Expression of constitutively active Ras (V12Ras) in cultured neonatal rat ventricular myocytes or targeted cardiac expression of V12Ras in transgenic mice induces myocardial cell growth and expression of genes that are markers of cardiac hypertrophy including atrial natriuretic factor (ANF) and myosin light chain-2. However, the signaling pathways that modulate the effects of Ras on acquisition of the various features of cardiac hypertrophy are not known. We identified the Ral guanine nucleotide exchange factor-like factor (Rlf) in a yeast two-hybrid screen of human heart cDNA library using Ras as bait, suggesting that Ras signaling in the heart may involve Rlf. We demonstrate here that Rlf is expressed in human heart. Expression of wild type Rlf or Rlf-CAAX, a membrane-targeted mutant of Rlf, trans-activated ANF and myosin light chain-2 promoters but did not activate canonical cAMP responsive elements or phorbol ester responsive elements, suggesting that Rlf expression does not lead to a generalized increase in transcription. Transfection of mutant ANF promoter-reporter gene constructs demonstrated that the proximal serum response element is both necessary and sufficient for Rlf-inducible ANF expression. Rlf-induced ANF promoter activation required Ral and Cdc42 but not RhoA, Rac1, ERK, or p38 kinase activation. In addition, Rlf potentiated α1-adrenergic receptor (α1-AR)-induced ANF expression. Prolonged activation of the α1-AR increasesRalGTP levels in neonatal rat ventricular myocytes, further emphasizing a role for Ral guanine nucleotide exchange factors in α1-AR signaling. Overall, this study supports the concept that Rlf and Ral are important previously unrecognized signaling components that regulate transcriptional responses in myocardial cells.

In response to hormones and mechanical stretch, ventricular myocytes exhibit a hypertrophic response characterized by induction of cardiac-specific genes (such as atrial natriuretic factor (ANF)) and myosin light chain (MLC-2) and increased myocardial cell size (1). Expression of activated Ras (V12Ras) induces cellular hypertrophy and ANF expression in cultured neonatal rat ventricular myocytes (NRVMs) (2). Cardiac-targeted (MLC-2v-driven) expression of V12Ras induces cardiac hypertrophy and diastolic dysfunction in transgenic mice (3). Further studies conducted in NRVM indicate that transcriptional and morphological features of myocardial cell hypertrophy mediated by α1-adrenergic (α1-AR) and M1 muscarinic cholinergic receptors require Ras activation (2, 4, 5). These observations imply an important role for Ras activation in development of cardiac hypertrophy; however, the role of specific Ras signaling pathways in cardiac hypertrophy has not been defined.

The best characterized effectors of Ras are serine/threonine kinases of the Raf family. Raf kinases regulate the activity of a kinase cascade that includes mitogen-activated/extracellular signal-regulated kinase kinase (MEK) and extracellular signal-regulated kinase (ERK) (6). The relative importance of the Ras-ERK pathway in cardiac hypertrophy is controversial. Some studies have shown that inhibition of ERK activation blocks α1-AR-induced ANF expression in cultured cells (7, 8). Other studies using either transfection of dominant negative forms of Raf and ERK or pharmacological blockade of ERK indicate that Raf and ERK are not required for α1-AR-induced increases in cell size (7) or ANF expression (9, 10). In support of the latter studies ERK activity is not elevated in the hearts of V12Ras transgenic mice, although significant ventricular hypertrophy is observed (10). Furthermore, whereas V12Ras induces myofilament disarray, cardiac fibrosis, and diastolic dysfunction in transgenic mice (3, 11), cardiac-targeted expression of activated MEK induces concentric hypertrophy and hyperdynamic contractile function, which is characteristic of...
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compensated cardiac hypertrophy (12). The difference in cardiac phenotype between V12Ras and MEK transgenic mice may reflect the ability of V12Ras to activate additional signaling pathways that lead to impaired cardiac function (13).

In addition to Raf, Ras directly activates phosphatidylinositoll-3-kinase (PI-3-kinase) and Ras guanine nucleotide exchange factors (RalGEFs), which activate the Ras. Several putative Ras effectors have been described, including a GTPase-activating protein for Ras (RasGAP) (14), AF6 (15), protein kinase Cζ (16), and Rin (17). Of these, RasGAP and RalGEFs have been implicated in mediating transcriptional responses to Ras in NRVMs (18, 19). Ras also regulates c-Jun N-terminal kinase (JNK) and p38 kinase through activation of MEK kinase or the Rho-related G proteins Rac1 and Cdc42 (reviewed in Ref. 16). JNK and p38 kinases are activated by the α1-AR agonist phenylephrine (PE) in NRVMs (20–22), and JNK is activated in hearts of V12Ras transgenic mice (10). Recent reports indicate that the balance between activation of ERK, JNK, and isoforms of p38 kinase may determine whether ventricular myocytes undergo hypertrophy or apoptosis (13, 22–25).

