The Ras superfamily at a glance

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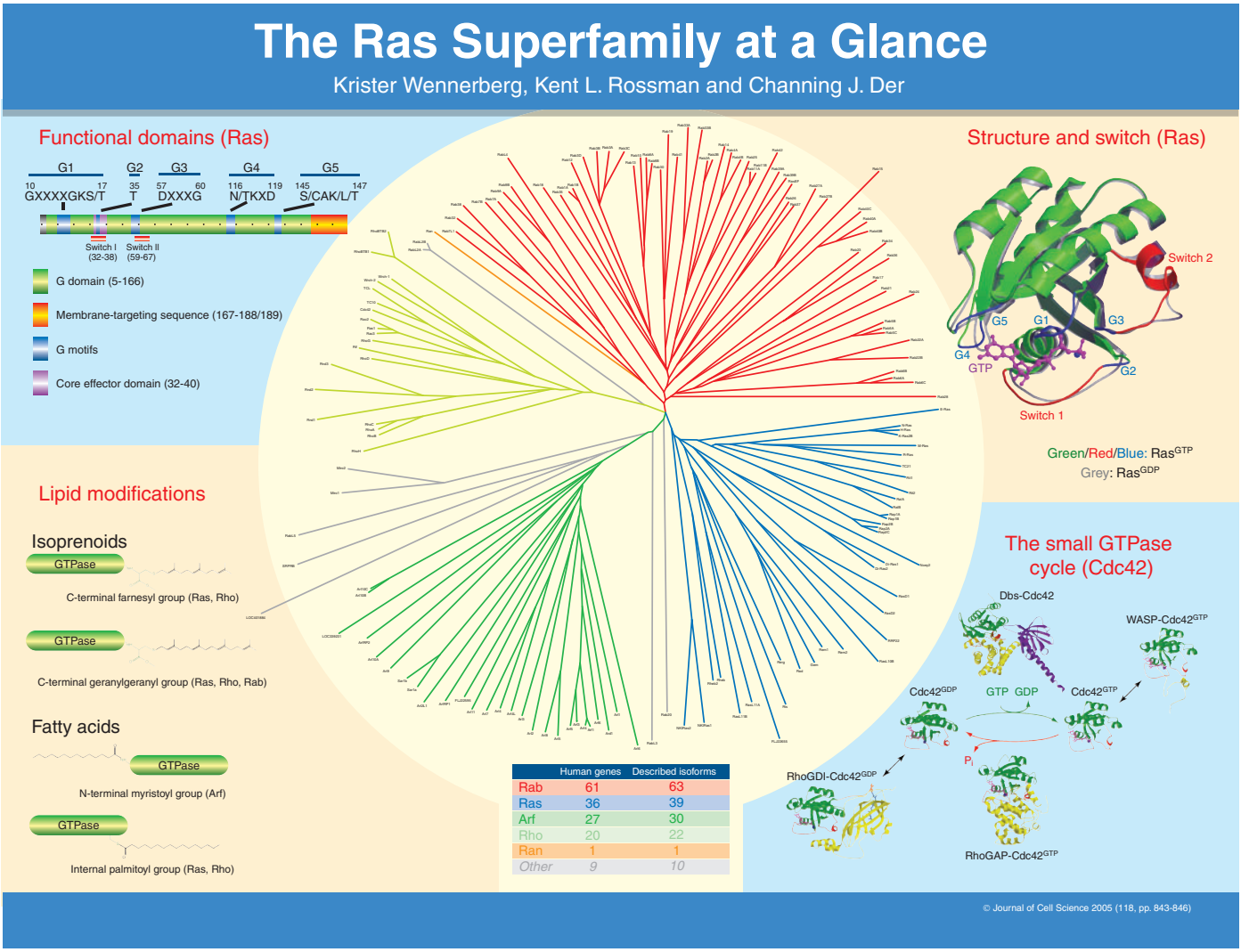
The Ras superfamily of small guanosine triphosphatases (GTPases) comprise over 150 human members (Table S1 in supplementary material), with evolutionarily conserved orthologs found in *Drosophila*, *C. elegans*, *S. cerevisiae*, *S. pombe*, *Dictyostelium* and plants (Colicelli, 2004). The Ras oncogene proteins are the founding members of this family, which is divided into five major branches on the basis of sequence (Fig. S1 in supplementary material) and functional similarities: Ras, Rho, Rab, Ran and Arf. Small GTPases share a common biochemical mechanism and act as binary molecular switches (Vetter and Wittinghofer, 2001). Although similar to the heterotrimeric G protein α subunits in biochemistry and function, Ras family proteins function as monomeric G proteins. Variations in structure (Biou and Cherfils, 2004), post-translational modifications that dictate specific subcellular locations and the proteins that serve as their regulators and effectors allow these small GTPases to function as sophisticated modulators of a remarkably complex and diverse range of cellular processes. Here, we present the basic structural features of Ras proteins, with respect to specific Ras sequences, to highlight the general properties of this family of proteins and discuss features that distinguishes the various branches of the superfamily from Ras.

