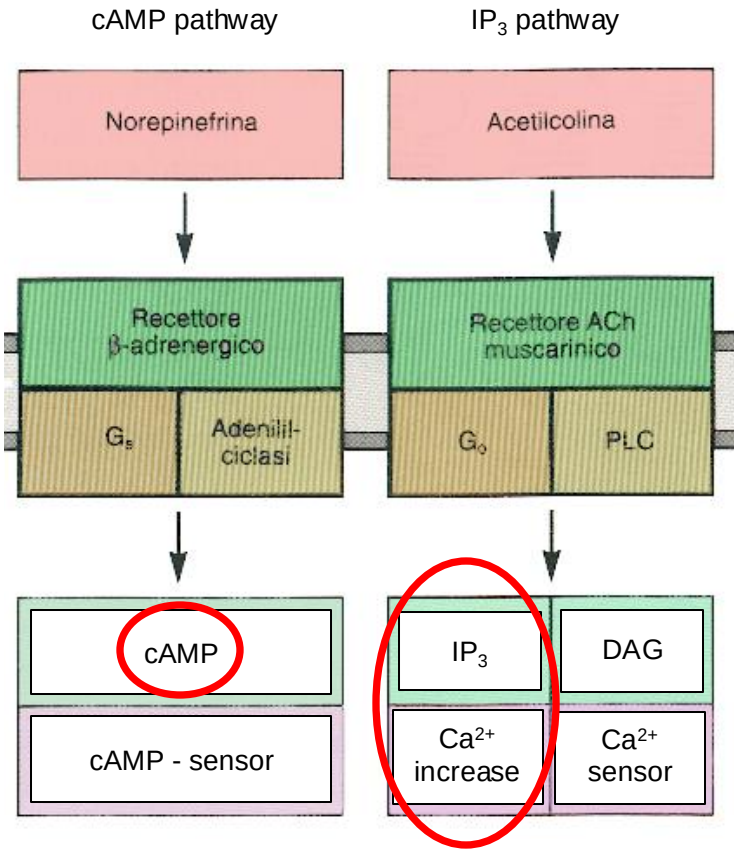
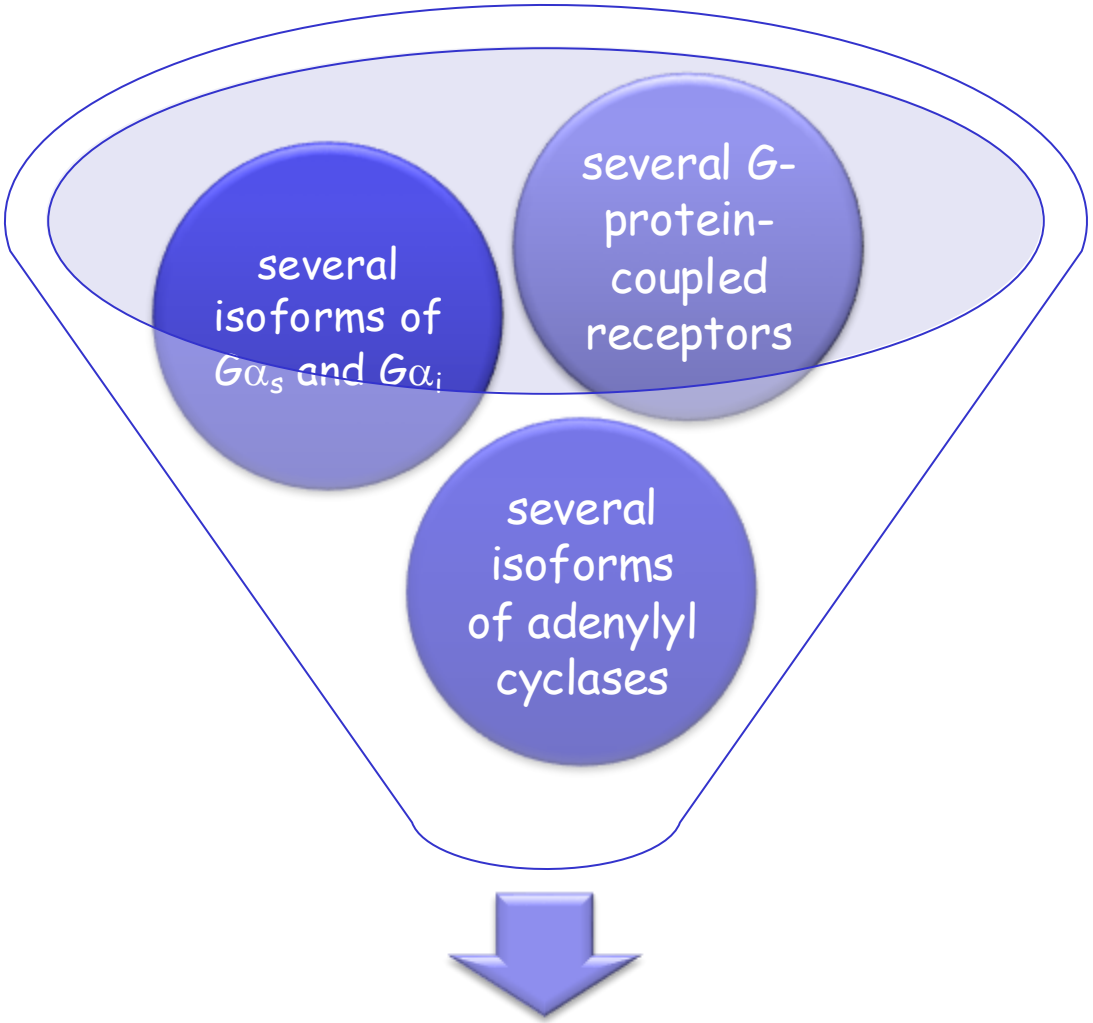


