Gene Expression, Vol. 4, pp. 345-356, 1995
Printed in the USA.

1052-2166/95 $5.00 + .00

Signal Transduction by Ras-Like GTPases: A Potential Target for Anticancer Drugs

MARCEL SPAARGAREN,[1] JAMES R. BISCHOFF, AND FRANK McCORMICK

ONYX Pharmaceuticals, 3031 Research Drive, Bldg. A, Richmond, CA 94806

Members of the *ras* family of GTPases are involved in a wide variety of cellular processes including cell proliferation, differentiation, apoptosis, and transformation. The *ras* oncogene is one of the most frequently mutated genes in human cancer. In addition, other oncogene and tumor suppressor gene products are components of the signal transduction pathways in which Ras or other Ras-like GTPases play key regulatory functions. Current progress in the elucidation of these signal transduction pathways will be reviewed and the potential use of these insights for the development of novel therapeutic agents for the treatment of cancer will be discussed.

Cancer Transformation Signal transduction GTPases Ras Raf

MEMBERS of the *ras* superfamily of small GTPases have key regulatory functions in a wide variety of cellular processes including cell proliferation, differentiation, apoptosis, and transformation (Bourne et al., 1990, 1991; Boguski and McCormick, 1993; Chardin, 1993). Based on homology, the *ras* superfamily of GTPases can be subdivided into three major families: the *rho* family, mainly involved in regulation of cytoskeletal organization; the *rab* family, in regulation of intracellular vesicle sorting; and the *ras* family, in regulation of proliferation and differentiation. The *ras* family, which will be the subject of this review, includes the H-, K (A and B)-, and N-*ras* proto-oncogenes encoding almost identical Ras proteins, as well as *rap* (1A, 1B, 2A, 2B), *ral* (A and B), TC21, and R-*ras* (Chardin, 1993).

Ras-like proteins contain a C-terminal prenylation site that is necessary for membrane localization and biological activity of Ras-like proteins. The Ras-like GTPases cycle between a biologically inactive GDP-bound state and active GTP-bound state. The rate of cycling between these two forms is regulated by GTPase activating proteins (GAPs), which enhance the intrinsic GTPase activity of Ras-like GTPases, and guanine nucleotide exchange factors (GEFs), which cause the exchange of bound GDP for GTP (Boguski and McCormick, 1993). In response to certain extracellular stimuli or intracellular events, the Ras-like GTPases become activated and bind to effector molecules, thereby transmitting the incoming signal to downstream pathways (Fig. 1).

In this review we will summarize some of the recent findings on the mechanism of signal transduction by Ras in particular, as well as by some of the other Ras-like GTPases, and will also discuss the possible use of these insights in the development of anticancer drugs.

SIGNAL TRANSDUCTION BY Ras

The *ras* oncogene [H-, K (A and B)-, and N-*ras*] is one of the most frequently occurring oncogenes in human cancer, being found in 40% of all human tumors (Bos, 1989). Oncogenic mutations of *ras* result in constitutively GTP-bound, and thus biologically active, Ras. Several lines of evidence established an important role of Ras in growth factor signaling and transformation (Lowy and Willumsen, 1993). First, most growth factors stimulate the activation of Ras in target cells (Satoh et al., 1992). Second, the expression of onco-

Accepted December 1, 1994.
[1]Address correspondence to Marcel Spaargaren at his present address: Laboratory for Physiological Chemistry, Utrecht University, P.O. Box 80042, 3508 TA Utrecht, The Netherlands.

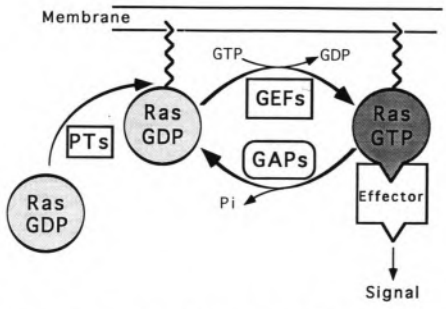

FIG. 1. Signal transduction by Ras-like GTPases. Ras-like GTPases (Ras) are posttranslationally modified by prenyltransferases (PTs), resulting in their membrane localization. Their activity is regulated by GTPases activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), resulting in their inactive GDP- or active GTP-bound state, respectively. In their biologically active GTP-bound state the Ras-like GTPases couple to effector molecules, which transmit the signal further downstream in the signal transduction pathway.

genic Ras mimics certain effects of oncogenic receptor and cytoplasmic tyrosine kinases (Feramisco et al., 1984; Stacey and Kung, 1984), whereas the expression of dominant negative mutants of Ras and microinjection of Ras-neutralizing antibodies decreases growth factor-induced proliferation of normal cells and suppresses transformation by oncogenic receptor and cytoplasmic tyrosine kinase (Mulcahy et al., 1985; Cai et al., 1990; Smith et al., 1986). Finally, genetic studies established a critical function of Ras in receptor tyrosine kinase signaling involved in development of the *Drosophila* eye and *C. elegans* vulva (Beitel et al., 1990; Simon et al., 1991).

The high frequency of mutation of Ras in tumors and its critical function in downstream signaling by oncogenic receptor and cytoplasmic tyrosine kinases renders Ras, its regulators, and its effectors very promising targets for anticancer treatment. In the next part of this review we will focus on some of the recent developments in our understanding of the regulation of Ras activity, the mechanism of signal transduction by Ras, and its effector molecules.

Posttranslational Modification of Ras

Posttranslational modification of Ras-like proteins involves prenylation of the C-terminal CAAX motif (where A is an aliphatic amino acid and X any C-terminal amino acid) (Hancock and Marshall, 1993). This prenylation is required for proper membrane localization and for their biological activity. Depending on the composition of the CAAX motif of the Ras-like proteins, the cysteine residue in the CAAX motif is farnesylated or geranylgeranylated. Geranylgeranylation occurs if the X position in the CAAX motif is a leucine (e.g., the Ras-related proteins Rap and R-*ras*), in most other cases the Ras-like protein will be farnesylated (e.g., H-, K-, and N-ras). After prenylation, the AAX residues are removed by proteolysis, as a consequence of which the cysteine residue becomes the C-terminal amino acid. Subsequently, this cysteine residue is carboxymethylated. Following these modifications, another localization signal is provided by the subsequent palmitoylation of additional cysteine residues near the C-terminus (H-ras, K-rasA, and N-ras), or by the presence of a polybasic domain (K-rasB) (Hancock and Marshall, 1993).

As a consequence of these posttranslational modifications the Ras proteins become specifically localized at the plasma membrane. Interestingly, farnesylation of the C-terminal CAAX motif and the resulting plasma membrane localization of oncogenic Ras proteins are essential for their transforming potential (Hancock and Marshall, 1993; Willumsen et al., 1984).

Regulation of Ras Activity by Exchange Factors and GAPs

The activity of Ras is regulated by GAPs, which stimulate the intrinsic GTPase activity of Ras resulting in GDP-bound inactive Ras, and by GEFs, which increase the dissociation of bound nucleotide, thereby promoting the formation of GTP-bound active Ras (Boguski and McCormick, 1993). So far two mammalian GAPs have been identified with activity for Ras: p120-GAP and NF-1 (Boguski and McCormick). Several proteins have been shown to possess GEF activity towards Ras; best characterized are Sos and RasGRF (Boguski and McCormick, 1993; Feig, 1994). In addition, Vav has been reported to have GEF activity towards Ras (Gulbins et al., 1993, 1994), but this has been disputed recently (Bustelo et al., 1994).

