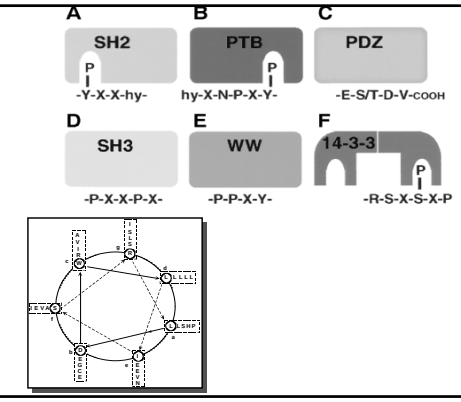
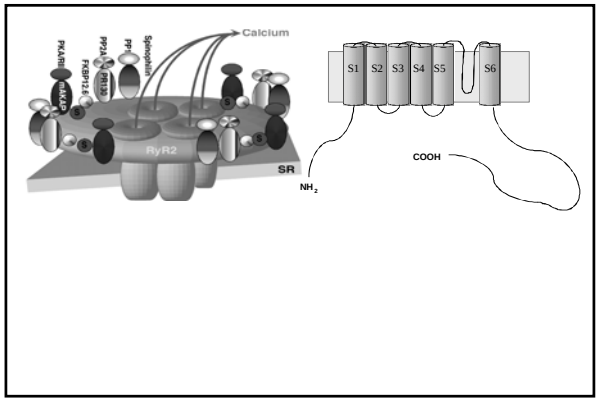
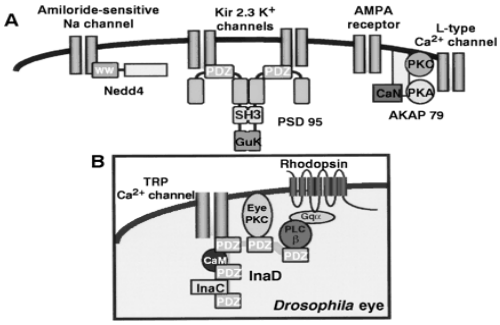
ION CHANNEL MACROMOLECULAR COMPLEXES







PDZ domains; recognition of short peptides with a COOH terminal hydrophobic residues and a free carboxylate group at the C-terminus of ion channels.
X (S/T) X Φ -stop
Φ-large hydrophobic residue

Originally recognized as ~ 90 amino acid long repeated sequence in the synaptic protein PSD-95/SAP90 (PSD for postsynaptic density/synapse associated protein; the Drosophila septate junction protein Discs-large and the epithelial tight junction protein ZO-1)

Can be detected frequently among GPCR

Recent study suggested that the C-termini of no less than 50 intracellular and membrane proteins have a high affinity for the PDZ domains of PSD-95, several of which are involved in G protein mediated signaling.
Considerable promiscuity between PDZ domains and their ligands and the identification of the right interaction partner for any putative PDZ ligand is not always straightforward.

Interactions can promote clustering of transmembrane receptors at specific subcellular sites. The domains play an important role in the spatial organization of voltage and ligand gated ion channels at synapses.

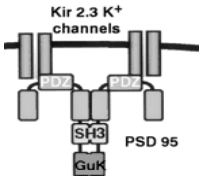
The Shaker type K^+ channels and all three classes of glutamate receptors are recognized by distinct PDZ domain proteins; specificity is conferred by ligand residues at the -2 to -4 position relative to the COOH terminus and may be regulated by phosphorylation because the -2 residue of the PDZ binding site is often a hydroxy-amino acid.

X (S/T) X Φ -stop
Φ-large hydrophobic residue

TABLE 1 Classification of PDZ domains according to specificity for C-terminal peptides[a]