Ras superfamily structure

Ras superfamily GTPases function as GDP/GTP-regulated molecular switches (Vetter and Wittinghofer, 2001). They share a set of conserved G box GDP/GTP-binding motif elements beginning at the N-terminus: G1,



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GXXXXGKS/T; G2, T; G3, DXXGQ/H/T; G4, T/NKXD; and G5, C/SAK/L/T (Bourne et al., 1991) (Fig. S1 in supplementary material). Together, these elements make up an ~20 kDa G domain (Ras residues 5-166) that has a conserved structure and biochemistry shared by all Ras superfamily proteins, as well as Gα and other GTPases.

Ras superfamily GTPase biochemistry and regulation

Small GTPases exhibit high-affinity binding for GDP and GTP, and possess low intrinsic GTP hydrolysis and GDP/GTP exchange activities. GDP/GTP cycling is controlled by two main classes of regulatory protein. Guanine-nucleotide-exchange factors (GEFs) promote formation of the active, GTP-bound form (Schmidt and Hall, 2002), whereas GTPase-activating proteins (GAPs) accelerate the intrinsic GTPase activity to promote formation of the inactive GDP-bound form (Bernards and Settleman, 2004). GTPases within a branch use shared and distinct GAPs and GEFs. GTPases in different branches exhibit structurally distinct but mechanistically similar GAPs and GEFs. The two nucleotide-bound states have similar conformations but these have pronounced differences corresponding to the switch I (Ras residues 30-38) and switch II (59-67) regions: the GTP-bound conformation possessing high affinity for effector targets (Bishop and Hall, 2000; Repasky et al., 2004). It is mainly through the conformational changes in these two switches that regulatory proteins and effectors 'sense' the nucleotide status of the small GTPases. Arf proteins contain additional N-terminal sequences, whereas Ran has additional C-terminal sequences that undergo significant conformational changes during GDP/GTP cycling. Although the GTP-bound form is the active form for all Ras superfamily GTPases, the cycling between the GDP-bound and GTP-bound states, in which distinct functions are associated with each nucleotide-bound form, is also critical for the activities of Rab, Arf and Ran GTPases. The core effector domain (Ras residues 32-40) includes the switch I domain and is critical for direct association with effectors (Herrmann, 2003).

Lipid modification and membrane targeting

A second important biochemical feature of a majority of Ras superfamily proteins is their post-translational modification by lipids. The majority of Ras and Rho family proteins terminate with a C-terminal CAAX (C=Cys, A=aliphatic, X=any amino acid) tetrapeptide sequence (Cox and Der, 2002). This motif, when coupled together with residues immediately upstream (e.g. cysteine residues modified by the fatty acid palmitate), comprises the membrane-targeting sequences that dictate interactions with distinct membrane compartments and subcellular locations. The CAAX motif is the recognition sequence for farnesyltransferase and geranylgeranyltransferase I, which catalyze the covalent addition of a farnesyl or geranylgeranyl isoprenoid, respectively, to the cysteine residue of the tetrapeptide motif. Rab family proteins terminate in a distinct set of cysteine-containing C-terminal motifs (CC, CXC, CCX, CCXX, or CCXXX) that are similarly modified by geranylgeranyltransferase II, which also attaches geranylgeranyl groups. Some members of the Arf family are modified at their N-termini by a myristate fatty acid. These modifications are essential for facilitating membrane association and subcellular localization critical for biological activities. Rho and Rab GTPases are regulated by a third class of proteins, guanine nucleotide dissociation inhibitors (GDIs), which mask the prenyl modification and promote cytosolic sequestration of these GTPases (Seabra and Wasmeier, 2004). Some Ras superfamily members do not appear to be modified by lipids, but still associate with membranes (e.g. Rit, RhoBTB, Miro and Sar1). Others (e.g. Ran and Rerg) are not lipid modified and are not bound to membranes.

