Available online at www.sciencedirect.com
ScienceDirect
European Journal of Cell Biology 85 (2006) 1079–1090
European Journal of Cell Biology
www.elsevier.de/ejcb

Identification and isolation of *Dictyostelium* microtubule-associated protein interactors by tandem affinity purification

Katrin V. Koch[a], Yvonne Reinders[b], Thi-Hieu Ho[a], Albert Sickmann[b], Ralph Gräf[a,*]

[a]*Adolf-Butenandt-Institut/Zellbiologie, Ludwig-Maximilians-Universität, Schillerstrasse 42, D-80336 München, Germany*
[b]*Protein Mass Spectrometry and Functional Proteomics Group Rudolf-Virchow-Center for Experimental Biomedicine Versbacher Str. 9, D-97078 Würzburg, Germany*

Abstract

Tandem affinity purification (TAP) is a method originally established in yeast to isolate highly purified protein complexes in a very gentle and efficient way. In this work, we have modified TAP for *Dictyostelium* applications and have proved it as a useful method to specifically isolate and identify microtubule-associated protein (MAP) complexes. MAPs are known to interact with other proteins to fulfill their complex functions in balancing the dynamic instability of microtubules as well as anchoring microtubules at the cell cortex, controlling mitosis at the centrosome and guiding transport along them. DdEB1 and the *Dictyostelium* member of the XMAP215 protein family, DdCP224, are known to be part of complexes at the microtubule tips as well as at the centrosome. Employing TAP and mass spectrometry we were able to prove an interaction between EB1 and the DdCP224. Additionally, among other interactions that remain to be confirmed by other methods, an interaction between DdCP224 and a TACC-family protein could be shown for the first time in *Dictyostelium* and was confirmed by colocalization and co-immunoprecipitation analyses.

Keywords: Tandem affinity purification; EB1; DdCP224; XMAP215; TACC; Microtubule; Centrosome; *Dictyostelium*

Introduction

Microtubules form a dynamic network throughout the cell and are required for many essential functions such as cell migration, organelle positioning and mitosis, which make them important targets for anticancer drugs (Wilson and Jordan, 2004). To fulfill such a multitude of different tasks, microtubules need the assistance of various microtubule-associated proteins (MAPs). Many MAPs are associated with microtubule plus and minus ends, where they are members of large protein complexes. The microtubule plus-end complex has a size of about 2–3 MDa (Karki and Holzbaur, 1999) and mediates the interaction between the cytoskeleton and the cortex. The microtubule minus ends emanate from the centrosome, which is the largest protein complex in a eukaryotic cell consisting of more than hundred different protein components (Gräf et al., 2004), the identification of which has been subject of several studies, e.g. in humans, yeast, *Chlamydomonas*, *Drosophila* and *Dictyostelium* (Andersen et al., 2003; Keller et al., 2005; Lange et al., 2000; Li et al., 2004a; Pazour et al., 2005; Reinders et al., 2005; Wigge et al., 1998).

Recently, we have characterized two MAPs, DdEB1 and DdCP224, which are associated with both microtubule plus ends and the centrosome (Gräf et al., 2000, 2003; Rehberg and Gräf, 2002). They are the *Dictyostelium* representatives of the EB1 and XMAP215 protein families, which comprise the two most universal

*Corresponding author.
E-mail address: rgraef@lrz.uni-muenchen.de (R. Gräf).

0171-9335/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2006.05.008

families of MAPs, since they are present not only in fungi and animals but also in plants. In *Dictyostelium*, both MAPs have already been shown to interact with each other in cytosolic complexes (Hestermann and Gräf, 2004). DdCP224 is involved in centrosome duplication, cytokinesis, microtubule growth and microtubule plus end/cell cortex interactions (Gräf et al., 2000, 2003; Hestermann and Gräf, 2004). The C-terminal 460 amino acids of DdCP224 are sufficient for centrosomal binding (Hestermann et al., 2002), whereas a construct consisting of the N-terminal 813 amino acids localizes to the cell cortex (Hestermann and Gräf, 2004). *Dictyostelium* EB1 (DdEB1) has also been thoroughly characterized (Rehberg and Gräf, 2002). Unlike other EB1-family proteins it does not play a major role in the interactions of growing microtubule ends with docking sites at the cell cortex, but similar to its ortholog in *Drosophila* (Rogers et al., 2002) it plays an important role in mitotic progression, i.e. the initiation of spindle formation.

