse ■ vesicle transport ■ cardiac hypertrophy ■ cardiomyopathy

Hypertrophy is the compensatory increase in myocardial mass in response to mechanical and toxic cardiac injury
or persistent increased hemodynamic loading. In undergoing any type of hypertrophic process, cardiac myocytes must
increase subcellular transport of constituent proteins. Indeed, an increase in myocyte synthetic machinery for proteins has
been observed in hypertrophied hearts.1,2 However, mechanisms regulating subcellular protein trafficking have not been
critically examined in the heart, and the possible pathological consequences of aberrant cardiomyocyte protein transport are
unknown. Absence of data in this area is especially noteworthy since regulation of subcellular intercompartmental protein
transport is governed by the Rab family of small G proteins,3 and related Ras, Rac, and Rho small G proteins have each
been thoroughly investigated in cardiac hypertrophy and failure.4 Perhaps, although they are the largest subfamily of
Ras-like small G proteins, Rab GTPases have been overlooked in this regard because they are not signaling mole-
cules. Unlike other Ras-like G proteins, which are integral members of cell-signaling cascades, Rab GDP/GTP exchange
regulates protein internalization and secretion by controlling vesicle docking and fusion throughout the cell.5 These func-
tions are critically important for all types of cellular growth, presumably including cardiomyocyte hypertrophy. Relevant
to the question of Rab G-protein function in cardiomyocyte hypertrophy, we recently found that expression of some Rab
family members is increased in a genetic model of dilated cardiomyopathy (see Results). Whether regulated Rab ex-
pression per se has any effects on cardiac function or simply represents a nonspecific response to myocardial growth is
unknown.

The present studies were undertaken to test the hypothesis that modified protein trafficking as a consequence of upregu-
lated Rab G-protein expression can alter cardiomyocyte and whole-heart structure and function. Since these are the first
investigations of any Rab G proteins in the heart, we chose to study the Rab family member with the broadest biological
effects, and which we therefore considered most likely to cause an in vivo overexpression phenotype. This is Rab1, the
most studied mammalian Rab member and the structural analogue of the prototypical Saccharomyces cerevisiae Rab
GTPase YPT1.6 In vitro expression studies have previously shown that Rab1 regulates the earliest stage of protein
trafficking through the secretory pathway, ie, vesicle trans-

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port from endoplasmic reticulum (ER) to and through Golgi stacks.7–9 Furthermore, we have found that a Rab1 isofrom is upregulated in murine dilated cardiomyopathy that occurs with β-adrenergic receptor overexpression10 (see Results). Herein, we report that cardiomyocyte-specific overexpression of Rab1a causes myocardial hypertrophy that progresses to dilated cardiomyopathy at a rate directly related to expression level. Hearts of these mice exhibit characteristic ultrastructural abnormalities of secretory organelles that anticipate functional deterioration.

Materials and Methods

Creation of Rab1 Transgenic Mice

A 1431-bp DNA fragment encompassing 28 bp of 5′-untranslated region, the mouse Rab1a coding region, and 785 bp of 3′-untranslated region (AF226673) was cloned into the mouse promoter region. Rab1a coding was then inserted into the mouse promoter region. After removal of the GST-Rab1a from the eluates by reincubation with glutathione-Sepharose, Rab-binding proteins were concentrated, size-separated by SDS-PAGE, and identified by MALDI mass spectrometric analysis at the University of Cincinnati Proteomics Core facility.

Assessment of Cardiac Hypertrophy and Function

Protein complexes eluted with high salt buffer (1.5 mmol/L NaCl). After removal of the GST-Rab1a from the eluates by reincubation with glutathione-Sepharose, Rab-binding proteins were concentrated, size-separated by SDS-PAGE, and identified by MALDI mass spectrometric analysis at the University of Cincinnati Proteomics Core facility.

Statistical Analysis

Data are expressed as mean ± SEM. Comparisons between nontransgenic (NTG) and transgenic mice were evaluated using Student’s t test; a value of P < 0.05 was considered statistically significant.

