Compartmentalized cAMP signalling regulates vasopressin-mediated water reabsorption by controlling aquaporin-2

V. Henn*, E. Stefan*, G.S. Baillie†, M.D. Houslay†, W. Rosenthal†‡ and E. Klussmann*‡

1Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Str. 10, 13125 Berlin, Germany, ‡Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Science, University of Glasgow, University Avenue, Glasgow G12 8QQ, Scotland, U.K., and Institut für Pharmakologie, Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Campus Benjamin Franklin, Thielallee 67–73, 14195 Berlin, Germany

Abstract

The cAMP/PKA (protein kinase A) signalling pathway is activated by a plethora of stimuli. To facilitate the specificity of a cellular response, signal transduction complexes are formed and segregated to discrete sites (compartmentalization). cAMP/PKA signalling compartments are maintained by AKAPS (A-kinase anchoring proteins) which bind PKA and other signalling proteins, and by PDEs (phosphodiesterases). The latter hydrolyse cAMP and thus limit its diffusion and terminate PKA activity. An example of a cAMP-dependent process requiring compartmentalization of cAMP/PKA signals is arginine-vasopressin-regulated water reabsorption in renal principal cells. A detailed understanding of the protein interactions within a signal transduction complex offers the possibility to design agents influencing PKA binding to a specific AKAP, the targeting of an AKAP or the interactions of AKAPS with other signalling molecules. The ability to specifically modulate selected branches of a signal transduction pathway would greatly advance basic research, and may lead to new drugs suitable for the treatment of diseases caused by dysregulation of anchored PKA signalling (e.g. renal and cardiovascular diseases).

Various hormones and other signals induce the generation of cAMP by activation of adenylate cyclase, most often via G-protein-coupled receptors. The most important effector molecule of cAMP is PKA (protein kinase A), a serine/threonine kinase that is involved in various processes such as growth, development, memory formation, metabolism and gene expression. Despite the great diversity of stimuli and effects, the cAMP/PKA-dependent signalling pathway is highly specific, reliably providing the adequate response to a given stimulus. One mechanism to achieve this specificity is to segregate spatially whole signal transduction complexes by concentrating them at discrete sites within the cell. This phenomenon is termed compartmentalization [1,2].

AKAPS (A-kinase anchoring proteins) play a central role in compartmentalization. AKAPS are a functionally related family of approx. 50 proteins, which are classified by their ability to bind to the regulatory subunits of PKA (Figure 1). The binding domain is structurally conserved amongst almost all AKAPS. It forms an amphipathic helix and was first characterized in a protein fragment designated Ht31 [3]. A synthetic peptide encompassing the PKA-binding region of Ht31, the Ht31 peptide, consists of 22 amino acids and competitively inhibits the binding of PKA to AKAPS. Introduced into a cell, the Ht31 peptide specifically prevents the compartmentalization but not the activation of PKA, thus making it an invaluable tool for analysing AKAP function [4–6]. Typically, AKAPS also contain a targeting domain that mediates the localization to subcellular compartments such as the plasma membranes, the cytoskeleton, nuclei or mitochondria. Many AKAPS contain several protein–protein interaction domains allowing them to bind directly to other signalling molecules. Some AKAPS function as scaffolding proteins, which organize whole signal transduction complexes. For example, Ht31/AKAP-Lbc [7,8], the full-length protein of the above mentioned Ht31 fragment, binds not only PKA but also PKC (protein kinase C) [9], PKD (protein kinase D) [9], 14-3-3 proteins [10], and the α-subunit of the heterotrimeric G-protein Gi2 [7]. In addition, Ht31/AKAP-Lbc functions as a GEF (guanine nucleotide-exchange factor) for the small G-protein RhoA. The GEF activity is regulated by the factors binding to Ht31/AKAP-Lbc. Activation of G12 by extracellular signals enhances the GEF activity, resulting in increased actin polymerization [7]. In contrast, an increase in cellular CAMP down-regulates the GEF activity through a complex mechanism: activated PKA phosphorylates Ht31/AKAP-Lbc thereby creating a binding site for a 14-3-3 protein. Binding of 14-3-3 down-regulates the GEF activity through a complex mechanism: activated PKA phosphorylates Ht31/AKAP-Lbc thereby creating a binding site for a 14-3-3 protein. Binding of 14-3-3 down-regulates the GEF activity most likely by interfering with the binding of RhoA [10]. Thus G12 and PKA have antagonistic effects on the GEF activity. Importantly, PKA phosphorylates the 14-3-3 binding site only if it is directly bound to Ht31/AKAP-Lbc, showing that proper localization of the components involved is a prerequisite for the functioning of a signalling pathway.

Key words: A-kinase anchoring protein (AKAP), aquaporin-2 (AQP2), cAMP compartmentalization, exocytosis, protein kinase A (PKA).

