AF397, AK652, AN193 and AV442 antibodies recognize a GFP-tagged Golgi protein by immunofluorescence

Anna Marchetti

Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

AF397, AK652, AN193 and AV442 antibodies against the GFP protein recognize a GFP-tagged human B4GT1 protein by immunofluorescence in paraformaldehyde-fixed HEK cells.

Introduction

The green fluorescent protein (GFP) (Uniprot P42212) is a 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 AF397, AK652, AN193 and AV442 recombinant antibodies detect a GFP-tagged human B4GT1 protein by immunofluorescence in HEK cells.

Materials & Methods

Antibodies: ABCD_AF397, ABCD_AK652, ABCD_AN193 and ABCD_AV442 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding domain fused to a rabbit IgG Fc. The synthesized antibody sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker $(GGGGS)_3$ (Table 1). HEK293 suspension cells (growing in HEK TF medium, Xell 861-0001, supplemented with 0.1% Pluronic F68, Sigma P1300) were transiently transfected with the vector coding for the mini-antibodies. Supernatants (see Table 1 for individual yields) were collected after 4 days.

Table 1: Clone number, epitope, reference and production yields for the antibodies used in this study.

ABCD	Clone	Binder type	Reference	Yield (mg/L)
AF397	LaG-2	VHH	Fridy *et al.*, 2014	140
AK652	BH-GBP2	VHH	Pellis *et al.*, 2012	120
AN193	3G86.32	DARPin	Brauchle *et al.*, 2014	50
AV442	N86/44.1	scFv	Andrews *et al.*, 2019	120

Antigen: HEK cells (growing in DMEM GlutaMAX™, Gibco 31966; supplemented with 8% Fetal Bovine Serum, Gibco 10270) cultured on glass coverslips (Menzel-Gläser, 22x22 mm), transiently transfected 2 days before the experiment with a C-terminally GFP-tagged human B4GT1 protein (Uniprot P15291), were used to detect the protein tag. The GFP-tagged B4GT1 protein is present mostly at the Golgi complex (Vernay *et al*., 2018).

Protocol: The whole procedure was carried out at room temperature. Cells were rinsed once with PBS, and fixed with PBS + 4% paraformaldehyde (PAF) (w/v) (Applichem A3013) for 30 min, blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem A3661) for 5 min, and then permeabilized in PBS + 0.1 % Triton X-100 for 3 min. Fixed cells were washed once (5 min) in PBS and once with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 30 min with the primary antibodies (final concentration 100 ng/mL in PBS). After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min with secondary goat anti-rabbit IgG conjugated to AlexaFluor-647 (1:400, Molecular Probes A21245). After 3 washes (10 min) with PBS-BSA, cells 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

AF397, AK652, AN193 and AV442 antibodies specifically detected a signal at the Golgi complex in cells transfected with the GFP-tagged B4GT1 protein (Fig. 1). The specificity of the signal was verified by the absence of anti-GFP staining in non-transfected cells (Fig. 1, "No B4GT1-GFP").

References

Andrews NP, Boeckman JX, Manning CF, *et al*. A toolbox of IgG subclass-switched recombinant monoclonal antibodies for enhanced multiplex immunolabeling of brain. Elife. 2019; 8:e43322. PMID: 30667360

Brauchle M, Hansen S, Caussinus E, *et al*. Protein interference applications in cellular and developmental biology using DARPins that recognize GFP and mCherry. Biol Open. 2014; 3(12):1252-61. PMID: 25416061.

Fridy PC, Li Y, Keegan S, *et al*. A robust pipeline for rapid production of versatile nanobody repertoires. Nat Methods. 2014; 11(12):1253-60. PMID: 25362362.

Pellis M, Pardon E, Zolghadr K, *et al*. A bacterial-two-hybrid selection system for one-step isolation of intracellularly functional nanobodies. Arch Biochem Biophys. 2012; 526(2):114-23. PMID: 22583807.

Tsien RY. The green fluorescent protein. Annu Rev Biochem. 1998; 67:509-44. PMID:9759496.

Vernay A, Lamrabet O, Perrin J, Cosson P. TM9SF4 levels determine sorting of transmembrane domains in the early secretory pathway. J Cell Sci. 2018; 131(21):jcs220830. PMID: 30301779.