Results

Regulation of Rab GTPases in β-Adrenergic Receptor–Induced Cardiomyopathy

As part of an ongoing comprehensive characterization of G-protein signaling in normal and diseased myocardium, we surveyed myocardial expression of Rab small G proteins. Since no published data exist on myocardial Rab-GTPases, we assayed mouse and human myocardium to determine which Rab family members were expressed in the heart. In both species, Rab1, 3, 4, 5, and 6 were readily detected (Figure 1A). Of the isoforms of Rab1, both 1a and 1b were present in human heart, but only Rab1b was detected in mouse heart (Figure 1A). Rabs 7, 8, and 11 were undetectable by Western blot in all cardiac tissues (not shown). Comparison of NTG mouse heart to myocardium from high and lower expressor (Figure 1B). Of the isoforms of Rab1, both 1a and 1b were present in human heart, but only Rab1b was detected in mouse heart (Figure 1A). Rabs 7, 8, and 11 were undetectable by Western blot in all cardiac tissues (not shown). Comparison of NTG mouse heart to myocardium from high and lower β2-adrenergic receptor (AR)–overexpressing hearts10,22 showed increased expression of some, but not all, Rab proteins in the cardiomyopathic hearts. Expression of Rab1, 4, and 6, but not Rab3 or 5, was significantly increased in the higher-expressing line, whereas only Rab6 was significantly upregulated in the lower expressor (Figure 1B). Rab1 and 6 regulate, respectively, antegrade and retrograde vesicle transport from endoplasmic reticulum (ER) to and through the Golgi stack,7–9.23,24 whereas Rab4 regulates protein recycling to plasma membranes.25 Thus, multiple Rab GTPases functioning at different steps in the vesicular transport process were regulated in the cardiomyopathy that develops as a consequence of β2-AR overexpression.

Hypertrophy Progression to Heart Failure in Rab1 Transgenic Mice

Since we observed increased expression of myocardial Rab proteins in the β2-AR–induced cardiomyopathy, we hypothesized that disordered protein trafficking caused by upregulated Rab proteins might be sufficient to cause some aspects
of cardiac pathology. In testing this hypothesis, we elected to overexpress Rab1 because it was upregulated in the \( \beta_2 \)-AR–expressing cardiomyopathic mouse. Rab1 is the mammalian analogue of the prototypical yeast Rab family member YPT1 (and is therefore the most thoroughly studied and best understood mammalian Rab family member); Rab1 also modulates the earliest and most generalizable events in protein transport and vesicular trafficking from ER to and through the Golgi apparatus. Since the two isoforms of this enzyme, Rab1a and 1b are \( \sim90\% \) identical and have identical functions, we took advantage of the observation that Rab1b accounted for virtually all Rab1 protein in mouse ventricles to express, instead, Rab1a. In this manner, the transgenic Rab1 could be specifically assayed in biochemical, immunofluorescence, and ultrastructural studies using an isoform-specific antibody, without introducing the potentially confounding functional changes that can occur with epitope tagging. Three lines of transgenic mice were identified with transgene copy numbers of 5, 7, and 15, designated as Rab1low, Rab1med, and Rab1high, respectively (Figure 2A). Ventricular Rab1a protein expression in the ventricles determined by Western blot analysis increased in proportion to transgene copy number (Figure 2A).

First-generation (F1) Rab1high transgenic mice died prematurely at \( 43 \pm 1 \) days of age; none of these mice was viable past the age of 47 days. Therefore, data reported herein for Rab1high mice are from six 6-week-old F1 transgenic mice (of 32 total pups) derived from the female founder. Hearts of Rab1a overexpressors, at the time that they developed heart failure, were grossly enlarged with dilated cardiac chambers and atria containing laminar thrombus (Figure 2B). Heart weight indexed to body weight was significantly increased compared with NTG sibling controls (Table 1). Consistent with morphological and gravimetric data, both end-systolic dimension (ESD) and end-diastolic dimension (EDD), measured by in vitro echocardiography, were increased (Table 1). Left ventricular wall thickness in Rab1 transgenic mice was similar to NTG controls, but the increase in ventricular wall thickness was modest compared to the marked increase in heart weight. Table 1 summarizes the morphological and echocardiographic data for 6-week-old and 1-year-old Rab1 transgenic mice compared to age-matched NTG controls.

