I. INTRODUCTION

The cardiovascular system is a complicated multiorgan and multicellular system that constantly and instantaneously integrates, with an extreme accuracy, a considerable amount of input information to adjust blood circulation and therefore nutrient and oxygen delivery to the whole organism according to its demand. This adjustment involves coordinated control of a great number of different functions of cardiac and vascular cells and requires an extremely high level of spatial and temporal regulation of signaling pathways driving these cellular processes. Over the past two decades, biochemical, functional, and genetic analyses as well as observations from model organisms and human pathology support the notion that monomeric G proteins of the Ras superfamily are essential elements of the spatiotemporal fine tuning of numerous cardiovascular functions and also of cardiovascular development.

Ras proteins act as binary molecular switches able to regulate a wide range of cellular processes including proliferation, differentiation, metabolism, contraction, motility, survival, and apoptosis. They form complex signaling networks that can be activated by a large array of external upstream stimuli and can achieve highly specific cellular effects thanks to a myriad of regulators and effectors. Accordingly, basal Ras protein activity is required for homeostatic functions in physiological conditions, but sustained overactivation of Ras proteins or spatiotemporal dysregulation of Ras signaling pathways has pathological consequences in the cardiovascular system. In this review, we focus on our current understanding of the roles the different subfamilies of Ras proteins in cardiovascular development, function, and diseases. The studies presented here will help reveal the major challenges in the field and the duality of Ras proteins, displaying both beneficial and deleterious effects in the cardiovascular system.

II. SMALL G PROTEINS OF THE RAS SUPERFAMILY

Ras proteins (H-, N-, and K-Ras) are the founding members of a large superfamily of monomeric small GTPases (20–25 kDa). These proteins are best known for their ability to serve as molecular switches regulating diverse cellular processes that include cell cycle progression, cell survival, actin cytoskeletal organization, cell polarity and movement, and vesicular and nuclear transport (62, 161, 266). Both unicellular and multicellular organisms express Ras proteins. The human Ras superfamily is composed of 166 members, which is divided into five major
branches: 36 Ras proteins, 20 Rho, 27 Arf/Sar, 1 Ran, 61 Rab, and 11 “unclassified” sequences (TABLE 1). Although the separation of these five protein families was an evolutionary event that predated the expansion of eukaryotes, all these proteins present sequence and functional similarities (201, 263, 397).

A. Structures and Domains (Figure 1)

The basic structure of Ras proteins consists of a central six-stranded mixed β-sheet surrounded by five α-helices. The ~20 kDa core G domain (corresponding to Ras residues 4–166) is conserved among all Ras superfamily proteins and is involved in GTP binding and hydrolysis (355). This domain is comprised of five polypeptides loops designated G1 through G5. The diaphosphate-binding loop G1 (also known as P-loop), with the consensus sequence GXXXXGKS/T, connects the β1 strand to the α1 helix and contacts the α- and β-phosphates of the guanine nucleotide. The connection between the α1 helix and the β2 strand corresponds to G2 and contains a conserved threonine residue (Thr35) involved in Mg2+ coordination. The G3 domain, at the NH2 terminus of the α2 helix, links the sites for binding Mg2+ and the γ-phosphate of GTP. The G4 domain that links the β5 strand and the α4 helix recognizes the guanine ring. The G5 loop, located between β6 and helix α5, reinforces the guanine base recognition site (195, 266, 397). Structurally, the Rho family proteins are distinguished from the Ras family by the presence of an extra 10–15 amino acids between helix α3 and strand β5 (47). The function of this insert region remains to be elucidated.

The transition between the GTP- and the GDP-bound form of Ras proteins is accompanied by conformational changes that dramatically affect its affinity for downstream signaling molecules. The crystal and NMR structures, in the active and inactive state, revealed that structural differences are primarily confined to two highly mobile regions, designated as switch I (corresponding to Ras residues 30–38) and switch II (corresponding to Ras residues 59–76) (195, 266, 397, 523). Most proteins of the Ras superfamily are localized to membranes by virtue of posttranslational modification with lipid moieties and posttranslational lipid processing (8). The only significant differences in the sequence of Ras proteins resides in COOH-terminal 25 amino acids of the hypervariable domain (HVR) and probably account for diversity of the biological functions of Ras proteins. The HVR contains the protein sequences that are involved in the association of Ras proteins with membranes. This domain commonly terminates with a CAAX motif that signals for farnesyl or geranylgeranyl isoprenoid addition to the cysteine residue. This process is catalyzed in the cytoplasm by either type I geranylgeranyltransferase (GGT) or farnesyltransferase (FT). Farnesyltransferase inhibitors (FTIs), by preventing isoprenylation of normally farnesylated Ras proteins inhibit their activity. Rab proteins contain a COOH-terminal HVR that terminates with cysteine-containing motifs that are modified by addition of geranylgeranyl lipids, with some undergoing carboxymethylation (8, 62, 195, 266, 397). Unlike other members of Ras superfamily, Ran protein is not lipid modified but contains a COOH-terminal extension that is essential for its functions (500).

B. The GDP-GTP Cycle and Regulatory Proteins

Ras proteins are transducers that couple cell surface receptors to intracellular effector pathways. Ras-superfamily small GTPases act by a conserved mechanism (FIGURE 2) (47, 266, 283, 284). Ras proteins cycle between “on” and “off” conformations that are conferred by the binding of GTP and GDP, respectively. Ras functions require the participation of distinct regulatory proteins to control the GDP/GTP cycling rate. Indeed, the extent and duration of Ras activation in cells depends on the interplay between a variety of negative and positive regulators of the Ras cycle. Guanine dissociation inhibitors (GDIs) maintain small GTPases in the inactive state. GTP exchange factors (GEFs) promote the active GTP-bound state by facilitating the exchange of GDP for GTP. Ras proteins have only a low intrinsic GTPase activity that is increased by interaction with GTGase activating proteins (GAPs).

1. GAPs

The basic function of GTPase-activating proteins is to promote GTP hydrolysis leading to inhibition of the active state of small G proteins. Based on the consensus sequence of the typical G domains, the human genome codes for ~150 potential GAPs. The sizes of these proteins range from 50 to 250 kDa. GAPs acting on Rho/Rac family of small G proteins are especially abundant. In humans, over 70 potential GAPs for Rho/Rac proteins have been identified, 38 GAPs for the Rab family, 15 GAPs for Ras proteins, 31 GAPs for Arf family, and 1 GAP for the Ran protein. It is interesting to note that in the Rho family, the number of GAPs is in almost threefold excess over Rho proteins, while in contrast, in the Ras and Rab families there are approximately two more G proteins than GAPs (266). In the native state, several GAPs are in a folded, autoinhibited conformation that limits the accessibility of the catalytic site. GAPs are regulated by several different mechanisms including phosphorylation/dephosphorylation, lipid-protein or protein-protein interactions, and degradation/resynthesis (262, 265, 266).

2. GEFs

GEFs provide a direct link between Ras protein activation and membrane receptors for soluble factors such as...
### Table I. Ras protein superfamily

<table>
<thead>
<tr>
<th>Family members</th>
<th>Ras (36)</th>
<th>Rho (20)</th>
<th>Rab (64)</th>
<th>Ran (1)</th>
<th>Arf (28)</th>
<th>Other (11)</th>
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<th>Myristoyl</th>
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<td>Number of GAPs</td>
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<td>&gt;70</td>
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<td>Main downstream effectors</td>
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<td>PIP3-kinase</td>
<td>Rho kinases</td>
<td>Ral-GDS</td>
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Number of proteins is given in parentheses. See text for definitions.
neurotransmitters, cytokines, or growth factors and adhesion molecules (62, 284). The first mammalian Rho GEF, Dbl, was identified as a transforming gene in an NIH3T3 focus formation assay using DNA from a cell line of human diffuse B-cell lymphoma. Since then, over 70 distinct members of this Dbl family have been identified in humans (115). GEFs for the large number of different Ras superfamily proteins vary drastically in structure, but there are significant similarities. GEFs constitute subfamilies specific for the different subclasses of Ras proteins, characterized by a 20- to 30-kDa catalytic domain responsible for the GEF activity. Examples of such GEF domains are CDC25 for the Ras family, Dbl-homology (DH) and Pleckstrin-homology (PH) for Rho family, and Sec7 for the Arf family GEFs.

GEFs are multidomain proteins, and the regulation of their activity occurs via a variety of strategies. They include protein-protein interactions, lipid binding, interaction with second messenger molecules [cAMP, diacylglycerol (DAG), Ca\(^{2+}\)], and posttranslational modification such as phosphorylation. Basically, when GEF proteins are activated, they interact with the switch I and switch II domains of Ras-related proteins, causing a series of side-chain rearrangements. These structural changes induce a 10,000-fold increase in the rate of GDP release and its replacement by GTP, the concentration of which in the cytosol is 10 times higher than that of GDP (400).

3. GDIs

Ras-related proteins are regulated by a third class of proteins, the guanine nucleotide dissociation inhibitors (GDIs) thus named as they were first identified as inhibitors of GDP dissociation (126). GDI proteins have been identified for geranylgeranylated Rab and Rho GTPases, but PDE\(\delta\) that preferentially solubilizes farnesylated Ras subfamily proteins, has been qualified as a GDI-like factor (71, 297, 330) (TABLE 1). The most important effects of GDI are their capacity to solubilize membrane-associated small G proteins in the cytosol (62, 132, 161, 283). By binding to their prenylated COOH terminus, GDIs are able to extract GDP-bound small G proteins from the membrane and to keep them inactive in the cytosol by protecting the hydrophobic lipid tail from aqueous solvent. Binding of GDIs, GEFs, and GAPs is mutually exclusive, suggesting that cytosolic GDI-bound G proteins are protected from nucleotide alternation regulated by GEFs and GAPs (78). The dissociation of GDI from the small G protein is therefore a prerequisite for GEF- and GAP-mediated regulation of the activity of small G proteins. Nevertheless, the mechanisms by which GDIs deliver their target G proteins to membranes remain largely unknown. Current knowledge suggests that a variety of different mechanisms may be involved, including regulation by candidate GDI-displacement factors (GDFs) for RabGDI and PDE\(\delta\) and phosphorylations for RhoGDIs (78). GDFs, through allosteric regulation, may squeeze the lipid out by shrinking the lipid-binding pocket of GDI, thereby facilitating the release of prenylated G proteins and its translocation to membranes. Contrary to RabGDI and PDE\(\delta\), there is no GDF candidate identified for RhoGDIs, and accumulating evidence suggests that the affinity between RhoGDIs and Rho proteins, and thus their release, is regulated by phosphorylations (132).
C. Subgroups of Ras Superfamily

1. Ras family

The history of Ras proteins started in the early 1980s by the discovery that a common set of genes, termed ras (for rat sarcoma virus), are responsible for the oncogenic properties of the Harvey and Kirsten sarcoma viruses (97). The Harvey and Kirsten sarcoma virus-associated oncogenes were thus named H-ras and K-ras, respectively. Together with the subsequently discovered neuroblastoma ras oncogene (N-ras), products of these genes constitute the founding members of the canonical Ras proteins (149, 478). Hormones, cytokines, and growth factors through the stimulation of plasma membrane receptors activate Ras proteins. This activation involves receptors tyrosine kinase and specific

![Activation cycle of small G proteins and their regulatory proteins. A: Ras GTPase cycle. Inactive Ras is GDP-bound and localized in the cytosol. Activation of Ras is mediated by a guanine nucleotide exchange factor (GEF). GTP loading induces the translocation of Ras from the cytosol to the plasmatic membrane and permits the interaction with effectors. To "turn off" the cycle, a GTPase-activating protein (GAP) accelerates the intrinsic GTPase activity of Ras, allowing Ras to return to its inactive state in the cytosol. Arf proteins have the same cycle of activation. B: the GDP-GTP cycle of Rho GTPases is similar to Ras proteins but a new regulator appears: the guanine nucleotide-dissociation inhibitor (GDI). This protein binds specifically to GDP-bound Rho, prolonging the inactive state and sequestering the GTPase in the cytosol. C: Ran protein is a regulator of nuclear import and export. Activation cycle of Ran occurs in the cytoplasm and in the nucleus. Nuclear Ran is maintained in the GDP-bound state through localization of GAP in the cytosol and the GEF RCC1 in the nucleus. Ran is the only small GTPase without posttranslational modification. D: Rab proteins include COOH-terminal prenylation signals. The Rab escort protein (REP) interacts with newly synthesized Rab and allows the Rab geranylgeranyl transferase (RabGGT) to add two geranylgeranyl lipid groups to the COOH terminus of Rab. Rab activation follows the prototypical GDP/GTP cycle of Rho proteins. Nevertheless, an additional step is needed to the localization of Rab. The insertion of Rab in the target membrane is mediated by a GDI dissociation factor (GDF) that releases the Rab from GDI.](http://physrev.physiology.org/)

![FIGURE 2.](http://physrev.physiology.org/)
GEFs (Sos, RasGRP, or RasGRF) (213). The effectors with which they interact determine the biological effects of Ras proteins, including gene expression, proliferation, survival, differentiation, cell cycle entry and cytoskeletal dynamics. The most important signaling pathways initiated by active Ras proteins are summarized in other reviews (116, 213, 374, 571).

Dysregulation of these cellular functions is a hallmark of cancer. In humans, dominant gain-of-function mutations of these Ras sequences were discovered in many cancers of the bladder, colon, and lungs (374). The resulting amino acid replacements were usually found to affect the hot spot residue 12, and also less frequently residues 13 and 61. Oncogenic substitutions in residues G12 and G13 prevent the formation of van der Waals bonds between Ras and the GAP proteins, which results in the pronounced attenuation of GTP hydrolysis. Similarly, oncogenic mutations of Q61 reduce the rate of GTP hydrolysis by affecting the coordination of a water molecule thereby protecting from the nucleophilic attack on the γ-phosphate (59, 424).

2. Rho family

The Ras homologous (Rho) family of small GTPases contains 20 members in humans. The most studied members are RhoA, Rac1, and Cdc42. Rho subfamily proteins act as molecular switches within extracellular factor-activated intracellular signaling pathways that regulate multiple cellular functions. RhoA, Rac1, and Cdc42 are ubiquitously expressed, and the major function of these proteins is the regulation of the cytoskeleton. However, the impact of each member of Rho family on the cytoskeleton could be different. RhoA activation leads to the formation of actin stress fibers and focal adhesion, as well as membrane protrusion by activating mDia and driving actin polymerization (162, 391). Stimulation of Rac1 induces actin polymerization at the cell periphery to generate protrusive actin-rich lamellipodia and membrane ruffling. Cdc42 initiates long parallel bundles of actin filaments to induce the formation of filopodia. These reorganizations of the actin cytoskeleton induced by Rho proteins have consequences on cell morphology, polarity, motility, and adhesion (47, 62, 161, 283).

In addition to their regulation of the cytoskeleton, Rho proteins could act as regulators of enzymatic activity. For example, Rac1 controls the enzyme complex responsible for superoxide formation (NADPH oxidase) (421), whereas RhoA regulates the Rho-kinases (Rock1 and Rock2) to modulate cell contraction (283, 284). Last but not least, Rho proteins play a major role in additional process such as gene transcription and cell cycle progression (306, 353, 524).

3. Ran family

The small GTPase Ran has functions in centrosome duplication, microtubule dynamics, chromosome alignment, kinetochoore attachment of microtubules, and nuclear-envelope dynamics, in addition to its characterized roles in nucleocytoplasmic transport.

Ran was first identified as an essential cofactor for nuclear import of nuclear localization signal (NLS)-containing proteins that interacts with transport factors from the importin-β superfamily, also known as the karyopherins (240). The NLS-containing protein-associated importin-β binds directly GTP-bound Ran protein in the nucleus, and this interaction triggers the release of the NLS-containing protein from the imported complex. The Ran-GTP-importin-β complex returns to the cytoplasm, and importin-β disassembles from Ran-GTP upon Ran-GAP1-mediated hydrolysis of GTP to GDP. Free Ran-GDP is then imported into the nucleus by a mechanism that involves the nuclear transport factor-2 (390, 453), where it is loaded with GTP by the Ran guanine nucleotide-exchange factor RCC1 (46). RCC1 is restricted to the nucleus, while the Ran-GAP1 is concentrated in the cytoplasm. Due to this compartmentalization of Ran regulators, the concentration of GTP-bound form of Ran is predominantly localized in the nucleus while the GDP-bound form is prevalently cytoplasmic and the energy supplied by GTP hydrolysis in the cytosol drives nuclear transport (147, 572).

4. Rab and Arf families

Originally described as Ras-like proteins in brain, Rab proteins constitute the largest family of small Ras-like GTPases with more than 60 members in humans. They are localized in the membrane of various subcellular organelles (463). Most intracellular vesicles contain more than one type of Rab protein. Analysis of Rab-GTP structures shows the greatest structural heterogeneity in their switch domains. These structural differences could explain how different Rab proteins recruit specific sets of effectors to regulate precisely their respective pathways. Rab proteins are associated with vesicular trafficking functions, including ensuring transport specificity and demarcating organelle identity (156, 185, 463).

Rab proteins use the classical GDP-GTP cycle, but use distinct additional regulatory protein. De novo synthesized Rab is GDP bound and inactive, with a very low affinity for RabGDT. Newly synthesized Rab protein associates with Rab escort protein (REP) to interact with RabGDT and to be prenylated. The insertion of the Rab-GDP into the target membrane is mediated by a GDF that releases the Rab from GDI (185).

A large body of evidence indicates that the Arf family is also implicated in membrane trafficking. The Arf family includes the Arf proteins (ADP-ribosylation factor), the Sar proteins (secretion and Ras-related protein), and the Arl (Arf-like) proteins. Arfs are ubiquitously expressed, but they are mainly concentrated in the Golgi apparatus. In this
Organelle, Arfs regulate the assembly of coat complexes around budding vesicles (195, 267).

Recent results suggest a cross talk between Rab and Arf proteins. For example, it is reported that the Arf6 GTPase negatively regulates Rab35 activation and hence the fast endocytic recycling pathway (79).

**III. RAS PROTEINS IN HEART FUNCTION AND DISEASES**

**A. Embryonic Heart Development**

The heart is the first organ to be formed during embryogenesis in vertebrates. The primordium of this organ is derived from mesodermal cells that are involved in numerous interactions with cells from several embryonic origins (352). The constitution of the multichambered heart is a multistep process, starting by linear heart tube formation from paired cardiac fields, then followed by the formation of the cardiac chambers, heart looping, and septation (352). The epithelial-mesenchymal transformation of the endocardial cells and invasion of the endocardial cells into the cardiac jelly are the major events involved in the formation of endocardial cushions that play a critical role in valve formation and cardiac septation, which separates the primitive heart tube into four chambers (352, 459). After cardiac looping, cardiac myocytes express muscle genes, the specific site of which is characteristic for each chamber, thereby leading to chamber-specific properties. These processes require precise temporal and region-specific regulation of cell proliferation, migration, death, and differentiation. Among the intracellular signaling molecules involved in this regulation, small G proteins and their effectors have been recognized as key actors of cardiac embryogenesis.

1. **Ras proteins**

A) RAS. In situ hybridization analysis showed that all three ras genes (H-ras, K-ras, N-ras) are uniformly expressed in the embryonic heart (embryonic day E13.5–E18.5) (329). While H-Ras-deficient mice and N-Ras-deficient mice are born and grow normally, the K-Ras-deficient embryos die progressively between E12.5 and term (230). K-Ras-deficient embryos display heart abnormality on E15.5, with thin ventricular walls that can be reduced to a one-cell-thick layer (230). However, the hearts of the K-Ras-deficient embryos were without septal and trabecular defects, and cardiomyocyte-specific markers were expressed in ventricular myocytes. It is likely that the thin ventricular walls in the K-Ras-deficient embryos are not related to abnormal differentiation or cell death but are due to a deficiency in the proliferation of the cardiomyocytes (230). This indicates that K-Ras is essential for normal heart development and that other Ras members H-Ras and N-Ras expressed in the heart cannot compensate for the loss of K-Ras function in K-Ras-deficient mice.

Nevertheless, the embryonic heart development is normal in mice in which K-Ras has been replaced by H-Ras (371) and the introduction of H-Ras transgene in K-Ras-deficient mice rescued mice from embryonic lethality in association with correction of thin ventricular walls of the heart of K-Ras-deficient mice (327). These observations thus suggest that intrinsic activities of H-Ras and K-Ras are similar and that H-Ras can functionally replace K-Ras in embryonic heart development and cardiomyocyte proliferation (327). However, during the adult life, K-Ras function is not entirely rescued by H-Ras as adult mice in which K-Ras has been replaced by H-Ras develop dilated cardiomyopathy associated with arterial hypertension, suggesting that K-Ras has a unique role in cardiovascular homeostasis (371).

Activation of the Raf/MEK/ERK1/2 signaling pathway in endothelial cells is a major effector of Ras during cardiac development. The hearts of endothelial specific Erk1/2 knockout mice are much smaller than that of control mice, with highly reduced cellularity, and the endocardial layer was detached from myocardial trabeculae indicating defective heart development and function (458). This phenotype is associated with a reduced migration and proliferation of endothelial cells.

The role of Ras in cardiac development has been further documented through indirect evidence arising from studies on neurofibrogen encoded by the Nf1 gene. Neurofibrin is a Ras GAP and thus downregulates Ras signaling (294). Loss of function mutation of Nf1 in human leads to neurofibromatosis type 1 (NF1) characterized by the appearance of benign and malignant tumors and cardiovascular defects such as pulmonary valvular stenosis (574). Mice in which the Nf1 gene has been inactivated have been useful to decipher the role of Ras signaling in the embryonic development of the heart, in particular valve formation. Homozygous deficient embryos die in mid gestation (E14) and exhibit signs of cardiac dysfunction with histological abnormalities such as an overabundance of tissue in endocardial cush-
ions of the outflow tract due to hyperproliferation and lack of normal apoptosis, and abnormal cardiac morphogenesis including a failure of proper conotruncal rotation resulting in double outlet right ventricle (247). Endothelial specific inactivation of Nf1 recapitulates the cardiovascular abnormalities involving endothelial cushions and myocardium (138). In contrast, endothelial specific expression of the Ras GAP-domain of neurofibromin rescued cardiac development in Nf1−/− embryos (190). With the use of explants of endocardial cushion tissue, it has been also demonstrated that Nf1−/− explants displayed enhanced epithelial-mesenchymal transformation that was corrected by the dominant negative Ras (Ras-N17) and mimicked by constitutively activated Ras (Ras-L61) in wild-type endocardial cushion explants (247). This indicates that Ras signaling is necessary and sufficient for endocardial-mesenchymal transformation and proliferation.

b) Rala and Ralb. RalA and RalB are both expressed in the myocardium and endocardial cushions of developing heart in mice from E9.5 to E16.5. Higher levels of expression were seen in the apical edge of endocardial cushions in the fusion seam of the septa at E12.5 and E13.5, a critical period for the formation of septa that involved mechanism of apoptosis in atrioventricular cushions (584).

c) Rheb. Rheb (Ras homolog enriched in brain)-deficient embryos die around midgestation because an impaired development of the cardiovascular system with thinning of the ventricular walls (141).

2. Rho proteins

Rho proteins have been found necessary for the development of normal heart. An original experimental approach consisting of cardiac overexpression of Rho GD1α using the α-myosin heavy chain promoter, active at embryonic day E8.0 during morphogenesis on the linear heart tube, has been used to analyze the role of Rho proteins in cardiac morphogenesis (520). Cardiac overexpression of RhoGD1α led to embryonic lethality. In these embryos, cardiac morphogenesis was disrupted, with incomplete looping, lack of chamber demarcation, hypocellularity with inhibition of cell proliferation, and lack of trabeculation. As the activities of RhoA, Rac1, and Cdc42 were all decreased in this model, the respective role of these three proteins cannot be determined.

a) Rhoa. Rhoa transcripts and proteins have been shown to be markedly upregulated in the heart forming region in chick embryo (204). siRNA-mediated Rhoa silencing inhibited the fusion of the bilateral heart primordia, suggesting that Rhoa is involved in the migration of cardiac precursors to the ventral midline and is necessary for the proper looping of the heart and the development of normal heart (204). During development, the initial myocardium-wide expression of Rhoa becomes restricted to the right side of the sinus venosus myocardium including the sino-atrial node, suggesting a role of Rhoa in the conduction system development (501). Rho-kinases appear as key effectors of Rhoa signaling during cardiogenesis. Rho-kinase transcripts are enriched in cardiac mesoderm and Rho-kinase inhibition induced cardia bifida and premature expression of early marker genes of cardiomyocytes differentiation (521). Rock1 and Rock2 genes are expressed in the atrioventricular endocardial cushions at all phases of cardiac development, and pharmacological blockade of Rho-kinases inhibits epithelial-mesenchymal transformation and cell migration, suggesting that Rho-kinases play an essential role in endocardial cell differentiation and migration during endocardial cushion development (521, 583). Rho-kinases are also involved in the formation of initial heart myofibrillogenesis by means of actin-myosin assembly, and focal adhesion/costamere and cell-cell adhesion (410). Upstream to Rhoa, the Rho GEF AKAP13 could account, at least in part, for the role of Rhoa activity in the development of the heart (302). Developing hearts of AKAP13-null mice display thin cardiac walls, with cardiomyocytes showing deficient sarcomere formation, and mice die at embryonic day 10.5–11.0. This phenotype is likely to be due to a reduced Rhoa-dependent activation of the transcription factors SRF and MEF2c and defect in actin assembly (302).

b) RhoB. RhoB is mainly localized on endosomes and regulates cytokine trafficking and cell survival. RhoB levels vary through the cell cycle, and the rhoB transcript has a half-life of 30 min, which is substantially shorter than rhoA or rhoC and indicates that RhoB function requires its expression to be highly regulated (526). During cardiac embryogenesis, RhoB is expressed in the developing atrioventricular and outflow tract endocardial cushions throughout their development (163). Transcripts can first be detected in the endocardial cells overlying the cushions, prior to the delamination of cushion mesenchyme. RhoB increases in expression as epithelium to mesenchyme transformation begins, and reaches a peak of expression as the mesenchymal cells migrate into the cardiac jelly. RhoB expression is downregulated at E11.5 as the endocardial cushions begin their differentiation to form the valves and septa of the heart (163). Expression of RhoB in migrating cells seems likely to be associated with its role in organization of the actin cytoskeleton.

c) Rac1. Rac1 is the only Rac gene expressed early in development. In mouse embryo, although the cardiac mesoderm was specified, the lack of Rac1 is associated with the absence of fusion of the heart at the midline, leading to cardia bifida (308). This defect has been ascribed to a defect in the actin remodeling required for the migration of the nascent mesoderm (308). Targeted deletion of Rac1 in the endothelial cells is sufficient to produce defects in cardiac development. Endothelial Rac1-deficient embryos lack a common
ventricular chamber of the heart but seemed to develop the atrial chamber and the atrioventricular canal (477).

