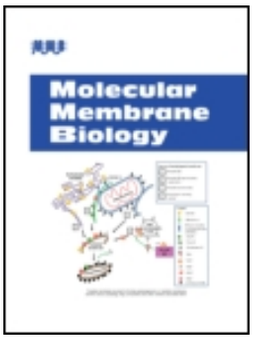

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Ras-induced cellular events (Review)

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Summary

Ras is a crucial regulator of cell growth in eukaryotic cells. Activated Ras can stimulate signal transduction cascades, leading to cell proliferation, differentiation or apoptosis. It is also one of the most commonly mutated genes in both solid tumours and haematologic neoplasias. In leukaemia and tumours, aberrant Ras signalling can be induced directly by Ras mutation or indirectly by altering genes that associate with Ras or its signalling pathways. A requisite for Ras function is localization to the plasma membrane, which is induced by the post-translational modification farnesylation. Molecules that interfere with this Ras modification have been used as anti-tumour agents. Ras is emerging as a dual regulator of cell functions, playing either positive or negative roles in the control of proliferation or apoptosis. The diversity of Ras-mediated effects may be related in part to the differential involvement of Ras homologues in distinct cellular processes or to the expanding array of Ras effectors.

Keywords: Ras effectors, proliferation, apoptosis, cancer, Ras homologues.

Introduction

Ras proteins are critical components of the signalling pathways that link the activation of cell surface receptors to the control of proliferation, differentiation or apoptosis (McCormick 1996, Hall 1998). The 21 kDa Ras family comprises H-Ras, K-Ras, N-Ras and other homologous proteins such as R-Ras, M-Ras, TC21, Rap and Ral (figure 1). The two K-Ras forms, K-Ras 4A and 4B, diverge in the C-terminal as a consequence of alternate exon utilization.

The activity of Ras proteins is controlled by a cycle between a GDP-bound inactive state and a GTP-bound active state. Ras proteins are activated transiently in response to diverse extracellular signals such as growth factors, cytokines, hormones, reactive free radicals, cellular redox stress and neurotransmitters. Activated Ras, in turn, stimulates a cascade of serine/threonine kinases that activate multiple signalling pathways.

The aim of this review is to analyse emerging aspects of Ras protein biology, focusing on novel mechanisms of Ras activation as well as on the involvement of Ras in cancer and in pro- or anti-apoptotic signalling pathways.

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Specific roles of Ras proteins

The mammalian Ras proteins are almost identical throughout most of their length, diverging only in the 20 C-terminal amino acids (figure 2). Ras isoforms are indistinguishable in most assays, leading to the speculation that they are redundant. Nonetheless, recent evidence has begun to accumulate for differential activities of the Ras isoforms.

The H-, N- and K-Ras genes are ubiquitiously expressed in mammalian cells. Mutation of specific Ras homologues is associated with different tumour types. K-Ras mutation was detected in mammary tumour progression (Liu *et al.* 1998) and activated K-Ras is involved in stimulation of human colon cancer cells (Okumura *et al.* 1999). H-Ras stimulates tumour angiogenesis (Arbiser *et al.* 1997) and mutation of H-Ras may be involved in pathogenesis of juvenile chronic myelogenous leukaemia (JCML) (Miyauchi *et al.* 1994), as well as in acute myelogenous leukaemia (AML) (Kiyoi *et al.* 1999). N-Ras mutations induce myeloproliferative disorders and apoptosis in bone marrow-repopulated mice (MacKenzie *et al.* 1999). Erythroid progenitor cells expressing mutated N-Ras exhibit a proliferative defect, resulting in an increased cell doubling time and a decrease in the proportion of cells in the S/G_2 cell cycle phase (Darley *et al.* 1999).

