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Novel Regulation of Adenylyl Cyclases by Direct Protein-Protein Interactions: Insights from Snapin and Ric8a

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Adenylyl cyclase · cAMP · Ric8a · Snapin · Calcium

Abstract
Adenosine 3′,5′-cyclic mononucleotide (cAMP) is one of the most important second messengers which govern cellular signal transductions. Adenylyl cyclases (ACs), which are cAMP-synthesizing enzymes, are responsible for cAMP production during extracellular stimulation or intracellular metabolic alteration. In mammals, 9 transmembrane ACs and 1 soluble AC have been identified and characterized. In the past 2 decades, the biochemical properties of these ACs have been extensively studied. Genetic knockout and transgenic overexpression mouse models of at least 6 ACs have been produced, revealing their specific in vivo functions. An awareness of the importance of microdomains and cellular compartmentation for selective AC regulation has also been fostered. Most intriguingly, a handful of novel AC-binding proteins have recently been reported. Selective binding of ACs to their binding partners allows the precise compartmentalization of ACs and permits unique regulation. Based on recent studies on AC-interacting proteins (particularly Snapin and Ric8a), this review focuses on the importance and possible involvement of AC-interacting proteins in (1) the association of the cAMP signaling pathway with various cellular machineries and (2) the coordination of tightly regulated cAMP signaling by other signaling molecules.

Introduction

Adenylyl cyclases (ACs) are a family of enzymes which produce adenosine 3′,5′-cyclic mononucleotide (cAMP) from ATP. To date, 9 transmembrane ACs (tmACs) and 1 soluble adenylyl cyclase (sAC) have been cloned and characterized in mammals [1–6]. All tmACs possess 3 cytosolic domains (designated the N, C1a/b and C2 domains; fig. 1) which are separated by 2 sets of 6 transmembrane clusters (M1 and M2) [1, 4, 7, 8]. The C1a and C2 domains form the catalytic core and are highly homologous (with 50–90% similarity in amino acids) among the 9 tmAC members [9]. The catalytic core complex can be activated by forskolin or Gsα proteins [10–12] by enhancing the interaction between the C1a and C2 domains and by stabilizing the C1a–C2 catalytic core complex [9]. In addition, several AC isoforms (type I AC, AC1; type V AC, AC5 and type VI AC, AC6), but not all, can be inhibited by Giα [13]. The tmACs can also be regulated by a wide variety of signaling molecules, including G protein βγ subunits, regulators of G protein signaling (RGSs), Ca^{2+}, calmodulin, protein kinases (e.g. protein kinase A, protein kinase C, tyrosine kinases and Ca^{2+}/calmodulin-dependent protein kinase II), phosphatases and nitric oxide in isozyme-specific manners [1, 14–29]. tmACs can thus function as molecular coincidence detectors which integrate positive and negative signals that act directly through stimulation of G protein-coupled receptors (GPCRs) or indirectly via intracellular signaling path-

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ways [30]. Besides classical regulatory mechanisms mediated by trimeric G proteins or phosphorylation, ACs can also be modulated by direct protein-protein interactions which highlight the importance of binding partners and microdomains in the selectivity of AC members.

Besides the 9 tmACs, only 1 mammalian sAC has been reported. Its structure is distinct from those of the tmACs, and it does not respond to standard activators (i.e. forskolin or Gsα) of tmACs. Instead, the sAC can be directly activated by CO_2/HCO_3 and Ca^{2+} [3]. Expression of the sAC was first identified in testes and was later also found to be expressed in many other tissues (e.g. in the brain and kidneys). It is of great interest that multiple proteins (including some involved in energy utilization and membrane signal transduction) are closely associated with the sAC [31]. Specifically, the coexistence of a sperm-specific Na^+/H^+ exchanger in the same signaling complex is vital for bicarbonate-mediated activation of the sAC [32]. In renal epithelial cells, the sAC is closely associated with the vacuolar proton-pumping ATPase and is thus believed to modulate renal distal proton secretion [33]. Similar to tmACs, direct protein interactions are also one of the key modulatory modes for the sAC.