The atypical Rac exchange factor Dock180 was found to have a major role during cardiovascular development. Deletion of Dock180 in mouse induces major cardiac abnormalities such as submembranous ventricular septal defect and double outlet right ventricle (413). Mitral valve leaflets were also thickened and sometimes fused, leading to blood retention in left atrium. A similar phenotype was obtained by specific Dock180 deletion in endothelial cells, suggesting the endothelial origin of the cardiac defect in Dock180-deficient mice. This effect has been ascribed to a major role of Dock180 in the regulation of Rac activation to control endothelial cell migration during cardiac development, but not in the epithelial-mesenchymal transformation of cardiac endothelial cells (413).

D) CDC42. Indirect evidence also suggested that Cdc42 is required for normal heart formation as the absence of the Cdc42 effector p21-activated kinase (Pak) 4 led to lethality by embryonic day 11.5, due to a defect in the fetal heart associated with a thinning of the myocardium and a dilatation of the atrium and sinus venosus, probably related to the role of Cdc42/Pak4 signaling in the regulation of cytoskeleton organization (377).

B. Cardiac Excitation/Contraction Coupling: Contractility

The transient change in tension and length in a working cardiac myocyte during the heart beat reflects the integrated effects of signaling cascades regulating mechanisms controlling the dynamics and intensity of rise in cytoplasmic Ca\(^{2+}\) as well as the responsiveness of the sarcomeric proteins to Ca\(^{2+}\).

The opening of cardiac voltage-dependent Na\(^{+}\) channel causes initial plasma membrane and t-tubule depolarization that induces activation of L-type voltage-dependent Ca\(^{2+}\) channel (Cav1.2) and Ca\(^{2+}\) entry into the cell. The resulting fast and localized Ca\(^{2+}\) rise in the subsarcolemmal space elicits the opening of sarcoplasmic reticulum Ca\(^{2+}\) release channels (ryanodine receptors, RyRs) leading to an amplified release of Ca\(^{2+}\) constituting the Ca\(^{2+}\) transient. The sarcoplasmic Ca\(^{2+}\)-ATPase SERCA2 is responsible for sarcoplasmic reticulum Ca\(^{2+}\) reuptake after systole.

The Ca\(^{2+}\)-dependent contractile response is regulated by the binding of cytosolic Ca\(^{2+}\) to a single site on cardiac troponin C (cTnC), which strengthens the affinity of cTnC for cardiac troponin I (cTnl). Through change in cardiac troponin T (cTnT), these Ca\(^{2+}\)-dependent conformational changes in cTnC-cTnI are transmitted to tropomyosin, favoring the displacement of the troponin complex/tropomyosin on the thin filament away from the actin-bindin sites for myosin. Myosin cross-bridges then bind actin, and cross-bridge cycling proceeds (229). Phosphorylation of myosin binding protein C (MyBP-C) and myosin regulatory light chain (RLCK, MLCK2) also control the Ca\(^{2+}\) sensitivity of the contraction (455).

Cardiac excitation-contraction coupling is regulated by a variety of signaling molecules and receptors, one of the most significant being the \(\beta\)-adrenergic stimulation coupled to cAMP pathway. Emerging evidence indicates that small G proteins, particularly by controlling intracellular Ca\(^{2+}\) signaling and phosphorylation/dephosphorylation processes, contribute to the regulation of cardiac excitation-contraction coupling and contraction.

1. Regulation of Ca\(^{2+}\) handling mechanisms

A) RAS PROTEINS. I) Ras. The hypertrophic effect of Ras is associated with changes in the contractile properties of the myocardium. The cellular mechanism involved in Ras-mediated cardiac contractile defects has thus been the subject of a number of studies. Expression of constitutively active Ras (H-Ras-V12) in cultured neonatal cardiac myocytes reduced the amplitude and prolonged the decay phase of the contractile Ca\(^{2+}\) transients (177). It also decreased the expression of SERCA2 and striated myofibrils. This Ras-mediated regulation of intracellular Ca\(^{2+}\) in cardiac myocytes was ascribed to the activation of the Raf-MEK-ERK cascade (177). However, it was unexpectedly observed that Ca\(^{2+}\) loading of the reticulum was increased in H-Ras-V12 cardiomyocytes, suggesting that H-Ras-V12 affects the excitation-Ca\(^{2+}\) release coupling. In support of this hypothesis, H-Ras-V12 was shown to reduce the amplitude of the L-type calcium channel current in cardiac myocytes through a Raf-MEK-ERK-dependent decrease in the expression of the pore-forming \(\alpha_{1C}\) subunit (also called Ca\(_{1,2}\)) of the Ca\(^{2+}\) channel (176).

In vivo, direct hemodynamic measurements in mice demonstrated that selective expression of H-Ras-V12 in cardiac myocytes significantly decreases cardiac contractility as well as causes diastolic dysfunction associated with interstitial fibrosis and a blunted response to a \(\beta\)-adrenergic agonist (585). At the ventricular myocyte level, these changes in contractility were associated with a reduction and a slowing of the decay of intracellular Ca\(^{2+}\) transient and a decrease of the sarcoplasmic reticulum Ca\(^{2+}\) content, confirming results obtained in cultured myocytes (402, 585). Only a modest reduction of the expression of SERCA2 was observed, and the expression and activity of the Na\(^+\)/Ca\(^{2+}\) exchanger were found to be increased (402, 585).

However, a strong decrease in Ser-16 phosphorylation of the SERCA2 regulator phospholamban (PLB) associated with an increase in the expression of protein phosphatase PP1\(\alpha\), the major PLB phosphatase in the heart, without change in PP2A level was observed in H-Ras-V12 ventricular myocytes (402, 585). These results provide evidence to implicate Ras signaling in the regulation of cardiac contractility.
through modulation of Ca2+ handling proteins expression and activity, including the L-type Ca2+ channel and SERCA2.

b) Rap Proteins. A role of Rap1 protein in cardiac myocyte excitation-contraction coupling became evident from recent studies. Besides the activation of the canonical PKA signaling, cAMP activates other signaling molecules, including Rap1 and Rap2 via the recruitment of cAMP-activated exchange factors Epac (52). In cardiac myocytes, activation of Epac increases electrically evoked Ca2+ transient via PLCε, the catalytic activity of which is directly regulated by Rap (350). Furthermore, inhibition of Rap activity by expressing RapGAP significantly inhibited β-adrenoceptor-dependent stimulation of Ca2+ release. Epac-mediated regulation of β-adrenergic-dependent stimulation of CICR appears to be mediated by PLCε-dependent stimulation of CAM kinase II and subsequent increase in the phosphorylation of the type 2 RyR (RyR2) and phospholamban (349, 362). Further evidence also suggested that Rap is involved in the electrical coupling of cardiomyocytes through the regulation of gap junctions which are essential to coordinate cardiac contractions; Epac-Rap1 signaling redistributes connexin 43, increasing the accumulation at cell-cell contacts, and leads to the formation of new gap junctions. This redistribution of connexin 43 is related to Rap-mediated N-cadherin accumulation at the cell-cell contacts, the major component of adherens junctions that are responsible for mechanical coupling between cardiomyocytes (456).

c) The RGK Subfamily of Ras Proteins. A common feature of the RGK subfamily members (Rad, Rem1, Rem2, Gem/Kir) is their ability to potently inhibit L-type Ca2+ channels in the heart and to reduce left ventricular systolic function (324, 509, 552). In addition, it has been recently shown that Rem prevents the stimulatory action of cAMP/cAMP-activated kinase (PKA) on L-type Ca2+ channel, suggesting a physiological and/or pathophysiological role of RGK proteins in the modulation of β-adrenergic regulation of cardiac function (551). This hypothesis is further supported by the observation that Rad overexpression negated β-adrenergic effects on L-type Ca2+ current and Ca2+ transients (509). One prevailing explanation of this effect is that RGK proteins interfere with the trafficking of pore forming subunit α1C to the sarcolemma, possibly by buffering endogenous β subunits (β1, β2, and β3), thereby reducing the surface density of the cardiac L-type Ca2+ channels (31, 367). However, recent data showing that Rem predominantly inhibits L-type Ca2+ channel current by arresting surface CaV1.2 channels in a low open probability gating mode emphasize the idea that RGK proteins use diverse mechanisms and determinants to inhibit L-type Ca2+ channel current (551). Similarly, whether or not the RGK proteins should be in the active GTP-bound state and targeted to the cell membrane to exert its effect on Cav1.2 remains controversial (89, 551, 561, 562).

d) Rho Proteins. 1) RhoA. Ventricular myocytes isolated from transgenic mice carrying a sixfold overexpression of Rho GD1α in the myocardium exhibited a 40% decrease in the density of the L-type Ca2+ channel current (565). As there was no change in the levels of mRNA and protein of the L-type Ca2+ channel, this inhibition is due to an effect on the activity of the channel. Expression of a dominant negative form of RhoA but not a dominant negative Rac1 or Cdc42 mimicked the effect of GD1α overexpression, indicating that the L-type Ca2+ channel is a downstream target of RhoA in cardiac myocytes (565). The exact molecular mechanism responsible for the positive action of RhoA on the L-type Ca2+ channel has not been precisely defined; however, as the density of channels in the plasma membrane was not modified, it seems to affect the activity of the channel through an actin-independent signal pathway (565). The progressive atrioventricular conduction defects displayed by GD1α overexpressing mice are in agreement with a loss of the positive regulatory role of RhoA on L-type Ca2+ channel activity (522). However, surprisingly, the ventricular contractile function was largely preserved in Rho GD1α transgenic hearts (522). In ventricular myocytes, L-type Ca2+ current constitutes the major pathway for Ca2+ entry that initiates excitation-contraction coupling, and pharmacological inhibition of L-type Ca2+ channel expression or activity affects myocyte contractility. This thus suggests that functional compensation exists in GD1α overexpressing mice, allowing a normal Ca2+ transient and contractility of ventricular myocytes despite a reduced L-type Ca2+ current (522).

RhoA/Rho-kinase signaling also affects intracellular Ca2+ store as in contracting neonatal ventricular myocytes, and pharmacological inhibition of Rho-kinase by Y27632 induces a 150% increase in SERCA2 mRNA expression (502). This effect has been shown to be due to a repressing effect of RhoA/Rho-kinase signaling on the SERCA2a promoter activity by transcription factor other than the well-known RhoA/Rho-kinase-dependent transcription factor SRF, myocardin, or GATA4 (502).

II) Rac and Cdc42. Although it has been suggested in other cell types that Rac and Cdc42 inhibit L-type calcium current, direct evidence for such an effect in the heart does not exist (529). Nevertheless, the identification of the Rac/Cdc42 Pak1 as a major regulator of the catalytic activity of the protein phosphatase 2A (PP2A) strongly argues for a role of Rac/Cdc42-Pak1 signaling in the regulation of cardiac contractility. It has been suggested that Pak1 forms a signaling module with PP2A in cardiomyocytes. Pak1 is highly expressed in the heart, and, as PP2A, localized to Z-disc, cell and nuclear membrane and intercalated disc of ventricular myocytes (220). In the resting state of the Pak1/PP2A complex, PP2A is inactive and phosphorylated on Tyr-307 (221, 525). Activation of Rac1/Cdc42 and the subsequent association of the small G protein with the Rho binding domain of Pak1 promotes its activation and its...
2. Regulation of contractile proteins

A) Ras proteins. Ras signaling through ERK1/2 to p90RSK (ribosomal S6 kinase) induces phosphorylation of cTnI on S23/S24 (194). In protein lysates from cardiomyocytes, phosphorylation of cTnT is inhibited by the Raf inhibitor GW5074, and in vitro kinase assays indicated that Raf-1 phosphorylated cTnT on T206 that has been linked to myosin filament function (363).

B) Rho proteins. 1) RhoA. RhoA through Rho-kinase increases the phosphorylation of the 20-kDa myosin regulatory light chain (RLC), via direct phosphorylation of myosin phosphatase targeting peptide (MYPT2) and consequently, inhibition of the myosin light chain phosphatase (MLCP) (428). This Rho-kinase-mediated increase in RLC phosphorylation could participate in the positive inotropic effects of RhoA activating agent such as α-adrenergic receptor or PGE2 receptor agonists (381). More recently, it has been demonstrated that Rho-kinase also directly phosphorylates cTnI (on S23, S24, and T144) and cTnT (on S278 and T287) (493). This effect is associated with a depression of the maximal tension and a decrease in the Ca2+ sensitivity (493). In addition, RhoA/Rho-kinase signaling has been proposed to participate in the maturation of myocardial contractile system through phosphorylation of its molecular targets in Z-discs and intercalated discs (464).

II) Rac1/Cdc42. More recent evidence also suggests that activation of the Rac1 and Cdc42 effector Pak1 leads to decreased phosphorylation of TnI and MyBP-C through interaction with the phosphatase PP2A and stimulation of PP2A activity (221, 440). In single cardiac myocytes, Pak1 increased the Ca sensitivity of tension, consistent with PP2A-mediated dephosphorylation Ser-23/24 of cTnI (441). Although through a distinct mechanism, Pak3 has been shown to also increase the Ca sensitivity of cardiac myofilaments (61). This effect could be related to direct phosphorylation of cTnI on a new phosphorylation site identified as Ser-149 in the inhibitory domain, and of cTnC. Taken together, these studies suggest that Paks form signaling complexes in the heart that are active in cTnI regulation.

C. Heart Rhythm and Arrhythmia (Table 2)

The normal mechanical functioning of the heart depends on proper rhythmic electrical activity, originating from specialized “pacemaker” cells in the sinoatrial node (SAN) in the right atrium. Propagation of this initial excitation wave through intercellular gap junctions leads to the depolarization of adjacent atrial myocytes, ultimately resulting in excitation of the atria. Next, the excitation wave propagates via the atrioventricular node (AVN) and the Purkinje fibers to the ventricles, where ventricular myocytes are depolarized, leading to excitation of the ventricles.

Rhythmic cardiac electrical activity is attributed to the generation of action potentials in individual cardiac cells. In atrial and ventricular myocytes, the resting potential between each action potential is stable and negative (around −85 mV) due to the high conductance of the IK1 channel (15). Electrical impulses received from adjacent cells induce opening of Na+ channels and the generation of Na+ current responsible for the rapid depolarization phase of the action potential. Transient outward K+ current (ITo) is then responsible for the early repolarization phase. The plateau of the action potential results from a balance between depolarizing L-type Ca2+ channel and outward delayed rectifying K+ currents (IKur, IKr, and IKs). The repolarization phase is due to the closing of L-type Ca2+ channels and the maintenance of outward rectifying K+ current and then to K+ efflux through IK1 channels. The P wave and the QRS complex of the surface electrocardiogram represent atrial and ventricular excitation, respectively, and the T wave represents ventricular repolarization.
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In contrast to atrial and ventricular myocytes, the absence of $I_{K1}$ in SAN and AVN myocytes allows a slow depolarization of the resting potential to occur between each action potential, via, though not exclusively, the activation of the inward pacemaker currents ($I_p$), the T-type Ca$^{2+}$ current, and Cav1.3-dependent L-type Ca$^{2+}$ current. As a consequence, most Na$^+$ channels are inactivated and, in SAN and AVN myocytes, the depolarization phase of the action potential is mainly achieved by the L-type Ca$^{2+}$ current (290).

Heart rate is physiologically regulated by the autonomic nervous system that modulates pacemaker currents in SAN cells. Release of norepinephrine and β-adrenergic stimulation following sympathetic stimulation increases the heart rate, primarily by modulating Ca$^{2+}$ and Na$^+$ permeability. In contrast, parasympathetic fiber stimulation decreases heart rate through the activation of $I_{K,Ach}$ (generated by Kir3.1 and Kir3.4) and the decrease of $I_p$ pacemaker current and Cav1.3-dependent L-type Ca$^{2+}$ current by acetylcholine-mediated M2 muscarinic receptor activation.

Modifications of the properties, regulation, and/or expression of cardiac ion channels can modify action potential waveforms, synchronization, and/or propagation, thereby increasing susceptibility to arrhythmias. Atrial fibrillation (AF) is the most common cardiac arrhythmia, often associated with many forms of cardiac pathology. However, ventricular arrhythmias are the most common cause of sudden cardiac death in hypertrophic cardiomyopathy and other cardiac diseases, including heart failure. Numerous studies have shown that small G protein regulation of electrical coupling, ion channel function, location, or expression participates in the generation and maintenance of normal and/or abnormal heart rhythms (2, 203, 324, 402, 407, 522, 552).

1. Ras proteins

A) RAS. Ras and Ras-GAP have been found to block $I_{K,Ach}$ activation by muscarinic receptor (566). This effect occurs by uncoupling of the receptor from the trimeric G protein rather than uncoupling of the G protein from the channel. It has been proposed that binding of GTP-bound Ras to Ras-GAP causes a conformational change that exposes the SH2-SH3 domain of the NH$_2$ terminus of Ras-GAP, allowing interaction with membrane phosphoproteins and finally uncoupling of muscarinic receptor from the trimeric G protein (295).

Ras is also a negative regulator of the inwardly rectifying K$^+$ channel Kir2.1 via a mechanism involving the MAPK cascade (137). Active Ras reduces $I_{K1}$ current by reducing the amount of available Kir2.1 channel at the cell surface membrane. This effect is not due to a transcriptional regulation but to an action of the Ras-MAPK pathway on the trafficking of Kir2.1 molecules.

In 1991, a tight linkage between H-ras gene on chromosome 11p and long QT syndrome in a large Utah family was reported (222). In agreement with its effect on $I_{K,Ach}$ H-Ras is considered as a candidate gene for this autosomal dominant cardiac arrhythmia. However, both sequencing and more precise definition of the chromosomal region containing the LQT gene has excluded H-ras from the candidate genes for LQT syndromes (401).

Nevertheless, some studies in transgenic mouse model described a role of Ras in heart rhythm regulation and arrhythmia. In addition to cardiac hypertrophy, transgenic mice with a tamoxifen inducible cardiac specific expression of H-Ras-V12 also gradually developed severe arrhythmia (402). Cardiac arrhythmia in H-Ras-V12 transgenic mice started at around day 7 after tamoxifen induction of the transgene expression and was more pronounced at day 14. Electrocardiogram recordings indicated sinus arrest, idioventricular rhythm, ventricular tachycardia, conduction block, and AF. Severity of arrhythmia in H-Ras-V12 mice was influenced by gender-specific factors as females demonstrated a more consistent arrhythmia phenotype. The onset of arrhythmia in H-Ras-V12 mice was temporally correlated with the increase of action potential duration, an altered sarcoplasmic reticulum Ca$^{2+}$ cycling leading to an increase in Na$^+$-Ca$^{2+}$ exchanger current, and a decrease in outward K$^+$ current (402). In addition to these changes in electrophysiological properties of cardiac cells, H-Ras-V12 expression also led to induction of G$_i$α1 expression and pertussis toxin (PTX) treatment, that blocks G$_i$α activity, normalized electrophysiological parameters, and reduced arrhythmias (402). This report thus suggests that contrary to hypertrophy, the electrophysiological remodeling induced by H-Ras-V12 expression in mouse heart does not seem to depend on a MAPK-dependent pathway, but strongly involves upregulation of G$_i$α signaling.

b) RGK FAMILY. The role of RGK family in the regulation of heart rhythm is directly related to their actions on the L-type Ca$^{2+}$ channel. In vivo adenovirus-mediated delivery of Gem, injected in the left ventricular cavity of guinea pig hearts, produces a significant shortening of the electrographic QT interval, due to decreased expression of L-type Ca$^{2+}$ channels and the resulting abbreviation of action potential duration (324). Focal delivery of Gem adenovirus to AV node in pig prolongs the PR interval of the surface electrocardiogram, indicating slowed conduction in the AV node. Moreover, overexpression of Gem in the AV node caused a 20% decrease in the ventricular rate during AF (324).

In contrast, cardiac α-myosin heavy chain promoter-driven overexpression of a dominant negative Rad mutant in mouse produced a prolongation of the QT interval on the surface electrocardiogram, without changes of others parameters. This was associated with an increased expression of the α$_{1c}$ subunit of the L-type Ca$^{2+}$ channel and a pro-
longed duration of action potentials recorded from papillary muscle (552). Diverse arrhythmias such as sinus node dysfunction, atrioventricular block, and ventricular extrasystoles were observed in Rad transgenic mice (552).

**c) RAP1A.** A microarray screening performed to search differentially expressed genes in samples of right appendages from patients with AF or sinus rhythm has identified Rap1a as a gene potentially involved in the pathological process. Rap1a transcript expression is negatively correlated with AF (223). Further supporting the potential effect of Rap1a in the regulation of heart rhythm is its role as an antagonist of the inhibitory effect of Ras on atrial muscarinic K⁺ channels (567).

2. **Rho proteins**

**a) RHOA.** Development of transgenic mouse models has allowed direct in vivo assessment of the role of RhoA in heart rhythm. Cardiac specific overexpression of RhoA induces a marked sinus and AV node dysfunction characterized by depression of heart rate, wandering pacemaker, prolonged PR intervals, second-degree AV block, and AF (407). While a marked positive chronotropic effect was induced by muscarinic receptor blockade by atropine in control mice, this effect was not observed in RhoA transgenic mice so that the bradycardia was not reversed by atropine (407). Since muscarinic receptor blockade by atropine in control mice, this indicates a marked positive chronotropic effect was induced by muscarinic receptor blockade by atropine in control mice, this effect was not observed in RhoA transgenic mice so that the bradycardia was not reversed by atropine (407). Since muscarinic receptors activate Iₖ,Acβ, and slow heart rate via hyperpolarization of pacemaker cells in SA and AV nodes, an hypothesis to explain this phenotype and the bradycardia observed in RhoA transgenic mice would be that RhoA overexpression led to constitutive activation of Iₖ,Acβ, which thus became independent of muscarinic regulation. Another potential mechanism could involve delayed rectifier potassium channels that participate in the repolarization of the action potential. Supporting this hypothesis, it has been shown that RhoA physically associates with and inhibits the delayed rectifier K⁺ channel Kv1.2, originally cloned from rat heart atrium and found to physically interact with RhoA in mouse atria and ventricles (65, 407). Overexpression of RhoA would therefore be predicted to suppress Kv1.2 current leading to prolongation of action potential thereby decreasing SA nodal rate.

AV conduction abnormalities at a young age, bradycardia, and severe atrial arrhythmia at old age were also observed in mice overexpressing the endogenous Rho protein inhibitor GDIs specifically in the heart (522). As the overexpression of GDIs resulted in the simultaneous reduction of the activity of RhoA, Rac1, and Cdc42, it is not possible to link the phenotype observed to a single Rho protein. Electrocardiography and intracardiac electrophysiological recordings revealed first-degree AV block in GDIs mice at 1 wk of age, which progressed into second-degree AV block at 4 wk of age (522). These conduction defects were associated with a dramatic decrease in the expression of connexin 40 (Cx40), a connexin specifically expressed in the atrium and the conduction system, the ablation of which resulted in AV and ventricular conduction abnormalities (225). GDIs mice displayed normal positive chronotropic response to β-adrenergic receptor stimulation, and the AV conduction defects could be partially rescued by β-adrenoceptor stimulation (522). This effect of RhoGDIs/Rho protein signaling on AV conduction was thus probably due to a primary effect on the expression and/or activity of cardiac proteins, including Cx40, involved in the propagation of the electrical activity.