A number of recent reports suggest that the different Ras homologues may preferentially mediate distinct cellular processes. K-Ras, but not H- or N-Ras, has an essential role in murine development (Johnson *et al.* 1997, Koera *et al.* 1997). K-Ras interacts specifically with microtubules (Thissen *et al.* 1997), and oncogenic K-Ras, but not N-Ras, disrupts basolateral polarity in epithelial cells (Yan *et al.* 1997). H-Ras is important in both the genesis and maintenance of solid tumours (Chin *et al.* 1999), and oncogenic H-Ras inhibits Fas ligand-mediated apoptosis by downregulating Fas expression via the phosphatidylinositol 3 kinase (PI3K) pathway (Peli *et al.* 1999). Cells with elevated levels of the serine/threonine phosphatase type 2A (PP2A) are more resistant to H-Ras-induced focus formation, which correlates with reduced H-Ras-stimulated expression of the c-*fos* promoter (Baharians and Schonthal 1999). Finally, activated H-Ras induces apoptosis by association with phosphorylated Bcl-2 in a mitogen-activated protein kinase-independent manner (Navarro *et al.* 1999).

Recent reports suggest that oncogenic N-Ras induces alterations in Golgi complex architecture and in constitutive protein transport (Babia *et al.* 1999). In keeping with this, the four Ras homologues differentially induce focus formation, cell migration or anchorage-dependent cell growth (Voice *et al.* 1999). Ras homologues vary in their ability to activate the Raf and PI3K effectors (Yan *et al.* 1998). The activation of these effectors has been related to induction or protection from apoptosis (Downward 1998). Results from the laboratory have shown distinct behaviours for Ras homologues in cells undergoing apoptosis or proliferation, with K-Ras present in mitochondria of IL-2-supplemented cells and H-Ras in mitochondria of IL-2-

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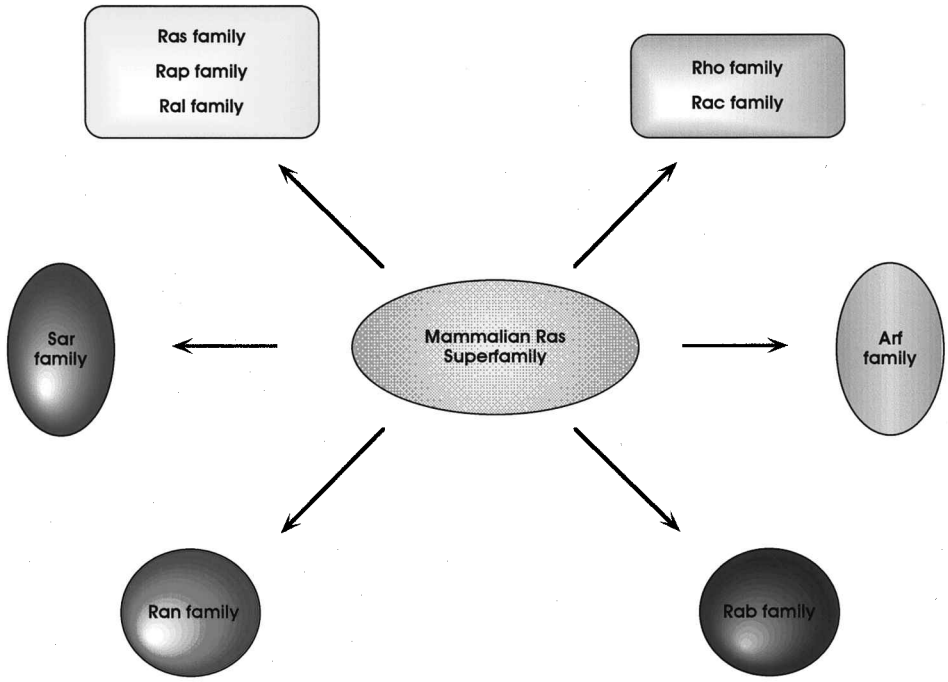

Figure 1. Schematic view of the mammalian Ras superfamily of monomeric G proteins. Ras proteins are involved in nuclear transport (Ran family), vesicular trafficking (Rab family), cytoskeleton organization/apoptosis (Rho and Rac family) and proliferation/differentiation/apoptosis (Ras, Rap and Ral family).

