RB481, RB482, RB483, RB484, RB485, RB486, RB498 and RB499 antibodies recognize the COPI complex by immunofluorescence

Anna Marchetti, Cheryl Pillonel, Philippe Hammel

Cell Physiology and Metabolism Dpt, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibodies RB481, RB482, RB483, RB484, RB485, RB486, RB498 and RB499 detect by immunofluorescence the COPI complex in paraformaldehyde-fixed cells COS7 cells.

Introduction

COPI (Coatomer or COat Protein complex I) is a large polypeptide complex (composed of seven different subunits) involved in retrograde traffic from the Golgi apparatus to the endoplasmic reticulum (Cosson and Letourneur, 1994). RB481, RB482, RB483, RB484, RB485, RB486, RB498 and RB499 antibodies were originally raised against a COPI complex bound to a specific peptide, and recognize the COPI complex by ELISA (Hammel, 2020). Here we analyzed the ability of these eight recombinant antibodies to successfully recognize the COPI complex by immunofluorescence.

Materials & Methods

Antibodies: ABCD_RB481, ABCD_RB482, ABCD_RB483, ABCD_RB484, ABCD_RB485, ABCD_RB486, ABCD_RB498 and ABCD_RB499 antibodies (ABCD nomenclature, web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al.*, 2014) as mini-antibodies with the antigen-binding scFv portion fused to a rabbit IgG Fc (RRB481, RRB482, RRB483, RRB484, RRB484, RRB485, RRB486, RRB498 and RRB499). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: COS7 cells (growing in DMEM GlutaMAX™ supplemented with 10% Fetal Bovine Serum) cultured on a glass coverslip (Menzel-Gläser, 22x22 mm) were used to detect the COPI complex.

Protocol: The whole procedure was carried out at room temperature. COS7 cells were 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 5 min, washed once (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 30 min with the antibody-containing supernatants (dilution 1:10). After 3 washes (5 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:400, Invitrogen, #A11034). After 3 washes (5 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 40x Neofluar oil immersion objective.

Results

The antibodies RRB481, RRB482, RRB483, RRB484, RRB485, RRB486, RRB498 and RRB499 label vesicular structures in a juxta-nuclear region, a staining typical of COPI peri-Golgi structures (Fig. 1). No signal was detected when the primary antibody was omitted (Fig. 1). Although the staining observed is what would be expected if these antibodies recognize COPI, it will be necessary to ascertain the specificity of this immunofluorescence signal by staining cells where COPI is depleted.

References

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014; 31(1):37-42. PMID:24100547

Cosson P, Letourneur F. Coatomer interaction with di-lysine endoplasmic reticulum retention motifs. Science 1994; 263(5153):1629-31. PMID:8128252

Hammel P. RB481, RB482, RB483, RB484, RB485, RB486, RB498 and RB499 antibodies recognize the coatomer COPI complex by ELISA. Antibody Reports 2020; 3:e139. doi:10.24450/journals/abrep.2020.e139

Conflict of interest

The authors declare no conflict of interest.

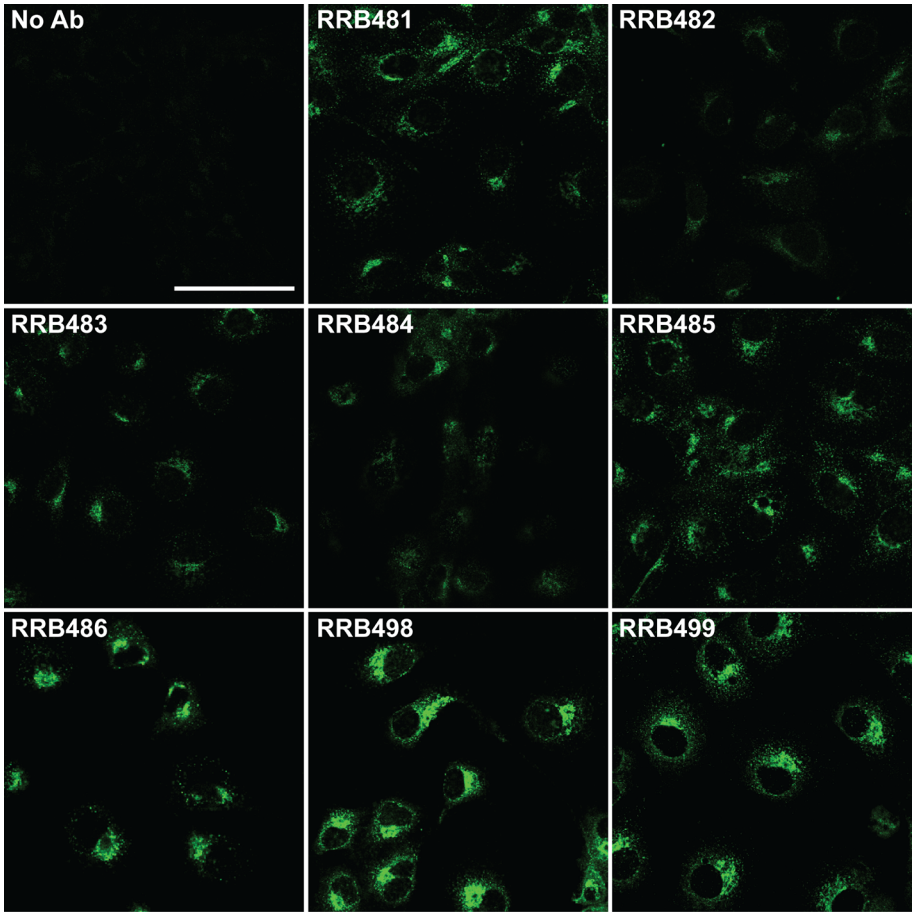

Fig. 1. RRB antibodies label vesicular structures in the juxta-nuclear region of COS7 cells. No labelling was seen when the primary antibody was omitted (No Ab panel). Scale bar: 10 µm.