In addition to in vivo studies in transgenic mouse models, several studies directly analyzed the effects of Rho proteins on ion channels involved in electrical cardiac activity. An example of these channels is the human ether-a-go-go related gene K⁺ channels (h-ERG encoded by KCNH2 gene), responsible for Iₖr, and the reduction of which leads to prolongation of cardiac repolarization resulting in the development of long QT syndrome. The constitutively active RhoA-L63 inhibits h-ERG channels, and it has been suggested that this effect was due to Rho-kinase either by directly affecting the channel or indirectly by modulation of protein phosphatase activity (467).

RhoA is also defined as a negative regulator of the Iₖ1 current. Constitutively active RhoA inhibited Kir2.1, and in contrast, dominant negative form of RhoA prevented muscarinic receptor-induced inhibition of Kir2.1 (203). This suggests that RhoA is involved in the physiological regulation of Kir2.1 by activation of muscarinic receptor and that abnormal overactivation could potentially result in ventricular arrhythmia. This inhibitory effect of RhoA on Kir2.1 could also participate in the loss of the positive chronotropic effect of muscarinic receptor blockade observed in mice overexpressing RhoA in the heart (407).

**b) RAC.** Evidence for a role of Rac1 in the regulation of heart rhythm comes from both in vivo and in vitro studies. One of the strategies used in mice was to overexpress a constitutively active Rac1 (Rac1-V12) under the control of the α-MHC promoter (2). Cardiac Rac1-V12 mice, showing a 30-fold increase in cardiac Rac1 activity, spontaneously developed AF with age. Electrocardiogram indicated that at the age of 10 and 16 mo, 44 and 75% of Rac1-V12 mice displayed AF, respectively. Aging of Rac1-V12 mice was associated with increased size of the atrium, but not the ventricles. A fourfold increase in atrial nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and a significant enhancement of atrial collagen content was observed in Rac1-V12 mice. Treatment with statin strongly reduced the cardiac Rac1 and NADPH activities of Rac1-V12 mice, without affecting the atrial dilation and interstitial fibrosis. Interestingly, a fourfold upregulation of Rac1 and NADPH oxidative activities was observed in samples of the left atrial appendage of patients with AF (2). A more recent study has suggested that the role of Rac1/NADPH...
signaling in AF involves upregulation of connective tissue growth factor (CTGF) and the resulting modulation of N-cadherin and Cx43 that were all upregulated in Rac1-V12 mice and in left atrial myocardium from patients with AF (3). This study thus suggests that Rac1 and NADPH oxidase contributes to signal transduction during AF. It can be speculated that statin-induced inhibition of Rac1 could contribute to the prevention of postoperative AF obtained by statin treatment (515).

Results obtained in guinea pig SAN pacemaker cells expressing constitutively active Pak1 indirectly suggest a role of Rac1 and/or Cdc42 in Ca2+ and K+ channels. Endogenous Pak1 protein is abundant in SAN, atrial, and ventricular tissue (219). Expression of active Pak1 does not modify the amplitude of the L-type Ca2+ current in SAN cells but strongly reduces the stimulatory effect of β-adrenergic stimulation (219). Similarly, in the resting condition, the outward K+ current was not changed in SAN cells expressing active Pak1, but the β-adrenergic stimulation-induced increase in K+ current amplitude and acceleration of the K+ current decay was almost abolished. In whole heart preparations, overexpression of active Pak1 attenuated the positive chronotropic action of β-adrenergic stimulation (219). As the PP2A inhibitor okadaic acid partially reversed the suppressing effect of active Pak1 on the response to β-adrenergic stimulation on Ca2+ and K+ currents, the effect of Pak1 is likely to be due to PP2A (219). These observations thus identify Pak1 as a potential target for therapeutic strategies against tachycardia triggered by catecholamines in different pathological conditions.

Several studies performed in non-cardiac cells can help to define the molecular identity of channels that underlie the effect of Rac1 on heart rhythm. For example, active Rac1 mutant stimulates ERG channel activity, via a stimulatory action on the serine-threonine phosphatase PP5 (134, 467). Dominant negative Rac1 has been shown to strongly increase Kir2.1 current, while the constitutively active Rac1 had no significant effect (54). This effect resulted from Rac1-mediated reduction of basal internalization of the channels, leading to an increase in the number of Kir2.1 channels at the plasma membrane, without change of its global expression level (54). These data thus suggest that Rac1-mediated regulation of ERG or Kir2.1 channels can participate in the arrhythmogenic effect of Rac1 activation.

D. Cardiac Hypertrophy/Remodeling and Heart Failure (Table 3)

The primary response of the heart to increased work load is cardiomyocyte growth in an attempt to enhance contractility and preserve cardiac function (170). Cardiac hypertrophy is a physiological adaptive response to normal postnatal maturation or physical exercise that is characterized by the maintenance of a normal myocardial structure and function. However, cardiac hypertrophy is also a common response to pathological stressors such as hypertension, myocardial infarction-induced defects in the efficiency of the contraction, neurohormonal stimulation, or oxidative stress. Although it is an initial compensatory response to maintain cardiac output, it evolves into a maladaptive process and a decompensated state with profound changes in cardiac structure and function, predisposing to arrhythmia and sudden death, and leading to the development of heart failure (121, 175). Pathological hypertrophy corresponds to an abnormal enlargement of the heart muscle originating from an increase in cell size of myocytes, interstitial fibrosis, and proliferation of nonmuscle cells. Cardiomyocyte hypertrophy is associated with the enhancement of myofibril organization and change in gene expression including increased expression of immediate early genes (c-jun, c-fos, egr1) and reexpression of fetal genes such as atrial natriuretic factor (ANF) and B-type natriuretic peptide (BNP) as well as genes involved in cardiac contractility [β-myosin heavy chain (β-MHC), α-skeletal actin).

Since heart failure is the leading cause of hospitalization and mortality, the signaling mechanisms underlying cardiac hypertrophy and, even more importantly, the processes responsible for the transition between compensated hypertrophy to decompensated heart failure remain the subject of extensive research. The following section focuses on the role of small G protein in cardiac hypertrophy and remodeling, in particular Ras and Rho subfamilies, shown to play a key role in the hypertrophic signaling pathways initiated by the activation of G protein-coupled receptor.

1. Ras proteins

A) Ras. Transient upregulation of H-Ras has been observed in association with reexpression of c-myc in a variety of experimental models of cardiac hypertrophy in adult rats, suggesting that it may participate in hypertrophy of heart (198, 232). In agreement with this observation in animal models, H-Ras is also upregulated in patients with hypertrophic cardiomyopathy and cardiomyocyte size correlated with the expression level of H-Ras (205). Moreover, patients suffering from “the RAS/MAPK syndromes” caused by mutations of molecules in the RAS/MAPK cascade (Noonan, LEOPARD, Costello, and CFC syndromes and neurofibromatosis type I) exhibit hypertrophic cardiomyopathy (18).

Both in vitro and animal studies support this role of H-Ras in cardiac hypertrophy (Table 3). In primary neonatal rat ventricular myocytes, microinjection of active H-Ras-V12 stimulates hypertrophic response, attested by an increase in cell surface and the induction of the expression of c-fos and ANF (483). Expression of a H-Ras-V12 mutant that selectively activates Raf or a constitutively active Raf mutant induces the same phenotype as H-Ras-V12, suggesting that Raf is the downstream effector involved in the hypertrophic...
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<tr>
<th>Target</th>
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<th>Transgene/Mouse</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
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<tbody>
<tr>
<td>Ras</td>
<td>Cardiac specific overexpression (α-MHC promoter)</td>
<td>Active H-Ras mutant (H-Ras-V12)</td>
<td>Cardiac muscle hypertrophy, with increased ventricular mass and myocardial cell size, Myofibrillar disarray, Fetal cardiac gene expression, Abnormal left ventricular diastolic function, Normal systolic functions remained normal</td>
<td>142, 184, 402</td>
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<tr>
<td>Nf1</td>
<td>Myocardial specific deletion</td>
<td>Progressive cardiac hypertrophy, fibrosis, and cardiac myocytes enlargement, Hyperactivation of Ras/Erk signaling in the heart</td>
<td>548</td>
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<tr>
<td>RASSF1A</td>
<td>Deficiency (KO)</td>
<td>Massive cardiac hypertrophy after transverse aortic constriction, Cardiac fibrosis, Left ventricular dilatation, Fetal cardiac gene expression</td>
<td>346</td>
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<td>Raf-1</td>
<td>Cardiac specific overexpression (α-MHC promoter)</td>
<td>Dominant-negative Raf1 mutant (DN-Raf1)</td>
<td>↓ of pressure overload-induced cardiac hypertrophy, ↓ of pressure overload-induced fetal cardiac gene expression, Increased cardiomyocyte apoptosis, Decreased ERK1/2 activity and cell growth, Left ventricular dysfunction and dilatation, Increased number of apoptotic cardiac cells</td>
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<tr>
<td>Rad</td>
<td>Deficiency (KO)</td>
<td>Increased susceptibility to cardiac hypertrophy, Increased CaM-kinase II activity, Enhanced cardiac fibrosis and increased CTGF expression</td>
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<tr>
<td>Rab1</td>
<td>Cardiac specific overexpression (α-MHC promoter)</td>
<td>Cardiac hypertrophy</td>
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<tr>
<td>RhoA</td>
<td>Cardiac specific overexpression (α-MHC promoter)</td>
<td>RhoA Active RhoA mutant (RhoA-L63)</td>
<td>Premature death, No increase in ventricular mass, No increase in cardiomyocyte size, Increased expression of ANF and βMHC, Cardiac conduction defects</td>
<td>407</td>
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<tr>
<td>Rock1</td>
<td>Haploinsufficiency (Rock1&lt;sup&gt;+/−&lt;/sup&gt;)</td>
<td>No change in heart structure and function, No change in ANG II- or L-NAME-induced cardiac hypertrophy, ↓ of ANG II, L-NAME- or pressure overload-induced cardiac fibrosis</td>
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<tr>
<td></td>
<td>Deficiency (Rock1&lt;sup&gt;−/−&lt;/sup&gt;)</td>
<td>↓ of pressure-overload-induced cardiac fibrosis, ↓ of pressure-overload-induced cardiomyocyte apoptosis</td>
<td>563, 582</td>
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Continued
effect of Ras (177). However, other evidence indicates that activation of Raf/MEK1/2/ERK1/2 alone is insufficient to initiate a complete hypertrophic response and that other Ras effectors are required. Indeed, it has been shown that in addition to Raf, the Ras effectors RalGDS, an exchange factor for RalA and RalB, and phosphatidylinositol (PI) 3-kinase participate in Ras-mediated hypertrophic response (128). RalGDS and Raf are mediators of specific hypertrophic transcriptional responses, whereas PI 3-kinase elicits a global effect on gene expression in cardiac myocytes (128). Other signaling molecules have been proposed to participate in Ras-mediated hypertrophy including MEKK/JNK pathway (383) and Ras-GAP (1).

Ras has emerged as a central signaling protein required for cardiac hypertrophy induced by different external stimuli such as mechanical overload or neurohumoral factors including phenylephrine (PhE) or angiotensin II (ANG II) (86, 404, 483, 558). Gaq, downstream of α1-adrenergic or type I ANG II (AT1) receptors is required for Ras activation associated with cardiomyocyte hypertrophic response to PhE and ANG II, in a protein kinase C-dependent or -independent manner (86, 171, 404). Furthermore, βγ-subunits of G protein-dependent activation of Ras has also been shown to be critical for ANG II-dependent cardiac fibroblast proliferation that also participates in cardiac hypertrophy in vivo (557). While Ras GEFs linking trimeric G protein to Ras activation following hypertrophic agonist stimulation of cardiomyocytes are unknown, α1-adrenergic receptor-mediated cardiac hypertrophy has been ascribed to reactive oxygen species-dependent Ras activation (512, 545). Stimulation of α1-adrenergic receptor causes the oxidative post-translational modification of Ras leading to the decrease in free Ras thiols and, thereby, Ras activation (243).

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<th>Target</th>
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<th>Transgene/Mouse</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
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<tr>
<td>Deficiency (Rock1&lt;sup&gt;−/−&lt;/sup&gt;)</td>
<td>In mice overexpressing Gαq in cardiomyocytes</td>
<td>No effect on cardiac hypertrophy</td>
<td>443</td>
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<td></td>
<td></td>
<td>Improved survival</td>
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<td>Preserved left ventricular structure and contractile function</td>
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<td>Decreased cardiomyocyte apoptosis</td>
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<tr>
<td>Cardiac specific overexpression (α-MHC promoter)</td>
<td>Rock1: in mice overexpressing Gαq in cardiomyocytes</td>
<td>Accelerate hypertrophic decompensation</td>
<td>442</td>
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<tr>
<td>Cardiac-specific overexpression (α-MHC promoter)</td>
<td>Active Cter-truncated Rock1</td>
<td>Extensive cardiac fibrosis</td>
<td>563</td>
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<tr>
<td>Rac1</td>
<td>Cardiac-specific overexpression (α-MHC promoter)</td>
<td>Active Rac1 mutant (Rac1-V12)</td>
<td>Neonatal lethal dilated cardiomyopathy</td>
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<td>Transient hypertrophy in young mice (hypercontractile heart)</td>
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<td></td>
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<td>↓ of ANG II-induced cardiac hypertrophy</td>
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<td>Cardiomyocyte-specific Rac1 deficiency (c-Rac1&lt;sup&gt;−/−&lt;/sup&gt;)</td>
<td>Tamoxifen-inducible Cre-mediated deletion of floxed Rac1 gene in cardiomyocytes</td>
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<td>Pak1</td>
<td>Cardiomyocyte-specific Pak1 deficiency (c-Pak1&lt;sup&gt;−/−&lt;/sup&gt;)</td>
<td>Cre-mediated deletion of floxed Pak1 gene in cardiomyocytes</td>
<td>of pressure-overload- or ANG II-induced cardiac hypertrophy</td>
<td>279</td>
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<td>of pressure-overload- or ANG II-induced cardiac fibrosis</td>
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<td>of pressure-overload-induced cardiomyocyte apoptosis</td>
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<td>of pressure-overload-induced cardiac fetal gene expression</td>
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<td>vulnerability to transition to heart failure</td>
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<td>Cdc42</td>
<td>Cardiomyocyte-specific Cdc42 deficiency (c-Cdc42&lt;sup&gt;−/−&lt;/sup&gt;)</td>
<td>Cre-mediated deletion of floxed Cdc42 gene in cardiomyocytes</td>
<td>of pressure-overload- or ANG II, PhE-induced cardiac hypertrophy</td>
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<td>of exercise-induced cardiac hypertrophy</td>
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<td>vulnerability to transition to heart failure</td>
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In mice, cardiac specific expression of H-Ras-V12 leads to cardiac muscle hypertrophy, with increased ventricular mass and myocardial cell size, myofibrillar disarray, and reactivation of ANF gene expression (142, 184) (TABLE 3). Functionally, mice expressing cardiac H-Ras-V12 display abnormal left ventricular diastolic function, but basal and β-adrenergic stimulated systolic functions remained normal (184). This hypertrophic action of H-Ras-V12 does not seem to secondarily result from the overactivation of Ras signaling during embryonic development because induction of H-Ras-V12 transgene expression in adult mice heart also leads to significant myocyte hypertrophy already observed 1 wk after induction as posterior wall thickness of the left ventricle, increased heart weight and single-cell capacitance, and fetal cardiac gene expression (402). Cardiac hypertrophy thus appears as an immediate downstream effect of Ras activation in adult hearts. This was confirmed by a recent study showing the reversibility of Ras-induced cardiac hypertrophy in a mouse model of temporary expression of H-Ras-V12 in myocardium (517).

Myocardial specific deletion of the Ras GAP Nf1 in mice promotes progressive cardiac hypertrophy, fibrosis, and cardiac myocytes enlargement associated with an hyperactivation of Ras/Erk signaling in the heart, suggesting a role of Nf1 to limit Ras activity in cardiomyocytes of the adult heart (548) (TABLE 3). These findings are in agreement with observations made in Ras-association domain family protein isoform 1A (RASSF1A) knockout mice, another mice model with defective Ras signaling (346). RASSF1A is a potent tumor suppressor ubiquitously expressed that inhibits the Ras-Raf1-ERK1/2 pathway in cardiomyocytes and prevents PhE-induced hypertrophy (346). RASSF1A knockout mice, after transverse aortic constriction, exhibited massive cardiac hypertrophy that was almost double that observed in control mice, associated with cardiac fibrosis and increased expression of fetal genes and left ventricular dilatation (346). A role of Ras signaling in cardiac hypertrophy is further supported by the discovery that Ras GAP and the Ras protein Rheb would be potential targets of miR1, a miRNA involved in cardiac hypertrophy (423). Taken together, these data underline the importance of endogenous molecular systems that maintain a low level of Ras activity under physiological conditions.

Transgenic mice with cardiac specific expression of a dominant negative form of Raf (DN-Raf-1) has been generated to define the requirement of Raf-1, downstream to Ras in cardiac hypertrophy in vivo (TABLE 3). Under basal conditions, DN-Raf-1 mice have normal cardiac structure and function (155). However, cardiac hypertrophy and hypertrophic gene induction in response to transverse aortic constriction-induced pressure overload are reduced in DN-Raf mice, associated with an increased cardiomyocyte apoptosis and a decreased ERK1/2 activity and cell growth (155). A more pronounced phenotype was obtained by cardiac specific deletion of Raf-1 in mice that, in absence of external stress, leads to left ventricular dysfunction and dilatation, with an increased number of apoptotic cells (554). These data are thus consistent with an antiapoptotic effect of Ras/Raf signaling, independent of ERK1/2 but related to the antagonistic action of Raf against the propapoptotic kinase ASK1 (554). These findings also indicate that the role of Ras/Raf signaling in cardiac hypertrophy is mainly due to its effect on cardiomyocyte hypertrophy and apoptosis resistance.

Very recently, a proteomic analysis of heart samples from the transgenic mouse model of cardiac specific expression of H-Ras-V12 has suggested that activation of the Wnt/β-catennin canonical pathway occurs secondary to Ras activation in the course of pathogenic myocardial hypertrophic transformation and may also participate in the hypertrophic effect of Ras activation (518).

b) RAD. In addition to its inhibitory role on Ca2+ channels, Rad has been shown to play a protecting role in cardiac hypertrophy. Rad is abundantly expressed in human heart, and its expression is strongly decreased in left ventricular myocardium samples from patients with heart failure that displayed severe hypertrophic morphology (73). Similarly, Rad has been shown to be decreased in the model of PhE-induced cardiomyocyte hypertrophy (73). Silencing of Rad expression by siRNA in rat neonatal cardiomyocytes strongly increased protein synthesis and ANF expression, whereas overexpression of Rad significantly reduced PhE-induced protein synthesis and ANF expression, and decreased CaM kinase II activity (73). In vivo, Rad-deficient mice were more susceptible than normal mice to cardiac hypertrophy, with increased CaM kinase II activity (73) and more severe cardiac fibrosis related to an increase in CTGF expression (579). Rad is thus identified as an endogenous cardioprotective mediator that prevents cardiac hypertrophy through the inhibition of CaM kinase II activity and cardiac fibrosis through the inhibition of CTGF.

c) RAP. In addition to their role in the regulation of excitation-contraction coupling, Rap proteins could also participate in signaling cascades involved in cardiac hypertrophy. Rap1 has been shown to mediate the activation of B-Raf/ERK1/2 induced by M1 muscarinic receptor activation, via the activation of the Rap1 GEF Ca2+ and diacylglycerol-regulated GEF1 (CaDAG-GEFI) (146). In neonatal ventricular myocytes, cAMP/PKA-dependent Rap1 activation is responsible for ERK1/2 activation and cell growth downstream of the PGE2 receptor EP4 (160). In the same cellular model, the role of Rap1 in hypertrophy has been ascribed to cAMP-mediated activation of the Rap1 GEF Epa1, leading to Rap1-dependent inhibition of ERK5 (106). Nevertheless, surprisingly, more recent in vivo and in vitro studies suggested that Epa1 hypertrophic effects, including those in-
duced by β-adrenergic receptor stimulation, are independent of Rap1 but rather involve Ras and Rac, calcineurin, and CaM kinase II (305, 319). Both Rap1 and Epac1 knockout mice will be useful to decipher the role of Rap1 signaling in cardiac hypertrophy.

D) RAB. Rab1 regulates protein transport, specifically from the endoplasmic reticulum to the Golgi apparatus. Overexpression of Rab1 induced hypertrophy of neonatal cardiomyocytes in culture (117). Transgenic expression of Rab1 in the myocardium promotes cardiac hypertrophy in mouse that has been ascribed to an abnormal expression and subcellular localization of proteins, such as PKCα (542).

E) RAL. RaLA and RaLB are among the closest relatives of Ras, and RaL proteins have been shown to be recruited by Ras. Activated Ras binds to the Ras-binding domain of RaL GEFs such as RaL-GDS, RaL guanine nucleotide exchange factor-like factor (Rlf), RGL1, and RGL2, leading to its translocation to the membrane where it produces GDP/GTP exchange on RaL (299). Since the identification of RaL-GDS as an effector of Ras involved in Ras-mediated ventricular myocytes hypertrophy (128), further studies supported the role of RaL signaling in cardiac hypertrophy. Overexpression of RaL-GDS, constitutively active RaL mutant, or Rlf stimulates promoter activity of early and embryonic genes in rat neonatal myocytes, synergistically with activated Ras (218, 370). Dominant-negative RaL partially inhibited active Ras-induced promoter activation (218). Stimulation of α1-adrenergic receptor induced activation of RaL in neonatal ventricular myocyte (370), and RaL activity is increased in hypertrophied heart (218).

2. Rho proteins

A) RHOA. RhoA has been implicated in the morphological changes and induction of gene expression associated with hypertrophic response in cardiac myocytes. In vitro, stimulation of cultured neonatal ventricular myocytes with hypertrophic factors such as PhE, endothelin-1, or ANG II induced activation of RhoA (16, 84). Transfection or infection of neonatal ventricular myocytes with active RhoA mutant (RhoA-V14) stimulates ANF expression and myofibrillogenesis (16, 181, 406, 484). Conversely, expression of the dominant negative RhoA-N19, Rho-GDI, or the selective RhoA inhibitor Clostridium botulinum C3 exoenzyme inhibits hypertrophic responses induced by soluble stimuli such as PhE, ANG II, or lysophosphatidic acid (10, 16, 168, 406) or by mechanical stress (12).

Mechanisms linking extracellular stimuli to RhoA activation in neonatal myocytes are still not firmly established. Trimeric G proteins play a key role in the activation of RhoA by agonists of G protein-coupled receptor, probably through activation of Rho GEFs that remain to be identified. Gαq was initially found to mediate RhoA activation following PhE-induced α1-adrenergic receptor stimulation (171, 406), but Go12/13 was also shown to be responsible for this activation (296). ANG II-induced RhoA activation depends on Go12/13 signaling (337), whereas activation of RhoA by lysophosphatidic acid involves Goi (168). In response to mechanical stretch, activation of RhoA depends on PKCα (356).

Rho-kinases have been identified as the RhoA effector mediating RhoA-dependent hypertrophic responses both in vitro and in vivo. In neonatal ventricular myocytes, pharmacological inhibition of Rho-kinase (fasudil or Y27632) or transfection of dominant negative forms of Rho-kinase prevents morphological and cytoskeleton changes, protein synthesis, and ANF expression associated with response induced by hypertrophic factors such as PhE, endothelin-1, or leptin (181, 245, 576). In neonatal ventricular myocytes, Ras activation by hypertrophic factors is also essential to their hypertrophic effects (see above), suggesting that Ras/MAPK and RhoA induce a hypertrophic response by separate, complementary, and synergistic pathways (58, 292). In fact, RhoA/Rho-kinase-mediated actin cytoskeleton remodeling has been shown to be necessary for the translocation of ERK to the nucleus, providing a mechanism for the synergy between RhoA and Ras signaling pathways regulating gene transcription and inducing hypertrophy. The precise downstream mechanisms by which RhoA/Rho-kinase activate the hypertrophic gene program remain obscure, however. Activation of the cardiac transcription factor GATA-4 is identified as downstream nuclear mediators of RhoA kinase during myocardial cell hypertrophy (560). Recently, myocardin-related transcription factor A (MRTF-A), and the subsequent transcriptional activation of serum response factor (SRF)-dependent fetal cardiac genes, have been defined as critical downstream mediators of RhoA/Rho-kinase-dependent hypertrophic response in cardiac myocytes (244). Another RhoA effector, protein kinase N (PKN), has also been involved in the SRF-dependent transcriptional responses associated with cardiomyocytes hypertrophy (321).

In vivo, pressure overload induces a rapid activation of Rho-kinases in the adult rat myocardium, suggesting that could participate in mechanisms of initial as well as chronic adaptive responses of cardiac myocytes to mechanical stress (488). While both Rock1 and Rock2 are expressed in the myocardium, the increased Rho-kinase activity induced by pressure overload has been ascribed to Rock1 (582). In Dahl salt-sensitive hypertensive rats, the ventricular hypertrophy and function are significantly ameliorated by Rho-kinase inhibition possibly through an upregulation of the downregulated endothelial nitric oxide synthase (eNOS) and the reduction of oxidative stress through the inhibition of NAD(P)H oxidase and LOX-1 expression (228, 314). Chronic Rho-kinase inhibition also markedly limits pathological cardiac hypertrophy induced by pressure overload in rats and improves diastolic function in rats (23, 364), but has no effect on chronic swimming-induced physiological...
cardiac hypertrophy (23). Several neurohormonal factors, such as ANG II, are suspected to play a role in ventricular hypertrophy and the transition to heart failure. Long-term treatment with the Rho-kinase inhibitor fasudil reduces the ANG II-induced cardiomyocyte hypertrophy in mice (166, 514). Rho-kinase activity is also involved in the pathogenesis of left ventricular remodeling after myocardial infarction (158). All these findings support the involvement of RhoA/Rho-kinase signaling in the development of cardiac hypertrophy in vivo.

