Corso di Laurea Magistrale in Genomica Funzionale e Biotecnologie Mediche

Segnalazione Cellulare

- ✓ 3 Crediti Formativi (CFU): 24 ore di lezione

International Centre for
Genetic Engineering and Biotechnology
(ICGEB), Trieste, Italy

Recapito docente
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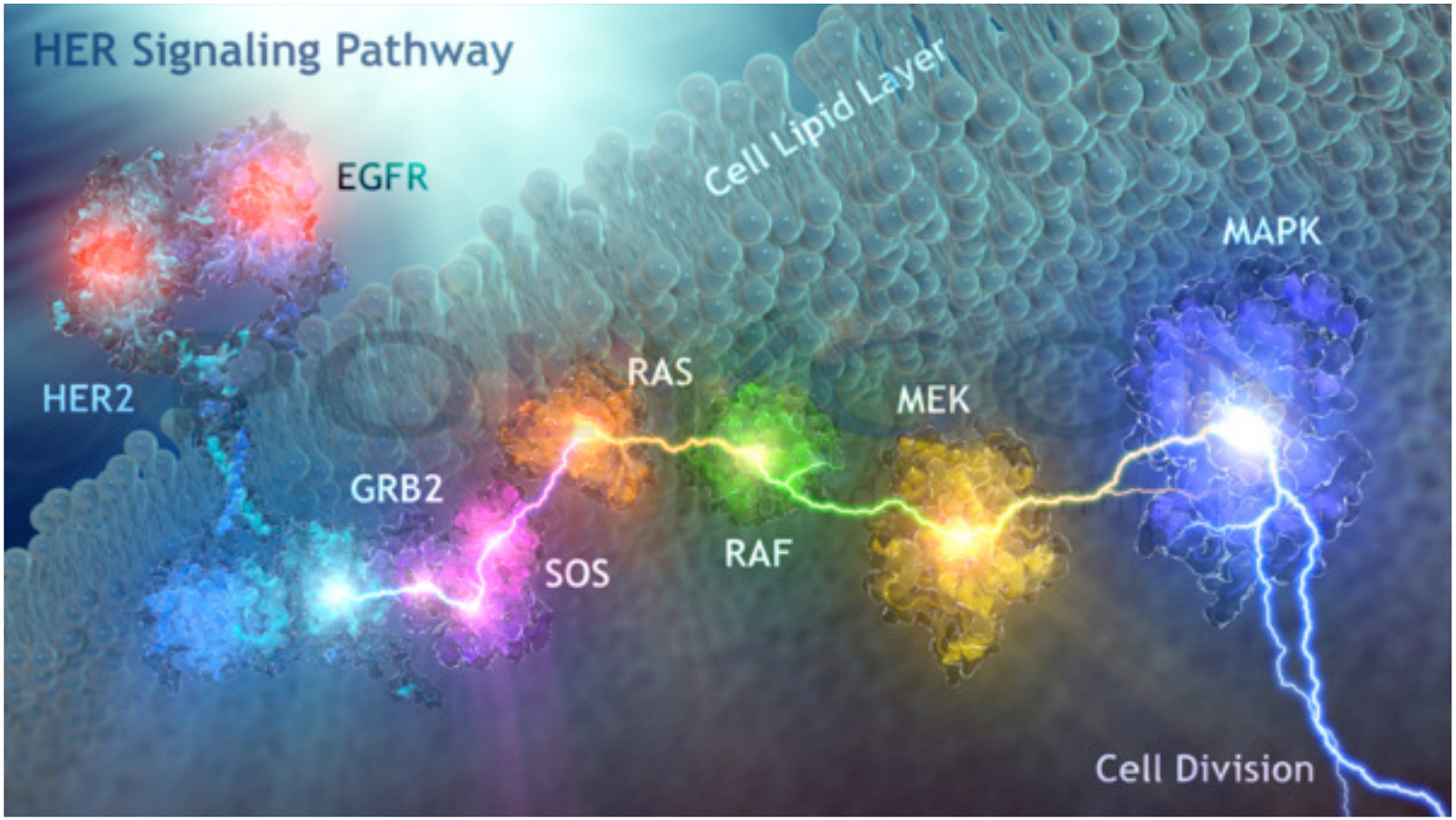
Padriciano 99, 34149 Trieste
e-mail: ccollesi@units.it
Tel: 040-3757375


HER Signaling Pathway
EGFR
Cell Lipid Layer
MAPK
HER2
RAS
MEK
GRB2
RAF
SOS
Cell Division

Struttura del corso

Parte introduttiva/propedeutica:

Basi della segnalazione inter-intra cellulare. Seven spanning transmembrane receptors. Recettori associati ad enzimi e recettori con attivita' enzimatica intrinseca. Integrazione e modulazione dei segnali in membrana.

Parte "core":

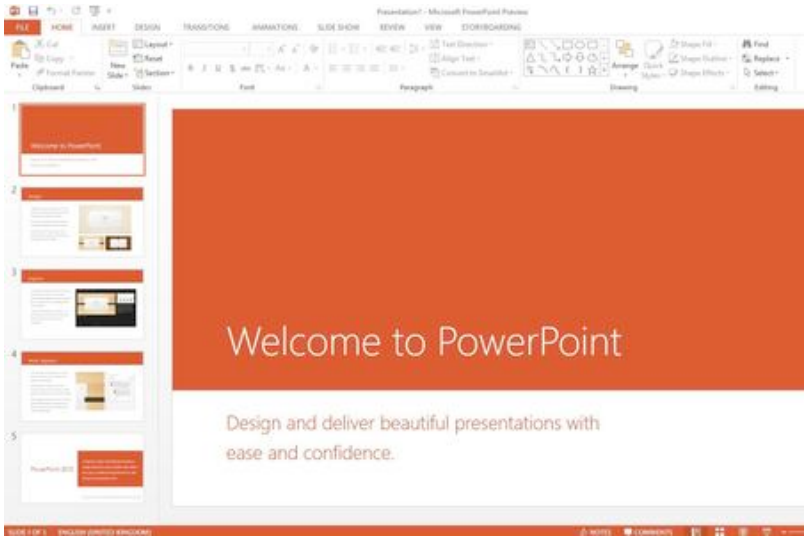
Il processo di "Rigenerazione" come modello di integrazione e crosstalk delle diverse vie di segnalazione: rigenerazione epatica, intestinale, cardiaca.

Parte "applicativa":

Come organizzare un journal club, caratteristiche e requisiti delle presentazioni. Journal Clubs su articoli selezionati (se avremo tempo….).

Moodle+MsTeams

- ✓ Presentazioni PPT (PDF) di ogni lezione



- ✓ Video-lezione registrata in audio e video

Testi consigliati

- ✓ F. Amaldi- BIOLOGIA MOLECOLARE- Casa Editrice Ambrosiana
- ✓ B. Alberts et al – Molecular Biology of the Cell– Garland Publishing
- ✓ B. Lewin – Genes XI– Oxford University Press

- ✓ Per alcune lezioni che tratteranno argomenti particolarmente innovativi e non sufficientemente descritti nei libri di testo, sara' fornito agli studenti opportuno materiale didattico.


NEWS & VIEWS

CANCER

T cells home in on brain tumours

Immunotherapies activate T cells to destroy tumours, but the approach has failed in some brain cancers. A strategy to improve migration of T cells across the blood–brain barrier could overcome this limitation.


nature biotechnology

REVIEW


Science
AAAS

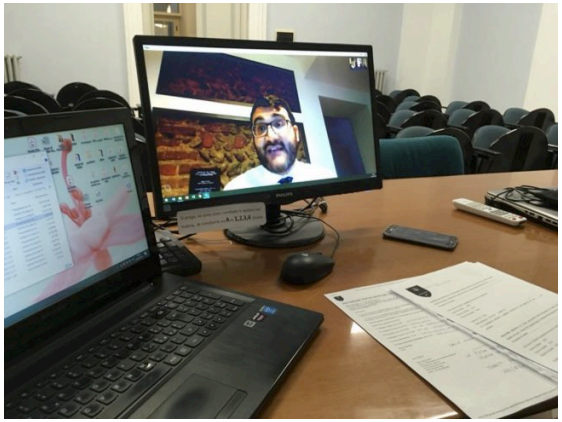
Cell Signaling in Space and Time: Where Proteins Come Together and When They're Apart
John D. Scott and Tony Pawson
Science **326**, 1220 (2009);
DOI: 10.1126/science.1175668

CRISPR-Cas systems for editing, regulating and targeting genomes

Jeffry D Sander[1,2] & J Keith Joung[1,2]

