Cellular Signalling 37 (2017) 1–11

Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Review

The many faces of compartmentalized PKA signalosomes

Omar Torres-Quesada, Johanna E. Mayrhofer, Eduard Stefan*

Institute of Biochemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria

ARTICLE INFO

Keywords:
Kinase
Molecular interactions
Second messenger
Dynamic complexes
Signaling nodes
Signaling circuits
Compartmentalization

ABSTRACT

Cellular signal transmission requires the dynamic formation of spatiotemporally controlled molecular interactions. At the cell surface information is received by receptor complexes and relayed through intracellular signaling platforms which organize the actions of functionally interacting signaling enzymes and substrates. The list of hormone or neurotransmitter pathways that utilize the ubiquitous cAMP-sensing protein kinase A (PKA) system is expansive. This requires that the specificity, duration, and intensity of PKA responses are spatially and temporally restricted. Hereby, scaffolding proteins take the center stage for ensuring proper signal transmission. They unite second messenger sensors, activators, effectors, and kinase substrates within cellular micro-domains to precisely control and route signal propagation. A-kinase anchoring proteins (AKAPs) organize such subcellular signalosomes by tethering the PKA holoenzyme to distinct cell compartments. AKAPs differ in their modular organization showing pathway specific arrangements of interaction motifs or domains. This enables the cell- and compartment- guided assembly of signalosomes with unique enzyme composition and function. The AKAP-mediated clustering of cAMP and other second messenger sensing and interacting signaling components along with functional successive enzymes facilitates the rapid and precise dissemination of incoming signals. This review article delineates examples for different means of PKA regulation and for snapshots of compartmentalized PKA signalosomes.

1. Introduction

Living cells respond to environmental cues using adaptive cellular networks of interacting signaling molecules. Distinct components of these signaling circuits sense and propagate the extracellular signaling input to coordinate the intracellular response [1]. A vast array of constantly received input signals is precisely processed through the compartmentalized interaction of a defined subset of signaling molecules among the huge number of various cellular molecules such as metabolites, amino acids, nucleic acids, second messengers, ions, lipids, and thousands of proteins [2–4]. It is necessary to accurately coordinate the spatiotemporal organization of the involved activators, effectors, enzymes, and substrates to ensure proper signal transmission. Besides the dynamics of second messenger fluxes the principle of physical assembly of macromolecular protein complexes has been proven to be directly relevant for the information flow [1,3,5]. In the mammalian cell system over 3000 signaling proteins and about 15 well-defined second messenger molecules are central components of cell-type specific and intertwined signaling pathways [5–10]. Transmission of the intracellular signal involves the interplay of second messenger fluxes/patterning, post-translational modifications (PTMs) and the formation of dynamic binary molecular interactions (protein-protein interactions, PPIs). The second messenger concept explains the dynamic wiring of membrane localized receptors with intracellular effector cascades and nuclear destinations. The spatiotemporal organization of discrete subsets of functionally interacting and second messenger-sensing signaling proteins is facilitated through scaffolding proteins [1,4,11]. The balance between diffusible second messengers in cytoplasm and nucleus along with the dynamic organization of these multiprotein complexes is a basic requisite for exact signal processing to guarantee the physiological output [1,12–14]. Scaffolding proteins are engaged in signal propagation by anchoring molecular switches such as GTPases and kinases. One of the best studied example for protein allostery and second messenger:protein interactions is the binding of the canonical and evolutionary conserved second messenger molecule 3′, 5′-cyclic adenosine monophosphate (cAMP) to its prototypical cellular effector molecule, the cAMP-sensing protein kinase A (PKA) [4,12,15,16]. In the course of evolution the concept of spatiotemporal coordination of functionally interacting proteins has been expanded by the creation of discrete compartmentalized PKA signalosomes. It became necessary to channel information through defined kinase signaling nodes which are organized by scaffolding proteins. Different scaffolding proteins sequester macromolecular kinase complexes into specific subcellular compartments to guarantee proper