cAMP and calcium are pleiotropic messengers

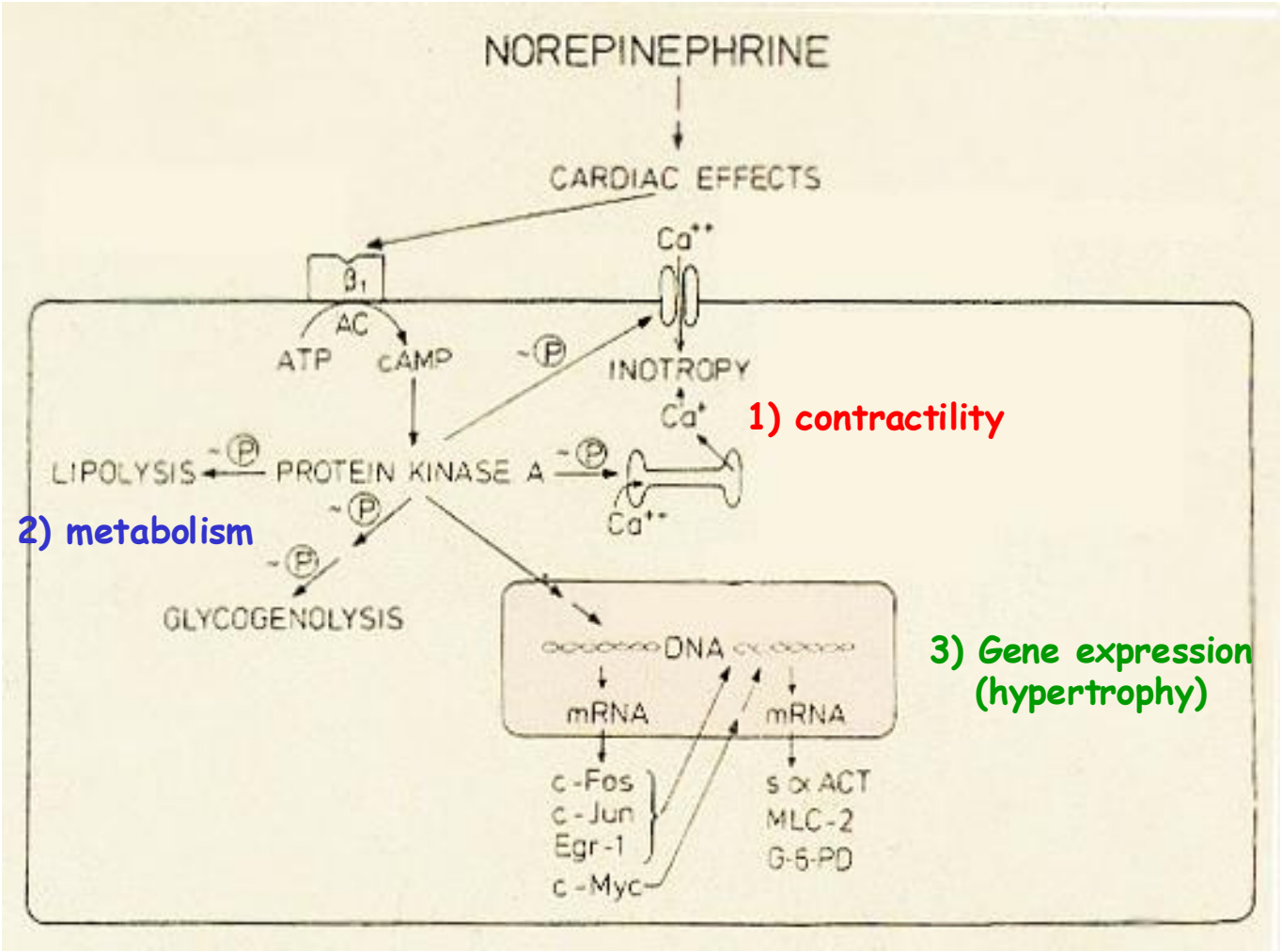
Different amplitude/duration of the cAMP signals



decoding

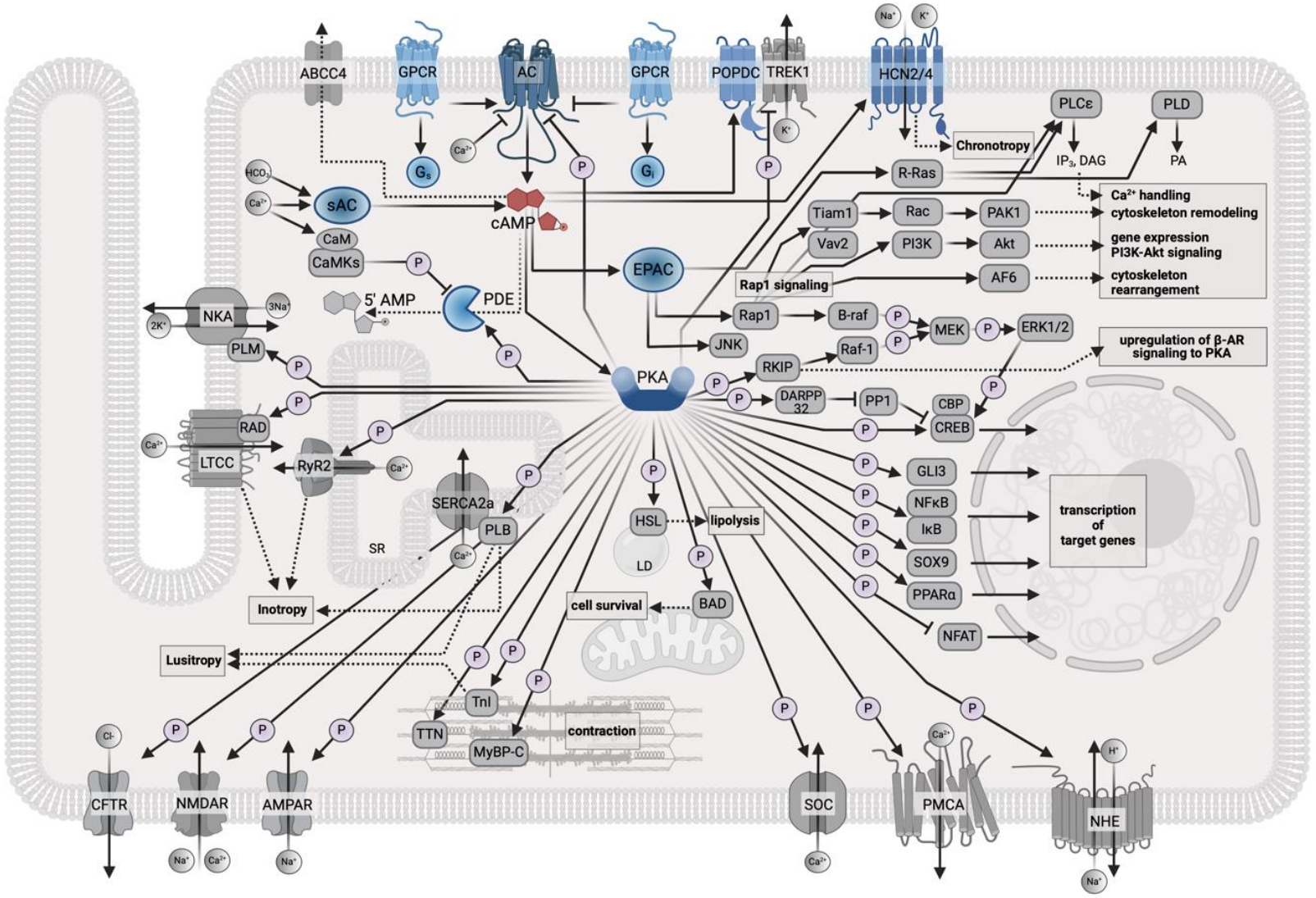
via different isoforms of cAMP sensors

Pleiotropy of cAMP in cardiac cells in the past



(Modificata da Zimmer, J. Mol. Med. 1997, **75**:849-859)

Pleiotropy of cAMP in cardiac cells at present

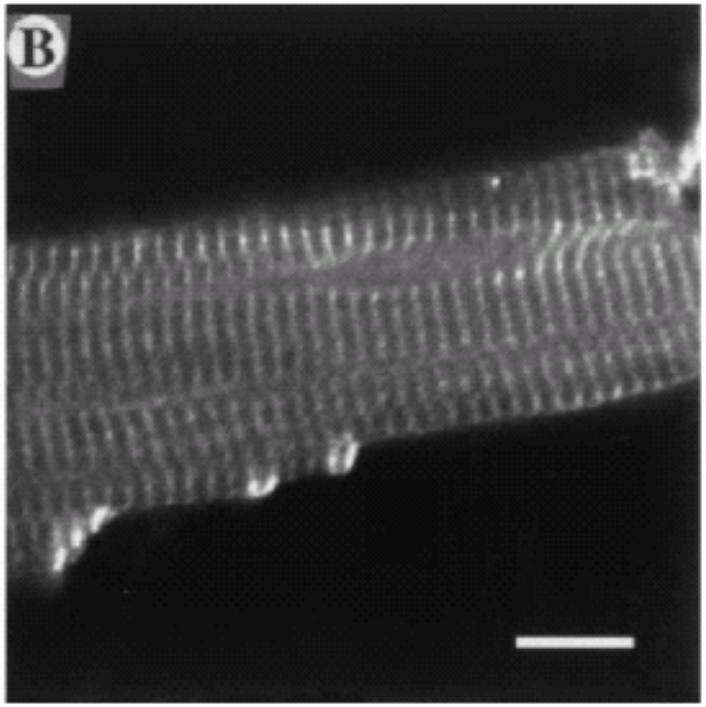


Zaccolo & Kovanich (2024) Physiol Rev, doi: 10.1152/physrev.00013.2024. Epub ahead of print.

