RA11 and RA12 antibodies recognize a peptide of the D. discoideum Mucolipin protein by western blot

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Abstract

Recombinant antibodies RA11 and RA12 detect by western blot a peptide of the *Dictyostelium discoideum* Mucolipin protein fused to a GST protein.

Introduction

Mucolipin (Mcln, DDB_G0291275, UniProt #Q54EY0) is a calcium channel involved in lysosomal calcium homeostasis in *D. discoideum* (Lima *et al*., 2012). Here we describe the ability of the RA11 and RA12 antibodies to detect by western blot a fragment of the Mucolipin protein fused to a GST protein.

Materials & Methods

Antibodies: ABCD_RA011 and ABCD_RA012 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc (MRA11 and MRA12). HeLa cells (growing in DMEM GlutaMAX™ (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~1 mg/L) were collected after 4 days.

Antigen: The antibodies were originally raised against a GST protein fused to the residues 529 to 669 of the Mucolipin protein. This chimeric GST-Mcln protein was used as antigen for detection. GST was used as a negative control.

Protocol: Expression of the GST-Mcln recombinant protein was induced in *E. coli* bacteria growing exponentially (OD_{600}, 0.5) at 37°C (in 50 ml of Luria-Bertani (LB) medium containing 20% glucose and 100 µM ampicillin) by addition of 1.5 mM IPTG. After 3 h, bacteria were pelleted and resuspended in lysis buffer (4 ml of PBS + 1% Triton X100 + aprotinin 10 µg/ml + leupeptin 20 µg/ml + iodoacetamide 1.8 mg/ ml + PMSF 18 µg/ml) and lysed by sonication. GST was purified on glutathione-coupled sepharose 4 Fast Flow beads (GE Healthcare Life Sciences #17-5132-01), then eluted in 500 µl of reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol). 15 µL of each sample was migrated (200 V, 30 min) in a 12% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1043), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked overnight at 4°C in PBS containing 0.1% (v/v) Tween20 and 5% (w/v) milk, and washed three times (5 minutes) in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the tested antibodies (undiluted), for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse (Biorad #170-6516, dilution 1:3000) for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) using a PXi-4 gel imaging systems (Syngene).

Results

MRA11 and MRA12 antibodies specifically recognize the GST-Mcln fusion protein (~43 kDa), as well as a probable partial degradation product at ~35kDa. The antibodies do not bind the GST negative control (Fig. 1).

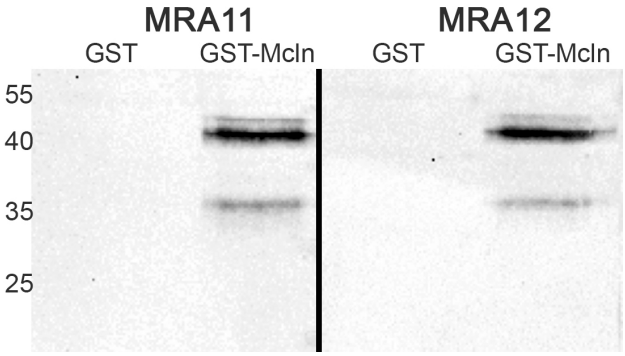

Fig. 1. Specific binding of MRA11 and MRA12 antibodies to the GST-Mcln protein (predicted molecular mass ~43 kDa).

References

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014;31(1):37-42. PMID:24100547

Lima WC, Leuba F, Soldati T, Cosson P. Mucolipin controls lysosome exocytosis in Dictyostelium. J Cell Sci. 2012; 125:2315-22. PMID:22357942

Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. Nucleic Acids Res. 2019; pii:gkz714. PMID:31410491

Conflict of interest

The authors declare no conflict of interest.