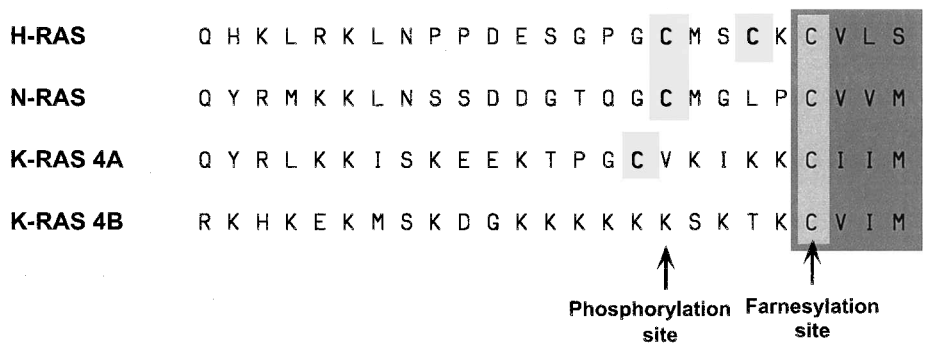

Figure 2. C-terminal amino acids of H-Ras, N-Ras, K-Ras 4A and K-Ras 4B proteins. A farnesyl group is added to the cysteine in the CAAX motif. The C-terminal tripeptide is removed by proteolysis and the newly-exposed cysteine residue is methylated. Ras proteins can be further palmitoylated (H-Ras, N-Ras and K-Ras 4A) or phosphorylated (K-Ras 4B). The palmitoylation sites of H-Ras, N-Ras and K-Ras 4A, the phosphorylation site of K-Ras 4B, the farnesylation sites and the CAAX motif of each of the Ras proteins are indicated.

deprived cells (Pérez-Sala and Rebollo 1999, Rebollo *et al.* 1999), suggesting that each Ras protein may mediate different signalling pathways.

Interestingly, the Ras-related protein M-Ras, which interacts with the Ras effector protein AF6 (Louahed *et al.* 1999, Quilliam *et al.* 1999), is regulated by upstream stimuli similar to those for H-Ras, but novel targets are responsible for its effects on cell transformation and differentiation. Constitutive M-Ras expression results in factor-independent growth of an IL-3-dependent cell line (Ehrhaedt *et al.* 1999). The Ras-

related protein R-Ras promotes cell adhesion and regulation of apoptotic responses in haematopoietic cells (Osada *et al.* 1999). It has been recently shown that Ras homologues differ significantly in their abilities to activate Raf and induce distinct biological responses. These studies, in addition to previous reports demonstrating that the four homologues can be differentially activated by upstream guanine nucleotide exchange factors, indicate that each Ras protein may participate qualitatively or quantitatively in distinct signalling cascades and have significantly different biological roles *in vivo* (Voice *et al.* 1999). These studies also suggest that the distinct, cooperative biological functions of K-Ras 4A and K-Ras 4B proteins may help explain why mutations of K-Ras, but not of N- or H-Ras, are frequently detected in human carcinomas (Voice *et al.* 1999).