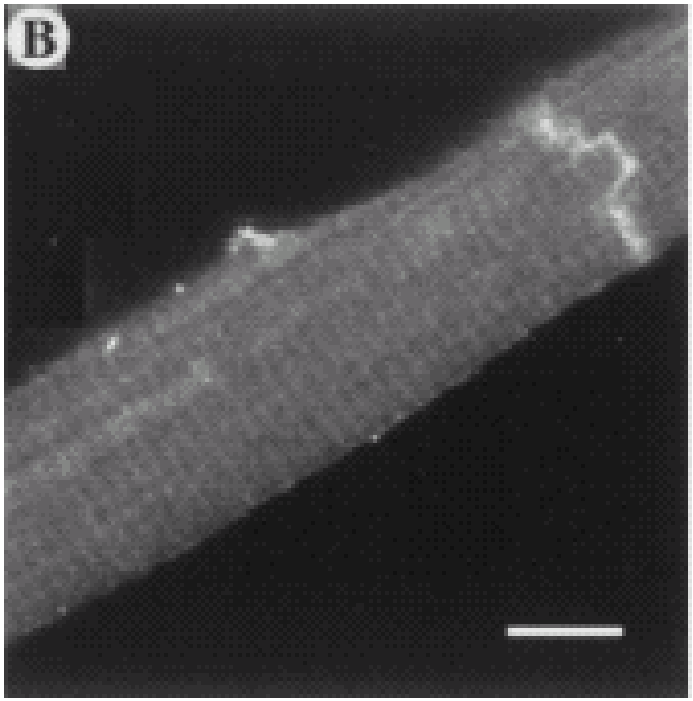
Ca^{2+} vs cAMP handling machinery

	Ca^{2+} signaling	cAMP signaling
ON mechanisms	Ion channels/exchangers	
	G-protein coupled receptors	G-protein coupled receptors
	Intracellular stores	
OFF mechanisms	PMCA	Phosphodiesterases (PDE)
	SERCA	
	Mitochondria	
	Cytosolic buffering proteins	
Experimental approaches	Immunolabelling Fluorescent probes	Immunolabelling FRET (PKA)

Localised synthesis of cAMP in cardiac cells



α subunit of G-protein

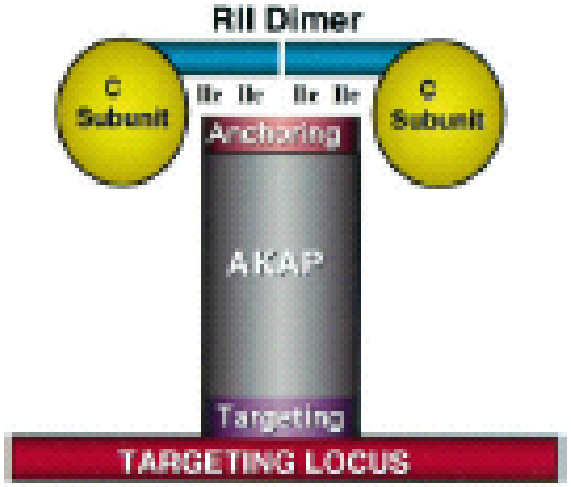


Adenyl cyclase

(Laflamme & Becker, *Am. J. Physiol* 1999, **277**:H1841-H1848)

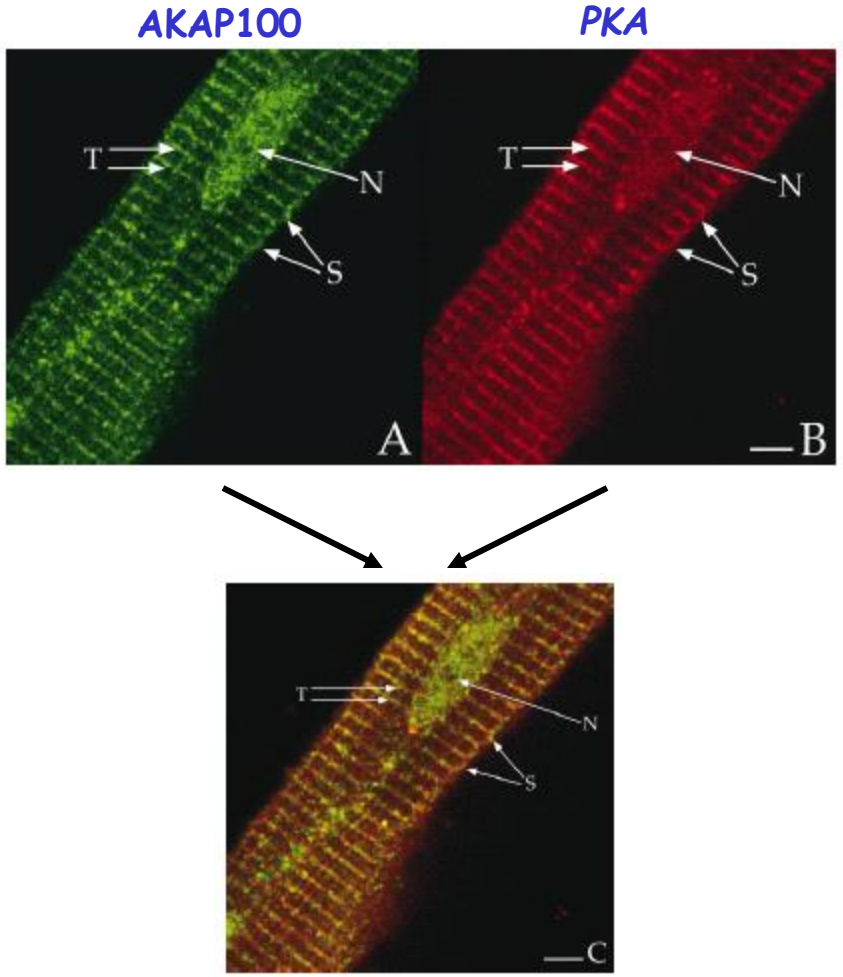
The family of AKAPs

(A-kinase-anchoring proteins)



"targeting hypothesis"

(in Dell' Acqua & Scott, *J. Biol. Chem* 1997, **272**:12881-12884)



(Yang *et al.*, *J. Cell. Biol.* 1998, **142**:511-522)

