

Synapses, Receptor Targeting

Synapses, a specialized cell junction between neuron-neuron, neuron-muscle.

Specialized for rapid unidirectional chemical signaling (presynaptic → postsynaptic),

Asymmetric in function and morphology, but ? evolved from a symmetric epithelial-epithelial type junction?

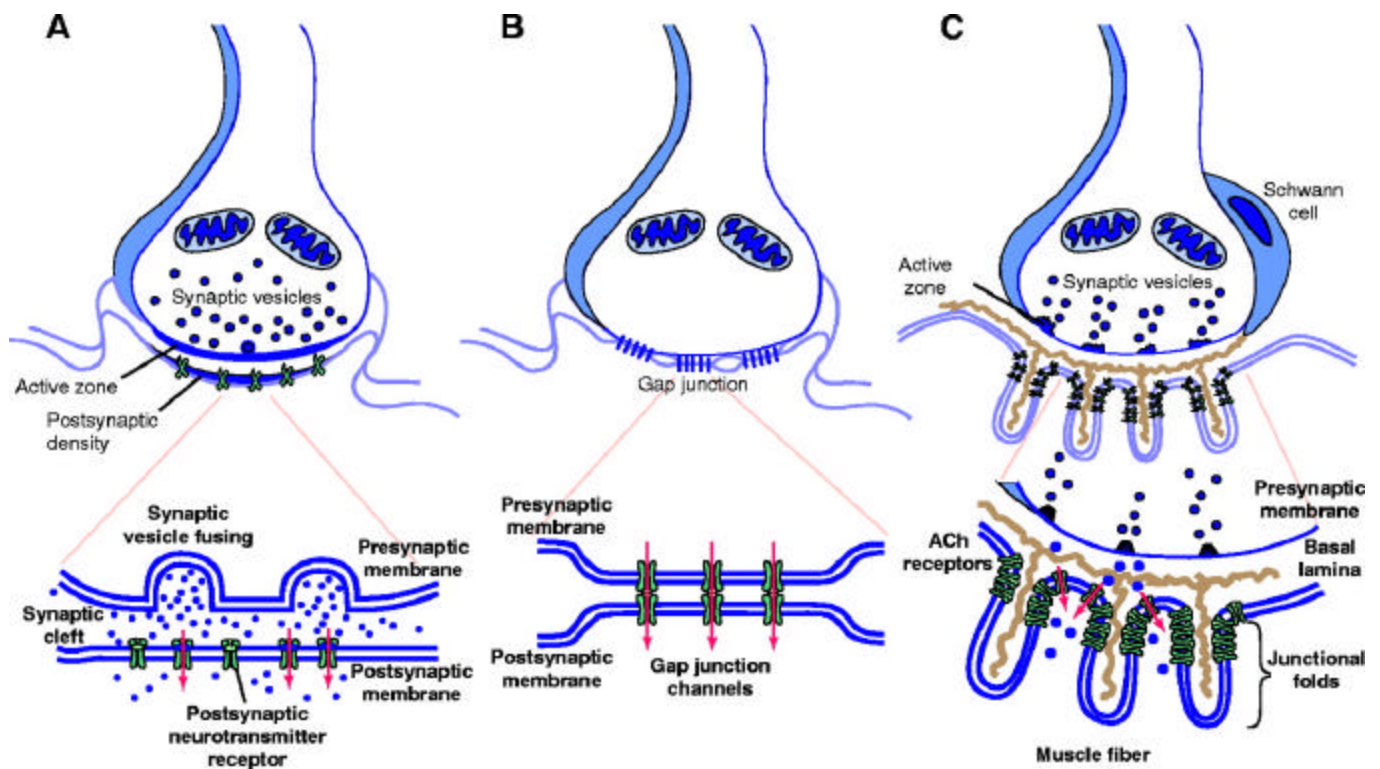
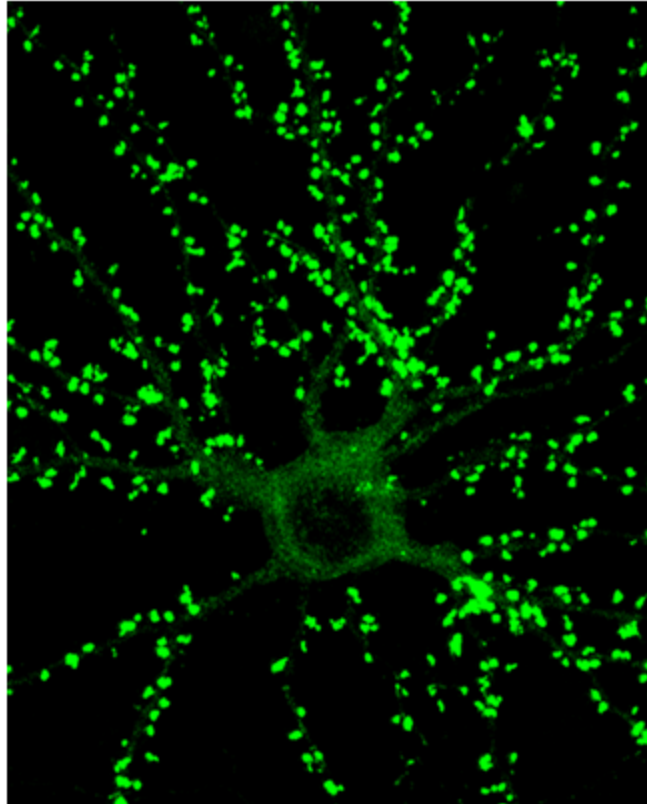