Interaction of Ras with the plasma membrane

Ras proteins are modified by post-translational modifications of the C-terminal CAAX motif (figure 2) (Clarke 1992, Zhang and Casey 1996), which is necessary and sufficient for recognition by a series of enzymes that modify the CAAX protein C-terminus (Reiss *et al.* 1990, Ashby 1998). The first modification, prenylation, is catalyzed by one or two soluble prenyltransferases that attach a farnesyl or geranylgeranyl lipid to the CAAX cysteine. Substrate specificity is determined by the residue in the X position of the CAAX motif (Casey and Seabra 1996). A prenyl group is added to the cysteine, a specific protease cleaves the AAX residues, and the modified prenylcysteine is recognized by a methyltransferase. In the case of N-, H-Ras and K-Ras 4A, but not K-Ras 4B, another cysteine is further modified by the addition of a palmitic acid (Hancock *et al.* 1989). Whereas the prenyltransferases are soluble, the prenyl-CAAX proteases and palmitoyl transferases are membrane-associated (Hancock *et al.* 1991). Protein palmitoylation may be particularly important for modulating protein function during cycles of activation and deactivation. Palmitoylation can affect the affinity of proteins for membranes, subcellular localization and interaction with other proteins (Dunphy and Linder 1998, Veit and Schmidt 1998). In addition to enzymatic-mediated palmitoylation a non-enzymatic catalyzed reaction of palmitoylation, has been described. Non-enzymatic, as well as enzymatic palmitoylation of proteins occurs predominantly on cysteine residues (Mollner *et al.* 1998, Veit *et al.* 1998). The observation that enzymes that modify prenylated Ras are localized in the endomembrane system suggests that Ras is not targeted directly from the cytosol to the plasma membrane. In addition, prenylation probably mediates specific association with the endoplasmic reticulum and Golgi, and further processing allows transport to the plasma membrane (Choy *et al.* 1999, Magee and Marshall 1999). Whereas, N- and H-Ras are connected on exocytic transport vesicles following association with endoplasmic reticulum and Golgi, K-Ras uses a route that may not involve the Golgi.

All the targeting information is contained in the variable domain of Ras proteins. The three Ras proteins have in common that the initial membrane interaction of their farnesylated forms occurs with the endoplasmic reticulum (Dai *et al.* 1998, Romano *et al.* 1998). It is even possible that protease and methyltransferase are physically associated in the membrane. Ras palmitoylation is required prior to farnesyl attachment. The distinct contributions of these two lipid modifications to Ras function have recently been explored using non-farnesylated Ras mutants. These mutants can be palmitoylated and trigger differentiation and transformation, suggesting that palmitate can support Ras membrane binding and two different biological functions (Booden *et al.* 1999). Finally, transient palmitoylation supports H-Ras membrane binding but only partial biological activity, suggesting that in some cellular models, farnesylation may be important for signalling, while palmitoylation may provide dynamic membrane regulation (Coats *et al.* 1999).

The association of Ras proteins with plasma membrane domains enriched in cholesterol and sphingolipids (rafts) (Simons and Ikonen 1997) or caveolae (Anderson 1998) has been reported. Recent studies show that raft disruption has different effects on the ability of activated H-Ras and K-Ras 4B to activate Raf. Expression of a dominant negative mutant of caveolin or extraction of cholesterol from rafts blocked H-Ras, but not K-Ras 4B activation of Raf, suggesting that H-Ras and K-Ras 4B may associate with different rafts/caveolae or that raft/caveolae association of H-Ras may be more sensitive to cholesterol content (Roy *et al.* 1999). Inhibition of Ras farnesyltransferase blocks proliferation by reducing the amount of functional Ras localized at the cytoplasmic membrane, as well as inhibiting activation of the MAPK pathway (Kouchi *et al.* 1999). Moreover, farnesylation of Ras is important in the interaction of the PI3K p110 gamma subunit with Ras (Rubio *et al.* 1999).