Genetically modified mouse models have then been useful to further refine the role of RhoA and Rho-kinases in cardiac remodeling and hypertrophy (TABLE 3). Surprisingly, although ventricular expression of some hypertrophic marker genes such as ANF and β-MHC are increased in mice that overexpressed Rhoa in a cardiac specific manner, they did not develop an obvious cardiac hypertrophy (407). In contrast, they display progressive left ventricular chamber dilation and decreased contractility. The role of Rhoa during cardiac development and the potential of compensatory mechanisms could explain why in vivo overexpression of Rhoa did not mimic its in vitro hypertrophic effect. Haploinsufficiency of Rock1 in mice does not alter cardiac structure and function under basal conditions (393). All indexes of cardiac hypertrophy, i.e., wall thickness, left ventricular mass, cardiomyocyte size, or expression of ANF, induced by ANG II or l-NAME were similar in control and Rock1+/- mice (393). However, a marked reduction of cardiac fibrosis is observed in Rock1+/- mice chronically treated with ANG II or l-NAME, or after transaortic constriction, compared with control mice (393). This decreased fibrosis is associated with a reduced expression of markers of fibrosis including transforming growth factor-β (TGF-β), CTGF, and type III collagen. Similar reduction of fibrosis has been observed in Rock1 knockout mice (582), together with a decreased cardiomyocyte apoptosis following pressure overload by aortic banding (563). These results thus indicate that in vivo, Rock1 contributes to the development of cardiac fibrosis but not hypertrophy in response to various pathological conditions. This was recently confirmed in a transgenic mice expressing a truncated activate form of Rock1 in cardiomyocytes (563). These mice displayed extensive cardiac fibrosis due to upregulation of TGF-β and cytokine release in cardiomyocytes that promoted myofibroblast differentiation (563).

In a mouse model with cardiac specific overexpression of Gαqα, that develops compensated cardiac hypertrophy at young ages and progressing to heart failure, transgenic overexpression of Rock1 increases cardiomyocyte apoptosis and accelerates hypertrophic decompensation (443). In contrast, although it does not prevent the development of cardiac hypertrophy, deletion of Rock1 improves survival, inhibits cardiomyocytes apoptosis, and preserves left ventricular structure and function in old Gαqα mice (442, 443). These data thus support the absence of a role of Rho-kinase in pathological hypertrophy but provide evidence for a crucial role of Rock1 in the transition from cardiac hypertrophy to heart failure.

The difference in the cardiac phenotype resulting from genetic modifications of Rhoa or Rock1 in mice could be related to the relative independence of Rock1 activation from Rhoa activity. Rock1 can be constitutively activated by caspase-3-mediated proteolytic cleavage of its inhibitory COOH terminus (432). Accumulation of this COOH terminus-truncated form of Rock1 has been described in failing human heart and genetic murine models of heart failure (72). This active truncated Rock1 stimulates caspase-3 and phosphatase and tensin homolog deleted on chromosome ten (PTEN) activities. The resulting repression of the PI-3-kinase/Akt cell survival pathway thus sets on a positive feed-forward regulatory loop that favors cardiomyocyte apoptosis (72). Because cardiomyocyte apoptosis is a major cellular event involved in the transition from compensated hypertrophy to dilated cardiomyopathy and failure, this mechanism can support the observed important role of Rock1 in hypertrophic decompensation (443).

Finally, the inhibitory effect of pharmacological inhibition of Rho-kinases on cardiac hypertrophy is not mimicked by Rock1 deletion. That might suggest that Rock2 could be involved in cardiac hypertrophy or the antihypertrophic effect of Rho-kinase inhibitors did not result from Rho-kinase inhibition. Cardiac specific deletion of Rock2 in mice would be useful to address this question.

b) Rac1. As for other small G proteins, first evidence for a role of Rac1 in cardiac hypertrophy was provided by in vitro analysis in neonatal ventricle myocytes. Expression of active V12-Rac1 results in sarcomeric reorganization, increase in cell size, and atrial natriuretic peptide (ANP) secretion (372). In contrast, the dominant negative N17-Rac1 expression inhibits the morphological changes and the increased protein synthesis induced by PhE (372). In agreement with this observation, hypertrophic factors including PhE, ANG II, and endothelin-1 have been shown to induce rapid Rac1 activation in cardiac myocytes, and N17-Rac1 inhibits the generation of reactive oxygen species (ROS) and the stimulation of ERK cascade, but not the activation of p38MAPK induced by these stimuli (84, 93, 172). Mechanical stretch also induces Rac1 activation in cardiomyocytes via a signaling involving PKCδ (84, 356). In contrast to that observed after stimulation with soluble hypertrophic factors, expression of dominant negative form of Rac1 prevents stretch-induced p38MAPK activation, increased protein synthesis, and intracellular ROS generation (13). Stretch-induced p38MAPK kinase activation is also inhibited by treatment with the antioxidant N-acetyl-l-cysteine (NAC), indicating that stretch-induced Rac1-dependent activation of p38MAPK was mediated by ROS production...
that Pak1 is not a major effector of Rac1 in cardiomyocytes and supports an important role of ROS in the hypertrophic effect of Rac1. This also suggests that upstream activators of Pak1 other that Rac1 regulate Pak1 activity in cardiomyocytes, with Cdc42 as a good candidate.

All these in vivo data, together with the threefold increase of Rac1 activity and the upregulation of NADPH oxidase-mediated ROS release observed in myocardium of patients with ischemic or dilated cardiomyopathy, suggest that Rac1/ROS signaling contributes to the pathophysiology (286).

c) Cdc42. Very little is known about the function of Cdc42 in cardiomyocytes. In fact, in vitro, Cdc42 was found to have hypertrophic effects in neonatal cardiomyocytes that appear morphologically distinct from those exerted by Rac1 or RhoA (328). Expression of dominant active mutant of Cdc42 promoted myofibril assembly in series and increased the length/width ratio of cardiomyocytes while RhoA and Rac1 induced parallel assembly of sarcomeric units and did not affect the length/width ratio (328). Dominant active mutant of Cdc42 expression also increased p38MAPK activity in neonatal cardiomyocytes (13). An increase in Cdc42 activity is rapidly observed (10 min) in cardiomyocytes stimulated by hypertrophic stimuli such as leukocyte inhibitory factor (LIF), ANG II, PhE, or isoproterenol (288), and expression of a dominant negative form of Cdc42 inhibits LIF-induced sarcomere organization in series and length-to-width ratio increase (328). Furthermore, silencing of Cdc42 by shRNA also reduces MEKK1/MKK4/7 and JNK activation induced by LIF or isoproterenol in neonatal cardiomyocytes (288). The observation that dominant negative Cdc42 suppressed stretch-induced p38MAPK activation indirectly suggests that mechanical stretch also activates Cdc42 (13). These data thus support pro-hypertrophic effect of Cdc42 that may involve MEKK1/MKK4/7/JNK and p38MAPK pathways. However, this pro-hypertrophic effect of Cdc42 observed in vitro has not been confirmed by in vivo studies. In fact, mice with cardiac-specific deletion of Cdc42 developed exacerbated pressure overload hypertrophy, greater cardiac hypertrophy in response to neuroendocrine agonist infusion (ANG II and PhE), and enhanced exercise-induced hypertrophy and sudden death (288). They transitioned more quickly from hypertrophy to heart failure than control mice. This phenotype is associated with the suppression of MEKK1/MKK4/7/JNK pathway activity, resulting in the upregulation of calcineurin-nuclear factor of activated T cells (NFAT) signaling (288). The causal role of the suppression of the JNK pathway in the cardiac phenotype of Cdc42−/− mice has been demonstrated by the reversion of the enhanced cardiac growth when they were crossed with Mkk7 transgenic mice (288). These results thus identify Cdc42 as an antihypertrophic and protective mediator during stress stimulation. Interestingly, the phenotype of

In vivo, transaortic constriction-induced cardiac hypertrophy in mice is associated with activation of Rac1 NADPH oxidase in left ventricular myocardium (93). To further address the role of Rac1 activation in the heart, transgenic mice that express constitutively activated Rac1 specifically in the myocardium have been developed (473). These mice display two different cardiac phenotypes: a neonatal lethal dilated cardiomyopathy and a transient hypertrophy in young mice that resolved with age. Hypertrophic hearts do not show signs of myofibril disarray and are hypercontractile in working heart analyses (473). These results thus unexpectedly suggest that both phenotypes result from deregulation of signaling by Rac1 and Pak, followed by alteration of cellular adhesion. The use of mice harboring cardiomyocyte-specific, tamoxifen-inducible Cre recombinase circumvented the problem of the embryonic lethality of Rac1 deletion to generate cardiomyocyte-specific Rac1 knockout (c-Rac1−/−) mice (415). Cardiac specific deletion of Rac1 prevents ANG II infusion-induced myocardial oxidative stress, NADPH activation, and cardiac hypertrophy. In agreement with in vitro studies, this mouse model supports the notion that Rac1-mediated NADPH oxidase activity and ROS production, but not some other effects of Rac1, are the primary regulators of cardiac hypertrophy in response to ANG II (415). This idea has been recently supported by data obtained in cardiomyocyte-specific Pak1 knockout mice (c-Pak1−/−) (279). These mice develop enhanced cardiac hypertrophy and fibrosis in response to pressure overload or ANG II infusion, compared with control, and they are more vulnerable to the transition to heart failure. This phenotype is associated with an increase in cardiomyocyte apoptosis and fetal gene expression. In c-Pak1−/− mice myocardium, pressure overload does not induce the activation of MKK4/MKK7-JNK pathway, whereas activation of p38MAPK and ERK1/2 are normal. In ANG II–treated mice, ROS production is comparable in control and c-Pak1−/− mice (279). These mice thus allow to uncover the cadioprotective role of Pak1/JNK pathway in attenuating cardiac hypertrophy and halting the transition to heart failure. In addition, the opposed phenotype of c-Rac1−/− and c-Pak1−/− mice provides further evidence that Rac1, are the primary regulators of cardiac hypertrophy in response to ANG II (167). This Rac1-Ros signaling pathway has been also shown to be upstream of JNK activation and cardiomyocyte death, in particular in the context of β-adrenoeceptor-stimulated apoptosis (192).

Indeed, the stimulation of the superoxide generating NADPH oxidase by Rac1 and the subsequent generation ROS generation are important mediators of the cellular responses downstream of Rac1. The use of Rac1-V12 and Rac1-N17 mutant expression in cardiomyocytes has demonstrated that, in addition to p38MAPK, Rac1-mediated ROS is responsible for the activation of the transcription factor NFκB. It was further shown that this activation involved ROS-mediated activation of apoptosis signal-regulating kinase 1 (ASK1) and that this pathway is essential for Rac1-induced hypertrophic effects (167). This Rac1-ROS signaling pathway has been also shown to be upstream of JNK activation and cardiomyocyte death, in particular in the context of β-adrenoeceptor-stimulated apoptosis (192).
c-Cdc42−/− mice is very similar to that of c-Pak1−/− mice, thereby supporting the idea that Cdc42 is the endogenous activator of Pak1 and that the Cdc42/Pak1/JNK signaling pathway is cardioprotective, in limiting hypertrophy and transition to heart failure.

Other clues speaking in favor of a role of Cdc42 in cardiac hypertrophy were recently supplied by studies of microRNAs. Cdc42 was identified as a target of, and negatively regulated by miR-1 and miR-133 (that also targets RhoA), and a decreased expression of both miR-133 and miR-1 was observed in mouse and human models of cardiac hypertrophy (66, 376).

E. Ischemia/Reperfusion Damage, Preconditioning

Myocardial ischemia, due to a reduced blood supply, induces severe tissue injury in the heart, and large effort has been made to understand the mechanisms causing cellular damage during ischemia. It is now recognized that upon reperfusion, exposure of ischemic myocardium to molecular oxygen initiates a cascade of events that paradoxically augments myocardial cell dysfunction and death, with ROS as essential mediators of ischemia/reperfusion injury. Nevertheless, the heart and cardiac myocytes have developed powerful endogenous mechanisms to protect themselves against ischemia/reperfusion injury which can be activated by brief period of ischemia and reperfusion preceding a more severe one through a phenomenon known as ischemic preconditioning. Signaling mechanisms mediating both ischemia/reperfusion injury and cardioprotection are the subject of intense investigation as they may represent molecular targets that could be used to mimic ischemic preconditioning by pharmacological interventions. Members of the Ras superfamily of small G proteins represent potential good candidates for such an objective. However, despite intensive research in this field, data are still inconsistent. Furthermore, studies are essentially performed in rat or mice models of cardiac ischemia/reperfusion while the temporal and spatial development of myocardial ischemia/reperfusion injury in these animal species is different from that seen in humans (165). Therefore, although small rodent heart models are useful to decipher molecular mechanisms and identify potentially interesting targets, the confirmation of such novel mechanisms in larger animal (such as pig or dog) models of myocardial ischemia/reperfusion while the temporal and spatial development of myocardial ischemia/reperfusion injury in these animal species is different from that seen in humans (165). Therefore, although small rodent heart models are useful to decipher molecular mechanisms and identify potentially interesting targets, the confirmation of such novel mechanisms in larger animal (such as pig or dog) models of myocardial ischemia/reperfusion that more closely resembles the human situation (124) appears mandatory before translation to the clinic.

1. Ras proteins (Figure 3)

A) Ras. Oxidative stress activates Ras in the cardiomyocytes and in the heart (85, 469). Numerous in vitro and in vivo studies clearly identified the Ras/Raf/MEK1/2/ERK1/2 pathway as a prosurvival pathway that protects cardiomyocytes from apoptotic death following oxidative stress, thus identifying ERK1/2 as major components of the reperfusion injury salvage kinase (RISK) pathway (11, 325, 399, 573). Results from pharmacological studies confirmed these data by showing that activation of ERK1/2 signaling significantly contributes to the cardioprotective effects of β-adrenergic receptor antagonists that are largely used in the clinical management of human ischemic heart diseases (237).

Genetic manipulations of MEK1/2, ERK1/2, and Raf-1 in mice nicely demonstrated the anti-apoptotic action of the Raf/MEK1/2/ERK1/2 signaling leading to cardioprotection during reperfusion (155, 269). However, cardiac specific disruption of the c-raf-1 gene indicated that the beneficial effect of Raf-1 on cardiomyocytes survival is not mediated by the MEK1/2-ERK1/2 pathway but involves repression of ASK1, Mst2, and JNK-p38MAPK activity (76, 325, 554).

More recent data also suggest that Ras, through the activation of its other downstream effector Rassf1A, has a pro-apoptotic effect through the kinase Mst1 already known to be activated by ischemia/reoxygenation in the heart (347, 556). Rassf1A has been identified as an endogenous activator of Mst1 in the heart, and the Rassf1A/Mst1 pathway promoted apoptosis in cardiomyocytes (98).

It therefore appears that although Ras signaling exerts potent cardioprotective effects through Raf-dependent and -independent mechanisms, stimulation of Raf-independent apoptotic signaling also occurred downstream of Ras. Accordingly, the dynamic balance of this dual signaling would be critical in determining cardiomyocyte fate subsequent to reperfusion injury. The observation that inhibition of Ras proteins by FTIs limits ischemia/reperfusion-induced injury even suggests that the Raf-independent pro-apoptotic Ras effects, probably through Mst1 and JNK, predominates...
over its Raf-ERK1/2-mediated cardioprotective action (35, 358).

b) RAD. Although its physiological or pathophysiological role has not been yet assessed, it has been recently shown in vitro in neonatal rat ventricular myocytes that Rad induces cardiomyocyte apoptosis through p38MAPK activation (472).

2. Rho proteins (Figure 4)

A) RHOA. RhoA has been initially described as both a mediator of survival and a trigger of cell death. High levels of expression of activated RhoA in cardiomyocytes initially elicit a hypertrophic response, but sustained expression leads to the development of apoptosis, via a mechanism involving Rho-kinase, p53-dependent upregulation of the pro-apoptotic protein Bax and the consequent induction of the mitochondrial death pathway (100). A special role has been ascribed to Rock1 as this isoform, cleaved by caspase 3, becomes irreversibly active and leads to a positive feedback on caspase 3 activity, activation of PTEN, and inhibition of Akt in cardiomyocytes (72). This truncated form is absent in normal heart but was found in human failing heart (72). In contrast to this pro-apoptotic effect of RhoA/Rho-kinase signaling, moderate expression of activated RhoA, at levels close to physiological levels of RhoA activation, protects cardiomyocytes from ROS-induced apoptosis through FAK phosphorylation, association of FAK with PI 3-kinase, and finally, Akt activation (99). Confirming these findings, inhibition of endogenous RhoA by C3 exoenzyme has been shown to decrease Akt activity and stimulate cardiomyocyte apoptosis (238). Thereafter, several studies have been designed to investigate in vivo whether RhoA signaling is beneficial or detrimental to cardiomyocyte survival during ischemia/reperfusion.

The first in vivo data reported were based on the effects of the pharmacological inhibition of the RhoA effector Rho-kinase on heart damages caused by ischemia/reperfusion in rats or mice. RhoA/Rho-kinase activity is not modified by ischemia, but an early and sustained activation is observed in the ischemic myocardium during reperfusion (25, 150). Rho-kinase inhibition with Y27632 or fasudil reduces ischemia/reperfusion-induced cardiomyocytes apoptosis and improves postischemic cardiac function through the activation of PI 3-kinase/Akt/NO signaling (25, 150, 538) and the inhibition of JNK (580). All these pharmacological data thus support a deleterious effect of Rho-kinase activity in myocardial ischemia/reperfusion injury. However, recent results obtained in mice with cardiomyocyte-specific conditional expression of low levels of activated RhoA (RhoA-L63) unexpectedly reveal that RhoA protects the heart against ischemia/reperfusion injury (544). Both in perfused heart experiments and in vivo, expression of active RhoA strongly reduced ischemia/reperfusion-induced myocardial cell damage, evidenced by a reduction of the infarct size and lactate dehydrogenase and cytochrome c release, thus showing that active RhoA in cardiomyocytes is protective against ischemia/reperfusion injury (544). Conversely, in mice with cardiomyocyte specific deletion of RhoA, infarct size and lactate dehydrogenase release following ischemia/reperfusion are significantly increased compared with control mice, thus confirming the beneficial effect of RhoA in cardiomyocytes (544). Downstream of RhoA, all the potential effectors involved such as Akt, FAK, ERK1/2, or PKN are not modified in hearts expressing active RhoA compared with control hearts, while PKD activity is markedly increased (544). A causal role of the PKD activation in the cardioprotective effect of RhoA is supported in vitro by the similar potentiation of H2O2-induced cardiomyocyte death by RhoA and PKD inhibition, and in vivo by the complete reversion of the cardioprotection conferred by active RhoA expression by PKD blockade (544). It is of interest to mention recent data regarding the other RhoA effector PKN. Hypotonic swelling of cardiomyocytes, a condition found in pathological contexts such as ischemia-reperfusion, leads to RhoA and PKN activation that mediates cardiac myocyte survival (206). In vivo, cardiomyocyte specific expression and deletion of the RhoA effector PKN in mice demonstrate that activation of PKN plays a cell-protective role in cardiac myocytes during ischemia/reperfusion (475). Although a direct link with RhoA was not made, this study is however
in agreement with a cardioprotective role of RhoA against ischemia/reperfusion injury in vivo.

This discrepancy between pharmacological studies and genetic manipulation of RhoA could result from multiple reasons, including 1) the targeting of Rho-kinase in pharmacological studies and RhoA in mice models, 2) the specific manipulation of RhoA signaling in cardiomyocytes in genetically modified mice while pharmacological inhibition affects every cell type with potential effects on neurohumoral feedback loops, and 3) the possible lack of selectivity of the pharmacological inhibitors.

Similarly, inconsistent results have been reported regarding the role of RhoA/Rho-kinase in preconditioning. Ischemic preconditioning reduces the activity of Rho-kinase in the myocardium, while, in contrast, the ERK1/2 signaling is increased (578). This downregulation of the RhoA/Rho-kinase signaling as well as the decrease in cardiomyocyte apoptosis induced by ischemic preconditioning is lost after ERK1/2 inhibition (578). These data suggest a relationship between ERK1/2 and RhoA/Rho-kinase, such that ERK1/2 activity is required in ischemic preconditioning to oppose the effect of RhoA/Rho-kinase signaling on apoptosis. This is in agreement with the observation that inhibition of Rho-kinase by Y27632 or high dose of fasudil mimics the effect of ischemic preconditioning in rats (101, 102). However, in opposition to these results, it has also been suggested that RhoA/Rho-kinase activity participates in the cardioprotective effect of ischemic preconditioning since inhibition of Rho-kinase by Y27632 in perfused hearts or by low doses of fasudil in vivo in rats reduces the protective effects of ischemic preconditioning (101, 409). This cardioprotective role of RhoA is also supported by its involvement in the preconditioning effect of adenosine, coupling A3 adenosine receptor to phospholipase D activation (259, 322).

In short, these data highlight the difficulties to definitively define whether RhoA signaling is deleterious or protective during ischemia/reperfusion and preconditioning, and much remains to be done before considering RhoA/Rho-kinase inhibition as a potential therapy of ischemia/reperfusion injury.

B) Rac. As a component of the NADPH complex, Rac1 has been suggested to play a dominant role in ROS generation after ischemia/reperfusion. Nevertheless, a limited number of studies have addressed the role of Rac in cardiac ischemia/reperfusion injury, and there are no data on cardiac preconditioning. Expression of the dominant negative Rac1-N17 mutant results in protection of cardiomyocytes from hypoxia/reoxygenation-induced cell death (224). In vivo, ischemia/reperfusion increases Rac1 in the heart and ROS production, and ischemia/reperfusion-induced ROS production and apoptosis are strongly decreased in hearts from mice with cardiac specific deletion of Rac1, compared with controls, resulting in a limitation of the infarct size and preservation of myocardial function (439). Pharmacological inhibition of Rac1 by NSC23766 gives the same results (439), thus providing evidence that Rac1 is involved in myocardial damage produced by ischemia/reperfusion and that strategy aiming at decreasing Rac1 activity in these circumstances could represent new potential therapy.

IV. RAS PROTEINS IN VASCULAR FUNCTION AND DISEASES

A. Embryonic Vascular Development (Vasculogenesis and Angiogenesis)

Vascular development in vertebrates is a complex phenomenon, initiated in the embryo at different places and times, and underlaid by two processes, vasculogenesis and angiogenesis. The first blood vessels of the mouse begin to form in the yolk sac at E6–6.5. Later in development, vasculogenesis is initiated with the embryo proper, with blood vessels appearing in the following order: endocardium, primary vascular network lateral to the midline, paired dorsal aorta, head, and cardinal vessels. Vasculogenesis corresponds to de novo formation of vessels from endothelial cell precursors or angioblasts that differentiate into endothelial cells and form lumenized tubes organized in primitive continuous vascular network (after E8.5 in mouse embryo). Angiogenesis is then responsible for the formation of secondary vessels by endothelial cells sprouting from the primary vessels. Endothelial cell proliferation, migration, and differentiation, essential to form mature vasculature are guided by extracellular cues that include soluble growth factors, extracellular matrix, and cell-cell interactions (249, 425). When the endothelial cells are assembled into vascular tubes, they become surrounded by mural cells of the smooth muscle cell lineage, referred to as pericytes (in capillaries, small venules, and immature blood vessels) and vascular smooth muscle cells (in mature and large blood vessels). Pericytes and smooth muscle cells differentiate from the mesenchyme and migrate around the growing blood vessels. Signaling molecules such as small G proteins that regulate differentiation, proliferation, and migration have been shown to play important role in vasculogenesis and angiogenesis.

1. Ras proteins

A) Ras. Mouse embryos lacking p120-Ras GAP exhibited major defects in their circulatory system organization. The yolk sac of p120-RasGAP deficient embryos was abnormal, with delayed organization of endothelial cells that aggregated into a honeycombed pattern but subsequently failed to organize into a vascular network (164). Within p120-RasGAP deficient embryos, the dorsal aorta was thinned with aberrant ventral branches that resemble intersegmental...
of cells of the endothelial lineage (537). The important role of p120-RasGAP in the regulation of Ras activity during vasculogenesis/angiogenesis has been further supported by the discovery of mutations in RASA1, the gene encoding p120-RasGAP in human (108). These mutations, leading to the loss of the GAP activity towards Ras proteins, cause capillary and arteriovenous malformations (CVM-AVM). This indicates that p120-RasGAP plays specific roles in the regulation of Ras signaling during vascular development that cannot be compensated for by other RasGAPs such as RAS2, RASAL, and NF1.