Class	C-term sequence	Interacting protein	PDZ domain	References
Class I				
X-S/T-X-V	E-S-D-V	NMDAR2A, B	PSD-95 (PDZ2)	Kornau et al 1995
	E-T-D-V	Shaker channel		Kim et al 1995
	Q-S-S-V	Citron	PSD-95 (PDZ3)	Zhang et al 1999
	Q-T-S-V	CRIPT		Niethammer et al 1998
	T-T-R-V	Neuroligin		Irie et al 1997
	E-T-X-V	PMCA4b	PSD-95 (PDZ1/2/3)	E Kim et al 1998
	E-S-L-V	Voltage-gated sodium channel	Syntrophin	Gee et al 1998
X-S/T-X-L	Q-T-R-L	GKAP	Shank	Naisbitt et al 1999
	S-S-T-L	mGluR5		Tu et al 1999
	D-S-S-L	β2-adrenergic receptor	NHERF (PDZ1)	Hall et al 1998
	P-T-R-L	GRIAA		Hall et al 1999
	D-T-R-L	CFTR		Wang et al 1998
Class II				
X-Φ-X-Φ	E-Y-Y-V	Neurexin	CASK	Hata et al 1996
	E-F-Y-A	Syndecan	CASK, syntenin	Hsueh et al 1998
	E-Y-F-I	Glycophorin C	p55	Marfatia et al 1997; Grootjans et al 1997;
	S-V-K-I	GluR2	GRIP (PDZ5), PICK-1	Dong et al 1997 Xia et al 1998
	S-V-E-V	EphB2	GRIP (PDZ6), PICK-1	Torres et al 1998
	G-I-Q-V	EphA7	GRIP (PDZ6), PICK-1 syntenin	
	Y-V-K-V	EphrinB1	GRIP (PDZ6), PICK-1 Syntenin	
Class III				
X-D-X-V	V-D-S-V	Melatonin receptor	nNOS	Stricker et al 1997

A COOH terminal motif (RRESAI) on the inwardly rectifying K^+ channel Kir2.3 binds to the second PDZ domain of a 95-kD postsynaptic density protein called PSD-95. PKA phosphorylation of the serine at -2 of the PDZ recognition site uncouples the channel from PSD-95 and results in inhibition of K^+ conductance.

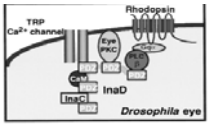


Some proteins have several PDZ domains; an individual PDZ protein can bind several subunits of a particular channel, thereby inducing aggregation/clustering. This can be enhanced by the PDZ containing protein to form oligomers (i.e. PSD-95). The individual PDZ domains can demonstrate distinct binding specificities, leading to the formation of a cluster that contains heterogenous groups of proteins.

Ability of third PSD-95 PDZ domain to bind the cell adhesion protein, neuroligin may direct the NMDA receptor (binds 1st PDZ domain) and K^+ channels (binds 2nd PDZ domain) to specific synaptic sites.

Sophistication shown with InaD, a polypeptide with 5 PDZ domains that regulates phototransduction in Drosophila photoreceptors. InaD associates through distinct PDZ domains with a calcium channel (TRP); phospholipase C-β and PKC.

InaD organizes these proteins into a signaling complex that allows efficient activation of the TRP channel by PKC-β in response to stimulation of rhodopsin and deactivation through phosphorylation of TRP by PKC. InaD acts a scaffold to organize a complex that mediates light activated signaling events.

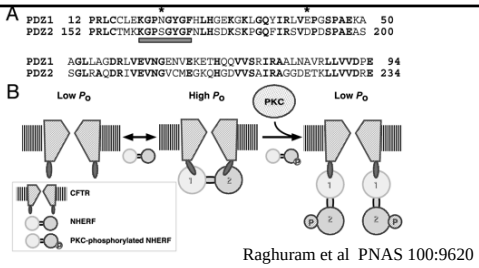


The cystic fibrosis transmembrane conductance regulator (CFTR)- cAMP-stimulated chloride ion channel expressed in the apical membrane of epithelial cells; critical for transepithelial salt and fluid transport.

Two homologous motifs, each consisting of six transmembrane helices followed by a cytoplasmic ATP binding domain. Two motifs connected by regulatory domain. PKA activates the channel by direct phosphorylation, but PKC does not activate CFTR substantially, but seems to increase responsiveness to PKA.

Binding of PDZ domains of the Na^+/H^+ exchanger regulatory factor (NHERF, also known as EBP50) directly regulates CFTR channel activity.

The binding and cross-linking of two C-terminal tails of CFTR by two PDZ domains in a bivalent molecule allosterically enhances CFTR gating, whereas PDZ domain binding in the absence of cross-linking does not.



Raghuram et al PNAS 100:9620

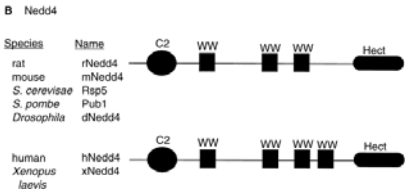
PKC phosphorylates S162 within the PDZ2 doman of NHERF, which lowers its affinity for CFTR C terminus and disrupts the bivalent PDZ domain interaction of NHERF. Phosphorylated NHERF is unable to cross-link the channel and stimulate the activity of CFTR.

