A-kinase-anchoring proteins and cytoskeletal signalling events

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Abstract

Targeting of protein kinases and phosphatases to the cytoskeleton enhances the regulation of many signalling events. Cytoskeletal signalling complexes facilitate this process by optimizing the relay of messages from membrane receptors to specific sites on the actin cytoskeleton. These signals influence fundamental cell properties such as shape, movement and division. Targeting of the cAMP-dependent kinase (protein kinase A) and other enzymes to this compartment is achieved through interaction with A-kinase-anchoring proteins (AKAPs). The present paper discusses recent progress on dissecting the biological role of WAVE1 (Wiskott–Alrich syndrome protein family verprolin homology protein 1), an AKAP that assembles a cytoskeletal transduction complex in response to signals that emanate from the low-molecular-mass GTPase, Rac.

Introduction

Protein phosphorylation is a tightly regulated and dynamic process that is controlled by the opposing actions of protein kinases and phosphatases. It is now accepted that targeting of these signalling enzymes to distinct intracellular locations provides a mechanism to ensure specificity of signal transduction events by dictating which substrates are phosphorylated [1,2].

The cAMP signalling pathway has been the most extensively characterized. This group of signalling proteins generate the second messenger cAMP, which mediates most of its cellular effects by activating a cAMP-dependent protein kinase A (PKA; reviewed in [3]). The tetrameric PKA holoenzyme consists of a regulatory (R) subunit dimer and two catalytic (C) subunits. Four genes that encode R subunits (RIα, RIβ, RIIα and RIIβ) and three genes that encode C subunits (Cα, Cβ and Cγ) have been identified. Tissue- and cell-specific combinations of these enzymes control key cellular processes such as metabolism, gene transcription, ion channel conductivity, cell growth, cell division and actin cytoskeleton rearrangements. Given the broad spectrum of signalling events that is controlled by this kinase, it is crucial to understand the biochemical mechanisms that dictate both spatial and temporal organization of the PKA holoenzyme.

Spatial orientation of PKA is controlled through association with A-kinase-anchoring proteins (AKAPs). This family of functionally related proteins is classified on the basis of their ability to associate with the PKA holoenzyme inside cells [3]. Each anchoring protein contains at least two functional motifs. The conserved PKA binding motif forms an amphipathic helix of 14–18 residues that interacts with hydrophobic determinants located in the extreme N-terminus of the regulatory subunit dimmer [4]. The subcellular address of each AKAP is encoded by a unique targeting motif [3]. A shared property of most, if not all, AKAPs is the ability to form multivalent signal transduction complexes. Through simultaneous interaction with multiple signalling enzymes, such as kinases or phosphatases, AKAPs have the capacity to integrate diverse signalling pathways and regulate the phosphorylation of specific cellular substrates [2]. Accumulating evidence suggests that AKAP-mediated organization of kinases and phosphatases is particularly important for the transduction of signals to the cytoskeleton [5].

Key words: A-kinase-anchoring protein, cytoskeleton, protein kinase A.

Abbreviations used: Abl, Abelson tyrosine kinase; AKAP, A-kinase-anchoring protein; PKA, protein kinase A; SH3, Src homology 3; VPH, verprolin homology; WASP, Wiskott–Aldrich syndrome protein; WAVE, WASP family VPH protein.

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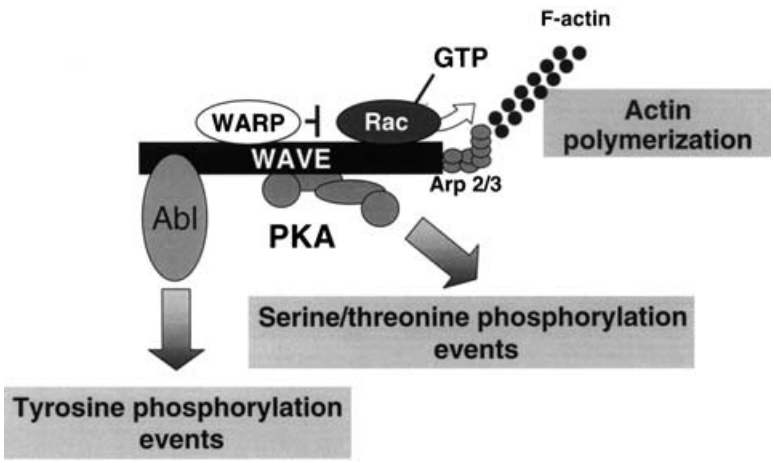
WAVE (Wiskott–Aldrich syndrome protein family verprolin homology) proteins