R-Ras GAP (also called GAP1AP4BP), which is membrane associated and displays a stronger GAP activity against R-Ras than H-Ras, has also been described as an important regulator of embryonic vascular development, in particular in blood barrier establishment (197). Homozygous mice expressing a R-Ras GAP mutant lacking its catalytic activity die at E12.5–13.5 of massive subcutaneous and intraparenchymal bleeding, probably due to underdeveloped adherens junctions between capillary endothelial cells, suggesting that Ras proteins are involved in the implementation of cell-cell junctions during vascular development.

Mechanisms and/or exchange factors upstream to Ras that ensure the control of Ras activity during the vascular development have been little addressed. Nevertheless, deletion of the H-Ras and K-Ras exchange factor Sos1 induces embryo death at mid-gestation with cardiovascular defects (508). In addition to an enlarged heart, the major vessels of Sos1-deficient embryos are distended, with areas of extensive hemorrhage. However, whether these changes are a secondary consequence of an earlier developmental failure or a direct result of loss of Sos1 protein has not been determined.

Effect of Ras during vascular development may involve at least in part its effector Braf identified as a critical signaling factor in the formation of the vascular system (537). Braf-deficient embryos die of vascular defects during mid-gestation. They had enlarged and irregularly shaped large blood vessels that were incompletely lined with endothelial cells. Apoptotic cells were particularly abundant in the endothelium of Braf-deficient embryos. Braf thus plays a critical and indispensable role downstream of Ras in the integration of mechanisms that regulate the differentiation of angioblasts, development and integrity of large vessels, and the survival of cells of the endothelial lineage (537).

In fact, inactivation of Erk1 and Erk2 in endothelial cells during embryonic development results in embryonic lethality due to strongly altered angiogenesis with a reduced vascular complexity of the vascular network, with only large unbranched vessels (458). This phenotype has been ascribed to a redundant role of ERK1 and ERK2 in the regulation of endothelial cell proliferation and migration, two processes that are critical for angiogenesis. The transcription factors Ets1 and Ets2, defined as targets of ERK signaling could mediate, at least in part, the effect of Ras/Raf/ERK1/2 signaling during angiogenesis (519). However, the rescue of the abnormal blood vessel organization in K-ras knock-down by Akt provided evidence that PI 3-kinase/Akt also plays an important role in mediating Ras signaling during embryonic vascular development (275).

Downstream to Ras, the Ras interacting protein 1 (Rasip1/Rain), identified as an endomembrane Ras effector, has been shown to be exclusively expressed in the endothelium of the developing vasculature in both frog and mice and involved in vasculogenesis (550). Although angioblasts emerged relatively normally, blood vessel development was severely inhibited in the Rasip1 knock-down frog embryo, due to a defect in branching. This suggests a role of Ras/Rasip1 signaling in angioblasts and endothelial cells after their initial angioblast specification, possibly during their migration, cord formation, or tubulogenesis. However, as rescue experiments demonstrate that failure of tubulogenesis in the absence Rasip1, can be fully restored by dominant-negative RhoA or siRhoA treatment, it appears that Rasip1 suppresses RhoA (known to inhibit lumen formation, see below) and promotes activation of Cdc42 and Rac1 (both known to positively mediate lumen formation, see below) probably secondary to the downregulation of RhoA activity (550).

b) Rapi. Total deletion of either Rap1a or Rap1b in mice leads to an inhibition of angiogenesis in vivo that has been ascribed to an endothelial cell defect (80, 81, 559). This defective angiogenesis in Rap1b-knockout mice resulted from an altered VEGF response as Rap1 has been shown to be required for VEGF-mediated VEGFR2 activation, in part via an integrin αβ2-dependent mechanism (248).

A role in the stabilization of endothelial cell-cell junctions is also ascribed to Rap1 and its specific effector KRT1 (274). Mouse homozygous null mutations of the Krt1 gene are embryonic lethal due to vascular defects including dilation of precursor vessels in the brain, enlargement and increased endothelial proliferation of the caudal dorsal aorta, as well as variable narrowing of the branchial arch arteries and proximal dorsal aorta (528). In humans, loss-of-function mutations of this gene causes autosomal dominant familial cerebral cavernous malformations (246). Mechanistically, it has been demonstrated that Rap1/KRT1 interaction leads to the translocation of microtubule-sequestrated KRT1 to cell-cell junctions, thereby supporting junctional integrity of endothelial cells and vascular development (139, 274). Interestingly, a recent study points to a role of

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the Rap-activated GAP ARAP3 in developmental angiogenesis (131). ARAP3 is a GAP for both Arf6 and RhoA, and its deletion in mice resulted in a major defect in developmental sprouting angiogenesis. ARAP3 could thus represent the molecular link between Rap1/KRIT1 and RhoA/Rho-kinase signaling, involving the repression of RhoA/Rho-kinase activity normally exerted by KRIT1 (see below).

Upstream to Rap1 protein, the guanine exchange factor C3G has been identified as a key regulator of vascular development. C3G stimulates guanine nucleotide exchange predominantly on Rap1 and, to a lesser extent, on R-Ras (187). C3G is a molecular link between Rap1 and cell-cell junction molecules such as cadherins, and induces activation of Rap1 upon dissociation of junctions (24). C3G mutant embryos died of vascular defect around E11.5 due to hemorrhage and loss of vascular integrity (503). Vascular endothelial cells appeared to be functioning normally, and the defects were related to abnormal blood vessel maturation with aberrant recruitment/migration, adhesion, and differentiation of mural cells. These data thus suggest that the role of Ras protein during vascular development is not restricted to the regulation of endothelial cell functions.

2. Rho proteins

A) RHOA. Part of the evidence for a role of RhoA in murine embryonic vascular development indirectly arises from the analysis of experimental models of cerebral cavernous malformation (CCM). CCM disease results from autosomal dominant loss of function mutations in the genes KRIT1 (CCM1), CCM2 (malcavernin, MGC4607), or PDCD10 (CCM3). Homozygous null mutations in KRIT1 or CCM2 results in lethal vascular and cardiac phenotype in mice and zebrafish, ascribed to essential dysfunction of endothelium causing embryonic lethality at midgestation stations (270, 408, 527). While initial vascular patterning is normal, targeted deletion of CCM2 in endothelial cells severely affects angiogenesis in mice, leading to morphogenic defects in the major arterial and venous blood vessels (53). KRIT1 and CCM2 (and also CCM3) physically interact, suggesting that they converge on the same downstream signaling effector and that the mechanisms altered by the mutations in these three genes causing the pathologtical phenotype are the same (504, 575). Indeed, KRIT1, CCM2, or CCM3 mutations induce activation of RhoA/Rho-kinase signaling, responsible for reorganization of endothelial cell actin cytoskeleton, loss of endothelial cell junctions, and increased vascular permeability (51, 466). The phenotype of endothelial cells from CCM2 mutant mice was rescued by pharmacological inhibition of Rho protein by simvastatin (466, 527). RhoA and Rho-kinase activity in endothelial cells thus appears to have a leading role during vascular morphogenesis and that a tight spatial and temporal inhibition of this pathway in endothelial cells is essential for normal cell migration, lumen formation, and normal angiogenesis. This concept is in agreement with in vitro studies that provide evidence that RhoA and Rho-kinase need to be actively inhibited during endothelial tube lumen formation and tube maintenance events (96). The role of RhoA/Rho-kinase axis in the developmental angiogenesis was further supported recently by the observation that homozygous mice deficient in both isoforms of Rho-kinase (Rock1−/−;Rock2−/−) display defective vascular remodeling in the yolk sac and die in utero before E9.5 (208). These results also suggest a redundant role of Rho-kinase isoform functions in vascular development during embryogenesis.

Loss-of-function studies in zebrafish and mouse point to a specific role of the RhoA GEF Syx (also called Tech, GEF720, PLEKHG5) in angiogenic sprouting in the developing vascular bed. Syx is not involved in vasculogenesis and angioblast differentiation, but in vascular sprouting. In zebrafish, Syx depletion induced the truncation and even the absence of intersomitic vessels (133). In syx knockout mice, arterial networks are sparse, with deficiency in small-diameter vessels and capillaries, whereas major arteries are intact (133). These phenotypes suggest evolutionary conservation of Syx function. At the molecular level, Syx has been identified as a regulator of the directional endothelial cell migration required for angiogenic sprouting through its interaction with angiomotin (Amot) to precisely localize RhoA activity to the leading front of migrating cells (110, 133).

Among RhoA activity regulators, a recent study pointed out a role of the RhoA GAP ARAP3, activated by PI 3-kinase and Rap. Deleting Arap3 in the mouse causes embryonic death in mid-gestation due to an endothelial defect in sprouting angiogenesis (131). However, as ARAP3 is also a GAP for Arf6, in addition to altered RhoA activity, change in the activity of Arf6 can also participate in the effect of Arap3 deletion on angiogenesis.

In addition to its role in endothelial cells, RhoA/Rho-kinase signaling in vascular smooth muscle cells is important for their recruitment to nascent vessels and vessel stabilization. Adapted regulation of RhoA and Rho-kinase activity is required for normal coverage of endothelial tubes with vascular smooth muscle, through the control of vascular smooth muscle cell contraction, spreading, polarity, and direct migration (317).

b) RHOB. RhoB plays a critical role in vascular development. Retinas of newborn RhoB-deficient mice display signs of retardation in the outgrowth of the primary plexus (6). The mechanism responsible for this effect is related to RhoB-mediated regulation of Akt stability and trafficking into the nucleus of endothelial cells. This regulation of Akt confers on RhoB a stage-specific role in the survival of sprouting endothelial cells that contributes to blood vessel assembly during development (6).
c) Rac1. The role of Rac1 in vascular development has been addressed thanks to the generation of mice with endothelial-specific deletion of Rac1. Endothelial-specific Rac1-deficient mouse embryos die at midgestation (around E9.5). They show an alteration of the development of major vessels, and small branched vessels were completely absent both in the embryos and their yolk sacs (477). In vitro functional studies of Rac1-deficient endothelial cells demonstrated the essential role of Rac1 in the transduction of the effects of vascular endothelial growth factor (VEGF) such as migration, tube formation, adhesion, and permeability. Rac1-deficient endothelial cells are not able to form lamellipodia structures and focal adhesions, or to reorganize their cell-cell contacts (477). Rac1 is not strictly required for the initial differentiation or recruitment of angioblasts from the mesoderm and blood islands but essential for subsequent migration of angioblasts to promote the establishment of capillary-like networks (477). These data support an important role of Rac1 in developmental angiogenesis rather than in vasculogenesis. Furthermore, Rac1 is necessary for the transition from blood endothelial cells to lymphatic endothelial cells required for endothelial cells to separate and form lymphatic vasculature and is therefore an essential part of lymphangiogenesis (94).

D) Cdc42. Ablation of Cdc42 in mouse embryonic stem cells does not affect endothelial lineage differentiation but completely blocks vascular network assembly because of an impaired directional migration (375). PKCα and GSK-3β have been identified as downstream effectors of Cdc42 during vascular morphogenesis (375). In zebrafish Cdc42 is involved in intracellular vacuole formation, the fusion of which plays a fundamental role in endothelial cell lumen formation of intersegmental vessels (207).

These data are in agreement with results obtained in mice lacking the specific Cdc42 effector Pak4 that showed abnormalities in vasculature throughout the extraembryonic tissue and the epiblast (486). In the knockout embryos, large vessels could still be detected, but smaller branching vessels were completely absent. The Pak4 knockout yolk sac did stain positively for endothelial cells, showing the presence of the vascular plexus, but did not have organized vessels. These data suggest that Cdc42/Pak4 signaling affects angiogenesis and branching, rather than the initial formation of vessels (486).

3. Other Rho signaling molecules

Pak2α, another member of the Pak family that can be activated by Cdc42 or Rac1, also participates in embryonic vessel formation. In zebrafish, Pak2α was identified as a downstream effector of the Rac1 and Cdc42 exchange factor βPix (273). Loss of βPix or Pak2α leads to loss of vascular integrity and hemorrhage in the head but not in other vascular bed. The βPix/Pak2α axis is thus involved in cerebrovascular stabilization, an effect that is likely to be due to a role in support cells (vascular smooth muscle cells and pericytes) surrounding the cranial vasculature (273).

B. Blood Pressure Regulation and Hypertension

In general, three major mechanisms contribute to long-term blood pressure control: the contraction of small arteries, the control of blood volume in which the kidney is a key player, and the regulation of the cardiac output. The first part of this section focuses on the involvement of Ras proteins and their regulators in the control of vascular tone, with a focus on their role in arterial smooth muscle cell contractility, either directly or indirectly through modulation of endothelial cell functions. The following sections will address the role of Ras proteins in the central control of blood pressure, and in the kidney-mediated regulation of water-electrolytes balance.

1. Role of Ras protein superfamily members in arterial smooth muscle cell contractility and vascular tone

The regulation of arterial contractility is essential for blood pressure control and represents a typical multicellular function regulated by Rho signaling pathways. Phosphorylation of 20-kDa myosin light chain (MLC) is the key event of the regulation of vascular smooth muscle contraction (373). MLC is phosphorylated by the Ca²⁺/calmodulin-activated MLC kinase (MLCK) and dephosphorylated by the Ca²⁺-independent MLC phosphatase (MLCP). Agonists (ANG II, norepinephrine, endothelin-1, thromboxane A₂ . . . ) that bind to G protein-coupled receptors produce contraction by increasing both 1) the cytosolic Ca²⁺ concentration and 2) the Ca²⁺ sensitivity of the contractile apparatus. The rise in cytosolic Ca²⁺ concentration results from the stimulation of Ca²⁺ entry through L-type voltage-dependent Ca²⁺ channels and Ca²⁺ release from the sarcoplasmic reticulum through IP₃-gated Ca²⁺ channels activated by PLC-generated inositol 1,4,5-trisphosphate (IP₃). The increased sensitivity of vascular smooth muscle toward Ca²⁺ results from inhibition of MLCP activity leading to increased MLC phosphorylation and tension at a constant Ca²⁺ concentration. In contrast, vasorelaxing agents decrease both the cytosolic Ca²⁺ concentration and the Ca²⁺ sensitivity of the contractile apparatus. Surprisingly, while Rho family proteins emerged as key regulators of smooth muscle cell contraction, the role of the other subfamilies of Ras proteins in the regulation of vascular smooth muscle contraction has not been extensively addressed.

2. Ras protein-mediated regulation of smooth muscle cell contraction, vascular tone and blood pressure (Figure 4)

a) Ras. The first demonstration of a role of Ras in the regulation of smooth muscle contraction was provided by the
observation that active H-Ras induces tension of permeabilized arteries by increasing the Ca$^{2+}$ responsiveness in a concentration-dependent manner (416). Downstream of Ras, ERK1/2 activation triggers MLCK phosphorylation, leading to an increase in its sensitivity to calmodulin and, consequently, its ability to phosphorylate MLC (227). In addition to this stimulation of MLCK activity, the Ras-ERK1/2 signaling was also shown to positively regulate the expression of MLCK in arterial smooth muscle by stimulating MLCK promoter (151, 152). This signaling pathway is activated by ANG II, both in vitro and in vivo in rats (152), and is also involved in the upregulation of MLCK in the arteries of spontaneously hypertensive rats (SHR) (151). The attenuation of high blood pressure produced by Ras FTIs in SHR or in ANG II-induced hypertension in rats suggested that activation of Ras signaling can contribute to the development of hypertension (34, 326).

All these data, suggesting a vasoconstricting and hypertensive action of Ras-ERK1/2 pathway, have been confirmed in vivo in genetically modified mice. Knock-in mice in which K-Ras has been replaced by H-Ras develop arterial hypertension (371). More recently, high blood pressure was also observed in a strain of genetically modified mice carrying the activating G12V mutation within their endogenous H-Ras locus (H-Ras-V12) (427). In contrast, H-Ras$^{-/-}$ mice show lower blood pressure than control animals, probably related to an upregulation of the NO-cGMP pathway (70).

b) Ral. Although a direct role of Ral proteins has not been provided, recent evidence suggests that RalA and RalGDS but not RalB is involved in the coupling of AT1 receptor to the activation of IP$_3$ signal transduction (140).

c) Rap. A recent study reported the existence of a cross-talk between Rap1 and RhoA signaling pathways in vascular smooth muscle. Activation of Rap1 causes relaxation by decreasing the Ca$^{2+}$ sensitization of force in smooth muscle through downregulation of RhoA activity (588). Rap1 activation is triggered by the exchange factor Epac, activated by the rise in cAMP induced by the stimulation of vasorelaxing receptors such as β-adrenergic or prostaglandine I$_2$ receptors. The mechanism by which Rap1 inactivates RhoA has not been identified but, by analogy to that found in endothelial cells and in neurons, it was suggested that a Rap1-activated RapGAP such as RA-RapGAP or ARAP3, shown to be directly stimulated by Rap1 (239, 553), may downregulate RhoA activity in smooth muscle cells (588).

3. Rho protein-mediated regulation of smooth muscle cell contraction, vascular tone and blood pressure (Figure 5)

A) RHOA. The major role of RhoA in smooth muscle contraction has been unequivocally demonstrated over the past 15 years. The Ca$^{2+}$-sensitizing effect of vasoconstrictors is ascribed to RhoA activation and the subsequent stimulation of its target Rho-kinase that phosphorylates MYPT-1, the regulatory subunit of MLCP, and inhibits its activity (490). While Rock1 and Rock2 are both expressed in vascular smooth muscle cells, Rock2 plays a prominent role in the regulation of vascular smooth muscle contractility (513). This mechanism of Rho-kinase-mediated Ca$^{2+}$ sensitize-

![Figure 5](http://physrev.physiology.org/)

**FIGURE 5.** Role of Ras proteins in the regulation of vascular smooth muscle cell contraction. Contraction is determined by the level of myosin light chain (MLC) phosphorylation (P-MLC), controlled by the activity of the MLC kinase (MLCK) and the MLC phosphatase (MLCP).
tion is thus responsible for the tonic component of vascular smooth muscle cell contraction. For in-depth coverage of RhoA/Rho-kinase signaling-mediated Ca\(^{2+}\) sensitization and regulation of smooth muscle contraction, the reader is referred to more comprehensive reviews (282, 373, 457).

Following the discovery of the key role of RhoA/Rho-kinase signaling in vascular smooth muscle cell contraction, numerous studies have addressed the role of this pathway in vivo in the control of blood pressure and hypertension (283). In SHR or in rats and mice treated with ANG II, L-NAME, or DOCA-salt, the development of hypertension is associated with an increase in rhoA and Rho-kinase activity in arteries (145, 216, 431, 435), thus showing that RhoA/Rho-kinase signaling activation is a common component for the pathogenesis of hypertension (282, 587). The causal relationship between the activation of RhoA and Rho-kinase and the increase in blood pressure has been indirectly addressed by the use of pharmacological Rho-kinase inhibitors. Rho-kinase inhibitors such as Y27632, fasudil, or SAR407899 reduce blood pressure in animal models of hypertension [ANG II (281); L-NAME (216, 281, 435); DOCA-salt (281, 490); SHR (241, 323)]. In addition to their blood pressure-lowering effect, Rho-kinase inhibitors also prevent the hypertension-associated cardiovascular remodeling, fibrosis, and inflammation (166, 189, 210, 216, 323).

All vasoconstrictors acting through G protein-coupled receptors induce RhoA activation, and different types of hypertension are all associated with an increased arterial RhoA activity. It is therefore likely that different Rho GEFs couple vasoconstrictor receptors to RhoA activation and are differently involved in the overactivation of Rho during hypertension. Nevertheless, only a few studies have addressed Rho GEFs expression, regulation, and activation in vascular smooth muscle cells. They mainly focused on the RGS-containing Rho GEF subfamily of Rho GEFs grouping Arhgef1 (p115-RhoGEF), Arhgef11 (PDZ-RhoGEF), and Arhgef12 (Larg) that are supposed to link G protein-coupled receptors to RhoA activation. All three RGS-containing Rho GEFs are expressed in arteries (169, 202, 569). Arhgef11 was described as the most expressed RGS-RhoGEF in rat aorta and mesenteric artery, while in mice aorta, the more abundant RGS-Rho GEF was Arhgef12 (531). Arhgef12 transcript and protein are expressed in vascular smooth muscle of murine embryos from E14 to later stages of development and in adulthood (30). The specific RhoA GEF p63RhoGEF and Arhgef11, have also been shown to participate in the early RhoA activation induced by ANG II stimulation of vascular smooth muscle cells (543, 568). The RhoGEF p63RhoGEF is also involved in RhoA activation and contraction induced by PhE and endothelin-1, by selectively coupling Goq/11 protein to RhoA activation (316). The RhoA exchange factors Lbc and Arhgef12 have been identified as mediator of RhoA activation in vascular smooth muscle cells stimulated by 5-HT and sphingosine 1-phosphate, respectively (28, 303) (FIGURE 6).

Genetic targeting of Rho protein signaling in mice shed light on the role of Rho GEFs and RhoA/Rho-kinase signaling in the regulation of blood pressure and hypertension. Rock1 haploinsufficient and deficient mice have normal basal blood pressure, and the rise in blood pressure induced by ANG II or L-NAME treatment is similar to that observed in control mice but vascular fibrosis is reduced (393, 582). Given the effect of Rho-kinase inhibitors in ANG II- and L-NAME-treated animals, this observation might suggest that Rock2 is the main isoform of Rho-kinase mediating the vasoconstricting effect of RhoA signaling in vascular smooth muscle cells. Unfortunately, since Rock2 deficiency is embryonically lethal, the cardiovascular phenotype of Rock2-deficient mice has not been assessed. Upstream to RhoA and Rho-kinase, genetic ablation of Arhgef1 and Arhgef12 has confirmed in vivo the role of these Rho GEFs in the regulation of vascular tone and blood pressure and highlighted their specific involvement in different pathways. While specific Arhgef1 deficiency in smooth muscle renders the mice resistant to ANG II- and L-NAME-induced hypertension (145), deletion of Arhgef12 induces insensitivity to salt-dependent hypertension (531).

b) Rac1. Rac1 is rapidly activated in vascular smooth muscle cells in response to stimulation with various vasoconstrictors such as ANG II, endothelin-1, and thromboxane A2. Nevertheless, in contrast to RhoA, its role in the regulation of smooth muscle contraction and the control of vascular tone remains unclear (452). The Rac effector Pak1 has been shown to phosphorylate and inhibit MLCK, thereby decreasing MLC phosphorylation and smooth muscle cell contraction (412, 532). This suggests that Rac action antagonizes the effect of RhoA on MLC phosphorylation, and negatively regulates vascular smooth muscle tone. However, opposite effects have also been reported as the other Rac effector Pak3 has been described to enhance smooth muscle cell Ca\(^{2+}\) sensitivity and contraction through phosphorylation of the thin filament regulatory protein caldesmon (119).

In vivo, chronic ANG II infusion induces Rac1 activation, essentially described in cardiac tissue (385, 415) but probably also in arteries (516). By triggering the activation of NADPH oxidase and redox signaling, Rac1 is a major mediator of the cardiac and vascular hypertrophic effect of...
ANG II (385, 421). Furthermore, it has been recently demonstrated that Rac1 is responsible for the oxidative stress and vascular dysfunction induced by mechanical stress of the arterial wall caused by high intraluminal pressure levels (499). Mechanical stress activates via integrin-linked kinase 1 and the Rac1 GEF betaPIX. Adenoviral transfection of a dominant-negative Rac1 decreases high blood pressure-induced NADPH oxidase activity and oxidative stress in carotid arteries, and normalizes vascular function. These data thus support a role of Rac1 in oxidative stress and its consequent vascular damages associated with hypertension (499). This role of Rac1 has been confirmed in mice with specific overexpression of the constitutively active mutant of Rac1 (Rac1-V12) in smooth muscle cells. These mice develop moderate hypertension compared with wild-type littermates, caused by enhanced superoxide production (157). Conversely, mice with heterozygous inactivation of Rac1 in endothelial cells (EC-Rac1<sup>+/−</sup>) develop moderate hypertension and display decreased eNOS expression and activity as well as impaired endothelium-dependent vasorelaxation (422). These observations suggest that endothelial Rac1 indirectly participates in the regulation of vascular tone and blood pressure through its effects on eNOS. Pharmacological strategies aiming to enhance endothelial Rac1 function might be of therapeutic interest for preserving endothelial function and normal blood pressure.

4. Rho protein-dependent endothelial-smooth muscle cells crosstalk

The maintenance of vascular homeostasis is largely dependent on the functional crosstalk existing between endothelial and smooth muscle cells within the arterial wall. The endothelium is a key determinant of vascular tone and resistance that acts by releasing vasoactive factors among which endothelium-derived nitric oxide (NO) is the most important. Both endothelial NO production and NO effects in vascular smooth muscle cells are regulated by RhoA and

**FIGURE 6.** RhoA GEFs involved in the activation of RhoA and Rock2 by the stimulation of G protein-coupled receptor by vasoconstrictors: ANG II and type 1 ANG II receptor (AT1R), phenylephrine (PhE) and α1-adrenoceptor (α1R), endothelin-1 (ET1) and endothelin receptor A (ETAR), serotonin (5HT), and sphingosine 1-phosphate. Contraction of vascular smooth muscle cells is determined by the level of myosin light chain (MLC) phosphorylation (P-MLC), controlled by the activity of the MLC kinase (MLCK) and the MLC phosphatase (MLCP).
Rac1 signaling (283). RhoA, through Rho-kinase activation, downregulates eNOS expression by destabilizing eNOS mRNA (256, 587) and decreases eNOS catalytic activity by inhibiting and stimulating PI 3-kinase/Akt pathway (538) and arginine activity (313), respectively. Rac1 antagonizes RhoA/Rho-kinase effects on eNOS. Rac1 upregulates eNOS expression by increasing eNOS mRNA stability and Pak1-mediated stimulation of transcription, and stimulates eNOS activity (422, 436).