Figure. Schematic representation of interneuronal and neuromuscular synapses. Synapses are asymmetric communication junctions formed between two neurons, or, at the neuromuscular junction (NMJ), between a neuron and a muscle cell. Chemical synapses enable cell-to-cell communication via secretion of neurotransmitters, whereas in less abundant electrical synapses signals are transmitted through gap junctions, specialized intercellular channels that permit ionic current flow. **(A)** At most interneuronal synapses, neurotransmitters are stored in synaptic vesicles and are released after synaptic vesicle fusion at the active zone (an event that is triggered by an action potential followed by a rapid influx of calcium into the presynaptic terminal). Neurotransmitter receptors and accessory molecules accumulate in the postsynaptic membrane directly opposite the active zone in a postsynaptic membrane specialization known as the postsynaptic density. **(B)** At electrical synapses, gap junctions between pre- and postsynaptic membranes permit current to flow passively through intercellular channels. In addition to ions, other molecules that modulate synaptic function (such as ATP and second messenger molecules) can diffuse through gap junctional pores. Electrical synapses synchronize electrical activity among populations of neurons. **(C)** At the mature NMJ, pre- and postsynaptic membranes are separated by a synaptic cleft containing extracellular proteins that form the basal lamina. Synaptic vesicles are clustered at the presynaptic release site, transmitter receptors are clustered in junctional folds at the postsynaptic membrane, and glial processes surround the nerve terminal.

Neuronal synapses

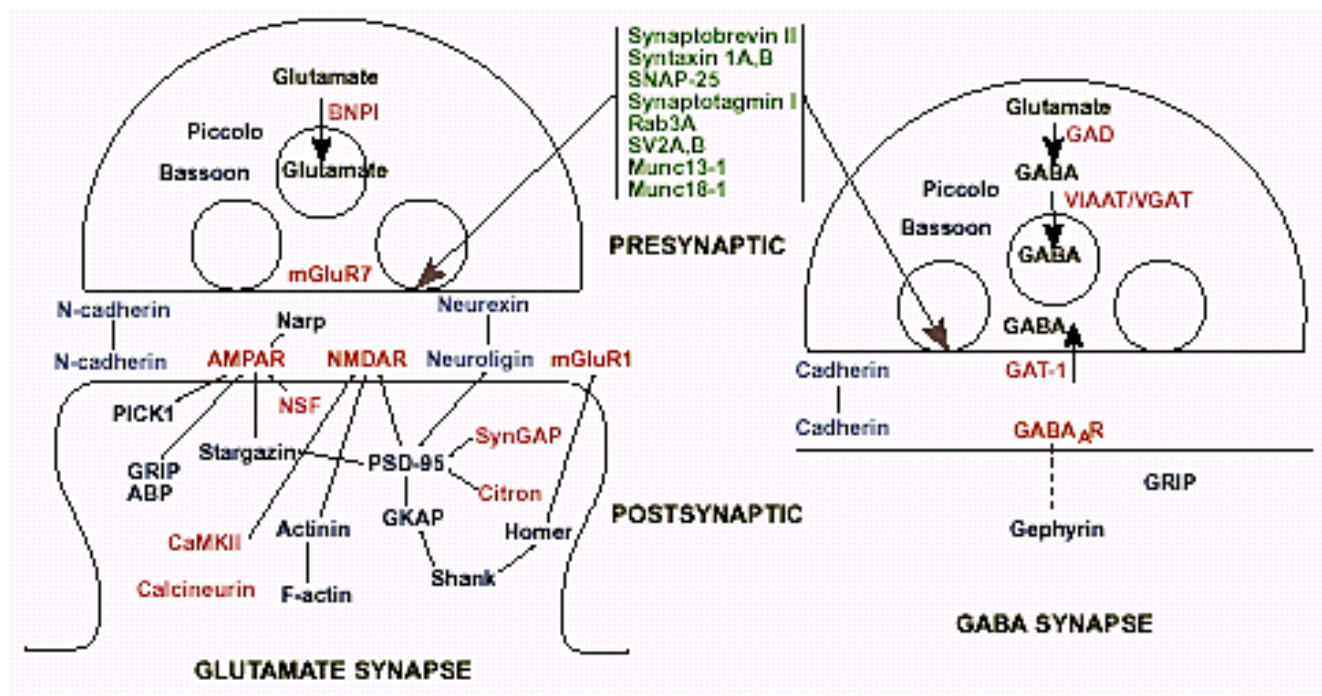


Heterogeneity within a single postsynaptic cell

between neurotransmitters e.g. glutamate and GABA synapses are segregated on same cell