Abbreviations used: AKAP, A-kinase anchoring protein; AQP2, aquaporin-2; AMP, adenosine monophosphate; GEF, guanine nucleotide-exchange factor; PDE, phosphodiesterase; PKA, protein kinase A.

*To whom correspondence should be addressed (email klussmann@fmp-berlin.de).
Figure 1 | Schematic representation of an AKAP

AKAPs are modular proteins comprising several different domains. The binding domain for the regulatory subunits of PKA (RBD) is conserved within the AKAP family. The ‘targeting’ domain mediates anchoring to subcellular compartments and is specific for each AKAP. ‘Docking’ domains bind to additional signalling molecules such as kinases, phosphatases or PDEs. A few AKAPs contain domains with catalytic activities such as a GEF activity (X → Y). R and C, regulatory and catalytic subunits of PKA respectively.

Localization of PKA by itself may not be sufficient to compartmentalize a pathway. As cAMP is a small molecule and readily diffuses throughout the cell, discrete cAMP signalling compartments are only conceivable if this diffusion is restricted. This is achieved by the action of PDEs (phosphodiesterases) that hydrolyse cAMP and thus prevent spreading of the signal [11–13]. There is a large PDE superfamily comprising enzymes that hydrolyse either cAMP or cGMP or both the cyclic nucleotides. Of these, there is currently considerable interest in the cAMP-specific PDE4 family as a therapeutic target. Four PDE4 genes (4A/B/C/D) give rise to some 18 different isoforms in mammalian cells. These variants share a common catalytic domain but differ in their N-terminal regulatory and targeting domains. As one consequence, they are distributed to different sites of the cell, enabling them to restrict cAMP diffusion in diverse compartments. Indeed, Zaccolo and Pozzan [14] visualized cAMP compartmentalization and accordingly to different sites of the cell, enabling them to restrict cAMP diffusion in diverse compartments. These variants share a common catalytic domain but differ in their N-terminal regulatory and targeting domains. As one consequence, they are distributed to different sites of the cell, enabling them to restrict cAMP diffusion in diverse compartments. Thus, it is conceivable that diffusion of the signal is restricted.

Inhibition of PKA activity is only efficient if PKA and PDE4D3 are tethered in close proximity to each other on mAKAP.

Up to now, most AKAPs have been characterized in vitro but little is known about their function in a physiological context. One exception is AKAP18α (also known as AKAP15) [21,22], an AKAP of 81 amino acids which is targeted to the plasma membrane by acylation of its N-terminus. In addition, it contains a leucine zipper motif that mediates the interaction with L-type Ca2+ channels in skeletal muscle cells and cardiac myocytes. The Ca2+ conductance of the channels is enhanced by PKA phosphorylation, which is facilitated by anchoring of PKA to the channel by AKAP18α [23].

Several exocytic events are regulated or modulated by cAMP, and for a few it is known that AKAPs are involved. For example, glucose-mediated insulin secretion from pancreatic β cells is potentiayed by the hormone GLP-1 (glucagon-like peptide 1) through activation of PKA. Displacement of PKA from an AKAP, presumably AKAP18α [5,21], abolished this enhancing effect, although cAMP generation was not disturbed. Pepsinogen secretion from gastric chief cells is induced by an increase in cellular cAMP. In these cells, a complex between PKA, AKAP150 and other signalling molecules was detected. Inhibition of AKAP–PKA interactions interfered with pepsinogen secretion [6].

A further exocytic process where AKAPs are known to be involved is vasopressin-regulated water reabsorption in the kidney [4]. A key role in this process is played by the water channel AQP2 (aquaporin-2) that shuttles between an intracellular compartment and the apical plasma membrane. This process is regulated by the antidiuretic hormone AVP (arginine-vasopressin) that activates adenylate cyclase via the G1-coupled vasopressin V2 receptor. Activated PKA phosphorylates AQP2 located on intracellular vesicles thereby inducing the insertion of AQP2 into the plasma membrane. This, in turn, facilitates water reabsorption from primary urine [24,25]. Epithelial cells expressing AQP2, the collecting duct principal cells, can be isolated from rat kidney and be used as a primary cell model for this process. Using this model we have shown that the Ht31 peptide (see above) that disrupts PKA–AKAP interactions strongly inhibits the insertion of AQP2 into the plasma membrane.

Phosphorylation of PKA of Ser15 increases the affinity of PDE4D3 for mAKAP [18] and allows more efficient recruitment to the signalling complex. Phosphorylation of Ser14 enhances the hydrolytic activity of PDE4D3 [19,20], resulting in accelerated degradation of cAMP and finally termination of PKA activity. Again, this feedback inhibition of PKA activity is only efficient if PKA and PDE4D3 are tethered in close proximity to each other on mAKAP.