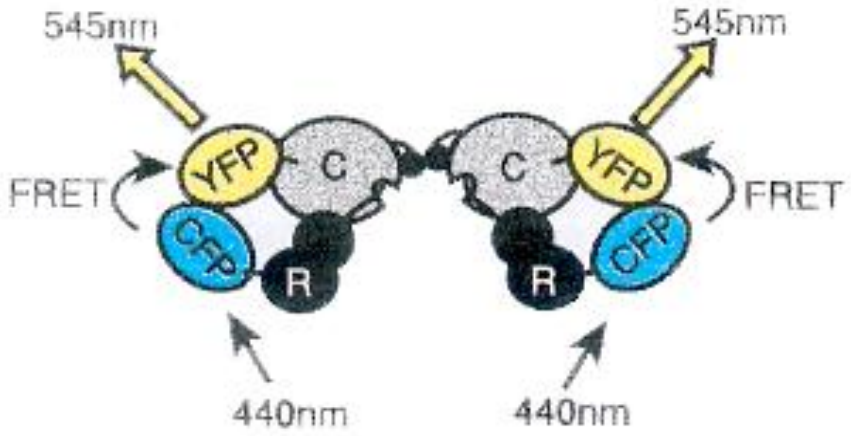
The AKAP family

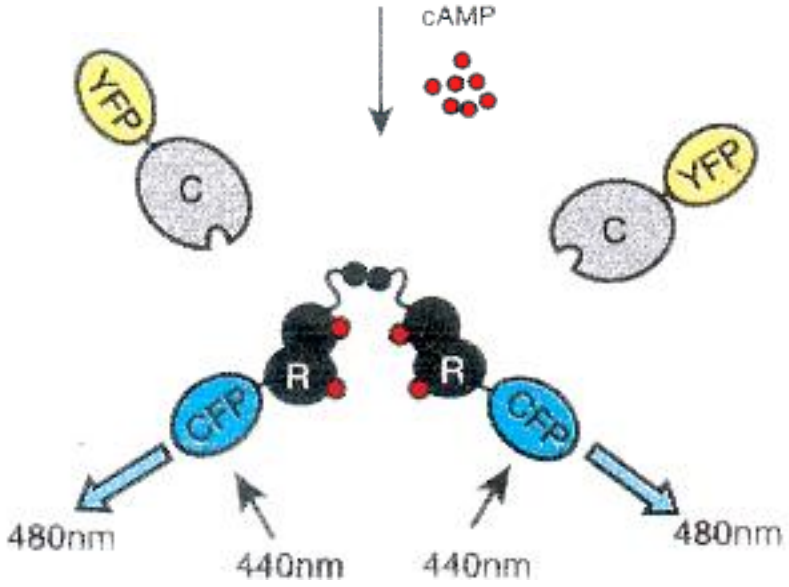
TABLE 1. *A kinase anchoring proteins*

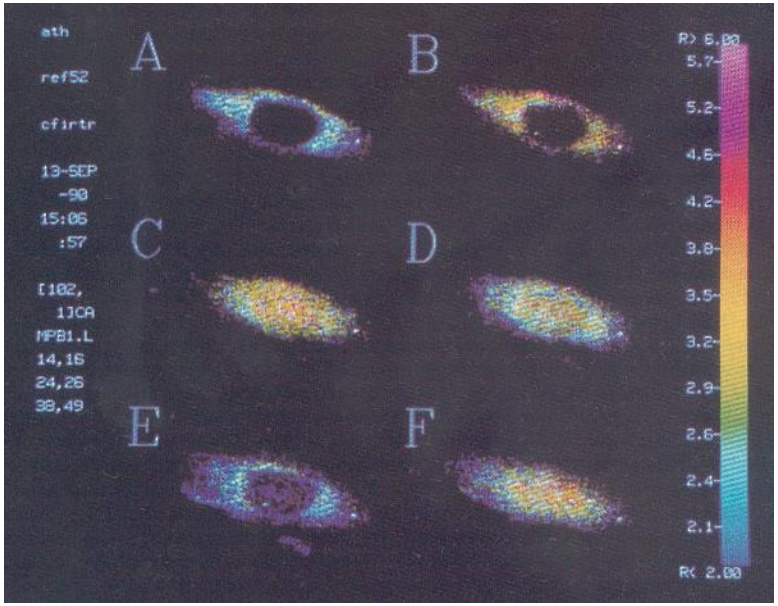
AKAP (Gene Nomenclature Committee Name)	Tissue	Subcellular Localization	Properties/Function	Reference Nos.
S-AKAP84/D-AKAP1/ AKAP121/AKAP149 (*AKAP1*)	Testis, thyroid, heart, lung, liver, skeletal muscle, and kidney	Outer mitochondrial membrane/endoplasmatic reticulum/nuclear envelope/sperm midpiece	Dual-specific AKAP; binds lamin B and PP1; multiple splice variants	57, 150, 151, 205, 313, 337
AKAP-KL (*AKAP2*)	Kidney, lung, thymus, and cerebellum	Actin cytoskeleton/apical membrane of epithellal cells	Multiple splice variants	88
AKAP110 (*AKAP3*)	Testis	Axoneme	Binds $G_{13}\alpha$	213, 345
AKAP82/FSC1 (*AKAP4*)	Testis	Fibrous sheath of sperm tail	Potential role in sperm motility and capacitation; multiple splice variants; binds both RI and RII	52, 228, 229
AKAP75/79/150 (*AKAP5*)	Bovine/human/rat orthologs; brain	Plasma membrane/postsynaptic density	Polybasic domains target to plasma membrane and dendrites; binds PKC, calcineurin (PP2B), β-AR, SAP97, and PSD-95	41, 51, 60, 62, 282
mAKAP (*AKAP6*)	Heart, skeletal muscle, and brain	Nuclear membrane	Binds PDE4D3; spectrin repeat domains involved in subcellular targeting	87, 167, 217, 220, 361
AKAP15/18 α,β,γ,δ (*AKAP7*)	Brain, skeletal muscle, pancreas, and heart	Basolateral (α) and apical (β) plasma membrane, cytoplasm (γ), secretory vesicles (δ)	Targeted to plasma membrane via fatty acid modifications; modulation of Na^+ and L-type Ca^{2+} channels (α); ADH-mediated translocation of AQP2 from vesicles to apical membrane in distal kidney tubules	114, 126, 127, 182, 338
AKAP95 (*AKAP8*)	Heart, liver, skeletal muscle, kidney, and pancreas	Nuclear matrix	Involved in initiation of chromosome condensation; binds Eg7/condensin; zinc-finger motif	59, 61, 95, 96, 312
AKAP450/AKAP350/ Yotiao/CG-NAP/ Hyperion (*AKAP9*)	Brain, pancreas, kidney, heart, skeletal muscle, thymus, spleen, placenta, lung, and liver	Postsynaptic density/neuromuscular junction/centromsomes/ Golgi	Binds PDE4D3, PP1, PP2A, PKN, and PKC; targets PKA and PP1 to the NMDA receptor; multiple splice variants.	18, 19, 46, 103, 120, 173, 204, 287, 297, 321, 323, 329, 357, 359
D-AKAP2 (*AKAP10*)	Liver, lung, spleen, and brain		Dual-specific AKAP	132, 149
AKAP220/hAKAP220 (*AKAP11*)	Testis and brain	Vesicles/peroxisomes/ centrosome	Binds PP1; dual-specific AKAP	196, 266, 284, 285
Gravin (*AKAP12*)	Endothelium	Actin cytoskeleton/cytoplasm	Binds PKC and β-AR; Xgravin-like (Xgl) is also a putative AKAP	124, 178, 241, 294
AKAP-Lbc/Ht31/Rt31 (*AKAP13*)	Ubiquitous	Cytoplasm	Ht31 RII binding site used in peptides to disrupt PKA anchoring; Rho-GEF that couples $G_{12}\alpha$ to Rho	49, 85, 179
MAP2B	Ubiquitous	Microtubules	Binds tubulin; modulation of L-type Ca^{2+} channels	76, 208, 282, 332
Ezrin/AKAP78	Secretory epithelia	Actin cytoskeleton	Linked to CFTR via EBP50/NHERF	92, 318, 319
T-AKAP80	Testis	Fibrous sheath of sperm tail		223
SSeCKS (Src-suppressed C kinase substrate)	Testis, elongating spermatids	Actin remodeling	Gravin-like	99
Pericentrin	Ubiquitous	Centrosome	Binds dynein and γ-tubulin; unique RII-binding domain	83
WAVE-1/Scar	Brain	Actin cytoskeleton	Binds Abl and Wrp; involved in sensorimotor and cognitive function	307, 356
Myosin VIIA	Ubiquitous	Cytoskeleton		189
PAP7	Steroid-producing cells (adrenal gland and gonads)	Mitochondria	Hormonal regulation of cholesterol transport into mitochondria; binds RI in vivo	201
Neurobeachin	Brain	Golgi		348
AKAP28	Primary airway cells	Ciliary axonemes	Modulation of ciliary beat frequency	188
Myeloid translocation gene (*MTG*) 8 and 16b	Lymphocytes	Golgi		115, 283
AKAP140	Granulosa cells and meiotic oocytes		Upregulated by FSH in granulosa cells; phosphorylated by CDK1 in oocytes; not cloned	47, 153, 184