These two proteins were good candidates to start a screen for further, possibly unknown members of the protein complexes at both ends of microtubules. The recent completion of the *Dictyostelium* genome project (Eichinger et al., 2005) opened up a good perspective for a proteomics approach to identify putative interactors of DdEB1 and DdCP224. Such proteomic approaches were strongly facilitated by the recent invention of tandem affinity purification (TAP). TAP is a chromatographic method that was originally developed in yeast (Puig et al., 2001; Rigaut et al., 1999) and successfully applied in describing novel protein interactions in this organism (Gavin et al., 2002; Gingras et al., 2005; Shevchenko et al., 2002), as well as in the closely related fission yeast (Gould et al., 2004; Horn et al., 2005; Tasto et al., 2001). For mammalian cell lines several approaches of modified TAP have been reported recently (Drakas et al., 2005; Li et al., 2004b) and the method was also successfully used in *Caenorhabditis elegans* and *Drosophila* (Rubio et al., 2005; Veraksa et al., 2005). The TAP tag is a C-terminal protein tag consisting of a calmodulin-binding peptide and the IgG-binding domains of protein A, which are separated by a cleavage site for the highly specific TEV protease. The tagged protein of interest within a cytosolic extract is purified by two subsequent affinity purification steps using IgG- and calmodulin-conjugated beads. One of the great advantages of TAP are the gentle physiological buffers and elution conditions that can be used throughout the whole purification process and prevent disruption of protein–protein interactions within the protein complexes of interest. This is made possible by the elution from the first column via TEV protease cleavage (Senger et al., 1998), which allows only the protein of interest to be eluted but not unspecifically bound cytosolic proteins containing no TEV protease recognition site. After binding of the eluate to calmodulin beads, EGTA is used to elute highly purified protein complexes, which are then subjected to gel electrophoresis followed by mass spectrometrical analysis of individual protein bands. To be able to assess protein interactions under conditions that most closely resemble the ones found in vivo, TAP was modified to be carried out in *Dictyostelium discoideum* in this study and successfully utilized to find interactors of known MAPs.

Materials and methods

Plasmid construction

A *Dictyostelium* C-terminal TAP tag vector containing a blasticidin resistance cassette was constructed by adding a BamHI/NsiI fragment from the yeast TAP tag vector pBS1539 (Puig et al., 2001) to p1ABsr8 (Gräf et al., 2000). This vector (pKK4) is available through the Dicty Stock Center (http://dictybase.org/StockCenter/StockCenter.html).

For pKK7 (EB1-TAP) full-length DdEB1 was cut from GFP-DdEB1 (Rehberg and Gräf, 2002) with BamHI and HindIII. pKK8 was generated utilizing a Kpn/BamHI fragment encoding amino acids 1-813 of DdCP224 from pTOG38Bsr6 (Gräf et al., 2000). In case of pKK9 a KpnI/BamHI fragment encoding amino acids 809–1392 (the C-terminal 584 aa) from pTOGCBsr3 (Gräf et al., 2000) was used.

Additionally, an DdEB1-TAP vector with G418 resistance was created (pKK16) using a Hind/Xho fragment from pKK7 in the vector pA15GFPV18Sac, that consists of an N-terminal GFP under control of the actin 15 promoter and a V18-promoter/G418 resistance cassette modified from pDiscGFPSSEB2 (Daunderer and Gräf, 2002).

For expression of a GFP-TACC-domain fusion (hereafter referred to as GFP-TACC), a *Dictyostelium* cDNA library was used as a template for the amplification of a PCR product, which corresponded to base position 3611–4529 and was flanked with Sal/BamHI restriction sites. It was cloned into a modified pA6PGFP-SSEB vector (Rehberg et al., 2005) with an additional GGSGG linker downstream from the GFP sequence.

Generation of *Dictyostelium* mutants

Plasmids described above were transformed into *Dictyostelium* strain AX2 except for pKK16 which was transformed into a DdEB1 knockout mutant (Rehberg and Gräf, 2002). Cells were cultured as described earlier (Gräf et al., 2000).