The RB530 antibody recognizes microtubules by immunofluorescence

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Abstract

The RB530 antibody detects microtubules by immunofluorescence in the green algae *Chlamydomonas reinhardtii* and in human cells in culture.

Introduction

Microtubules are polymers of α- and β-tubulin essential for many biological fundamental processes ranging from cytoskeleton organization to cell division (Janke and Bulinski, 2011). Here, we describe the ability of the RB530 antibody to recognize microtubules by immunofluorescence.

Materials & Methods

Antibodies: ABCD_RB530 antibody (ABCD nomenclature, web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (Blanc *et al.*, 2014; https://www.unige.ch/antibodies/) as a mini-antibody with the antigen-binding scFv fused to a mouse IgG2A Fc (MRB530). HEK293 suspension cells (growing in FreeStyleTM 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatant (100 mg/L) was collected after 4 days.

Antigen: The antibodies were originally raised against microtubules polymerized *in vitro*. Microtubules were polymerized in the presence of pure tubulin (Cytoskeleton Inc. #T240) with 5% of tubulin biotin (Cytoskeleton Inc. #T333P). To do so, the tubulin mix (10 µl at 5 mg/ml) was dialyzed in MES buffer pH 6.9 (MES 10mM, $MgCl_2$ 1mM, EGTA 1mM) during 1 hour at 4 °C. To produce microtubules without C-terminal tails (Schmidt-Cernohorska *et al.*, 2019), tubulin was incubated at 25 °C during 45 min in the presence of subtilisin (0.2 µl at 1 mg/ml). The reaction was stopped using 0.1 µl PMSF (10 mM). Microtubules were then polymerized using taxol (final concentration 60 µM) for 40 min at 37 °C.

Protocol: U2OS cells were grown on 12 mm coverslips at 37 °C with 5% CO_2 in DMEM supplemented with GlutaMAX (Life Technology), 10% tetracycline-negative fetal calf serum (Brunschwig), penicillin and streptomycin (100 µg/ml). *Chlamydomonas reinhardtii* cells were grown in liquid Tris-acetate-phosphate medium (TAP medium containing TRACE; Hutner *et al.*, 1950) at 22 °C. Before fixation, *Chlamydomonas reinhardtii* cells were sedimented 30 min on 12 mm coverslips in a humid chamber. Cells on coverslips were fixed in cold methanol (-20 °C) for 7 min and blocked with PBS + 2% (w/v) BSA (PBS-BSA) for 15 min. Cells were then incubated for 1 hour with MRB530 diluted in PBS-BSA (1:250) and rabbit anti-beta tubulin (1:500, Abcam #Ab18251). Next, the coverslips were washed three times for 10 min in PBS-Tween 0.1% and incubated for 1 hour with secondary antibodies goat anti-mouse coupled to Alexa 488 (1:500, Invitrogen #A11029) and goat anti-rabbit coupled to Alexa 568 (1:500, Invitrogen #A11036). After 3 washes (10 min) with PBS-Tween 0.1%, coverslips were mounted in glycerol mounting medium containing DAPI (Abcam). Imaging was done on a Zeiss LSM780 microscope, using a 63x oil immersion objective.

Results

The MRB530 antibody labels both the axoneme of *Chlamydomonas reinhardtii* flagella as well as the basal body at its base (Fig. 1, inset). MRB530 also recognizes microtubules of the cytoskeleton of both *Chlamydomonas reinhardtii* and U2OS cells (Figs. 1 and 2).

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Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

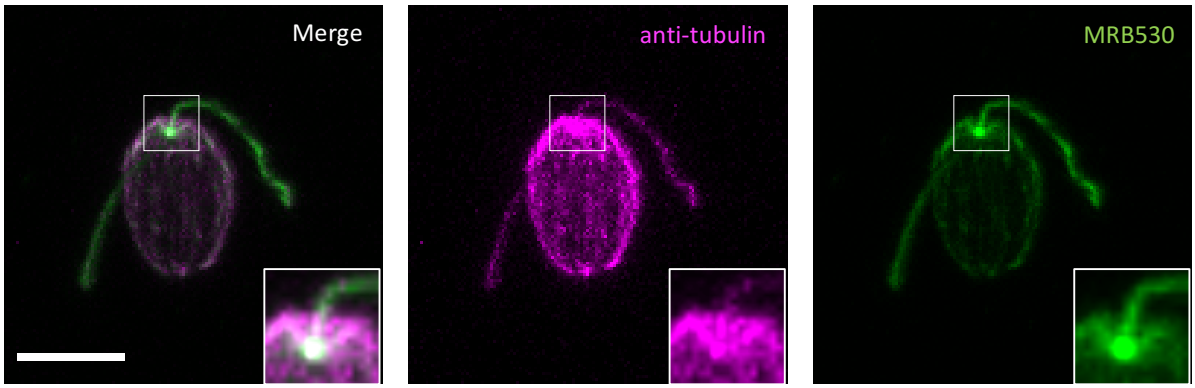

Fig. 1. MRB530 antibody labels microtubules, the flagellum and the basal body in *C. reinhardtii* cells. Representative confocal image of a *Chlamydomonas* cell co-stained with the antibody MRB530 (green) and anti-beta tubulin Ab18251 (magenta). The inset shows the basal body. Scale bar: 5 µm.

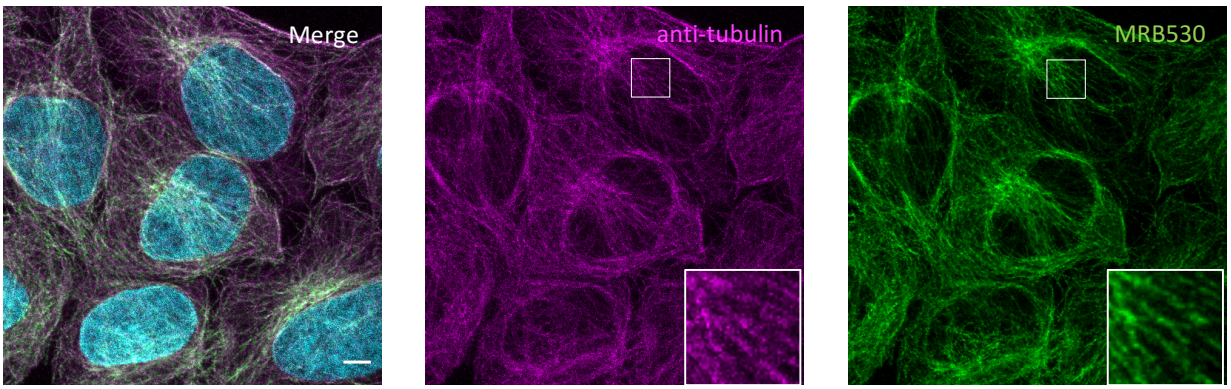

Fig. 2. MRB530 antibody recognizes microtubules in human U2OS cells. Representative confocal image of U2OS cells co-stained with MRB530 (green), anti-beta tubulin Ab18251 (magenta) and DAPI (blue). Note the colocalization between the control anti-tubulin Ab18251 antibody and MRB530. Scale bar: 5 µm.