Esame

✓ Colloquio orale



✓ Video-colloquio

✓ Commento e/o interpretazione articoli scientifici


articles

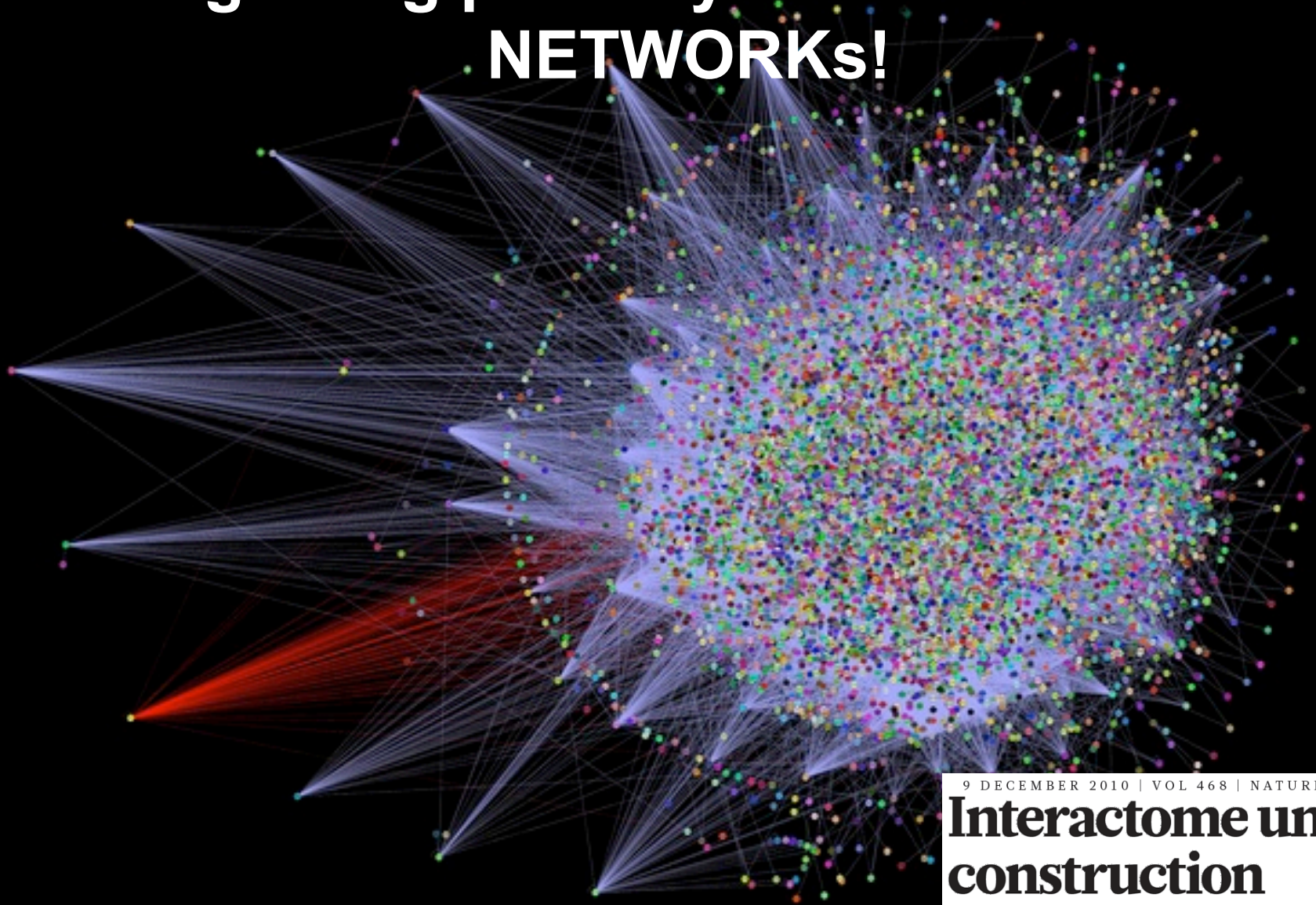
Structure of importin-β bound to the IBB domain of importin-α

news feature

Reservoirs dog AIDS therapy

Pools of latent HIV, lurking in the cells of infected people, remain u
even by powerful drug combinations. Paul Smaglik reports on how
forcing researchers to rethink their strategies for fighting the virus.

Signaling pathways are linked into NETWORKs!



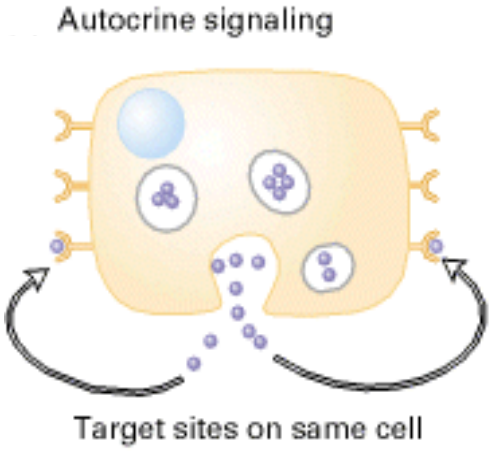
9 DECEMBER 2010 | VOL 468 | NATURE | 851

Interactome under construction

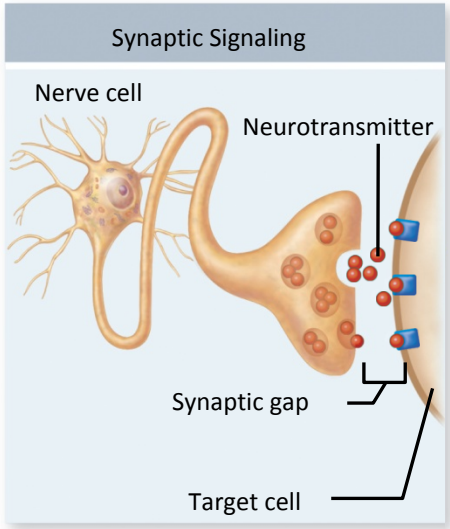
Developing techniques are helping researchers to build the protein interaction networks that underlie all cell functions.

Cellular Messaging:
Inter-cellular signaling

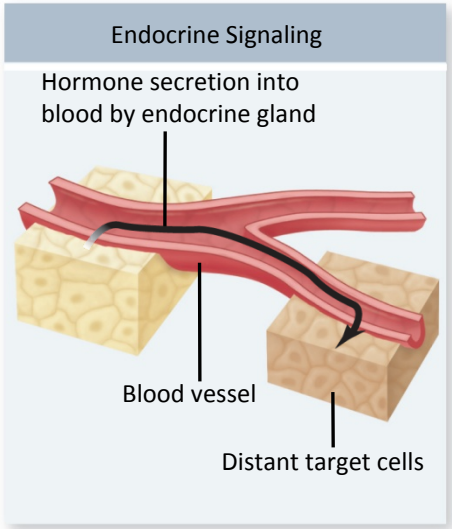
In multicellular organisms, intercellular exchanges of signals are performed in various ways:



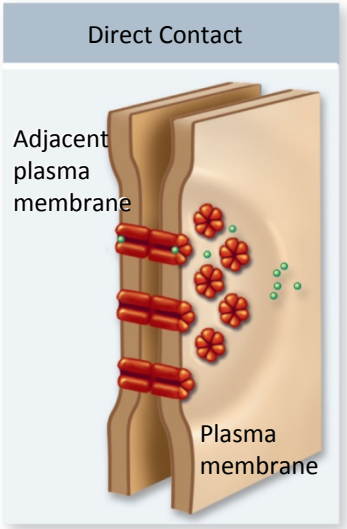
a.



b.



c.



d.

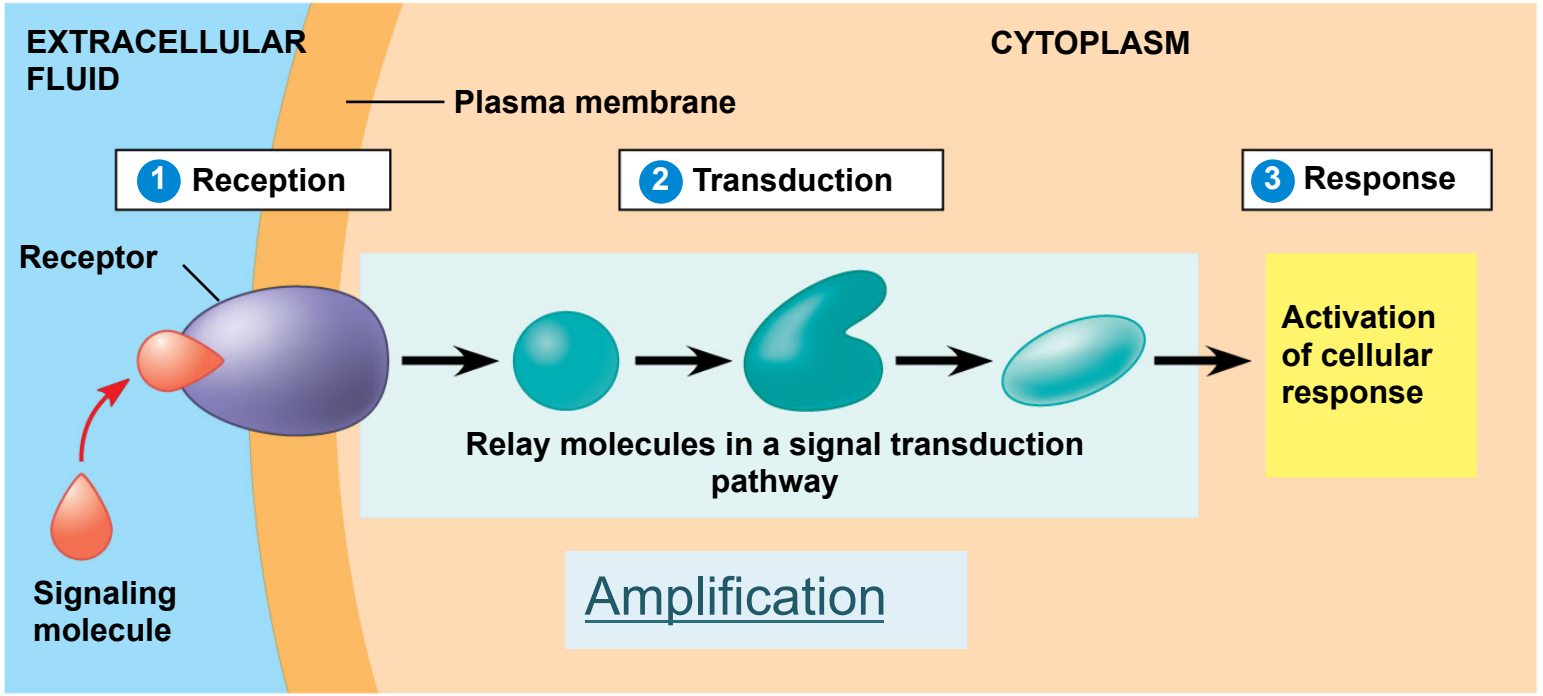
Inter-cellular signaling

Intercellular signaling molecules include hormones, growth factors and cytokines.

