

RAS family

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DNA/RNA

The establishment of an in vitro assay to screen for active transformation of mouse NIH 3T3 fibroblasts has revealed transforming genes identified as human homologs of Harvey or Kirsten murine sarcoma virus oncogenes (v-Ha-ras, or v-Ki-ras, respectively). Thereupon, an additional transforming gene was found in human neuroblastoma and fibrosarcoma cell lines that has been identified as the third functional member of the ras gene family. This gene have been termed N-ras. Thus, the human ras family consists of three proto-oncogenes, [c-Harvey \(H\)-ras](#), [c-Kirsten \(K\)-ras](#), and [N-ras](#), no c-prefix was added because no viral counterpart was found. Additionally, genes were found in the genome of human and other mammalian species that display high homology to the functional ras genes but lack intervening sequences (introns). These genes were identified as processed and inactivated pseudogenes. Functional ras genes differ greatly in length due to large differences in the size of their introns ranging from about 6kb to 50kb, but they each have 4 coding exons. The human K-ras gene contain an alternative fourth coding exon. Alternative RNA splicing specifies either of two isomorphous proteins differing by 25 amino acid residues at their carboxy-terminus. Ras genes are expressed in all tissues, have a promotor region with multiple GC boxes, but lack a TATA- or CCAT-box; features resembling the promoters of housekeeping genes (11).

A comparison of human H-, K-, and N-ras nucleotide sequences with the corresponding regions in other mammalian species reveals a remarkable sequence similarity (29). All differences are synonymous changes with no effect on the amino acid sequence of RAS proteins, indicating a strong evolutionary pressure on the amino acid sequence of these genes.

The ras oncogenes in various human tumors harbor point mutations that confer transforming activity. Mutations leading to an amino acid substitution at the positions 12, 13, and 61 are the most common in naturally occurring (i.e. non-experimental) (9) neoplasms and experimentally induced animal tumors (12, 27) (see following sections).

In addition to the most frequent mechanism of point-mutational activation, overexpression of non-mutated ras genes can also convert normal ras genes into oncogenes. The increased amount of the corresponding mRNA derives either from high transcriptional activity of heterologous promoters and enhancers (7) or results from amplified ras genes located either intrachromosomally as homogeneously staining regions or extrachromosomally as double minute chromosomes (18).

Functional aspects of RAS proteins

Mammalian ras genes code for closely related, small proteins of 189 amino acids with a molecular weight of 21,000 Daltons (p21). When the alternative exon of H- or K-ras is used, proteins of 170 or 188 amino acids are synthesized. The molecular weight of the H-ras protein variant is 19,000 Daltons and that of the K-ras variant is not distinguishable from the normal RAS proteins.

RAS proteins are localized in the inner plasma membrane, bind GDP and GTP and possess an intrinsic GTPase activity, implicated in the regulation of their activity. Because of the functional resemblance to G-Proteins, p21RAS have been hypothesized to be also involved in different types of ligand-mediated signal transduction pathways. Later, RAS proteins were shown to influence proliferation, differentiation, transformation, and apoptosis by relaying mitogenic and growth signals into the cytoplasm and the nucleolus (10). In a normal cell most of the RAS molecules are present in an inactive GDP-bound conformation. An extracellular stimulus initiates the release of GDP and the subsequent binding of GTP. This conformational change enables the interaction with the putative effector molecules and permits the transmission of signals. Finally, the active GTP-bound state is turned off by hydrolysis of GTP to GDP and inorganic phosphate.

The intrinsic GTPase activity is rather weak, not sufficiently effective for signal transduction pathways where rapid inactivation is required. In order to accelerate this low rate of hydrolysis (which is about 10^{-2} min⁻¹) and to enable a transient burst of signalling activity, regulatory proteins like GAPs (GTPase activating proteins) (1) or [NF-1](#) (Neurofibromatosis Type 1) protein (13), bind to the GTP-containing conformation and stimulate the GTPase activity more than 100-fold. The decreasing level of RAS-GTP, and hence, increasing level of RAS-GDP complexes result in a loss of the biological activity of RAS (see Fig. 1). In a normal cell, GAPs help to keep most of p21RAS in an inactive GDP-bound state. The finding that overexpression of non-mutated ras genes can also transform cells supports the idea that the abundance of GAPs is limited. Overexpression of p21RAS could lead to saturation of the regulatory proteins, resulting in a constitutive, deregulated activation of RAS proteins and oncogenic transformation.

Another group of regulatory proteins involved in stimulating the transition of RAS proteins from the inactive- to the active GTP-bound state are designated Guanine Nucleotide Exchange Factors (GEFs) or RAS-GRFs (guanine nucleotide releasing factors) (22). Normally, the release of GDP is regulated by the intracellular concentration of GTP. An increase in the GTP concentration leads to an enhanced dissociation of GDP. GEFs catalyze the dissociation of GDP (see Fig.1). A ligand-free RAS protein immediately binds GTP, because it is 10-fold more abundant in the cytosol than GDP.

