Genes and mechanisms involved in the development of solid tissue cancers

MED 213

The Genetic Bases of Cancer

Oncogenes
Tumor suppressor genes
Repair genes

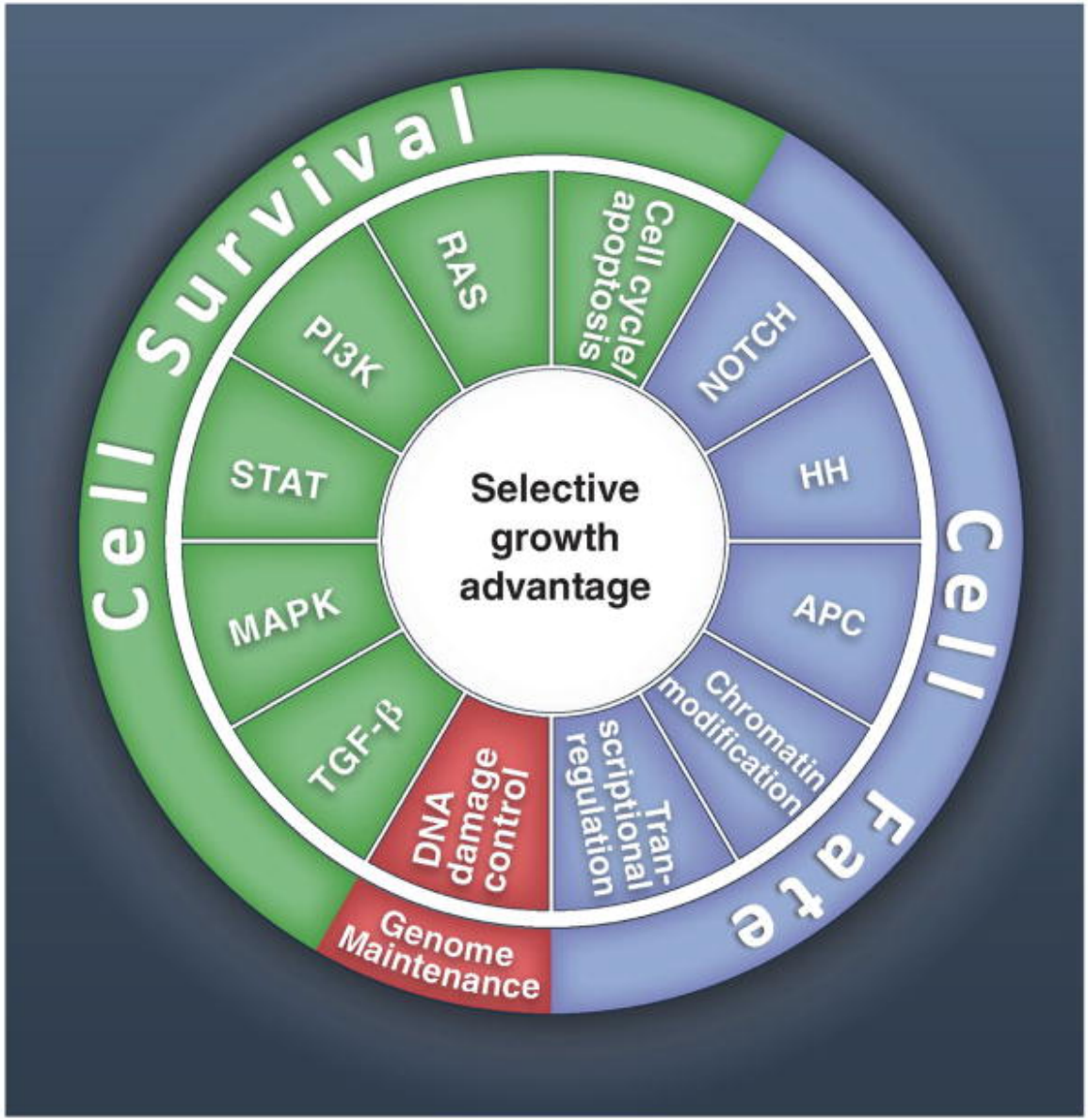
Environmental mutagens
(biological, chemical, physical agents)

Genetic mechanisms in Familial vs Sporadic Cancers

Genes and mechanisms involved in the development of solid tissue cancers

Epigenetics and Cancer

Molecular targets for Cancer Therapy


Cell Survival
Cell Fate
Genome Maintenance
RAS
PI3K
STAT
MAPK
TGF-β
Cell cycle/ apoptosis
NOTCH
HH
APC
Chromatin modification
Tran-scriptional regulation
DNA damage control
Selective growth advantage

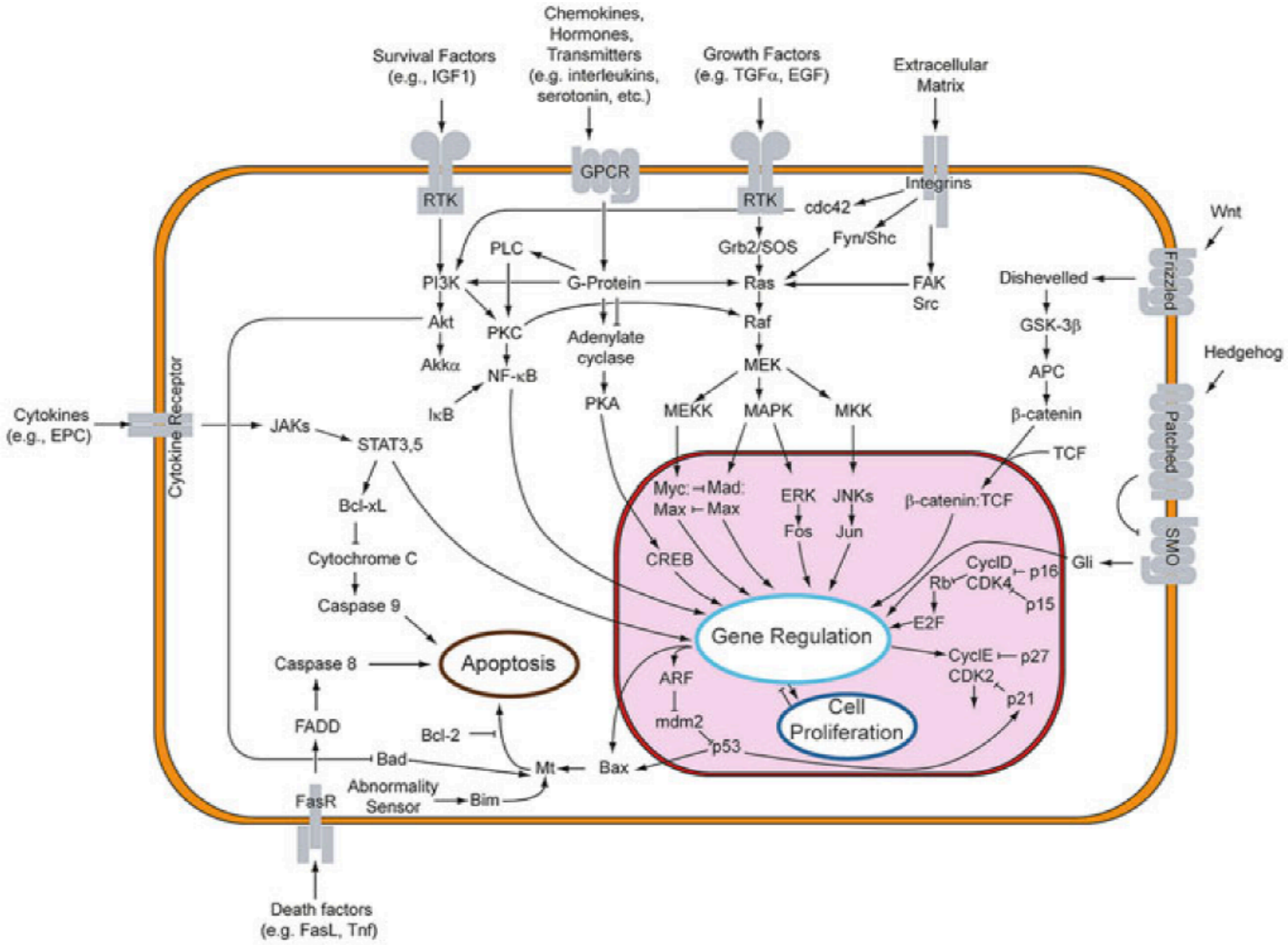
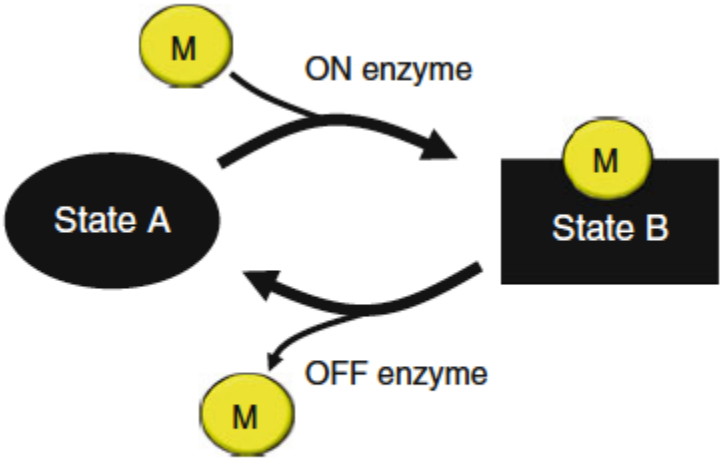