**TABLE 1. Characteristics of High- and Low-Expressing Rab1 Mice**

<table>
<thead>
<tr>
<th></th>
<th>6-Week-Old Mice</th>
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<th>1-Year-Old Mice</th>
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<tbody>
<tr>
<td></td>
<td>NTG (n=8)</td>
<td>Rab1 High (n=6)</td>
<td>NTG (n=5)</td>
</tr>
<tr>
<td>Morphometry, mg/g</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Heart/body wt</td>
<td>5.23±0.19</td>
<td>15.41±0.90*</td>
<td>5.06±0.41</td>
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<tr>
<td>Lung/body wt</td>
<td>7.1±0.32</td>
<td>7.7±0.94</td>
<td>6.19±0.23</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>FS, %</td>
<td>51.9±1.9</td>
<td>26.1±5.5*</td>
<td>56.4±2.56</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>1.4±0.1</td>
<td>3.2±0.3*</td>
<td>1.2±0.08</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>2.8±0.1</td>
<td>4.3±0.1*</td>
<td>2.87±0.04</td>
</tr>
<tr>
<td>SWT, mm</td>
<td>0.58±0.02</td>
<td>0.61±0.04</td>
<td>0.76±0.07</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>0.61±0.01</td>
<td>0.62±0.07</td>
<td>0.67±0.06</td>
</tr>
</tbody>
</table>

NTG indicates nontransgenic; ESD, end-systolic dimension; EDD, end-diastolic dimension; SWT, septal wall thickness; PWT, posterior wall thickness; and FS, fractional shortening. Values are mean±SEM. *P<0.05 vs Rab1 transgenic mice.
dimension indicated a dilated cardiomyopathy phenotype. Similar to the β2-AR overexpressor and other cardiomyopathy models, RNA dot-blot analysis of cardiac genes showed increased atrial natriuretic peptide (ANF), β-MHC, and α-skeletal actin expression (13.4-, 2.6-, and 4.4-fold increase over NTG, respectively) and decreased α-MHC, sarcoplasmic reticulum ATPase (SERCA), and phospholamban (PLB) expression (2.1-, 3.9-, and 3.2-fold decrease over NTG, respectively) (Figure 2D). However, unlike the β2-AR cardiomyopathy mouse, histological examination of high-expressing Rab1a overexpressors revealed no cardiomyocyte dropout or myocardial fibrosis (not shown). Furthermore, TUNEL staining showed no apoptosis in ventricular cardiomyocytes of these mice (not shown). Therefore, development of cardiac enlargement and heart failure in Rab1high mice was not from loss of cardiomyocytes.

Whereas higher level Rab1a expression proved lethal, the intermediate- and lower-expressing lines generated viable transgenic mice used for longitudinal studies and analysis of the “dosage” effects of Rab1a expression. Heart weight and ventricular function were assessed in medium and low Rab1a expressors at 6, 12, 24, and 52 weeks. As with the high expressors, cardiac mass of medium expressors was significantly increased, compared with NTG siblings, at the earliest time studied (Figure 2C). Echocardiographic left ventricular fractional shortening, which was normal at 6 and 12 weeks, progressively deteriorated over time and, when evaluated at 24 and 52 weeks, showed development of the cardiomyopathy phenotype (Figure 2C). Likewise, whereas the low expressors were apparently normal in all respects at the early time points (not shown), delayed development of the dilated phenotype was observed after 1 year (Table I).

Increased expression of ANF, β-MHC, and α-skeletal actin in Rab1a transgenic hearts could represent a primary effect of the transgene, a secondary event related to developing contractile dysfunction or a combination of both. To distinguish between these possibilities, cardiac gene expression in high-expressing Rab1a transgenics was compared with medium Rab1a overexpressors. Expression of hypertrophy-associated genes was higher in Rab1high compared with Rab1med mice of the same age (ANF and α-skeletal actin expression increased 3.3- and 1.5-fold, respectively, over Rab1med at 6 weeks of age) and abnormally increased even in younger Rab1med transgenic mice in which ventricular contractile function was normal (ANF, β-MHC, and α-skeletal actin expression increased 4.1-, 2.3-, and 2.9-fold at 6 weeks and 13.2-, 5.5-, and 6.5-fold at 12 weeks, respectively) (Figure 2D).

Automated analysis of cell volume was used to determine whether the increase in cardiac mass of Rab1med overexpressors was associated with cardiomyocyte hypertrophy. Rab1a transgenic cardiomyocyte volume was increased by 11% and 13% in cells from left and right ventricles, respectively (Table 2; also see Figure 3D). To determine if increased Rab1 expression altered cardiomyocyte function as well, isolated left ventricular cardiomyocytes were studied. Ventricular myocyte contractile function, measured as either the extent or maximal rate of unloaded shortening of 12-week-old Rab1a medium mice, was significantly depressed compared with that of NTG siblings (Figure 3A, Table 2). The amplitude of the Ca2+ transient was normal, but calcium reuptake, measured as the time for 50% (T50) and 80% (T80) normalization, was depressed in Rab1mice compared with control (Figure 3B, Table 2). Furthermore, whole-cell patch-clamp studies performed to measure Ca2+ channel activity showed that L-type Ca2+ channel current (Ica) was significantly depressed in myocytes from Rab1 transgenic compared with NTG mice (Figure 2C, Table 2). Myocyte size, measured as cell capacitance, was significantly increased in Rab1mice, confirming hypertrophy at the cellular level (Table 2). Thus, progressive functional deterioration in ventricular systolic function in Rab1a transgenic mice seems to be a consequence of cardiomyocyte hypertrophy and an intrinsic contractile defect that, in Rab1med mice, precedes overt chamber dysfunction.