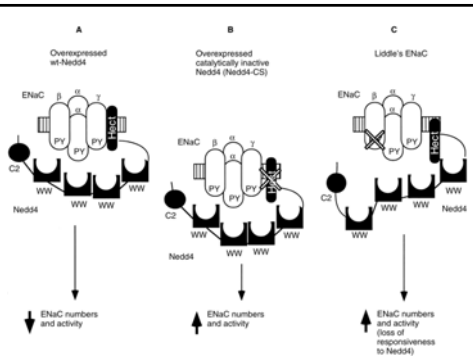
The epithelial Na^+ channel (ENaC) is comprised of three subunits; α, β, and γ and plays an essential role in Na^+ and fluid absorption in the kidney, colon and lung.
EnaC is a short-lived protein with a $t_{1/2}$ of ~ 1 hour of its total cellular pool as well as a short half-life of protein at the cell surface.

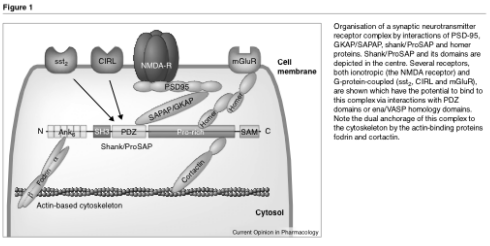
Ubiquitination serves to tag proteins for rapid degradation. The E3 ubiquitin protein ligase such as Nedd4 is responsible for the attachment of ubiquitin onto lysine residues of target proteins, targeting them for degradation via the proteasome.

WW domains- small modules of 35-40 residues that bind proline rich motifs, commonly with the consensus PPXY or PPLP.

WW domain of the E3 ubiquitin protein ligase Nedd4

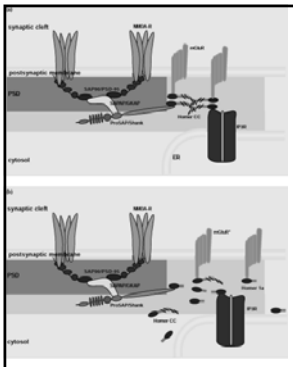






Organisation of a synaptic neurotransmitter receptor complex by interactions of PSD-95, GKAP/SAPAP, shank/ProSAP and homer proteins. Shank/ProSAP and its domains are depicted in the centre. Several receptors, both ionotropic (the NMDA receptor) and G-protein-coupled (ssl$_2$, CIRL and mGluR), are shown which have the potential to bind to this complex via interactions with PDZ domains or ena/VASP homology domains. Note the dual anchorage of this complex to the cytoskeleton by the actin-binding proteins fodrin and cortactin.

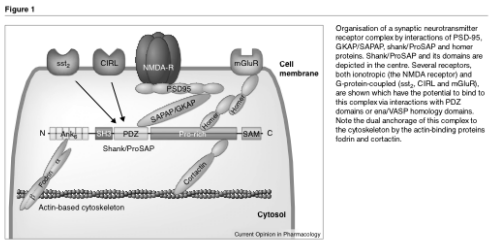
Homer
mGlu1 and mGlu5 receptors; both signal by activation of PLC and mobilization of intracellular calcium via IP3R.
mGluR contain PPxxF motif (Homer ligand), which binds the Ena/VASP homology domain of homer protein.



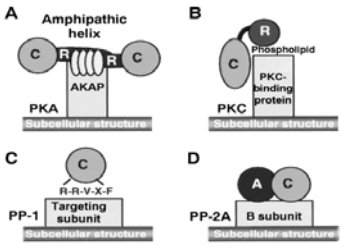
Most homer isoforms contain a coiled coil motif, by which they dimerize and form a complex with two binding sites for homer ligands.
The findings that IP3R contained a homer ligand motif led to the discovery that mGluR are physically coupled to their IP3R effector via the homer dimer.

Disruption of the homer dimer by the expression of a naturally occurring form of homer (homer 1a, that lacks coiled coil region).