Molecules released by cells occurs over distances from a few microns [autocrine (a) and paracrine (b) signaling] to several meters in endocrine (c) signaling. In some instances, receptor proteins attached to the membrane of one cell interact directly with receptors on an adjacent cell (d).

The molecules involved in signal transduction between cells are called first messengers, while the diffusible molecules engaged in intracellular signal transduction in response to first messengers are known as second messengers (e.g., Ca2+ and cAMP).

The basic mechanism of *Intra*-cellular signal transduction



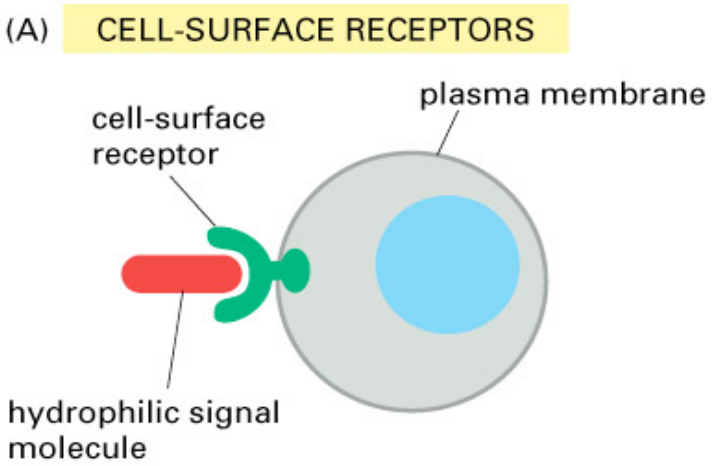
Cells have many receptor proteins on their plasma membrane surface, and molecules that bind specifically to these receptors are called signaling molecules. When bound with signaling molecules, receptors go through structural changes and are activated, which then changes the shape, movement and functions of the cell by activating intracellular signal transduction proteins, and regulates gene expression through the relocation of intracellular signaling molecules to the nucleus.

Which are the key players?

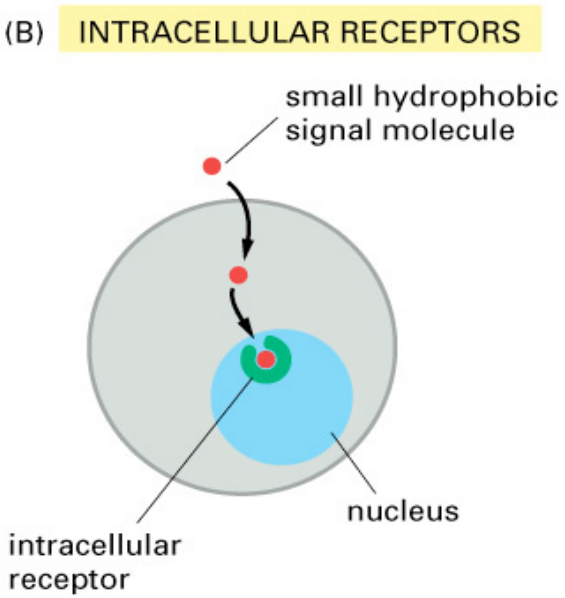
- *Ligands*
  - Hormones, growth factors, cytokines, gases
- *Receptors*
  - Specifically bind signaling molecules
  - Either on cell surface or intracellular
- *Second messangers*
  - Molecules which amplify the intracellular response

Extracellular signaling molecules fall into 2 classes:

(A) Molecules that are too large or too hydrophilic to cross the plasma membrane - rely on <u>membrane receptors</u>



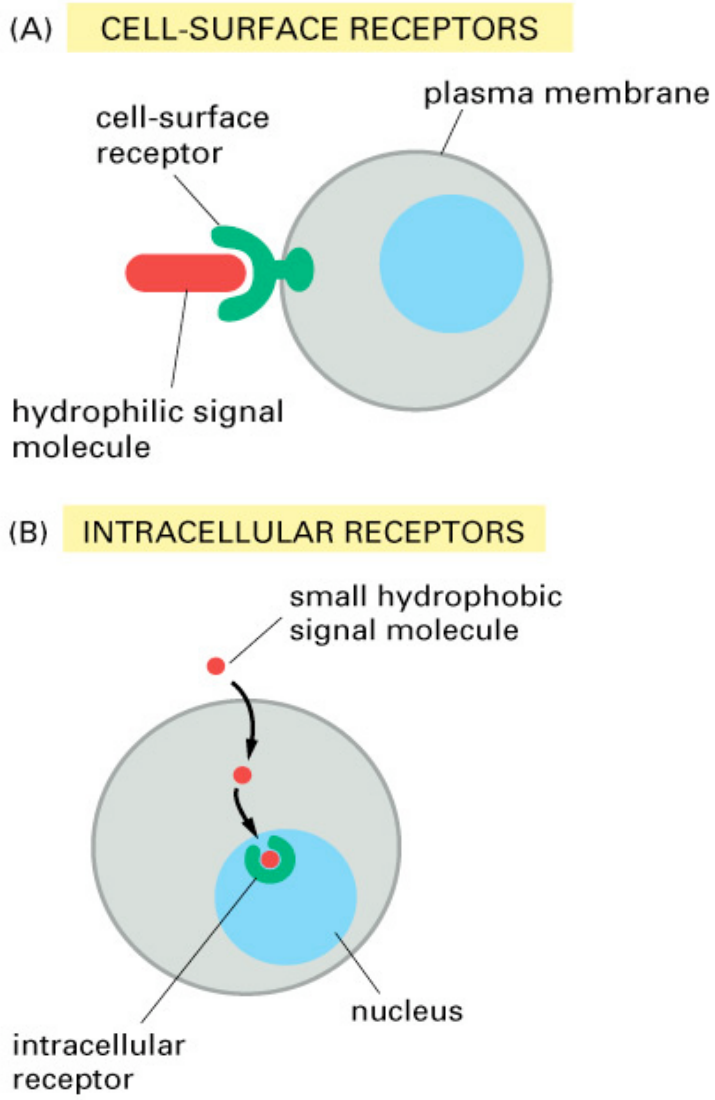
(B) Molecules that are small enough or hydrophobic and pass through the membrane - directly activate <u>intracellular receptors</u> in the cytoplasm or nucleus of target cell



Receptor molecules fall into 2 classes:

Cell surface receptors:

- Signaling molecules include peptide hormones, growth factors, cytokines etc
- The binding and subsequent events trigger (**a**) an ↑ or ↓ in the cytosolic concentration of a *second messenger* (***b***) the activated receptor acts as a *scaffold* to recruit and activate other intracellular proteins

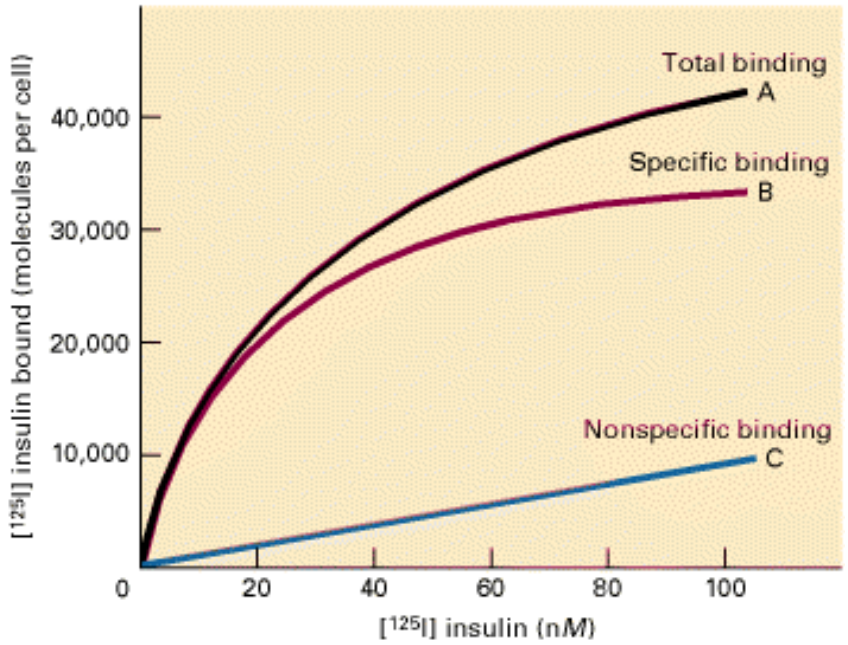


Intracellular receptors

- signaling molecules include steroid hormones, retinoids, thyroxine etc
- receptor-hormone complex acts a ***transcription factor*** to alter the transcription of target genes