Subgrouping of the Ras superfamily

The Ras superfamily has traditionally been divided into five different major branches. The classification of some less-studied proteins into these major subfamilies is arbitrary, and sequence comparisons of the G domains suggest that they may define distinct subfamilies. In the absence of any functional data, a definitive classification of these GTPases is not yet possible. Here, we group the proteins that, on the basis of structure, function or both, clearly belong to a specific subfamily. In cases where neither structural nor functional data support putting a protein in one of the major subfamilies, we leave the protein as 'Unclassified' even though some of these proteins have previously been labeled as belonging to a certain subfamily. In the human genome, there are also a large number of *Ras* superfamily pseudogenes. We have chosen not to include gene sequences from databases where no evidence of transcription has been found. Furthermore, in addition to the proteins listed here, there are many genes that have regions predicted to encode sequences similar to parts of a small GTPase domain, but we have chosen only to include proteins that contain complete Ras-like GTPase domains.

The Ras family

The Ras sarcoma (Ras) oncoproteins are the founding members of the Ras family (36 members) and have been the subject of intense research scrutiny, in large part because of their critical roles in human oncogenesis (Repasky et al., 2004). Ras proteins serve as signaling nodes activated in response to diverse extracellular stimuli. Activated Ras interacts with multiple, catalytically distinct downstream effectors, which regulate cytoplasmic signaling networks that control gene expression and regulation of cell proliferation, differentiation, and survival.

The best characterized Ras signaling pathway is activation of Ras by the epidermal growth factor receptor tyrosine kinase through the RasGEF Sos (Repasky et al., 2004). Activated Ras binds to and promotes the translocation of the Raf serine/threonine kinase to the plasma membrane, where additional phosphorylation events promote full Raf kinase activation. Raf phosphorylates and activates the MEK1/2 dual specificity protein kinase, which phosphorylates and activates the ERK1/2 mitogen-activated protein (MAP) kinase. Activated ERK translocates to the nucleus, where it

phosphorylates Ets-family transcription factors, which in turn activate Ets-responsive promoters.

Other Ras family proteins, including Rap, R-Ras, Ral and Rheb proteins, also regulate signaling networks. Finally, although biochemically similar to Ras, several Ras family proteins appear to act as tumor suppressors, rather than as oncogenes (e.g. Rerg, Noey2 and D-Ras), in cancer development (Colicelli, 2004).

The Rho family

Like Ras, Ras homologous (Rho) proteins also serve as key regulators of extracellular-stimulus-mediated signaling networks that regulate actin organization, cell cycle progression and gene expression (Etienne-Manneville and Hall, 2002). Twenty members have been identified, RhoA, Rac1 and Cdc42 being the best studied. Rho GTPases are key regulators of actin reorganization. RhoA promotes actin stress fiber formation and focal adhesion assembly; Rac1 promotes lamellipodium formation and membrane ruffling; and Cdc42 promotes actin microspikes and filopodium formation. Consequently, Rho GTPases have been implicated in the regulation of cell polarity, cell movement, cell shape, and cell-cell and cell-matrix interactions, as well as in regulation of endocytosis and exocytosis (Ridley, 2001). Reflecting their involvement in such a diversity of cellular processes, RhoA, Rac1 and Cdc42 proteins are each regulated by a surprising diversity of GEFs and GAPs (Schmidt and Hall, 2002; Moon and Zheng, 2003) and utilize a similarly diverse set of downstream effectors (Bishop and Hall, 2000). Actin reorganization functions have also been observed for other Rho family GTPases, in particular Rnd proteins, which antagonize RhoA.