The mechanism by which growth factor stimulation of cells results in Ras activation has been the subject of intensive research for several years. Most progress has been made with the elucidation of the Ras-activation mechanism by the EGF receptor tyrosine kinase (McCormick, 1993; Schlessinger, 1993; Feig, 1993) (Fig. 2). Upon binding of EGF to the receptor, the receptors dimerize (Ulrich and Schlessinger, 1990), resulting in activation of the receptor tyrosine kinase (Spaargaren et al., 1990) and subsequent intermolecular cross-phosphorylation (Ulrich, 1990). A major breakthrough was achieved when it was shown that acti-

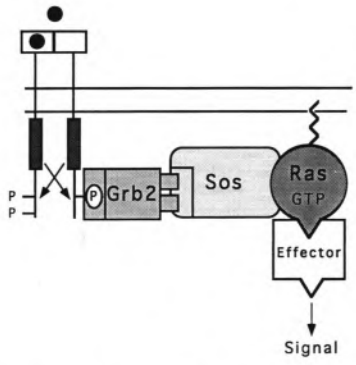

FIG. 2. Growth factor-induced Ras activation. The mechanism by which EGF activates Ras. Upon binding of EGF to the EGF receptor, the receptors dimerize as a consequence of which the intrinsic tyrosine kinase of the receptor becomes activated, resulting in autophosphorylation of the receptor by intermolecular cross-phosphorylation. The phosphorylated tyrosine residues of the EGF receptor provide a binding site for the SH2 domain of Grb2. Grb2 is complexed with the RasGEF Sos by its SH3 domains that bind a proline-rich sequence in Sos. Probably as a consequence of the recruitment of Grb2 by the autophosphorylated receptor tyrosine kinase, Sos is translocated to the plasma membrane in the proximity of Ras, thereby resulting in Ras activation.

vation of the EGF receptor tyrosine kinase results in the translocation of a complex of Sos and an adapter protein Grb2 to the plasma membrane (McCormick, 1993; Schlessinger, 1993; Feig, 1993). Grb2 does not have any catalytic activity and consists of one SH2 domain and two SH3 domains. These domains are involved in protein–protein interaction, the SH2 domain binding to specific phosphotyrosine residues and SH3 domains to proline-rich sequences. The translocation of the Grb2-Sos complex is mediated by the interaction of the SH2 domain of Grb2 with certain autophosphorylation sites of the EGF receptor, whereas the interaction between Sos and Grb2 involves the SH3 domains of Grb2 interacting with proline-rich regions in Sos (Rozakis-Adcock et al., 1993). Interestingly, Sos becomes phosphorylated upon EGF treatment (Burgering et al., 1993; Rozakis-Adcock et al., 1993). Thus far, however, no growth factor-induced activation of Sos has been observed. Moreover, using a chimerical Sos protein containing the C-terminal plasma membrane localizing CAAX motif of Ras, it was shown that plasma membrane localization of Sos is sufficient to mimic a ras-transformed phenotype in NIH 3T3 cells (Quilliam et al., 1994). Therefore, the translocation of Sos to the plasma membrane appears to be involved in, and may even be sufficient for, activation of Ras. However, the possibility that Grb2 alters the intrinsic exchange factor activity of Sos cannot be excluded.

The mechanism of Sos translocation and Ras activation by the EGF receptor cannot be generalized for all receptor tyrosine kinases, nor for the EGF receptor itself (Rozakis-Adcock et al., 1992; Pelicci et al., 1992). Many different scenarios exist for the recruitment of the Grb2-Sos complex to the plasma membrane. Other proteins involved in Ras activation, varying from different growth factor receptor signaling pathways and tissues, include Shc (Rozakis-Adcock et al., 1992; Ravichandran et al., 1993; Pelicci et al., 1992; Pronk et al., 1994; Skolnick et al., 1993), IRS-1 (Myers et al., 1994), and Syp/SH2-PTP (Li et al., 1994). In general these proteins function as intermediates between receptors and Grb2. They are substrates of receptor tyrosine kinases and/or cytoplasmic tyrosine kinases, and their phosphorylated tyrosine residues substitute the autophosphorylated receptor sites for interaction with the SH domain of Grb2 (Rozakis-Adcock et al., 1992; Ravichandran et al., 1993; Pellici et al., 1992; Pronk et al., 1994; Skolnick et al., 1993; Myers et al., 1994; Li et al., 1994). The Syp and Shc proteins were shown to bind autophosphorylated growth factor receptors by means of their SH2 domains (Pelicci et al., 1992; Li et al., 1994) and via its SH2 domain Shc also binds phosphorylated tyrosines of cytoplasmic tyrosine kinases as well as of noncatalytic receptors (Ravichandran et al., 1993). If and how IRS-1, which is a major substrate of the insulin receptor, localizes to the plasma membrane remains to be established.

The mechanism of action of the other rasGEFs, Vav and RasGRF, remains largely unknown (Feig, 1993). The Vav protein, which contains an SH2 and SH3 domain, becomes phosphorylated on tyrosine residues by Lck and its phosphorylation state correlates with its GEF activity (Gulbins et al., 19993, 1994). However, as mentioned before, controversy exists as to whether Vav indeed functions as a RasGEF (Bustelo et al., 1994). Furthermore, Vav is only expressed in haemapoietic cells (Gulbins et al., 1993), and RasGRF is only expressed in neurons in the brain (Shou et al., 1992), suggesting these are tissue-specific regulators of Ras.

The proteins identified so far that are able to enhance the intrinsic GTPase activity of Ras are p120-GAP and neurofibromin (Boguski and McCormick, 1993). Different oncogenic mutations of Ras result in constitutively active GTP-bound Ras as a consequence of the inability of GAPs to stimulate their intrinsic GTPases activity, even though the GAP can still bind Ras. So far these rasGAPs are not believed to play an active regulatory role

in growth factor-induced Ras activation because no regulatory mechanisms for the activity of these GAPs have been elucidated. However, translocation mechanisms that are either able to bring Ras and GAPs in the proximity of each other or to prevent them from interacting, may very well play an important role in regulation of Ras activity. Noteworthy, in T-lymphocytes, the activation of the T-cell receptor results in a decreased GAP activity, which correlates with Ras activation (Downward et al., 1990).

The p120-GAP protein, in addition to its ras-GAP-domain, contains two SH2 domains, one SH3 domain, and a pleckstrin homology (PH) domain (3). Noteworthy, p120-GAP associates to autophosphorylated growth factor receptor tyrosine kinases and cytoplasmic tyrosine kinases by means of its SH2 domain (Kaplan et al., 1990; Kazlaukas et al., 1990). However, mutation of the (phospho-)tyrosine binding site for p120-GAP in several growth factor receptor tyrosine kinases does not affect stimulation of Ras activity (Fantl et al., 1992). Because SH2, SH3, and PH domains are involved in protein–protein interactions, this indicates that p120-GAP may have additional roles besides being a rasGAP. Indeed, as will be discussed later, several studies suggest that p120-GAP may be an effector molecule for Ras (Martin et al., 1992; Duchesne et al., 1993; Medema et al., 1992). The neurofibromin protein contains a region with homology to the rasGAP domain of p120-GAP, and was shown to exert GAP activity towards Ras (Boguski and McCormick, 1993). Interestingly, neurofibromin is a tumor suppressor gene found to be deficient in patients with von Recklinghausen neurofibromatosis (NF-1), thereby suggesting the involvement of Ras in the pathology of this disease (Boguski and McCormick, 1993).

Downstream Signaling of Ras by Raf

Several lines of evidence have supported a role for the Raf-1 serine/threonine kinase downstream of Ras (Roberts, 1992; Moodie and Wolfman, 1994). The expression of oncogenic, active, Ras mutants results in the activation of Raf (Morrison et al., 1988; Wood et al., 1992), whereas dominant negative mutants of Ras blocked the growth factor-induced activation of Raf (Wood et al., 1992). Furthermore, the expression of v-raf overcomes the effect of dominant negative Ras mutants or Ras-neutralizing antibodies (Smith et al., 1986), and the expression of dominant negative Raf mutants or antisense Raf abolishes the transforming effect of oncogenic Ras (Kloch et al., 1991). Finally, genetic studies on *Drosophila* eye and *C. elegans* vulva development demonstrated a critical role for Raf in signaling downstream of Ras (Dickson and Hafen, 1994).