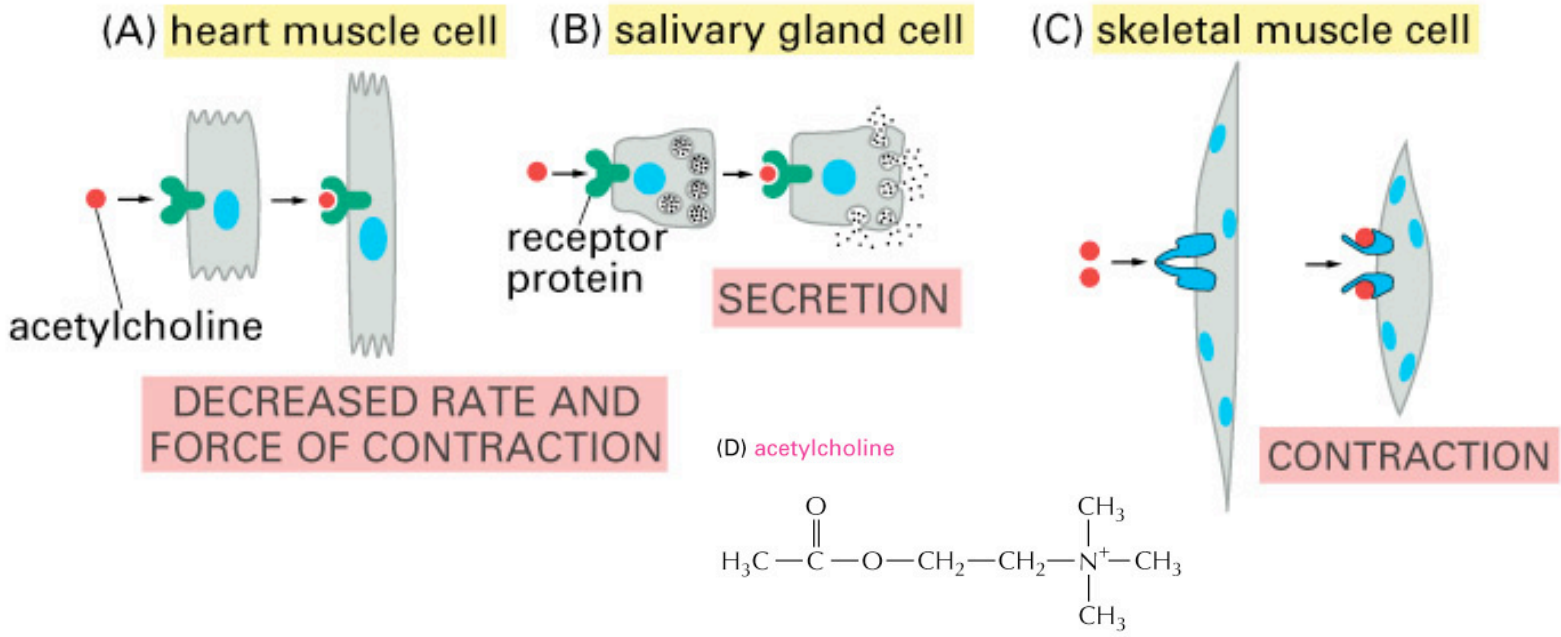
Receptor biochemical features

1. Participates in transduction of the signal from the external messenger to some component of the metabolic machinery
2. Has at least one additional functional site which is altered by ligand binding (allosteric site)
3. Ligand binding to receptors is saturable, resembling Michaelis-Menten kinetics



4. Ligand-receptor interaction characterized by tight binding (K_d = pM - μM)

ADVANTAGES



1. Each cell is programmed to respond to specific combinations of signaling molecules.

2. Different cells can differently respond to the same chemical signal.

Intracellular Signal Transduction

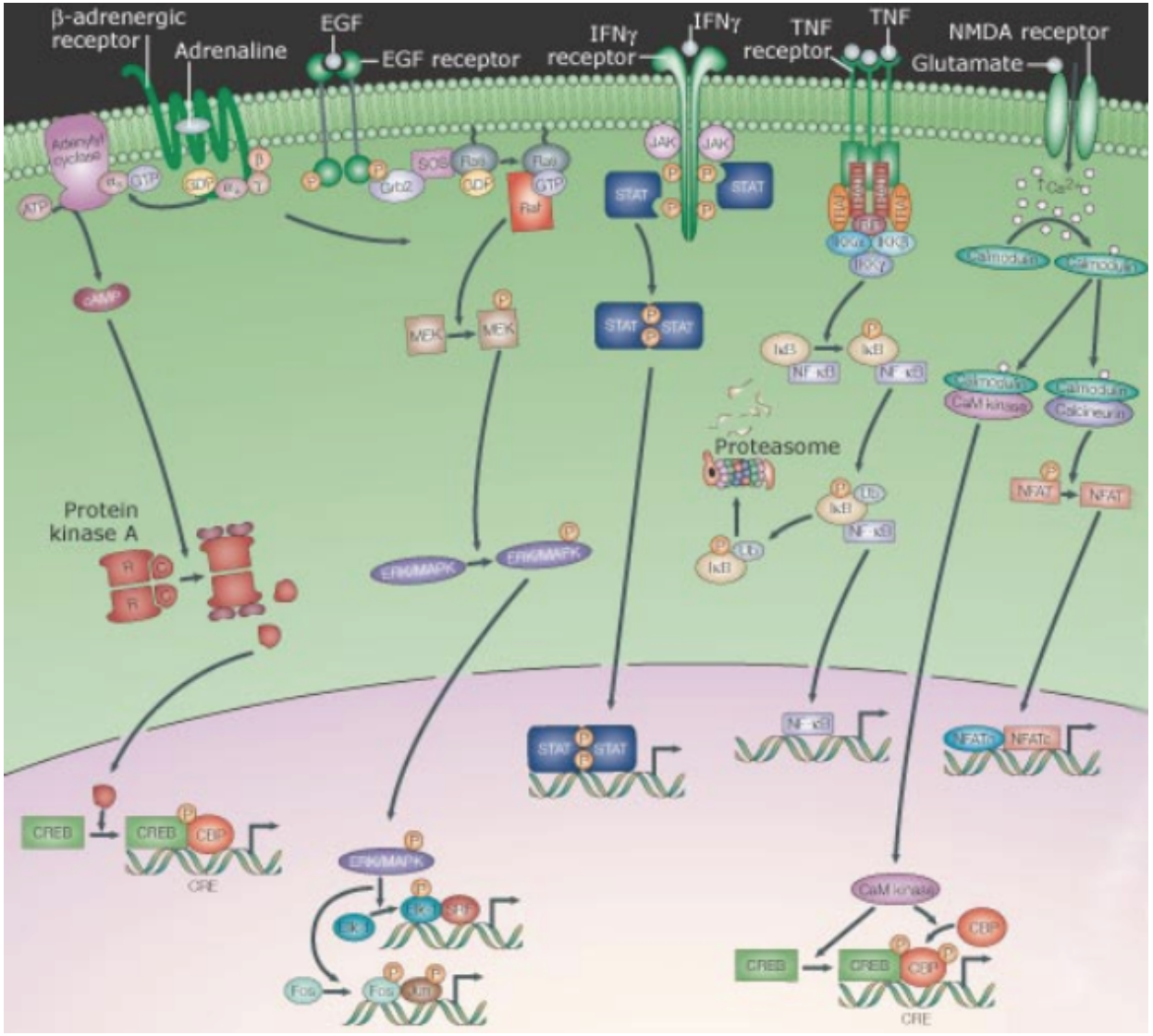
Intracellular signal transduction is a communication system in which signaling molecules bind to the receptors on the cell surface, triggering signaling within a cell.

nature REVIEWS MOLECULAR CELL BIOLOGY

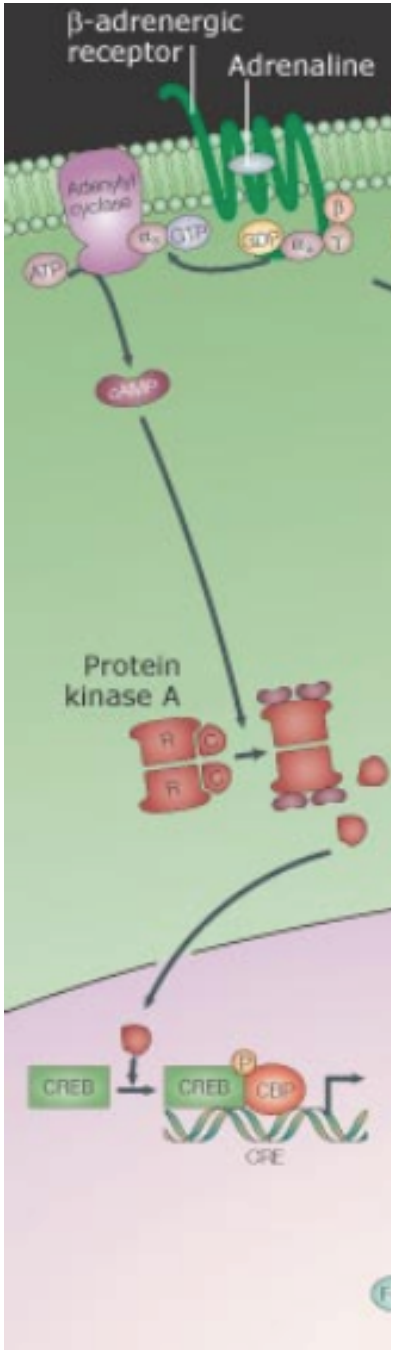
Signalling from the membrane to the nucleus

Signalling from cell-surface receptors through relay systems

This group includes receptors that, after engaging their extracellular ligand, undergo a conformational change, which induces them to oligomerize. This results in the production or release of second messengers (ions, lipids) or the post-translational modification (phosphorylation, proteolysis) of cytoplasmic proteins. A series of protein interactions and modifications then relays the signal - sometimes amplifying
and diversifying it - to the nucleus, whereupon the activity of transcription factors is altered.