To understand the role of Ras signaling pathways in cardiac hypertrophy, we performed a yeast two-hybrid screen of a human heart cDNA library. This screen identified the human homologue of a protein previously shown to associate with the GTP-bound form of Ras and its close relative Rap1 (26, 27). The murine homologue, termed Rlf for Ral guanine nucleotide dissociation stimulator (RalGDS)-like factor, is a specific activator of Ras (28). In addition to interacting in the yeast two-hybrid system, Ras and Rlf interact as recombinant proteins in in vitro binding assays (29). In fibroblasts Rlf mediates Ras-induced transcriptional activation of the c-fos promoter through a pathway independent of Raf-MEK-ERK (30). The role of Rlf in cardiac hypertrophy has not been explored.

The aim of this study was to investigate the role of Rlf in transcriptional responses in NRVMs. We report that expression of either wild type or a membrane-targeted form of Rlf induces transcriptional activation of genes that are markers of cardiac hypertrophy but do not elicit a global effect on transcription. Rlf-mediated activation of the ANF promoter requires the proximal serum response element (SRE), Ras, and Cdc42 but not Rho, Rac, ERK, or p38 kinase activation. Consistent with a role for Ral guanine nucleotide exchange factors in hypertrophic signaling, Rlf potentiates the transcriptional response to α1-AR activation. In addition Ral is activated following prolonged stimulation of the α1-AR in NRVMs. These findings suggest that Rlf is an important previously unrecognized signaling component in myocardial cell growth responses.

EXPERIMENTAL PROCEDURES

Two-hybrid Screening—For library screening, the yeast reporter strain Y190 (obtained from Dr. S. Elledge, Baylor College of Medicine) was transformed with a bait plasmid that expressed a fusion protein containing 1.5-kb inserts that were sequenced. BLAST searches revealed that one clone was identical to the Ras binding domain of human RGL2, a partial cDNA sequence identified as a Rap1B-interacting protein and homologue of RalGDS (26). The second clone also overlapped the Ras binding region of RGL2. The full-length murine homologue of RGL2 was isolated and termed Rlf for Ral guanine nucleotide exchange factor-like factor (27).

Northern Analysis—RNA was prepared from several human tissues by the single-step guanidinium thiocyanate method. Twenty micrograms of total RNA was electrophoresed on a 1% agarose gel and blotted to nylon membrane. The Northern blot was processed using a previously described procedure (28). The 1.5-kb fragment of human RGL2 cDNA was isolated in the yeast two-hybrid screen and used as a positive control for in vitro mating. The EcoRI fragment of human RGL2 was cloned into pGAD10 (Matchmaker) according to the manufacturer’s instructions (CLON-TECH). Transformants capable of growth on synthetic media lacking uracil, leucine, tryptophan, and histidine containing 2 μg/mL 3-amino- zole were assayed for β-galactosidase activity using a filter lift assay. The vector pGAD10 containing potential Ral-interacting cDNAs were rescued from yeast and used to transform HB101 Escherichia coli. Two plasmids containing ~1.5-kb inserts were sequenced. BLAST searches revealed that one clone was identical to the Ras binding domain of human RGL2, a partial cDNA sequence identified as a Rap1B-interacting protein and homologue of RalGDS (26). The second clone also overlapped the Ras binding region of RGL2. The full-length murine homologue of RGL2 was isolated and termed Rlf for Ral guanine nucleotide exchange factor-like factor (27).