Survival Factors
(e.g., IGF1)
Chemokines,
Hormones,
Transmitters
(e.g. interleukins,
serotonin, etc.)
Growth Factors
(e.g. TGFα, EGF)
Extracellular
Matrix
RTK
GPCR
RTK
Integrins
cdc42
Fyn/Shc
Grb2/SOS
PLC
PI3K
G-Protein
Ras
FAK
Src
Wnt
Frizzled
Dishevelled
GSK-3β
APC
β-catenin
Hedgehog
Patched
SMO
Akt
PKC
Adenylate
cyclase
Raf
Akkα
NF-κB
IκB
MEK
PKA
MEKK
MAPK
MKK
Cytokines
(e.g., EPC)
Cytokine Receptor
JAKs
STAT3,5
TCF
Myc: ⊣ Mad:
Max ⊢ Max
ERK
JNKs
β-catenin:TCF
Fos
Jun
Bcl-xL
CREB
Gli
CyclD
CDK4
p16
p15
Rb
E2F
Cytochrome C
Caspase 9
Gene Regulation
CyclE
CDK2
p27
p21
Caspase 8
Apoptosis
ARF
mdm2
p53
Cell
Proliferation
FADD
Bcl-2
Bad
Mt
Bax
FasR
Abnormality
Sensor
Bim
Death factors
(e.g. FasL, Tnf)

Table 5.1 Reversible protein modifications

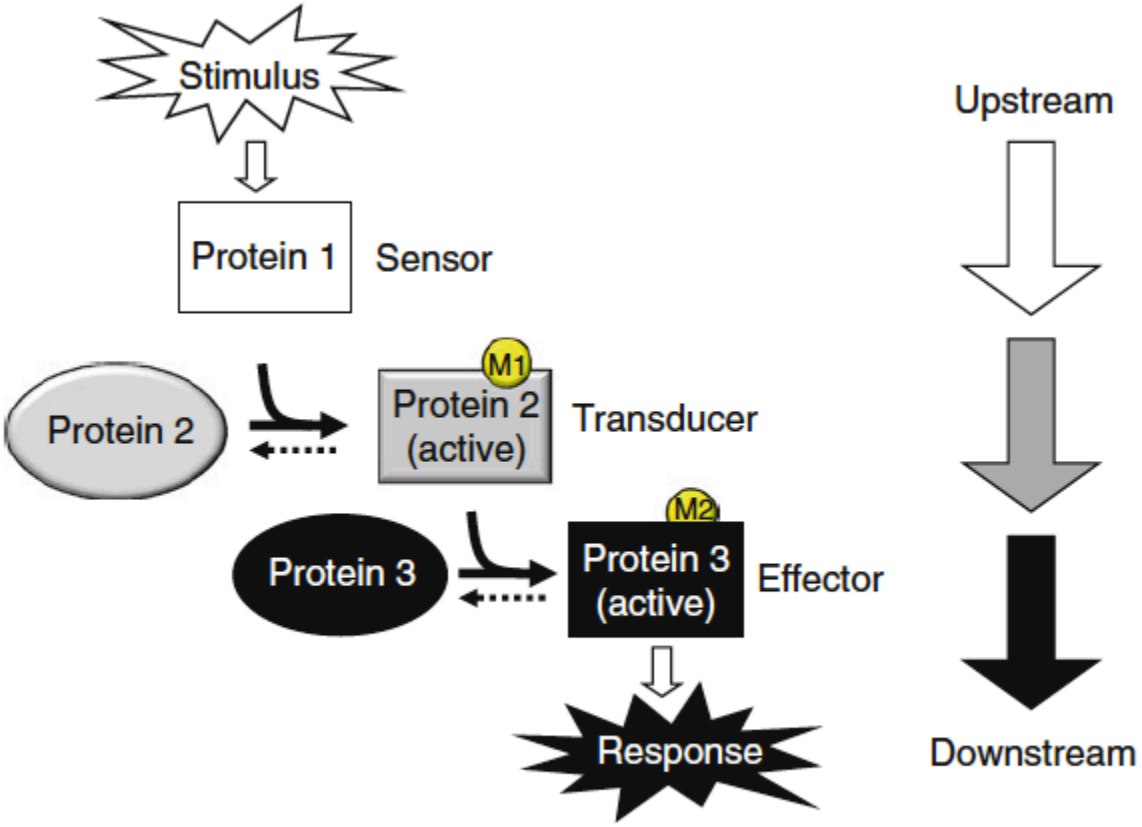
Molecule	Size	Target	'ON' enzyme	'OFF' enzyme
Phosphate group–PO_3	79 Da	Ser, Thr, Tyr	Protein kinase	Protein phosphatase
Methyl group–CH_3	15 Da	Arg, Lys	Protein methyl-transferase	Protein demethylase
Acetyl group–$COCH_3$	43 Da	Lys	Protein acetylase	Protein deacetylase
Ubiquitin–polypeptide	8.5 kDa	Lys	Multiple sequential enzymes	De-ubiquitinase
Small ubiquitin-related modifier (SUMO) – polypeptide	10–11 kDa	Lys	Multiple sequential enzymes	SUMO isopeptidases

The covalent modification of a protein reversibly alters its functional state.