In characterizing the Rab phenotype, we considered that counterregulation of one or more of the other Rab GTPases expressed in the heart could occur as a compensatory event in the Rab1a transgenic heart. This was not the case, however, as myocardial content of Rabs1b, 2, 3, 4, 5, and 6 did not change in the Rab1a transgenic mouse hearts (Figure 4A). Another possibility that might affect the phenotype is collateral regulation of Rab-associated proteins, particularly those that modulate Rab GTPase function. To determine whether this was occurring, it was first necessary to identify Rab-associated proteins in the heart, which was accomplished by affinity chromatography of rat myocardial extracts with GST-Rab1a fusion protein coupled to Sepharose. As shown in Figure 4B, several proteins specifically bound to the GST-Rab1a column, compared with GST-Sepharose alone. Two of these were identified by MALDI mass spectroscopy as GDP-dissociation inhibitor (GDI) 1 and 2. Having determined that these GDI s were myocardial Rab1a-interacting
proteins, we compared their expression in NTG and Rab1med mouse hearts by immunoblot analysis. Of the two proteins, GDI1 was present in greater amounts and did not appear to be regulated, whereas GDI2 was very faint and appeared to increase in Rab1a-overexpressing hearts (Figure 4C). Overall, however, these studies demonstrate little effect of Rab overexpression on myocardial content of related Rabs or of Rab1-associated proteins.

PKC Regulation in Rab1 Transgenic Mice
Myocardial hypertrophy and lethal cardiac failure in Rab1a overexpressors suggested that conventional hypertrophy signaling pathways might be activated. In particular, it was possible that overexpressed Rab1a could be promiscuously activating pathways downstream of related Ras, Rac, or RhoA small GTPases. Western blot analysis of ERK, p38, and JNK MAP kinase expression and activation (phospho-specific antibodies) showed no differences between Rab1a overexpressors and controls (not shown), suggesting that this was not the case. However, analysis of cardiac PKC isoforms in ventricular tissue from medium expressors showed marked increases in the content of the α, δ, and ζ isoforms by 1.7-, 3.6-, and 1.9-fold, respectively, with no change in content of β, ε, or μ isoforms (Figure 5A). PKCε subcellular partitioning to the particulate subcellular fraction was strikingly augmented in Rab1 transgenic mice (particulate to cytosol ratio: 5.52±1.02 versus NTG 1.53±0.25, n=8; P<0.05), whereas subcellular partitioning of PKCα (Rab1 1.05±0.05 versus NTG 1.06±0.25, n=8; P=NS) and δ (Rab1 0.96±0.14 versus NTG 1.05±0.16, n=8; P=NS) was not altered (Figure 5B). Nevertheless, the amount of “active” α and δ PKC was increased 2- to 3-fold simply as a function of increased particulate PKC content.

To determine if increased PKCα expression was associated with abnormal subcellular localization, as might be expected from altered Rab-mediated vesicular transport, confocal mi-
crosscopy was used to localize Rab1a and PKCα. (Since the available antibodies for these two proteins are both rabbit polyclonal, simultaneous colocalization was not possible.) In NTG myocytes, PKCα labeled cytoskeletal structures where it colocalized with vimentin (Figure 5C). In Rab1a transgenic myocytes, however, PKCα was seen also in punctate cytoplasmic inclusions and no longer completely colocalized with cytoskeletal vimentin (Figure 5C). These inclusions likely represent abnormal Golgi structures and transport vesicles (see next paragraph), and PKCα mapping in these structures may represent a more generalized abnormality of protein trafficking. Together, these biochemical and immunohistological studies indicate that one consequence of Rab1 overexpression is altered PKC isoform expression and subcellular localization.