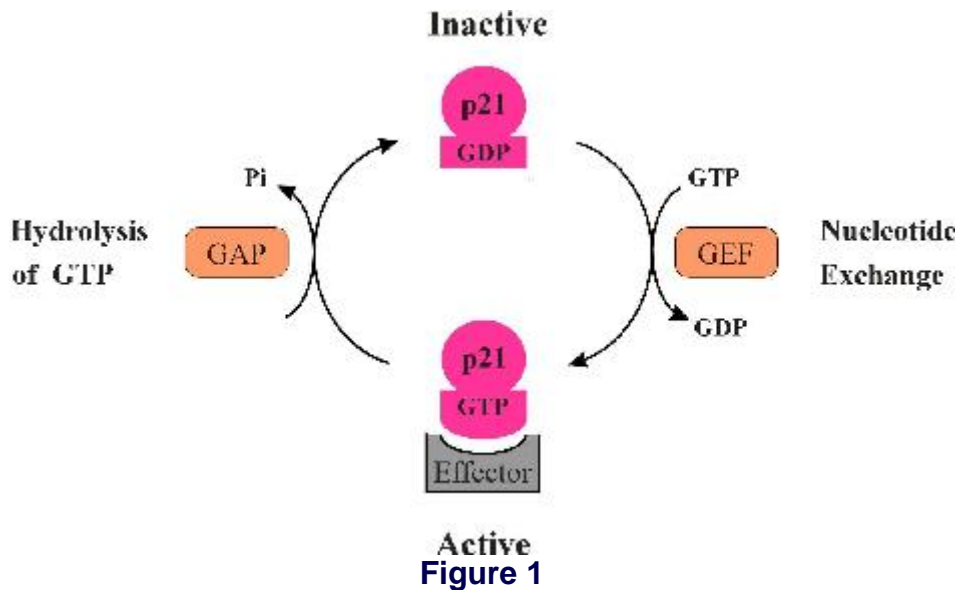


Figure 1 Mechanism of RAS regulation

The activity of RAS proteins is regulated by a cycle of guanine nucleotide binding and hydrolysis. In the active state p21 is bound to GTP, in the inactive to GDP. GEF (Guanine Nucleotide Exchange Factor) promotes dissociation of GDP and acts as a positive regulator; GAP (GTPase activating protein) promotes hydrolysis of GTP and acts as a negative regulator. Pi, inorganic phosphate.

Structure of RAS proteins

The alignment of their primary amino acid sequence clearly indicates the presence of four domains within the RAS molecules. The first domain includes 85 amino acids at the N-terminus which are found to be identical in H-, K-, and N-ras, demonstrating a high degree of conservation. The following 80 amino acids form a second domain, showing less conservation (70-80%) within the RAS proteins. The third domain spans the rest of the molecule, except for the last four amino acids, and represents a hypervariable region. The highly conserved carboxy-terminal motif CAAX (where C stands for cysteine, A for any aliphatic residue, and X for any uncharged amino acid) is the result of posttranslational modifications and forms the last domain.

For more accurate identification of biologically relevant regions of p21RAS and for the interpretation of activating mutations, X-crystallographic analysis of GDP- and GTP-bound RAS molecules and in vitro mutagenesis studies with mutated or truncated RAS proteins were performed. As a result of these studies, the catalytic domain was identified between residues 1 to 171, including the region involved in guanine nucleotide binding where residues 10-16 and 56-59 interact with b- and c-phosphate, and residues 116-119 and 152-165 interact with the guanine base., The so called core effector region (located between residues 32-40), represents an essential element for all interactions with putative downstream effectors and the GAPs. A region encompassing the last four amino acids at the C-terminus (residues 186-189), was shown to be essential for the attachment of p21RAS to the plasma membrane.

Comparison of the crystal structure of RAS-GTP (as indicated in Fig.2) and RAS-GDP complexes revealed that switching between the active and the inactive state is associated with a conformational change of two regions, designated as switch I (residues 30-38), overlapping with the core effector region, and switch II (60-76). Binding of the neutralizing antibody Y13-259 to residues 63-73, inhibits the GTP-GDP change, indicating that this conformational change is necessary for the transition of RAS from the GDP- to the GTP-bound state and vice versa (14, 23).