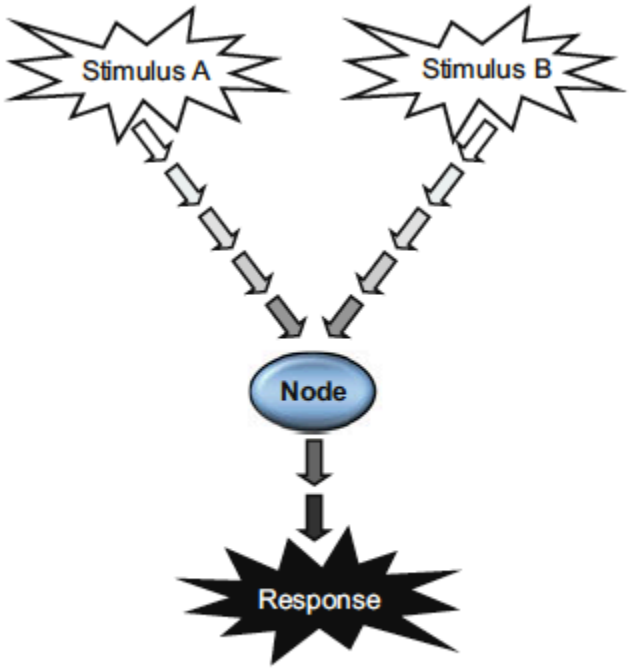
A hypothetical protein exists in two states, A and B. The transition from state A to B is mediated by the addition of a modifying group (M, shown in yellow) to an amino acid residue. This reaction is catalyzed by a hypothetical ‘ON’ enzyme. The reverse reaction, resulting in the removal of the modification and the transition from state B to state A, is catalyzed by a distinct ‘OFF’ enzyme. Either state A or state B can be the activated state, depending on the protein and the modifying molecule

A generic pathway

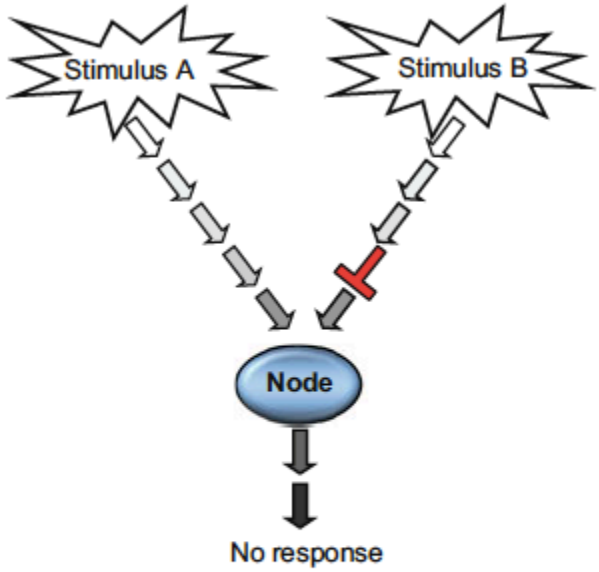


Several features that are common to many cellular pathways are shown. All pathways are characterized by directionality; signals are said to be transduced from an upstream stimulus to a downstream response. This hypothetical pathway is activated by a stimulus that activates a sensor (Protein 1). This sensor protein then adds a modification (M1, shown in yellow) to a second protein (Protein 2), causing it to become catalytically active. Once activated, Protein 2 serves as a transducer of the signal to a downstream effector (Protein 3) by catalyzing the addition of a modifying group (M2). Protein 3 then directly catalyzes a response. In many pathways, the intermediate transducer proteins can amplify upstream signals. The removal of a modifying group at any stage (dotted arrows) can result in the deactivation of the entire pathway. The hypothetical pathway shown has a single sensor, transducer and effector. Actual pathways can have multiple upstream and downstream proteins

Multiple stimuli end up with different responses

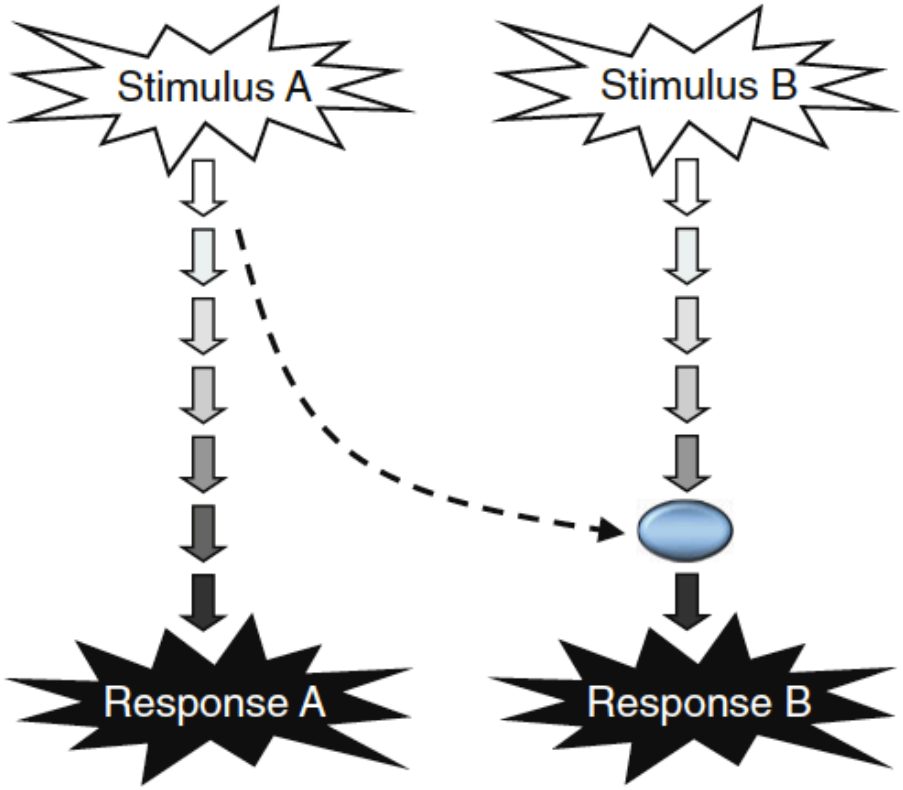


Convergent pathways. Distinct upstream signals can lead to a common response. Here, two pathways, triggered by stimulus A and stimulus B, converge at a single point and join a common downstream pathway. Points at which pathways intersect are sometimes referred to as nodes



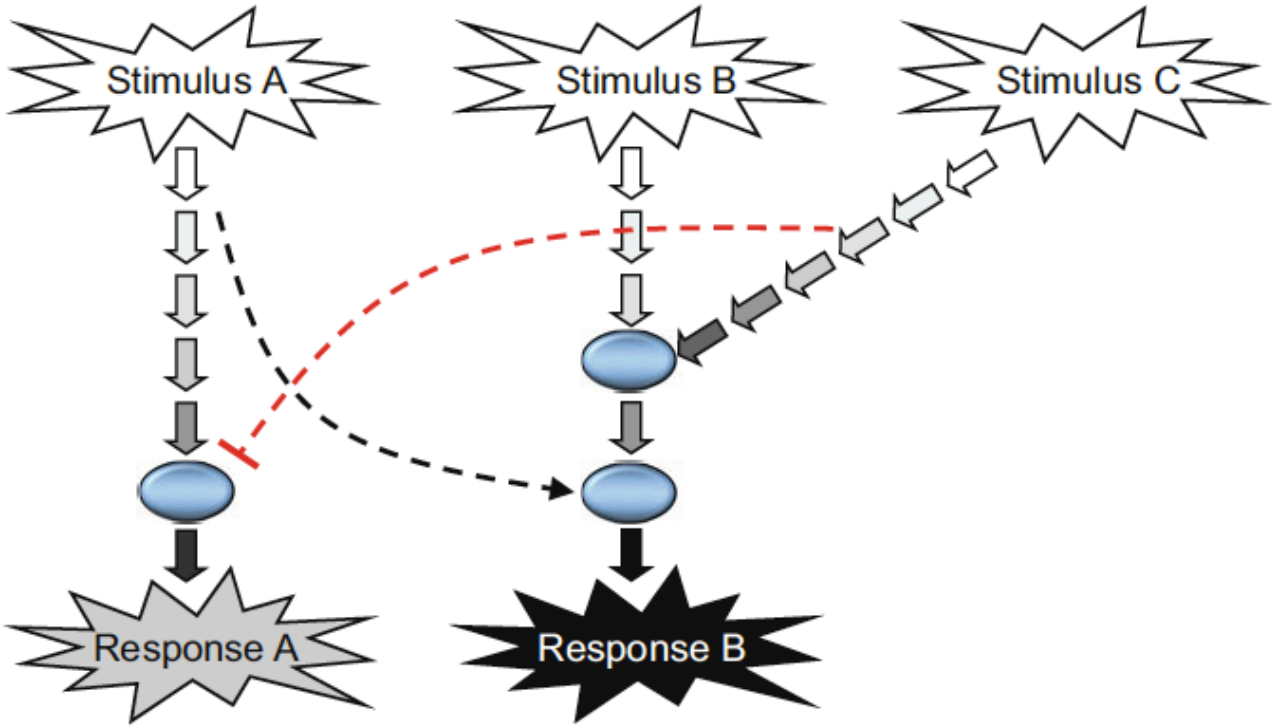
Inhibitory and stimulatory pathways can converge. Some pathways can trigger reactions (shown in red) that inhibit downstream signaling events. In this example, the response that would be triggered by stimulus A is attenuated by the pathways activated by stimulus B