Besides the observation that Ras is required for growth factor-induced activation of Raf-1, by means of dominant negative and constitutively active point mutants of Ras in mammalian cells as well as genetic studies in *Drosophila* and *C. elegans*, it was also established that Ras is required for activation of MAP-kinase and MEK (Wood et al., 1992; Thomas et al., 1992; de Vries-Smits et al, 1992; Leevers and Marshall, 1992) (Fig. 3). The Ras-mediated activation of Raf-1 is the first step in a kinase cascade in which Raf-1 phosphorylates and activates MEK (Dent et al., 1992; Howe et al., 1992; Kyriakis et al., 1992; Macdonald et al., 1993), which then phosphorylates and activates MAP kinase (Macdonald et al., 1993; Crews et al., 1992; Blumer and Johnson, 1994; Johnson and Vaillancourt, 1994) (Fig. 3). A number of substrates of MAP kinase have been identified, including the EGF receptor, phospholipase A2, ribosomal S6 kinase 90, as well as several transcription factors such as c-myc, c-jun, c-fos, and elk-1 (Johnson and Vaillancourt, 1994).

The question remained as to how activation of Ras results in Raf activation. A major breakthrough came with the demonstration of a direct

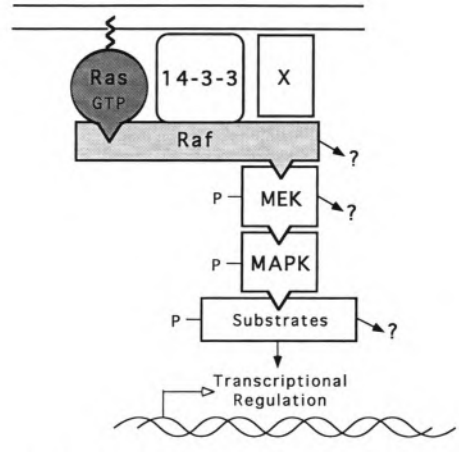

FIG. 3. Ras-mediated activation of Raf and signaling downstream of Raf. Activated Ras recruits Raf to the plasma membrane by a direct interaction. At the plasma membrane Raf is activated, a process that may involve the protein 14-3-3 and/or another unknown factor (X). As a consequence of the activation of Raf, Raf phosphorylates and activates MEK (and other substrates?), which in its turn phosphorylates and activates MAP kinase (and other substrates?). The MAP kinase can phosphorylate a number of substrates, including other kinases as well as several transcription factors, thereby regulating the transcriptional control of their target genes.

interaction between H-ras and Raf (Moodie and Wolfman, 1994; Avruch et al., 1994). Several studies showed the formation of a complex containing activated Ras and Raf in cell lysates (Koide et al., 1993; Moodie et al., 1993), in the yeast two-hybrid system (van Aelst et al., 1993; Vojtek et al., 1993; Zhang et al., 1993), and in mammalian cells (Finney et al., 1993), and using purified proteins the interaction was shown to be direct and GTP dependent (Vojtek et al., 1993; Zhang et al., 1993; Warne et al., 1993). This interaction was shown to involve the effector domain of Ras (amino acids 32–40) and the N-terminal regulatory domain of Raf, in particular the domain encompassing amino acids 51–131 of Raf-1 (Vojtek et al., 1993; Spaargaren and Bischoff, 1994). However, these studies did not reveal the mechanism by which Raf is activated, as the interaction between Ras and Raf as such is not sufficient for activation of Raf in vitro (Zhang et al., 1993; S. G. Macdonald and F. McCormick, unpublished observations).

The function of Ras in Raf activation appears to be to recruit Raf to the plasma membrane where it can subsequently be activated by as yet unknown factors (Fig. 3). Evidence for this was recently obtained, as it was shown that a chimeric Raf protein, containing at its C-terminus the CAAX box motif and polybasic domain of K-ras, translocates to the plasma membrane and becomes activated independently of Ras (Stokoe et al., 1994; Leevers et al., 1994). Furthermore, once Raf activation has occurred, the interaction with Ras is no longer required to maintain its activity (Leevers et al., 1994). A phosphatidyl-specific phospholipase C has been found to function downstream of Ras but upstream of Raf, suggesting it may be involved in Ras-mediated Raf activation (Cai et al., 1993). Recently, it has been observed that 14-3-3 proteins bind to Raf without competing with Ras, cotranslocate with Raf to the plasma membrane as a consequence of the expression of active Ras, are able to activate Raf in yeast as well as in vitro, and a *S. cerevisiae* homologue of 14-3-3 is required for Ras-induced Raf activation in yeast (Freed et al., 1994; Irie et al., 1994). These data strongly suggest a role for these 14-3-3 proteins in the regulation of Raf activity. However, how Raf becomes activated upon translocation by Ras to the plasma membrane, whether the 14-3-3 proteins are actively involved in this process or rather serve as an adapter molecule, what the nature is of the Raf activator (e.g., a protein kinase or certain lipid molecules), whether recruitment of Raf to the plasma membrane is the only function of Ras, and whether Raf is the only effector molecule for Ras in mammalian cells still remain to be established.

Interestingly, cAMP was recently shown to inhibit the signaling from Ras to MAP kinase in certain mammalian cells (Burgering et al., 1993; Cook and McCormick, 1993; Wu et al., 1993). The inhibitory effect of cAMP was pinpointed to occur between Ras and Raf (Burgering et al., 1993; Cook and McCormick, 1993). It was shown that in vitro phosphorylation of Raf by PKA reduces its affinity for binding by Ras (Wu et al., 1993). This suggests that the inhibitory effect of cAMP is mediated by PKA-induced phosphorylation of Raf, resulting in decreased translocation of Raf to the plasma membrane by Ras, thus suppressing Raf activation.

Putative Effector Molecules: RalGDS and p120-GAP

By means of a yeast two-hybrid screen and subsequent two-hybrid experiments, RalGDS was recently identified as a Ras binding protein (Spaargaren and Bischoff, 1994). RalGDS had been characterized as an exchange factor for the Ras-like GTPase Ral without any detectable exchange factor activity towards Ras, R-ras, and Rho (Albright et al., 1993). This suggested that the interaction between RalGDS and Ras does not occur by virtue of its exchange factor activity. In agreement with this, a 127 amino acid C-terminal fragment of RalGDS, which is distinct from its conserved catalytic exchange factor region, was identified as the Ras binding domain. Moreover, RalGDS was shown to interact specifically to activated but not inactive point mutants of Ras-like proteins. Subsequent in vitro binding experiments using purified proteins demonstrated the direct GTP-dependent binding of RalGDS by Ras, as well as competition between RalGDS and Raf for binding to Ras (Spaargaren and Bischoff, 1994). These data indicate that RalGDS is a putative effector molecule of Ras.

