0031-3998/07/6206-0645
PEDIATRIC RESEARCH
Vol. 62, No. 6, 2007
Printed in U.S.A.

SCIENCE – IN THE NEWS

Which Way is Up? Sorting Membrane Proteins to the Apical Plasma Membrane

The role of the small GTPase Rab8 in sorting plasma membrane proteins in polarized epithelial cells has been difficult to precisely determine. In vitro studies in MDCK cells expressing constitutively active Rab8GTPase (unable to hydrolyze GTP) gave rise to mislocalization to the apical surface of basolateral proteins that depend on interaction with the epithelial-specific clathrin adaptor AP-1B (1). Expression of dominant negative Rab8 (unable to load GTP) did not affect localization of basolaterally-targeted proteins. The specificity of disruption of only AP-1B dependent trafficking, along with its high homology to the yeast protein Sec4p, led investigators to conclude that this protein is involved in AP-1B-dependent basolateral sorting of proteins at the level of the trans-golgi network. Analogy from the yeast system, however, suggests a more complex function, one in which the actin substructure might be involved.

A recent paper in Nature, in which Rab8 knockout mice were generated, sheds considerable light on this puzzle (2). Sato and colleagues generate a general Rab8 null mouse line and a gut epithelial specific (using Villin-Cre) Rab8 knockout line. Both generalized Rab8 null and gut-specific Rab8 null mice were viable, but rapidly developed severe diarrhea and wasting 3 wk after birth and died by 5 wk due to severe malabsorption. To their surprise, the localization of basolateral proteins was indistinguishable between the wild type and null mice. However, all apical proteins (by fluorescence microscopy and western blot), including important carbohydrate and dipeptide transporters and disaccharidases, were severely mislocalized to large apical intracellular vacuoles (colocalizing with lysosomal markers) and were globally diminished along the brush border membrane by 3 wk after birth. Moreover, when functional transport studies of specific carbohydrates and dipeptides were performed on intestines of the Rab8 null mice, their transport was severely diminished by 3 wk. These authors show this clear apical localization of Rab8 in wild-type animals and that this phenomenon is not restricted to mice—they show Rab8GFP localized to apical poles of the roundworm C. elegans and the temperature sensitive knock-down recapitulated the phenotype of mislocalized apical membrane proteins to intracellular vacuoles.

On closer electron-microscopic examination of intestinal cells at 3 wk post birth, the findings of short stubby microvilli and large vacuoles containing microvilli were reminiscent of those found in human microvillus inclusion disease (MVID). MVID is an autosomal recessive disorder of unknown etiology and is a common cause of intractable diarrhea of infancy. The authors then went on to sequence the exons of the Rab8 gene in 3 human patients and found no genetic differences; in one patient, they show a dramatic decrease in immunostaining for Rab8 in apical compartments of small intestinal cells.

Despite some similarities of the murine model to MVID, several subtle, but important distinctions should be noted. While the appearance of the microvilli are similar to what is seen in most patients with MVID, the mice do not have the "flat intestine" or villous atrophy that is characteristic of this disorder. It is this villous atrophy that likely accounts for the combined secretory and malabsorptive diarrhea that is a classic feature seen in children with MVID. Nevertheless, the Sato study indicates a clear role of Rab8 in microvillus formation and targeting of apical proteins to the brush border membrane, and provides some long awaited clues into the pathogenesis of MVID. – *J. Adrian Lunn and Martín G. Martín*

REFERENCES

1. Ang AL, Fölsch H, Koivisto UM, Pypaert M, Mellman I 2003 The Rab8 GTPase selectively regulates AP-1B-dependent basolateral transport in polarized Madin-Darby canine kidney cells. J Cell Biol 163:339–350
2. Sato T, Mushiake S, Kato Y, Sato K, Sato M, Takeda N, Ozono K, Miki K, Kubo Y, Tsuji A, Harada R, Harada A 2007 The Rab8 GTPase regulates apical protein localization in intestinal cells. Nature 448:366–369