Thomas J Neurochem 2002 81:407



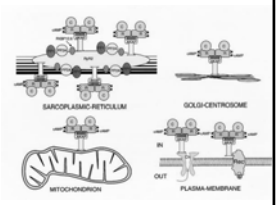
NMDA receptors are anchored by PSD-95 proteins and PSD-95 is anchored by guanylate-kinase-associated proteins (GKAPs). Shank proteins (consist of ankyrin repeats, SH3 domain, a PDZ domain and long proline-rich domain) provide a scaffold for several neurotransmitter complexes. The proline rich part, consists of a homer ligand motif, enabling the binding a homer-mGluR complex. Shank may recruit IP3R into the synapse as well.



PKA-serine threonine kinase composed of two catalytic subunits held in an inactive state by association with regulatory dimer.

Binding of two cAMP molecules to each regulatory subunit relieves PKA autoinhibitory contact, and allows phosphorylation of substrate

Two forms of PKA holoenzyme exist based upon type of regulatory subunit (type I and II)

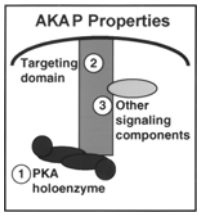


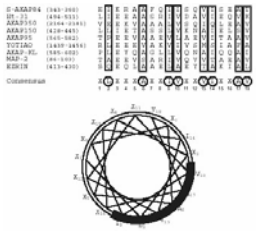
Feliciello et al; J. Mol Biol 2001 308:99

Discrete localization of type II PKA is due to association of regulatory subunit with scaffold protein, A Kinase Anchoring Protein-AKAP; family now includes > 50 members

All contain an amphipathic helix of 14-18 residues that functions to bind to the N-termini of the PKA-RII dimer

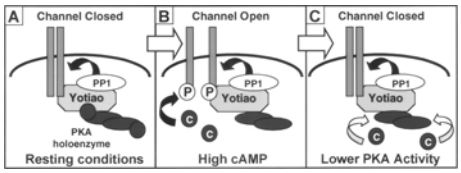
Each AKAP contains a unique subcellular targeting domain that restricts its location within the cell





Michel and Scott Annu. Rev. Pharmacol Tox 2002; 42:235

AKAP's can coordinate a more extensive signaling complex to include: Phosphatases- PP1 binds to yotiao which is targeted to NMDA receptor and KCNQ1; yotiao targets active PP1 to the NMDA receptor; consistent with yotiao targeting to KCNQ1



Michel and Scott Annu. Rev. Pharmacol Tox 2002; 42:235

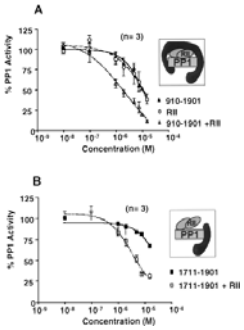
Determination of ion channels partners is critical for elucidation of signaling pathways

AKAP's can also inhibit phosphatase activity

AKAP220 was found to bind PP1 and PKA

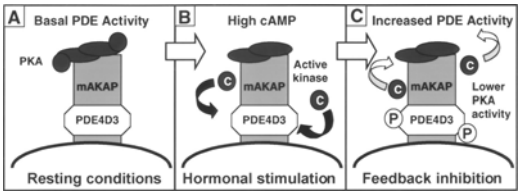
Determinant for binding PP1 to AKAP220 between residues 385-530. However, residues 1711 and 1901 also affect binding and is a competitive inhibitor of PP1.

RII directly binding to PP1 further inhibits PP1 activity.



Michel and Scott Annu. Rev. Pharmacol Tox 2002; 42:235

mAKAP coordinates a signaling complex that contains phosphodiesterase (PDE4D3) and PKA; thus mAKAP provides a framework for assembly of a PKA/PDE negative feedback loop. PKA phosphorylates Ser-13 and Ser-54 of PDE4D3 which increases PDE4D3 activity, which would increase cAMP metabolism and attenuate PKA activity.



