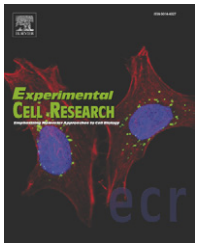
EXPERIMENTAL CELL RESEARCH 315 (2009) 127–140

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yexcr



Research Article

Microarray phenotyping places cyclase associated protein CAP at the crossroad of signaling pathways reorganizing the actin cytoskeleton in *Dictyostelium*

Hameeda Sultana[a,b], *Girish Neelakanta*[b], *Ludwig Eichinger*[a,c,d], *Francisco Rivero*[a,c,e,*], *Angelika A. Noegel*[a,c,d,*]

[a]*Center for Biochemistry, Medical Faculty, University of Cologne, 50931 Köln, Germany*
[b]*Section of Infectious Diseases, Department of Internal Medicine, Yale University School of Medicine, 300 Cedar Street, New Haven, CT 06511, USA*
[c]*Center for Molecular Medicine Cologne (CMMC), University of Cologne, 50931 Köln, Germany*
[d]*Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, 50931 Köln, Germany*
[e]*The Hull York Medical School and Department of Biological Sciences, University of Hull, Hull HU6 7RX, UK*

ARTICLE INFORMATION

Article Chronology:
Received 16 April 2008
Revised version received
29 August 2008
Accepted 14 October 2008
Available online 31 October 2008

Keywords:
Cyclase associated protein
cAMP signaling
PI3-kinases
RhoGDI
cAMP dependent protein kinase A
Endocytosis
Microarray

ABSTRACT

Large-scale gene expression analysis has been applied recently to uncover groups of genes that are co-regulated in particular processes. Here we undertake such an analysis on CAP, a protein that participates in the regulation of the actin cytoskeleton and in cAMP signaling in *Dictyostelium*. Microarray analysis revealed that loss of CAP altered the expression of many cytoskeletal components. One of these, the Rho GDP-dissociation inhibitor RhoGDI1, was analyzed further. RhoGDI1 null cells expressed lower amounts of CAP, which failed to accumulate predominantly at the cell cortex. To further position CAP in the corresponding signal transduction pathways we studied CAP localization and cellular functioning in mutants that have defects in several signaling components. CAP showed correct localization and dynamics in all analyzed strains except in mutants with deficient cAMP dependent protein kinase A activity, where CAP preferentially accumulated in crown shaped structures. Ectopic expression of CAP improved the efficiency of phagocytosis in Gβ-deficient cells and restored the pinocytosis, morphology and actin distribution defects in a PI3 kinase double mutant (*pi3k1/2* null). Our results show that CAP acts at multiple crossroads and links signaling pathways to the actin cytoskeleton either by physical interaction with cytoskeletal components or through regulation of their gene expression.

Introduction

Despite the advances of systems biology approaches, the functions of a significant number of genes and, more important, the interactions between gene products remain unknown. Large-scale gene expression analysis has been applied recently to uncover groups of genes that are co-regulated in particular processes in several organisms [1,2]. In *Dictyostelium* this approach has been used to address, for example, chemotaxis, development, spore germination, the effects of bacterial infection and the

* *Corresponding authors.* Center for Biochemistry, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 52, 50931 Köln, Germany. Fax: +49 221 478 6979.
E-mail addresses: francisco.rivero@hyms.ac.uk (F. Rivero), noegel@uni-koeln.de (A.A. Noegel).

0014-4827/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2008.10.023

response to osmotic stress [3–7], but microarray phenotyping has not been applied specifically to the study of cytoskeleton components.

The actin cytoskeleton is subject to constant remodeling in response to a diversity of extracellular cues whose effects are mediated through a complex array of signaling pathways. In *Dictyostelium* cAMP is a key regulator controlling movement during aggregation, mound and slug formation as well as cell-type specific gene expression and morphogenesis [8]. The signaling pathways leading to the activation of the adenylyl cyclase (ACA) during aggregation are well investigated. Extracellular cAMP activates second messenger pathways via a seven transmembrane receptor, cAR1, which is coupled to a heterotrimeric G-protein consisting of the Gα2 subunit and the Gβγ subunits, the latter being the direct activator of ACA. Activated ACA then produces cAMP that activates cAMP-dependent protein kinase A (PKA) and is also released into the surroundings relaying the signal to which the cells respond by chemotaxis. Through activation of PI3-kinases the cAMP gradient causes localized production of 3-phosphorylated phosphoinositides such as $PI(3,4)P_2$ and $PI(3,4,5)P_3$ which are responsible for localized actin polymerization [9]. Actin polymerization then results in directed movement of the cells along the chemotactic gradient so that they aggregate and eventually form multicellular fruiting bodies [10–13].