The finding of another putative effector molecule supports the notion that Ras may not solely exert its biological effects by modulation of Raf activity. However, the functional outcome and biological effect of the interaction between RalGDS and Ras remains to be established. Although the function of the interaction may be to suppress Raf binding and activation, it is tempting to speculate that by binding to RalGDS, Ras is able to regulate its exchange factor activity towards Ral (Fig. 4). For example, the interaction between Ras and Ral-

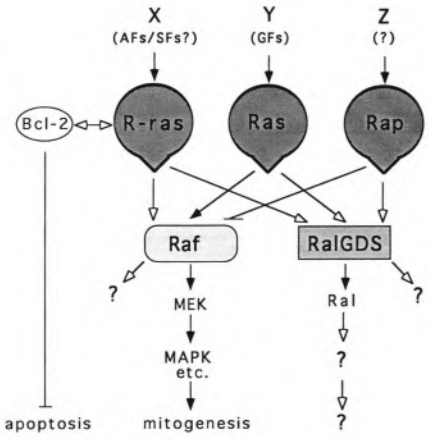

FIG. 4. Cross-talk between different Ras-like GTPases. Different stimuli (X, Y, and Z) may result in the activation of different, or a particular combination of, Ras-like GTPases (R-ras, Ras, and Rap). The final biological outcome of these stimuli will be determined by which of the effector molecules (Raf and RalGDS) of these GTPases are subsequently activated or inactivated. For example, activation of Ras stimulates (closed arrow) Raf, whereas activation of Rap inhibits (blunt arrow) activation of Raf. The effect of the activation of R-ras on Raf activity is unknown (open arrow). Whereas activation of the Raf pathway is involved in mitogenesis, the biological effect of the interaction of the Ras-like GTPases with RalGDS, as well as the effect of the interaction between Bcl-2 (which suppresses apoptosis) and R-ras, remains to be established. AFs are apoptosis-inducing factors, SFs are survival factors, and GFs are growth factors.

GDS as such may directly modulate the catalytic exchange factor activity of RalGDS. Furthermore, the interaction may result in translocation of RalGDS to the plasma membrane, as a consequence of which RalGDS either can be activated, can activate Ral, or can be prevented from exerting its activity towards Ral. Finally, as RalGDS can be phosphorylated on serine and threonine residues (Albright et al., 1993), the interaction may regulate its catalytic activity by modulating the phosphorylation state of RalGDS (by bringing it in the proximity of Raf?). Therefore, the interaction between Ras and RalGDS reveals the intriguing possibility that Ras can regulate the activity of yet another member of the Ras family, Ral, by directly modulating the activity of the exchange factor for Ral. Unfortunately, however, little is known about the biological function of Ral (Chardin and Tavitian, 1986; Feig and Emkey, 1993). It has been reported that Ral is rather ubiquitously expressed (Olofsson et al., 1988; Wildey et al., 1993), and that constitutively active Ral mutants are nontransforming and do not impose a striking phenotype on the cell (Feig and Emkey, 1993). Based upon its cellular localization, it has been proposed that Ral may have a role in endocytotic clathrin vesicle function (Feig and Emkey, 1993).

It has been beleived for a long time that p120-GAP partially functions as an effector molecule. Initially this was mainly based on the interaction of p120-GAP with the effector domain of Ras, the domain that is crucial to the mitogenic signaling and transformation by Ras, and on the GTP dependency of this interaction (McCormick, 1989). The p120-GAP protein, in addition to its C-terminal rasGAP domain, contains two SH2 domains, one SH3 domain, and a PH domain in its N-terminal part. Several studies have indicated a role for the SH2 and SH3 domains of p120-GAP for its function in signaling downstream of Ras (Lowy and Willumsen, 1993; Martin et al., 1992; Duchesne et al., 1993; Medema et al., 1992). However, p120-GAP is not required for signaling by the receptor tyrosine kinase *sevenless* and Ras involved in *Drosophila* eye development (Roberts, 1992). One of the proteins interacting with the N-terminus of p120-GAP is p190, which itself is a GAP for the Ras-related protein Rho (Settleman, 1992). The association between p190 and the N-terminal domain of p120-GAP correlates with the control of cell shape and cell adhesion (McGlade et al., 1993), thereby suggesting that p120-GAP may connect Ras to the Rho family of small GTPases and thus to cytoskeletal effects.

The proteins identified so far as (putative) effector molecules for Ras-like GTPases (i.e., Raf-1, RalGDS, and p120-GAP) do not seem to share any structurally conserved domains of homology in their Ras binding domains.

SIGNAL TRANSDUCTION BY THE Ras-LIKE GTPases Rap AND R-ras

Little is known about the function of the other Ras-like GTPases: TC21, Ral (A and B), Rap (1A, 1B, 2A, and 2B) and R-ras. All these Ras-like GTPases, except for Ral, have a domain identical to the core effector domain of H-Ras (amino acids 32–40). TC21 was recently shown to be the first Ras-like protein with transforming potential (Graham et al., 1994). Ral was reported to be a nontransforming Ras-related protein (Chardin and Tavitian, 1986; Feig and Emkey, 1993), having its own specific exchange factor RalGDS (Albright et al., 1993), and so far its function, as well of that of TC21, is unknown. A little more is known about the signal transduction of the Ras-like GTPases Rap and R-ras. In the next section we will review and discuss some recently obtained insights on their possible function and signaling mechanism.

Signal Transduction by Rap and Its Suppressing Effect on Ras Signaling

Rap1A (also known as Krev-1, i.e., K-ras revertant-1) has approx. 50% homology with H-ras, and is closely related to other members of the Rap subfamily, such as Rap1B, Rap2A, and Rap2B (Katayama and Noda, 1993). Rap1A becomes geranylgeranylated and has a polybasic domain (Hancock and Marshall, 1993). Subcellular localization studies indicated that Rap1A is associated with the Golgi complex (Beranger et al., 1991). Little is known about its normal biological function, although its copurification with NADPH oxidase cytochrome b558 from stimulated neutrophils (Quinn et al., 1989), as well as recent functional studies (Maly et al., 1994), suggests a possible role in the oxidative burst of superoxide radicals in neutrophils. Interestingly, another member of the Ras superfamily, Rac, has also been implicated in this process (Abo et al., 1991; Diekmann at al., 1994). Rap1A is insensitive to rasGAP (although it can bind to it) and has its own specific rapGAP (Rubinfield et al., 1991). Furthermore, Rap1A is phosphorylated in vivo and is a substrate for PKA in vitro, the phosphorylation resulting in increased affinity for smgGDS (Hata et al., 1991). Rap1A was identified by virtue of its sequence similarity to H-ras (Pizon et al., 1988), and independently by its ability to reverse oncogenic Ras-induced transformation (Kitayama et al., 1989). Later Rap1A was shown to exert its reverting effect on Ras transformation by interfering with Ras signaling between Ras and Raf (Cook et al., 1993; Sakoda et al., 1992). Interestingly, the formation of a complex containing Rap and Raf-1 was recently shown in the yeast two-hybrid system (Zhang et al., 1993), and the direct GTP-dependent interaction of Rap with the 81 amino acid Ras binding domain of Raf was shown in vitro using purified proteins (Spaargaren and Bischoff, 1994). These observations combined indicate that Rap may exert its antagonistic effect on Ras-induced transformation by the formation of a complex with Raf, thereby interfering with the binding of Ras to Raf and thus preventing Raf from being activated. In addition, Rap was also shown to bind in a GTP-dependent manner with RalGDS, both in vivo in the two-hybrid system, as well as in vitro using purified proteins (Spaargaren and Bischoff, 1994).

Signal Transduction by R-ras and Its Possible Role in Apoptosis

R-*ras* has 55% amino acid identity and a 26 amino acid N-terminal extension compared with H-ras (Lowe et al., 1987). Based on its C-terminal sequence, R-ras probably becomes geranylgeranylated and palmitoylated, but no data are available yet as to its subcellular localization. R-ras appears to be nontransforming (Lowe and Goeddel, 1987; Lowe et al., 1988) and is sensitive to p120-GAP (Garrett et al., 1989; Tsai et al., 1989) and neurofibromin (Rey et al., 1994), but not to the exchange factor Sos (Buday and Downward et al., 1993). Furthermore, the formation of a complex of R-ras with the Raf-1 kinase was shown in vivo in the two-hybrid system (Spaargaren et al., 1994), as well as in vitro using purified proteins (Rey et al., 1994; Spaargaren et al., 1994), and this interaction was shown to be GTP dependent and only requires an 81 amino acid region of the N-terminal regulatory domain of Raf (Spaargaren et al., 1994). In addition, R-ras was also shown to interact with RalGDS, in the two-hybrid system as well as in in vitro binding experiments using purified proteins (Spaargaren and Bischoff, 1994). Interestingly, the C-terminus of R-ras was shown to associate with the apoptosis-suppressing proto-oncogene product Bcl-2 (Fernandez-Sarabia and Bischoff, 1993), suggesting a role for R-ras in the regulation of apoptosis.