Crosstalk between parallel pathways



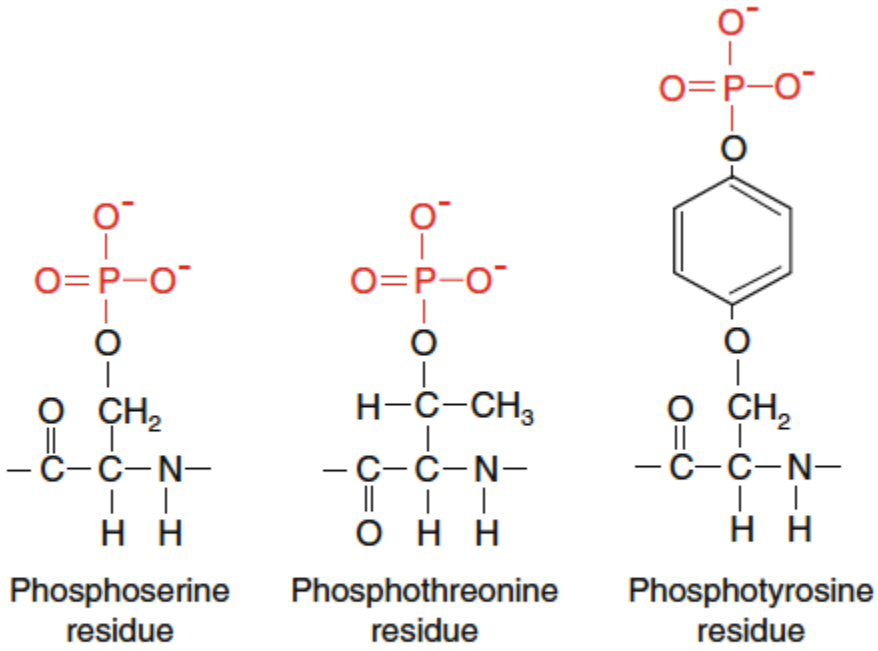
Stimulus A leads primarily to Response A. Stimulus B leads to Response B via a distinct pathway. The 'A' pathway is interconnected (dotted line) with the 'B' pathway at a node. Stimulus A can thus affect Response B to some degree. Crosstalk can increase or decrease the signals transduced by pathways that are otherwise parallel in structure

Interconnected pathways form signaling networks



Multiple upstream signals affect multiple downstream responses. The activation of pathways that are influenced by crosstalk pro-vides highly modulated signals that can effect nuanced responses. Shown is a simple multi-nodal network in which responses are stimulated by three activating pathways that are influenced by both stimulatory (black dashed line) and inhibitory (red dashed line) crosstalk. Responses A and B can thus be modulated with high precision

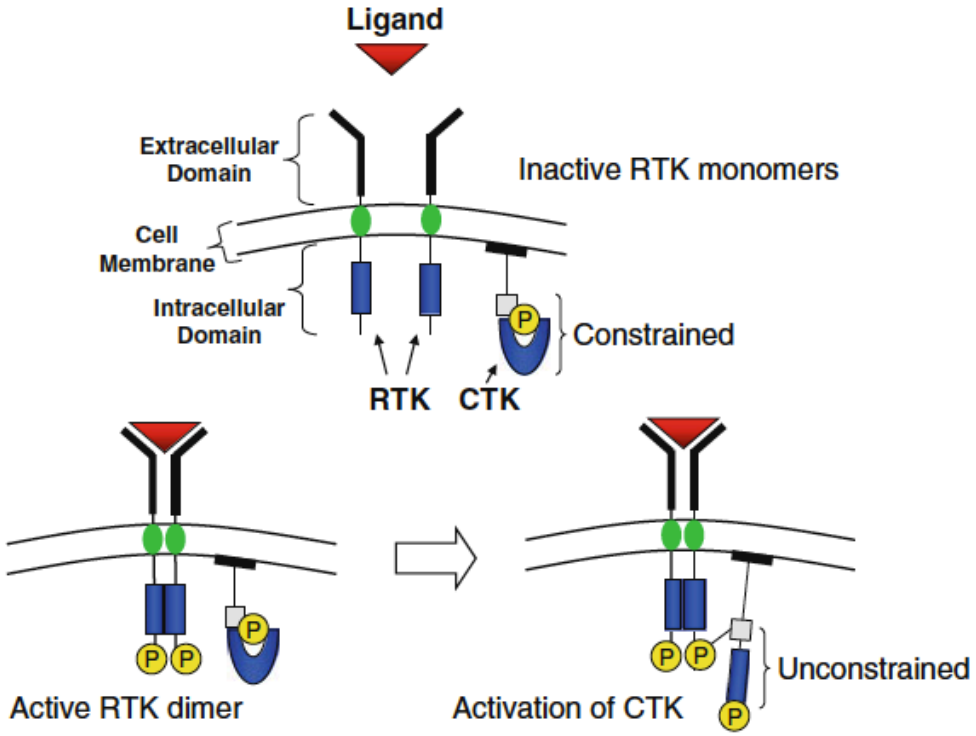
Protein Phosphorylation is a Common Regulatory Mechanism



Phosphorylated derivatives of serine, threonine and tyrosine. The addition of a phosphate group (red) adds a negatively charged moiety to a protein, altering its hydrophobicity and structure

Kinases catalyze the transfer of the γ–phosphate group from adenosine triphosphate (ATP) to protein residues, while phosphatases catalyze the removal of this phosphate group. It is thought that up to 30% of the proteins encoded by the human genome variably contain covalently bound phosphate. The human genome encodes approximately 1,000 kinases and 500 phosphatases that mediate these transactions. The reversible phosphorylation of proteins affects virtually every cellular activity and function.

Activation of a protein tyrosine kinase by an extracellular ligand.