Fluorescent protein kinase A as cAMP indicator

(FRET, fluorescence resonance energy transfer)

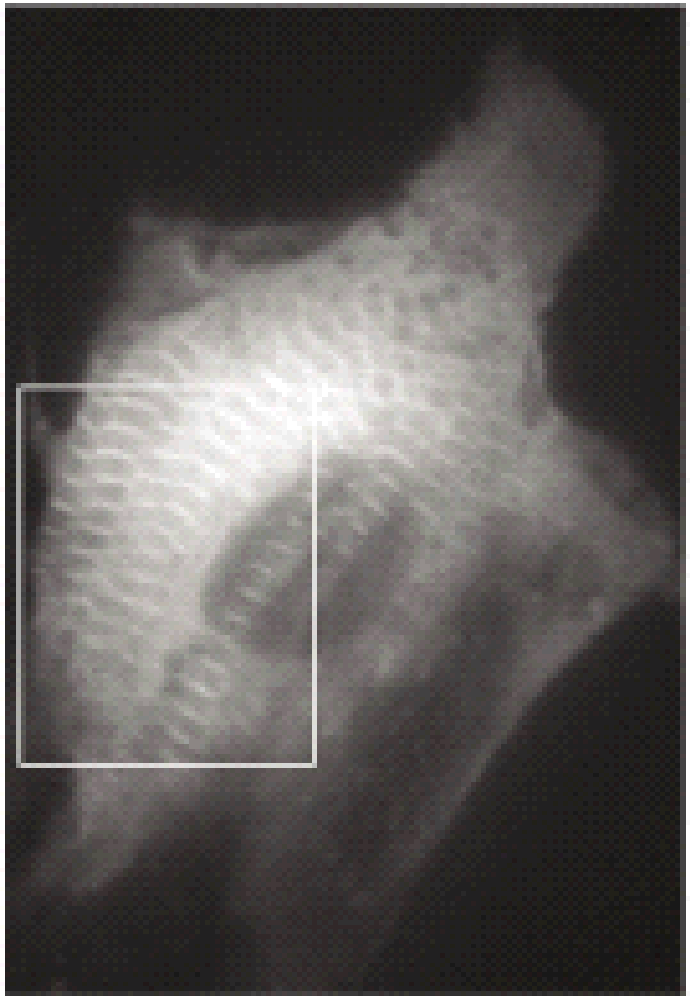


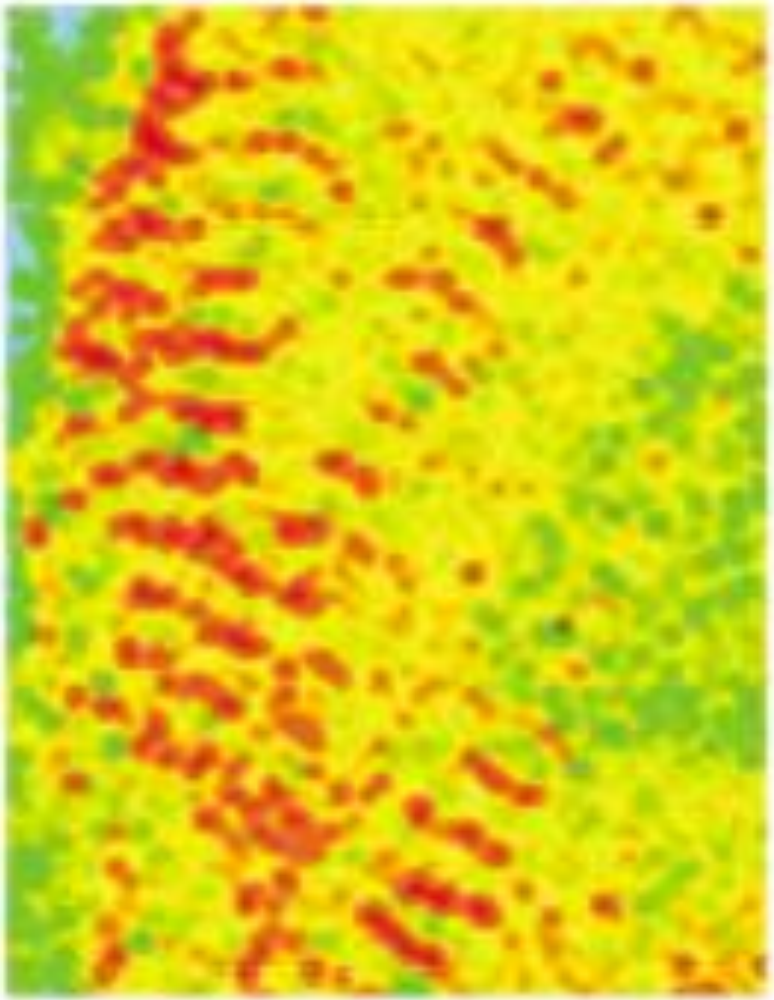




(Adams *et al.*, *Nature* 1991, **349**:694-697)

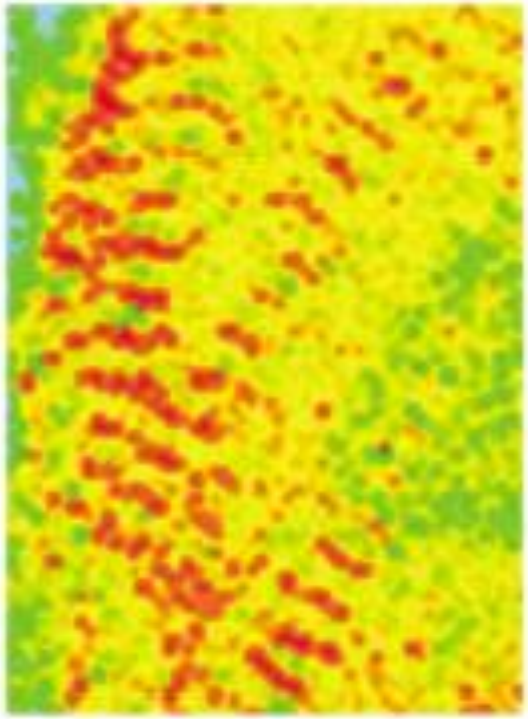
Localised cAMP (PKA) signals in rat cardiomyocytes