It is generally accepted that microdomains and cellular compartmentalization are critical factors determining the specific functions of ACs [14]. In the past few years, several intriguing AC-binding proteins, which provide novel associations with either new signaling molecules or components of other cellular machineries, have been reported. This review focuses on recent findings of novel AC-binding proteins which permit novel regulation and functions of ACs via direct protein-protein interactions.

Pathophysiological Functions of ACs

Due to the availability of transgenic overexpression or knockout mice of several AC isozymes, knowledge regarding the specific functions of AC isozymes has rapidly accumulated in the past decade. Selective functions of ACs revealed by genetic mouse models are listed in table 1. This is by no means a comprehensive list, but an illustration of how discriminating the functions of these ACs can be in vivo.

It has long been believed that diverse tissue expression profiles and/or selective subcellular localizations of ACs enable AC isozymes to mediate their specific pathophysiological functions. For example, type III AC (AC3) is mainly expressed in olfactory neurons [34–36] and was found to mediate the odorant-induced transduction cascade and behavior [37–39]. AC5 is heavily expressed in the striatum. It is therefore not surprising that genetic removal of AC5 impairs the function of a striatal GPCR (the D2 dopamine receptor) [40]. Likewise, the sAC was found to be highly expressed in testes. To date, the most prominent function of sAC found so far is in sperm fertilization [41–43]. Another interesting example is the apparent overlapping roles of AC1 and type VIII AC (AC8) in learning and memory. AC1 and AC8 are Ca^{2+}/calmodulin (CaM)-activated ACs, both of which exist in the hippocampus and have been demonstrated to critically contribute to long-term memory [44]. More recent studies showed that AC1 and AC8 are selectively located in the postsynaptic density and presynaptic active zones, respectively [45]. Consistent with the postsynaptic distributions, genetic removal of AC1 (but not AC8) eliminates the cortical lesion induced by an intracortical injection of N-methyl-D-aspartic acid [46]. Taken together, AC1 and AC8 play different functional roles due to their distinct localizations.

Table 1. Selective pathophysiological functions of ACs revealed by genetic mouse models

AC isozyme	Pathophysiological functions	Reference(s)
AC1	Learning and memory	116–118
	Glutamate-induced neuronal death	46
	Insertion of the AMPA receptors into synapses	119
	Opiate dependence	120
	Pain	121
	Pattern formation in the cortex	122
	Circadian rhythms	123
AC3	Odorant-induced transduction cascade	37–39
	Male fertility	124
AC5	Dopaminergic signals and motor functions	40, 125
	Cardiac stress	49, 50, 56
	Sympathetic and parasympathetic regulation of the heart	126
	Action of morphine	127
	Pain	128
	Life span	129
AC6	Left ventricular function	51–54
AC8	Learning and memory	130
	Opiate withdrawal	120
	Pain	121
	Anxiety	131
sAC	Male fertility	42, 132, 133

Table 2. Interacting proteins of ACs

AC isoform	Interacting protein	Interacting domain(s) of AC	Function	Reference(s)
AC5	PAM	C2	Inhibiting AC activity	73, 134
	RGS2	C1	Inhibiting AC5 activity	19, 74
	AKAP79/150	n.d.	Mediating the PKA-evoked inhibition of AC5	75
	Ric8a	N	Inhibiting AC5 activity	89
AC6	Snapin	N	Preventing the PKC-mediated inhibition of AC6 activity	80
	AKAP79/150	n.d.	Mediating the PKA-evoked inhibition of AC6	75
AC8	Calmodulin	N, C	Mediating the CCE-evoked stimulation	78, 114
	PP2A-C	N	Coordinating Ca^{2+} and cAMP signals	81
sAC	Na^+/H^+ exchanger	n.d.	Mediating the bicarbonate-mediated activation of sAC	32
	Vascular proton-pumping ATPase	n.d.	Modulating renal distal proton secretion	33

Trimeric GTP-binding proteins bind multiple ACs and are therefore not listed here. n.d. = Not defined.