The *bcl-2* gene was identified as an oncogene overexpressed by the translocation t(14;18) in non-Hodgkin's B-cell lymphomas. It was found that Bcl-2 prolongs cell survival without stimulation of cell proliferation by suppressing apoptosis, thereby identifying it as the first genetic regulator of apoptosis in mammalian cells (Vaux et al., 1988; Reed, 1994). Apoptosis, or programmed cell death, is the physiological mechanism by which cells can actively regulate their own and/or each others' death, thereby regulating life span and cell numbers, and preventing damaged cells from harming the organism. Apoptosis plays an important role in normal development (embryonic and adult) and homeostasis, whereas its dysregulation is involved in the pathogenesis of many diseases, including cancer (Reed, 1994; Harrington et al., 1994; Raff et al., 1993; Vaux, 1993; Barr and Tomei, 1994). Obviously, tumor formation can be due to either enhanced proliferation or suppressed apoptosis. Furthermore, one of the major roles of apoptosis is to eliminate damaged or precancerous cells, and failure of an organism to do so may finally result in oncogenesis. The cellular and biochemical aspects underlying the mechanism of apoptosis are still largely unknown. Apoptosis can be induced by many different extracellular stimuli as well as by several oncogenes, tumor suppressor genes, and viral genes (Reed, 1994; Harrington et

al., 1994; Raff et al., 1993; Vaux, 1993; Barr and Tomei, 1994). These genes have been shown to exert a direct effect on the survival and death of cells by regulating the process of apoptosis, which plays an important role in their transforming potential (Harrington et al., 1994). The *bcl-2* protooncogene is able to suppress a wide variety of naturally occurring or induced forms of apoptosis (Reed, 1994; Harrington et al., 1994; Raff et al., 1993; Vaux, 1993); however, the underlying mechanism of action remains to be established.

The interaction between R-ras and Bcl-2 suggests the intriguing possibility that R-ras may have a key regulatory role in a signal transduction pathway involved in the regulation of apoptosis (Fernandez-Sarabia et al., 1993). It is tempting to speculate that the interaction between Bcl-2 and R-ras either serves as a mechanism for R-ras to regulate the apoptosis-suppressing ability of Bcl-2, or as a mechanism for Bcl-2 to exert its apoptosis-suppressing effect via modulation of R-ras activity. Interestingly, the *v-raf* oncogene was shown to suppress apoptosis without stimulation of Bcl-2 expression (Cleveland et al., 1994), and Raf and Bcl-2 were shown to coimmunoprecipitate from mammalian cells, suggesting they are present in a protein complex (Wang et al., 1994). Given the observed interaction between R-ras and Raf (Spaargaren et al., 1994), these observations provide further support for the intriguing possibility that R-ras may play a regulatory role in the process of apoptosis, although it remains to be established what the functional outcome is of the interaction between R-ras and Raf. Furthermore, it will be interesting to determine whether Bcl-2 has any GEF or GAP activity towards R-ras, whether activated or dominant negative mutants of R-ras modulate the apoptosis-suppressing activity of Bcl-2, can influence the effect of apoptosis-inducing stimuli, or can induce apoptosis by themselves, and by what extra- or intracellular stimuli R-ras becomes activated.

Cross-Talk Between Different Ras-Like GTPases

Several Ras-like GTPases can bind to Raf as shown by the in vivo complex formation in the two-hybrid system of Raf with H-ras (van Aelst et al., 1993; Vojtek et al., 1993; Zhang et al., 1993), Rap (Zhang et al., 1993), and R-ras (Spaargaren et al., 1994), and by the direct GTP-dependent interaction in vitro with H-ras (Vojtek et al., 1993; Zhang et al., 1993; Warne et al., 1993), Rap (Spaargaren and Bischoff, 1994), and R-ras (Spaargaren et al., 1994). In addition, using both the two-hybrid system and in vitro binding experiments, the direct GTP-dependent interaction of R-ras, H-ras, K-ras, and Rap with RalGDS has been shown as well (Spaargaren et al., 1994). These binding abilities of H-ras, Rap, and R-ras are especially intriguing given the different biological effects of these Ras-like GTPases; H-ras, being involved in regulation of proliferation and differentiation and having transforming abilities (Boguski and McCormick, 1993; Lowy and Willumsen, 1993), Rap being able to revert oncogenic Ras-induced transformation (Katayama et al., 1989; Cook et al., 1993; Sakoda et al., 1992), and R-ras, which is possibly involved in the regulation of apoptosis (Fernandez-Sarabia and Bischoff, 1993). However, it is not surprising because the effector domain, which has been proposed to mediate the interactions with Raf (Koide et al., 1993; Moodie et al., 1993; van Aelst et al., 1993; Vojek et al., 1993; Zhange et al., 1993; Warne et al., 1993; Spaargaren et al., 1994) and probably also with RalGDS (Spaargaren and Bischoff, 1994), is conserved between Ras, Rap, and R-ras (Marshall, 1993; Polakis and McCormick, 1992). Given the recent data, which indicate that the Ras-like GTPases may mainly function as a recruitment factor (Stokoe et al., 1994; Leevers et al., 1994), the cellular localization, besides their affinity, may determine which Ras-like GTPases and effector molecules will interact in vivo, and what the functional outcome of the interaction will be. Noteworthy in this respect is that H-ras becomes farnesylated and palmitoylated, Rap becomes geranylgeranylated and has a polybasic domain, and R-ras probably becomes geranylgeranylated and palmitoylated (Hancock and Marshall, 1993). Therefore, it will be interesting to see which GTPase will turn out to be the most efficient activator or suppressor of either Raf or RalGDS, in particular with respect to their cellular localization.

In contrast to binding of the Ras-like GTPases with effector molecules, more specificity appears to exists on the level of the exchange factors (Boguski and McCormick, 1993; Feig, 1994). For example, the exchange factor for Ras, Sos, has no activity towards RalA (Chardin et al., 1993) or R-ras (Buday and Downward, 1993), RasGRF has no activity towards Rap1A (Orita et al., 1993) or Ral (Shou et al., 1992), and Vav has no activity towards Ral (Kohl et al., 1993), whereas RalGDS has no activity towards H-ras, R-ras, or Rap (Albright et al., 1993). Therefore, it is likely that different ligand–receptor interactions and/or intracellular events activate a different Ras-like GTPases or a particular combination of these GTP-

ases, which, in combination with the cellular context, determine the biological outcome of the activating signal. For example, Ras is activated by most growth factors, resulting in either proliferation or differentiation, whereas possibly R-ras activity may be regulated by apoptosis-inducing stimuli such as TNF and the Fas-ligand and/or apoptosis-suppressing survival factors such as IGF-I, NGF, or erythropoietin (Raff et al., 1993), some of which are also able to activate Ras. Because the Ras-like GTPases appear to be able to influence each others' downstream signaling by binding to the same effector molecules, the identification of their upstream regulatory components (i.e., activating stimuli and GEFs) may reveal an intriguing network of extensive cross-talk between these different signal transduction pathways, in which the Ras-like GTPases are key regulatory elements (Fig. 4).