within same neurotransmitter (e.g. GABA(A)R $\alpha 1$, $\alpha 6$, $\beta 2/3$, $\gamma 2$ concentrated in GABAergic Golgi-cerebellar granule synapses, while δ subunit absent from synapses and present abundantly in extrasynaptic membranes (Nusser et al (1998) J. Neurosci 18: 1693-1703); GluR4 and mGluR1a present only at auditory nerve synapse on basal dendrites of fusiform cell of dorsal cochlear nucleus, but not at parallel fiber synapses on apical dendrites (Rubio and Wenthold (1997) Neuron 18: 939-950) [immunogold studies]

In addition to different neurotransmitter receptors, different synapses have distinct and characteristic molecular composition



Molecular heterogeneity and similarity of glutamate synapses and GABA synapses.

Components are color coded according to function. Red, neurotransmitter receptors; pink, enzymes; blue, scaffolds; turquoise, cell adhesion proteins; green, vesicle fusion machinery. Gray lines, protein-protein interactions. Not all synaptic components are shown.

In general the presynaptic components (required for SV exocytosis) are more similar between different kinds of synapses than the postsynaptic. Classical cell adhesion proteins such as cadherin are also found in both inhibitory and excitatory synapses. Glutamate receptors and their binding proteins (e.g. PSD-95) are often used as “markers” for glutamatergic synapses, and GABA_A receptors and gephyrin as markers for GABAergic synapses.

Neurotransmitter receptors are concentrated at their respective postsynaptic sites:

e.g. NMDARs and AMPARs in the PSD of glutamatergic synapses,
GlyRs at glycinergic synapses,
GABA(A) receptors at GABAergic synapses.

[What experimental approaches can be used to investigate the molecular mechanisms underlying the specific targeting of receptors to postsynaptic sites?]

Looking for proteins interacting with (neurotransmitter) receptors

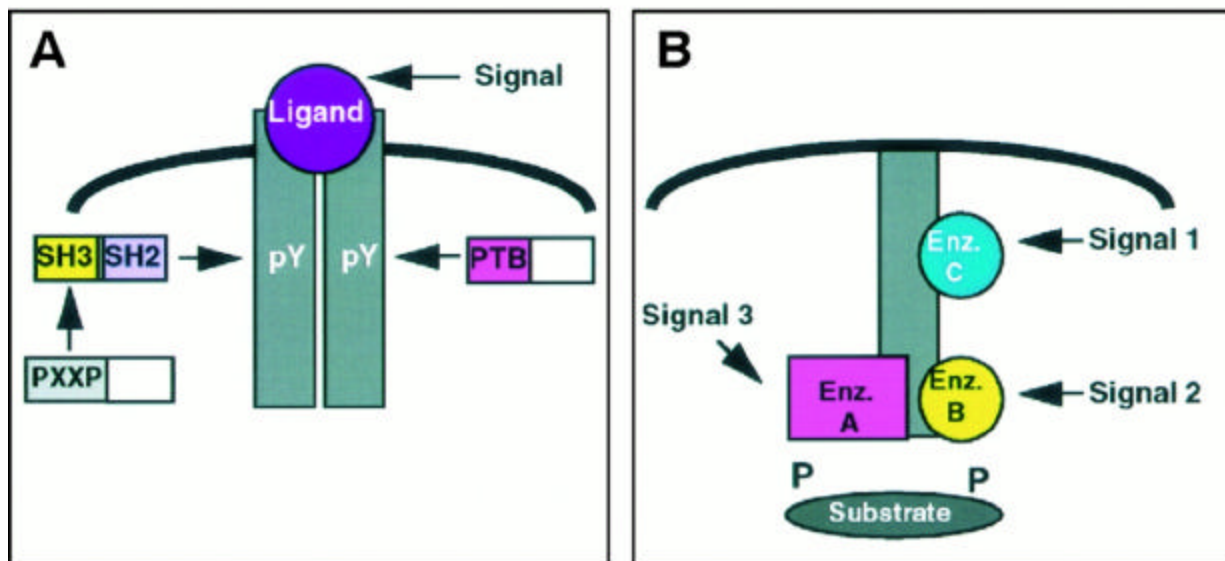
	Example	Advantages	Disadvantages	Bias
<i>Biochemistry Conventional</i>	PSD purif'n: eg PSD-95, densin (Mary Kennedy) Receptor copurification: AchR + rapsyn, GlyR + gephyrin	Straightforward, Informs about size, biochemical properties	Impurities eg PSD Cloning requires peptide seq (but now mass spec); labor intensive ; synapses heterogeneous; receptors not abundant (except Torpedo E.O.) No function No localization	Abundant proteins
<i>Antibody screening</i>	Raise antibodies against receptor enriched preparation such as PSD; eg SAP90/PSD-95; picollo	Screen for synaptic localization prior to cloning; Obtain useful Ab reagent	Labor intensive ; Antibodies can be used to screen for cDNA clone (but not trivial); Impurities of prep	Abundant proteins; Immunogenic proteins; Non-genome coded epitopes
Affinity purification	Purify receptor binding prot;	Informs about protein-protein interaction; affin purification can work for ternary complex	Need purified affinity ligand (ie cloned receptor, abundantly expressed) Interacting protein needs to be soluble and "folded" May get false positive	Abundant proteins; Biased by choice of affinity ligand
<i>Yeast two-hybrid system (expression cloning)</i>	Screen for direct receptor interacting proteins (screen library for binding partners)	Informs about protein-protein interaction; less labor intensive ; Obtain cDNA directly; Amenable to high thruput screening; Does not require solubility of ligand or interactor	Doesn't always (usually) work False positives Requires known cloned protein as bait (affinity ligand), but Y2H does not need purification of ligand; Can only isolate direct bimolecular interactors;	Abundant mRNAs; Intracellular domains;
<i>Forward genetics</i>	Screen for synaptic transmission phenotypes; Cell biological screens with GFP-tagged receptor	Gives you "function" at cellular and organismal level; Not biased by abundance or biochemical properties	Needs sophisticated phenotypic screen; Could be very indirect; Screening labor intensive ; Cloning easier in post-genomic era; Has to be done in genetic organism	Biased by phenotype, non-lethality, redundancy