The diverse functions of 2 calcium-inhibitable ACs (i.e. AC5 and AC6), however, are rationalized with greater difficulty. AC5 and AC6 are 2 major cardiac ACs. They both produce cAMP during stimulation of β-adrenergic receptors and have been implicated in cardiac functions for years [47–49]. Homology between AC5 and AC6 is strikingly high. The identity in amino acids of the catalytic cores (C1 and C2) of rat AC5 and AC6 is nearly 85%. Surprisingly, studies using knockout and transgenic overexpression mouse models have suggested that AC5 and AC6 function in opposite directions during cardiac stress [50, 51]. Overexpression of AC6 enhances the left ventricular function in heart failure [52–54]. Moreover, expression of AC6 in cardiomyocytes elevates cAMP production, ameliorates heart function and prolongs survival in cardiomyopathy [55]. Consistent with the beneficial roles of AC6 in the heart, the genetic inactivation of AC6 impairs the calcium response and causes severer cardiac defects [51]. In contrast, genetic removal of AC5 is beneficial and suppresses myocardial apoptosis by elevating Bcl-2 during heart failure [49, 56]. The effects of AC6 and AC5 deletion on several cardiac signaling parameters [e.g. SERCA2a affinity for Ca^{2+}, phosphorylation of phospholamban and protein kinase A (PKA) activity] also stunningly differ [51]. It is noteworthy that the beneficial effects of AC6 on failing hearts cannot be explained by simply elevating the intracellular cAMP level because chronic elevation of the cAMP content has been shown to increase mortality in patients with heart failure [57, 58]. AC6 thus might exert its advantageous effect via a pathway which is independent of cAMP generation [51, 55, 59]. It was proposed that the beneficial effect of AC6 in the heart might be mediated by regulating the activating transcription factor 3 and phospholamban in a cAMP-independent but phosphatase-dependent pathway [48, 55]. Selective cellular compartmentalization of cardiac AC6 might also contribute to its cAMP-independent functions. In the latter case, further investigation of the signal complexes that (1) comprise the binding partners (e.g. Snapin) of AC6 and (2) regulate the cAMP and Ca^{2+} interplay might provide important clues [6, 59–61].

Interacting Proteins of ACs

The 2 best-characterized binding proteins of tmACs are the α subunits of trimeric GTP-binding proteins. The stimulatory Gsα and inhibitory Giα respectively bind to the C2 and C1 domains of ACs [9, 62]. The heterotrimeric Gβγ complex also regulates the activities of tmACs. Specifically, Gβγ inhibits AC1 and AC8, while activating type II AC (AC2) and type IV AC (AC4) [21, 63]. One of the major binding domains of Gβγ in tmACs appears to be the variable C1b domain (fig. 1) [21, 64–66]. Gβγ is also associated with the C2 domain of AC2 [67, 68]. Although Gβ2 clearly binds to a relatively homologous region in the regulatory N terminus of both AC5 and AC6, the effects of Gβγ on AC5 and AC6 remain controversial and might depend on the cellular signaling conditions [69–72].

Besides the trimeric GTP-binding proteins, additional binding proteins of tmACs have been reported (table 2).

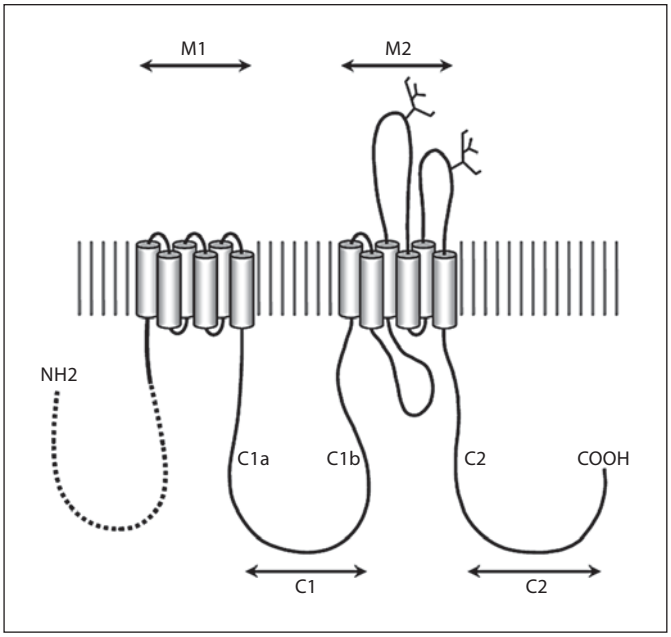

Fig. 1. Schematic representation of tmACs. All tmACs possess 3 large cytosolic domains (designated are the N, C1a/b and C2 domains) that are separated by 2 sets (M1 and M2) of 6 transmembrane clusters. The C1a and C2 domains form the catalytic core. The N terminus domains are diverse in length (ranging from 28 to 239 aa, table 3) and in amino acid sequence.