In vascular smooth muscle cells, it has been shown that cGMP-dependent protein kinase (PKG) inhibits RhoA/Rho-kinase signaling by phosphorylating Ser188 of RhoA (417). This inhibitory phosphorylation, by decreasing the Ca\(^{2+}\) sensitivity of contractile proteins, plays a major role in the vasodilator effect of NO (417). However, recent findings indicate that Rac1 participates in the control of cGMP level in vascular smooth muscle cells. Activation of Rac1 increases intracellular cGMP concentration via Pak1-mediated inhibition of the activity of type 5 phosphodiesterase (PDE5) (418). The reduced cGMP and vasodilatory response to NO donors observed in arteries from Vav2-deficient mice supports a role of the Rho GEF Vav2 and the tyrosine kinase Src as the upstream regulators of Rac1 in vascular smooth muscle cells (418).

5. Role of Rho protein superfamily members in central blood pressure regulation

The baroreceptor reflex is one of the main physiological mechanisms that contributes to the stability of blood pressure (365). The specific areas involved in this nervous regulation of blood pressure are located in the brain stem (365). Afferent fibers from arterial baroreceptors project in the nucleus tractus solitarii (NTS). Signals are transmitted from the NTS to the rostral ventrolateral medulla (RVLM) from which sympathetic nerves conduct them to heart and vessels (365).

In vivo studies in the rat revealed that RhoA/Rho-kinase signaling in the NTS is involved in the sympathetic nervous system-dependent regulation of basal blood pressure. Inhibition of Rho-kinase in the NTS by adenoviral transfection of dominant-negative Rho-kinase or local infusion of the Rho-kinase inhibitor Y27632 reduces mean blood pressure and urinary excretion of norepinephrine (191). Blockade of Rac1 signaling in the NTS does not affect mean blood pressure or urinary norepinephrine excretion, although it decreases NADPH oxidase activity (344). These data suggest that RhoA- but not Rac1-dependent pathways in the NTS participate in the physiological regulation of blood pressure via the central nervous system. In contrast, both proteins play a role in ANG II-dependent hypertension. ANG II infusion increases NADPH oxidase activity and superoxide production and activates RhoA/Rho-kinase in the brain stem through an AT1 receptor-dependent mechanism (405, 590). Transfection into the brain of adenovirus vector encoding a dominant-negative Rac1 reduces the increase in blood pressure induced by ANG II (589). Similarly, the intracisternal infusion of the Rho-kinase inhibitor Y27632 reduces the central pressure action of ANG II (405). These observations thus provide evidence that ANG II-mediated Rac1 and RhoA/Rho-kinase activation in the brain stem contributes to hypertension induced by ANG II infusion.

6. Role of Ras protein superfamily members on kidney-mediated blood pressure regulation

The kidney has a central role in the control of blood pressure by regulating the maintenance of water-electrolyte balance, mainly through the reabsorption of filtered Na\(^{+}\). Na\(^{+}\) flux through epithelial Na\(^{+}\) channels (ENaCs) expressed in the apical plasma membrane of the renal collecting duct is the final renal adjustment step for Na\(^{+}\) balance. ENaC thus plays a critical role in modulating the homeostasis of Na\(^{+}\) and blood pressure. This channel is an end-effector in the renin-angiotensin-aldosterone system, and several regulatory pathways, some of them involving Ras proteins, modulate its activity and its expression (FIGURE 7).

7. Role of Ras proteins in the regulation of ENaC

A) RAS. In renal epithelia, aldosterone induces expression and activation of K-Ras (465). Moreover, K-Ras is both necessary and sufficient for activation of ENaC (465). K-Ras increases the activity of channels already present in the...
membrane by increasing channel open probability (461, 462, 463). Inhibition of PI 3-kinase by wortmannin blocks, while in contrast overexpression of active PI 3-kinase mimics the stimulation of ENaC induced by K-Ras (461, 462). This wortmannin-sensitive stimulation of ENaC is also observed in cells expressing an effector-specific mutant of Ras capable of only activating PI 3-kinase (462). These data indicate ENaC is activated by K-Ras in a PI 3-kinase signaling-dependent manner and that this K-Ras-dependent pathway may participate in the control of systemic Na⁺ balance and blood pressure.

b) RAB. Rab proteins have been recently identified as a component of aldosterone-dependent ENaC trafficking, thus regulating the ENaC density the apical membrane of epithelial cell of the collecting duct and Na⁺ absorption (264). Under basal conditions, the nonphosphorylated Rab-GAP AS160, by maintaining Rap proteins in an inactive GDP-bound state, stabilizes ENaC within an intracellular compartment (264). Aldosterone stimulation of Na⁺ transport, associated with increased apical ENaC density, involves the transcriptional induction of serum- and glucocorticoid-induced kinase (SGK1), which phosphorylates AS160 and inhibits its GAP activity leading to Rab activation. This relieves the AS160-mediated intracellular retention of ENaC and permits forward trafficking of the channel to the apical surface to augment Na⁺ absorption (264).

As ENaC recycles in polarized kidney epithelial cells, it is likely that the channel traverses a number of Rab-dependent compartments en route to the apical membrane, involving different Rab proteins. In fact, Rab 4, 11, and 27 have been shown to participate in ENaC regulation, and recent data indicate the absolute requirement for Rab11b to deliver ENaC to the apical membrane (63, 64).

B. Role of Rho proteins in the regulation of ENaC

A) RHOA. As for Ras proteins, evidence for a role of RhoA in the regulation of ENaC has been obtained in cell systems in vitro. Expression of wild-type RhoA and constitutively active V14-RhoA markedly increases ENaC activity, while in contrast the dominant negative RhoA-N19 decreases it (460). The effects of RhoA are primarily to increase the density of ENaC on the plasma membrane. Activation of ENaC in response to RhoA is sensitive to inhibition of Rho-kinase and disruption of PI(4,5)P₂ synthesis and is mimicked by overexpressing phosphatidylinositol-4-phosphate 5-kinase [PI(4)P5-kinase], indicating that activation of Rho-kinase and its known effector PI(4)P5-kinase, and the consequent increase in PI(4,5)P₂ levels mediate the increase in membrane levels of ENaC (460). It was further demonstrated that activation of RhoA rapidly increases the membrane levels of ENaC by promoting translocation and insertion of the channel. In addition to PI(4,5)P₂, RhoA-induced targeting of vesicles containing ENaC to the plasma membrane also involves cytoskeletal actin and tubulin filaments (214, 366).

b) RAC1. Expression of wild-type Rac1 or constitutively active Rac1 significantly increases ENaC activity, while CdC42 has no effect (360, 460). In contrast, the Rac inhibitor NSC23766 markedly decreases ENaC activity in cortical collecting duct (215). Knock-down of Rac1 also decreases ENaC-mediated Na⁺ reabsorption by decreasing the number of channels at the apical membrane (215). The effect of Rac1 on ENaC depends on WAVE1 and WAVE2, two members of the Wiskott-Aldrich syndrome proteins known to stimulate Arp2/3-mediated actin polymerization (215). The Rac1/WAVE signaling complex might thus play a central role in blood pressure control in the kidney, although the exact mechanisms involved are not defined yet. In support of such a role of Rac1, however, it has been recently suggested that Rac1 in kidneys is essential for salt-sensitive hypertension, via a mineralocorticoid receptor-dependent pathway (123, 446). High-salt loading triggers Rac1 activation in the kidneys of the Dahl rat model of salt-sensitive hypertension, and NSC23766 treatment significantly reduces the blood pressure of the rats, indicating a causal role of Rac1 activity in the salt-dependent elevation of blood pressure (446). Along with the repression of Rac1 activity, the treatment of Dahl rats with NSC23766 also significantly reduces salt loading-induced increase in mineralocorticoid receptor expression as well as SGK1 upregulation, indicating that the effect of Rac1 activation on blood pressure involves the activation mineralocorticoid receptor signaling in the kidney (446, 447).

9. Role of Rho proteins on sodium-hydrogen exchanger

In addition to the role of ENaC in the renal collecting duct, the sodium-hydrogen exchanger isoform 3 (NHE3) expressed in the apical membrane of the proximal convoluted tubules is one of the major transporters involved in the renal reabsorption of sodium under physiological conditions.

Inhibition of Rho proteins by toxin B from Clostridium difficile or Rho-kinase by Y27632 results in reduced NHE3 activity, due to a marked decrease in the surface expression of NHE3 and relocation of the exchanger to an intracellular compartment (159). In fact, RhoA and Rho-kinase, by controlling the phosphorylation of MLC and, ultimately, the organization of the actin cytoskeleton, are important for NHE3 association with ezrin and actin, and this interaction is required for maintenance of the exchanger at the apical surface (159, 474). In SHR, hypertension is associated with a marked increase in NHE3 activity and a decreased Na⁺ excretion (338). Inhibition of Rho-kinase by Y27632 reduces apical NHE3 activity and enhances Na⁺ excretion and total urine volume (338). Because, in general, a natriuresis in patients with hypertension reduces blood pressure, it has been suggested that the natriuresis induced by Y27632 may contribute to its blood pressure lowering effect (474).
10. Evidence for a role of Rho protein signaling in high blood pressure in humans

Both pharmacological data and genetic studies indirectly support a potential role of Rho proteins in the generation of high blood pressure in humans. The first data suggesting a role of RhoA/Rho-kinase in the pathogenesis of human hypertension were obtained by showing that the increase in forearm blood flow and the decrease in forearm vascular resistance produced by the Rho-kinase inhibitor fasudil are significantly stronger in patients with essential hypertension than in the normotensive group of patients, suggesting that hypertension-associated vascular dysfunction depended, at least in part, on Rho-kinase activation (298). The altered vasodilation and increased resistance of the forearm observed in patients with heart failure has been also ascribed to Rho-kinase activation (226). More recently, data obtained in healthy male subjects indicated that the Rho-kinase activity correlates with oxidative stress and aortic stiffness (340).

In addition to these functional evidences, genetic analyses have shown that Rho-kinase polymorphism influences blood pressure. This has been demonstrated for the nonsynonymous Thr431Asn Rock2 variant (430). A high basal blood pressure and increased systemic vascular resistance is associated with the Asn/Asn genotype (compared with Asn/Thr or Thr/Thr). More recently, a lower risk of high blood pressure is associated with a major haplotype block at the Rock2 locus in a recessive manner (384). In addition, recent data from genome-wide association studies have identified the association between diastolic blood pressure and common variants in eight loci, with one of them containing two genes related to Rho protein signaling: 1) RTKN2 encoding the RhoA effector rhotekin-2 and 2) RHOBTB1 encoding RhoBTB (333). Nevertheless, the potential role of these two gene products in the regulation of blood pressure is completely unknown.

C. Vascular Remodeling

Vascular remodeling occurs during normal development and participates in various physiological processes. However, vascular smooth muscle cell hypertrophy, proliferation, and migration also occur in hypertension, atherosclerosis, and restenosis after angioplasty or stent implantation, leading to pathophysiological remodeling of the arterial wall. These processes are possible because, in contrast to the majority of differentiated cells, smooth muscle cells retain the capacity to modulate their phenotype, to lose their contractile properties, to proliferate, and to migrate in response to a variety of pathological stimuli. By making analogies between vascular proliferative disorders and cancer, studies of Ras proteins have led to the discovery of their roles in cell processes underlying pathological arterial wall remodeling, and suggested that targeting these small G proteins may prevent or limit vascular remodeling associated with cardiovascular diseases.

1. Ras proteins

A) Ras. A role for Ras protein in the regulation of vascular smooth muscle cell proliferation has been proposed more than 20 years ago. H-Ras is expressed in smooth muscle cells, with a peak expression in proliferating cells in mid to late G1 phase (403). Growth factors such as PDGF activate the Ras-MAPK pathway in vascular smooth muscle cells, and Ras inhibition by FTI or expression of dominant negative H-Ras mutant reduces proliferation (235, 491). In contrast, introduction of the dominant active H-Ras-V12 in vascular smooth muscle cells induces a growth arrest with phenotypic characteristics of cellular senescence, suggesting that H-Ras signaling contributes to age-related vascular disorders (312). The other member of the Ras family, KiRas2A, is identified as a mediator of ANG II-induced vascular smooth muscle cell proliferation and senescence (310, 311). Accordingly, it has been proposed that different levels of Ras expression and activity may be one of the mechanisms that regulate the switch between growth/proliferation and senescence signaling in vascular cells (309).

Evidence for a role of Ras signaling in the regulation of vascular smooth muscle cell in vivo was then provided by studies showing that plasmid- or virus-mediated local expression of H-Ras dominant negative mutant significantly reduced neointimal formation in balloon-injured rat carotid arteries (188, 491) or pig coronary artery (541). In the same in vivo models, prevention of posttranslational modifications and thereby, inhibition of Ras activity by FTIs or S-adenosylhomocysteine hydrolase inhibitors (433, 540), or S-trans,farnesylthiosalicylic acid (136) has the same inhibitory action on balloon injury-induced neointimal proliferation.

Genetically modified mice models have further confirmed the in vivo role of Ras signaling in vascular remodeling and vascular cell proliferation, in particular models of haploinsufficiency or tissue specific deletion. Haploinsufficient nf1 mice (nf1+/−) have increased neointima formation, vascular inflammation, excessive vessel wall cell proliferation, and ERK activation after vascular injury (252, 253). A similar phenotype is observed in mice with smooth muscle cell specific deletion of nf1 but is rescued by smooth muscle cell expression of the GAP domain of NF1, indicating the causative role of excessive Ras activity in the increased vascular smooth muscle cell proliferation produced by nf1 deletion (549).

Upstream to Ras, the Grb2 adaptor, linking growth factor receptors to the Ras exchange factor Sos, has been shown to be expressed in vascular smooth muscle cells and recruited to the membrane by stimulation with PDGF, ANG II, or mechanical stretch, suggesting that Grb2/Sos could be re-
sponsible for the activation of Ras (33, 182, 268). This hypothesis is supported in vivo by the resistance to the development of neointima thickening in injured carotid of heterozygous Grb2<sup>+/−</sup> mice (581).

The Ras/MAPK pathway is also the target of physiological processes that inhibit vascular smooth muscle cell proliferation and maintain vascular wall homeostasis. Vascular smooth muscle cells expressed endogenous negative regulator of Ras that, under normal conditions, maintain a low activity of the Ras-Raf-MAPK signaling, thus preserving differentiation and quiescence. Such a regulation is responsible for the antiproliferative effect of the contractile phenotype marker SM22α. SM22α binds Ras, resulting in downregulation of Ras-Raf-MAPK signaling and blockade of G<sub>1</sub>-S phase cycle progression in vascular smooth muscle cells (107). In vivo, this mechanism is responsible for SM22α overexpression-induced inhibition of neointimal hyperplasia induced by balloon injury (107). In the same way, R-Ras is a member of Ras protein family that antagonizes H-Ras signaling (88, 438). R-Ras is abundantly expressed in normal mature blood vessels but significantly downregulated in hyperplastic neointimal smooth muscle cells (231). R-Ras knockout mice develop exaggerated neointimal thickening in injured femoral artery due to sustained smooth muscle cell proliferation (231). These results thus indicate the in vivo role of R-ras as a negative regulator of vascular smooth muscle cell hyperplasia, probably through inhibition of H-Ras signaling. A similar role has been ascribed to mitofusin-2, initially called hyperplasia suppressor gene (HSG). Mitofusin expression is decreased in hyperplastic smooth muscle cells from SHR arteries and from balloon-injured rat carotids compared with controls (77). It is also reduced in vitro upon stimulation of vascular smooth muscle cells by mitogenic factors such as PDGF, FGF, or ET-1 (77). Overexpression of mitofusin-2 by adenoviral infection almost totally prevents balloon injury-induced neointima formation and restenosis in rat carotid arteries (77). Mechanistically, mitofusin-2 binds Ras leading to inhibition of Raf-MAPK signaling and G<sub>1</sub>/G<sub>0</sub> cell cycle arrest (77).

**b) RAD.** Rad expression has been shown to be increased during vascular lesion formation after balloon injury (122). Adenovirus-mediated in vivo Rad gene delivery in carotid artery induces a marked reduction in neointimal formation after balloon injury, while in contrast, expression of a dominant negative form of Rad increases neointimal thickening. Rad was thus identified as an endogenous inhibitor of vascular lesion formation, acting by reducing migration of vascular smooth muscle cells, through at least in part, inhibition of Rho-kinase signaling (122).

**c) RAP.** Rap1a and Rap1b proteins have been shown to be upregulated by PDGF in vascular smooth muscle cells, and this upregulation during the smooth muscle cell cycle is directly linked to cell proliferation (378). PDGF and FGF-2 also induce an increase in Rap2 during late S phase and G<sub>2</sub>/M phase of the smooth muscle cell cycle (368). Silencing of Rap2 indicated that Rap2 is required for migration but not for vascular smooth muscle cell proliferation. Despite these data, the role of Rap proteins in vascular smooth muscle cells and vascular remodeling is not known, and in vivo studies are now necessary to address this question.

### 2. Rho proteins

**A) RHOA.** Numerous studies provide evidence for a direct link between RhoA/Rho-kinase signaling and pathological vascular remodeling, by interfering with several processes such as vascular smooth muscle cell differentiation, migration, and proliferation. RhoA/Rho-kinase signaling has been demonstrated to be a critical mechanism for controlling smooth muscle differentiation through the regulation of the actin cytoskeleton that selectively controls differentiation marker gene expression by modulating SRF-dependent transcription (272, 287).

In vitro, most mitogen factors for vascular smooth muscle cells activate RhoA and Rho-kinase, and inhibition of Rho-kinase reduces vascular smooth muscle cell proliferation induced by mechanical stretch or by several growth factors and vasoconstrictors including PDGF, thrombin, ANG II, and 5-HT (209, 210, 280, 345, 420, 429, 555, 577). RhoA/Rho-kinase activation accelerates cell cycle progression through downregulation of p27<sup>Kip1</sup> expression (257, 420). Conversely, the upregulation of p27<sup>Kip1</sup> expression induced by Rho-kinase inhibitors accounts for their antiproliferative effects (210, 420). In addition to this effect on p27<sup>Kip1</sup>, the necessary role of Rho-kinase for the nuclear translocation of ERK1/2 may also participate in Rho-kinase-mediated regulation of smooth muscle cell proliferation (280, 345, 577). As RhoA and Rho-kinase positively regulate two incompatible processes, differentiation and proliferation, it is possible that functions of RhoA/Rho-kinase signaling depend on the differentiation status of smooth muscle cells. This hypothesis is supported by the differential effect of Rho-kinase inhibition on the migration of proliferative and differentiated arterial smooth muscle cells. Inhibition of the RhoA/Rho-kinase signaling by pharmacological agents or expression of dominant negative limits vascular smooth muscle cell migration induced by several factors such as PDGF, thrombin, or lysophosphatidic acid (9, 420, 431), while in contrast, it increases cell motility of differentiated aortic smooth muscle cells (74).

In vivo studies have confirmed the role of RhoA/Rho-kinase in vascular remodeling. Rho-kinase inhibitors reduce neointimal formation induced by balloon injury in rat (420, 444) and pig (114, 332) or by stent implantation in pig (301). In these models, both an antiproliferative action through downregulation of the cyclin-dependent kinase inhibitor p27<sup>Kip1</sup> (420) and stimulation of apoptosis (444,
445) have been involved in the beneficial effect of Rho-kinase inhibitors. Hypertension-associated medial hypertrophy and perivascular fibrosis of small coronary artery of SHRs are also suppressed by long-term treatment with Rho-kinase inhibitors (323). Heterozygous deletion of Rock1 and Rock2 alleles further demonstrated the major role of Rock2 in arterial remodeling. Neointima formation induced by carotid ligation was markedly reduced in Rock1+/− mice compared with wild-type or Rock2+/− mice (341). This correlates with decreased vascular smooth muscle cell migration, proliferation, and survival and reduced leukocyte infiltration (341).

The RhoA/Rho-kinase signaling also participates in age-induced arterial wall remodeling and stiffening developing with ageing. A threefold increase in RhoA expression and activity has been observed in artery of old (19 mo) compared with young (2 mo) rats (307). Indirect evidence suggested that this upregulation also exists in human artery as it has been observed that RhoA/Rho-kinase-mediated cold-induced cutaneous vasoconstriction is augmented with age (482). Increased RhoA and Rho-kinase activity could thus be part of the mechanisms underlying the hypersensitive response of the vessel wall to injury and its increased susceptibility to develop hypertension and advanced atherosclerotic lesions with age. Indeed, it has been recently demonstrated that age-dependent increase in extracellular matrix stiffness enhances vascular cell contractility through RhoA/Rho-kinase activation (186). In endothelial cells, this process leads to mechanical destabilization of cell-cell junctions, increasing endothelial permeability and thus susceptibility to atherosclerosis.

B) RHOB. While RhoB is very similar to RhoA and shares its downstream effectors, in particular Rho-kinase, the role of RhoB in the vascular wall is, so far, almost not investigated. However, recently a role of RhoB in vascular smooth muscle cell migration induced by PDGF has been reported. Stimulation of the PDGF receptor-β on primary vascular smooth muscle cells results in RhoB-dependent trafficking of endosome-bound Cdc42 from the perinuclear region to the cell periphery, where the Rho GEF Vav2 and Rac are also recruited to drive formation of circular dorsal and peripheral ruffles necessary for cell migration (183). Analysis of vascular remodeling in RhoB-deficient mice would be certainly of great interest.

C) RAC1. Compared with RhoA, the function of Rac1 in vascular remodeling is less well understood. However, through its role in NADPH oxidase-dependent ROS generation, Rac1 plays an important role in the development of vascular smooth muscle hypertrophy and migration (180, 285). This role of Rac1 is involved, in particular, in vascular remodeling associated with increased oxidative stress as observed in high blood pressure (499). Moreover, Pak1 also appears as a downstream effector of Rac1 in vascular smooth muscle as expression of a dominant negative Rac1 suppresses ANG II-induced Pak1 activation, hypertrophy (539), and migration (173). Activation of the Rac1 and the subsequent engagement of Pak1 and JNK also participate in the EGF-induced vascular smooth muscle cell proliferation (32). Only a few studies have addressed the role of Rac1 in vascular remodeling in vivo. Transgenic overexpression of dominant active Rac1-V12 confirmed the role of Rac1 as a regulator of the redox state of blood vessel (157). In the balloon injury model of vascular remodeling, Pak1 is found to be activated in neointima of injured rat carotid artery, and a marked reduction of the neointimal thickening is observed in dominant-negative Pak1 or Rac1 adenovirus-treated artery (49, 173). In diabetes-induced vascular injury linked to an increased oxidative stress and Rac1/Pak1 activity, dominant-negative Rac1 gene transfer has also been shown to be protective (498). In short, all these data suggest that Rac1/Pak1 activation participates in pathophysiological vascular remodeling.

While Rac2 was initially found to have a restricted expression in hematopoietic cells, Rac2 expression and activity are induced upon stimulation of vascular smooth muscle cell with inflammatory cytokines (485). Retroviral overexpression of Rac2 in vascular smooth muscle cells leads to increased migration, activation of the NADPH oxidation cascade, and increased activation of Pak1 and its proximal effectors, ERK1/2, and p38MAPK. Rac2 was thus identified as a potential mediator of inflammation-driven vascular smooth muscle response to injury (485). Further studies, in particular in vivo analyses, are now required to clarify the role of Rac isoforms in arterial remodeling.

D) CDC42. Almost nothing is known regarding the role of Cdc42 in the arterial wall. A very recent work reports that the regulation of collagen I secretion by PKCδ in arterial smooth muscle cells is dependent on Cdc42 (261). Collagen type I is the most abundant component of extracellular matrix in the arterial wall, and a tight regulation of collagen is critical to homeostasis of the arterial wall. These data thus suggest that Cdc42, in addition to RhoA and Rac proteins, could also participate in the control of arterial wall structure.

D. Vascular Permeability

In addition to its role in the regulation of vascular tone and blood flow, a major function of the vascular endothelium is to constitute a semi-permeable barrier that controls passage of macromolecules and fluids between circulating blood and the body tissues. Endothelial barrier integrity is governed by the junctions between adjacent endothelial cells that line blood vessels. Both tight junctions that are particularly developed in the blood-brain barrier and adherens junctions are responsible for endothelial cell-cell adhesion. Most of the factors affecting vascular permeability modify
interacts with the actin cytoskeleton through both transient breakdown of the endothelial barrier followed by sealing as such as acute inflammation or angiogenesis (see sect. IVA). In contrast, dysregulated endothelial barrier function characterizes a pathological situation such as chronic inflammatory diseases and atherosclerosis (see sect. IVE).