RESULTS

Yeast Two-hybrid System Identifies Rlf as a Ras-interacting Protein in the Heart—To identify proteins that interact with Ras and therefore contribute to Ras-induced myocardial cell growth, we used the yeast two-hybrid system. We screened a human heart cDNA library (107 independent clones, CLON-TECH) using Ras with a mutated CAAX sequence as bait. We obtained two clones that displayed growth on His− plates and...
Expression of Rlf in Human Tissues—To investigate the pattern of expression of RGL2 in various tissues we performed Northern analysis using the 1.5-kb fragment of RGL2 isolated in the yeast two-hybrid screen with total RNA prepared from adult human tissues. In agreement with the predicted size of the full-length Rlf transcript (27), a transcript of ∼3 kb was detected in the heart, brain, lung, spleen, and kidney (Fig. 1). Despite equal amounts of total RNA (determined by optical density), glyceraldehyde-3-phosphate dehydrogenase levels were modestly higher in heart and lung tissue. Furthermore, an additional smaller transcript was detected in the heart. This smaller transcript may represent another RalGDS homologue, a splice variant of Rlf, or a related mRNA. Nonetheless, the expression of RGL2 in the human heart and its ability to interact with Ras in the yeast two-hybrid system suggests that RGL2 may be an important component of Ras signaling in myocardial cells.

Rlf Activates the ANF and MLC-2 Promoters in Cardiac Myocytes—Expression of Ral mutants that preferentially activate Raf and RalGEFs up-regulates genes associated with cardiac hypertrophy and increases myocardial cell size, suggesting that exchange factors for Ral are important regulators of Ral-induced cardiac hypertrophy (19). To examine the role of Rlf in cardiac-selective gene expression, NRVMs were transiently transfected with the cDNA for wild type Rlf together with ANF or MLC-2 promoter-luciferase constructs and maintained in serum-free medium. Targeting of Rlf to the plasma membrane by replacement of the C-terminal Ras binding domain with the Ras membrane localization signal (CAAX) eliminates the requirement for Ras binding in activation (30). We tested the effect of wild type Rlf and Rlf-CAAX on the induction of ANF and MLC-2 promoter activity. As shown in Fig. 2, expression of either wild type Rlf or Rlf-CAAX is sufficient to activate ANF and MLC-2 promoter-luciferase reporter genes. Expression of the Rlf-CAAX construct in which a large region of the Ral guanine nucleotide exchange domain was deleted (Rlf-ΔCAT-CAAX) (30) did not induce ANF promoter activity (not shown), indicating that the ability of Rlf to induce nucleotide exchange on Ral is required for transcriptional activation of cardiac-specific genes.

The promoter of the ANF gene contains regulatory sequence elements that function as binding sites for a variety of transcription factors. In parallel experiments, we tested the effect of Rlf on induction of promoter-luciferase reporter genes containing cAMP responsive elements (CREs) or phorbol ester (TPA) responsive elements (TREs) (Fig. 2). In contrast to its effects on the ANF and MLC-2 promoters, expression of Rlf or Rlf-CAAX did not trans-activate reporter genes driven by tandem repeats of canonical CREs or containing AP-1 binding sites (2× TRE). This observation indicates that Rlf does not induce a generalized effect on gene transcription in ventricular myocytes.

The SRE Is Necessary and Sufficient for Rlf-mediated ANF Expression—In previous studies it has been shown that nearly all information for α1-AR induction of ANF transcription resides in the 638-base pair region just 5′ to the transcriptional start site (38). In addition to CRE- and TRE-like sequences, this region of the ANF promoter contains two SREs. To further examine sequences present in the ANF promoter required for Rlf-mediated transcriptional regulation of ANF, we used a series of truncated and mutated ANF promoter luciferase constructs (34). NRVMs were transfected with backbone vector (pMT2) or Rlf and maintained in serum-free medium for 48 h prior to determination of luciferase activity. As shown in Fig. 3, Rlf-induced luciferase activity was similar in cells transfected with reporter plasmids containing either 638 or 134 base pairs of the 5′-flanking sequence of the ANF promoter. However, deletion of sequences between −134 and −65 abolished Rlf-stimulated ANF promoter activity, indicating that Rlf-responsive elements are situated between −134 and −65 base pairs of the ANF promoter. This region of the ANF promoter contains an SRE-like sequence without a 3′ Ets binding motif (38). To evaluate whether the ANF SRE is necessary for Rlf-mediated ANF induction, a mutation that disrupts serum response factor binding to the SRE was tested (ANF 134 (M ANF SRE)). Rlf did not transactivate this mutated ANF promoter-reporter construct. When the ANF SRE was inserted 5′ to the minimal ANF 65 promoter (ANF 65 (ANF SRE)), full responsiveness to Rlf was regained (Fig. 3). These results suggest that the SRE is necessary and sufficient for Rlf-inducible ANF expression.