Table 3. Distinct sizes of the N termini of tmACs

AC isozyme	Length of the N terminus (aa)	Accession No. (GenBank)
AC2	44	M80550. Gb_Ro
AC4	28	M80633. Gb_Ro
AC7	33	U12919. Gb_Ro
AC5	239	M96159. Gb_Ro
AC6	163	M96160. Gb_Ro
AC1	62	M25579. Gb_Om
AC8	178	L26986. Gb_Ro
AC3	77	M55075. Gb_Ro
AC9	120	U30602. Gb_Ro

For example, the protein associated with Myc (PAM) was found to interact with AC5. By binding to its C2 domain, PAM inhibits the catalytic activity of AC5 and a few other ACs, but not all ACs (e.g. not AC2) [73]. In addition, regulators of the G protein signaling 2 (RGS2) were shown to bind to the C1 domain of AC5 and inhibit its activity [19]. Since RGS2 also interacts with other ACs, this RGS2-mediated inhibition might be a general regulatory mode for other tmACs [74]. Using an affinity purification method, an A-kinase anchoring protein (AKAP79/150) was found to form a complex with AC5 and AC6 [75]. AKAP79/150 is a multivalent scaffolding protein which is associated with many signaling molecules (such as PKA and PKC) that are capable of regulating tmACs [76]. In particular, both AC5 and AC6 can be suppressed by PKA [18, 77]. Interacting with AKAP79/150 brings PKA close to these 2 ACs and creates a negative feedback loop for the tight control of cAMP production by AC5 and AC6 during stimulation [75].

The N-terminal domains of the 9 tmACs are diverse in both length and amino acid sequences [72]. AC4 has the shortest N terminus [28; amino acids (aa)], while AC5 has the longest N terminus (239 aa) (table 3; fig. 1). Accumulating evidence has demonstrated that N termini of tmACs usually serve as regulatory domains [3, 78–81]. To date, a handful of N-terminus-interacting proteins which bind to 3 tmACs with long N termini (i.e. AC5, AC6 and AC8) have been characterized. Due to the divergent nature of these N termini, regulation mediated by N-terminus-binding proteins is usually isozyme specific [79, 80].

Interaction with Ric8a Allows the Receptor-Independent Regulation of AC5

AC5 has the longest N terminus among all 9 tmACs (table 3). The first reported binding partner of AC5′s N terminus was Gβ2 as described above [71]. Interestingly, the binding region (aa 77–151) of Gβ2 is where the N-terminal domains of AC5 and AC6 share some homology [72]. Binding to Gβ1 therefore affects the activities of both AC5 and AC6 [71].

The second reported protein which binds to the N terminus of AC5 is Ric8a, a guanine nucleotide exchange factor [79]. Ric8a was first reported to mediate the Gqα-responsible signaling pathway for neurotransmitter release in nematodes and was subsequently shown to function as a receptor-independent guanine exchange factor by binding to numerous Gα proteins [82]. Briefly, Ric8a is known to bind to the GDP-bound form of the Gα protein and promote the exchange of GDP and GTP. Interactions between AC5 and Ric8a lead to suppression of the cyclase activity of AC5 via a Giα protein-dependent pathway. Most intriguingly, this inhibition of AC5 by Ric8a requires no activation of the GPCR. In addition, Ric8a has been shown to enhance βγ-evoked signaling [47]. Since Gβγ is also known to modulate AC5 [69, 71],

it will be of great interest to further elucidate the functional interactions between Ric8a and Gβγ in regulating AC5.