Interference with the post-translational modifications of Ras may be an important therapeutic strategy for cancer. One of the most successful Ras activity-blocking drugs is the farnesyltransferase inhibitor (FTI). This enzyme catalyzes the transfer of a farnesyl group to a cysteine residue located near the C-terminus of the protein. FTI blocks Ras farnesylation, antagonizes its cell transforming activities, and can, therefore, be used as an antitumour agent (Gibbs and Oliff 1997). Several groups of FTI have been described, and some seem to be very effective (Perrin *et al.* 1996, Quian *et al.* 1997). Peptide analogues were designed to inhibit farnesyl protein transferase activity, on the basis of the last four amino acids of the Ras proteins. When comparable studies were performed in cells transformed by K-Ras or N-Ras, FTIs appears to be less potent at inhibiting Ras farnesylation (Cox and Der 1997). Whether the FTI have an effect on cells expressing wild-type Ras is unknown. FTI can be used not only in treating tumours expressing mutated Ras, but also for tumours in which Ras is deregulated. The effect of these compounds on T cell signalling is not known. Ras has an important role in signal transduction via IL-2R and TCR, among others. FTI prevent many changes associated with neoplastic transformation in mouse fibroblasts, including anchorage-independent growth, morphologic transformation and cytoskeletal alterations (Gibbs and Oliff 1997). Many of the FTI-induced cellular effects may be considered cytostatic, as suggested by the return of H-Ras-transformed fibroblasts to the transformed phenotype once FTI is removed from the culture medium (James *et al.* 1993, Pendergast *et al.* 1994).

The three Ras homologues differ in their susceptibility to inhibition of farnesyltransferase. H-Ras is uniquely sensitive to FTI and K-Ras is highly resistant to inhibition due to its ability to be alternatively modified by geranylgeranyl in cells treated with FTI (Lerner *et al.* 1997, Whyte *et al.* 1997). Novel mechanisms for regulation of Ras processing have recently been proposed. Induction of isoprenoid biosynthetic pathway by lipoprotein depletion can upregulate the farnesylation and membrane association of Ras (Gadbut *et al.* 1997). The modification of Ras post-translational status may contribute to the potentiation of growth factor induced DNA synthesis by insulin, since insulin can activate FTase and augment levels of farnesylated Ras (Goalstone *et al.* 1998). It should also be taken into account that FTI can target proteins other than Ras, including RhoB, nuclear lamin A and B, and TC21 (Cox and Der 1997). Ras palmitoylation also requires prior farnesyl attachment. The distinct contribution of these two lipid modifications to Ras function have been explored using non-farnesylated mutants of H-Ras (Booden *et al.* 1999). These mutants can be palmitoylated and trigger differentiation and transformation, suggesting that palmitate can support H-Ras membrane binding and two different biological functions.

Treatment of H-Ras transformed cells with FTI inactivates the Raf/MAPK cascade by preventing binding of Raf to membrane-bound Ras-GTP (Lerner *et al.* 1995). The incomplete correlation between Ras status and sensitivity to FTI suggests that not all cells with Ras mutations depend on Ras for transformed growth. These cells may have other mutations that make mutant Ras redundant. Alternatively, farnesylation of other proteins, in addition to Ras, is important for cancer cell growth.

Ras and cancer

Mutated Ras oncogenes were initially identified by their ability to transform NIH3T3 cells (Perucho *et al.* 1981, Boss 1989). These mutations render Ras proteins resistant to GTPase-activating proteins (GAPs) and prevent hydrolysis of GTP into GDP (Lowy and Wilumsen 1993). This continuously activated Ras protein autonomously stimulates cell growth or differentiation by stimulating its downstream effectors. Analysis of a variety of tumour samples revealed that, in some human tumours, one of the three Ras genes had a point mutation. As a result, the protein product has an altered amino acid, most commonly at one of the critical positions (12, 13 or 61) that lock the GTP binding protein into a state of permanent activation. In human tumours, mutation at residue 12 is the most common (Krontiris and Cooper 1981, Perucho *et al.* 1981, Shib and Weinberg 1982); with regard to Ras genes, K-Ras is the most frequently found in human tumours, whereas H- and N-Ras are rarer. In addition, the type of Ras mutation seems to correlate with tumour type (Bollag and McCormick 1991, Bogusti and McCormick 1993, Lowy and Wilumsen 1993). Although activating Ras mutations are particularly associated with myeloid malignancies and carcinomas of colon, pancreas, lung and thyroid, they have also been detected in other cancer types (Beaupre and Kuzrock 1999, Rowinsky *et al.* 1999, Weijzen *et al.* 1999).

