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RAS Genes

The ras superfamily of genes encodes small GTP-binding proteins that are responsible for the regulation of many cellular processes.

Oncogenic ras genes in human cells include H-ras, N-ras, K-ras, R-ras and M-ras genes.

The N- and H-ras genes are located at the short arm of human chromosomes 1 and 11, respectively, whereas the K-ras gene is located on the short arm of chromosome 12.

RAS Proteins

Ras proteins are normal intracellular proteins involved in **signal transduction** through RTK (receptors with intrinsic tyrosine kinase activity) signaling pathway.

Ras proteins are small membrane-bound **GTP-binding proteins** (G proteins). This family consists of at least **20** proteins, the most important of which are H-, N-, K-RAS, R-RAS, R-RAS2, R-RAS3, RAP1(A and B), RAP 2(A and B), and Ral (A and B).

RAS Proteins

They are synthesized in the cytosol and become associated with the **inner side of the plasma membrane** via posttranslational modifications. Inactive **hydrophilic** precursor proteins must undergo modification to be **lipophilic** molecules that anchor into the cell membrane.

Ras Proteins Functions:

RAS appears to have different cellular functions:

1) Cell **cycle entry** and **DNA synthesis** in some cell types, such as fibroblasts.

2) Terminal **differentiation** in others.

3) In myoblasts, activated ras **downregulates** expression of muscle-specific mRNA transcription.

4) Promotes **survival** in some cell types, such as those of hematopoietic lineages, upon cytokine withdrawal, and sympathetic neurons upon removal of NGF.

5) **Cytoskeletal organization and cell motility.**

Ras and Cancer:

The initial evidence for ras involvement in cancer came from the discovery of transforming retroviruses, Harvey and Kirsten sarcoma viruses, which contained H- and K-ras cellular-derived oncogenes.

It was one of the first human oncogenes that were identified by transfecting genomic DNA from human tumor cell lines into mouse fibroblasts and isolating the DNA fragments from the transformed foci. These were shown to be the human homologs of the viral ras genes.

Ras is activated in about **30%** of human cancers.

Ras mutations in human cancers

Pancreas	**K**	**90 %**
Colorectal	**K**	**44**
Thyroid		
Follicular	H, K, N	53
Undifferentiated	**H, K, N**	**60**
Papillary	**H, K, N**	**00**
Seminoma	K,N	43
Liver	N	30
Kidney	H	10
Acute myelogenous leukemia	N	30
Myelodysplastic syndrome	N, K	30

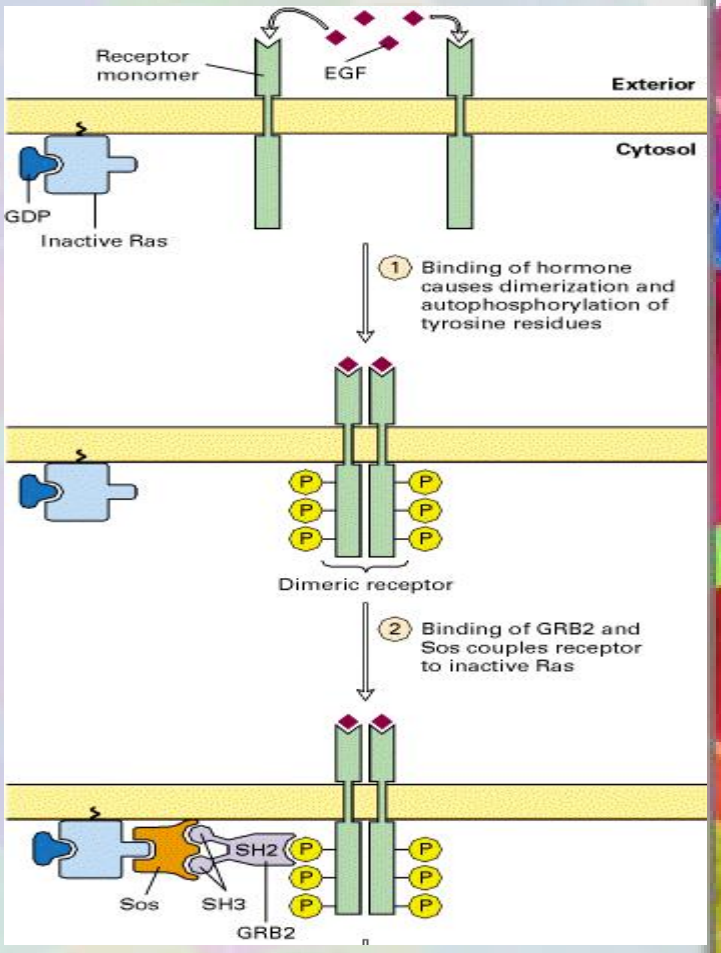
Ras protein activation:

Ras proteins alternates from an **inactive GDP-bound** state to an **active GTP-bound** state.

Ras activation involves the following steps:

1) Binding of **growth factor** to **RTK**. Growth factor binds two receptor molecules leading to their dimerization and autophosphorylation of tyrosine residues of the receptors.

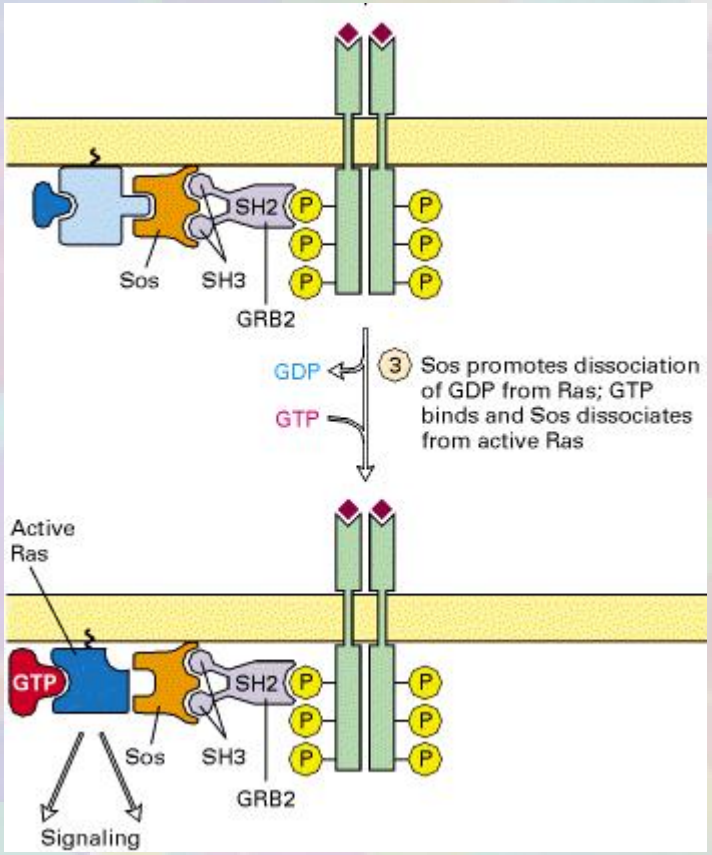
2) An **adapter** protein called **Grb2** binds to activated RTK. Grb2 contains one SH2 domain that binds to RTKs, including the PDGFR and the EGFR. Grb2 also has two SH3 domains.



Ras activation:

3) The third step involves the recruitment of a guanine nucleotide exchange factor (**GNEF**) to the cell membrane. GNEFs bind to Grb2 via the SH3 domains. The best examples of a ras GNEF are **SOS** (son of sevenless), GRF1 and 2 and ras GRP.