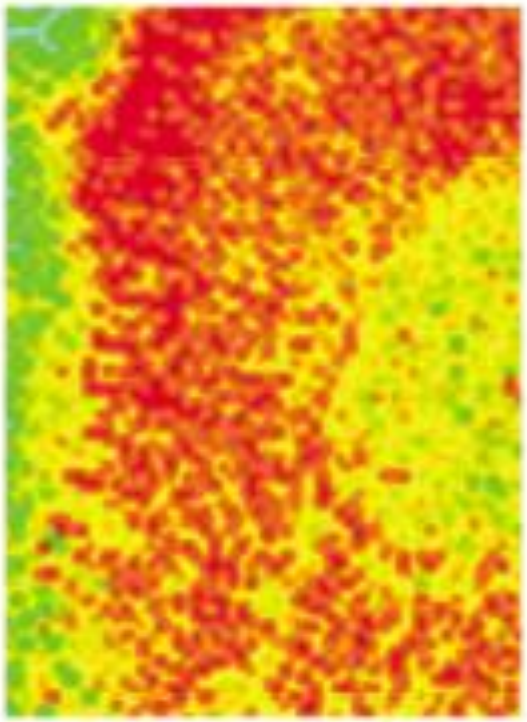


(Zaccolo & Pozzan, *Science* 2002, **295**:1711-1715)

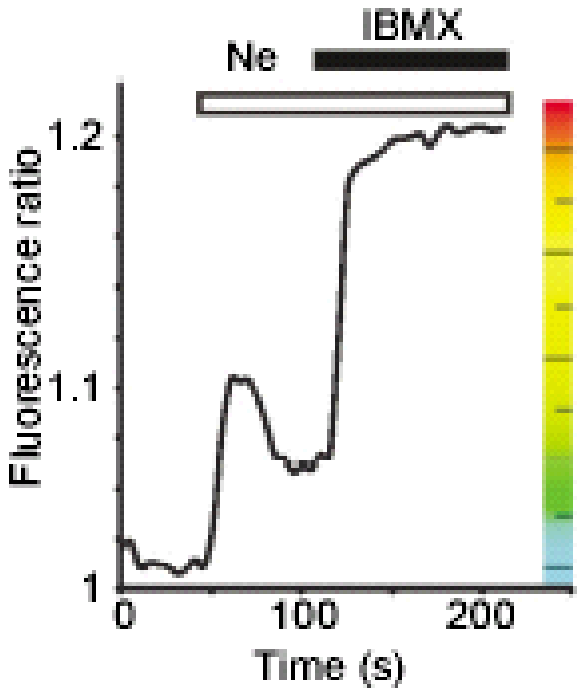
Rapid degradation of cAMP in cardiomyocytes: the role of cyclic nucleotide phosphodiesterase (PDE)



Stimulated
(+NE)



+ Inhibitor of PDE
(+IBMX)



(Zaccolo & Pozzan, *Science* 2002, **295**:1711-1715)

AKAP-PKA-PDE complex: cAMP synthetizing unit

(Davare *et al.*, *Science* 2001, **293**:98-101; Dodge *et al.*, *EMBO J.* 2001, **20**:1921-1930)

"Molecular channeling hypothesys"

Compartmentalisation of the cAMP signals

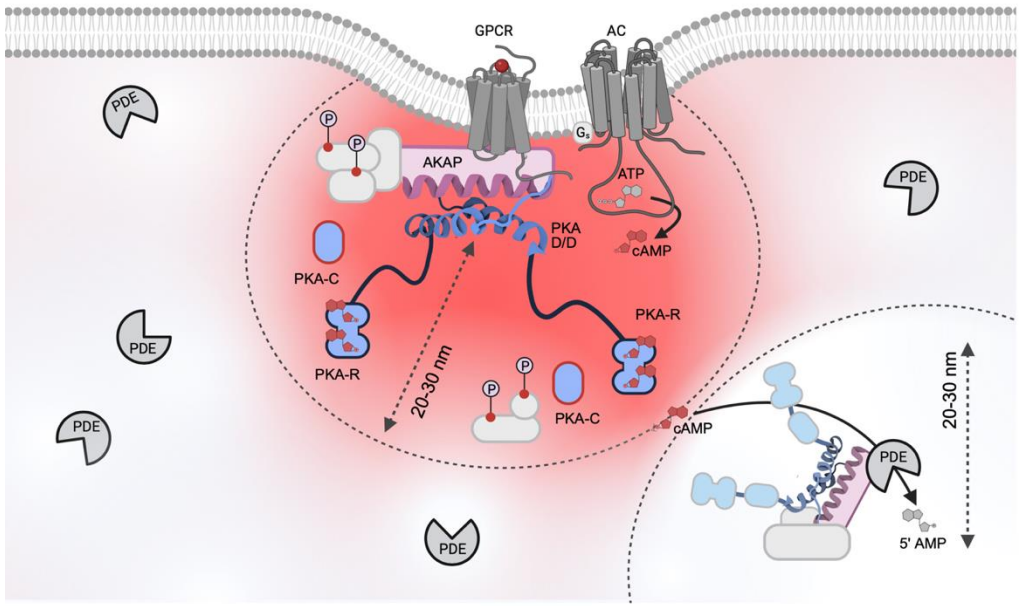
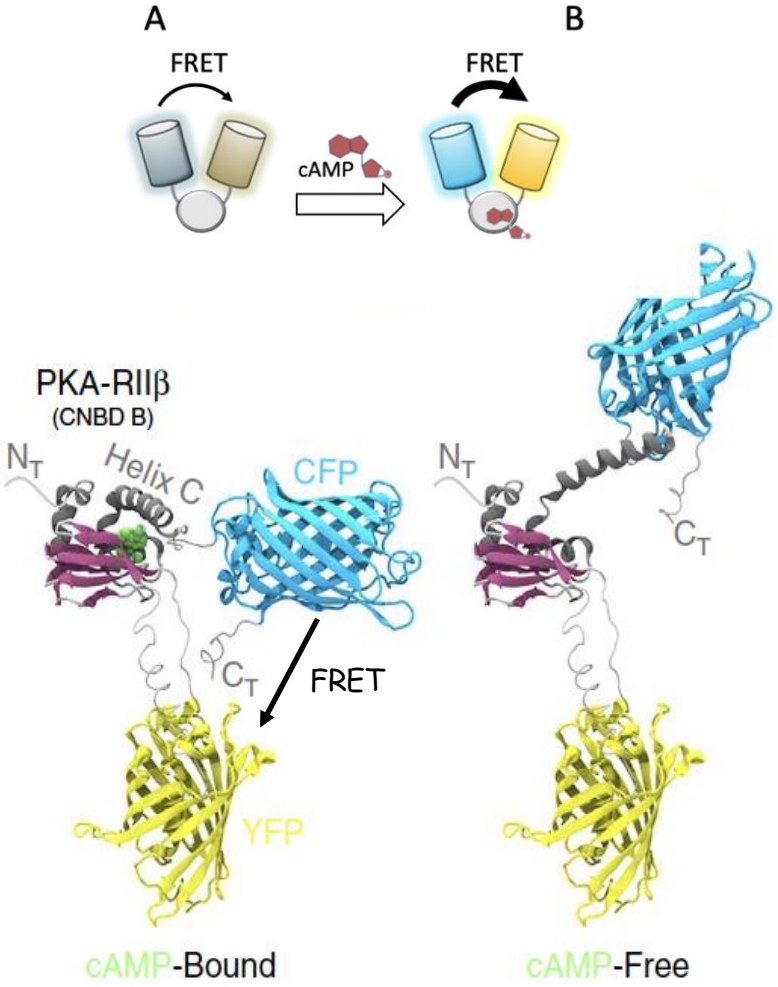