Apart from Ras gene mutations, other events affect Ras regulation. Constitutive activation of guanine nucleotide exchange factor (GEF) Sos leads to a persistent activation of Ras (Sanchez-Garcia and Martin-Zanca 1997, Boriack-Sjodin *et al.* 1998). In addition, loss of GAPs can result in constitutive association of Ras with GTP, followed by activation (Skorski *et al.* 1994, Chuang *et al.* 1995, Largaespada *et al.* 1996). Disturbance of proteins upstream of Ras can also affect Ras activation, i.e. overexpression or truncation of certain growth factor receptors (Gibbs *et al.* 1990, Satoh *et al.* 1990, 1993, Sawyers and Denny 1994). To prevent neoplasia, cells from multicellular organisms activate cellular programmes such as apoptosis in response to deregulated oncogene expression, making the suppression of such programmes an essential step in the establishment of neoplastic cells as tumours. It has been suggested that cells may activate a non-apoptotic cell death programme; accordingly, it has been shown that oncogenic Ras triggers cell suicide through the activation of a caspase-independent cell death programme in human cancer cells (Chi *et al.* 1999). The activation of this non-apoptotic cell death programme may become a potential cancer therapy, complementing apoptosis-based therapies.

Activation of effector proteins

Two mechanisms have been proposed to explain how Ras-GTP activates its downstream effectors. In the recruitment model, Ras is anchored to the plasma membrane, where it binds to the cytoplasmic effectors. In the allosteric model, Ras binding induces a conformational change in the effector molecule. Both mechanisms may be involved, depending on which effector protein is activated.

In its activated state, Ras can stimulate several downstream effector pathways, the best characterized of which is the serine/threonine kinase c-Raf-1 (Campbell *et al.* 1998, Bonni *et al.* 1999). Ras/c-Raf-1 association induces MEK1 and 2 kinase activation and, in turn, ERK1 and 2 kinases. ERKs phosphorylate cytoplasmic targets such as RSK, Mnk and phospholipase A2 (Waskiewicz *et al.* 1997, Sturgill *et al.* 1998, Wang *et al.* 1998) and translocate to the nucleus, where they phosphorylate a variety of substrates such as the transcription factor Elk1. In addition to ERK 1 and 2 and MEK, an important contribution of the ERK5/MEK5 pathway to Ras/c-Raf-1 signalling and growth control has recently been described (English *et al.* 1999). Ras interacts with two distinct parts of the c-Raf-1 N-terminal region (Fabian *et al.* 1994, Brtva *et al.* 1995), and the strength of this Ras/c-Raf-1 interaction determines the response of c-Raf-1 to Ras (Okada *et al.* 1999). Ras isoforms vary in their ability to activate c-Raf-1, suggesting that activation of different Ras isoforms can have distinct biochemical consequences for the cell (Yan *et al.* 1998).

Among other candidate Ras effectors is the phosphatidylinositol 3 kinase (PI3K). The PI3K p110 subunit interacts with Ras-GTP through the domain located between amino acids 133-314. Mutants of this region show differential impairment of effector interaction (Winkler *et al.* 1997). PI3K-dependent Ras activation also controls the activity of Akt/PKB, Rac and p70s6k (Marte and Downward 1997). In