CAP was identified in yeast as a component of the adenylyl cyclase [14]. Further analysis showed that CAP was not only involved in aspects of adenylyl cyclase signaling but also affected the actin cytoskeleton. This latter proposal was supported by biochemical data which indeed showed that CAP can bind to actin and, moreover, influence F-actin polymerization [15]. CAP is composed of two domains separated by a central proline-rich stretch. The C-terminal domain is responsible for binding to ADP-G-actin [16] whereas the N-terminal domain interacts with the G-actin–cofilin complex [17]. The proline-rich region interacts with SH3-domain proteins like Abp1 and also with profilin [18]. The function of CAP appears to be more complex than simple monomer sequestering. Recent biochemical and *in vivo* data indicate that in yeast CAP recycles cofilin and actin for further depolymerization and polymerization, respectively, and mammalian CAP can stimulate nucleotide exchange on actin monomers, relieving the inhibitory effects of cofilin [19].

In *Dictyostelium* CAP is encoded by a single gene (*cap*) that gives rise to a 50 kDa protein present abundantly throughout development. It localizes diffusely in the cytosol, accumulates at the cell cortex [20,21] and displays partial colocalization with components of the endocytic pathway, like V-ATPase, N-ramp1 and vacuolin [22]. Using GFP fusions of CAP and several domain combinations it was shown that targeting to the cell cortex is mediated by the N-terminal domain. The proline-rich central region is dispensable for targeting [23]. This is in contrast to the yeast protein, which is targeted to the cortical cytoskeleton through binding of Abp1 to this region. The proline-rich region of *Dictyostelium* CAP is less prominent than in yeast or mammalian homologs: there is only one motif that could potentially bind SH3 domains, but this has not been analyzed. A direct interaction with profilin reported in the yeast homolog is unlikely in *Dictyostelium* CAP.

The roles of CAP were revealed during analysis of a *Dictyostelium* mutant, CAP bsr, expressing less than 5% of the endogenous protein. The mutant cells show a cytokinesis defect, a growth and developmental defect, defects in cell polarity and chemotaxis, and in the synthesis and relay of cAMP, although it remains to be clarified whether the link with the adenylyl cyclase is a direct one, like in the yeast homolog, or is mediated by other components involved in cAMP signaling [23–25]. Whether these deficiencies are a result of CAP's involvement in the regulation of the actin cytoskeleton or through separate activities of the protein has not been clarified.

Here we used DNA microarrays to characterize genes that are differentially regulated in the CAP bsr mutant. We found that expression of several genes encoding proteins directly involved in actin cytoskeleton remodeling was reduced in CAP bsr. One of these, *rdiA*, encodes a Rho GDP-dissociation inhibitor and when disrupted results in defects in cytokinesis, actin reorganization and the contractile vacuole system that in part resemble those of CAP bsr [26]. In a complementary approach to identify the position of CAP in signaling processes we moderately overexpressed CAP as a GFP–CAP fusion protein in mutants that lacked components of the G-protein-dependent signal transduction chains and tested its effect on the properties of the mutants. We show that ectopic expression of CAP in these mutants alleviated several actin-associated defects particularly in the gβ and *pi3k1/2* deficient cells. Taken together our results suggest that CAP acts at multiple crossroads and links signaling pathways to the actin cytoskeleton either by physical interaction or through regulation of gene expression of cytoskeletal components.

Materials and methods

Strains and growth conditions

Wild type strain AX2, the CAP deficient mutant CAP bsr [24], mutants deficient for cAR1/3, Gα2 [27], Gβ [28,29], ACA [30], Pia [31], PI3K1/2 [32], RhoGDI1 [26], strain HSB101 (a mutant of Pia lacking in addition ACA and ACR adenylyl cyclases) [33], strains that overexpress either the wild type (PKA-R) or a mutated (no longer binding cAMP) PKA regulatory subunit ($PKA\text{-}R^{AB}$) that inhibit the activity of the catalytic subunit [34], and strains that overexpress the Q61L mutant of Rac1a or the Q64L mutant of RacC that render the proteins constitutively active [26] were cultured as described in the respective references. GFP–CAP was expressed under the control of the constitutively active actin15 promoter [24]. The amount of protein expressed varied from one to two fold in comparison to wild type protein level. Only cells expressing moderate amounts were subcultured for the analysis.

Microarray analysis

We employed cDNA microarrays that carry a non-redundant set of 5,423 EST clones that were selected as part of the *Dictyostelium* cDNA project [35]. In addition, appropriate positive and negative controls as well as partial sequences of 450 selected genes were present on the array [36]. All probes were spotted in duplicate. A complete description of the microarray dataset is available at the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo; accession number GPL1972). Microarray production, expression profiling and data analysis have been performed essentially as described [3]. Briefly, total RNA was isolated from axenically growing AX2 and CAP bsr cells ($2–3 \times 10^6$ cells/ml) using the RNeasy Midi/Mini Kits (Qiagen, Hilden, Germany). We analyzed eight