The cytoplasmic Acetoacetyl CoA Thiolase (ACAT2), a novel Rab5 effector, regulates endocytic membrane transport.

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ABSTRACT

Endocytosis is a complex process via which segments of the plasma membrane and extracellular nutrients are internalized via vesicular carriers towards the endolysosomal system. This trafficking pathway is subject to regulation by Rab GTPases.

The small GTPase Rab5, regulates membrane traffic towards and between early endosomes, and is necessary for the biogenesis of the endolysosomal system [1]. To regulate the above processes, Rab5 functions as a molecular switch by alternating between active GTP- and inactive GDP-bound states. An appealing question in the field is how Rab5 regulates the multiple complex steps of endocytosis. In a previous study, a large set of over 40 proteins interacting with Rab5-GTP were isolated using Rab5-affinity chromatography and identified by mass spectroscopy [2], while only one protein was found to interact with Rab5-GDP. By Mass-spectrometry, we identified this new protein as the cytoplasmic Acetoacetyl-CoA Thiolase 2 (ACAT2), an enzyme that catalyzes the conversion of acetyl-CoA to acetoacetyl-CoA, the first reaction of the mevalonate pathway. Besides, ACAT2 has been recently characterized as a new cytoplasmic protein acetyl-transferase [3].

To get insights into the significance of the interaction between Rab5 and ACAT2, at first, we confirmed the GDP-dependent binding between these two proteins in intact cells. To this end, we found that targeting of wild type Rab5 to the cytoplasmic surface of mitochondria (using a mitochondrial targeting signal) was able to relocate endogenous ACAT2 from the cytoplasm to the mitochondrial membrane. Furthermore, using an *in vitro* endosome fusion assay, we found that ACAT2 inhibits Rab5-mediated endosome fusion, suggesting that it exerts an inhibitory role on Rab5. Consistently, we found that knock down of ACAT2 accelerates the internalization and degradation of VEGFR2 and EGFR. Moreover, knock down of ACAT2 augments the number of early endosomes and lysosomes. Finally, by mass spectrometry we identified that ACAT2 acetylates Rab5-GDP in vitro. Taken together, these data suggest that ACAT2 is a critical regulator of Rab5 in the endocytic pathway. Ongoing work in the lab aims to shed light on the mechanism via which the interaction between Rab5 and ACAT2 regulates the activity of these two enzymes and the implications in the endocytic trafficking.

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