* Corresponding author at: Institute of Biochemistry, University of Innsbruck, Innrain 80/82, A-6020 Innsbruck, Austria.
E-mail address: eduard.stefan@uibk.ac.at (E. Stefan).

http://dx.doi.org/10.1016/j.cellsig.2017.05.012
Received 4 April 2017; Received in revised form 16 May 2017; Accepted 17 May 2017
Available online 18 May 2017

signal transmission. Typically the scaffold proteins contain a modular organization with pathway specific arrangements of interaction motifs or domains [1,17]. One prominent example for functionally related scaffolding proteins with a common interaction domain but, in other respect, with different targeting and effector domains are A-kinase anchoring proteins (AKAPs) [4,18–20]. They represent a family of diverse proteins which physically interact with the cAMP-dependent PKA and coordinate second messenger responsive events by tethering the kinase to distinct subcellular compartments.

In this review we will discuss how distinct macromolecular PKA complexes act as multilayered sensors for extracellular and intracellular input signals. Moreover, we want to advance the notion that PKA activation is more than the dynamic assembly and disassembly of PKA regulatory and catalytic subunits. Recent progress in analyzing PKA structures, binary interaction partners, and signal-dependent formation of cell-type and compartment-specific PKA signaling units underlines the complexity of PKA signaling and positions PKA at the crossroad of central signaling pathways. We illustrate selected examples how PKA complexes function as multivalent and cell/compartment specific signal integrator. For example we discuss how the modular organization of compartmentalized PKA complexes reshapes the signaling output. Hereby PKA activities differ in several aspects of organizing the cellular information flow. We discuss selected features of kinase signaling by highlighting examples of PKA isoform abundance and specificity (#2), the modular scaffolding concept (#3), second messenger dependent kinase activation and inactivation (#4 & 5), PKA dynamics upon signal integration and small molecule sensing (#6), and finally selected and differentially organized/localized AKAP:PKA complexes (#7).

2. cAMP-dependent PKA holoenzyme complexes

The PKA holoenzyme is a hetero-tetramer consisting of two catalytic (C, PKAc) subunits that bind a dimer of identical regulatory (R) subunits. Binding of the cAMP-sensing R dimer to C subunits keeps the enzyme in its inactive state. Multiple genes for PKA R and C subunits have been identified; three genes for the C subunit gene products Cα, Cβ, and Cγ, and four R genes, which have been subdivided into two classes of R subunits, RI (RIα, RIβ) and RII (RIIα and RIIβ) respectively [21,22]. Cα isoforms are divided into three splice variants, Cα1, Cα2 and Cα3 respectively. The canonical Cα1 form is ubiquitously expressed throughout human tissue, while Cα2 is primarily expressed in sperm cells. Cα3 is the third variant which has been reported and still needs to be characterized [23]. The Cβ isoform is widely expressed in human tissues and presents also several spliced variants (Cβ1, Cβ2, Cβ3, Cβ4, Cβ3ab, Cβ3b, Cβ3abc, Cβ4ab, Cβ4b, and Cβ4abc) which may bind to R subunits independently of cAMP availability. The Cγ isoform has been isolated from testis tissues but its function remains still to be elucidated [24]. C subunits have a bi-lobal subdomain organization, consisting of an N-terminal (small) and C-terminal (large) lobe. Each lobe is flanked by a short N- and C- terminal tail. The N-terminal tail contains a myristoylation motif. The small lobe is followed by a non-conserved α-helix spanning both lobes. The active site of the enzyme is located in the cleft between the two lobes, with the residues critical for catalysis located on the surface of the large lobe at the active site cleft. The binding site for adenosine tri-phosphate (ATP) is also located in the cleft between the large and small lobes. Each lobe includes two hydrophobic motifs which are classified as regulatory spine (R-spine) and catalytic spine (C-spine) spanning both lobes. The position of the small lobe relative to the large lobe along with the ordering of the R and C spines contributes to the opening and closing of the active site cleft and are essential features for the catalytic process of kinases in general [12,15,25,26].