addition to Raf and PI3K, other Ras effectors have been described, including Rin1, p120GAP, AF6, RalGDS, Nore1, Rfl and protein kinase C (PKC) ζ (Diaz-Meco *et al.* 1994, Han and Colicelli 1995, Feig *et al.* 1996, Kuriyama *et al.* 1996, McCormick 1998, Vavvas *et al.* 1998, Watari *et al.* 1998, Vetter *et al.* 1999, Wolthuis and Bos 1999, Yamamoto *et al.* 1999). p120GAP was the first molecule to be proposed as a Ras effector. Although it acts as a negative regulator of Ras, recent evidence suggests that p120GAP regulates Rho functions. PKCζ associates with Ras-GTP, suggesting that Ras localizes PKCζ to the plasma membrane. Another Ras effector candidate is the mitogen-activated protein kinase 1 (MEKK1), a serine/threonine kinase that is an upstream activator of SEK and may also serve as a cdc42 and Rac effector (Fanger *et al.* 1997). Ras is also reported to interact with REKS and Bcl-2 (Shimizu *et al.* 1994, Chen and Faller 1996, Rebollo *et al.* 1999). In addition, it has been shown that Ras interacts with the transcription factor Aiolos. This association results in inhibition of Bcl-2 expression, consequently leading to apoptosis (Romero *et al.* 1999). Finally, other Ras effectors have been identified that may contribute to Ras regulation; Ras interacts with N-Jun amino-terminal kinase (JNK), MEK kinase and kinase suppressor of Ras (KSR) (Shimizu *et al.* 1994, Adler *et al.* 1995, Russell *et al.* 1995, Therrien *et al.* 1995, Zhang *et al.* 1997).

Ras-GTP also activates Rac and Rho. The activation of Rac and Rho by oncogenic Ras may lead to morphologic changes that increase the invasive properties of transformed cells. Cells expressing constitutively activated Rac show an increase in membrane ruffling, whereas cells expressing constitutively activated Rho show cytoskeletal reorganization and increased numbers of focal adhesions (Tanaka *et al.* 1999).

Role of Ras in proliferation and aptosis

Ras proteins have been implicated in both protection from and promotion of apoptosis, due to the ability of Ras to regulate multiple signalling pathways through its interaction with different effectors (Downward 1998). Expression of oncogenic forms of Ras proteins leads to the induction of cell cycle progression, causing exit of quiescent cells from G_0 and passage through G_1 and S phase (Downward 1997). Cyclin D1 is one of the earliest cell cycle regulators affected by Ras. Ras activation induces cyclin D1 expression and downregulation of the cdk inhibitor p27kip, probably through a mitogen activated protein kinase (MAPK)-mediated pathway (Jarpe *et al.* 1998). Ras function has also been associated to the retinoblastoma (Rb) cell cycle checkpoint (Mittnacht *et al.* 1997, Lee *et al.* 1999, You *et al.* 1999). Ras may also act at many other later regulatory points in the cell cycle, for instance, regulation of E2F transcription factor release from Rb following its phosphorylation. Cellular Ras and cyclin D1 are required at similar times of the cell cycle in quiescent NIH3T3 cells that have been induced to proliferate, but not in the case of cycling cells. Continuous cell cycle progression in NIH3T3 cells requires Ras activity to promote cyclin D1 synthesis during G2 phase. Cyclin D1 expression then continues through G1 phase independently of Ras activity and drives the G1-S phase transition, suggesting that Ras-dependent induction of cyclin D1 expression beginning in G2 phase is critical for continuous cell cycle progression in NIH3T3 cells (Hitomi and Stacey 1999a, b).