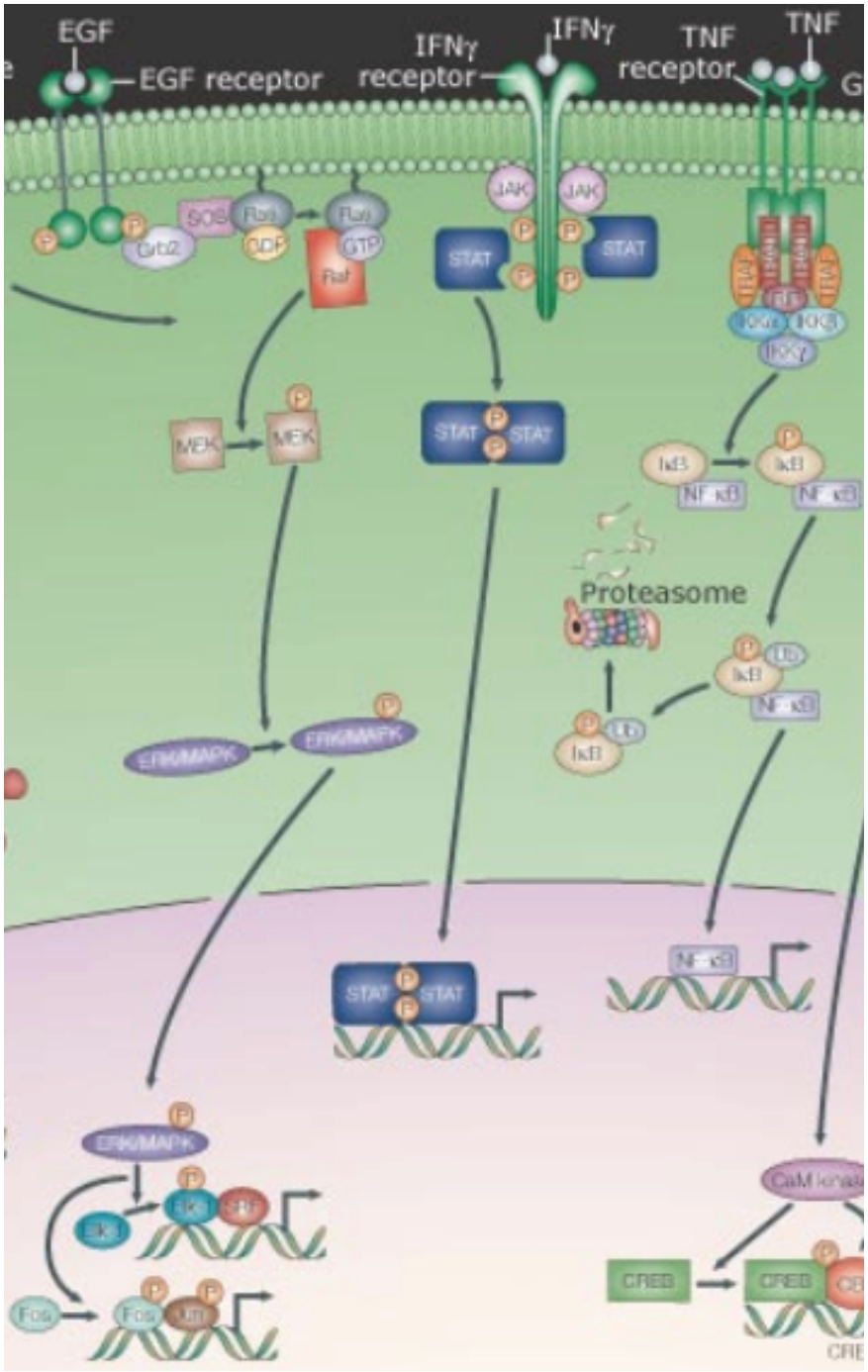
Signalling from the membrane to the nucleus
Signalling from cell-surface receptors through relay systems



1- Seven-transmembrane (7TM) receptors

Ligand binding to the extracellular domain of 7TM receptors induces a conformational change, which causes a heterotrimeric G-protein that is associated with the receptor to dissociate into an α monomer and a $\beta\gamma$ dimer. The monomer and the dimer can both induce downstream signalling events.

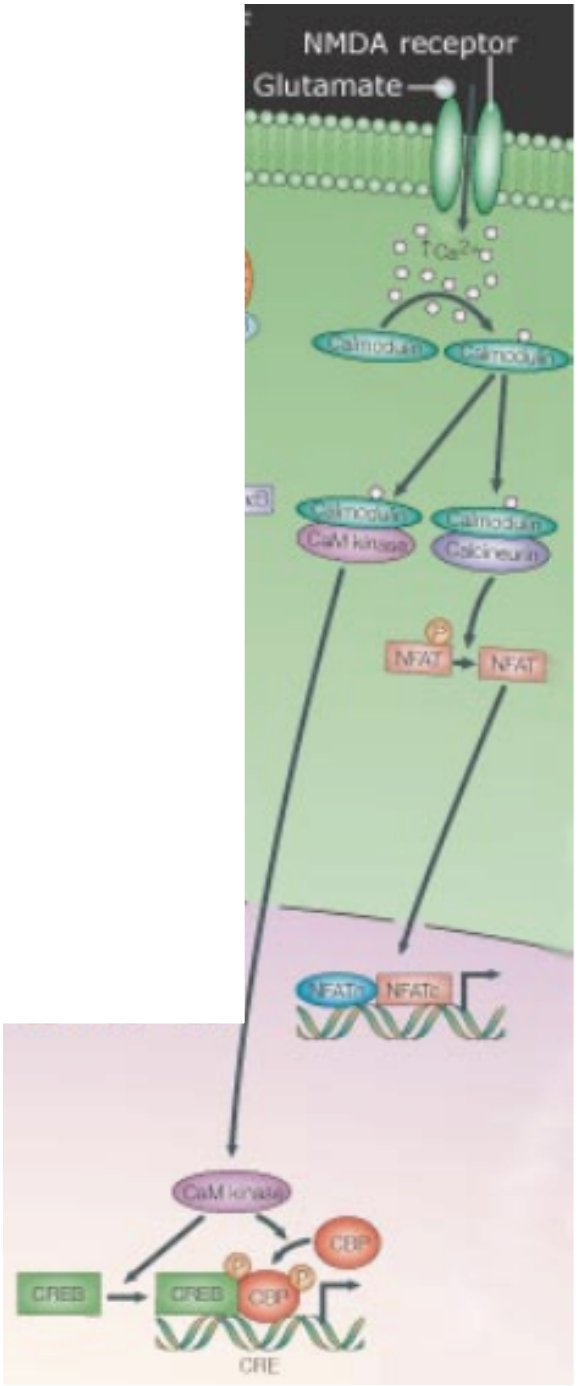
The example shown above is the β-adrenergic receptor, but other examples of G-protein-coupled receptors (GPCRs) include receptors for diverse ligands such as odorants, hormones and neurotransmitters.



2- Enzyme-linked cell-surface receptors

These receptors either have intrinsic enzymatic activity, or they can recruit proteins - either individually or as a complex -that have catalytic function. On binding their ligands, the receptors undergo a conformational change that activates or represses their intrinsic activity, or the activity of the proteins that they recruit.

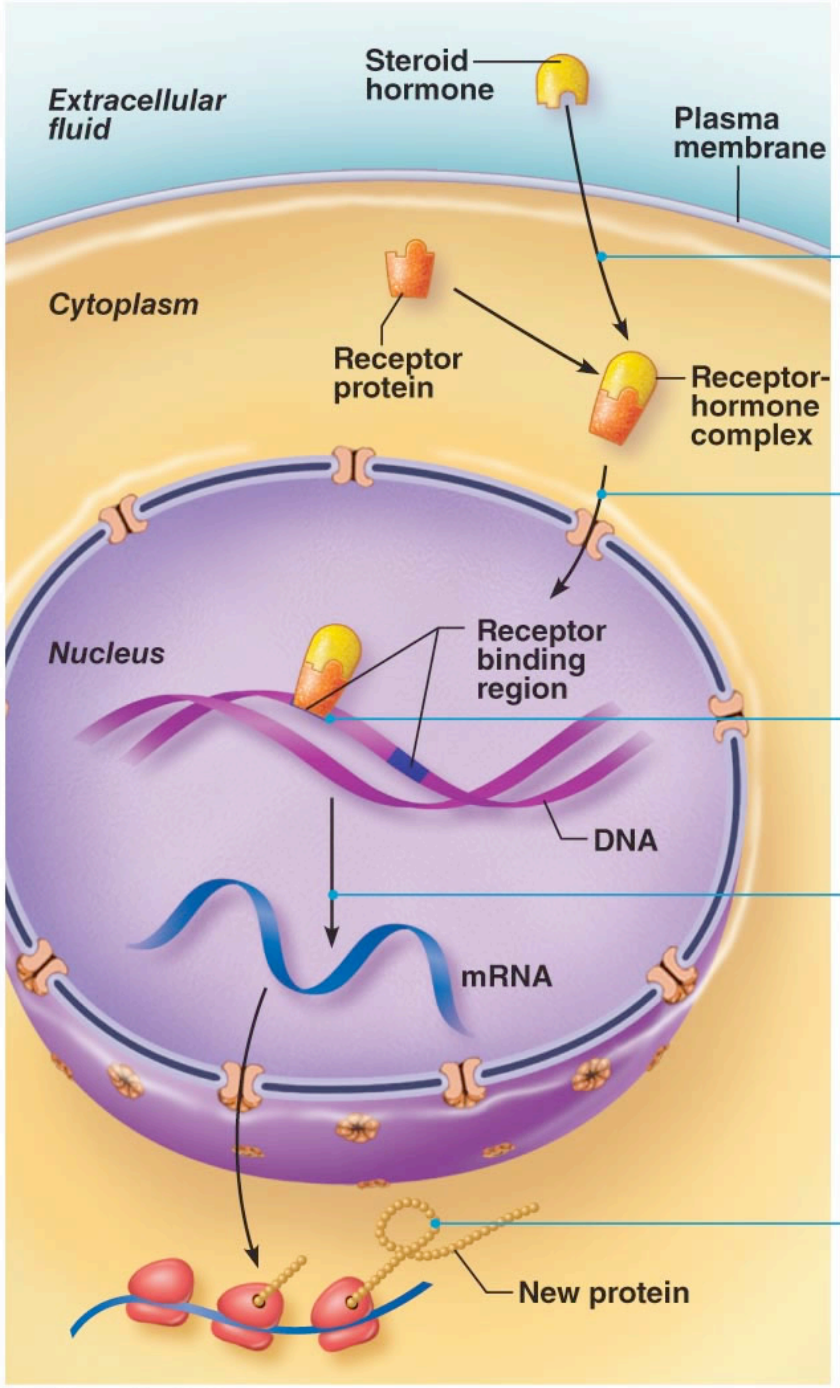
Selected examples include receptor tyrosine kinase receptors (such as the EGF receptor); TGFβ receptors; guanylyl cyclase receptors; receptor tyrosine phosphatases; integrin receptors; T-cell receptors; B-cell receptors; class I and II cytokine receptors (such as the IFNg receptor, shown above); scavenger receptors; Toll-like receptors; and death receptors (such as the TNF receptor).