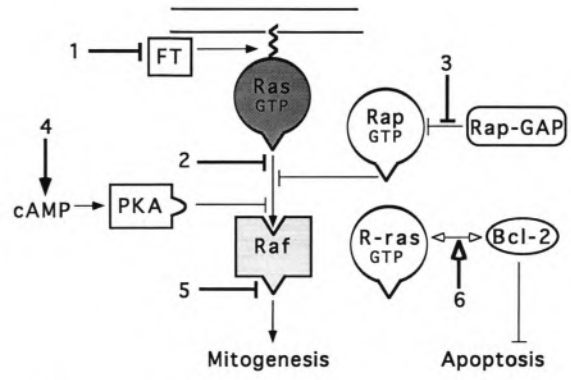

FIG. 5. Potential target sites in the signal transduction pathways of Ras-like GTPases for the development of anticancer drugs. Schematic representation of anticancer drug targets in the Ras-like GTPase signaling pathways, focusing on drugs that counteract the effect of oncogenic mutants of Ras and upstream tyrosine kinases. For an explanation of the potential anticancer drug targets and the underlying biological principle, see the text. A stimulatory effect is indicated by the closed arrow, a suppressing effect by a blunted arrow, and an unknown effect by the open arrow. FT is farnesyltransferases, PKA is protein kinase A.

TARGETS FOR ANTICANCER DRUGS

Given the high frequency at which activating mutations of the Ras proteins are found in different tumors, and the key role of the Ras proteins in downstream signaling from oncogenic receptor tyrosine kinases and cytoplasmic tyrosine kinases, the Ras signal transduction pathway provides an interesting target for anticancer drugs. The recent developments in the elucidation of the mechanisms of signal transduction by Ras and the Ras-like GTPases provide some promising insights as to how this knowledge can be used to modulate these signaling events, in a way that may be useful for the treatment of cancer. We will summarize some of the potential target sites for anticancer drugs, focusing on suppression of the signaling downstream of Ras (Fig. 5).

First of all, because farnesylation of the C-terminal CAAX motif and the resulting plasma membrane localization of Ras are essential for its transforming potential (Hancock and Marshall, 1993; Willumsen et al., 1984), the enzyme farnesyltransferase provides a promising target for inhibitory compounds. Indeed, attempts are already being made to develop specific farnesyltransferase inhibitors for use as anticancer drugs (Kohl et al., 1993; James et al., 1993; Hancock, 1993) [Fig. 5(1)].

The critical role of Raf in the downstream signaling of receptor tyrosine kinases, cytoplasmic tyrosine kinases, and Ras makes it an excellent target for anticancer drugs, as these agents may be able to reverse oncogenic Ras-induced and oncogenic receptor and cytoplasmic tyrosine kinase-induced transformation. The direct interaction between Ras and Raf is of major importance in signaling downstream of Ras and in activation of Raf (Moodie and Wilfman, 1994; Avruch et al., 1994). Therefore, small molecule drugs that can prevent the interaction between Ras and Raf [Fig. 5(2)] may prove to be powerful tools in the treatment of cancer. In addition, activated Rap is able to revert ras-induced transformation (Kitayama et al., 1989; Cook et al., 1993; Sakoda et al., 1992), probably by interfering with the interaction of Ras with Raf by binding to Raf itself (Zhang et al., 1993; Spaargaren and Bischoff, 1994). This indicates that, by small molecule drugs that inhibit either the activity of rapGAP towards Rap or the interaction between Rap and RapGAP [Fig. 5(3)], it may be possible to reverse oncogenic Ras (and receptor and cytoplasmic tyrosine kinase)-induced transformation. Furthermore, elevation of cellular cAMP levels and the subsequent PKA activation suppresses Ras signaling in certain cell types, probably by phosphorylation of Raf, which may prevent Ras from binding to Raf (Burgering et al., 1993; Cook and McCormick, 1993; Wu et al., 1993). Stimulation of cAMP levels in tumor cells (a response elicited by certain G-protein-coupled receptors) or the use of small molecule drugs that mimic its effect may provide an additional way to suppress signaling downstream of Ras [Fig. 5(4)]. Obviously, specific Raf kinase inhibitors, able to inhibit the kinase activity of Raf directly, may

prove to be very potent inhibitors of downstream signaling by Ras [Fig. 5(5)]. Furthermore, although the biological function of RalGDS as a downstream effector of Ras-like GTPases remains to be established (Spaargaren and Bischoff, 1994), if involved in the transforming signal from Ras, the interaction between Ras and RalGDS may prove to be a potential target as well.

Less well understood, but equally intriguing, is the interaction between Bcl-2 and R-ras (Fernandez-Sarabia and Bischoff, 1993). This interaction is a potential target for anticancer drugs, as either prevention or stimulation of the interaction may result in the stimulation or restoration of the apoptotic response, which may have an antitumorigenic effect in certain tumors (Barr and Tomei, 1994) [Fig. 5(6)].

Finally, given the key role of Ras in the mitogenic signaling by receptor tyrosine kinases and cytoplasmic tyrosine kinases, several targets upstream of Ras may prove to be excellent targets for drugs in the treatment of cancer caused by oncogenic tyrosine kinases. These agents, however, will not be able to counteract tumorigenesis caused by oncogenic mutations of Ras. Examples of potential targets for these anticancer drugs are the interaction between Sos and Ras, between Grb2 and Sos, between autophosphorylation sites on receptor tyrosine kinase and Grb2 or other docking proteins such as IRS-1, Shc, and Syp, as well as the critical dimerization sites of receptor tyrosine kinases.

As may be clear from this short summary, our current knowledge of the signal transduction pathways involving these Ras-like GTPases provides us with a number of potential targets for small molecule drugs for the treatment of cancer or other diseases caused by hyperproliferation or suppressed apoptosis. Whether it will indeed be possible to discover and/or develop small molecule drugs that specifically modulate the activity of the most promising targets in these signal transduction pathways and whether this will result in a substantial decrease of the growth rate of cancerous cells while leaving normal cells unscathed, thereby proving its potential value in the battle against cancer, remains to be established. However, future research will inevitably gain new insights in oncogenesis and is likely to provide novel means of prevention, diagnosis, and treatment of cancer.

ACKNOWLEDGEMENTS

We would like to thank our colleagues at ONYX Pharmaceuticals for sharing their knowledge and insights with us. M.S. is supported by a fellowship from the Dutch Cancer Society.

REFERENCES

A. Abo, E. Pick, A. Hall, N. Totty, C. G. Teahan, and A. Segal (1991), Nature 353, 668-670.

C. F. Albright, B. W. Giddings, J. Liu, M. Vito, and R. A. Weinberg (1993), EMBO J 12, 339-347.

J. Avruch, X.-F. Zhang, and J. M. Kyriakis (1994), Trends Biochem Sci 19, 279-283.

P. J. Barr and L. D. Tomei (1994), Biotechnology 12, 487-493.

G. J. Beitel, S. G. Clark, and H. R. Horvitz (1990), Nature 348, 503-509.

F. Beranger, B. Goud, A. Tavitian, and J. Gunzburg (1991), Proc Natl Acad Sci USA 88, 1606-1610.

K. J. Blumer and G. L. Johnson (1994), Trends Biochem Sci 19, 236-240.

M. S. Boguski and F. McCormick (1993), Nature 366, 643-654.

J. L. Bos (1989), Cancer Res 49, 4682-4689.

H. R. Bourne, D. A. Sanders, and F. McCormick (1990), Nature 348, 125-132.

H. R. Bourne, D. A. Sanders, and F. McCormick (1991), Nature 349, 117-127.

L. Buday and J. Downward (1993), Cell 73, 611-620.

B. M. T. Burgering, E. Freed, L. van der Voorn, F. McCormick, and J. L. Bos (1994), Cell Growth Differ 5, 341-347.

B. M. T. Burgering, G. J. Pronk, P. C. van Weeren, P. Chardin, and J. L. Bos (1993), EMBO J 12, 4211-4220.

X. R. Bustelo, K-L. Suen, K. Leftheris, C. A. Meyers, and M. Barbacid (1994), Oncogene 9, 2405-2413.

H. Cai, P. Erhardt, J. Troppmair, M. T. Diaz-Meco, G. Sithanandam, U. Rapp, J. Moscat, and G. M. Cooper (1993), Mol Cell Biol 13, 7645-7651.