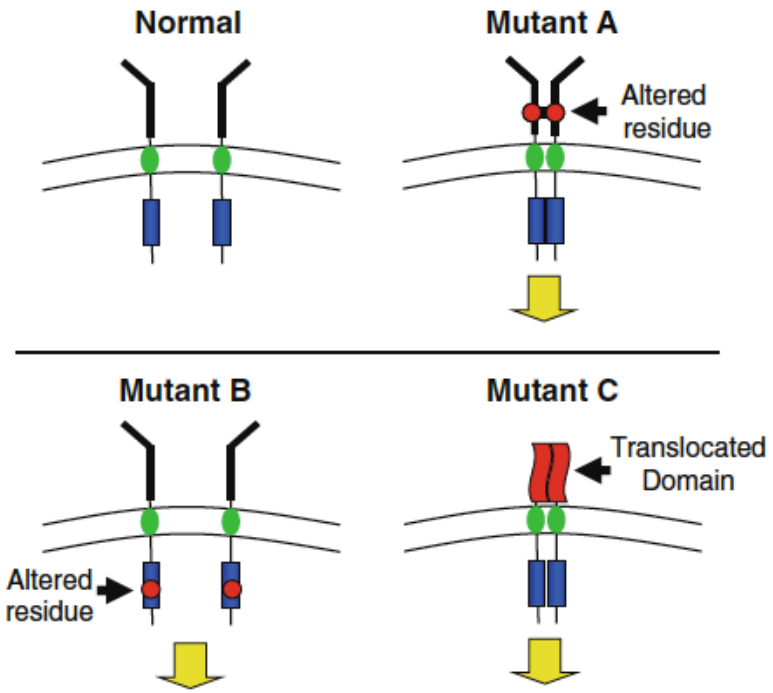


A generic receptor protein tyrosine kinase (RTK) is composed of an extracellular domain (black) that directly interacts with ligands (red), a transmembrane domain (green) and an intracellular domain that contains a conserved catalytic region (blue). A membrane-associated cytoplasmic tyrosine kinase (CTK) is maintained in inactive form by intramolecular constraints that inhibit its catalytic domain. Upon ligand binding, the RTK molecules dimerize, and activate their catalytic domains by autophosphorylation. The intramolecular constraints that keep the CTK inactive are relieved when the SRC-homology domain (gray) preferentially associates with the phosphorylated form of the RTK dimer. Thus activated, RTK and CTK can trigger downstream pathways

RTK genes altered in cancers

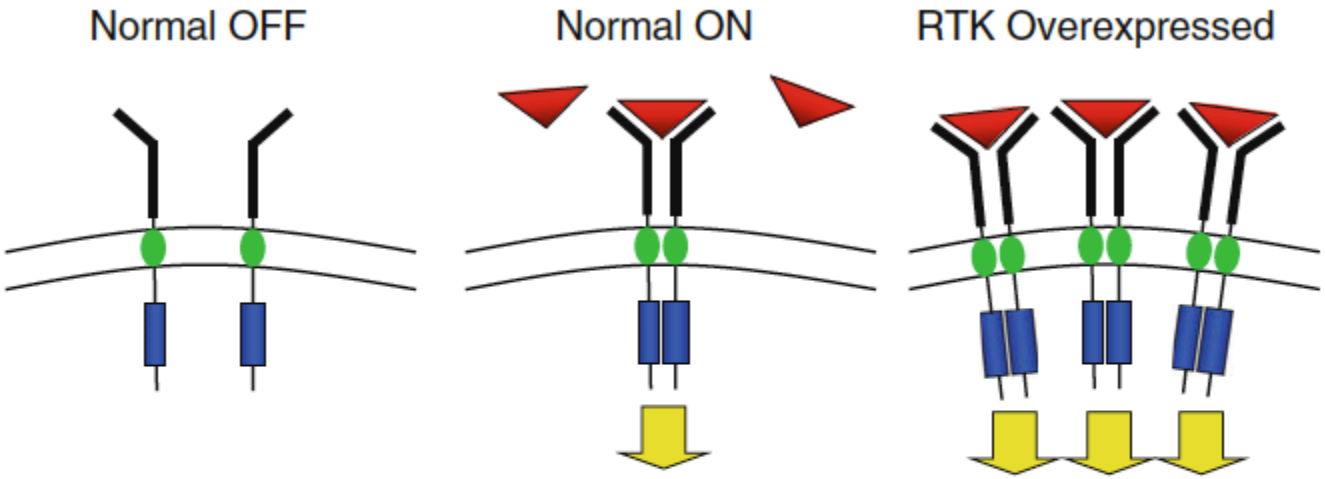
Proto-oncogene	Ligand	Oncogenic alteration	Cancers
EGFR (ERBB1)	Epidermal growth factor (EGF)	Point mutation, deletion	Lung, colorectal, and breast carcinoma
	Transforming growth factor β (TGFβ)	Amplification	Glioblastoma
ERBB2 (HER2/neu)	None	Amplification	Breast, ovarian, gastric, cervical, and lung carcinoma
		Point mutation	Neuroblastoma
MET	Hepatocyte growth factor	Amplification	Medulloblastoma
		Point mutation	Esophageal and gastric carcinoma
			Hereditary papillary renal cell carcinoma
RET	Glial-derived neurotropic factor	Complex rearrangement	Thyroid carcinoma
		Point mutation	Multiple endocrine neoplasia syndromes 2A & 2B
C-KIT	Stem cell factor	Point mutation	Acute myeloid leukemia, germ cell tumors
		Amplification	Glioblastoma
FGFR1	Fibroblast growth factor	Point mutation	Glioblastoma
		Translocation	Acute myelogenous leukemia, lymphoma

Point mutations can result in RTK dysregulation.



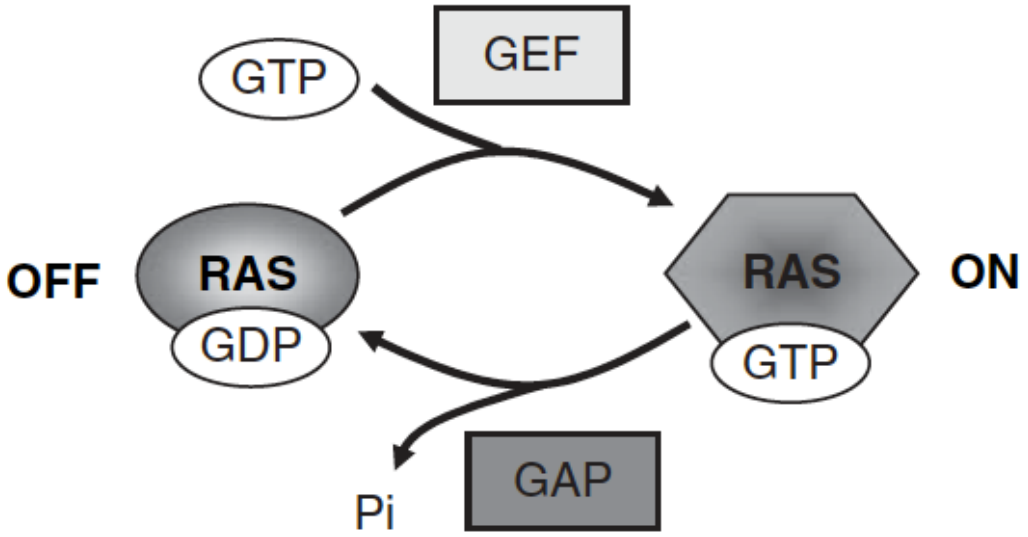
Mutant A contains an amino acid substitution (shown in red) in the extracellular domain that causes RTK molecules to have an increased affinity for one another and to dimerize. Mutations in the transmembrane domain can have a similar effect (not shown). Mutant B carries an amino acid substitution mutation in the activation loop of the catalytic domain, increasing the basal kinase activity of RTK monomers. Mutant C is a fusion protein in which the extracellular domain derived from an unrelated protein that is normally 'sticky' and therefore participates in protein–protein interactions. In all cases, signaling is ligand-independent

Amplification of RTK genes can cause cells to become hypersensitive to ligand.