Signaling complexes

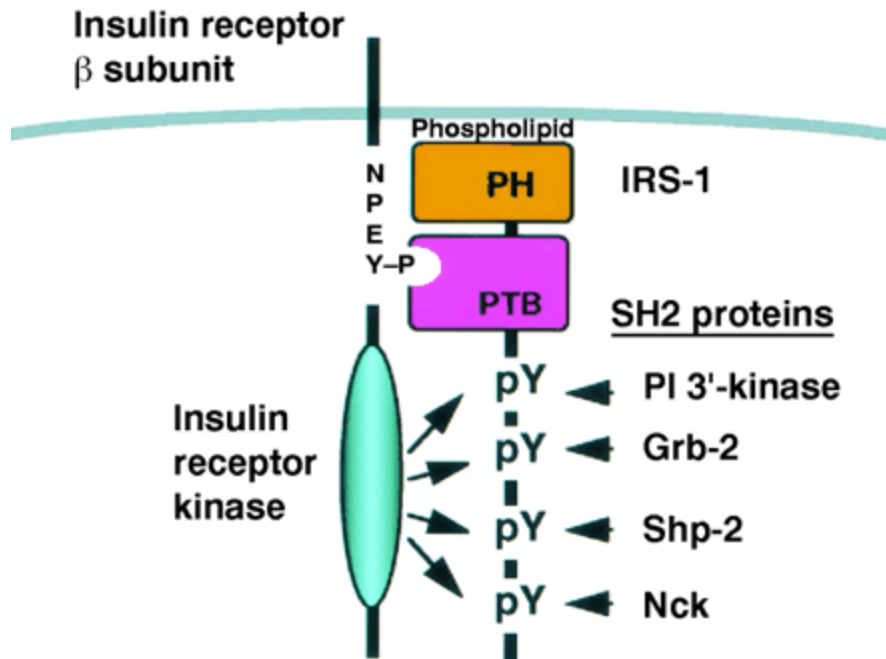
The concept of efficiency and specificity of signaling via the organization of protein complexes

Scaffold proteins organize “signaling cascades”

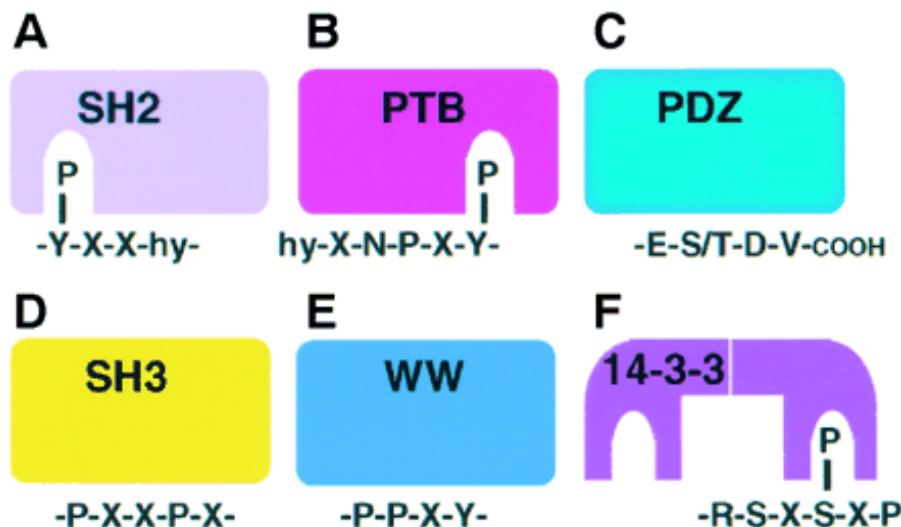
Bring into physical proximity substrate and enzyme, or a series of enzymes working in a pathway – increases speed (no need for diffusion) and confers specificity of coupling.