The WASP (Wiskott–Aldrich syndrome protein) family of proteins currently consists of six members: WASP, N-WASP, Scar-1 and three WAVE isoforms. WASP, the founding member of the family, is mutated in Wiskott–Aldrich syndrome, an X-linked human immunodeficiency disease. The WASP homologue N-WASP is expressed ubiquitously in vertebrate cells and causes the formation of filopodia when co-expressed with GTPase cdc42. Scar-1 was discovered in *Dictyostelium* in a genetic screen for proteins downstream of the chemotaxis receptor for cAMP, cAR2. More recently, three mammalian orthologues of Scar-1, termed WAVE1, WAVE2 and WAVE3, that are involved in Rac1-induced actin reorganization have been cloned. WASP family members provide a molecular bridge that functionally couples individual Rho GTPases to the Arp2/3 complex, a group of seven related proteins that function to nucleate actin polymerization [6–8]. The N-terminal regions of WASP and N-WASP contain a CRIB (cdc42/Rac interactive binding) domain that mediates the direct interaction with the activated form of cdc42, whereas the three WAVE isoforms are coupled with Rac through an as yet undefined mechanism. The central portion of each WASP family member contains

Figure 1 | WAVE1 signalling complex

A schematic diagram of protein components of the WAVE-signalling complex. Individual enzymes and effector proteins are labelled. Boxed areas highlight the potential functions of individual WAVE-associated proteins.



proline-rich sequences that interact with SH3 (Src homology 3) proteins, the G actin-binding protein profilin and other actin-associated proteins. Direct attachment to the actin cytoskeleton occurs through two conserved binding motifs: a verprolin homology (VPH) domain and a C-terminal acidic module that binds to the Arp2/3 complex.

WAVE1 binds both PKA and the Abelson tyrosine kinase (Abl) [9] (Figure 1). Abl binding appears to be a common characteristic of the WAVE family since WAVE2 and WAVE3 also interact with the Abl SH3 domain. In contrast, only the WAVE1 isoform anchors PKA. Interestingly, the RII-binding region of WAVE1 overlaps with a VPH domain that has been characterized previously as a binding site for G-actin. *In vitro* competition experiments show that actin competes for the RII-binding site [9]. This might provide a mechanism for the regulation of PKA-anchoring at sites of actin reorganization, where the local actin concentration may be sufficient to displace the anchored PKA. Another fascinating property of the WAVE isoforms is their ability to homo- and heterodimerize. This provides an additional level of organization since signalling units containing various WAVE isoforms may be nucleated at distinct sites of actin reorganization. Assembly of the WAVE1 signalling complex is dependent upon extracellular stimuli. Activation of Rac upon application of platelet-derived growth factor (PDGF) results in a rapid redistribution of WAVE1, PKA and Abl to lamellipodia and actin ring structures at the periphery of the cell [9]. Dynamic assembly of WAVE-signalling complexes may represent a sophisticated mechanism to co-ordinate the location and action of PKA and Abl, in response to extracellular signals [9] (Figure 1).

WAVE-interacting proteins

Using a combination of biochemical and proteomic approaches, a spectrum of WAVE-binding partners have been identified. WAVE-signalling complexes were isolated from rat brain extracts by immunoprecipitation. The WAVE-interacting proteins were separated by SDS/PAGE. Specific bands were excised from the gel, trypsinized and sequenced by nanoelectrospray tandem (MS/MS) mass spectrometry. So far ten or so potential binding partners have been identified. These proteins included cytoskeletal components such as a-tubulin, spectrins αII and βIII, SH3-containing adaptor proteins such as the Abl-kinase-interacting proteins, Abi-1 and Abi-2, and SNIP [SNAP-25 (synaptosomal-associated protein 25)-interacting protein]. One novel protein was identified which encoded a Rac selective GTPase activating protein (GAP) named WARP (Figure 1). WARP binds directly to WAVE1 through its SH3 domain and specifically inhibits Rac function *in vivo*. It has been suggested that WARP functions as a signal termination factor for Rac signalling and interacts with WAVE1. Similar biochemical approaches have been used to identify other WAVE1-interacting partners, including bovine orthologues of RIR121 (a p-53 inducible mRNA), Nap125 (a protein that associates with the adaptor protein Nck) and HSPC30023. It has been proposed that Rac regulation of actin polymerization involves the dissociation of active WAVE1 from this heterotetrameric complex. In light of this new information, I would propose that the WAVE-signalling complex consists of numerous signalling enzymes, cytoskeletal components and effector proteins. One of its binding partners, WARP, may provide a signal-termination component to shut down Rac-mediated actin reorganization events that proceed through the WAVE1 complex (Figure 1).

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