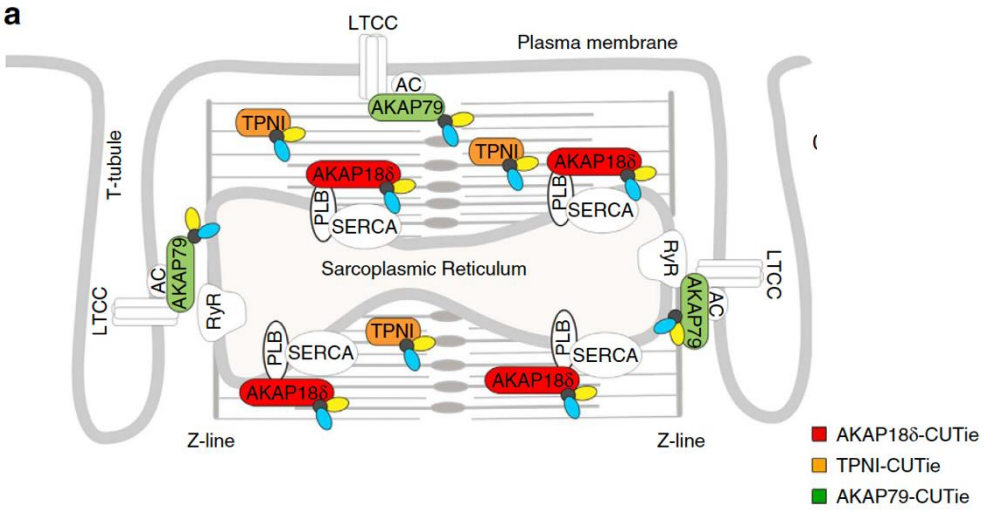
Figure 4 - AKAP-dependent signalosome activated by a cAMP nanodomain. *The schematic illustrates a generic AKAP-dependent signalosome localized in proximity of a GPCR and AC at the plasma membrane. Activated PKA is shown in blue. The interaction between the AKAP amphipathic helix and the R D/D domain is modelled on the AKAP18:PKA-RII crystal structure (PDB* 4ZP3) (186). *Activation of the receptor generates a pool of high [cAMP] that remains confined and, depending on the nature and amplitude of the stimulus, can be limited to a radius of about 20-30 nm. This distance also approximately equates to the range within which the AKAP-anchored PKA R subunits can extend. PKA targets (light grey), are phosphorylated only if they reside within the active cAMP nanodomain. PDEs hydrolyse cAMP in their immediate neighborhood (20-30 nm), creating region within which [cAMP] remains below the activation threshold of PKA. Within this domain PKA targets are not phosphorylated. Thus, PDEs contribute to compartmentalise cAMP and protect targets from inappropriate phosphorylation. AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; D/D, dimerization/docking domain; GPCR, G protein coupled receptor; PKA, protein kinase A; PDE, phosphodiesterase. This figure was generated using Biorender.*

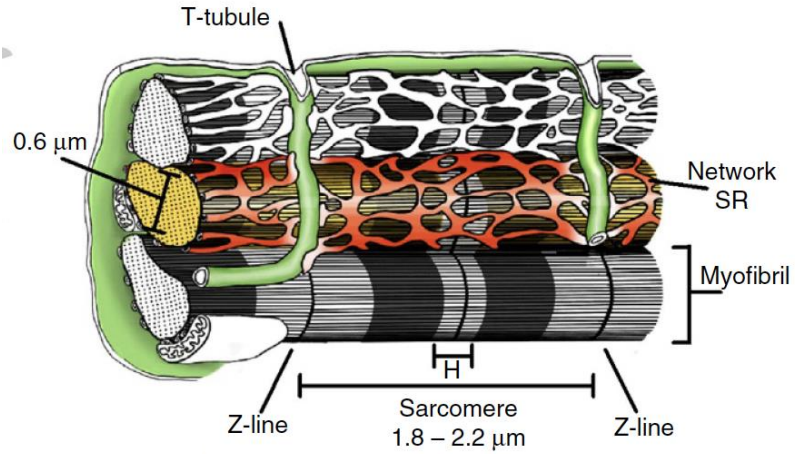
Zaccolo & Kovanich (2024) Physiol Rev, doi: 10.1152/physrev.00013.2024. Epub ahead of print.

Targeted FRET based reporters for cAMP



Genetically encoded
FRET basedreporters





Surdo *et al.* (2017) *Nat Commun* 20;8:15031

cAMP nanodomains and signalosomes

Open question

How do different GPCRs precisely couple with the intracellular molecular machinery to ensure cAMP levels rise only in the appropriate compartment, especially when the signalosomes activated by cAMP are distant from the cell surface where the receptors are located?

