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SHORT COMMUNICATION

A phototaxis signalling complex in *Dictyostelium discoideum*

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Abstract

Phototaxis has been studied in a variety of organisms belonging to all three major taxonomic domains – the bacteria, the archaea and the eukarya. *Dictyostelium discoideum* is one of a small number of eukaryotic organisms which are amenable to studying the signalling pathways involved in phototaxis. In this study we provide evidence based on protein coimmunoprecipitation for a phototaxis signalling complex in *Dictyostelium* that includes the proteins RasD, filamin, ErkB, GRP125 and PKB.

Keywords: *Dictyostelium*; phototaxis; RasD; filamin; ErkB; GRP125; PKB

Introduction

In a variety of organisms, including mammals (Treisman, 1996), signalling cascades involved in the transduction of extracellular signals into cellular responses have been characterized. The cells of both unicellular and multicellular organisms need to communicate with each other and to gather information from their surroundings. Cell movement, differentiation, growth, and apoptosis are some examples of events that can be regulated by signals that cells receive from their environment. The signals themselves can be chemical or physical in nature and one such stimulus is light. Phototactic movement towards or away from light is important for many organisms to guide their movement to locations that are better suited for acquiring nutrients or finding prey, avoiding predators or photochemical damage, growing photosynthetically or dispersing spores (Fisher, 2002).

Dictyostelium discoideum exhibits phototaxis and thermotaxis in both the unicellular and the multicellular stages of its life cycle (Fisher, 2001). Because of its well developed biochemistry and molecular genetics, its fully sequenced haploid genome (Eichinger et al., 2005) and its experimental tractability as a microorganism, *Dictyostelium discoideum* is a useful model to study eukaryotic photomovement. Classical genetic studies of phototaxis in the multicellular "slug" stage of the *Dictyostelium* life cycle suggested that as many as 55 genes are important for this process and that most of them are also required for normal thermotaxis (Darcy et al., 1994). The encoded proteins regulate signal transduction pathways involving intracellular messengers cAMP, cGMP, IP3, Ca^{2+}, heterotrimeric and small GTP-binding proteins as well as cytoskeletal proteins such as filamin (actin-binding protein (ABP)-120 or gelation factor) and myosin II (Fisher, 2001). Among the proteins known to be involved in phototaxis are RasD (Wilkins et al., 2000; Chubb and Insall, 2000), filamin (Fisher et al., 1997), and the gelsolin-related protein GRP125 (Stocker et al., 1999). Null mutants lacking any of these proteins exhibit impaired orientation towards light and deranged thermotaxis.

Many eukaryote signalling pathways are characterized by the involvement of large multiprotein complexes that mediate the necessary protein–protein interactions

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involved (Treisman, 1996). However, a role for such signalling complexes in eukaryotic photomovement has not yet been described. In this paper we report evidence based on coimmunoprecipitation for a protein complex involved in signal transduction for phototaxis in *Dictyostelium*, a complex including the proteins RasD, filamin, GRP125, PKB, and ErkB (also known as ERK2).

Materials and methods

Dictyostelium strains and culture conditions

In addition to the axenic parental strain AX2, the following transformants were used: HPF616 (transformant of AX2 ectopically expressing wild-type RasD under control of the actin-15 promoter), HPF619 (transformant of AX2 ectopically expressing wild-type RasD under control of the actin-15 promoter as well as aequorin under control of the actin-6 promoter), HG1264 (filamin null mutant, Brink et al., 1990), HPF620 (transformant of HG1264 ectopically expressing filamin under control of the actin-6 promoter) and HPF401 (AX2 transformant ectopically expressing aequorin under control of the actin-6 promoter).

Cells were either grown in shaken suspension (150 rpm) at 21 °C in HL5 axenic medium (supplemented where appropriate with 15 μg/ml G418 (Promega, Annandale, Australia) for transformed cell lines), or on SM agar plates on *Klebsiella aerogenes* lawns containing 15 μg/ml G418 (Wilczynska and Fisher, 1994; Sussman, 1966; Franke and Kessin, 1977).

Molecular cloning and vectors

A full-length *rasD* cDNA was cloned into pZErO^{TM}-2 bacterial vector (Invitrogen, Mount Waverley, Australia) using EcoRI and subcloned into pA15GFP (Fey et al., 1995) using Xho I and ClaI sites in order to create a construct expressing wild-type RasD. For antibody production, the *rasD* cDNA was cloned in frame with the FLAG epitope at the C terminus using the EcoRI site of the pFLAG-MAC vector (Sigma, Castle Hill, Australia). For production of the anti-filamin antibody, the rod domain of filamin was cloned with a hexahistidine tag at the C-terminus into the pQE32 vector (Qiagen, Doncaster, Australia) using the BamHI and SacI restriction enzymes. The FLAG-tagged RasD and His-tagged rod domain of filamin were expressed in *Escherichia coli* DH5α and M15 cells, respectively, and purified for use in antibody production from *E. coli* inclusion bodies for RasD and filamin from the soluble fraction.

The filamin-deficient mutant strain HG1264 was kindly provided by A. Noegel, having been created originally via chemical mutagenesis of strain AX2-214 with 1 mg/ml MNNG (1-methyl-3-nitro-1-nitrosoguanidine) as described in (Brink et al., 1990). No filamin protein can be detected in this mutant and the mutant phenotype can be rescued through transformation of a construct (pPROF389) encoding the full-length filamin protein.

The full-length filamin cDNA was amplified via RT-PCR using RNA derived from wild-type strain AX2 as template and 1 μg of each primer (AWF: GCGGCGGATCCTTCGAAAAGCTTCAAAAATGGCTGCCTGCTCCAAGGTGGA and AWR: GCGGCGAGCTCCTCGAGTTAATTGGCCAGTACGAGTAGTAG). After verification of its sequence, the PCR product was cloned into the pDNeO-2 (Witke et al., 1987) vector using BamHI and SacI restriction enzyme sites.

Transformation

AX2 cells were transformed with 20 μg of the RasD overexpression construct and 30 μg of the filamin construct were transformed into the filamin null strain HG1264 via $Ca(PO_4)_2$ DNA precipitation method (Nellen et al., 1984). Following 15 days of selection on *Micrococcus luteus* lawns on SM agar plates containing 15 μg/ml G418 (Wilczynska and Fisher, 1994) transformants were subcultured on *Klebsiella* lawns.

Antibody production

Polyclonal antibodies directed against RasD and filamin were produced commercially by the Institute of Medical and Veterinary Science (IMVS, Adelaide, Australia) in rabbits after subcutaneous immunization with either the recombinant His-tagged rod domain of filamin or the Flag-tagged-RasD using a standard immunization protocol with Freund's adjuvant. We affinity purified the antibody from the serum by the method of Smith and Fisher (1984).

Specific peptides were synthesized commercially (Chemicon, Boronia, Australia) for the purpose of producing antibodies reactive against the following peptides – CSGGGSGNKWNKVEE (for GRP125) and CDFEGFTYVAESEHLR (for PKB). Rabbit polyclonal antibodies against these peptides were produced by IMVS.

The anti-ErkB antibody was a kind gift from Dr. Mineko Maeda.