The AJ520 antibody recognizes the Dictyostelium vacuolar H$^+$-ATPase subunit A by immunofluorescence

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Abstract

The AJ520 antibody, derived from the 221-35-2 hybridoma, detects by immunofluorescence the full-length vacuolar H$^+$-ATPase subunit A from *Dictyostelium discoideum*.

Introduction

The vacuolar H$^+$-ATPase subunit A protein (VatA, DDB_G0287127, UniProt #P54647) is a membrane protein present in the contractile vacuole and endosomal compartments in *D. discoideum*, recognized by the 221-35-2 monoclonal antibody (Jenne *et al*., 1998; Neuhaus *et al*., 1998). Here we describe the ability of the AJ520 antibody, a single chain fragment (scFv) derived from the 221-35-2 hybridoma, to label the contractile vacuole and endosomes by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AJ520 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 $(GGGGS)_3$. The sequencing of the 221-35-2 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 5 days.

Antigen: 5x10^5 *D. discoideum* DH1 cells, sedimented on a 22x22 mm glass coverslip (Menzel-Gläser) for 90 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) (Applichem, #A3013) for 30 min, and blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in methanol at -20 ºC for 2 min, washed once (5 min) with PBS, and once (15 min) with PBS + 0.2% (w/v) BSA (PBS-BSA). Cells were then incubated for 30 min with the original mouse hybridoma 221-35-2 supernatant (dilution 1:3 in PBS-BSA) or with the reformatted scFv antibody (dilution 1:10 in PBS-BSA). After 3 washes (5, 5, 15 min) with PBS-BSA, cells were incubated for 30 min with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (hybridoma) or AlexaFluor-647 (scFv) (1:300, Molecular Probes #A11029 and #A21235, respectively). 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 Möwiol (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 descriptions of the 221-35-2 antibody (Jenne *et al*., 1998; Neuhaus *et al*., 1998), the AJ520 antibody labels the tubules and vacuoles of the contractile vacuole system and endosomal compartments (Fig. 1).

References

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Conflict of interest

Pierre Cosson and Wanessa Cristina Lima are editors of the Antibody Reports journal.

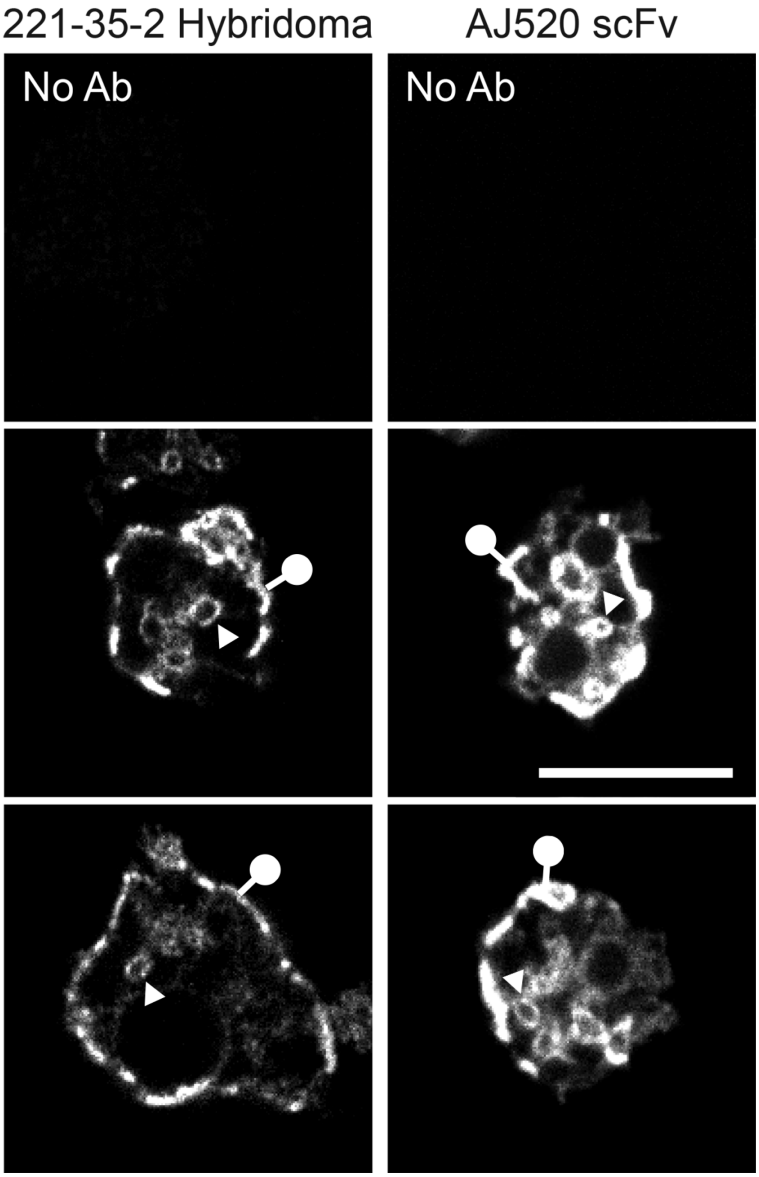

Fig. 1. The 221-35-2 hybridoma and the AJ520 scFv antibody label the contractile vacuole system (pinheads) and endosomal compartments (arrowheads) in *Dictyostelium* cells. No labelling was seen when the primary antibody was omitted (No Ab). Scale bar: 10 μm.

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