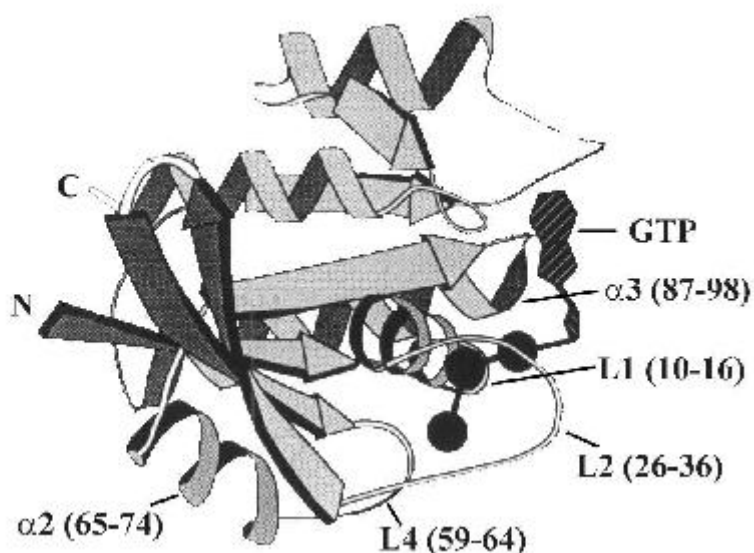


Figure 2

Figure 2 Topological structure of p21

The polypeptide chain of RAS p21 consists of six b-strands and five a-helices. Loop 1, alias phosphate-binding (P-) loop (residues 10 to 16), switch regions I (30 to 37), including loop 2 with adjacent residues, and II (60 to 67), including loop 4 and a-helix 2, represent the active center of the molecule and are involved in the binding interaction between p21RAS and GTP. N stands for the amino terminal, C for the carboxy terminal end.

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Activating point mutations have been localized in codons 12, 13, 59, 61, 63, 116, 117, 119, and 146 (4). All of these alterations occur at or near the guanine nucleotide binding sites. The effects of point mutations are either reduced GTPase activity (if amino acids 12, 13, 59, 61, 63 are involved), so that oncogenic RAS mutants are locked in the active GTP-bound state, or decreased nucleotide affinity, and hence, increased exchange of bound GDP for cytosolic GTP (if amino acids 116, 117, 119 or 146 are affected). The inefficient deactivation of the active GTP-bound RAS proteins is intensified by the inability of GAPs to stimulate the conversion to the inactive, GDP-bound state. All point mutations cause an accumulation of activated RAS-GTP complexes, leading to continuous signal transduction by facilitating accumulation of constitutively active, GTP-bound RAS protein, and thus contributing to a malignant cell phenotype.

Incidence of ras mutations

(see also the appendix)

Activating ras mutations can be found in human malignancies with an overall frequency of 15-20%. A high incidence of ras gene mutations has been reported in malignant tumors of the pancreas (80-90%, K-ras) (2, 24), in [colorectal carcinomas](#) (30-60%, K-ras) (5, 26), in non-melanoma skin cancer (30-50%, H-ras) (4, 21), in hematopoietic neoplasia of myeloid origin (18-30%, K-and N-ras) (5, 16, 17, 22), and in [seminoma](#) (25-40%, K-ras) (15, 19). In other tumors, a mutant ras gene is found at a lower frequency: for example, in [breast carcinoma](#) (0-12%, K-ras) (20, 25), glioblastoma and [neuroblastoma](#) (0-10%, K- and N-ras) (3, 6, 8).

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Appendix : RAS mutations in various cancers and bibliography

H-RAS mutations

Tumor	Frequency (%)	Reference
Stomach	0-40	(6, 11, 17, 18, 28)
Urinary Bladder	0-65	(9, 16, 19, 21, 29)
Prostate	0-10	(4, 8, 12, 23)
Skin	0-45	(2, 26)
Thyroid	0-60	(1, 3, 7, 14, 15, 25)
Breast	0-10	(13, 24)
Head and neck	0-30	(5, 10, 20, 22)
Endometrium	5	(27)

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K-RAS mutations

Tumor	Frequency (%)	Reference
Pancreas	80-90	(2, 52, 61)
Colon and rectum	25-60	(5, 6, 8, 19, 24, 38, 53, 60, 62)
Lung	25-60	(20, 42, 43, 45, 55)
Prostate	0-25	(9, 11, 18, 25, 26, 49)
Skin	0-25	(1, 50)
Thyroid	0-60	(10, 17, 27, 28, 54)
Liver	10-25	(12, 30, 56, 57)
Ovary	0-50	(15, 21, 58)
Endometrium	10-40	(14, 16, 22, 31, 46, 47, 59)
Kidney	0-50	(34, 51)
Brain	0-15	(3, 4, 7, 23, 29)
Testis (Seminoma)	10-45	(32, 33, 41)
Leukemia (ANLL, MDS)	5-15	(35, 36, 39, 48)
Urinary Bladder	5	(37)
Head and neck	10	(40, 44)
Breast	10	(13)

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N-RAS mutations

Tumor	Frequency (%)	Reference
Leukemia ANLL, MDS	20-40	(7, 17, 18, 19, 22, 24)
Leukemia CML, ALL	0-10	(28, 29)
Brain	0-15	(2, 3, 4, 9, 13)
Skin	0-20	(1, 14, 21, 27)
Thyroid	0-60	(5, 8, 11, 12, 23)
Testis	0-40	(15, 16, 20)
Stomach	(gastric tumors) 5	(10)
Liver	0-15	(6, 25, 26)

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