Two mechanisms for recruiting or localizing signal transduction components. (A) Assembly of modular signaling molecules on an activated receptor tyrosine kinase. An extracellular signal input drives dimerization and autophosphorylation of the receptor and leads to the recruitment of cytoplasmic proteins that contain various protein modules that bind to phosphotyrosine motifs. pY, phosphotyrosine. (B) A localized signaling complex of three anchored signaling enzymes. Each enzyme is inactive when bound to the anchoring protein but is released and activated by different signals. Enz., enzyme. Example is AKAP, where enzyme A is protein kinase A, and enz B is often protein phosphatase PP1 or PP2A. AKAPs in addition bind to receptors/ion channels or other subcellular structures, thereby targeting PKA and opposing phosphatases to specific substrates.



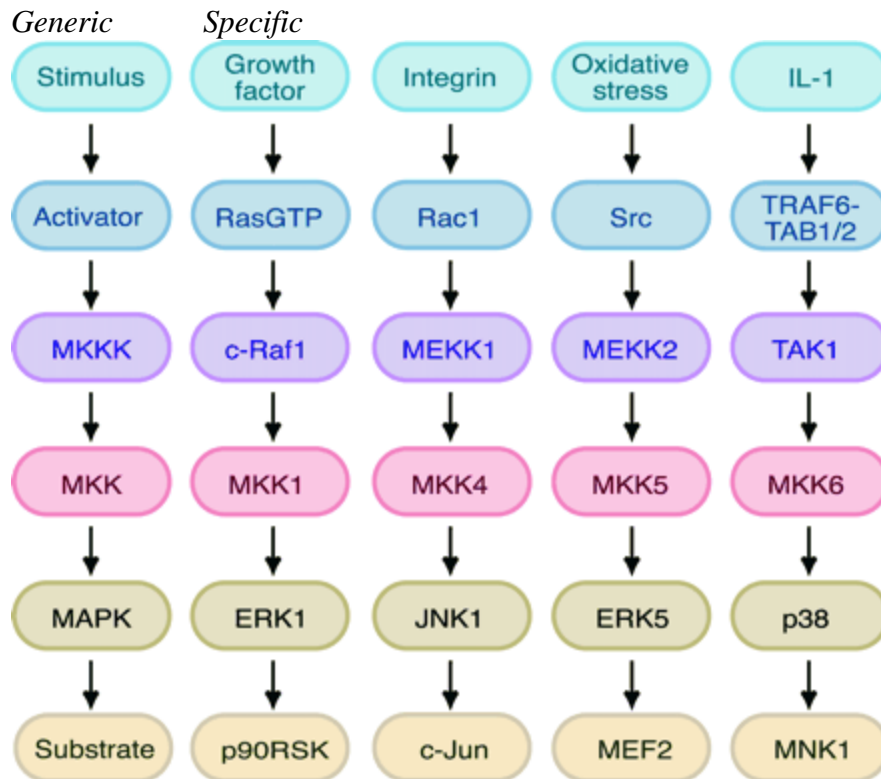
A receptor binding adaptor protein can be a scaffold protein. The IRS-1 docking protein contains an NH_2 -terminal PH domain that potentially mediates interactions with the membrane and a PTB domain that binds a specific juxtamembrane Tyr autophosphorylation site in the insulin receptor. The kinase domain of the activated insulin receptor phosphorylates Tyr residues in IRS-1 that act as docking sites for multiple SH2-domain signaling proteins.



Protein modules for the assembly of signaling complexes. Several modular domains have been identified that recognize specific sequences on their target acceptor proteins. These sequences, in single-letter code, are indicated for (A) SH2 domains, (B) PTB domains, (C) PDZ domains, (D) SH3 domains, (E) WW domains, and (F) 14-3-3 proteins. hy indicates hydrophobic residues.