4) GNEFs promote the release of GDP from the catalytic pocket of ras, and the relative abundance of intracellular GTP ,as compared to GDP, allows binding of GTP to ras leading to **ras activation.**



Signaling Downstream of Ras :
(Ras > Raf > MAP Kinase Cascade)

1) The most well-studied **effector** of ras is the **serine/threonine kinase raf**. There are three known mammalian raf isoforms, designated A-, B-, and C-raf.

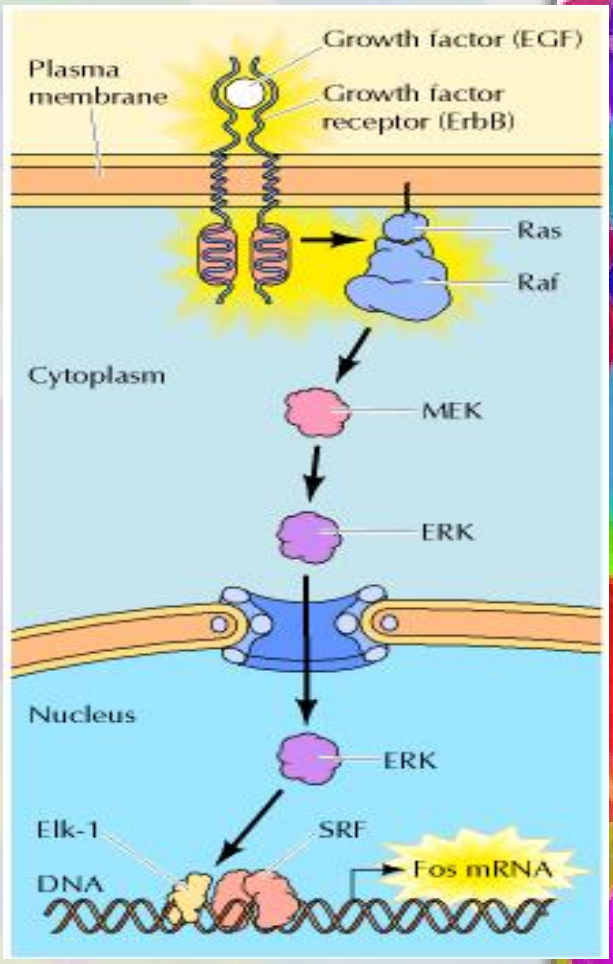
Interaction of ras with the ras-binding domain of raf allows **translocation of raf to the membrane**, where additional steps leading to its full activation can occur. These steps include **several phosphorylation** events on both serine-threonine and tyrosine residues.

Signaling Downstream of Ras :
(Ras > Raf > MAP Kinase Cascade)

2) Once activated, raf can phosphorylate **MEK** (mitogen /extracellular-signal-regulated kinase kinase), also known as **MAP (mitogen activated protein) Kinase Kinase (MKK),** present in the cytosol leading to its activation. There are two isoforms of MEK, designated **MEK1 and MEK2**.

Signaling Downstream of Ras : (Ras > Raf > MAP Kinase Cascade)

3) MEK, once activated, can, in turn, activate **MAP Kinase** or extracellular signal-regulated kinase (ERK). Activation occurs via **phosphorylations** on both threonine and tyrosine. There are two ERK isoforms (1 and 2). These proteins, 44 and 42 kDa, respectively, **translocate to the nucleus**, where they can activate a variety of proteins through phosphorylation on serine or threonine.

