Supplemental Materials

Novel Isotypic γ/ζ-Subunits Reveal Three Coatomer Complexes in Mammals

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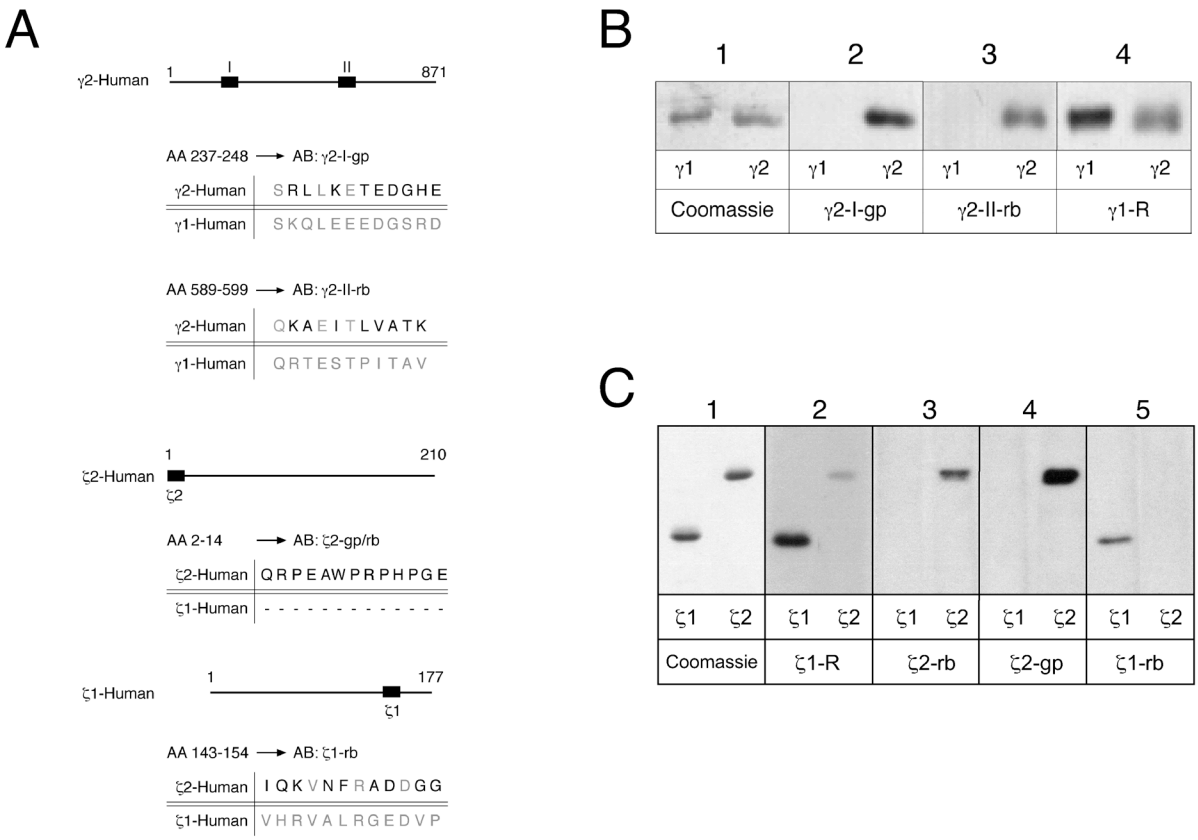
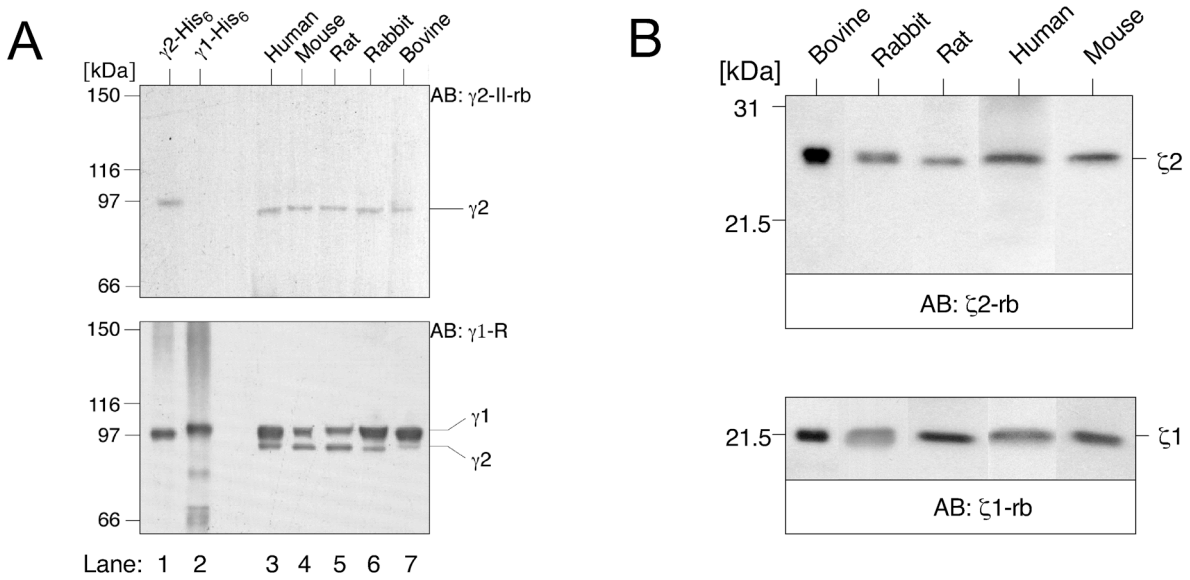
Figure A 1. Antibodies that distinguish between γ- and ζ-isotypes.