PDE4D3 can bind directly to both AKAP450 [16] and mAKAP [17]. The interactions between PDE4D3, mAKAP and PKA are subject to complex regulation by cAMP. Under resting conditions, inactive PKA and basally active PDE4D3 are bound to mAKAP. Elevation of cAMP causes the activation of PKA, which phosphorylates PDE4D3 on two different sites. Phosphorylation of Ser15 increases the affinity of PDE4D3 for mAKAP [18] and allows more efficient recruitment to the signalling complex. Phosphorylation of Ser14 enhances the hydrolytic activity of PDE4D3 [19,20], resulting in accelerated degradation of cAMP and finally termination of PKA activity. Again, this feedback inhibition of PKA activity is only efficient if PKA and PDE4D3 are tethered in close proximity to each other on mAKAP.

©2005 Biochemical Society
smaller portion is attached to AQP2-bearing vesicles and co-
translocates with AQP2 to the plasma membrane in response to
AVP. It is unclear how AKAP18δ associates with the vesicles.
Moreover, we identified a PDE4 variant on AQP2-
bearing vesicles (E. Stefan, G.S. Baillie, M.D. Houssloy,
W. Rosenthal and E. Klussmann, unpublished work). It in-
teracts with AKAP18δ and is involved in the AQP2 trans-
location. Thus central components of the signalling pathway
controlling the localization of AQP2 are compartmentalized
on these vesicles.

The studies of AVP-mediated water reabsorption in the
kidney clearly show that activation of PKA by itself is not
sufficient to trigger a cellular response, PKA activation has
to occur in a proper environment. Studies analysing insulin
secretion from pancreatic islets and pepsinogen secretion
from gastric chief cells support this conclusion (see above).
Analyses of anchored signalling events are likely to yield the
most reliable results if compartments are largely undisturbed.
Overexpression of proteins in a model system may lead to
saturation of a compartment resulting in overflow of the
overexpressed protein into other compartments. Therefore,
analyses of primary cell culture systems (e.g. kidney principal
cells, see above) with endogenously expressed proteins
will be of invaluable help to understand the relevance of
compartmentalization for a given cellular response.

A detailed understanding of the protein interactions within
a signal transduction module such as the ternary complex
comprising AKAP18δ, PKA and PDE4 offers the possibility
to design agents influencing PKA binding to a specific AKAP,
the targeting of an AKAP or the interactions of AKAPs with
other signalling molecules. This is particularly important for
the cAMP/PKA-dependent pathway, where direct inhibition
of PKA will affect a multitude of cellular responses. The ability to specifically modulate selected branches of a signal
transduction pathway through identification of ligands
modulating the function of a specific AKAP would greatly
advance basic research. In addition, it may lead to a new class
of drugs interfering with the function of specific AKAPs.
For example, such drugs may act as aquaretics, promoting water
excretion by inhibiting the AVP-induced redistribution of
AQP2 from intracellular vesicles to the plasma membrane.

This work was supported by grants from Deutsche Forschungs-
gemeinschaft (Ro597/9-1 and KL1415/2-1), the European Union
(QLK3-CT-2002-02149) and the Medical Research Council (U.K.;
G8604010).

References
3 Carr, D.W., Haasen, Z.E., Fraser, I.D., Stolfo-Hahn, R.E. and Scott, J.D.
Liver Physiol. 281, G1051–G1058
7 Diviani, D., Soderling, J. and Scott, J.D. (2001) J. Biol. Chem. 276,
44247–44257
8 Klussmann, E., Edemir, B., Pepperle, B., Tamma, G., Henn, V.,
FEBS Lett. 507, 264–268
9 Carnegie, G.K., Smith, F.D., McConnachie, G., Langeberg, L.K. and Scott,
2811–2820
J. Biol. Chem. 278, 5493–5496
15 Mongillo, M., McSorley, T., Evelin, S., Sood, A., Lissandron, V., Terrin, A.,
Circ. Res. 95, 67–75
16 Tasken, K.A., Collins, P., Kemmnier, W.A., Witzczak, O., Conti, M. and
17 Dodge, K.L., Khouangsiathene, S., Kapillot, M.S., Mouton, R., Hill, E.V.,
Houslay, M.D., Langeberg, L.K. and Scott, J.D. (2001) EMBO J. 20,
1912–1930
18 Carlisle Michel, J.J., Dodge, K.L., Wong, W., Mayer, N.C., Langeberg, L.K.
19 Hoffmann, R., Baillie, G.S., Mackenzie, S.J., Yarwood, S.J. and Houssloy,
M.D. (1999) EMBO J. 18, 893–903
21 Fraser, I.D., Tavalin, S.J., Lester, L.B., Langeberg, L.K., Westphal, A.M.,
22 Gay, P.C., Tilsch, V.C., Catterall, W.A. and Murphy, B.J. (1997)
J. Biol. Chem. 272, 6297–6302
37, 625–631
687–698
Biochem. Pharmacol. 141, 33–95
26 Henn, V., Edemir, B., Stefan, E., Wiebner, B., Lorenz, D., Theilig, F.,
J. Biol. Chem. 279, 26654–26665

Received 21 June 2005