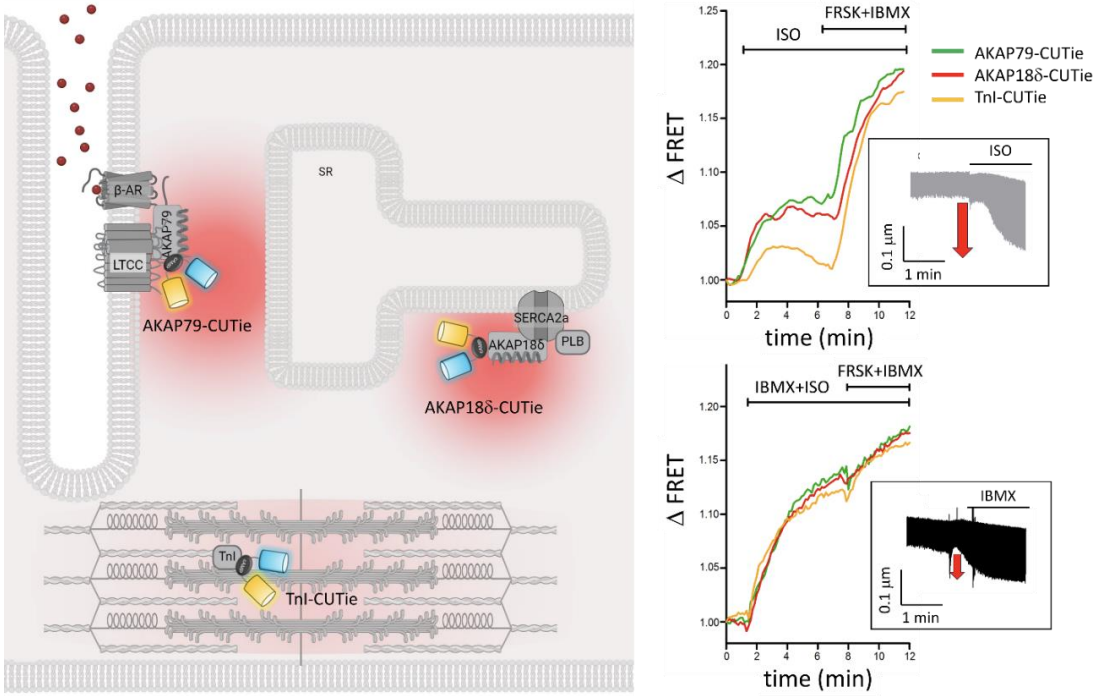


Figure 7 - The heterogeneous cAMP signal generated by β-AR stimulation is required to achieve optimal inotropic response. *Detection of local cAMP signals at specific locations is possible by using targeted FRET reporters generated by fusing the sensor to a protein component of a specific signalosome. In the example illustrated here, the FRET reporter CUTie was fused to AKAP79 for targeting to the plasmalemma, to AKAP18δ for targeting to the sarcoplasmic reticulum (SR), or to the myofilament protein troponin I (TnI) for targeting to the sarcomere. Activation of the β-AR with isoproterenol generated distinct responses at these locations. As illustrated in the top right panel, the cAMP level detected at the plasma membrane and SR were comparable and showed sustained kinetics, while the cAMP response at the sarcomere was delayed and significantly smaller than at the other sites and transient in nature. The compartmentalisation of the cAMP signal was dependent on the presence of active PDEs as, on application of the PDE inhibitor IBMX, the signal equilibrated at the three locations (bottom right panel). The insets show a measure of cardiac myocyte contractility, as determined by quantification of sarcomere fractional shortening (μm) and illustrate that compartmentalized cAMP elevation achieves significantly larger inotropic effect compared to homogeneous cAMP increase. Modified from (202).*

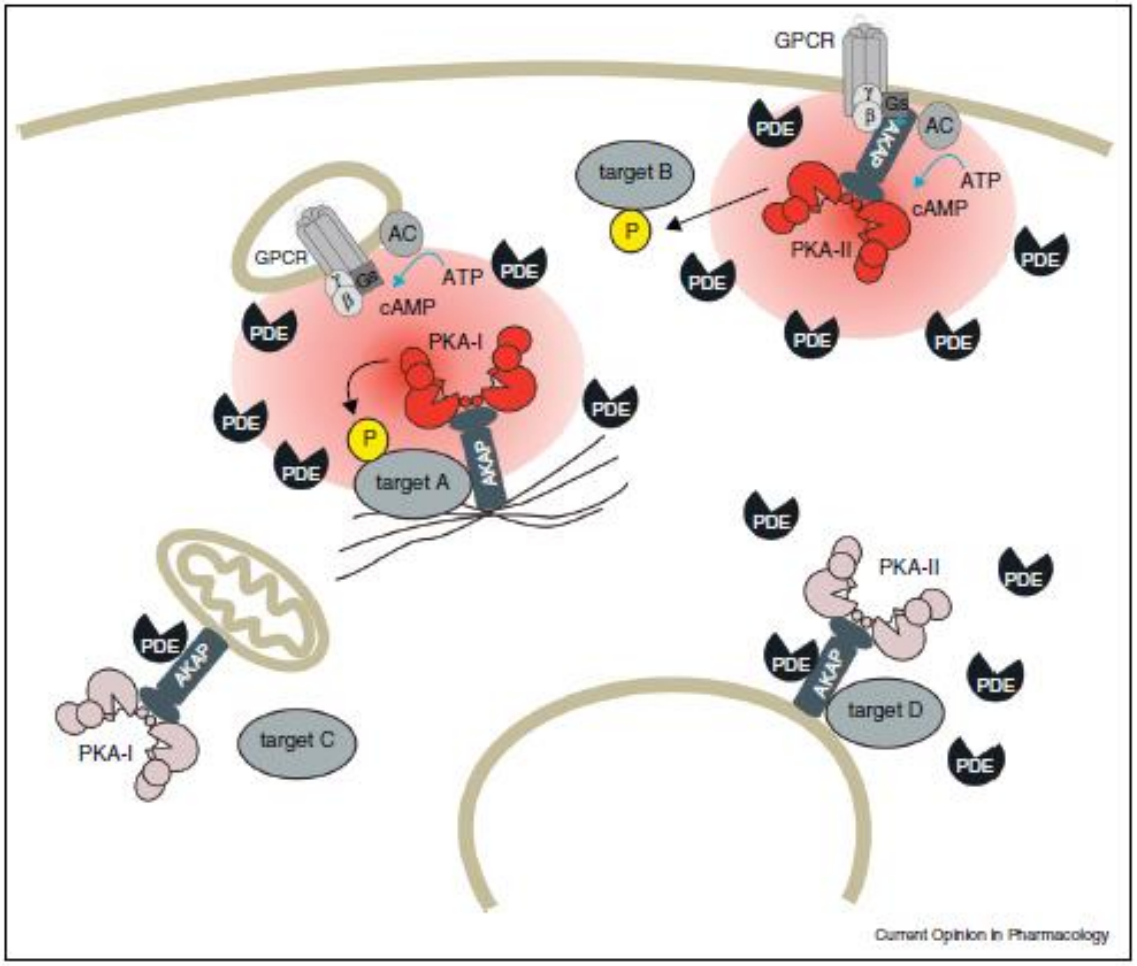
Zaccolo & Kovanich (2024) Physiol Rev, doi: 10.1152/physrev.00013.2024. Epub ahead of print.

Internalization of the receptors?

Current Opinion in Pharmacology 2011, 11:649–655

Spatial control of cAMP signalling in health and disease

Manuela Zaccolo



Compartmentalised cAMP signalling. The schematic shows two distinct cAMP pools (illustrated by a red shaded oval area) generated by an AC anchored at the plasma membrane and activated by a GPCR exposed to the extracellular environment and an AC associated to an internalised GPCR on the cytoplasmic face of an endosome. PDEs, by degrading cAMP, limit its diffusion outside a spatially confined microdomain and contribute to define the boundaries of the cAMP pools. The two pools of cAMP activate distinct subsets of PKA anchored to different AKAPs. It is interesting to note that PKA-I and PKA-II have different sensitivity to cAMP activation, thus providing a further opportunity for signal discrimination. Activation of an individual subset of PKA results in the selective phosphorylation of the target that is coupled with the specific microdomain. PKA subsets localised outside the cAMP pools do not sense an increase in cAMP concentration and therefore are not activated. GPCR = G protein-coupled receptor; AC = adenylyl cyclase; PKA-I, PKA-II = isoforms I and II of protein kinase A; AKAP = A kinase anchoring protein; PDE = phosphodiesterase; P = phosphate group.

cAMP cascade elements are spatially organised also in other cell models

1) Hippocampal neurons:
co-localisation of β2 adrenergic receptors
and voltage-dependent Ca^{2+} channels (Ca_v 1.2)
(Davare *et al.*, *Science* 2001, **293**:98-102)

2) Human embryonic kidney cells (HEK-293):
co-localisation of ion channels and adenyl cyclase
(Rich *et al.*, *Proc. Natl. Acad. Sci. USA* 2001, **98**:13049-13054)