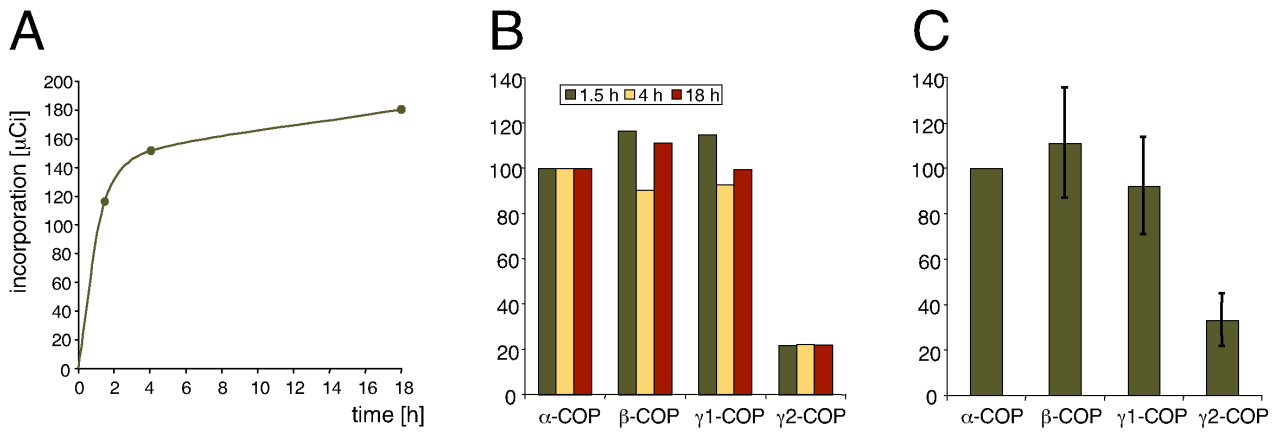
(A) Locations and sequences of peptides used for immunization to generate γ2-, ζ1- and ζ2-COP specific antibodies. Peptides were synthesized as indicated and used to raise antibodies in rabbits and guinea pigs. (B and C) Recombinant subunits γ1-, γ2-, ζ1-, and ζ2-COP were expressed in E. coli and used for Western blot analyses (7.5% and 12% acrylamide, respectively). Similar amounts of the recombinant proteins were loaded on the gel as shown by Coomassie staining (panels B 1 and C 1), and the specificity of the γ2-antibodies (B, panels 2 and 3), and the ζ1- and ζ2-COP antibodies (C, panels 3 to 5) was probed. Note that antisera rose against either recombinant γ1- or ζ1-COP recognize both isotypes (B, panel 4 and C, panel 2).

Figure A 2. Ubiquitous expression of γ2- and ζ2-COP in various mammalian species.



(A) In the upper panel, a Western blot (7.5% acrylamide) is shown of recombinant γ1- and γ2-COP as a control (lanes 1 and 2), and of immunoprecipitates with an antibody against intact coatomer from cell extracts of human, mouse, rat, rabbit, and bovine livers (lanes 3-7), all stained with the γ2-specific antibody. In the lower panel, the blot was developed with the polyclonal antibody against recombinant γ1-COP (γ1−R). (B) In a similar way, the presence of ζ−COP isotypes was probed (12% acrylamide-gel) with the antibodies specific for either ζ2-COP (upper panel) or ζ1-COP (lower panel).

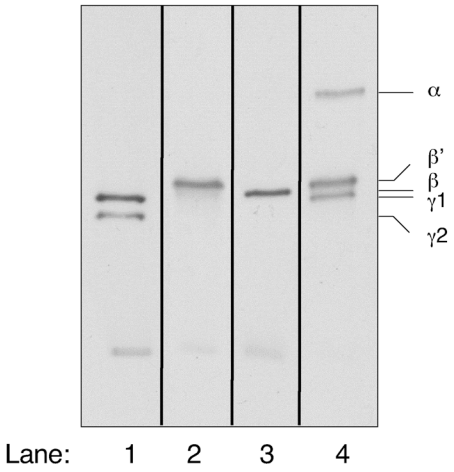
Figure A 3. Stoichiometry of coatomer subunits is independent of the labeling times.



(A) Proteins of confluent grown HepG2 cells were labeled with ^{35}S-methionine for 1.5, 4 or 18 h and the incorporation of the radioactivity was determined. (B) Quantitative evaluation of immunoprecipitations with the anti-β'-COP antibody 891 after metabolic labeling for the different

time periods as in A. α-COP was set to 100% and individual protein masses were calculated, taking into account the number of Met-residues in each subunit. (C) Quantitative evaluation of five independent experiments with the anti-β'-COP antibody 891 and a labeling period of 1.5 h.

Figure A 4. Characterization of the anti-coatomer antibody 883.



Coatomer was immunoprecipitated from a rat liver cytosol and the corresponding Western blot (7.5% acrylamide) was analyzed with anti-coatomer antibodies: γ1-R (lane 1), anti-β'-COP (C1PL, (57), lane 2), anti-β-COP (M3A5, lane 3), and anti-coatomer antibody 883 that recognizes α-, β'- and γ1-COP (lane 4).