PKA specificity is among other means mediated by cAMP-sensing R subunits which simultaneously associate with AKAPs and C subunits and therefore link phosphotransferase activities to space-restricted substrate phosphorylation (Fig. 1). All four R subunits have been shown to be functionally non-redundant. In general, RIα and RIIα are ubiquitously expressed, while the RIβ and RIIβ isoforms show a more tissue specific expression pattern [27]. RIβ has been mainly detected in neuronal tissues, while RIIβ has the highest expression in neuronal, adipose, testes and heart tissues [16,20,28–30]. The amino acid sequence conservation between R types varies in sequence (they share 75% identity), molecular weight, isoelectric point, and cAMP-binding affinities. The most significant difference between RI and RII isoforms is the auto-phosphorylation potential of the inhibitor site (IS) of RII subunits, which is relevant for affecting RII:C interaction and consequently for signal amplification (see below). Moreover, PKA type I complexes have an absolute requirement for two magnesium ions and ATP to form the holoenzyme complex, while type II PKA can form a high affinities complex (< 1 nM) in the absence of this nucleotide [31]. Originally it has been proposed that RII subunits have higher affinity for type II AKAP scaffolding proteins than RI subunits for type I selective ones. Macromolecular type II AKAP complexes are often found localized to subcellular organelles, especially to the nuclear envelope, the Golgi, the cytoskeleton, and to the plasma membrane [4,16,19,32,33]. However, two type I selective AKAPs with low nM affinity for RI have recently been identified [34–36]. PKA R subunits contain a conserved domain organization. It starts at the N-terminus with the dimerization and docking domain (D/D domain). The D/D domain is essential for the assembling of the R subunit dimer and it is also indispensable for AKAP binding. For R-subunit dimerization the approximately 50-residue large D/D segments form an anti-parallel X-type helical bundle thereby creating a hydrophobic surface for AKAP docking [37–39]. The flexible linker comprises the IS and is located between D/D and the C terminal cAMP-binding domains. The IS contains the 5 residues of the PKA substrate recognition sequence, which acts as substrate/pseudosubstrate sequence by binding to the active site of C subunits [12,40]. RIα and RIβ miss the IS phosphorylation motif found in RII [41,42]. The 20 residue linker between the IS and cAMP binding domains is only partially ordered in the free R-subunit, and becomes fully ordered in the presence of the C-subunit [43,44]. Two cyclic-nucleotide binding domains A and B (CNBA, CNBB) are located C-terminally to the flexible linker region. The general CNB domain organization consists of an eight beta-stranded sandwich that forms a basket-like structure. cAMP docks to a functional phosphate in the bottom of this basket. The Phosphate Binding Cassette (PBC) forms the core of the cAMP binding module. PBC consists of two β-strands attached by a small helical turn containing the central arginine and glutamic acid residues for cAMP binding [12]. Conformational changes that occur upon cAMP binding to the PBC lead to PKA holoenzyme disassembly. The N-terminal CNBA has extended interactions with the C-subunit, while CNBB plays a role in adjusting the cAMP affinity of the CNBA and it acts as a secondary holoenzyme binding surface [12,40,45]. Structures of PKA complexes have been generated with and without the dimerization relevant N terminus of R subunits. This needs to be considered for deriving interpretations for a macromolecular AKAP:R^2:C^2 complex. The structures of PKA type I and type II holoenzymes differ considerably: The RIIβ^2:C^2 tetramer has a compact doughnut shape and shows a close conformation of the inhibited C subunit even in absence of ATP. On the contrary, the RIα^2:C^2 hetero-tetramer requires binding of two Mg^{2+} ions and ATP to the activation cleft of each C subunit leading to the closed conformation. Moreover, varying dissociation constants for the binding of the C subunit to the different R subunit have been determined for indicated R:C complexes: 0.19 nM for RIα:C, 0.1 nM for RIβ:C, > 1 nM for RIIα:C, and 0.2 nM for RIIβ:C [12,31,46–49].

3. A-kinase anchoring proteins

In this chapter we present the general AKAP concept for the organization of macromolecular PKA complexes before we discuss the different means of kinase interactions and regulations. The different PKA holoenzymes are typically sequestered to specific intracellular