3- Ligand-gated ion channels

These receptors function as channels that open or close in response to ligand binding. When they are open - or in the active state - they allow the passage of selected ions through the pore. This is a transient process, as the channels become desensitized. Such receptors are often present on post-synaptic membranes.
Examples include glutamate receptors (shown above is the NMDA receptor); and the nicotinic acetylcholine receptor.

4- Intracellular receptors



① The steroid hormone diffuses through the plasma membrane and binds an intracellular receptor.

② The receptor-hormone complex enters the nucleus.

③ The receptor-hormone complex binds a specific DNA region.

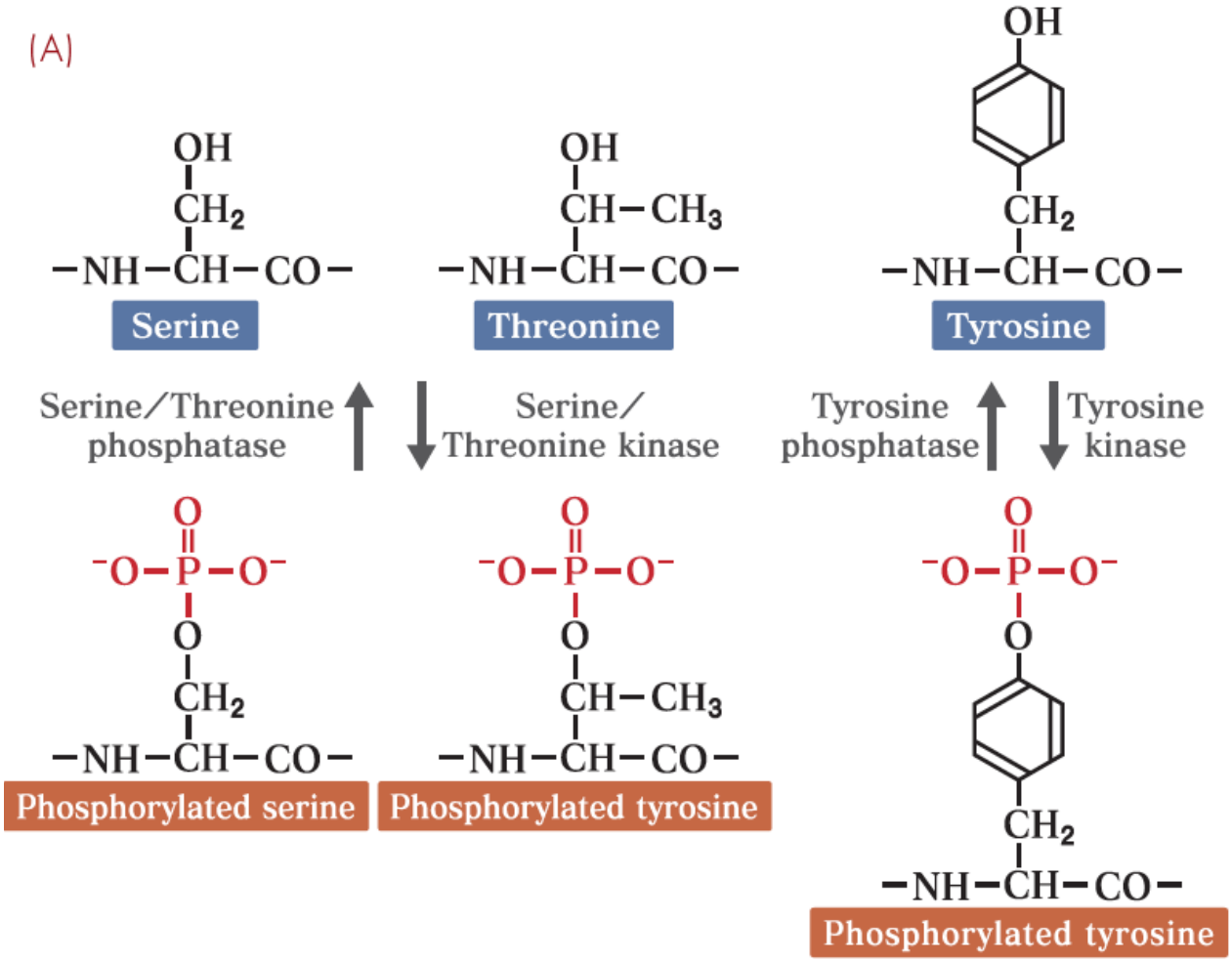
④ Binding initiates transcription of the gene to mRNA.

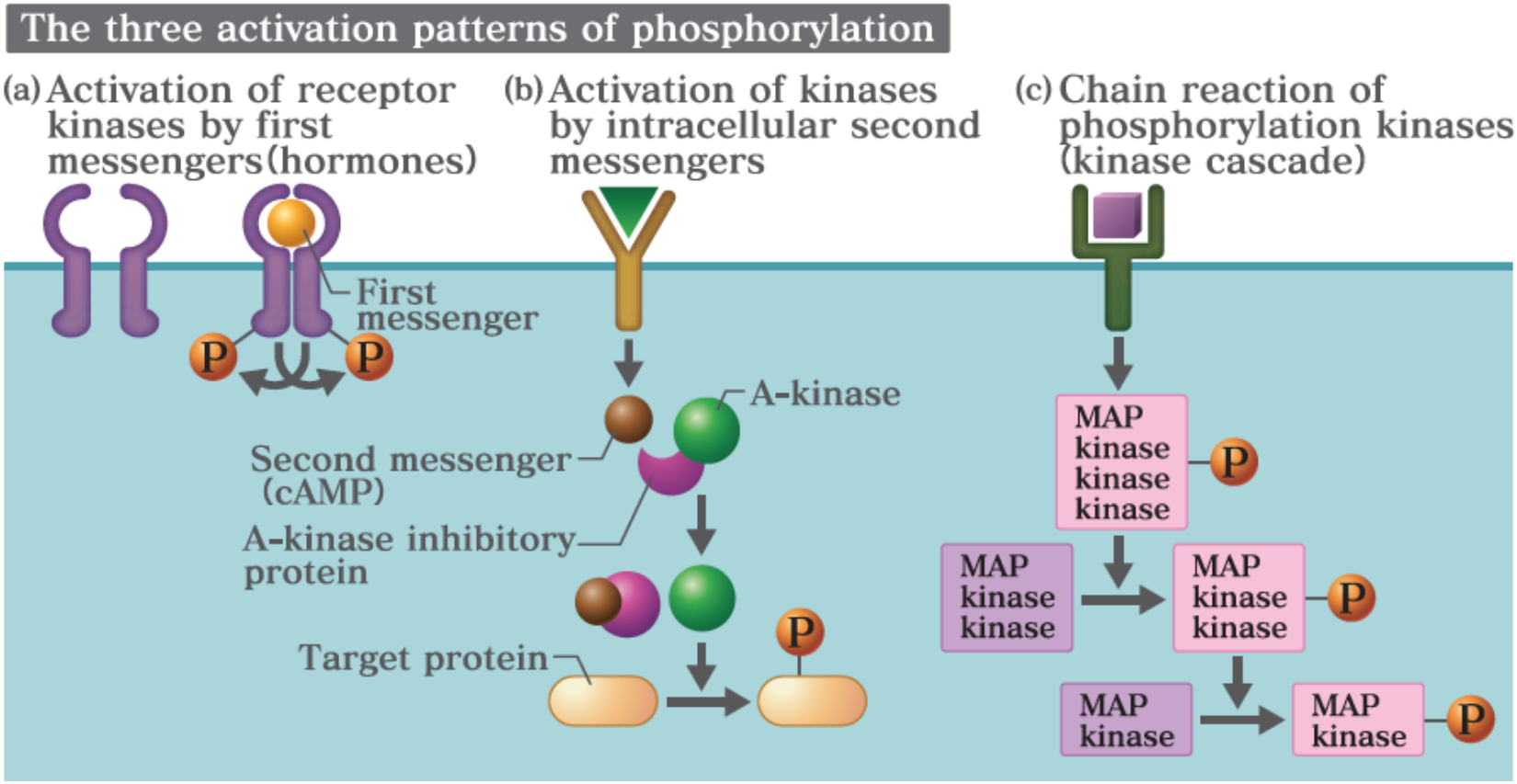
⑤ The mRNA directs protein synthesis.

Phosphorylation and Dephosphorylation of Proteins

The most important chemical modification among the intracellular signal transduction mechanisms is the **phosphorylation** of the side chains of tyrosine, serine and threonine in proteins.

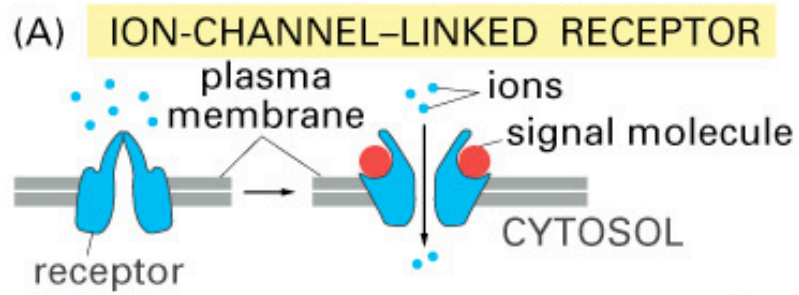
Phosphorylation is one of the most effective ways of **changing the structure of proteins** due to the large size and negative charge of the phosphate group; for the same reason, it is also effective as a **recognition marker** for other proteins.