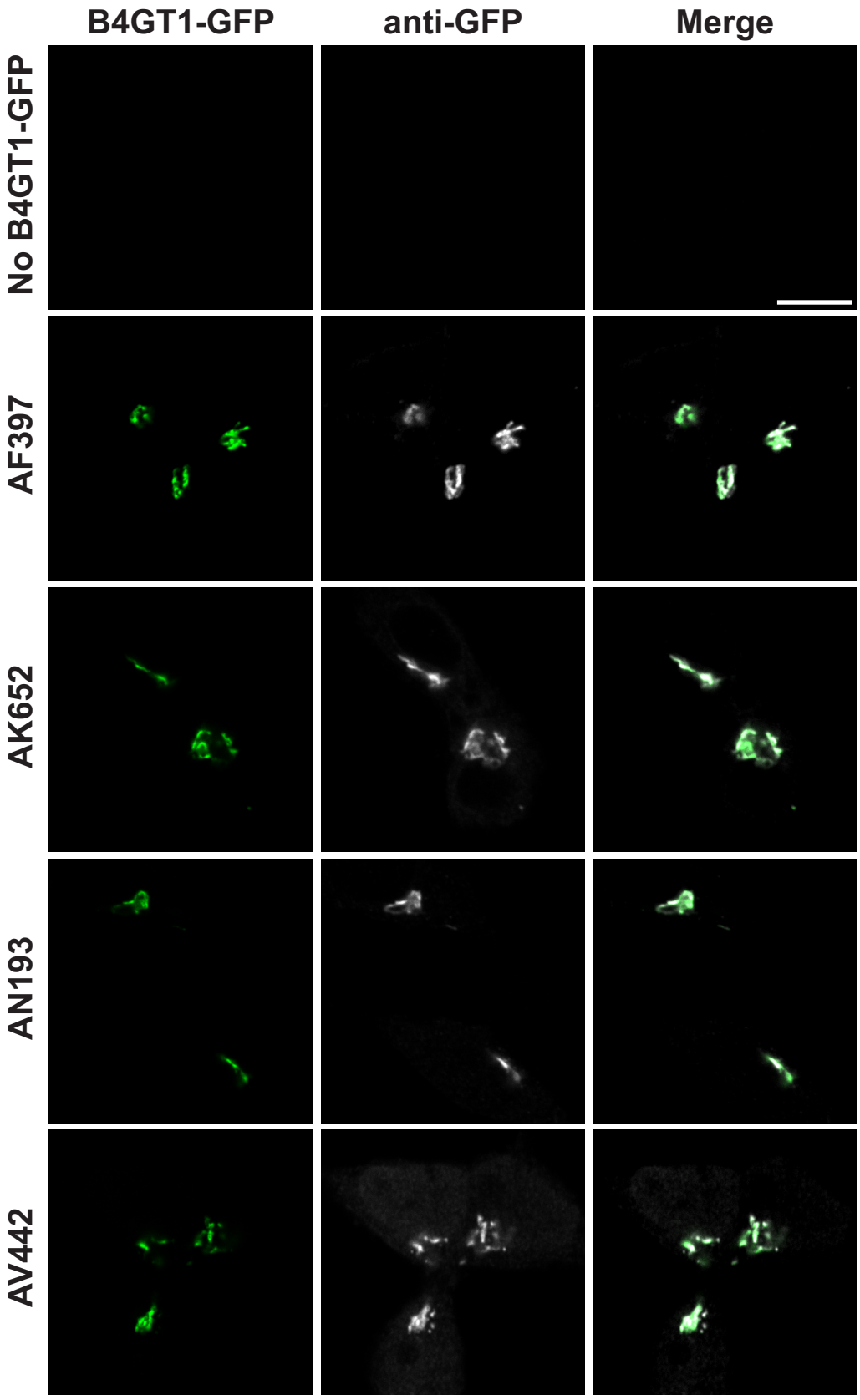

Fig. 1. AF397, AK652, AN193 and AV442 labeled the Golgi complex of HEK cells expressing the GFP-tagged B4GT1 protein (in white); the signal co-localized with the signal generated by the GFP reporter (in green). No labelling was seen in non-transfected cells ("No B4GT1-GFP"). Scale bar: 10 µm.