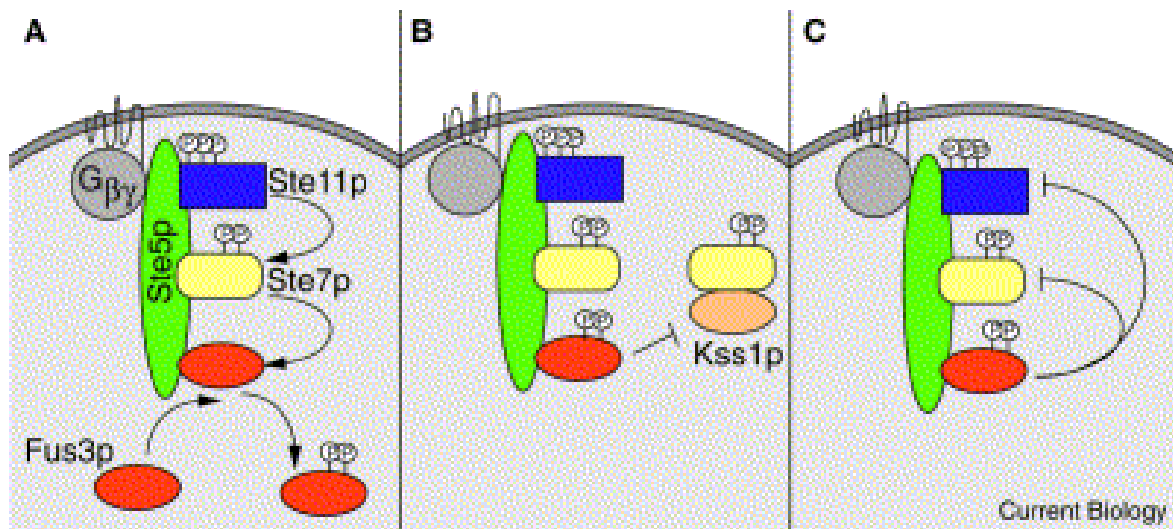
Example of specific MAP kinase cascades organized by scaffold proteins

MAPK phosphorelay systems.



Representative modules of pathway connections for the respective MAPK phosphorelay systems. There are multiple component MKKKs, MKKs, and MAPKs for each system. For example, there are three Raf proteins (c-Raf1, B-Raf, A-Raf), two MKKs (MKK1 and MKK2), and two ERKs (ERK1 and ERK2) that can compose MAPK phosphorelay systems responsive to growth factors. Our understanding of the connections within the MAPK systems is incomplete and often controversial and continues to be defined in different cell types. p90RSK, 90-kD ribosomal protein S6 kinase, Src, an oncogenic tyrosine kinase; MEF2, myocyte enhancer factor 2; IL-1, interleukin 1; TRAF6, tumor necrosis factor receptor-associated factor 6; TAK1, transforming growth factor- β -activated protein kinase 1; and MNK1, MAPK-interacting kinase 1.

How do you get specificity?

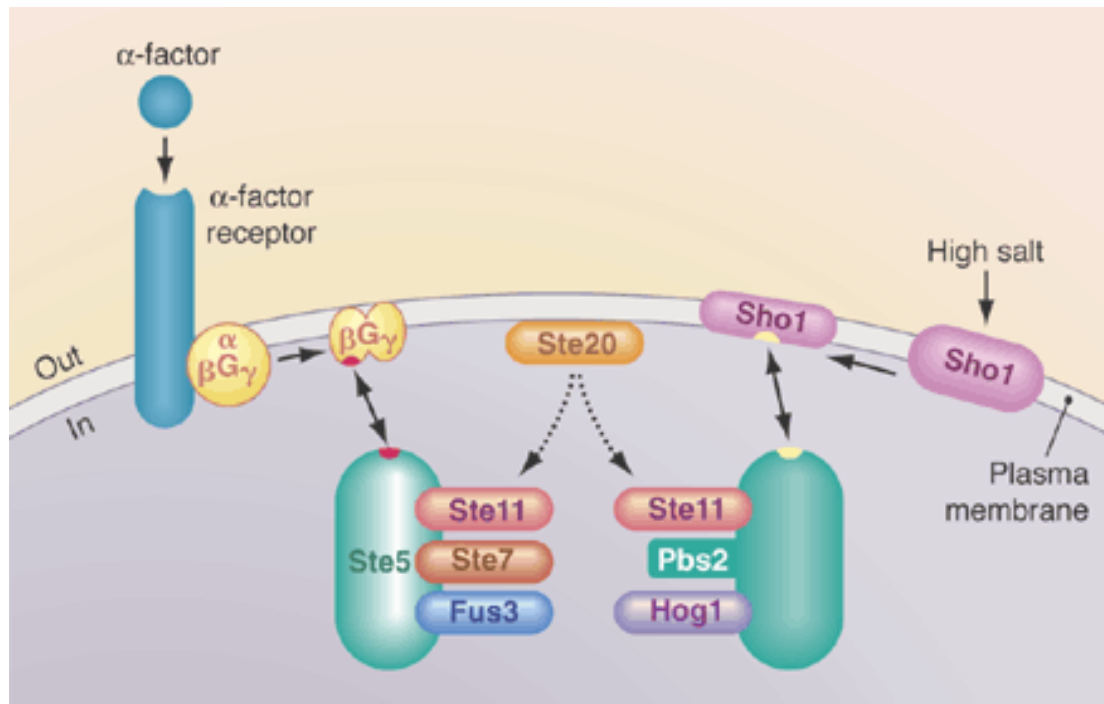


Distinct mechanisms contribute to pathway specificity. (A) Most important, scaffolds interact with components of MAP kinase cascades and channel the activity towards specific MAP kinases. (B) Activated Fus3p (MAP kinase) inhibits activation of Kss1p (another MAP kinase) after prolonged exposure to α -factor, thereby reducing crosstalk. (C) Negative feedback loops operate on the upstream kinases Ste7p and Ste11p. These feedback mechanisms may temporally limit MAP kinase activation, and possibly prevent crosstalk between different MAP kinase pathways.

