

The AJ156 antibody recognizes the Dictyostelium Talin A protein by immunofluorescence

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Abstract

The AJ156 antibody, derived from the 169-477-5 hybridoma, detects by immunofluorescence the full-length Talin A protein from *Dictyostelium discoideum*.

Introduction

Talin A (Tala, Filopodin, DDB_G0290481, UniProt #P0CE95) is a cytoskeletal protein, present mostly in the tips of filopods of *D. discoideum*, recognized by the 169-477-5 monoclonal antibody (Kreitmeier *et al.*, 1995). Here we describe the ability of the AJ156 antibody, a single chain fragment (scFv) derived from the 169-477-5 hybridoma, to label filopods by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AJ156 antibody (ABCD nomenclature, web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibody with the antigen-binding scFv fused to a mouse IgG2A Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions joined by a peptide linker (GGGS)₃. The sequencing of the 169-477-5 hybridoma was performed by the Geneva Antibody Facility. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (~50 mg/L) were collected after 4 days.

Antigen: 5x10⁵ *D. discoideum* DH1 cells, sedimented on a 22x22 mm glass coverslip (Menzel-Gläser) for 120 minutes at room temperature in HL5 medium, were used to detect the full-length protein.

Protocol: Cells were fixed with HL5 + 4% paraformaldehyde (w/v) (Appllichem, #A3013) for 30 min, then washed once in PBS for 5 min. Cells were then

permeabilized in methanol at -20 °C for 2 min, washed once (5 min) with PBS, and blocked for 30 min with PBS + 0.5% (w/v) BSA. Cells were then incubated overnight with the original mouse hybridoma 169-477-5 supernatant or with the reformatted AJ156 scFv antibody (dilution 1:10 in PBS + 0.2% (w/v) BSA [PBS-BSA]). After 4 washes (5, 5, 15, 5 min) with PBS-BSA, cells were incubated for 60 min with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes #A11029). After 3 washes (5, 5, 15 min) with PBS-BSA and one wash (5 min) with PBS, coverslips were mounted on slides (Menzel-Gläser, 76x26 mm) with Mowiol (Hoechst) + 2.5% (w/v) DABCO (Fluka, #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results

In agreement with the original description of the 169-477-5 antibody (Kreitmeier *et al.*, 1995), the AJ156 antibody labels filopods, with a strongest signal seen at their tips. A faint cytosolic labelling is also seen, but the same pattern could be distinguished in cells with the primary antibody omitted (Fig. 1)

References

Kreitmeier M, Gerisch G, Heizer C, Müller-Taubenberger A. A talin homologue of Dictyostelium rapidly assembles at the leading edge of cells in response to chemoattractant. *J Cell Biol.* 1995; 129(1):179-88. PMID:7698984

Conflict of interest

Wanessa Cristina Lima is an editor of the Antibody Reports journal.

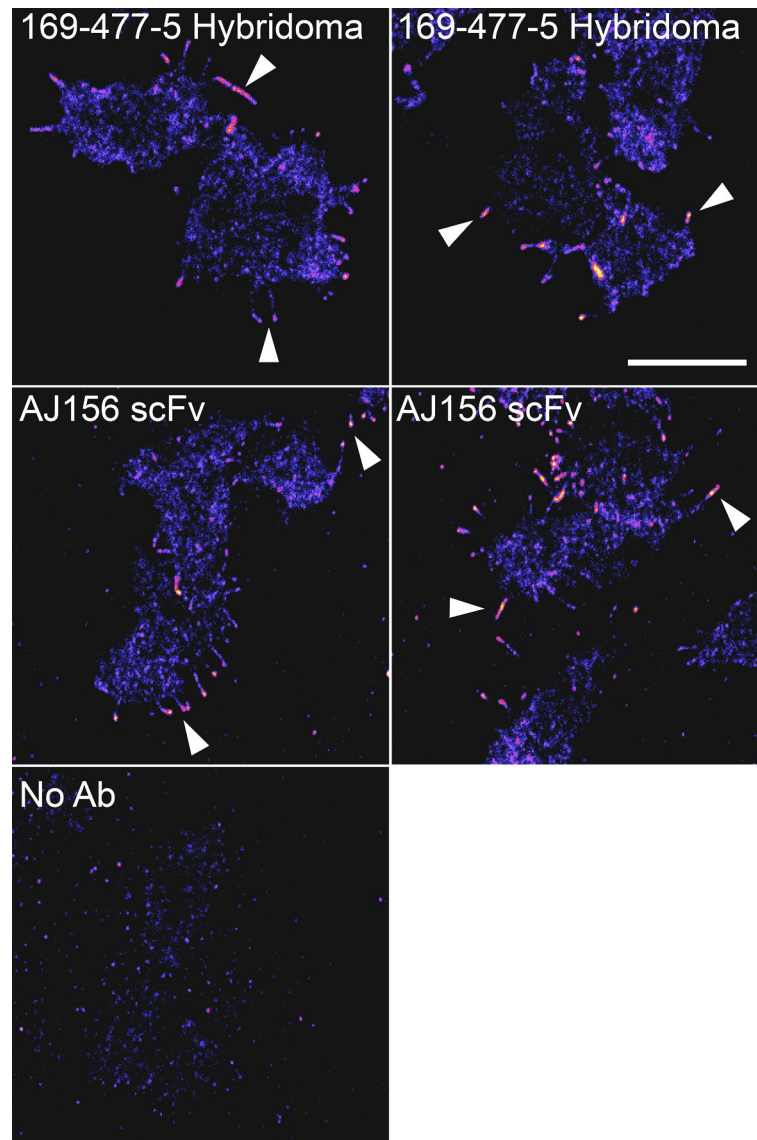


Fig. 1. The 169-477-5 hybridoma and the AJ156 scFv antibody label the tips of filopods (some examples are indicated by arrowheads) in *Dictyostelium* cells. A faint cytosolic labelling is seen also when the primary antibody was omitted (No Ab). Scale bar: 10 μ m.