H. Cai, J. Szeberenyi, and G. M. Cooper (1990), Mol Cell Biol 10, 5314-5323.

P. Chardin (1993), in The ras Superfamily of GTPases (J. C. Lacal and F. McCormick, eds.), CRC Press, Boca Raton, pp. 203-229.

P. Chardin, J. H. Camonis, N. W. Gale, L. van Aelst, J. Schlessinger, M. H. Wigler, and D. Bar-Sagi (1993), Science 260, 1338-1343.

P. Chardin and A. Tavitian (1986), EMBO J 5, 2203-2208.

J. L. Cleveland, J. Troppmair, G. Packham, D. S. Askew, P. Lloyd, M. Gonzalez-Garcia, G. Nunez, J. N. Ihle, and U. R. Rapp (1994), Oncogene 9, 2217-2226.

S. J. Cook and F. McCormick (1993), Science 262, 1069-1072.

S. J. Cook, B. Rubinfield, I. Albert and F. McCormick (1993), EMBO J 12, 3475-3485.

C. M. Crews, A. Alessandrini and R. L. Erikson (1992), Science 258, 478–480.
A. M. M. de Vries-Smits, B. M. T. Burgering, S. J. Leevers, C. J. Marshall, and J. L. Bos (1992), Nature 357, 602–604.
P. Dent, W. Haser, T. A. J. Haystead, L. A. Vincent, T. M. Roberts, and T. W. Sturgill (1992), Science 257, 1404–1407.
B. Dickson and E. Hafen (1994), Curr Opin Genet Dev 4, 64–76.
D. Diekmann, A. Abo, C. Johnson, A. W. Segal, and A. Hall (1994), Science 265, 531–533.
J. Downward, J. D. Graves, P. H. Warne, S. Rayter, and D. A. Cantrell (1990), Nature 346, 719–723.
M. Duchesne, F. Schweighoffer, F. Parker, F. Clerc, T. Frobert, M. N. Thang, and B. Tocque (1993), Science 259, 525–528.
W. J. Fantl, J. A. Escobedo, G. A. Martin, W. Turck, M. Del Rosario, F. McCormick, and L. T. Williams (1992), Cell 69, 413–423.
L. Feig (1993), Nature 260, 767–768.
L. A. Feig (1994), Curr Opin Cell Biol 6, 204–211.
L. A. Feig and R. Emkey (1993), in The ras Superfamily of GTPases (J. C. Lacal and F. McCormick, eds.), CRC Press, Boca Raton, pp. 247–257.
J. R. Feramisco, M. Gross, T. Kamata, M. Rosenberg, and R. W. Sweet (1984), Cell 38, 109–117.
M. J. Fernandez-Sarabia and J. R. Bischoff (1993), Nature 366, 274–275.
R. E. Finney, S. M. Robbins, and J. M. Bishop (1993), Curr Biol 3, 805–812.
E. Freed, M. Symons, S. G. Macdonald, F. McCormick, and R. Ruggieri (1994), Science, 265, 1713–1716.
M. D. Garrett, A. J. Self, C. van Oers, and A. Hall (1989), J Biol Chem 264, 10–13.
U. Gaul, G. Mardon, and G. M. Rubin (1992), Cell 68, 1007–1019.
S. M. Graham, A. D. Cox, G. Drivas, M. G. Rush, P. D'Eustachio, and C. J. Der (1994), Mol Cell Biol 14, 4108–4115.
E. Gulbins, K. M. Coggeshall, G. Baier, S. Katzav, P. Burn, and A. Altman (1993), Science 260, 822–825.
E. Gulbins, K. M. Coggeshall, C. Langlet, G. Baier, N. Bonnefoy-Berard, P. Burn, A. Wittinghofer, S. Katzav, and A. Altman (1994), Mol Cell Biol 14, 906–913.
J. Hancock (1993), Curr Biol 3, 770–772.
J. Hancock and C. J. Marshall (1993), in The ras Superfamily of GTPases (J. C. Lacal and F. McCormick (eds.), CRC Press, Boca Raton, pp. 65–84.
E. A. Harrington, A. Fanidi, and G. I. Evan (1994), Curr Opin Gen Dev 4, 120–129.
Y. Hata, K. Kaibuchi, S. Kawamura, M. Hiroyoshi, H. Shirataki, and Y. Takai (1991), J Biol Chem 266, 6571–6577.
L. R. Howe, S. J. Leevers, N. Gomez, S. Nakielny, P. Cohen, and C. J. Marshall (1992), Cell 71, 335–342.
K. Irie, Y. Gotoh, B. Yashar, B. Errede, E. Nishida, and K. Matsumoto (1994), Science 265, 1716–1719.
G. L. James, J. L. Goldstein, M. S. Brown, T. E. Rawson, T. C. Somers, R. S. McDowell, C. W. Crowley, B. K. Lucas, A. D. Levinson, and J. C. Marsters, Jr. (1993), Science 260, 1937–1942.
G. L. Johnson and R. R. Vaillancourt (1994), Curr Opin Cell Biol 6, 230–238.
D. R. Kaplan, D. K. Morrison, G. Wong, F. McCormick, and L. T. Williams (1990), Cell 61, 125–133.
A. C. Kazlaukas, C. Ellis, T. Pawson, and J. A. Cooper (1990), Science 247, 1578–1581.
H. Kitayama and M. Noda (1993), in The ras Superfamily of GTPases (J. C. Lacal and F. McCormick, eds.), CRC Press, Boca Raton, pp. 231–245.
H. Kitayama, Y. Sugimoto, T. Matsuzaki, Y. Ikawa, and M. Noda (1989), Cell 56, 77–84.
N. Kohl, S. D. Mosser, S. J. deSolms, E. A. Giuliani, D. L. Pompliano, S. L. Graham, R. L. Smith, E. M. Scolnick, A. Oliff, and J. B. Gibbs (1993), Science 260, 1934–1937.
H. Koide, T. Satoh, M. Nakafuku, and Y. Kaziro (1993), Proc Natl Acad Sci USA 90, 8683–8686.
W. Kolch, G. Heidecker, P. Lloyd, and U. R. Rapp (1991), Nature 349, 426–428.
J. M. Kyriakis, H. App, X. F. Zhang, P. Banerjee, D. L. Brautigan, U. R. Rapp, and J. Avruch (1992), Nature 358, 417–421.
S. J. Leevers, H. F. Paterson, and C. J. Marshall (1994), Nature 369, 411–414.
S. L. Leevers and C. J. Marshall (1992), EMBO J 11, 569–574.
W. Li, R. Nishimura, A. Kashishian, A. G. Batzewr, W. J. H. Kim, J. A. Cooper, and J. Schlessinger (1994), Mol Cell Biol 14, 509–517.
D. G. Lowe and D. V. Goeddel (1987), Mol Cell Biol 7, 2845–2856.
D. G. Lowe, D. J. Capon, E. Delwart, A. Y. Sakaguchi, S. L. Naylor, and D. V. Goeddel (1987), Cell 48, 137–146.
D. G. Lowe, M. Ricketts, A. D. Levinson, and D. V. Goeddel (1988), Proc Natl Acad Sci USA 85, 1015–1019.
D. R. Lowy and B. M. Willumsen (1993), Annu Rev Biochem 62, 851–891,
S. G. Macdonald, C. M. Crews, L. Wu, J. Driller, R. Clark, R. L. Erikson, and F. McCormick (1993), Mol Cell Biol 13, 6615–6620.
F.-E. Maly, L. A. Quilliam, O. Dorseuil, C. J. Der, and G. M. Bokoch (1994), J Biol Chem 269, 18743–18746.
M. S. Marshall (1993), Trends Biochem Sci 18, 250–254.
G. A. Martin, A. Yatani, R. Clark, L. Conroy, P. Polakis, A. M. Brown, and F. McCormick (1992), Science 255, 192–194.
F. McCormick (1989), Cell 56, 5–8.
F. McCormick (1993), Nature 363, 15–16.
J. McGlade, B. Brunkhorst, D. Anderson, G. Mbmalu, J. Settleman, S. Dedhar, M. Rozakis-Adcock, L. B. Chen, and T. Pawson (1993), EMBO J 12, 3073–3081.
R. H. Medema, W. L. De Laat, G. A. Martin, F. McCormick, and J. L. Bos (1992), Mol Cell Biol 12, 3425–3430.