Vascular endothelial (VE)-cadherin plays a key role in the regulation of adherens junctions. VE-cadherin is a transmembrane protein that forms homotypic interactions between neighboring endothelial cells. Its intracellular part interacts with the actin cytoskeleton through both α- and β-catenin and p120-catenin (29). Because Rho proteins are potent regulators of the actin cytoskeleton organization, numerous studies have addressed the role of RhoA, Rac1, and Cdc42 in the control of endothelial permeability (369, 536). Rap1 has also been recognized as a regulator of the endothelial barrier, and more recently, a role of other small G proteins such as Ras has also been described (359, 369).

1. Ras proteins

A) RAS. In endothelial cells, endogenous R-Ras physically interacts with filamin A, a member of the nonmuscle actin binding protein family (143). Knockdown of either R-Ras or filamin A promotes increased vascular permeability and disorganization of VE-cadherin at adherens junctions, through an increase in Src-dependent phosphorylation of VE-cadherin at Tyr731 (143). R-Ras, through its interaction with filamin A, is therefore required for maintaining endothelial barrier function. An analysis of tumor blood vessel formation in R-Ras knockout mice has confirmed its critical role in vessel integrity and function and particularly in the regulation of vascular permeability (419). Plasma leakage is increased, endothelial cell-cell junctions are disrupted, and VE-cadherin immunostaining is decreased in tumor vessels in R-Ras knockout mice. It was further demonstrated that expression of the constitutively active R-Ras-V38 in confluent endothelial cell cultures induced accumulation of VE-cadherin and β-catenin at the cell-to-cell interface, thus improving endothelial barrier function (419). This effect has been ascribed to the suppression of VE-cadherin internalization through inhibition of its phosphorylation on Ser665. Expression of the dominant negative R-Ras-N43 or knockdown of endogenous R-Ras has opposite effects (419).

In contrast, in vitro in human umbilical endothelial cells, H-Ras signaling has been shown to disrupt endothelial barrier integrity (148, 437). H-Ras/Raf1-dependent activation of MEK/ERK signaling results in increased phosphorylation of MLC, which leads to the recruitment of Src to the VE-cadherin, Src-mediated VE-cadherin phosphorylation, and dissociation of adherens junctions (148). Nevertheless, activation of PI 3-kinase/Akt pathway downstream of H-Ras is also described to be sufficient for H-Ras-induced vascular permeability (437).

B) RAP1. In vitro activation of Epac1/Rap1 in endothelial monolayer decreases basal and thrombin-induced permeability by potentiating VE-cadherin-mediated endothelial cell-cell contacts (92, 125, 234). This Epac1/Rap1 signaling pathway participates in the cAMP-dependent increase in endothelial cell-cell contacts induced by physiological factors such as prostaglandins or ANP (44, 45). In vivo, activation of the Epac/Rap1 signaling strongly reduces the baseline and platelet-activating factor-induced acute increase in endothelial permeability in intact microvessels and stabilizes the endothelial-endothelial junction complex (5). Through this regulation of the endothelial barrier function, the Epac1/Rap1 signaling pathway was thus identified as an essential mediator of the anti-inflammatory effects of cAMP and cAMP-elevating agents.

In addition to the AMPc/Epac1 activating system, Rap1 is also activated by homophilic VE-cadherin interaction upon cell-cell contact formation by the Rap1 GEF PDZ-GEF1 via the protein adaptor MAGI (411). This result thus reveals a reciprocal interaction between Rap1 and VE-cadherin, with Rap1 stabilizing VE-cadherin-dependent adhesion and cell-cell contact inducing Rap1 activation. Recently, it has been described that Rap1 isoforms differentially regulated endothelial cell junctions. Although Rap1B accounts for more than 90% of the total Rap1 expressed in endothelial cells, Rap1A is the predominant isoform involved in the formation and maintenance of endothelial cell barrier properties, and Rap1B is not able to substitute to Rap1A for these functions (534).

Downstream of Rap1, several different effectors are involved in the effects on endothelial barrier function. Among them is Rac1 as activation of Epac1/Rap1 in endothelial cells results in the activation of the Rac1 GEFs Tiam1 and Vav2 (44, 45). The resulting activation of Rac1 leads to enhancement of peripheral actin cytoskeleton and adherens junctions. CCM1 was also identified as a Rap1 effector for endothelial cell-cell junction regulation (139). Activated Rap1 interacts with CCM1, which targets CCM1 to cell-cell contacts (92, 125, 234). This Epac1/Rap1 signaling participates in the cAMP-dependent increase in endothelial barrier function (437, 438). Interestingly, AF6 is also considered as a Rap1 effector as it specifically binds Rap1-GTP (468) and preferentially the Rap1A isoforms (534).

Recently, Raf1 has been identified as an essential component of Epac1/Rap1-mediated endothelial cell cohesion. Upon Rap1 activation, Raf1 is recruited to VE-cadherin complexes, which is required to bring Rock2 to VE-cadherin containing junctions to provide a relevant control of actomyosin activity in these structures (530).
2. Rho proteins

A) RhoA. RhoA was first described for its role in endothelial cell contraction and hyperpermeability. Both in vitro and in vivo data demonstrated that RhoA activation plays an essential role in the barrier-disrupting effect of various agents such as thrombin, tumor necrosis factor (TNF)-α, lipopolysaccharide (LPS), and lysophosphatidic acid (LPA) (83, 111, 112, 495, 496, 535). In addition, RhoA and its target Rho-kinase also have a negative role in the regulation of basal endothelial barrier function. Inhibition of Rho-kinase reduced baseline vascular permeability in vivo and in different cultured endothelial cells in vitro (4). Generation of actomyosin contractility and the resulting isometric tension in endothelial cells are the main mechanisms through which RhoA/Rho-kinase signaling induces junctional breakdown and induction of permeability. RhoA-stimulated Rho-kinase activity phosphorylates and inhibits MLCP, leading to an increased phosphorylation of MLC and acto-myosin interactions (111).

In contrast, it was also reported in confluent endothelial cell cultures that intrinsic basal activity of Rho-kinase is required for barrier maintenance through an action on VE-cadherin endosomal recycling (494). Nevertheless, the relevance of these data has not been demonstrated in vivo.

Several mechanisms have been proposed to trigger the activation of RhoA by agents that increase endothelial permeability. It has been first described that thrombin/PAR-1-induced RhoA activation resulted from PKCα-mediated Rho-GDI phosphorylation and inhibition of its activity, leading to increased RhoA-GDP/GTP turnover and RhoA activation (304). Arhgef1 has then been identified as the RhoA GEF mediating activation of RhoA in response to thrombin/PAR-1 receptor stimulation in a Gα12/13- and PKCα-dependent fashion (40, 179). This first phase of Arhgef1-mediated RhoA activation is followed by a second phase, dependent on microtubule disassembly. It has been proposed that, in addition to its role on actomyosin contractility, Rho-kinase phosphorylates the microtubule regulatory protein tau, causing peripheral microtubule disassembly and the subsequent release of microtubule-associated GEFs such as p190-RhoGEF and Arhgef2 (GEF-H1) (39, 40). These events induce a more sustained RhoA activation, cytoskeletal changes, and endothelial barrier dysfunction in response to thrombin (39, 40). More recently, Arhgef1 was also identified as the RhoA GEF mediating-RhoA activation and permeability induced by TNF-α (361) and LPS (546). Activation of Arhgef1 by TNF-α is mediated by PKCα phosphorylation of Arhgef1 (361).

On the other hand, concomitantly to Rac1/Cdc42 activation, downregulation of RhoA activity is essential for both the maintenance of basal endothelial barrier function and recovery after disruption. The GAP p190-RhoGAP that inactivates RhoA has been recognized as a key signaling molecule for this process. The activity of p190-RhoGAP is regulated by Src-mediated tyrosine phosphorylation, and in the basal condition, the tyrosine phosphatase SHP2 participates in the maintenance of a low RhoA activity by regulating p190-RhoGAP phosphorylation (144). Phosphorylation of p190-RhoGAP on serine/threonine residues also participates in the regulation of its activity (318), and kinases such as PKCδ and FAK also positively control p190-RhoGAP activity to limit basal RhoA activity and to stop RhoA activation during thrombin stimulation, respectively (154, 178). Regulation of p190-RhoGAP expression provides an additional level of regulation of RhoA activity as the enhancement of endothelial barrier function induced by cAMP elevating agents involves downregulation of RhoA activity through stimulation of p190RhoGAP-A expression at a transcriptional level (75).

B) Rac1 and Cdc42. Contrary to RhoA, Rac and Cdc42 reinforce endothelial barrier function. Rac1 is required for the formation and the maturation of endothelial junctions. Rac1 activity is increased during junction assembly, and Rac1-deficient endothelial cells are unable to form cell-cell contacts (250, 343, 477). Consistent with this role of Rac1 for maintaining cell-cell junctions, expression of dominant negative Rac1 increases endothelial permeability and disturbs tight and adherens junctions (27, 535). Inhibition of Rac1, in association with RhoA activation, is involved in the increase in endothelial permeability and loss of cell-cell junctions induced by thrombin (505, 535). Thrombin-induced Rac1 inhibition results from proteinase-activated receptor 1/Gαq-mediated inactivation of adenylate cyclase, decrease of cAMP level, and subsequent inhibition of the Epac1/Rap1/Vav2, Tiam/Rac1 cascade (41, 507). On the other hand, factors that increase endothelial barrier function such as mechanical forces, sphingosine 1-phosphate, ANP, prostacyclin, oxidized phospholipids, or hepatocyte growth factor are thought to act via activation of Rac1 downstream of either Epac1/Rap1 or PI3-kinase signaling pathways (42, 45, 271, 449, 506). In addition to these mechanical and soluble factors, “outside-in” signaling from VE-cadherin also regulates Rac1 activity by a positive feedback mechanism that is essential for maximal endothelial barrier function and recovery of endothelial cell monolayer integrity (43).

Cdc42 does not participate in the effect of thrombin on endothelial permeability, but it contributes to the increased actomyosin contractility subsequent to thrombin stimulation as dominant negative Cdc42 prevents thrombin-induced stress fiber formation (535). Moreover, Cdc42 is activated at sites of junctional complex formation, suggesting its role in the regulation of cadherin junctions (343). Cdc42 has then been shown to promote association of α-catenin with E-cadherin/β-catenin complex and its consequent interaction with the actin cytoskeleton (56). In vivo expression of a dominant active Cdc42 prevents RhoA-dependent increase in endothelial permeability, thus protecting barrier...
Adherens junctions themselves control Cdc42 activity (369). Following loss of homotypic interaction, the cytoplasmic domain of VE-cadherin triggers the activation of Cdc42 (369). The mechanisms responsible for this VE-cadherin effect are not identified but might involve either a direct activation via a specific Cdc42 GEF or an indirect mechanism involving the recruitment of p190-RhoGAP by p120-catenin in disassembled junctions, the inactivation of RhoA followed by the activation of Cdc42 (57, 236). This activation of Cdc42 by junction disassembly plays an essential role in the restoration of endothelial barrier integrity.

The mechanisms by which Rac1 and Cdc42 contribute to junction assembly are still speculative but probably involve IQGAP1. Active Rac and Cdc42 bind IQGAP1, thus releasing β-catenin from IQGAP and allowing β-catenin/VE-cadherin association and stabilization of the junctional protein complex (242, 369). Another possibility is that Rac1 and Cdc42 indirectly control adherens junction through local actin cytoskeleton regulation (271, 369). Active Rac1 recruits Pak and its target Lim kinase that phosphorylates and inactivates coflin, which finally leads to accumulation of cortical actin (506). Focal adhesion proteins have also been proposed to participate in Rac1/Cdc42-mediated regulation of endothelial permeability. Rac1/Cdc42 activation allows redistribution of focal adhesion proteins and association with adherens junction protein complexes through paxillin/β-catenin interactions (42). Although the molecular mechanism involved is not completely understood, it might involve Pak-induced phosphorylation of paxillin on Ser273 (331).

E. Atherosclerosis, Atherogenesis (Figure 8)

In recent years, great efforts have been devoted to unraveling the basic mechanisms of atherosclerosis, the underlying pathology of cardiovascular diseases. An imbalanced lipid metabolism with increased circulating levels of low-density lipoprotein (LDL) and a maladaptive immune response entailing a chronic inflammation of the arterial wall are clearly identified as major triggering factors. Atherogenesis typically starts with dysfunction and activation of endothelial
cells that triggers recruitment of proinflammatory circulating cells. The resulting local inflammation induces leukocyte chemotaxis and adhesion to the damaged endothelium, leading in turn to an increased vascular permeability for plasma lipids. Monocytes located in the arterial intima accumulate these lipids and transform into macrophage-derived foam cells, thus constituting early plaques. By secreting cytokines and growth factors that stimulate vascular cells, these early plaques generate their own growth and progress into mature plaques. Therefore, in addition to vascular wall cells, circulating immune cells including monocytes, lymphocytes, and platelets contribute to the disease initiation and progression. By regulating numerous functions in these different cell types, members of Ras protein superfamily, in particular Rho proteins, are involved in this complex pathological process.

1. Ras proteins

A) RAS. Cellular senescence and inflammation are classical features of atherosclerosis. The enhancement of vascular inflammation and senescence induced in vivo by adenovirus-mediated expression of dominant active H-Ras in rat arteries has led to the suggestion of a potential role of Ras in atherogenesis (312). This is in agreement with the attenuation of atherosclerotic plaques in ApoE−/− mice treated by FTI, in association with a reduced Ras activity in arteries (135, 471). FTI has no effect on plaque T lymphocyte and macrophage content, but vascular cell adhesion protein-1 (VCAM-1) and NFκB expression, and oxidative stress are markedly reduced in treated ApoE−/− mice compared with controls (135, 471). This suggests that Ras activity has a pro-atherogenic action, via mechanisms that are not elucidated. It has been proposed that the downstream Ras effectors early growth response-1 (Egr-1) that regulates transcription of proinflammatory and procoagulant genes could be involved. Oxidized LDL induces expression of Egr-1 in endothelial and smooth muscle cells and in macrophages at least in part via the Ras-ERK1/2 pathway (153).

Recently, pieces of evidence supporting a role of Ras proteins in atherosclerosis in human have been provided by genome-wide association studies (109). They identified a coronary artery disease locus in M-Ras. The gene encodes for the M-Ras protein that is widely expressed in all tissues, with a very high expression in the cardiovascular system. Although these data do not allow anticipating the role of M-Ras in atherogenesis, it could be related to its involvement in adhesion signaling (570).

2. Rho proteins

Given the key role of Rho proteins in the regulation of vascular cell migration and proliferation, the recognition of their involvement, in particular RhoA and its effector Rho-kinase, in atherosclerosis is not surprising. However, recent data also revealed that Rho proteins contribute to vascular inflammation, leukocytes infiltration, and lipid uptake/eflux in macrophages.

A) RHOA. RhoA expression and activity increase with cellular infiltration during the progression of atherosclerotic lesions (351). Downstream RhoA, Rho-kinases have been also shown to be upregulated at inflammatory arteriosclerotic lesions in both a porcine model of coronary artery spasm (211) and arteriosclerotic human arteries (212). In agreement with this observation, in vitro studies have shown that oxidized LDL and its compound LPA activate RhoA in vascular smooth muscle and endothelial cells, suggesting that hyperlipidemia could contribute to the increased RhoA activity in the wall of arteriosclerotic arteries (130, 470). A causal role of RhoA/Rho-kinase signaling in the progression of plaques was first provided by pharmacological analyses that showed that in vivo chronic treatment with Rho-kinase inhibitors strongly reduced arteriosclerotic lesions and spams in pig (320) and in ldlr−/− mice (289). NF-κB activation downstream to RhoA/Rho-kinase and modulation of T-lymphocyte proliferation have been suggested to contribute to the pro-atherosclerotic effect of RhoA/Rho-kinase signaling (289). Together with the indirect evidence that inhibition of Rho protein isoprenylation mediates, at least in part, the anti-inflammatory and anti-arteriosclerotic properties of statins, these observations thus confirm the pro-inflammatory and pro-arteriosclerotic effects of RhoA/Rho-kinase signaling (334).

Upregulation of RhoA in arteriosclerotic vascular wall could also account for endothelial cell activation and dysfunction associated with atheroma plaque formation. Activation of RhoA/Rho-kinase signaling decreases expression and activity of eNOS and, by increasing endothelial cell contractile forces, enhances permeability, thus allowing easier passage of molecules, such as LDL, and leukocytes from the bloodstream into the vessel wall intimal layer (186, 254, 533). Many different mechanisms are likely to be involved in the overactivation of RhoA, including the increased oxidative stress and ROS production in atherosclerotic arteries. Effects of ROS on Rho proteins may be cell specific and depend on the localization and the activity of a particular Rho protein. Indeed, both ROS-mediated activation and inhibition of RhoA have been described (7, 336). Under conditions where ROS are generated at increased pathological levels, ROS can directly activate RhoA by oxidation of cysteine 16 and 20 (7).

Leukocyte adhesion is further favored by RhoA-mediated direct positive regulation of leukocyte adhesion to the endothelium. RhoA controls both the expression of leukocyte adhesion receptors such as E-selectin, VCAM-1, and intercellular adhesion molecule-1 (ICAM-1) on endothelial cell membrane at least in part through NF-κB signaling, and downstream signals from these receptors (69). In fact, en-
gagement of ICAM-1 following leukocyte adhesion induces RhoA activation and recruitment of ERM (ezrin/radixin/moesin) to sites of ICAM-1 clustering. ERM proteins thus participate to further increase RhoA activity in endothelial cells by binding to and recruiting the Rho GEF Dbl, and by binding to RhoGDI which releases its inhibition of RhoA (333). Furthermore, RhoA/Rho-kinase activation also favors recruitment of circulating cells into atherosclerotic lesions by inducing release of cytokines and chemokines such as monocyte chemotactic protein-1 (MCP-1, also known as chemokine ligand 2, CCL2) and interleukin-6 by vascular cells (129, 193).

On the other hand, adhesion to the endothelium also induces upregulation of Rock1 in leukocytes (120), and accumulating evidence also suggests that RhoA signaling in infiltrating leukocytes, in particular in monocyte/macrophages, plays a key role in the progression of atheroma plaques. Indeed, atherosclerosis is decreased in mutant mice with specific Rock1 deficiency in bone marrow-derived cells on a LDL receptor deficiency background (510). Lack of Rock1 in bone marrow-derived cells decreases macrophages, T cells, and lipid accumulation in the atherosclerotic lesions. In vitro, Rock1-deficient macrophages exhibit impaired chemotaxis and reduced oxidized LDL uptake and foam cell formation. By promoting macrophages chemotaxis and lipid accumulation, Rock1 in macrophages is therefore critical to the development of atherosclerosis. Whether or not Rock2 plays a similar role remains to be determined. Recently, although the involvement of RhoA activation has not been addressed, it has been shown that the Vav family of Rho GEFs regulate CD36-dependent uptake of oxidized LDL in macrophages and foam cell formation (380).

The pro-atherosclerotic effect of RhoA/Rho-kinase signaling and its positive action of foam cell formation could also be related to its inhibitory effect on macrophage cholesterol efflux. RhoA, by inhibiting PPARγ activity, negatively controls ABCA1 expression and ApoA1-mediated cholesterol efflux, thus favoring foam cell formation (21, 293, 564).

b) RAC1. As RhoA, Rac1 expression and activity is increased in arteries from atherogenic animal models (196, 255, 351). Interestingly, Rac1 expression and activity are reduced by estrogen in vascular smooth muscle and monocytes, suggesting that this effect could participate in the atheroprotective action of estrogen (255). The involvement of Rac1 activation in atherogenesis has not been directly assessed, although a number of results suggest it could play a role. Rac1 is part of the NADPH oxidases NOX1 and NOX2, which play a major role in ROS formation and subsequent processes involved in atheroma plaque formation such as vascular smooth muscle hypertrophy and migration (180, 285) and VE-cadherin-dependent endothelial cell-cell adhesion (29, 497). Rac1 also regulates ICAM-1 and VCAM-1 expression in endothelial cells and could thus participate in the recruitment of inflammatory cells (69). Moreover, VCAM-1 activation induces Rac1 signaling in endothelial cells leading to NADPH-derived ROS-dependent loss of cell-cell junction that facilitates paracellular leukocyte transendothelial migration (497, 533).

A tight control of temporal and spatial activation of RhoA and Rac proteins is essential to coordinate leukocyte and macrophage migration. Active Rac at the leading edge of lamellipodia mediates local actin reorganization, producing lamellar extension and forward cell movement, while active RhoA at the rear produces contraction and detachment of the tail. The balance between the opposing activities of these proteins is therefore a key determinant of cell morphology and movement that is also regulated by Rac1-mediated ROS generation (336). Rac1 induces downregulation of RhoA activity through production of ROS that inhibits the low-molecular-weight protein phosphatase and then increases the tyrosine phosphorylation and activation of p190-RhoGAP which in turn inactivates RhoA (336). This mechanism is essential for the induction and maintenance of lamellipodia in migrating cells (336).

Macrophage chemotaxis is induced by chemokines and cytokines produced during local inflammation at sites of plaque formation. Macrophage migration triggered by such chemotactic factors, including MCP-1 and macrophage colony-stimulating factor (M-CSF), is dependent on Rac1 activation with Pak1 and ERK1/2 as downstream Rac1 effectors (392, 454).

Although local activation of Rac1 in the lamellipodia is required for cell movement, strong enhancement of Rac activity and imbalanced Rac1/RhoA activities disrupt cell migration (14). Such a mechanism has been recently identified as responsible for the inability of macrophage foam cells to leave the artery, thus causing progression of atherosclerotic lesions. Cholesterol loading of macrophages leads to increased plama membrane localization and activation of Rac1, and abrogation of migration toward various chemotactic stimuli (354). Conversely, it has been suggested that the ability of high-density lipoproteins (HDL) to promote regression of atherosclerosis resulted from the inhibition of this mechanism and consequent emigration of macrophages from lesions. HDL, in conjunction with the ATP-binding cassette transporters ABCA1 and ABCG1, serve to prevent formation of cholesterol-rich domains on the inner side of the plasma membrane of cholesterol-loaded macrophages and thus limit Rac1 activity (354). This antiatherogenic effect is further reinforced by the inhibitory action of HDL lysosphingolipids and type 3 sphingosine 1-phosphate receptors on Rac1 activity, ROS generation, and MCP-1 production by vascular smooth muscle.
cells, which limits macrophage entry into atherosclerotic lesions (487).

A role for Rac signaling in human atherosclerosis has been recently provided by a genetic association mapping study showing associations of single nucleotides polymorphisms (SNPs) in the KALRN gene with early onset of coronary arterial disease (511). Kalirin, the product of the KALRN gene, is a GEF for Rac (and RhoG). The strongest association was found for the SNP rs9289231 that resides in the first intron of an alternative transcript of the KALRN gene. Furthermore, the risk allele of rs9289231 was significantly correlated with atherosclerosis burden in human aortas (511). Strikingly, the same study also identified a SNP in the CDGAP gene encoding for the Rac/Cdc42 GAP CDGAP that displays evidence of association with coronary artery disease. These data thus strongly suggest that dysregulation of Rac signaling contributes to the coronary artery disease susceptibility linked to KALRN SNP. Functional analyses of the consequence of kalirin mutations are now required to understand its role in the development of atherothrombotic diseases.

c) Cdc42. Again, like for Rac, the role of Cdc42 in atherosclerosis has never been directly assessed, although several observations suggest that it could participate in various processes involved in atherogenesis. First, Cdc42 activity is essential for endothelial barrier repair, adherens junctions stability, and restoration of permeability (57). Therefore, dysregulated Cdc42 activity might contribute to the failure to restore endothelial barrier function associated with atherosclerosis. The mechanisms involved in this Cdc42 function are not fully understood, although it probably involves its effect on actin remodeling. Upstream receptors as well as Cdc42 regulators and effectors engaged during endothelial barrier repair remain to be identified. Second, although in contrast to Rac and Rho, Cdc42 is not required for cell locomotion, it is essential for polarizing in the direction of the chemotactic gradient. Macrophages expressing a dominant negative Cdc42 mutant are able to migrate but are unable to polarize in the direction of the gradient of MCSF and move randomly (14). Cdc42 activity is thus probably involved in chemottractant-induced monocyte recruitment to sites of vascular inflammation during the formation and the progression of atherosclerosis. Third, Cdc42 is involved in cholesterol movement in macrophages. As mentioned above, HDL exerts atheroprotection via multiple mechanisms, but one of the most important is cholesterol efflux. Cholesterol efflux from the cells is the rate-limiting step in regulating the intracellular cholesterol content as well as raft structure in the plasma membrane. By this mechanism, that involved ABCA1 and ABCG1, excess cellular cholesterol is released from cells and transferred to HDL particles. This is the first step of reverse cholesterol transport that carries cholesterol from peripheral tissues to the liver for eventual elimination. The role of Cdc42 in cholesterol efflux has been discovered thanks to studies on Tangier disease, a hereditary HDL deficiency caused by Abca1 mutations characterized by the presence of defective cellular cholesterol efflux and development of premature atherosclerosis (174). Expression of Cdc42 is markedly decreased in macrophages, and also in fibroblasts from patients with Tangier disease. Expression of a dominant negative Cdc42 mutant reduces while in contrast, expression of an active Cdc42 mutant increases lipid efflux, suggesting that defective Cdc42 signaling is enough to alter lipid efflux (174). It was further demonstrated that binding of apoA-I, the major HDL apolipoprotein, to the plasma membrane induced activation of Cdc42 and JNK has been identified as a downstream Cdc42 effector involved in apoA-I/HDL-induced cholesterol efflux (174, 339). By promoting rearrangement of actin cytoskeleton and activation of JNK, Cdc42 activation could allow trafficking of cholesterol vesicles from the intracellular site to the plasma membrane where ABCA1 assembles apoA-I and cholesterol for its efflux from cells. Cdc42 thus plays an important role in cellular cholesterol efflux, and its dysregulation might lead to the development of atherosclerotic cardiovascular disease.