At least 4 MAP kinase cascades in yeast, including some that share component kinases.

Yeast mating (pheromone response) and osmolality response involve two distinct MAPK pathways that share two common kinases (Ste 20, and Ste11).

Three kinases of the respective cascades are attached to a protein scaffold: Ste5 (mating) and Pbs2 (osmolality).

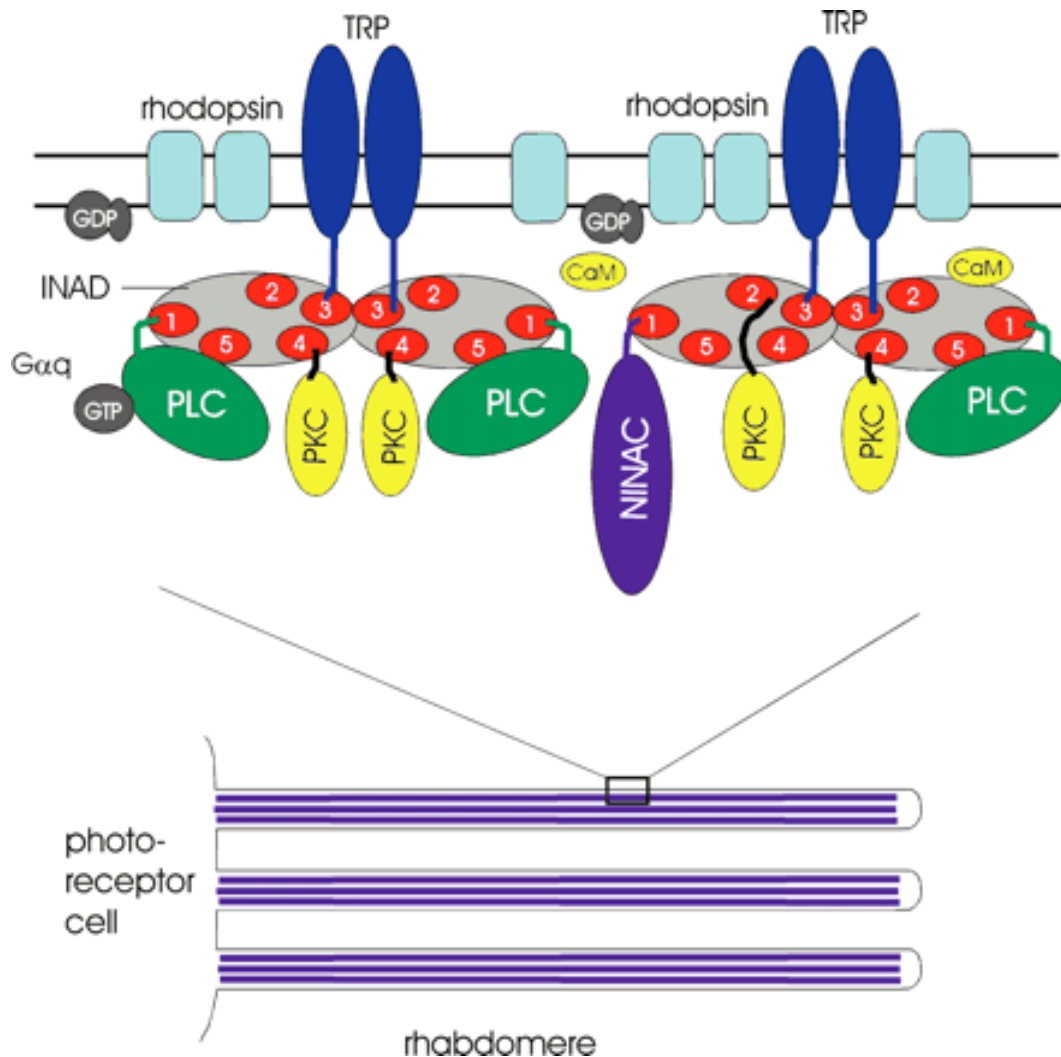


Activation of two MAPK cascades in yeast. (Left) The mating cascade is activated when the cell's α -factor receptor receives the α -factor pheromone from an expectant partner. The receptor is associated with a G protein, and interaction with pheromone frees the G $\beta\gamma$ protein (also called Ste4/18). G $\beta\gamma$ exposes a surface that binds to the scaffold Ste5. **(Right)** The osmolality cascade is activated when the membrane protein Sho1 senses a high salt concentration in the medium. Under high-salt conditions, Sho1 evidently exposes a surface (indicated by the change in shape of Sho1) that binds to the scaffold Pbs2 (which is both a kinase and a scaffold in that it binds to Ste11 and Hog1). **(Center)** Ste20 is an active kinase tethered to the membrane (7, 8). G $\beta\gamma$ recruits Ste5 to the membrane, where Ste20 triggers the mating cascade. Sho1 recruits Pbs2 to the membrane, where Ste20 triggers the osmolality cascade. The Pbs2 scaffold has two bound kinases and an intrinsic kinase domain, as indicated. Fus3 and Hog1 are called MAPKs, Ste7 and Pbs2 (the intrinsic kinase) are MAPKKs, and Ste11 is a MAPKKK. By extension, Ste20 is sometimes called a MAPKKKK.

Specificity comes from recruitment of the right scaffold protein to the membrane, where the cascade is initiated by Ste20 kinase.

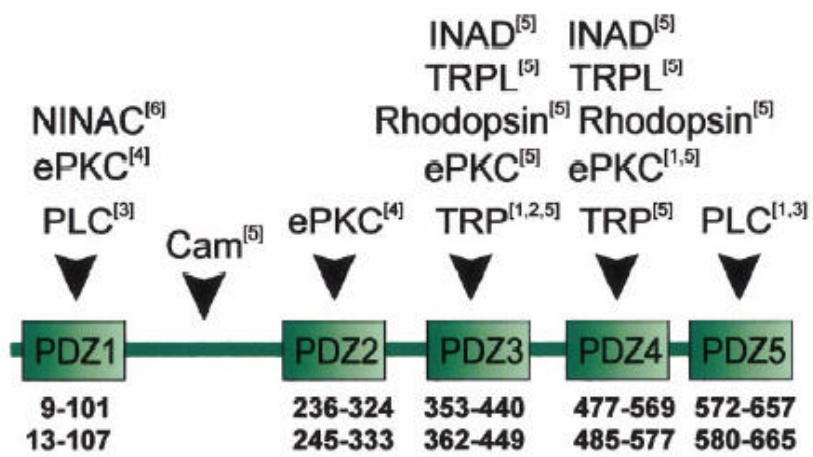