Ultrastructural Alterations of Cellular Organelles in Rab1 Transgenic Mice

Since Rab proteins are critical regulators of subcellular protein transport, and Rab1a overexpression caused cardiac hypertrophy that decompensated over time, it was reasonable to speculate that disordered cardiomyocyte protein transport in the transgenic mice could contribute to the development of hypertrophy and heart failure. Abnormal vesicular transport can be manifested by formation of bizarre intracellular organelles, as assessed by electron microscopy.27–29 Ultrastructural studies were therefore performed on 8-week-old Rab1med atrial and ventricular specimens, i.e., before functional deterioration of these mice. As shown in Figure 6B, Golgi stack and surrounding transitional vesicles were markedly enlarged and increased in number in left ventricular cardiomyocytes from Rab1med compared with those from NTG mice (Figure 6A). Characteristic degenerative myelin figures were observed in both ventricular and atrial cardiomyocytes (Figures 6B and 6C). Atrial myocytes contained overabundant ANF granules (Figure 6C). To determine whether these ultrastructural features were specific effects of Rab1a or represented nonspecific degenerative changes, immunogold labeling of ultrathin sections with anti–Rab1a antibody was used, which localized Rab1a protein within the abnormal vesicular structures (Figure 6D). These ultrastructural studies suggest that direct effects of Rab1a overexpression on cardiomyocyte vesicle transport cause the observed pathology.

Figure 5. A, Comparative immunoblot analysis of PKC expression in Rab1 transgenic mice. Representative of 4 individual immunoblots is shown. B, Subcellular partitioning of PKCα indicated by translocation from cytosolic (C) to particulate (D) fractions. C, Confocal examination of PKCα subcellular localization. Top panels show PKCα associated with cytoskeletal elements, labeled by vimentin, in NTG. Bottom panels show accumulation of PKCα in cytoplasmic vesicles, not labeled by vimentin.

Figure 6. Ultrastructural studies. A, Longitudinally sectioned cardiomyocyte from NTG left ventricle. Golgi apparatus (arrow) is surrounded by a few electron dense granules. m indicates mitochondria. B, Representative subsarcolemmal section of left ventricular cardiomyocytes from Rab1med mice at 12 weeks old. Golgi stacks (arrow) are surrounded by numerous vesicles. A myelin figure (asterisk) near the Golgi complex is observed. C, Representative perinuclear area of left ventricular cardiomyocytes from Rab1med, showing numerous ANF granules (a), transitional vesicles, and degenerative myelin figures (asterisk). D, Immunogold labeling of Rab1a in left ventricular cardiomyocytes from Rab1med transgenic mice. The gold particles are concentrated in abnormal vesicular structures (asterisk).
Discussion

Both normal cellular homeostasis and cellular growth require continuous packaging, compartmentalizing, secretion, and recycling of cellular proteins. Tight control is necessary to regulate protein transport to and from specialized intracellular organelles to prevent promiscuous interactions between transport vesicles and cellular organelles that would result in deleterious mixing of organelle proteins. Coordination of transcompartmental protein movement through a complex cascade of vesicular budding, docking, and fusion events is largely mediated by the Rab family of Ras-like small G proteins.3 Mutual recognition of a transport vesicle and its target organelle is the critical process controlled by the Rab GTP/GDP exchange cycle, and this initial interaction between vesicle and target membrane is followed by vesicle fusion and transfer of protein contents from donor vesicle to acceptor organelle.3,5

We observed that myocardial expression of several Rab family members was increased in cardiomyopathic mouse hearts overexpressing β2-AR.10,11 Upregulation of Rabs1, 4, and 6, but not Rabs3 and 5, suggested that something other than nonspecific increases in protein “traffic cops” was operative. Why would these Rab GTPases be upregulated in β2-AR-induced cardiomyopathy? Based on their differential subcellular localization and results of transfection and gene deletion experiments, the various Rab family members are believed to regulate protein trafficking through different cellular organelles. For instance, Rabs1 and 6 are located primarily at the cis-Golgi network. Consistent with this localization, Rabs1 and 6 regulate, respectively, antegrade and retrograde trans-Golgi transport in the protein secretory pathway.7,9,23,24 Since the majority of cell proteins transit through the Golgi apparatus, upregulation of these two Rab family members may generally facilitate cardiomyocyte protein trafficking. On the other hand, Rab4, which is localized adjacent to plasma membranes in the early endosomal compartment, has as its most relevant cardiac effect coordination of β2-AR insertion into plasma membranes.35 Increased Rab4 expression therefore has obvious implications in a model of β2-AR overexpression. It is interesting that Rab5, which is the functional antithesis of Rab4 in that it controls β2-AR internalization through the endocytic pathway,27,30,31 was not similarly upregulated in the β2-AR overexpression model. Thus, increased levels of Rab4 may have been a selective response to the greatly increased requirement for β2-AR insertion into plasma membranes.