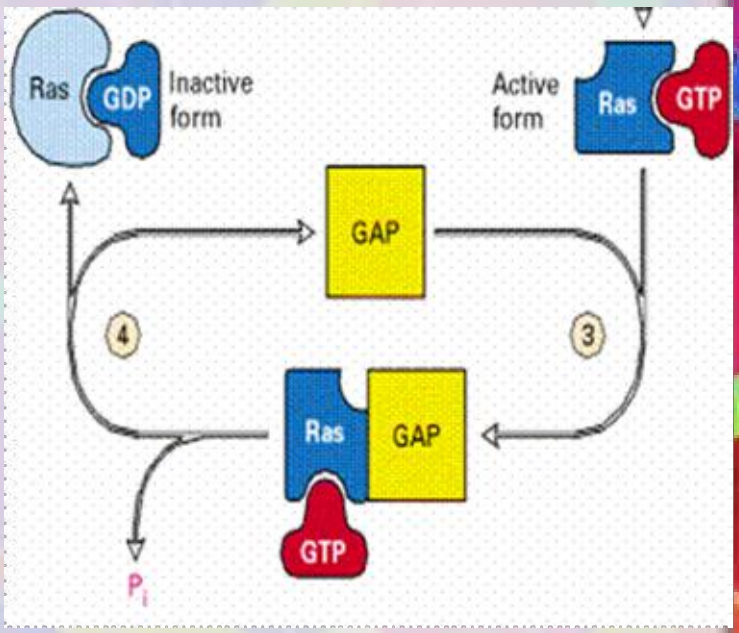


Nuclear targets of ras:

ERK (MAP) can phosphorylate several **transcription factors including c-fos, c-myc and c-jun**. Fos interacts with Jun to produce the **AP1 complex**, a nuclear transcription factor that binds to specific sequences near the myc gene. Signals converge to induce **D-type cyclins**, which are proteins that form complexes with cyclin-dependent kinases (cdk) and induce **G1 progression.**

Ras protein inactivation:

Normally after signal transduction, ras is inactivated. Although ras has an **intrinsic GTPase activity**, this activity is low and requires additional proteins, known as **GTPase-activating proteins** (GAPs), to promote GTP hydrolysis.



GAPs for ras include **p120 GAP** (act as scaffolding protein), **NF1-GAP/neurofibromin** (product of tumor suppressor gene neurofibromatosis I).

Target of ras mutation:

Single **point mutations** in the **ras** oncogene have been localized in codons 12, 13, 59, and 61. **Overexpression** of normal ras is less common in malignant tumors.

The major hot spot for ras protein mutations is located **near the bound guanine nucleotide**.

Target of ras mutation:

Ras mutations may remain silent for substantial periods until **proliferative** stimuli or additional **genetic alterations** trigger neoplastic proliferation (eg, exposure to viruses, smoking, etc.). **Premalignant** cells, such as those in human colon polyps, that show no signs of malignancy can harbor ras mutations.

Effect of ras mutation:

The majority of these mutations **decrease the intrinsic rate of GTP hydrolysis** by ras and make the molecule significantly **less sensitive to GAP-stimulated GTP hydrolysis.** The outcome of ras mutation is a molecule that is predominantly **GTP bound** and therefore **permenantly active** without growth factor stimulation. Mutated ras continues to activate downstream pathways **in the absence of any stimulation.**

Other ras related mutations:

Ras alone is unable to transform primary mouse or human fibroblasts. When oncogenic ras is introduced into such cells by retroviral mediated gene transfer, the cells undergo permanent growth arrest termed **replicative senescence**. This senescence response appears to be dependent on the function of certain genes such as p53, which act as tumor-suppressor genes. Inactivation of p53 genes is always present in tumors containing ras oncogenic mutations. Tumors that do not carry mutated ras genes may be dependent on Ras signaling for proliferation due to **overexertion of RTKs or enhanced secretion of growth factors**. Also activated forms of **Raf** cause tumorigenic transformation simulating oncogenic mutants of Ras.

Inhibitors of ras signaling:

The improved understanding of the molecular mechanisms of ras has provided an important background for the development of **Ras-targeted therapies** in attempts to control human tumor proliferation and metastasis. **Ras inhibitors** may also be used for the treatment of other diseases, including skin diseases (Psoriasis), vascular disorders (neovascularization), and autoimmune diseases (diabetes mellitus, multiple sclerosis, systemic lupus erythematosus).

Inhibition of **Ras** protein expression can be through:

Gene therapy

Antisense oligonucleotides

Intracellular antibodies

Thank You