AF394, AF395 and AF396 antibodies recognize a GFP-tagged recombinant protein by immunofluorescence

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Abstract

AF394, AF395 and AF396 antibodies against the GFP protein recognize a GFP-tagged human TAC protein by immunofluorescence in paraformaldehyde-fixed HeLa cells.

Introduction

The green fluorescent protein (GFP) (Uniprot #P42212) is a large (~235 aa) protein tag, originally isolated from the jellyfish *Aequorea victoria*, widely used as a fluorescent reporter to detect and visualize GFP-fused proteins (Tsien, 1998). Here, we show that the AF394, AF395 and AF396 recombinant antibodies detect a GFP-tagged human TAC protein by immunofluorescence in HeLa cells.

Materials & Methods

Antibodies: ABCD_AF394, ABCD_AF395 and ABCD_AF396 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the nanobodies GBP1, GBP4 (for AF394 and AF395, Kirchhofer *et al.*, 2010) and VHH (for AF396, Rothbauer *et al.*, 2006). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (50, 80, 100 mg/L for AF394, AF395 and AF396, respectively) were collected after 4 days.

Antigen: HeLa cells (growing in DMEM GlutaMAX™, Gibco #31966; supplemented with 8% Fetal Bovine Serum, Gibco #10270) cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and transiently transfected 2 days before the experiment with a C-terminally GFP-tagged TAC protein (Uniprot #P01589), were used to detect the protein tag. The GFP-tagged TAC protein is expected to be mostly present at the cell surface.

Protocol: The whole procedure was carried out at room temperature. Transfected HeLa cells were rinsed once with PBS, fixed with PBS + 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 PBS + 0.2% saponin (w/v) (Sigma, #S7900) for 3 min, incubated in PBS + 0.2% (w/v) BSA (PBS-BSA) for 30 min, and then with the tested anti-GFP antibodies (final concentration 5 mg/L in PBS-BSA) for 1 h. After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-647 (1:300, Molecular Probes, #A21235). After 3 washes (10 min) with PBS-BSA, cells were incubated during 10 min with DAPI (1:500, Molecular Probes, #D1306), washed twice with PBS-BSA and once with PBS, and 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

AF394, AF395 and AF396 antibodies specifically detected a signal at the plasma membrane in cells transfected with the GFP-tagged TAC protein (Fig. 1). The specificity of the signal was further verified by the absence of anti-GFP staining in the few non-transfected cells (Fig. 1, arrowheads). No staining was observed when the primary antibody was omitted (Fig. 1, No Ab).

The signal revealed by the antibodies mostly co-localized at the plasma membrane, with the signal generated by GFP fluorescence (Fig. 1, arrows). We note however that a GFP signal can also be detected intracellularly, and is mainly not recognized by the anti-GFP antibodies (red arrows). It is possible that part of the GFP protein is either slightly modified or not accessible to the antibodies, accounting for this observation.

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Conflict of interest

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

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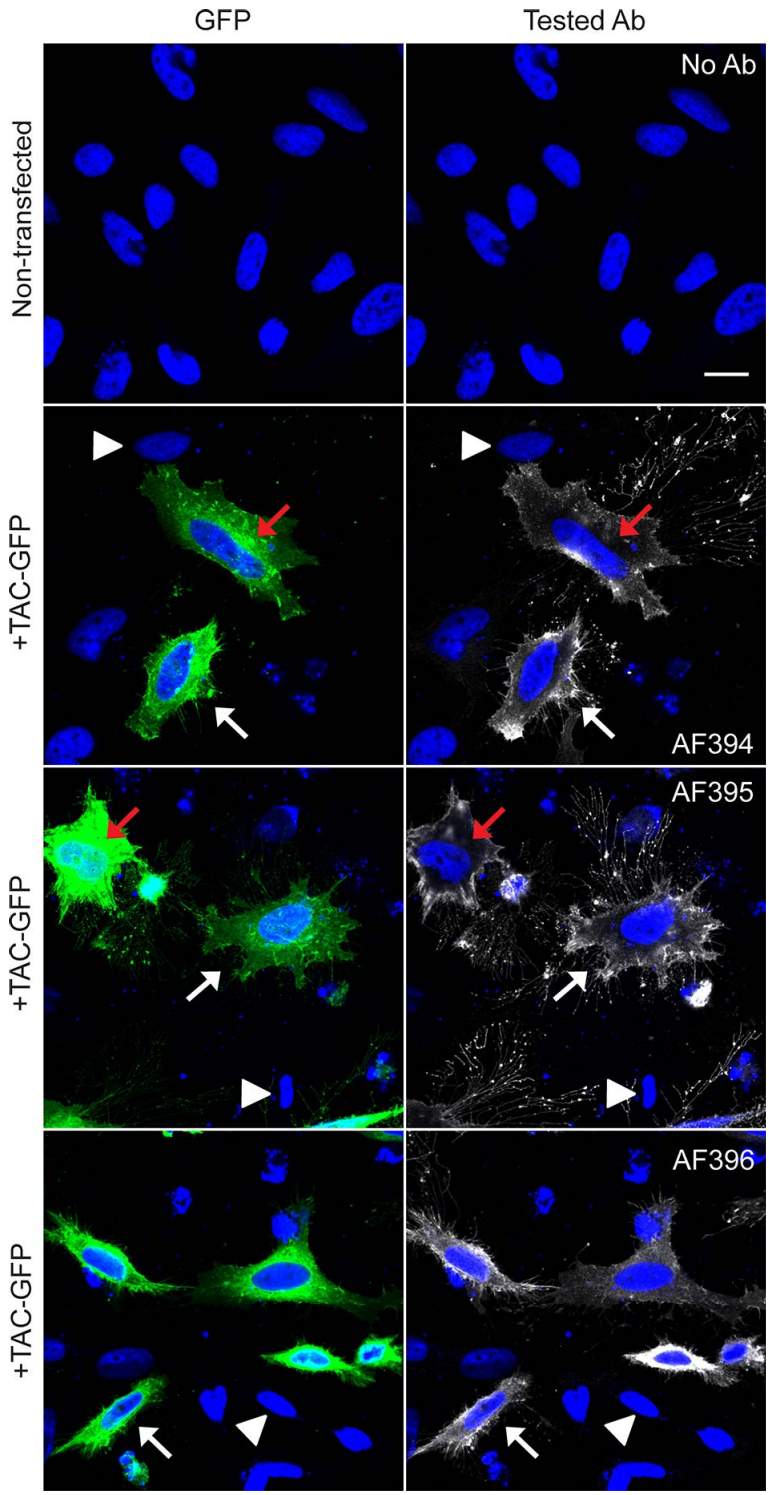

Fig. 1. AF394, AF395 and AF396 labeled the plasma membrane of HeLa cells expressing the GFP-tagged TAC protein (in white); the signal co-localized (arrows) with the signal generated by the GFP reporter (in green); however, intracellular GFP signal is mainly not recognized by the anti-GFP antibodies (red arrows); in blue, nuclei were stained with DAPI. No labelling was seen when the primary antibody was omitted, or in non-transfected cells (arrowheads). Scale bar: 20 μm.