Although the Miro proteins were first described as Rho proteins, these atypical GTPases instead appear to form their own subgroup of the Ras superfamily (Wennerberg and Der, 2004). In addition to their N-terminal GTPase domain, they contain EF-hand domains and one C-terminal GTPase-like domain. They lack the insert domain that is characteristic of Rho GTPases (Fig. S1 in supplementary material). The Miro proteins do not regulate the cytoskeleton; instead they are localized to mitochondria and regulate the integrity of these cellular compartments.

The Rab family

First described as Ras-like proteins in brain (Rab), Rab proteins comprise the largest branch of the superfamily, with 61 members (Pereira-Leal and Seabra, 2001). Rab GTPases are regulators of intracellular vesicular transport and the trafficking of proteins between different organelles of the endocytic and secretory pathways (Zerial and McBride, 2001). Rab proteins facilitate vesicle formation and budding from the donor compartment, transport to the acceptor compartment, and vesicle fusion and release of the vesicle content into the acceptor compartment.

Rab proteins localize to specific intracellular compartments consistent with their function in distinct vesicular transport processes (Zerial and McBride, 2001). This localization is dependent on prenylation, and specificity is dictated by divergent C-terminal sequences. For example, Rab1 is located in the intermediate compartment of the cis-Golgi network and is involved in ER-to-Golgi transport. By contrast, Rab5 is located in early endosomes and regulates clathrin-coated-vesicle-mediated transport from the plasma membrane to early endosomes. Similar distinct intracellular locations and roles in vesicular transport have been established for other Rab members.

The Ran family

The Ras-like nuclear (Ran) protein is the most abundant small GTPase in the cell and is best known for its function in nucleocytoplasmic transport of both RNA and proteins (Weis, 2003). Although related to the Rab proteins in sequence, it has features that distinguish it. Unlike other small GTPases, Ran function is dependent on a spatial gradient of the GTP-bound form of Ran. There is a single human Ran protein that is regulated by a Ran-specific nuclear GEF and cytoplasmic GAP activities. This results in a high concentration of Ran-GTP in the nucleus, which facilitates the directionality of nuclear import and export. Nuclear Ran-GTP interacts with importin to promote cargo release, and with exportin-complexed cargo, to facilitate nuclear import and export of cargo, respectively. By a similar mechanism, Ran GDP/GTP cycling also regulates mitotic spindle assembly, DNA replication and nuclear envelope assembly (Li et al., 2003).

The Arf family

Like the Rab proteins, the ADP-ribosylation factor (Arf) family proteins are involved in regulation of vesicular transport, Arf1 being the best characterized (Memon, 2004). Arf GDP/GTP cycling is regulated by distinct GEFs and GAPs (Nie et al., 2003). Arf-GTP, the active form, interacts with effectors including vesicle coat proteins. Conformational differences between the two nucleotide-bound forms include not only the switch I and II regions, but also changes in the N-terminal region that allow the myristate group to interact with membranes in their GTP-bound state (Pasqualato et al., 2002).

Arf1 regulates the formation of vesicle coats at different steps in the exocytic and endocytic pathways (Nie et al., 2003; Memon, 2004). GTP- and donor-membrane-bound Arf associates with and activates coat proteins. The Arf–coat-protein complex then facilitates cargo sorting and vesicle formation and release. GAP-mediated formation of Arf-GDP is required for dissociation of the Arf–coat-protein complex and subsequent vesicle fusion with acceptor membranes. In contrast to Rab proteins, which function at single steps in membrane trafficking, Arf proteins can act at multiple steps. For example, Arf1 controls the formation of coat protein I (COPI)-coated vesicles involved in retrograde transport between the Golgi and ER, of clathrin/adapter protein 1 (AP1)-complex-associated vesicles at the trans-Golgi network (TGN) and on immature secretory vesicles, and of AP3-containing endosomes. Arf6 is functionally distinct from Arf1 and can regulate actin organization as well as endocytosis. Regulation and function of Sar1 is

similar to that of Arf1, controlling the assembly of the COPII-coated vesicles at the ER. Arl1 also functions in membrane trafficking. Other family members exhibit different or poorly characterized cellular functions.