As detailed above, AC5 is considered to be harmful to cardiac function during cardiac stress because the hearts of AC5-null mice express higher levels of Bcl-2 during pressure overload [49]. Suppression of AC5 is considered to be beneficial by inhibiting myocardial apoptosis during heart failure. AC5-selective inhibitors are therefore potential drugs for treating cardiac myocyte apoptosis evoked by excessive β-adrenergic stimulation [56]. Although the physiological relevance of the AC5/Ric8a complex is currently unknown, it was interesting that the level of Ric8a was elevated 48 h after an acute myocardial infarction induced by left coronary artery ligation [83]. Since Ric8a suppresses the overactivation of AC5 [79], the increase in Ric8a expression might play a protective role during cardiac stress. Potential clinical implications of the AC5/Ric8a complex are worthy of further exploration.

Snapin Links AC6 to a Wide Variety of Cellular Apparatuses

The N terminus of AC6 is a relatively large cytosolic domain of 160 aa. It interacts with the C1a domain and plays an important role in the Giα-mediated inhibition of AC6 [84]. Moreover, phosphorylation of the N terminus of AC6 at Ser^{10} by PKC is responsible for the PKC-evoked inhibition of AC6 [16, 72]. The first protein identified to interact with the N terminus of AC6 was Snapin [60]. Snapin was originally found to be located on synaptic vesicle membranes and to primarily be involved in the exocytosis process for neurotransmitter release [85]. It interacts with the assembled soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) core complex as well as with isolated SNAP25 through its C-terminal coiled-coil domain [85]. Moreover, Snapin is implicated as being a synapse-specific PKA target with an important regulatory role in neurotransmitter release [85–88]. It is interesting to note that Snapin might have a more general role in SNARE-mediated fusion events, since it has a broad tissue distribution and predominantly cytoplasmic localization [89, 90]. More recent studies have suggested that Snapin may also be involved in intracellular vesicle trafficking. The interaction between AC6 and Snapin provides a fine-tuning mechanism for the cAMP production system via the vesicle-transporting machinery in the brain.

It is intriguing that at least 20 binding proteins of Snapin were found in the past decade, indicating that Snapin might be a critical scaffold protein linking a wide variety of cellular machineries with the vesicle-transporting system (table 4). Some of these Snapin-interacting proteins are associated with neurosecretion and biogenesis of the endosomal-lysosomal system [88, 91–93]. Other Snapin-interacting proteins include a regulator of G protein signaling 7 [94], TPR/MET tyrosine kinase [95], dysbindin [96], casein kinase 1δ [97], a UT-A1 urea transporter [98], the Exo70 subunit of the exocyst [99], members of the transient receptor potential canonical (TRPC) family [93, 100] and ryanodine receptors (RyRs) [61]. Among the Snapin-interacting proteins, RyRs are of greatest interest because they are calcium channels located in the endoplasmic reticula (ER) and are major players in calcium-induced calcium release in animal cells. Snapin binds to all 3 forms of RyRs [61]. The AC6/Snapin/RyR complex might allow AC6 to be efficiently suppressed by extracellular calcium influx as well as by intracellular calcium release (fig. 2). By binding to Snapin, AC6 might regulate the trafficking of important membrane proteins in multiple organs. The role of AC6 in Snapin-mediated vesicle fusion might be complex and needs to be carefully investigated in each specific cell type. It is possible that AC6 might manipulate vesicle fusion events and the tight interaction between Ca^{2+} and cAMP by (1) producing cAMP and (2) tethering a group of calcium-sensitive molecules to the site of action. In cells expressing AC6 (fig. 2a), the stimulation of a Gsα protein-coupled receptor (Gsα-R) activates AC6, triggers cAMP production and subsequently activates PKA. It is noteworthy that AC6 was shown to be associated with AKAP150 and likely coexists with PKA [75]. Activation of PKA then leads to the phosphorylation of (1) Snapin enhancing its ability to interact with SNAP25 [85] and modulating vesicle fusion events, (2) receptors or channels (e.g. AMPA GluR1 and the L-type calcium channel) increasing their activities and triggering more calcium to enter the cells [101–104], (3) the RyR, prolonging its activity [105] and thus promoting greater calcium release from ryanodine-sensitive intracellular stores and (4) AC6 itself, efficiently terminating cAMP production [18]. In the presence of AC6 signalosomes, activation of the Gsα-R might efficiently potentiate calcium-mediated responses at multiple steps by PKA-evoked phosphorylation. The impaired calcium response found in cardiac myocytes that contain no AC6 supports the above-mentioned concept [51]. Due to the apparent increase in efficiency, this fine-tuning mode of