Pharmacological Inhibitors of ERK Activation and p38 Kinase Activity Do Not Block Rlf-mediated ANF Expression—The mitogen-activated protein kinase family members (ERK, JNK, and p38 kinase) mediate transcriptional activation induced by a variety of hypertrophic agents. To test for the involvement of ERK in Rlf-induced ANF expression, NR- and backbone-transfected myocardial cells were treated with the cell-permeable inhibitor of ERK activation (39) or Me2SO for 48 h. At a concentration (10 μM) that inhibits α1-AR-induced ERK activation (9), the MEK inhibitor did not inhibit Rlf-induced ANF expression (Fig. 4). The lack of inhibition by PD98059 was consistent with a previous study showing that Rlf-induced c-fos expression occurs through an ERK-independent pathway in fibroblasts (30).

The ANF SRE is activated by p38 kinase (34). To determine whether p38 kinase mediates the transcriptional responses of Rlf in myocardial cells, we examined Rlf-induced activation of the ANF promoter in the presence of 5 μM SB 203580, a selective inhibitor of p38/αβ kinases (40). Although this concentration of SB 203580 blocks PE-induced ATF6-induced transcriptional responses (Ref. 34 and data not shown), SB 203580 did not block Rlf-induced ANF expression (Fig. 4). Together, these
results demonstrate that activation of ERK and p38 kinase is not required for transcriptional effects of Rlf.

**Dominant Negative Ral Blocks Rlf-induced Activation of the ANF Promoter**

We investigated the role of Ral in Rlf-induced ANF expression by co-expressing RalN28, a dominant negative mutant of Ral (DNRal) that is constitutively GDP-bound (30). As shown in Fig. 5, DNRal inhibited Rlf-induced ANF promoter activity. This result implies that Ral mediates Rlf-induced activation of the ANF promoter.

**Dominant Negative Cdc42, but Not Rac or Rho, Blocks Rlf-induced Activation of the ANF Promoter**

The Rho family of G proteins (RhoA, Rac1, and Cdc42) regulates transcriptional activation by the serum response factor and the SRE through mitogen-activated protein kinase-independent pathways (41). To investigate the role of Rho-related G proteins in Rlf-induced ANF expression, NRVMs were transfected with Rlf alone or with dominant negative mutants of either RhoA (N19RhoA), Rac (N17Rac1), or Cdc42 (N17Cdc42). Expression of N17Cdc42 but not N17Rac or N19RhoA attenuated Rlf-induced ANF expression (Fig. 6). This result suggests that Cdc42 is required for Rlf-induced ANF gene expression.

**Rlf Enhances α1-AR-induced ANF Promoter Activation**

To examine the role of Rlf in α1-AR-mediated activation of the ANF promoter.

**FIG. 2.** Rlf and Rlf-CAAX stimulate transcription activation of the ANF and MLC-2 promoter but do not lead to generalized increases in gene expression. Myocardial cells were cotransfected with 5 μg of the cDNA-encoding backbone vector (pMT2), wild type Rlf (Rlf-wt), a membrane-targeted Rlf (Rlf-CAAX) together with 3 μg of the ANF303 promoter-luciferase construct, the MLC-2 reporter gene, a cAMP response element (CRE), or a phorbol ester responsive element (2XTRE). Cells were maintained in serum-free medium (SFM) for backbone, Rlf, and Rlf-CAAX-transfected cells or treated with the α1-AR agonist phenylephrine (100 μM) plus 2 μM propranolol (to block β-AR) for 48 h. Luciferase activity in cell lysates was determined, and results were normalized to protein content. Data are the mean ± S.E. of three experiments performed in triplicate.

**FIG. 3.** The SRE is necessary and sufficient for Rlf-inducible ANF expression. Myocytes were transfected with 5 μg of wild type Rlf or backbone vector (pMT2) together with a series of truncated and mutated ANF promoter-luciferase constructs. The ANF-638, ANF-134, and ANF-65 promoter constructs contain 638, 134, or 65 base pairs of the 5'-flanking region of the rat ANF promoter, respectively. ANF-134 (M ANF SRE) is the ANF-134 promoter with a mutation that disrupts binding of the serum response factor to the SRE. The ANF-65 (ANF SRE) contains the ANF SRE inserted 5' to the ANF-65 base pair promoter. Myocytes were maintained in SFM for 48 h, and luciferase activity was determined and normalized to protein content. Data expressed are the average-fold activation induced by Rlf relative to control ± S.E. from three experiments performed in triplicate.