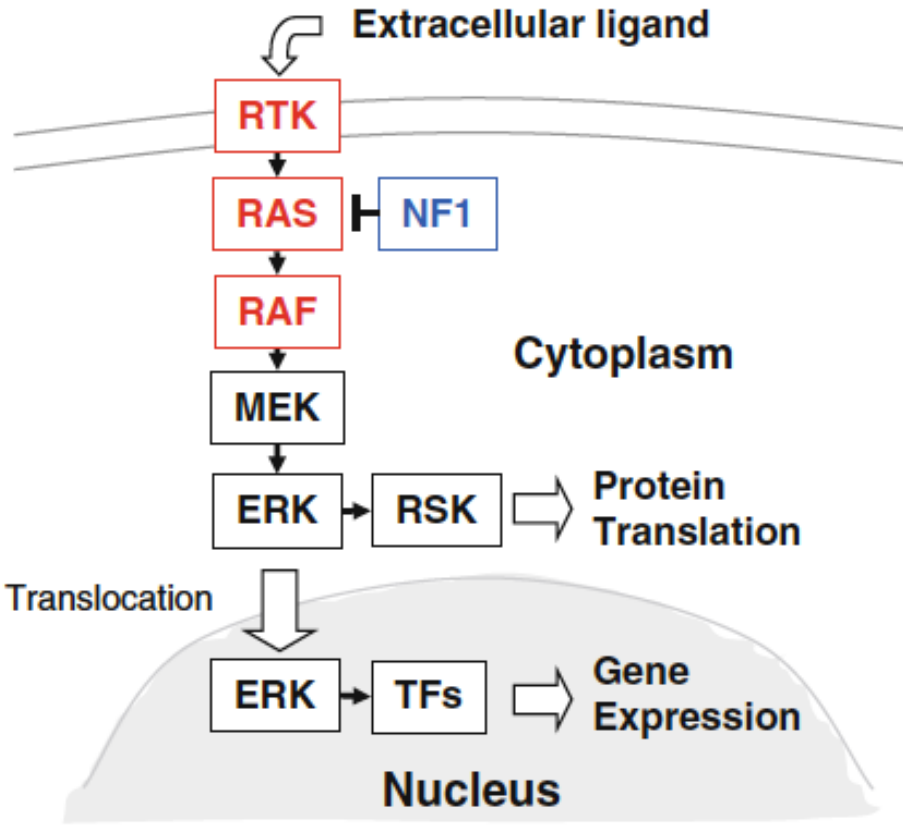
RTK proteins encoded by wild type genes normally trigger downstream responses (yellow arrow) that depend upon the presence of ligand (red). Amplification of RTK genes leads to overexpression of RTK receptors, and their increased numbers at the cell surface. Although each receptor is normal in structure and function, cells are hypersensitive to ligand

Regulation of RAS-mediated GTP binding and GTP hydrolysis.



RAS proteins have low intrinsic GTP-binding activity, which can be greatly stimulated by physical association with a guanine nucleotide exchange factor (GEF). Similarly, the hydrolysis of GTP by RAS is stimulated by a GTPase activating proteins (GAP). Thus the binary mode of signaling of RAS (ON and OFF) is highly regulated

RAS signaling connects RTKs with kinase cascades that alter gene expression and protein translation.

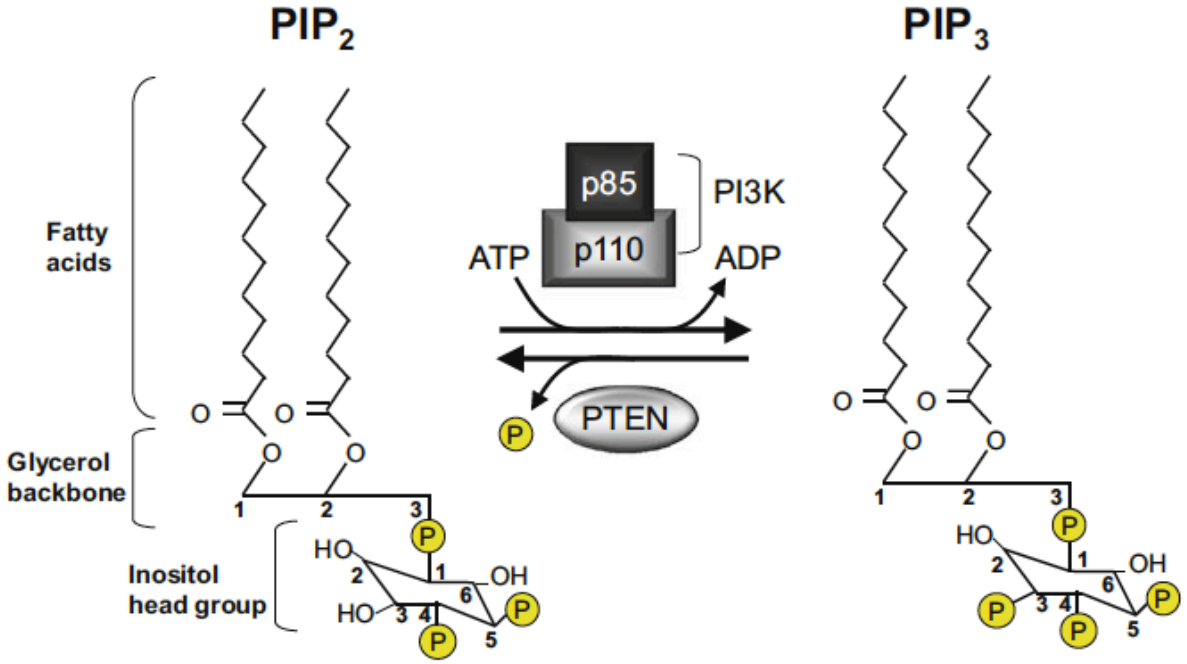


In response to RTK signaling, RAS proteins activate RAF family members. RAS can be deactivated by the GAP proteins, which include the product of the NF1 gene. RAF proteins phosphorylate and activate the MEKs, which in turn phosphorylate and activate the ERKs. The ERK proteins can activate the ribosome-associated RSK proteins, thereby affecting protein synthesis.

ERKs can also translocate into the nucleus and regulate numerous transcription factors (TFs). Thus, RAS signals are transmitted throughout the cell. Proteins that can be constitutively activated via oncogenic mutations are shown in red. NF1 is the product of a tumor suppressor gene and is shown in blue

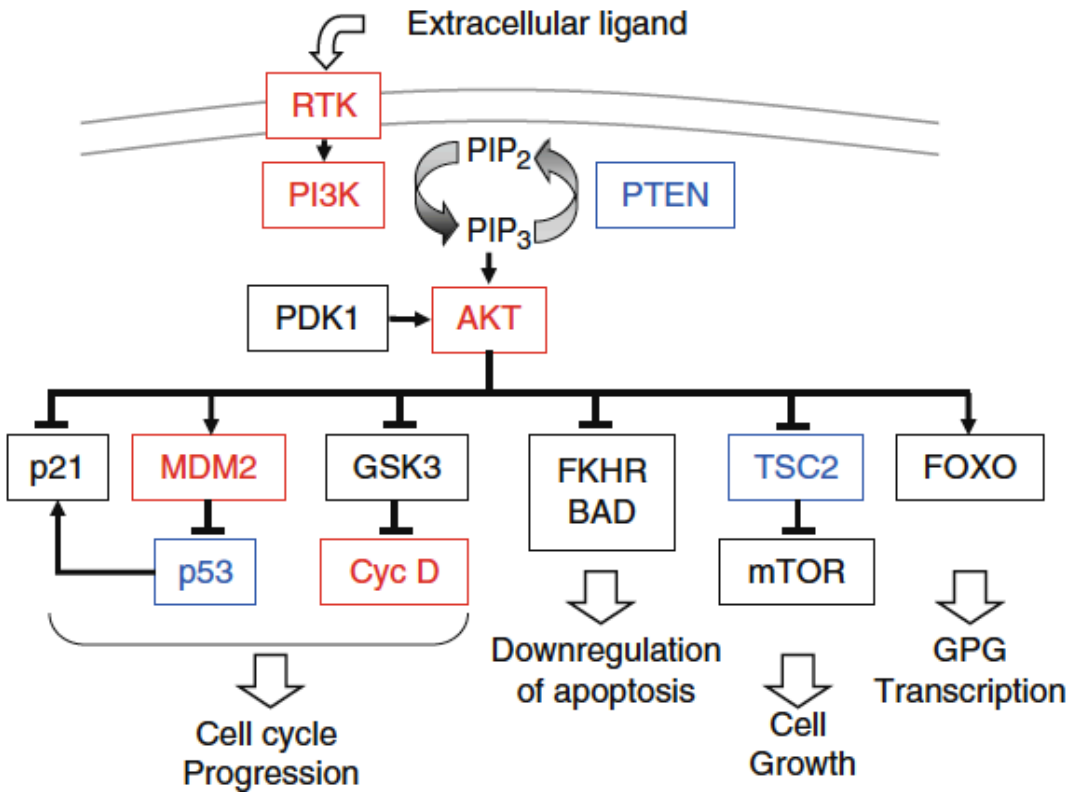
Membrane-Associated Lipid Phosphorylation: The PI3K/AKT Pathway