A *hybrid scaffold protein* (“diverter”) that fuses *mutant Ste5 scaffold* [defective in Ste 7 binding and hence unable to mediate pheromone response] to *Pbs2 scaffold* mediates an osmolality response to mating pheromone. [Park et al Science (2003) 299, 1061.]

Organization of the phototransduction complex by INAD in *Drosophila* photoreceptors

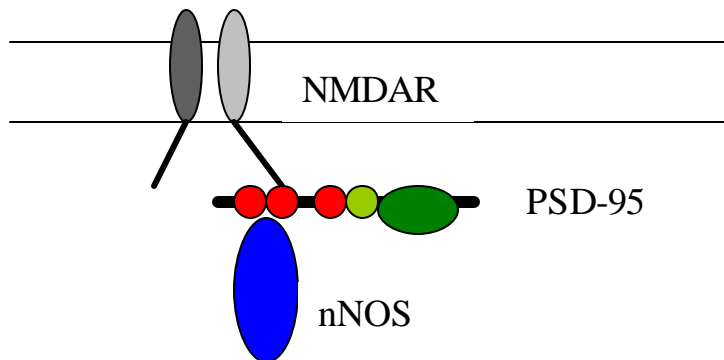


INAD, a scaffold protein with 5 PDZ domains, binds to multiple key components of the phototransduction cascade in *Drosophila* photoreceptor rhabdomeres.

A schematic of actin-filled microvilli of rhabdomeres is shown at bottom. INAD is depicted beneath the plasma membrane multimerized via its PDZ3/PDZ4 domains. The major interacting proteins are shown binding to specific PDZ domains of INAD. G protein is depicted in dark gray, associated with the membrane in its GDP-bound form. Activated (GTP-bound) Gαq subunit interacts with phospholipase C (PLC). PDZ domains (numbered) are represented by red ovals, F-actin by purple lines.



Example of PSD-95 mediated “functional complex” of NMDAR and nNOS



nNOS is calcium/calmodulin regulated enzyme
Specifically coupled to calcium entry through NMDA receptors

Binds specifically to PDZ2 of PSD-95 (PSD-95 also binds to NMDA receptors via PDZ1 and PDZ2). PSD-95 is believed to physically link and functionally couple nNOS to NMDA receptor.

nNOS-PSD-95 interaction: an example of a PDZ-PDZ interaction, rather than involving C-terminus

Antisense knockdown of PSD-95 reduces NO production induced by NMDA receptor activation in neuron culture.

Inhibition of PDZ2 interactions of PSD-95 inhibits nNOS activation by NMDA receptors and reduces excitotoxicity following ischemia/stroke in culture and in rodents (M. Tymianski and colleagues)