Table 4. Snapin-interacting proteins

Protein	Interacting domain of Snapin	Function	Reference
SNAP25	C terminus (aa 79–136)	Synaptic vesicle exocytosis	85
SNAP23	C terminus (aa 79–136)	Nonneuronal vesicle exocytosis	89
RGS7	n.d.	Synaptic vesicle exocytosis	94
AC6	aa 33–51	Prevention of the PKC-mediated inhibition of AC6	60
TRPV1	n.d.	Inhibition of the PKC-induced potentiation of TRPV1 channel activity	135
BLOS1 BLOS2 BLOS3	n.d.	n.c.	92
Dysbindin-1	n.d.	Synaptic vesicle exocytosis	96
Cypin	C terminus (aa 81–126)	Modulation of neuronal processes	136
Collectrin	n.d.	Modulation of insulin exocytosis	137
EBAG9	N terminus (aa 21–82)	Exocytosis	91
TPR/MET	n.d.	n.c.	95
CK1δ	C terminus (aa 37–136)	Phosphorylation of Snapin	97
GCSF-R	n.d.	n.c.	138
RyR	n.d.	Sensitization of the RyR at submicromolar concentrations of Ca^{2+}	61
Urea transporter UT-A1	C terminus (aa 81–126)	Enhancement of urea transport activity	98
TRPM7	n.d.	Modulation of postsynaptic EPSPs	93
α_{1A}-AR	n.d.	Enhancement of α_1a-adrenoceptor-mediated calcium influx through TRPC6	100
TRPC6	n.d.	Enhancement of α_1a-adrenoceptor-mediated calcium influx through TRPC6	100
Exo70	C terminus (aa 84–118)	Modulation of GLUT4 trafficking	99

The functional consequence of the corresponding interaction is listed.

SNAP25 = Synaptosome-associated 25-kDa protein; RGS7 = regulators of G-protein signaling 7; AC6 = type VI adenylyl cyclase; TRPV1 = vanilloid receptor-1; BLOS1–3 = BLOC subunits 1, 2 and 3 (BLOC-1 = lysosome-related organelle complex-1); EBAG9 = estrogen receptor-binding fragment-associated gene 9; CK1δ = casein kinase 1δ; GCSF-R = granulocyte colony-stimulating factor receptor; RyR = ryanodine receptor; TRPM7 = transient receptor potential cation channel, subfamily M, member 7; α_{1A}-AR = α_{1A} adrenergic receptor; EPSP = excitatory postsynaptic potential; TRPC = transient receptor potential canonical; GLUT4 = glucose transporter 4; aa = amino acids; n.d. = not defined; n.c. = not characterized.

AC6 signalosomes might be critical for the suboptimal stimulation of the Gsα-R. In cells expressing other AC isoforms, the Snapin/RyR complex is not in the proximal position of AC and might not be effectively phosphorylated by PKA, except when activation of the Gsα-R is maximal.

When the actions of Ca^{2+} are considered, the cAMP/PKA pathway is expected to be negatively regulated at multiple steps in cells expressing AC6 (fig. 2b). Stimulation of receptors and/or depolarization allows calcium to enter through a membrane receptor/channel (R_2). An increase of cellular Ca^{2+} (1) triggers vesicle fusion through the SNARE complex, (2) evokes RyR-mediated Ca^{2+}-induced Ca^{2+} release, further increasing the cellular Ca^{2+} level and thus suppressing AC6, and (3) directly suppresses the activity of AC6, reducing cAMP production and PKA activation. Collectively, the presence of the AC6/Snapin complex ensures that the cAMP/PKA pathway activated by AC6 is efficiently suppressed by Ca^{2+} at multiple steps, thereby creating a precisely controlled interplay between cAMP and Ca^{2+}.