In response to mitogenic ligands, receptor tyrosine kinases (RTKs) can trigger the activation of a class of enzymes known as phosphatidylinositol 3-kinases (PI3Ks). This unique class of enzymes catalyzes the phosphorylation of inositol-containing lipids. These phospholipids then act as second messengers that stimulate down-stream signaling molecules. PI3K activation represents a distinct pathway that is triggered by receptor tyrosine kinase (RTK) signaling.



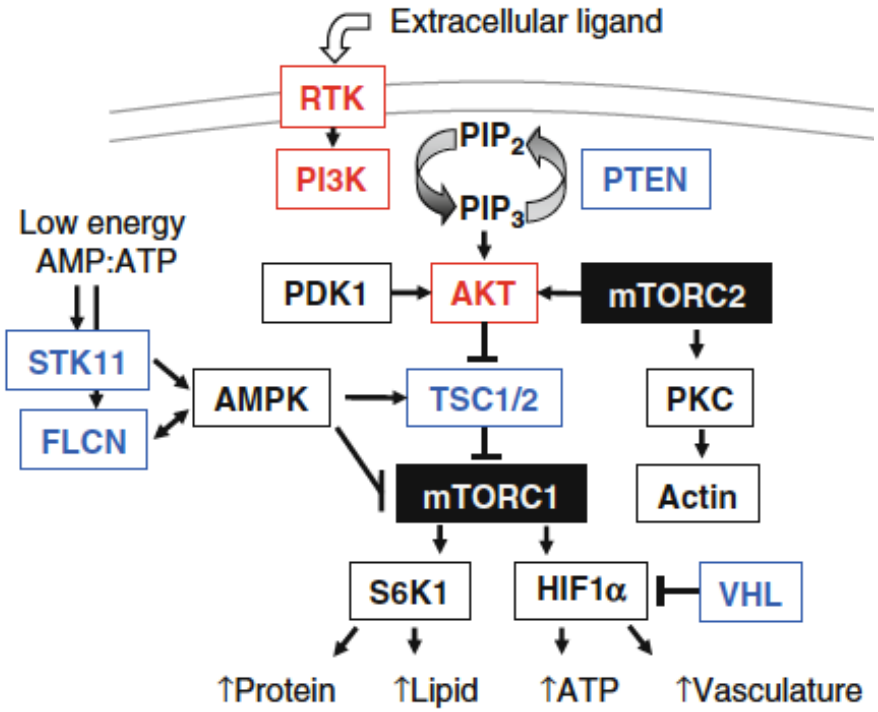
Regulation of the PIP2–PIP3 cycle by PI3K and PTEN. The heterodimeric PI3K complex catalyzes the ATP-dependent phosphorylation of phosphatidylinositol (4,5) bisphosphate (PIP2) at the D3 position of the inositol moiety to generate phosphatidylinositol (3,4,5) triphosphate (PIP3). The reverse reaction is catalyzed by the lipid phosphatase encoded by the PTEN tumor suppressor gene. Relevant phosphate groups are shown in yellow

The PI3K/AKT pathway.



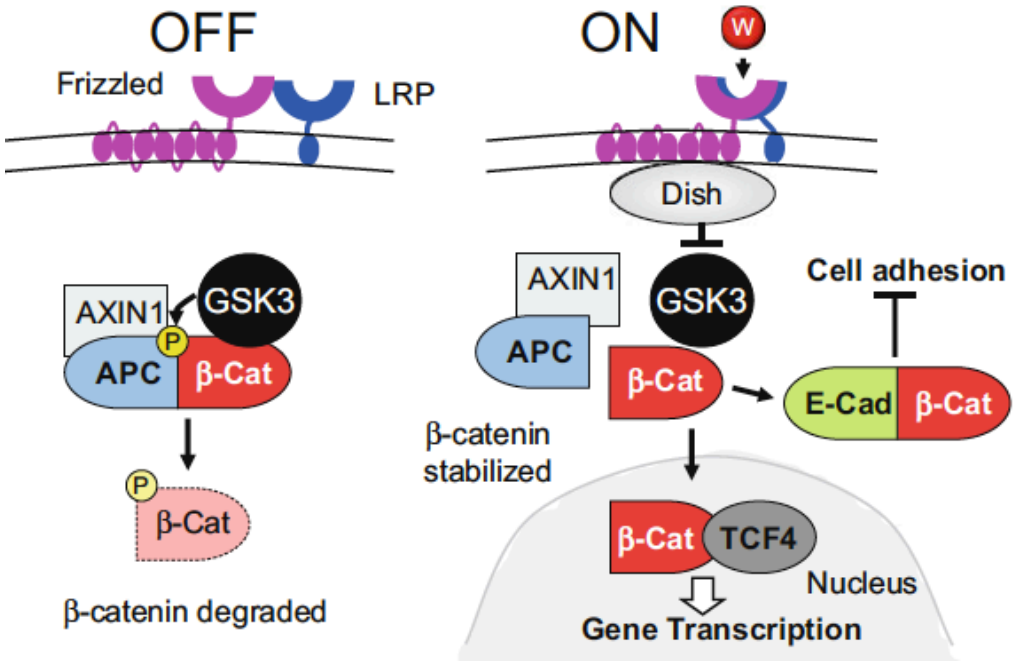
Ligand-dependent activation of RTK signaling causes the activation of PI3K, and the generation of PIP3. PTEN - a tumor suppressor - catalyzes a reversible dephosphorylation reaction. AKT binds PIP3 and is thus recruited to the inner surface of the cell membrane. AKT is activated by a dual regulatory mechanism that requires translocation and subsequent phosphorylation by PDK1. Active AKT phosphorylates numerous downstream substrates; only the representative ones are shown. Cell cycle progression is stimulated by the AKT-dependent phosphorylation of the cyclin-dependent kinase inhibitor p21. Expression of p21 is also inhibited by the MDM2-dependent inhibition of p53. The activity of cyclin D is increased by the AKT dependent inhibition of glycogen synthetase kinase 3B. Apoptosis is downregulated by inhibitory signaling to several proapoptotic proteins. AKT inhibits the mTOR pathway via inhibition of TSC2, and thereby promotes protein biosynthesis. The expression of growth-promoting genes is increased by the activation of the FOXO family of transcription factors. Proteins encoded by proto-oncogenes are shown in red; tumor suppressor gene products are shown in blue