**FIG. 4.** Inhibitors of ERK or p38 kinase activity do not block Rlf-induced activation of the ANF promoter. Myocytes were transfected with 2 μg of wild type Rlf or backbone vector (pMT2) and the full-length ANF promoter. Transfected cells were washed and then incubated with either Me2SO (0.1%; control), 10 μM PD98059, or 5 μM SB 203580 for an additional 48 h. Luciferase activity was determined and normalized to protein content. Data are expressed as fold activation mean ± S.E. of four experiments performed in triplicate.
ANF promoter, myocardial cells were transfected with backbone vector (pMT2) or wild type Rlf (5 μg) together with the ANF reporter gene construct. Cells were then incubated in the presence or absence of the α1-AR agonist PE (100 μM) and propranolol (2 μM) for 48 h (to block endogenous β-adrenergic receptors). Activation of the ANF promoter by PE was enhanced in Rlf-transfected cells (Fig. 7), suggesting that PE and Rlf activate sequential signaling pathways that converge on the ANF promoter. Alternatively, Rlf expression may be limiting for activation of the ANF promoter and thus increasing expression of Rlf enhances α1-AR-Rlf signaling in myocardial cells.

**PE Activates Ral**—To examine PE-induced regulation of endogenous Ral in ventricular myocytes we used an affinity precipitation assay for activated Ral (37). Myocytes were treated with PE or 15% serum for 5 min to 24 h, and GTP-Ral was isolated from cell lysates using a GST-Ral binding domain fusion protein. The bound proteins were immunoblotted with an anti-Ral A antibody (Fig. 8). In contrast to serum-treated myocardial cells, Ral activation in PE-treated cells was detected at 24 h. Western blots of whole cell lysates indicate that PE-mediated increases in RalGTP at 24 h were not associated with an increase in Ral protein levels.

**DISCUSSION**

Despite numerous studies in NRVMs and transgenic mouse models of cardiac hypertrophy, the signaling cascade linking G protein-coupled receptors to activation of Ras and the role of Ras effectors in induction of genetic and morphologic changes characteristic of cardiac hypertrophy remains uncertain. Although α1-AR-induced cardiac gene expression and changes in morphology require Ras activity (2), earlier studies suggested that activation of the Ras-ERK kinase cascade is not sufficient to induce cardiac gene expression in ventricular myocytes or Ras transgenic mice (9, 10). These findings indicate that activation of additional Ras effectors is necessary for the characteristic changes in gene expression that occur in response to hypertrophic agonists or Ras. In agreement Fuller et al. (19) demonstrated that effector domain mutants of Ras that preferentially activate Raf and RalGEFs but not PI 3-kinase induce characteristic changes in gene expression that occur in response to hypertrophic agonists or Ras. In agreement Fuller et al. (19) demonstrated that effector domain mutants of Ras that preferentially activate Raf and RalGEFs but not PI 3-kinase induce characteristic changes in gene expression that occur in response to hypertrophic agonists or Ras. In agreement Fuller et al. (19) demonstrated that effector domain mutants of Ras that preferentially activate Raf and RalGEFs but not PI 3-kinase induce characteristic changes in gene expression that occur in response to hypertrophic agonists or Ras. In agreement Fuller et al. (19) demonstrated that effector domain mutants of Ras that preferentially activate Raf and RalGEFs but not PI 3-kinase induce characteristic changes in gene expression that occur in response to hypertrophic agonists or Ras.
clude RalGDS (42), Rgl (43), and RPM/RGL3 (44, 45). The biological role of RalGEFs in gene expression and cell growth is just beginning to be elucidated. When transfected into fibroblasts, Rlf mediates Ras-induced c-fos gene induction through a signaling pathway distinct from the Raf-MEK-ERK pathway (27, 30). Similarly, expression of RalGDS complements the activities of Raf and PI 3-kinase on cell growth and transformation in NIH3T3 fibroblasts (46). However, in PC12 cells expression of RalGDS opposes the action of Raf and PI 3-kinase and inhibits neurite outgrowth (47). Expression of RalGDS also inhibits expression of muscle-specific reporter genes and differentiation of C3H10T1/2 mouse fibroblasts to skeletal muscle (48). We show that Rlf is expressed in the heart (Fig. 1), induces the expression of genes associated with cardiac hypertrophy (Fig. 2), and potentiates the transcriptional response to PE (Fig. 7). Therefore, Rlf may play a role in regulating hypertrophic growth signals in terminally differentiated cardiac myocytes.

