The AK422 antibody recognizes Dictyostelium SctA protein by Western blot

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Abstract
The AK422 antibody, derived from the B4.2 hybridoma, detects by Western blot the full-length SctA protein from Dictyostelium discoideum.

Introduction
SctA (Secreted Protein A, Protein p17, DDB_G0278725, UniProt #O77257) is the most abundant protein in secreted pycnosomes (Sabra et al., 2016). Here we describe the ability of the AK422 antibody, a single chain fragment (scFv) derived from the B4.2 hybridoma, to detect the full-length Dictyostelium SctA protein by Western blot.

Materials & Methods
Antibodies: ABCD_AK422 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 rabbit IgG 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 B4.2 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 were collected after 4 days; this antibody has a low production yield on our system (<10 mg/L).

Antigen: D. discoideum DH1 cells, cultivated in HL5 medium, were used to detect the full-length protein.

Protocol: 10^6 and 10^7 cells were pelleted, resuspended in 200 µL of 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 was added for reducing condition; reduced samples were also boiled for 15 min at 95 °C. 10 µL of each sample (5x10^4 and 5x10^5 cells) was migrated (150 V, 45 min) in a 4-20% acrylamide gel (Genscript, SurePAGE Bis-Tris, #M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 7 minutes (iBlot gel transfer device, Invitrogen #IB23001). The membranes were blocked during 60 min in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed once for 15 minutes in PBS + 0.1% (v/v) Tween20 (PBS-Tween). The membranes were then incubated overnight at RT with either the B4.2 mouse monoclonal or the AK422 scFv antibody (diluted 1:5 or 1:2 in PBS-Tween, respectively). The membranes were then washed three times (15+15+10 min) and incubated for 1 hour with the horseradish peroxidase-coupled goat anti-mouse IgG (Biorad#170-6516, dilution 1:3000) or anti-rabbit IgG (Sigma #A8275, dilution 1:3000) and washed three times (15 min) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results
Despite the fact the AK422 antibody is produced at low yield, it specifically recognizes the SctA protein, detecting a single band around 15 kDa, similarly to the original B4.2 hybridoma (Fig. 1) (Sabra et al., 2016).

Fig. 1. The B4.2 hybridoma and the AK422 scFv antibodies specifically recognize the SctA protein (predicted molecular mass ~15 kDa). (NR: non-reducing conditions; RB: reducing and boiled conditions)

References

Conflict of interest
The authors declare no conflict of interest.