Oncogenic Ras also causes growth arrest and premature senescence associated with upregulation of p53 and p16 ink (Serrano *et al.* 1997). Ras is involved in phosphorylation and activation of the cdc25 phosphoserine phosphatases (Galaktionov *et al.* 1996). Finally, it has been shown that Ras regulates c-*myc* expression and its stability, suggesting that one aspect of the Myc/Ras collaboration is the ability of Ras to enhance the accumulation of transcriptionally active Myc protein (Kerkhoff and Rapp 1998, Sears *et al.* 1999). The extent of Ras activation in PC12 cells determines proliferation or differentiation. Treatment of cells with epidermal growth factor (EGF) leads to transient activation of Ras and proliferation, while stimulation with nerve growth factor (NGF) results in sustained Ras activation, which leads to differentiation (Yan and Ziff 1995, Yao and Cooper 1995). The antiapoptotic activity of Ras has been linked to its ability to activate PI3K (Wennstron and Downward 1999). The PI3K-mediated survival signal is triggered by activation of the serine/threonine kinase Akt/PKB (Franke *et al.* 1997, Cerezo *et al.* 1998), although Akt/PKB can be activated in a PI3K-independent fashion (Konishi *et al.* 1996). Activated PI3K induces cyclin D1 transcription and E2F activity, mediated at least in part by Akt/PKB. Akt/PKB activation correlates with inhibition of JNK2 activity and prevention of apoptosis in IL-4-stimulated T cells (Cerezo *et al.* 1998). One mechanism for Akt/PKB protection against apoptosis is the phosphorylation and inactivation of Bad, a pro-apoptotic molecule (Datta *et al.* 1997, del Peso *et al.* 1997). Taken together, these results suggest that multiple cooperating pathways mediate the effect of Ras on progression through the cell cycle (Gille and Downward 1999). Finally, IL-2- and IL-3-dependent cells are protected from starvation-induced apoptosis by activated Ras through upregulation of Bcl-2 and Bcl-x expression (Kinoshita *et al.* 1995, Gómez *et al.* 1997). Ras-mediated JNK activation may promote different cellular consequences, depending on the cell type or the activation of complementary pathways (Xia *et al.* 1995, Lenczwski *et al.* 1997). IL-2 deprivation correlates with an increase in JNK1 activity directly related to induction of apoptosis (Cerezo *et al.* 1999). On the contrary, activation of the ERK pathway suppresses JNK activity and promotes cell survival (Xia *et al.* 1995).

Multicellular organisms may protect against the oncogenic potential of Ras by mounting an anti-proliferative activation of Ras; this can be in the form of inducing expression of cyclin-dependent kinase inhibitor p21 and p16ink (Serrano *et al.* 1997, Wood *et al.* 1997) or the induction of apoptosis in some cell types (Downward 1998). Ras activation has also been implicated in apoptosis induction. Ras is reported to mediate signals triggered by activation of the cell death receptor Fas (Gulbins *et al.* 1995). Ras is also linked to apoptosis induction in the phaechromocytoma cell line PC12 (Ferrari and Greene 1994). Ras is activated following both IL-2-stimulation or -deprivation in T cells, leading to cell proliferation or apoptosis, depending on complementary stimuli (Gómez *et al.* 1996, 1997). In this context, the interaction described between Aiolos and Ras delineates a novel Ras-mediated pro-apoptotic pathway (Romero *et al.* 1999). Finally, oncogenic Ras down-modulates the expression of Par-4, a transcriptional repressor

protein, that is essential, but not sufficient to induce apoptosis (Barradas *et al*. 1999, Goufang Qiu *et al*. 1999, Nalca *et al*. 1999).

Concluding Remarks

The functional spectrum of the Ras superfamily covers almost every cellular process, and recent data suggest that they are functionally interconnected. Thus, a major part of the choice between cell life and death may be assumed by Ras superfamily proteins. The nature of the signals they transmit may be modulated by other simultaneously triggered parallel signalling pathways that may result in the final order that allows the cell to keep on living or to die. Ras is a crucial regulator of cell growth in eukaryotic cells. The importance of a fine regulation of commitment to apoptosis during the cell cycle is critical for the prevention of the tumorogenic state. Ras is one of the most commonly mutated genes in solid tumours and haematologic neoplasms. Aberrant Ras signalling can be induced directly by mutation of the Ras gene or indirectly by altering genes that associate with Ras or its signalling pathways. New molecules that interfere with Ras activity by inhibiting the enzyme FTase have been described. Clinical studies suggest that these molecules have significant antitumour activity, which appears to be mediated through both apoptosis and cell cycle regulation. In addition to FTIs, other approaches have been used to block Ras function, such as antisense Ras, retrovirus therapy and neutralizing anti-Ras antibodies

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