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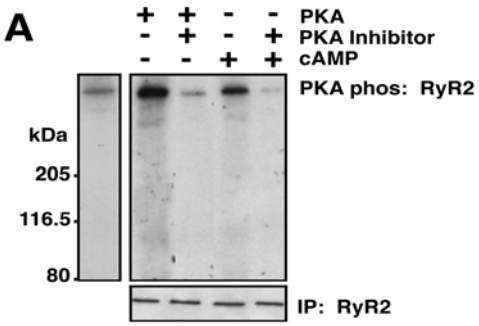
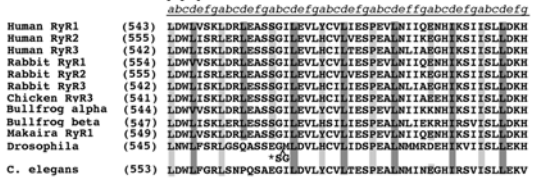
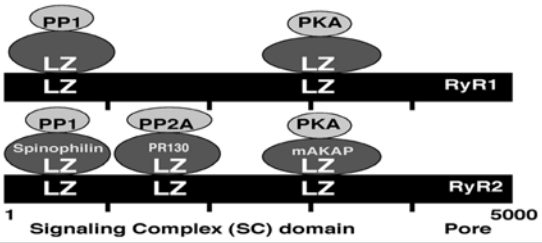
RyR2/calcium release channel phosphorylated by addition of cAMP alone



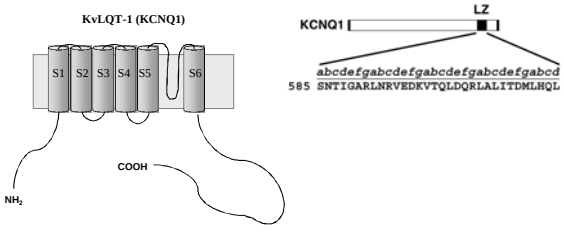
LZ motifs are evolutionary conserved

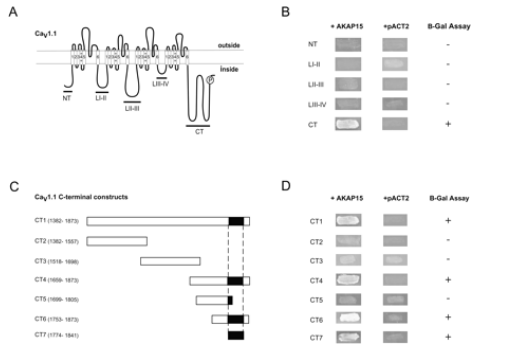


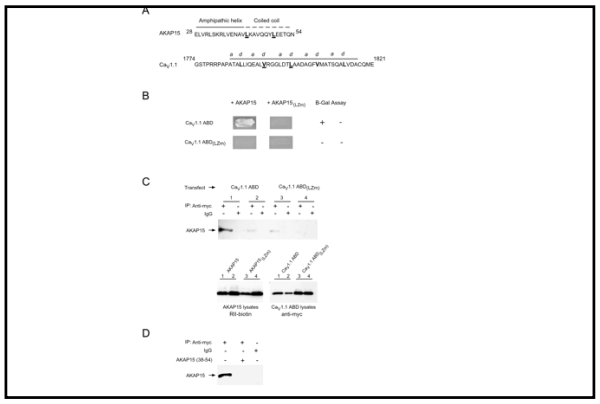
RyR macromolecular complexes are held together by leucine/isoleucine zippers

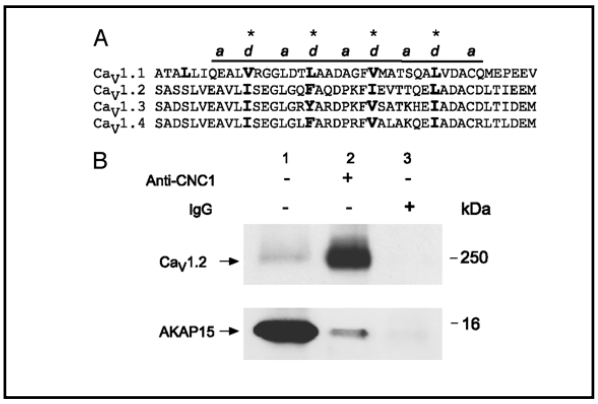


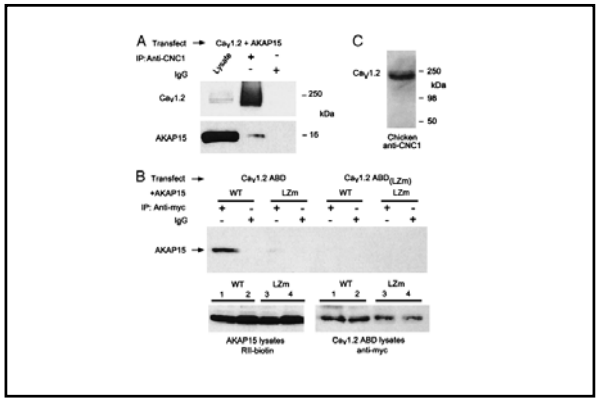
KCNQ1 has a leucine zipper in C-terminus