Another very interesting class of Snapin-binding partners is the TRPC family (fig. 3). In sympathetic neurons, Snapin binds to a TRPC member (TRPM7) which is located in synaptic vesicles. TRPM7 is a nonselective cationic channel and is inactive unless synaptic vesicles are fused with plasma membranes via the SNARE machinery [93]. In addition, Snapin directly interacts with TRPC6 (a TRPC member) and the α_1-adrenergic receptor (a GPCR) and subsequently enhances the calcium

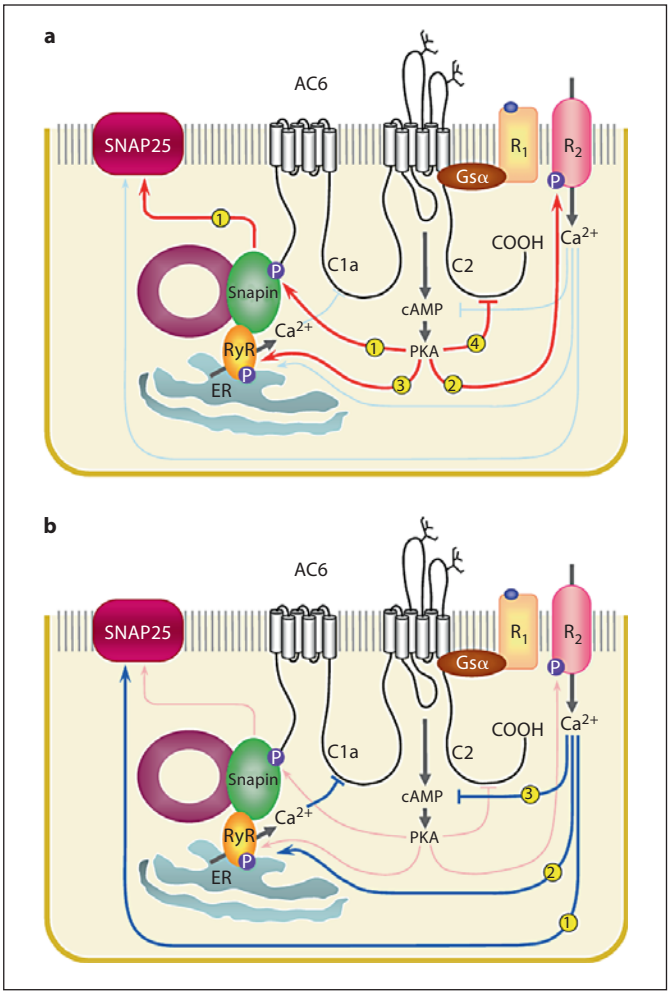

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Fig. 2. Schematic diagrams of the potential roles of AC6/Snapin/RyR complex in the calcium–cAMP interplay. **a** Actions of PKA: in the presence of an AC6 signalosome, the activation of a Gsα protein-coupled receptor (Gsα-R) efficiently modulates calcium-mediated responses at multiple steps by PKA-evoked phosphorylation (1–3, please see the text for details). Activated cAMP/PKA might also phosphorylate AC6 itself to rapidly terminate cAMP production (4). This fine-tuning mode of the AC6 signalosome might play a significant role during suboptimal stimulation of the Gsα-R. Only the actions of PKA are illustrated here. **b** Actions of Ca^{2+}: when the actions of Ca^{2+} are considered, the cAMP/PKA pathway is expected to be negatively regulated at multiple steps in cells expressing AC6. Stimulation of receptors and/or depolarization allows calcium entry through a membrane receptor/channel (R_2). Increase of cellular Ca^{2+} (1) triggers vesicle fusion through the SNARE complex, (2) evokes RyR-mediated Ca^{2+}-induced Ca^{2+} release, further increasing cellular Ca^{2+} levels and thus suppressing AC6, and (3) directly suppresses the activity of AC6, reducing cAMP production and PKA activation. The presence of the AC6/Snapin/RyR complex ensures that the cAMP/PKA pathway activated by AC6 is efficiently suppressed by Ca^{2+} at multiple steps. ER = Endoplasmic reticula; P = phosphorylation site; R_1 = Gsα-coupled receptor; R_2 = receptors or channels which allow the entry of extracellular calcium.