Induction of ANF is one of the most conserved features of the hypertrophic response. The promoter of the ANF gene contains several regulatory sequence elements that bind a variety of transcription factors. Using a series of truncated and chimeric ANF promoter-reporter gene constructs, we found that Rlf does not transactivate promoters containing canonical CREs and TREs. Furthermore, deletion of these regulatory motifs in the ANF promoter does not affect Rlf-mediated transcriptional activity. In contrast, the proximal SRE is both necessary and sufficient for Rlf-mediated transactivation of the ANF promoter (Fig. 3). This region of the promoter is a target for multiple intracellular signaling pathways in myocardial cells, including p38 kinase (34, 49), calcium calmodulin kinase (50), electrical pacing (51), RhoA, and the protein kinase C (PKC)-related kinase PKN (52). We found that Rlf-induced transcriptional activation of the ANF promoter requires Ral and Cdc42 but not Rho, Rac, ERK, or p38 kinase activation. An earlier study using transfection of dominant inhibitory and activated RhoA and Ras suggests that RhoA transactivates the ANF promoter through a pathway that is parallel and complementary to Ras (53). The interrelationship between Ras, Ral, Rac, and Cdc42 with respect to hypertrophic growth responses in myocardial cells has not been established. However, our data are consistent with a model that places Rlf on a pathway parallel to both RhoA and Rac.

An important function of Rho-related G proteins is control of actin polymerization and the assembly of integrin complexes. In addition to its impact on cellular morphology and movement, actin polymerization and/or stabilization induce the expression of SRE target genes (54). A recent study has shown that RhoA-mediated organization of the actin cytoskeleton facilitates hypertrophic gene induction (55). Like the Rho family of G proteins, Ral has been linked to changes in cellular morphology. For example, expression of a dominant-interfering mutant of Ral blocks developmental shape changes in Drosophila melanogaster (56) whereas activated Ral induces filopodial outgrowth in fibroblasts (57). A link between Rlf and the actin cytoskeleton is formed through Ral and an effector protein of Rac, Rac-binding protein 1 (RabBP1). RabBP1 binds to Cdc42 and Rac through a GTPase domain (58). Together Rlf signaling to the SRE may involve Ral and/or Cdc42-induced regulation of actin polymerization, an area of investigation that we are actively pursuing.

The regulation of RalGEF activity is just beginning to be elucidated. Several G protein-coupled receptors and receptor tyrosine kinases induce rapid and transient activation of Ral (37, 59, 60). Studies have demonstrated that insulin and EGF-induced Ral activation in fibroblasts is blocked by dominant negative Ras, providing evidence that the RalGEF-Ral pathway is downstream of Ras (60). Ral can also be activated through Ras-independent pathways involving calcium (37, 59). In addition, the activity of RalGDS can be negatively regulated by phorbol esters (61). We show here for the first time that α1-ARs are coupled to signaling pathways that increase the levels of GTP-bound Ral. Although serum-induced Ral activation peaks at 5 min, PE-induced Ral activation occurs only following prolonged PE exposure (24 h). This contrasts with the kinetics of PE-induced ERK activation, which peaks at 5–20 min, returns to basal levels at 1 h, and remains low for 24 h (9). In a similar fashion, nerve growth factor-induced PKC activation enhanced ERK signaling and prevented Ras-mediated activation of RalGEF (61). Whether PKC mediates α1-AR-induced activation of ERK and/or Ral in NRVMs is not known. However, our findings are consistent with a model whereby prolonged exposure to PE (24 h) down-regulates a pathway that inhibits RalGEF activation (such as PKC or RalGAP) or up-regulates an activator of Ral (possibly Rlf). In support of the latter mechanism, we found that Rlf overexpression enhances PE-induced ANF promoter activity (Fig. 7). The significance of Rlf expression and delayed Ral activation on myocardial cell hypertrophy is currently under investigation.

In summary, we identified the human homologue of Rlf as a Ras-interacting protein in the heart using the yeast two-hybrid system and demonstrated its expression in human heart. Expression of wild type and membrane-targeted Rlf is sufficient to activate the promoters of genes that are markers of cardiac hypertrophy but does not lead to generalized increases in gene expression in myocardial cells. Our studies show that Rlf-mediated transcriptional regulation of the ANF promoter requires Ral, Cdc42, and the proximal SRE but not ERK, p38 kinase, Rac1, or RhoA. Co-expression of Rlf and treatment of myocardial cells with PE enhances ANF promoter activity. We also show that PE increases RalGTP levels, further emphasizing a role for Rlf and Ral in α1-AR signal transduction. Overall, these findings also suggest that Rlf and Ral signaling may be an important determinant in the development of myocardial hypertrophy.

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