RB447, RB448, RB449, RB450, RB451 and RB453 antibodies recognize the Dictyostelium *AlyL protein by Western blot*

Otmane Lamrabet, Tania Jauslin

Cell Physiology and Metabolism Dpt, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibodies RB447, RB448, RB449, RB450, RB451 and RB453 detect by Western blot the full-length AlyL protein from *Dictyostelium discoideum*.

Introduction

AlyL (Amoeba LYsozyme Like, DDB_G0286229, UniProt #Q54M35) is a member of the amoeba lysozyme family in the amoeba *D. discoideum*. Here we describe the ability of six recombinant antibodies (RB447, RB448, RB449, RB450, RB451 and RB453) to detect the full-length AlyL protein by Western blot.

Materials & Methods

Antibodies: ABCD_RB447, ABCD_RB448, ABCD_RB449, ABCD_RB450, ABCD_RB451 and ABCD_RB453 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) 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 Fc (MRB447, MRB448, MRB449, MRB450, MRB451 and MRB453). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: RB447 was raised against a GST protein fused to the residues 18 to 84 of the AlyL protein. RB448, RB449, RB450, and RB451 were raised against a GST protein fused to the residues 18 to 84 followed with residues 479 to 572. RB453 was raised against a GST protein fused to the C-terminal residues 534 to 572. *D. discoideum alyL* knockout (KO) cells expressing a 6xHis-tagged AlyL protein (AlyL-His, 6xHis-tag fused to the C-terminus) were used to detect the full-length AlyL protein. *AlyL* KO cells were used as a negative control.

Protocol: $5x10^6$ *D. discoideum* cells were pelleted and resuspended in 200 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). 20 µL of each sample was migrated (200 V, 30 min) in a 7.5% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1023), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked during 1 hour in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed three times for 15 minutes in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the six MRB antibodies (dilution 1:10 in PBS-Tween), overnight at 4°C, then washed three times for 15 minutes. The membranes were then incubated during 1 hour with horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170-6516, dilution 1:3000) and washed twice for 15 minutes and once for 5 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results

Antibodies MRB447, MRB448, MRB449, MRB450, MRB451 and MRB453 specifically recognize the AlyL protein in *D. discoideum* cells overexpressing a 6xHis-tagged AlyL protein; the protein was not detected in *alyL*-KO cells (Fig. 1).

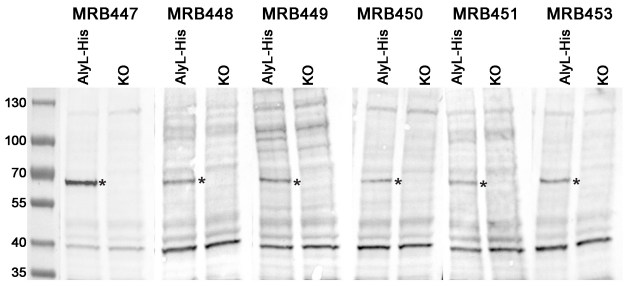

Fig. 1. Specific binding of MRB antibodies to cells overexpressing AlyL-His. AlyL-His was successfully detected by all six antibodies (position indicated by an asterisk). No band was observed in *alyL* KO cells.

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

Conflict of interest

The authors declare no conflict of interest.