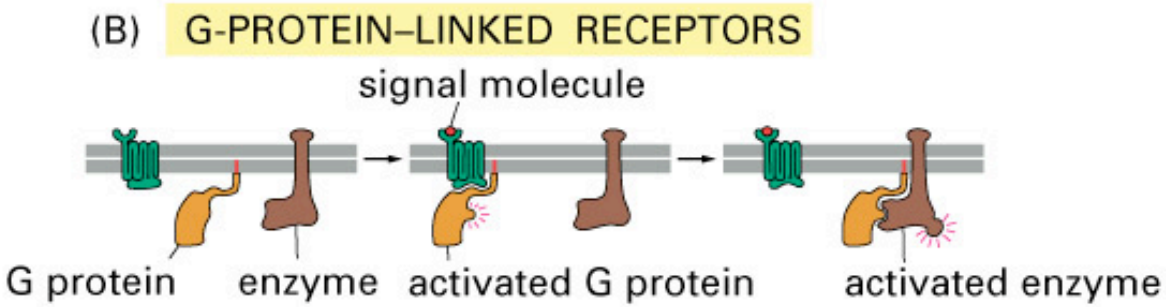


- Receptors themselves may have kinase activity. In such cases, receptors on the plasma membrane bind a signaling molecule (i.e., a first messenger), oligomerize, thereby activating their kinase activity within the cell.
- A kinase may bind with a second messenger located in the cell and become activated. A-kinase, which is activated by cAMP, is normally inactive when bound to an inhibitory protein. When cAMP binds PKA, the kinase and the inhibitor are separated, thereby activating PKA.
- Kinases activated in the cell may induce chain reactions. This phenomenon is called a kinase cascade, since a series of reactions occur in the same way as a waterfall flowing over a cliff. A well-known example is that of MAPK (mitogen-activated protein kinase),signaling cascade, which results in the activation of various transcription factors.

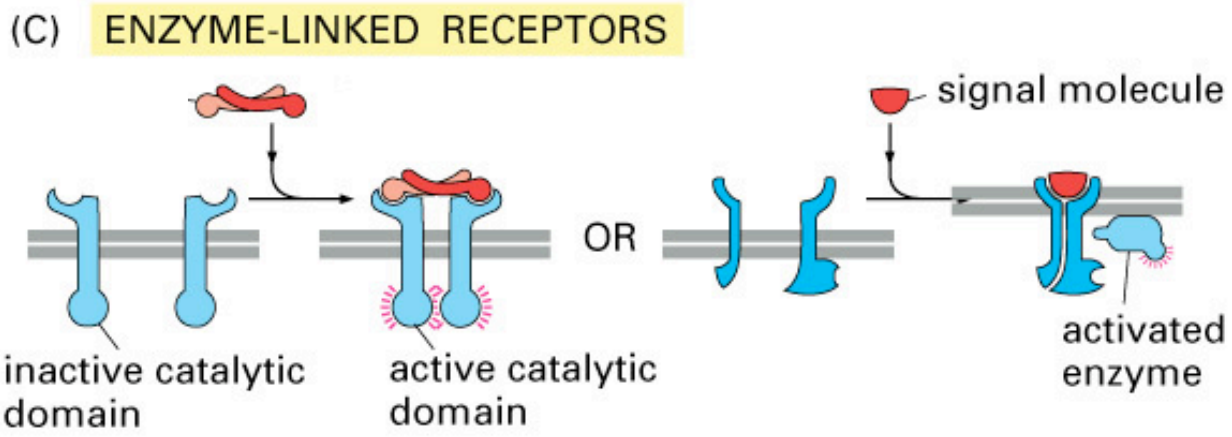
Plasma Membrane receptors fall into 3 main classes



- Major use in the Nervous System
- Ach NICOTINIC receptor



- 7 trans-membrane receptors; Ach MUSCARINIC receptor; involved in **light** and **odorant** detection, detection of certain hormones and neurotransmitters

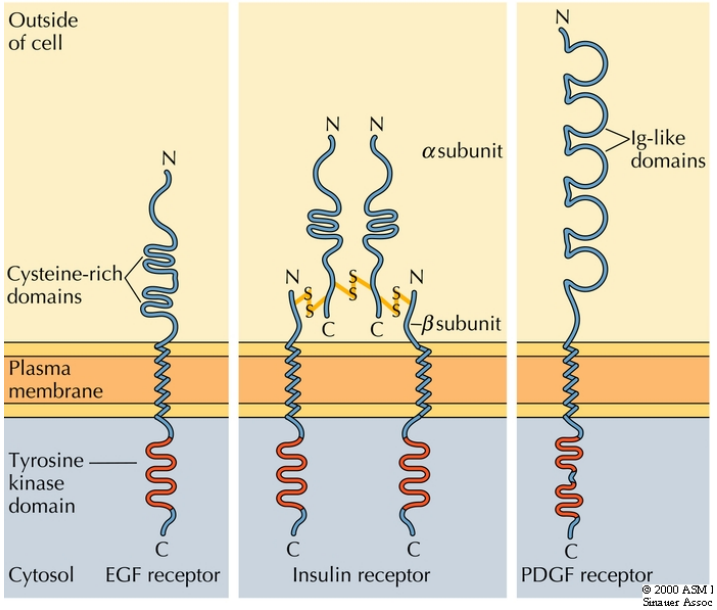


- Genes involved in 30% of human cancers are activated by these types of receptors

Enzyme-linked receptors fall into 3 categories:

1- Tyrosine Kinase Receptors

- Not only a receptor
- Also an enzyme: Tyrosine kinase

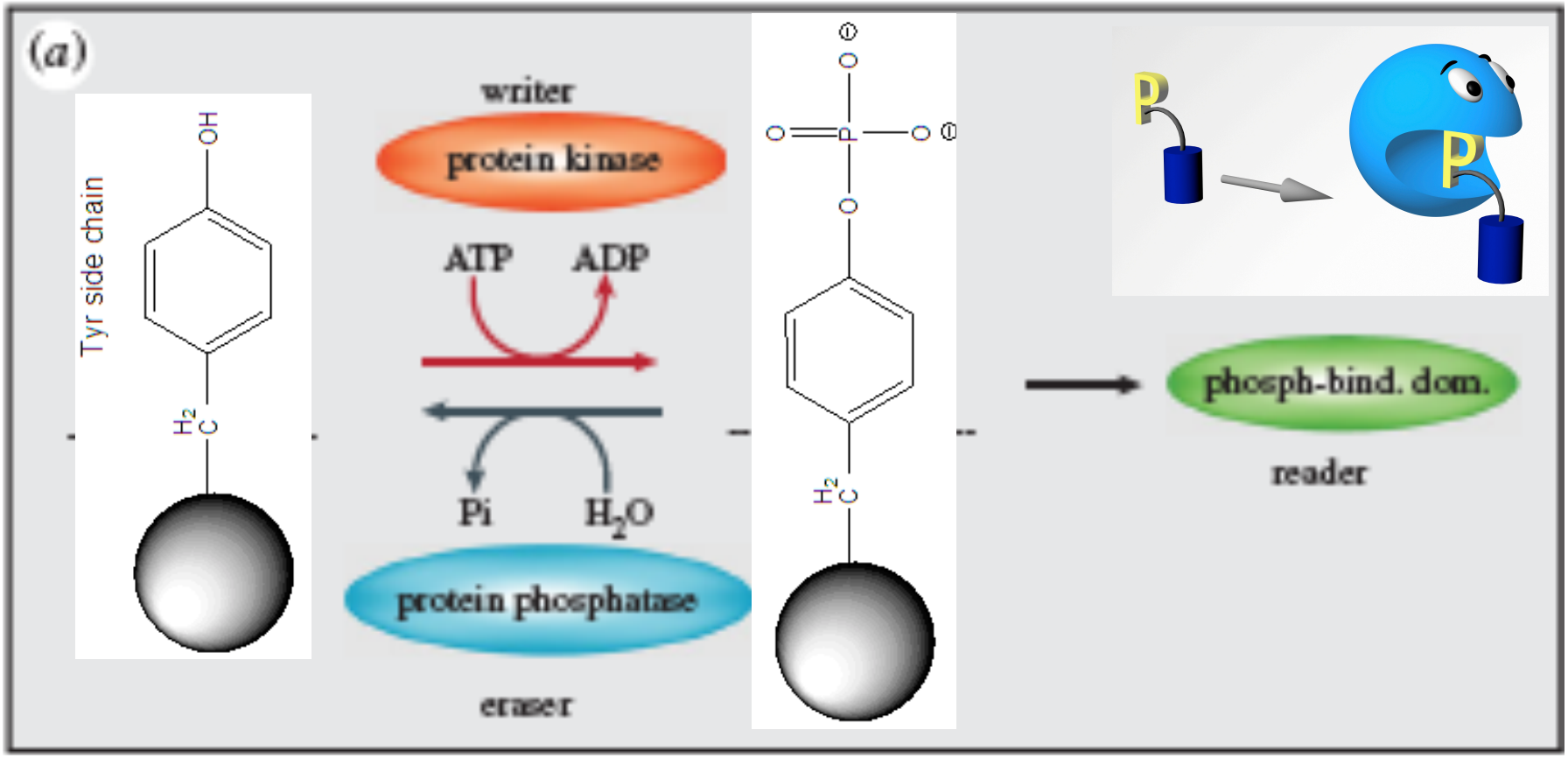


2- TGF-β receptors

3- Cytokine superfamily receptors

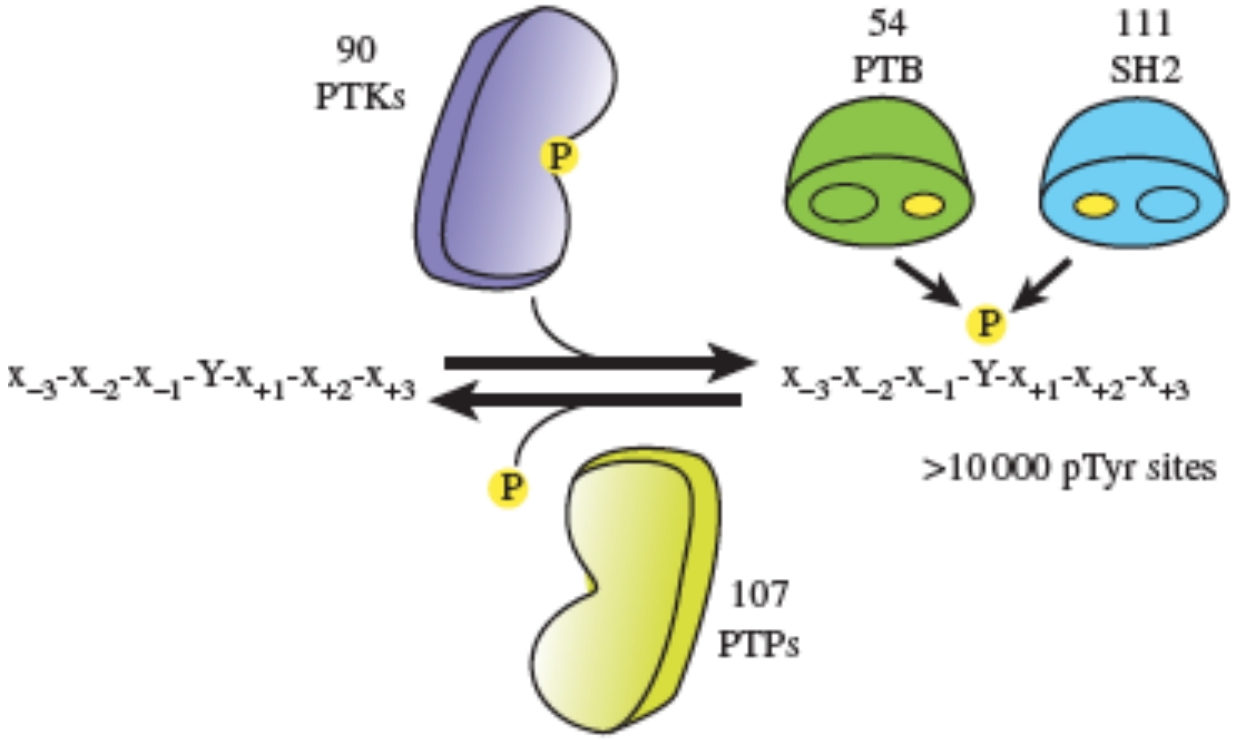
- No catalytic domain
- Interact with non receptor protein-tyrosine kinases
  - Src family
  - JAK family

Key concepts

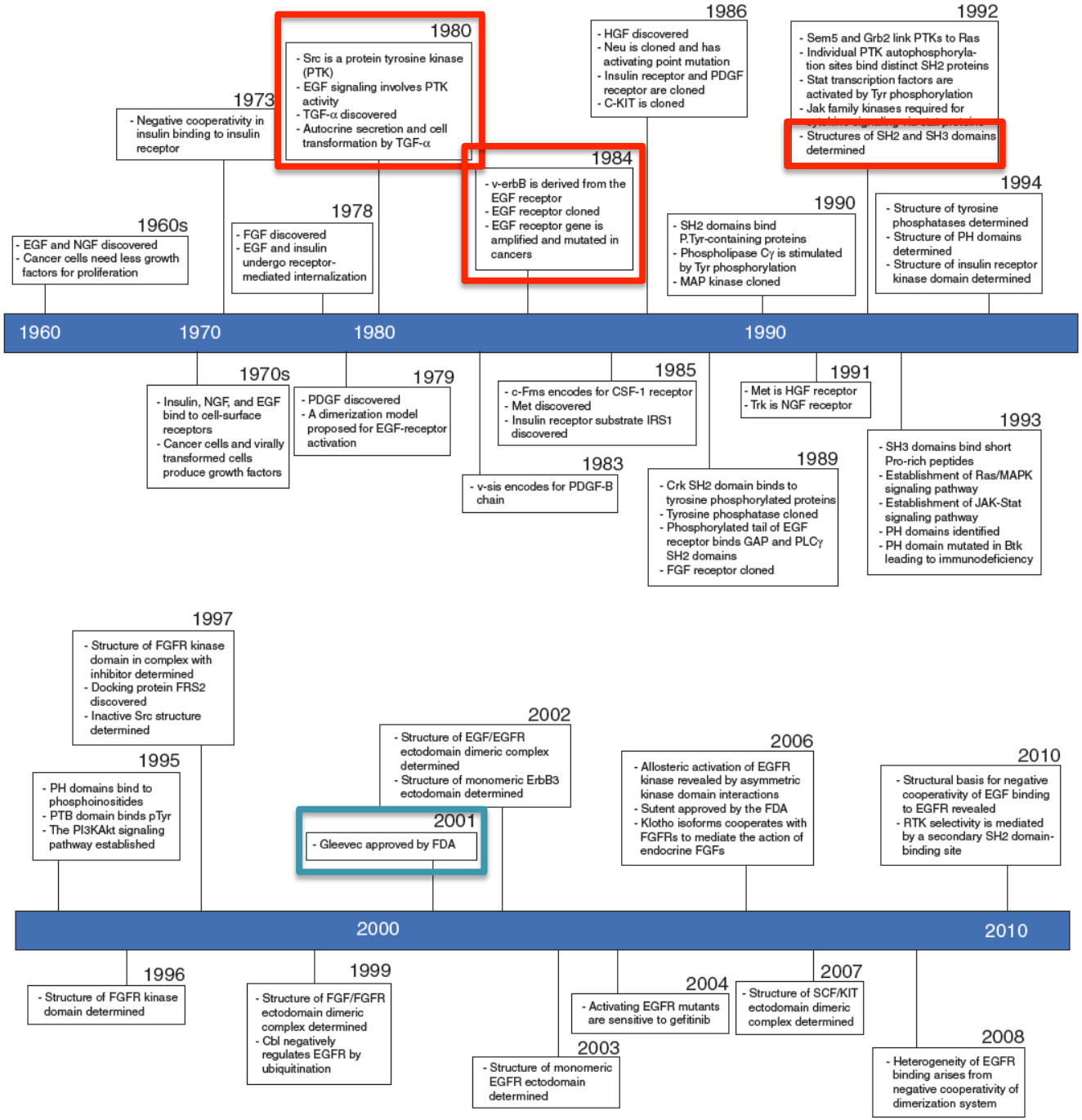


Key concepts

The eukaryotic phosphorylation-based network is operated by a modular kinase-phosphatase-interaction domain toolkit



Key people


1960s
- EGF and NGF discovered
- Cancer cells need less growth factors for proliferation
1973
- Negative cooperativity in insulin binding to insulin receptor
1978
- FGF discovered
- EGF and insulin undergo receptor-mediated internalization
1980
- Src is a protein tyrosine kinase (PTK)
- EGF signaling involves PTK activity
- TGF-α discovered
- Autocrine secretion and cell transformation by TGF-α
1984
- v-erbB is derived from the EGF receptor
- EGF receptor cloned
- EGF receptor gene is amplified and mutated in cancers
1986
- HGF discovered
- Neu is cloned and has activating point mutation
- Insulin receptor and PDGF receptor are cloned
- C-KIT is cloned
1990
- SH2 domains bind P.Tyr-containing proteins
- Phospholipase Cγ is stimulated by Tyr phosphorylation
- MAP kinase cloned
1992
- Sem5 and Grb2 link PTKs to Ras
- Individual PTK autophosphorylation sites bind distinct SH2 proteins
- Stat transcription factors are activated by Tyr phosphorylation
- Jak family kinases required for
- Structures of SH2 and SH3 domains determined
1994
- Structure of tyrosine phosphatases determined
- Structure of PH domains determined
- Structure of insulin receptor kinase domain determined
1960
1970
1980
1990
1970s
- Insulin, NGF, and EGF bind to cell-surface receptors
- Cancer cells and virally transformed cells produce growth factors
1979
- PDGF discovered
- A dimerization model proposed for EGF-receptor activation
1983
- v-sis encodes for PDGF-B chain
1985
- c-Fms encodes for CSF-1 receptor
- Met discovered
- Insulin receptor substrate IRS1 discovered
1989
- Crk SH2 domain binds to tyrosine phosphorylated proteins
- Tyrosine phosphatase cloned
- Phosphorylated tail of EGF receptor binds GAP and PLCγ SH2 domains
- FGF receptor cloned
1991
- Met is HGF receptor
- Trk is NGF receptor
1993
- SH3 domains bind short Pro-rich peptides
- Establishment of Ras/MAPK signaling pathway
- Establishment of JAK-Stat signaling pathway
- PH domains identified
- PH domain mutated in Btk leading to immunodeficiency
1995
- PH domains bind to phosphoinositides
- PTB domain binds pTyr
- The PI3KAkt signaling pathway established
1997
- Structure of FGFR kinase domain in complex with inhibitor determined
- Docking protein FRS2 discovered
- Inactive Src structure determined
2001
- Gleevec approved by FDA
2002
- Structure of EGF/EGFR ectodomain dimeric complex determined
- Structure of monomeric ErbB3 ectodomain determined
2006
- Allosteric activation of EGFR kinase revealed by asymmetric kinase domain interactions
- Sutent approved by the FDA
- Klotho isoforms cooperates with FGFRs to mediate the action of endocrine FGFs
2010
- Structural basis for negative cooperativity of EGF binding to EGFR revealed
- RTK selectivity is mediated by a secondary SH2 domain-binding site
2000
2010
1996
- Structure of FGFR kinase domain determined
1999
- Structure of FGF/FGFR ectodomain dimeric complex determined
- Cbl negatively regulates EGFR by ubiquitination
2003
- Structure of monomeric EGFR ectodomain determined
2004
- Activating EGFR mutants are sensitive to gefitinib
2007
- Structure of SCF/KIT ectodomain dimeric complex determined
2008
- Heterogeneity of EGFR binding arises from negative cooperativity of dimerization system

Tyrosine Kinase Receptors