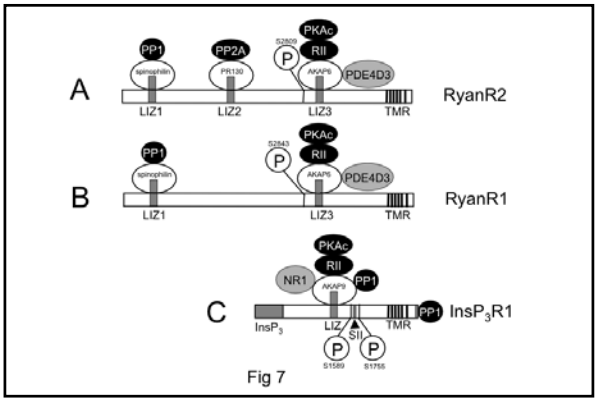
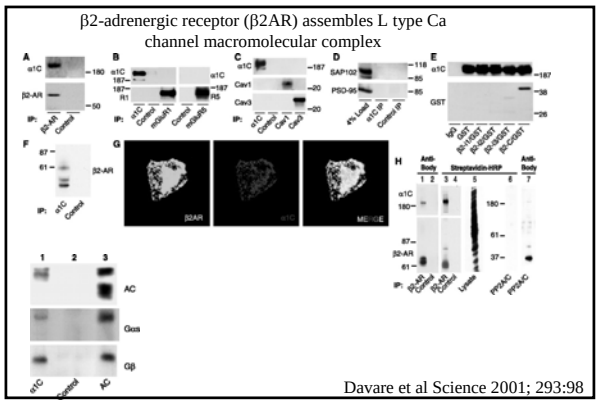
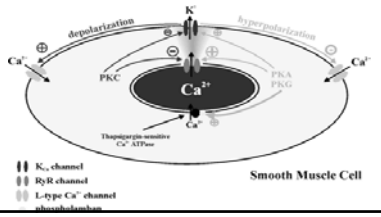


Fig 7

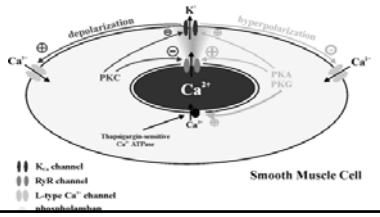


Ion channels in smooth muscle

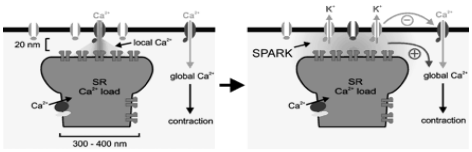
- Excitation-contraction coupling in smooth muscle is believed to occur by two mechanisms- electromechanical and pharmacomechanical coupling.
- Electromechanical coupling operates through changes in surface membrane potential; typically resting membrane potential= -40 to -70 mV.
- Primary drive for the rise in intracellular calcium is membrane depolarization, with the consequential opening of voltage operated calcium channels; neurotransmitters or hormones acting to depolarize the membrane will cause contraction while those producing membrane hyperpolarization will cause relaxation.
- Like cardiac muscle, the influx of Ca^{2+} likely causes release of Ca^{2+} from sarcoplasmic reticulum.



- Drugs that block calcium entry through VOCC will inhibit electromechanical coupling- thus the use of calcium channel blocking agents to relax vascular smooth muscle, thus producing vasodilatation and a decrease in blood pressure.
- Cell-type dependent; for instance, in asthma, Ca^{2+} blocking drugs are not effective in promoting relaxation of muscle.
- Electromechanical coupling appears to play a predominant role in phasic smooth muscle in which the membrane potential often displays marked oscillations upon which are superimposed calcium spikes
- The plasma membranes contain numerous ion channels and the distribution and properties vary among different tissues, contributing to the diversity of smooth muscle.



Proposed functional roles of Ca^{2+} sparks in smooth muscle cells



dihydropyridine-sensitive (L-type) calcium channel
Ca^{2+}-sensitive potassium channel
ryanodine-sensitive Ca^{2+} release channel
sarcoplasmic reticulum Ca^{2+}-ATPase
phospholamban

Unanswered questions: role of Ca^{2+} entry activating Ca^{2+} sparks