The complex modes of regulation of Ras superfamily small GTPases facilitate their key involvement in an amazingly diverse spectrum of biochemical and biological processes. The extent of this superfamily, when combined with Gα subunits and up to 50 other human GTPases (Colicelli, 2004), reveal the versatile role of GTPase switches in the control of cellular processes.

References

Bernards, A. and Settleman, J. (2004). GAP control: regulating the regulators of small GTPases. *Trends Cell Biol.* **14**, 377-385.
Biou, V. and Cherfils, J. (2004). Structural principles for the multispecificity of small GTP-binding proteins. *Biochemistry* **43**, 6833-6840.
Bishop, A. L. and Hall, A. (2000). Rho GTPases and their effector proteins. *Biochem. J.* **348**, 241-255.
Bourne, H. R., Sanders, D. A. and McCormick, F. (1991). The GTPase superfamily: conserved structure and molecular mechanism. *Nature* **349**, 117-127.
Colicelli, J. (2004). Human RAS superfamily proteins and related GTPases. *Sci. STKE* **2004**, RE13.
Cox, A. D. and Der, C. J. (2002). Ras family signaling: therapeutic targeting. *Cancer Biol. Ther.* **1**, 599-606.
Etienne-Manneville, S. and Hall, A. (2002). Rho GTPases in cell biology. *Nature* **420**, 629-635.
Herrmann, C. (2003). Ras-effector interactions: after one decade. *Curr. Opin. Struct. Biol.* **13**, 122-129.
Li, H. Y., Cao, K. and Zheng, Y. (2003). Ran in the spindle checkpoint: a new function for a versatile GTPase. *Trends Cell Biol.* **13**, 553-557.
Memon, A. R. (2004). The role of ADP-ribosylation factor and SAR1 in vesicular trafficking in plants. *Biochim. Biophys. Acta* **1664**, 9-30.
Moon, S. Y. and Zheng, Y. (2003). Rho GTPase-activating proteins in cell regulation. *Trends Cell Biol.* **13**, 13-22.
Nie, Z., Hirsch, D. S. and Randazzo, P. A. (2003). Arf and its many interactors. *Curr. Opin. Cell Biol.* **15**, 396-404.
Pasqualato, S., Renault, L. and Cherfils, J. (2002). Arf, Arl, Arp and Sar proteins: a family of GTP-binding proteins with a structural device for 'front-back' communication. *EMBO Rep.* **3**, 1035-1041.
Pereira-Leal, J. B. and Seabra, M. C. (2001). Evolution of the Rab family of small GTP-binding proteins. *J. Mol. Biol.* **313**, 889-901.
Repasky, G. A., Chenette, E. J. and Der, C. J. (2004). Renewing the conspiracy theory debate: does Raf function alone to mediate Ras oncogenesis? *Trends Cell Biol.* **14**, 639-647.
Ridley, A. J. (2001). Rho family proteins: coordinating cell responses. *Trends Cell Biol.* **11**, 471-477.
Schmidt, A. and Hall, A. (2002). Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev.* **16**, 1587-1609.
Seabra, M. C. and Wasmeier, C. (2004). Controlling the location and activation of Rab GTPases. *Curr. Opin. Cell Biol.* **16**, 451-457.
Vetter, I. R. and Wittinghofer, A. (2001). The guanine nucleotide-binding switch in three dimensions. *Science* **294**, 1299-1304.
Weis, K. (2003). Regulating access to the genome: nucleocytoplasmic transport throughout the cell cycle. *Cell* **112**, 441-451.
Wennerberg, K. and Der, C. J. (2004). Rho-family GTPases: it's not only Rac and Rho (and I like it). *J. Cell Sci.* **117**, 1301-1312.
Zerial, M. and McBride, H. (2001). Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.* **2**, 107-117.

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