All these data point out the need to combine in vitro and in vivo approaches using relevant animal models to advance our understanding of Cdc42 role in the initiation and the progression of atherosclerosis.

V. "RASOPATHIES"-ASSOCIATED CARDIOVASCULAR DYSFUNCTIONS

The Rasopathies form a group of nine developmental syndromes caused by germline mutations in genes encoding Ras proteins as well as Ras protein regulators or effectors, leading to Ras/MAPK pathway dysregulation. (Table 4 is available online as supplementary data due to its size.) Therefore, although each syndrome displays unique phenotypic features, they share a common pathophysiology and many clinical signs including characteristic facial features, heart defects, skin anomalies, mental retardation or learning difficulties, growth deficit, and an increased risk of developing tumors. This part thus deals with the cardiovascular features of the major rasopathies.

A. Noonan Syndrome

1. Clinical features

Noonan syndrome (NS; OMIM 163950) is a relatively common autosomal dominant inherited disorder with an estimated prevalence of 1 in 1,000 to 1 in 2,500 live births.
The NS phenotype, which becomes less pronounced with age, includes a typical dysmorphic appearance, developmental delay and learning problems, visual abnormalities, auditory problems, orthopedic defects, and short stature (103, 398, 481). Congenital heart defects are found in 50–80% of patients with NS (60, 291, 398). Newborns and children suspected of developing NS need a careful cardiac evaluation. Pulmonary valve stenosis and hypertrophic cardiomyopathy are the most common forms of cardiac disease. Atrial septals and partial atrioventricular canal defects are also associated with the NS. Fifty percent of patients with NS have an unusual electrocardiographic pattern characterized by left-axis deviation, an abnormal R/S ratio over the left precordial precordium, and an abnormal Q wave (379).

2. Molecular pathogenesis

Tartaglia et al. (479) identified missense mutations in the Ptpn11 gene in ~50% of NS patients (479). It is more prevalent among NS patients with pulmonary valve stenosis and short stature, and less common in subjects with hypertrophic cardiomyopathies and/or severe cognitive deficits. The Ptpn11 gene encodes Src homology 2 containing protein tyrosine phosphatase 2 (SHP2), a nonreceptor protein tyrosine phosphatase. This ubiquitous protein is involved in the effects of numerous physiological mediators such as growth factors, hormones, or cytokines. In particular, by modulating phosphorylation of proteins within multiple signaling pathways, including the Ras/MAPK cascade, SHP2 controls intracellular transduction mechanisms that regulate cell cycle progression, growth, and migration (38, 95). Most of the mutations of Ptpn11 in NS patients disrupt an autoinhibitory interaction between the NH2-terminal domain and the catalytic phosphatase domain. These amino acid substitutions promote SHP2 gain-of-function leading to increased binding affinity for signaling partners of Ras/MAPK pathway. Various strains of mice have been generated to understand the role of SHP2 in cardiac development and physiology. Mice expressing a constitutively active form of SHP2 (Ptpn11 D61G allele) exhibit defects similar to those seen in NS patients including short stature, facial dysmorphism, valvular hyperplasia, and septal defects (20). By studying an inducible Ptpn11 allele, they demonstrated that the expression of mutant SHP2 only in endocardium is sufficient to induce all cardiac defects in NS (19). The cardiac-specific deletion of Ptpn11 rapidly induces a compensated dilated cardiomyopathy in mice without an intervening hypertrophic phase (233). In this study, the authors demonstrated in vitro and in vivo an abnormal ERK/MAPK and RhoA/Rho-kinase activation in SHP2-deleted cardiomyocytes. Ex vivo, inhibition of these signaling pathways rescues the dilated phenotype, suggesting a critical role of RhoA and ERK/MAPK in the dilated cardiomyopathy of NS patients. Recently, Langdon et al. (251) have confirmed this hypothesis by introduction of Noonan SHP-2 mutation (SHP-2N308S) into Xenopus embryos. Embryos expressing this mutation are characterized by an over activity of Rho-kinase leading to abnormal arrangement and polarity of cardiac actin fibers, and consequently cardiac development defects.

The second most frequent gene mutated identified in NS patient (20%) is Sos1 (258, 395, 480). The Sos1 gene encodes the Ras and Rac GEF Son of Sevenless 1 protein. Mutations in Sos1 affect principally two domains, the Pleckstrin homology (PH) and the Ras exchanger motif (REM) domains, which are not implicated in the exchange activity but rather in the intrinsic autoinhibitory function of Sos1. These mutations lead to the maintenance of Sos1 in a noninhibited form, resulting in a permanent activation of the Rac and Ras/ERK pathways. Although some GEFs have been found to be mutated or aberrantly expressed in human cancer, NS-associated germline mutations of Sos1 were the first described example of activating GEF mutations linked with a human genetic disease (395, 480). The extent to which Sos1 gain-of-function mutations affect different Ras-dependent or Rac-dependent signals remains to be elucidated.

In 2006, a germline K-Ras mutation was found in a NS patient who presented a severe clinical phenotype. Subsequently, K-Ras mutations were identified in <2% of NS patients but generally associated with more significant learning issues and developmental delays (68, 426). Cardiac involvement was present in up to 85% of affected individuals, including pulmonary stenosis, hypertrophic cardiomyopathy, atrial septal defect, patent ductus arteriosus, and ventricle septal defect. Most mutations of K-Ras cluster in the first and second coding exons, which are shared by the two K-Ras isoforms K-RasA and K-RasB. Mutations affecting exon 6 encoding residues at the COOH terminus of K-RasB but not K-RasA have also been reported. NS-associated K-Ras mutations affect highly conserved amino acid residues and are assumed to confer mild gain of function.

In 2007, heterozygous missense mutations in Raf1 were reported in 20% of NS patients (357). Whereas hypertrophic cardiopathies are rare (18%) in NS caused by Ptpn11 or Sos1 mutations, they are frequently observed (76%) in association with Raf1 mutations. These observations highlight differences in genotype/phenotype correlations, in particular for hypertrophic cardiopathies, and suggest an important role of Raf1 in pathophysiological modulation of cardiac hypertrophy. However, the role of Raf1 in cardiac hypertrophy obtained with animal experimental models remains controversial. In mice, cardiac expression of dominant-negative Raf1 was shown to prevent ERK activation and to strongly reduce pressure overload-induced cardiac hypertrophy (155). In contrast, cardiac-specific deletion of Raf1 had no effect on ERK expression/activation or heart growth (554).
B. LEOPARD Syndrome

1. Clinical features

LEOPARD syndrome (LS; OMIM 151100) is an acronym for multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, growth retardation, and sensorineural deafness. LS was first reported by Zeisler and Becker in 1936 (577). Diagnostic clues to LS include multiple lentigines, café-au-lait macules, deafness, and hypertrophic cardiomyopathy. LS patients show retardation of growth and a delayed puberty. Electrocardiographic anomalies and progressive conduction anomalies are the most common heart defects, including left or biventricular hypertrophy, often in association with Q waves, prolonged QTc, and repolarization abnormalities. Hypertrophic cardiomyopathy is the most frequent anomaly (80%), which in general is asymmetric. Less frequent heart defects are pulmonary stenosis 25% and aortic/mitral valve abnormalities in some LS patients (414).

2. Molecular pathogenesis

It has recently been reported that LS is related to a missense mutation in the Ptpn11 gene (105). As described in the previous paragraph, germlinal mutations in the Ptpn11 gene are also responsible for ~40–50% of NS. But biochemical in vitro studies point to a decrease of SHP-2 catalytic activity for LEOPARD-associated mutations, in contrast to the enhanced SHP-2 activity found for NS. Curiously, LS and NS are associated with the same gene and lead to similar clinical phenotype. To date, no explanation is provided to understand how the gain of function or the loss of function of SHP-2 could induce the same phenotype. In addition, Raf1 gene mutations were identified in LS patients (357, 426), indicating that LEOPARD syndrome cannot simply be understood by invoking reduced Ras/MAPK signaling.

C. Costello Syndrome

1. Clinical features

Costello syndrome (CS; OMIM 218040) was first described in 1971 by Costello. The clinical manifestations included postnatal growth retardation, distinct facial appearance with large head, curly hair, nasal papillomata, hyperextensible fingers with loose integuments of the hands and feet, and hyperpigmented skin (22, 90, 91). This rare congenital disorder affects multiple organ systems encompassing severe failure to thrive, cardiac anomalies, tumor predisposition, and cognitive impairment. More than 60% of patients develop cardiac defects including hypertrophic cardiomyopathies, congenital malformations, usually pulmonary valve stenosis, and arrhythmia, especially atrial tachycardia (103).

2. Molecular pathogenesis

Aoki et al. (17) identified heterozygous de novo missense mutations of H-Ras gene in CS patients. The patient missense mutations result in amino acid substitutions of a glycine residue in position 12 or 13 of the protein product. These particular amino acids are located at the GTP binding site, and mutations at these sites have previously been shown to cause constitutive activation of H-Ras, in turn causing increased activation of downstream effectors in MAPK signaling pathways controlling cell proliferation and differentiation. Interestingly, these H-Ras germine mutations affect the same amino acid residues that are mutated in cancer.

A strain of mice carrying a germline G12V mutation within their endogenous H-Ras locus has been generated to shed light on the role of this gain of function of H-Ras in the developmental and physiological defects associated with CS (427). H-Ras<sup>G12V</sup> mice are viable and closely phenocopy some of the defects observed in CS patients, including a number of cardiovascular dysfunctions. Four-month-old mutant mice develop cardiomyopathy characterized by general enlargement of all heart chambers, concentric left ventricle hypertrophy, and fibrosis. The authors have observed consistent thickening of the aortic valve without any alterations in the pulmonary or atroventricular valves. In addition, H-Ras<sup>G12V</sup> mice exhibit systemic arterial hypertension resulting from activation of the ANG II pathway. High blood pressure is not common in CS patients, but it has been described in 10% of CS patients with the mutation 34G (113). H-Ras<sup>G12V</sup> mice thus appear as a good animal model to understand the etiology of cardiovascular CS symptoms and to evaluate potential therapeutic strategies.

D. Cardio-Facio-Cutaneous Syndrome

1. Clinical features

The cardio-facio-cutaneous syndrome (CFCS; OMIM 115150) is a rare sporadic occurring disorder (389). Short stature, mental retardation, global developmental defects, and delayed language acquisition are found in most patients (>75%) (103, 217, 394). Children with CFC have typical facial characteristics similar to those noted in NS: high forehead with bitemporal constriction, palpebral ptosis, and a short nose with relatively broad nasal base. Cutaneous and adnexal abnormalities detected in CFCs are similar to those reported in the NS and CS. Cardiac abnormalities are detected in 75% of patients. Affected individuals often present with pulmonary stenosis (45%), hypertrophic cardiomyopathy (40%), and atrial septal defect (22%). Life expectancy of CFC patients is probably shortened on average, due to the severe cardiovascular defects.
2. Molecular pathogenesis

CFCS has been first considered as a severe form of NS and CS because these three syndromes share several manifestations, and “borderline” cases do exist, usually in infants. However, Ptpn11 and H-Ras mutations were not found in CFC patients, indicating a specific etiology and genetic entity of this syndrome. The final evidence came in 2006, with the discovery among 23 patients with the CFC, of 11 different mutations of the B-Raf gene in 18 of them, mutations of MEK1 in 2 and of MEK2 in 1 patient (396). These mutations have then been rapidly confirmed by other authors (335). Two clusters of mutations were identified in the B-Raf gene: in the cysteine-rich domain in the exon 6 and in the protein kinase domain. All of them are missense mutations, suggesting a gain-of-function effect and a consequent over-activation of the B-Raf/MEK1/2 signaling.

A mouse strain that expresses a constitutive active form of B-Raf (B-RafV600E) has been recently generated (492). This animal model mimics some characteristic defects found in CFC patients, including reduced life span, small size, facial dysmorphism, and epileptic seizures. In addition, B-RafV600E mice develop cataracts and upregulation of some sympathetic functions. With regard to the cardiovascular system, no gross alterations in the histological cardiac structure (auricles, ventricles, aortic and pulmonary valves) and signs of tissue fibrosis are observed in mutant mice. However, a cardiomegaly is detected, due to an increase in the total number of cardiomyocytes. Heart function from B-RafV600E mice is altered, including lower end-systolic and end-diastolic volumes. Systolic arterial pressure is not modified in the mutant animals, despite a significant increase in the heart ejection fraction. The other cardiovascular alterations typical of CFC patients, such as pulmonary valve stenosis as well as sepal and aortic defects are not observed in the mutant mice. B-RafV600E mice are a suitable animal model of the RASopathy CTC and may serve as a tool to evaluate the potential therapeutic efficacy of B-Raf inhibitor, such as PLX4032 described recently as a potent inhibitor of B-Raf kinase activity (48, 118).

E. Neurofibromatosis 1

1. Clinical features

Neurofibromin is encoded by a large gene spanning over 350 kb in the chromosomal region 17q11.2 (26, 434). The protein product of this gene functions as a Ras GTPase-activating protein (GAP) to negatively regulate Ras activity (294, 547). More than 240 different mutations have been described within the Nf1 gene (386). The majority of mutations lead to a truncated or no protein product. Reduction or complete loss of neurofibromin induces activation of Ras, which in turn activates mitogenic signaling leading to increased cellular proliferation or differentiation. For this reason, neurofibromin is considered as a tumor suppressor (260). Neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease (OMIM 611431), is the condition most commonly associated with mutations in the Nf1 gene. It is a dominant disorder affecting 1 in 3,000 individuals. Most adults with NF1 develop café-au-lait macules, neurofibromas, optic glioma, osseous lesions, Lisch nodules, and axillary or inguinal freckling. In addition, NF1 patients present prominent cognitive impairments (attention deficit, hyperactivity disorder, spatial deficit, and language delay) and musculoskeletal abnormalities (scoliosis, long bone dysplasia, and osteopenia). NF1 is also remarkable for its association with occlusive (stenoses) or aneurysmal arterial disease affecting predominantly the aorta and renal, mesenteric and carotid-vertebral arteries. One of the more common vascular lesions in NF1 patients is renal artery stenosis with consequent hypertension (200, 253, 348). The frequency of vasculopathy among NF1 patients in general has not been determined because most of the time NF1-associated vascular lesions are asymptomatic.

2. Molecular pathogenesis

NF1 vasculopathy lesions are characterized by an accumulation of vascular smooth muscle cells within the intima layer of the blood vessel resulting in neointima formation. The pathogenesis of this vascular lesion formation remains to be understood, whereas they contribute to excess morbidity and mortality of young NF1 patients (387).

To shed light on the role of neurofibromin in vascular lesions, murine models of NF1 vasculopathy have been generated. Homozygous deficient embryos die in mid gestation (approximately E13.5). Mutant embryos display hyperplasia of neural crest-derived sympathetic ganglia and exhibit signs of cardiac dysfunction including over abundant tissue that accumulates and obstructs blood flow in the hearts due to hyperproliferation and lack of normal apoptosis of endocardial cushions (55, 199, 247). Endothelial-specific inactivation of Nf1 recapitulates the complete null cardiovascular phenotype providing evidence that neurofibromin is essential for endocardial cushion formation by modulating Ras activity in endothelial cells (138). In contrast, no apparent defects were observed in mice with specific Nf1 deletion in smooth muscle, and no structural abnormalities were detected in the vascular system (549). Nevertheless, the loss of Nf1 in vascular smooth muscle leads to an exaggerated vascular remodeling in response to vascular injury (carotid artery ligation) ascribed to an over-activation of Ras and its downstream effectors, ERK, S6K, and mTOR (549). Surprisingly, the same results were obtained in Nf1−/− mice that also display vascular inflammation (252) but not in heterozygous inactivation of Nf1 in vascular smooth muscle cells or endothelial cells alone (253). These results suggest the role of another cell lineage in NF1 vascular diseases. This hypothesis has been confirmed by showing that specific heterozygous inactivation of Nf1 in bone
marrow-derived cells was sufficient for increased neointima formation in response to vascular injury (253). In vitro studies demonstrate that direct contact of Nf1+/- macrophages with vascular smooth muscle cells stimulates vascular smooth muscle cell proliferation through a Ras/Erk-dependent pathway, and this effect is further increased with Nf1+/- vascular smooth muscle cells (253). This observation underlies the potential role of inflammatory cells in the pathogenesis of NF1 vasculopathy. This hypothesis is reinforced by the fact that NF1 patients have increased levels of circulating monocytes and proinflammatory cytokines known as biomarkers linked to human vasoocclusive disease (IL-1β and IL-6) compared with the healthy population (253). In short, these recent data provide evidence for an important role of vascular inflammation in NF1 patients and provide a framework for understanding the pathogenesis of NF1 vasculopathy and developing potential therapeutic and/or diagnostic strategies.

VI. THE RAS SUPERFAMILY PROTEINS AND EFFECTORS AS PHARMACOLOGICAL TARGETS

As frequently mentioned throughout this review, pharmacological compounds that with a more or less good specificity targeting Ras proteins or their regulators have been extensively used in vitro and in animal models and have proven efficient to demonstrate the involvement of Ras protein signaling in numerous studies. Among these compounds, some of them are already used against cardiovascular diseases in humans, and development of new inhibitors targeting these signaling pathways for a potential therapeutical application is the subject of intensive research.

A. Targeting Ras Protein Lipidation

Ras protein activity absolutely requires their anchoring to cellular membranes via a lipophilic prenyl group covalently bound to the COOH terminus of these proteins (FIGURE 9). For instance, enzymatic attachment of farnesyl isoprenoid by the action of FT is required for Ras to bind membranes, transform cells, and induce tumorigenesis. Over the past decade, intensive efforts have been devoted to developing FTIs as anticancer agents (36, 489). To date, four types of FTIs have been developed: peptidomimetics, farnesylpyrophosphate analogs, bisubstrate analogs that combine the two former features, and small-molecule inhibitors. In cells, FTIs inhibit H-Ras farnesylation and membrane association, block the activation of mitogen-activated protein ki-
nase and PI 3-kinase/Akt pathways, and reduce the growth of a wide variety of human cancer cell lines (388). However, results obtained from nearly 75 clinical trials of 4 different FTIs (lonafarnib, tipifarnib, BMS-214662, and L-778123) reveal that these agents have no or minimal efficacy in the treatment of hematopoietic cancers or solid tumors (36). With regard to the cardiovascular system, unwanted cell growth is involved in the pathogenesis of cardiovascular diseases. Proliferation of vascular smooth muscle cells participates in the development of atherosclerotic lesions and is the main mechanism leading to restenosis of the coronary artery after balloon angioplasty. Thus inhibition of vascular smooth muscle cell proliferation would logically have beneficial effects in these pathological contexts. Although Ras proteins are important, geranylgeranylated proteins such as Rho proteins may also be required for the proliferation of these cells (188). Accordingly, GGT has also been defined as a relevant target for the development of inhibitors of smooth muscle cell proliferation (FIGURE 9). While a great number of preclinical studies showed that FTI and GGTI inhibit vascular smooth muscle growth, and despite the possibility of local delivery with drug releasing stents in the case of prevention of restenosis (540), they did not lead to clinical trials (87). These compounds thus appear to be far from reaching clinical application for cardiovascular diseases.

Nevertheless, the therapeutic interest of inhibition of Ras protein lipidation for cardiovascular diseases is strongly supported by the effect of statins (FIGURE 9). Statins are considered as the gold standard therapy for lowering serum LDL-cholesterol and improving cardiovascular morbidity/mortality. Interestingly, results from recent experimental and clinical studies suggest that their cholesterol-lowering effects could not account for all their beneficial effects on the cardiovascular system (50). Actually, inhibition of the isoprenoid synthesis and the resulting downregulation of Ras protein signaling pathways are responsible for most of the cholesterol-independent pleiotropic effects of statins (586). Although the involvement of inhibition of the Ras subfamily in the beneficial effect of statins is not documented, numerous studies point out the important role of Rho protein inhibition. Indeed, statins, at clinically relevant concentrations that are used to reduce LDL-cholesterol, have been shown to inhibit RhoA isoprenylation (82) and Rho-kinase activity in humans (476). Furthermore, a recent clinical study demonstrates that therapeutic doses of statins mainly exhibit a pleiotropic effect through the inhibition of Rac1 (385). The analogies between the effects of RhoA/Rho-kinase and Rac1 inhibition observed in preclinical/basic studies and the effects of statins in humans, including increased expression of atheroprotective genes, inhibition of pro-inflammatory mediators, endothelial protective effects, enhanced stability of atherosclerotic plaques, and inhibition of vascular smooth muscle proliferation, strongly argue of an important role of statin-mediated inhibition of Rho proteins in the benefits of statin treatments. In fact, measurement of leukocyte Rho-kinase activity is currently being utilized to identify patients at cardiovascular risk and to gauge statin efficacy on vascular function that is independent of cholesterol lowering effects (276–278).

On the basis of these observations, Rho protein inhibitors are thus considered as promising treatment for cardiovascular diseases, and the current search for small-molecule inhibitors of RhoA could be highly valuable for developing clinically useful agents (104).

B. Rho-Kinase Inhibitors

As the major downstream RhoA effector and a key regulator of vascular smooth muscle cell contraction but also cell migration, proliferation, differentiation, apoptosis and survival, and gene transcription, Rho-kinase is identified as an interesting target for cardiovascular pharmacology, and strong efforts are made for the development of Rho-kinase inhibitors.

Numerous studies have demonstrated the potential beneficial effects of Rho-kinase inhibitors in animals and humans (282, 451). They have been shown to be effective for the treatment of vascular smooth muscle hyperconstriction-induced disorders such as coronary and cerebral vasospasm as well as systemic and pulmonary hypertension. Some data suggested that in addition, Rho-kinase inhibitors might be useful for treating arteriosclerotic diseases, including arteriosclerosis, restenosis, cardiac allograft vasculopathy and vein graft disease ischemia-reperfusion injury, CCM, myocardial hypertrophy, and heart failure.

The Rho-kinase inhibitor fasudil was initially developed as an intracellular calcium antagonist. Fasudil has been approved in Japan since 1995 for the treatment of cerebral vasospasm after aneurysmal subarachnoid hemorrhage and to improve blood flow after acute ischemic stroke (448). More recently, clinical trials have been performed to assess the effectiveness of fasudil in other cardiovascular diseases such as stable effort angina pectoris where it was found to reduce myocardial ischemia (315, 450). Intra-arterial administration of fasudil was also shown to decrease the forearm vascular resistance in patients with heart failure (226). Moreover, intravenous fasudil treatment significantly reduces pulmonary vascular resistance in patients with severe pulmonary hypertension (127).

Clinical trials with Rho-kinase inhibitors are in progress in the United States, Europe, and Japan, in various indications, including angina, pulmonary hypertension, atherosclerosis, and reperfusion injury (451).

VII. CONCLUDING REMARKS

Recent findings have considerably increased our understanding of the role of Ras proteins in cardiovascular phys-
iole and physiopathology. These proteins are not only oncogenes but, in physiological situation, are major regulators of cardiovascular cell functions, so long as activation of Ras proteins occurs at the right time and at the right place. Sustained or dysregulated spatiotemporal activation of Ras proteins is most of the time deleterious, leading to pathological situations. Thus, while these proteins are identified as potential targets for the development of new therapeutic strategies in cardiovascular medicine, efforts should be made to understand the mechanisms and identify the molecular partners that regulate their activity. They are likely to be different according to the cell types, upstream activating factors, and even not the same in diverse subcellular compartments. Much remains to be done to understand the role of the numerous Ras proteins expressed in the cardiovascular system. However, thanks to the rapidly increasing availability of mouse models of deletion or expression of active mutants of Ras proteins and regulators with inducible and tissue-selective targeting, our knowledge on the role of Ras protein signaling in cardiovascular functions and diseases should continuously improve, with the potential discovery of targets for the development of new therapeutic strategies.

ACKNOWLEDGMENTS

We thank Dr. Flavien Charpentier (UMR_S1087, Nantes) for critical reading of the manuscript and Dr. Agnès Quemener (UMR_S892, Nantes) for the Ras superfamily proteins 3D structure.

Address for reprint requests and other correspondence: G. Loirand, Inserm UMR_S1087, IRS-UN, 8 quai Moncousu, 44007, Nantes, France (e-mail: gervaise.loirand@univ-nantes.fr).

GRANTS

This work was supported by French Agence Nationale de la Recherche Grants ANR-08-GENO-040-01, ANR-09-JCJC-0115-01, and ANR-11-BSV1-013-01 and by Fondation pour la Recherche Médicale Grant DEQ20090515416.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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