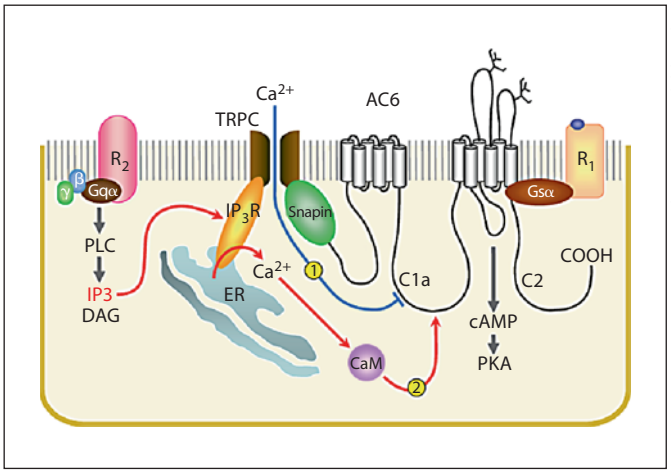

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Fig. 3. Schematic diagram of the potential roles of AC6/Snapin/TRPC complex in a calcium–cAMP interplay. By binding to Snapin, AC6 is expected to be close to TRPC (a major component of CCE) [110, 119] and can be effectively inhibited by CCE [23]. In addition, since IP_3R is also a binding partner of TRPC [111], the AC6/Snapin complex might therefore be associated with TRPC and IP_3R, which allows AC6 to be selectively potentiated during activation of Gαq-coupled receptors [112]. R_1 = Gsα-coupled receptor; R_2 = Gqα-coupled receptor; ER = endoplasmic reticula.

influx evoked by receptor-operated Ca^{2+} channels [100]. Two other TRPC family members (TRPC1 and TRPC3) were also found to interact with Snapin-interacting proteins (e.g. SNAP-25, SNAP-23 and syntaxin-3) [106–108]. Taken together, the direct binding of Snapin at its N terminus brings AC6 to a position proximal to TRPC family members which are involved in capacitative calcium entry (CCE) [109, 110]. This is of great importance because AC6 can be selectively inhibited by CCE in non-excitable cells [23]. As TRPC also interacts with inositol 1,4,5-triphosphate receptors (IP_3Rs) [111], it is likely that the AC6/Snapin complex might be associated with TRPC and IP_3R. Noteworthy is that since several ACs (e.g. AC5, AC6 and AC8) with different N termini can be regulated by CCE [112, 113], Snapin-mediated signalosomes (AC6, Snapin, TRPC and IP_3R) are less likely to play a major role in CCE-mediated inhibition. Instead, such a protein complex might significantly contribute to Gαq-mediated selective potentiation of AC6 (but not AC5) [112].

In addition to the RyR and TRPC family, there are at least 18 more proteins which interact with Snapin (table 4). Through binding to Snapin, AC6 might move through the vesicular transport system to closely associ-

ate with a wide variety of cellular machineries. The AC6-AKAP79/150-PKA complex allows Snapin to tightly control PKA-mediated regulation of the apparatuses that interact with Snapin. Although the most important functions of Snapin signalosomes appear to provide a platform for the interplay between cAMP and Ca^{2+} as described above, novel cross-talk mechanisms among Snapin-binding proteins are expected to be rapidly unraveled in the near future.

The N Terminus of AC8 Brings Together the Key Elements for a cAMP-Signaling Module

The N terminus of AC8 is of great interest because it is the longest among those of the 3 Ca^{2+}-responsive ACs (AC1, AC3 and AC8; table 3) and plays a critical role in CCE's regulation of AC8 [114]. At least 2 important signal molecules (calmodulin and phosphatase 2A) bind to AC8's N terminus in a mutually exclusive manner [78, 82, 114]. Dynamic regulation among AC8, calmodulin and phosphatase 2A might represent a central step in coordinating cAMP, Ca^{2+} and protein phosphorylation of major components in the surrounding microdomains [6].

Concluding Remarks

Almost 2 decades after the first AC was cloned [115], tremendous amounts of information regarding ACs have accumulated. The most exciting findings to date include specific in vivo functions of ACs revealed by genetic mouse models, and the awareness that AC signalosomes tether major players of cAMP modules to appropriate cellular compartments. Searching for novel AC-interacting proteins, particularly those which bind to regulatory domains, has therefore set the stage for identifying novel functions and regulatory mechanisms of ACs. In addition, new genetic mouse models of tissue/cell-specific AC knockout, conditional AC knockout and knockin of AC mutants lacking regulatory domains should provide further understanding of the pathophysiological roles of ACs in the coming decade.

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