In considering whether increased Rab expression in cardiomyopathy has pathophysiological significance, it was decided that cardiac-specific expression of one of the upregulated Rabs would best reveal the direct effects of increased Rab expression on cardiomyocyte structure and cardiac function. Since this was the first examination of Rab effects in the heart, we chose to express the regulated Rab family member that controls the broadest array of protein trafficking functions, ie, Rab1. Although Rab1 regulation in the β2-AR cardiomyopathic heart was less striking than its functional opposite, Rab6, it was believed that activation, through overexpression, would be most enlightening in the protein synthetic, rather than the protein catabolic, pathway. To more closely reproduce intrinsic Rab upregulation as seen in the β2-AR cardiomyopathy model, Rab1a was not mutationally activated, as were small G proteins in previous cardiac transgenic studies.32–34

Rab1a overexpression caused development of cardiac hypertrophy that progressed to cardiomyopathy at a rate that was proportional to expression level. Although the general characteristics of increased atrial and ventricular mass, cardiac dilation, and increased expression of embryonic heart genes are similar to other hypertrophy/cardiomyopathy mouse models, Rab1a effects on cardiomyocyte structure were strikingly unique. Ultrastructural examination showed none of the previously described derangements in myofibrillar or cytoskeletal structures, but instead revealed bizarre and enlarged Golgi structures, transitional vesicles, and ANF granules. Furthermore, immunogold labeling localized transgenically expressed Rab1a in myelin figures consisting of degenerated Golgi apparatus. These findings are consistent with the notion that enhanced Rab1 activity increased antegrade protein transport to, and through, Golgi structures in transgenic cardiomyocytes.7,9 Furthermore, the observed effects on Golgi structure, and localization of Rab1a to these abnormal vesicles, argue against the notion that these are nonspecific effects of overexpressed protein.

It was surprising that increased Rab1a, which is not a signaling molecule notwithstanding its similarity to Ras, Rac, and Rho, would produce a cardiac phenotype that was similar in many respects to transgenic overexpression of those other small G proteins. In fact, since the Rab1a expressed herein was not mutationally active, and the previously described lethal Ras and Rho transgensics were, the Rab1a phenotype is especially noteworthy. How then did altered protein trafficking stimulate cardiomyocyte hypertrophy? The possibility that Rab1a could promiscuously activate the conventional Ras, Rho, or Rac hypertrophy signaling pathways35 was eliminated by the absence of activated MAP kinases, which are downstream of the small signaling G proteins. Unexpectedly, however, we found that several PKC isoforms were dramatically upregulated in the Rab1a-expressing hearts, and that the subcellular localization of PKCα was sequestered in cytoplasmic vesicular structures. The pattern of PKC isoform expression observed herein is not seen in other hypertrophy/dysfunction models such as the β2-AR and Gq overexpression models,10,12,20,22 suggesting that it does not represent a nonspecific response. Therefore, although these associative data should not be interpreted as showing that PKC is a major effector of Rab1-mediated cardiac hypertrophy and failure, they serve to demonstrate the broad effects of this highly targeted intervention and suggest a plausible link between abnormal protein trafficking induced by increased Rab expression and stimulation of cardiac hypertrophy from disturbed subcellular localization and accumulation of PKC.

The following experimental limitations deserve comment. First, although the data suggesting a specific effect of Rab1a on Golgi are strong, it is not possible to exclude a nonspecific effect of the transgene as well. Second, despite in vitro studies demonstrating functional identity between Rab1a and Rab1b, there may be an undetected difference that could change the experimental interpretation. Finally, overexpression of Rab1a represents a pure gain-of-function approach, and complementary
loss of function studies could reveal additional aspects of cardiac Rab1 function. In the context of these limitations, we have shown that several Rab family members are upregulated in a model of cardiac failure, and that increased myocardial Rab1a expression can be profoundly deleterious to the heart. It is therefore of interest that two human diseases are now known to be the consequence of abnormal Rab function, caused by genetic defects in Rab escort protein 1 (X-linked choroideremia)36 or Rab GDP-dissociation inhibitor (X-linked nonspecific mental retardation).37 Whether myocardial Rab proteins are regulated in human cardiac hypertrophy and failure merits investigation.

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References
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