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The AN703 antibody recognizes *Dictyostelium discoideum* PDI by immunofluorescence

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Abstract

The AN703 antibody, derived from the 221-42-1 hybridoma, detects PDI-containing endoplasmic reticulum of *Dictyostelium discoideum* by immunofluorescence.

Introduction

Protein disulfide isomerase (PDI, DDB_G0276141, UniProt Q86IA3) is a protein present in the endoplasmic reticulum (ER) in *D. discoideum*, and it is recognized by the 221-42-1 monoclonal antibody (Monnat *et al.*, 1997). Here we describe the ability of the AN703 antibody, a single chain fragment (scFv) derived from the 221-42-1 hybridoma, to label the endoplasmic reticulum by immunofluorescence.

Materials & Methods

Antibodies: The ABCD_AN703 antibody (ABCD nomenclature, <http://web.expasy.org/abcd/>) was produced by the Geneva Antibody Facility (<http://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 221-42-1 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. The supernatant (20 mg/L) was collected after 4 days.

Antigen: 5x10⁵ *D. discoideum* DH1 cells, sedimented on a 22x22 mm glass coverslip (Menzel-Gläser) for 90 minutes at room temperature in HL5 medium, were used.

Protocol: Cells were fixed either (i) with HL5 + 4% paraformaldehyde (w/v) (Appllichem, #A3013) for 30 min, blocked with PBS + 40 mM ammonium chloride (NH₄Cl)

(Appllichem, #A3661) for 5 min, and then permeabilized in PBS + 0.1 % Triton X-100 for 5 min; or (ii) with methanol at -20 °C for 2 min. Cells were then washed once (1 min) with PBS, and once (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA). Cells were then incubated for 30 min with the original mouse hybridoma 221-42-1 supernatant (dilution 1:1 in PBS-BSA) or with the AN703 antibody (5 µg/ml 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 (1:400, 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

The AN703 antibody, like the 221-42-1 antibody (Monnat *et al.*, 1997), labels the endoplasmic reticulum of *Dictyostelium discoideum* (Fig. 1). For both antibodies, cytosolic elements as well as a perinuclear staining are visible (Fig. 1).

References

Monnat J, Hacker U, Geissler H, Rauchenberger R, Neuhaus EM, Maniak M, Soldati T. Dictyostelium discoideum protein disulfide isomerase, an endoplasmic reticulum resident enzyme lacking a KDEL-type retrieval signal. FEBS Lett. 1997;418(3):357-62. PMID: 9428745.

Conflict of interest

The authors declare no conflict of interest.

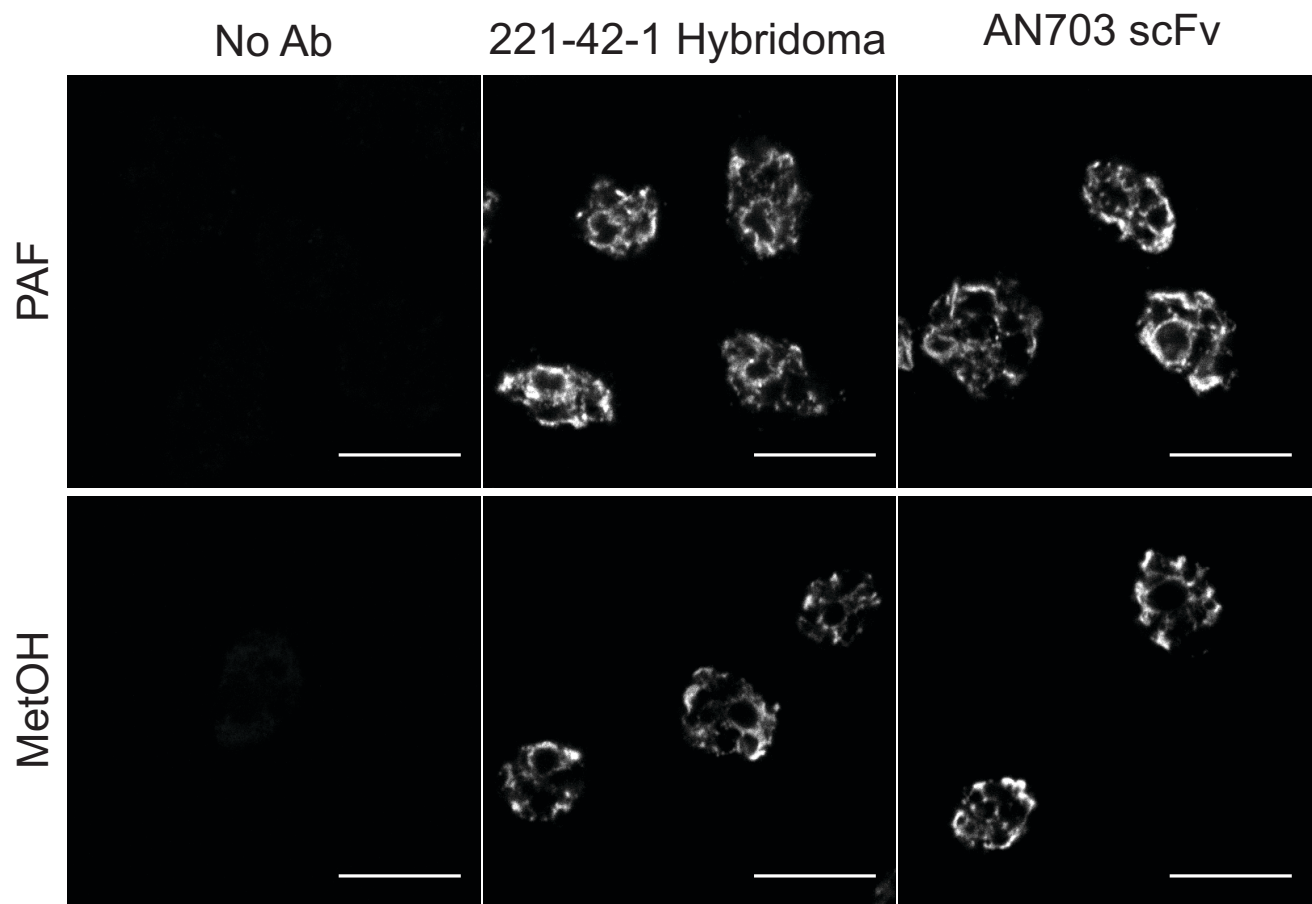


Fig. 1. The 221-42-1 and the AN703 antibodies label the endoplasmic reticulum of *Dictyostelium* cells, using two different fixation/permeabilization methods. No labelling was seen when the primary antibody was omitted (No Ab). Scale bar: 10 µm.