The control of biosynthetic and energetic pathways by mTOR complexes



mTOR activity is controlled by multiple regulatory proteins. AKT promotes activation of the mTORC1 complex via the inactivation of the TSC1/2 heterodimer. A second complex, mTORC2, promotes the activity of AKT. mTORC2 also interacts with the cytoskeleton by a promoting an interaction between protein kinase C (PKC) and actin. The TSC1/2 complex is activated in response to the 5′-adenosine monophosphate-activated protein kinase (AMPK), an evolutionarily conserved metabolic master switch that senses fluctuations in the AMP:ATP ratio via signals from the STK11 kinase. A complex of proteins containing foliculin (FLCN) also appears to be involved in energy and nutrient sensing by AMPK. Downstream targets of mTORC1 include S6K1, which promotes the biosynthesis of proteins and lipids, and HIF1α, which promotes ATP generation by glucose metabolism. HIF1α is negatively regulated by the tumor suppressor VHL. Proteins encoded by proto-oncogenes are shown in red ; tumor suppressor gene products are shown in blue

Morphogenesis and cancer: WNT/APC pathway

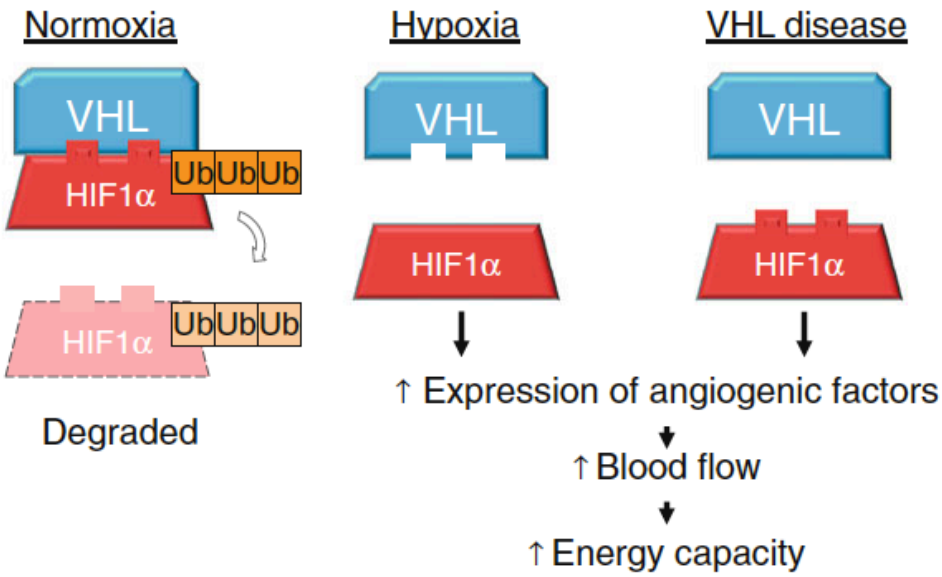


In the absence of WNT ligand (OFF; left panel), phosphorylation of β-catenin by the GSK3 kinase favors the formation of a complex composed of APC and AXIN. β-catenin is targeted for degradation when the WNT pathway is OFF.

When the pathway is turned on by ligand, Frizzed and LRP cooperatively activate Disheveled at the cell membrane, which functions to inactivate GSK3. In the absence of GSK3-mediated phosphorylation, the degradation complex is dissociated and β-catenin is stabilized, translocates to the nucleus and, in cooperation with the TCF family of transcription factors, activates the expression of growth promoting genes .

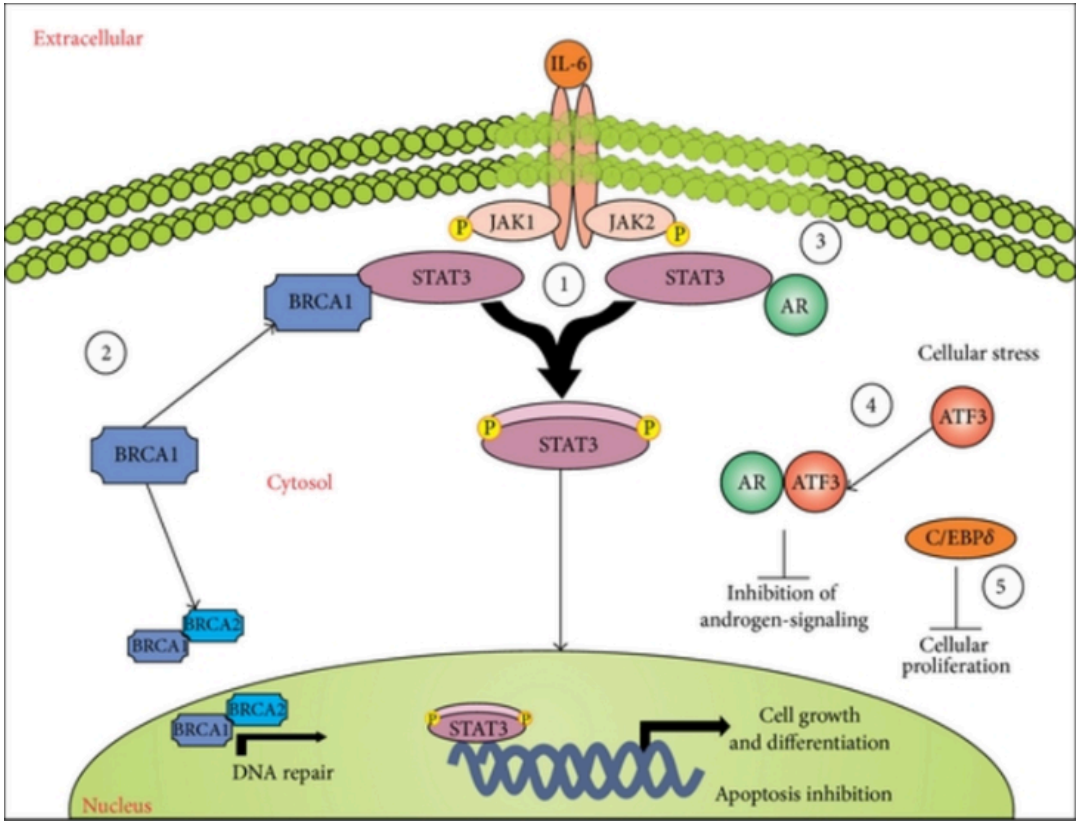
Cytoplasmic β-catenin can also associate with E-cadherin, which mediates cell adhesion

The regulation of HIF1α by VHL



HIF1α is a transcription factor that is highly responsive to the microenvironmental oxygen concentration. At normal oxygen levels (normoxia), two proline residues on HIF1α are covalently modified and facilitate its interaction with a specific site on VHL. This interaction results in the poly-ubiquitination of HIF1α, and its subsequent degradation by the proteasome. Under conditions of hypoxia, which are frequently encountered in growing tumors, the specific proline residues on HIF1α are unmodified and VHL is therefore not bound. The mutations that cause VHL disease commonly alter the HIF1α binding site. In the stable VHL-unbound state, HIF1α induces the expression of genes, including VEGFA, that promote angiogenesis and increase local blood flow

JAK/STAT pathway: Transduces cytokine signals to nucleus (intracellular tyrosine kinase / signal transduction activator)



The Signal Transducer and Activator of Transcription (STAT) pathway is an important component of innate and adaptive immune regulation. This evolutionarily conserved signaling system is highly responsive to cytokines, small secreted proteins that transmit signals to and from cells of the immune system. Mutations that affect upstream RTKs can cause constitutive STAT signaling.

Molecular Targets for Cancer Therapy