S. A. Moodie, B. M. Willumsen, M. J. Weber, and A. Wolfman (1993), Science 260, 1658–1661.
S. A. Moodie and A. Wolfman (1994), Trends Genet 10, 44–48.
D. K. Morrison, D. R. Kaplan, U. Rapp, and T. M. Roberts (1988), Proc Natl Acad Sci USA 85, 8855–8859.
L. S. Mulcahy, M. R. Smith, and D. W. Stacey (1985), Nature 3131, 241–243.
M. G. Myers, X. J. Sun, and M. F. White (1994), Trends Biochem Sci 19, 289–293.
B. Olofsson, P. Chardin, N. Touchot, N. Zahraoui, and A. Tavitian (1988), Oncogene 3, 231–236.
S. Orita, K. Kaibuchi, S. Kuroda, K. Shimizu, H. Nakanishi, and Y. Takai (1993), J Biol Chem 268, 25542–25546.
G. Pelicci, I. Lanfrancone, F. Grignani, J. McGlade, F. Cavallo, G. Forni, I. Nicoletti, F. Grignani, T. Pawson, and P. G. Pelicci (1992), Cell 70, 93–104.
V. Pizon, P. Chardin, I. Lerosey, B. Olofsson, and A. Tavitian (1988), Oncogene 3, 201–204.
P. Polakis and F. McCormick (1992), J Biol Chem 268, 9157–9160.
G. J. Pronk, A. M. M. de Vries-Smits, L. Buday, J. Downward, J. A. Maassen, R. Medema, and J. L. Bos (1994), Mol Cell Biol 14, 1575–1581.
L. A. Quilliam, S. Y. Huff, K. M. Rabun, W. Wei, W. Park, D. Broek, and C. J. Der (1994), Proc Natl Acad Sci USA 91, 8512–8516.
M. T. Quinn, C. A. Parkos, L. Walker, S. H. Orkin, M. C. Dinauer, and A. J. Jesaitis (1989), Nature 342, 198–200.
M. C. Raff, B. Barres, J. F. Burne, H. S. Coles, Y. Ishizaki, and M. D. Jacobson (1993), Science 262, 695–700.
K. S. Ravichandran, K. K. Lee, Z. Songyang, L. C. Cantley, P. Burn, and S. J. Burakoff (1993), Science 262, 902–904.
J. C. Reed (1994), J Cell Biol 124, 1–6.
I. Rey, P. Taylor-Harris, H. van Erp, and A. Hall (1994), Oncogene 9, 685–692.
T. M. Roberts (1992), Nature 360, 534–535.
M. Rozakis-Adcock, R. Fernley, J. Wade, T. Pawson, and D. Bowtell (1993), Nature 363, 83–85.
M. Rozakis-Adcock, J. McGlade, G. Mbamalu, G. Pelicci, R. Daly, W. Li, A. Batzer, S. Thomas, J. Pelicci, J. Schlessinger, and T. Pawson (1992), Nature 360, 689–691.
B. Rubinfield, S. Minumitsu, R. Clark, L. Conroy, K. Watt, W. J. Crosier, F. McCormick, and P. Polakis (1991), Cell 65, 1033–1042.
T. Sakoda, K. Kaibuchi, K. Kishi, S. Kishida, K. Doi, M. Hoshino, S. Hattori, and Y. Takai (1992), Oncogene 7, 1705–1711.
T. Satoh, M. Nakafuku, and Y. Kaziro (1992), J Biol Chem 267, 24149–24152.
J. Schlessinger (1993), Trends Biochem Sci 18, 273–275.
J. Settleman, C. F. Albright, L. C. Foster, and R. A. Weinberg (1992), Nature 359, 153–154.
C. Shou, C. L. Farnsworth, B. G. Neel, and L. A. Feig (1992), Nature 358, 351–354.
M. A. Simon, D. D. Bowtell, G. S. Dodson, T. R. Laverty, and G. M. Rubin (1991), Cell 67, 701–716.
E. Y. Skolnick, A. Batzer, N. Li, C. H. Lee, E. Lowenstein, M. Mohammadi, B. Margolis, and J. Schlessinger (1993), Science 260, 1953–1955.
M. R. Smith, S. J. DeGudicubus, and D. W. Stacey (1986), Nature 320, 540–543.
M. Spaargaren and J. R. Bischoff (1994), Proc Natl Acad Sci USA 91, 11089–11093.
M. Spaargaren, L. H. K. Defize, J. Boonstra, and S. W. De Laat (1990), J Biol Chem 266, 1733–1739.
M. Spaargaren, G. A. Martin, F. McCormick, M-J. Fernandez-Sarabia, and J. R. Bischoff (1994), Biochem J 300, 303–307.
D. W. Stacey and H.-F. Kung (1984), Nature 310–508–511.
D. Stokoe, S. G. MacDonald, K. Cadwallader, M. Symons, and J. F. Hancock (1994), Science 264, 1463–1467.
S. M. Thomas, M. DeMarco, G. D'Arcangelo, S. Halegoua, and J. Brugge (1992), Cell 68, 1031–1040.
M-H. Tsai, A. Hall, and D. W. Stacey (1989), Mol Cell Biol 9, 5260–5264.
A. Ullrich and J. Schlessinger (1990), Cell 61, 203–212.
L. van Aelst, M. Barr, S. Marcus, A. Polverino, and M. Wigler (1993), Proc Natl Acad Sci USA, 90, 6213–6217.
D. L. Vaux (1993), Proc Natl Acad Sci USA 90, 786–789.
D. L. Vaux, S. Cory, and J. Adams (1988), Nature 335, 440–442.
A. B. Vojtek, S. M. Hollenberg, and J. A. Cooper (1993), Cell 74, 205–214.
H. G. Wang, T. Miyashita, S. Takayama, T. Sato, T. Torigoe, S. Krajewski, S. Tanaka, L. Hovey, III, J. Troppmair, U. R. Rapp, and J. C. Reed (1994), Oncogene 9, 2751–2756.
P. H. Warne, P. Rodriguez Viciana, and J. Downward (1993), Nature 364, 352–355.
G. M. Wildey, M. Viggewarapu, S. Rim, and J. K. Denjer (1993), Biochem Biophys Res Commun 194, 552–559.
B. M. Willumsen, K. Norris, A. G. Papageorge, N. L. Hubber, and D. R. Lowy (1984), EMBO J 3, 2581–2586.
K. W. Wood, C. Sarnecki, T. M. Roberts, and J. Blenis (1992), Cell 68, 1041–1050.
J. Wu, P. Dent, T. Jelinek, A. Wolfman, M. J. Weber, and T. W. Sturgill (1993), Science 262, 1065–1069.
X.-F. Zhang, J. Settleman, J. M. Kyriakis, E. Takeuchi-Suzuki, C. J. Elledge, M. S. Marshall, J. T. Bruder, U. R. Rapp, and J. Avruch (1993), Nature 364, 308–313.