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Intestinal Brush Border Assembly Driven by Protocadherin-Based Intermicrovillar Adhesion

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SUMMARY

Transporting epithelial cells build apical microvilli to increase membrane surface area and enhance absorptive capacity. The intestinal brush border provides an elaborate example with tightly packed microvilli that function in nutrient absorption and host defense. Although the brush border is essential for physiological homeostasis, its assembly is poorly understood. We found that brush border assembly is driven by the formation of Ca^{2+}-dependent adhesion links between adjacent microvilli. Intermicrovillar links are composed of protocadherin-24 and mucin-like protocadherin, which target to microvillar tips and interact to form a *trans*-heterophilic complex. The cytoplasmic domains of microvillar protocadherins interact with the scaffolding protein, harmonin, and myosin-7b, which promote localization to microvillar tips. Finally, a mouse model of Usher syndrome lacking harmonin exhibits microvillar protocadherin mislocalization and severe defects in brush border morphology. These data reveal an adhesion-based mechanism for brush border assembly and illuminate the basis of intestinal pathology in patients with Usher syndrome.

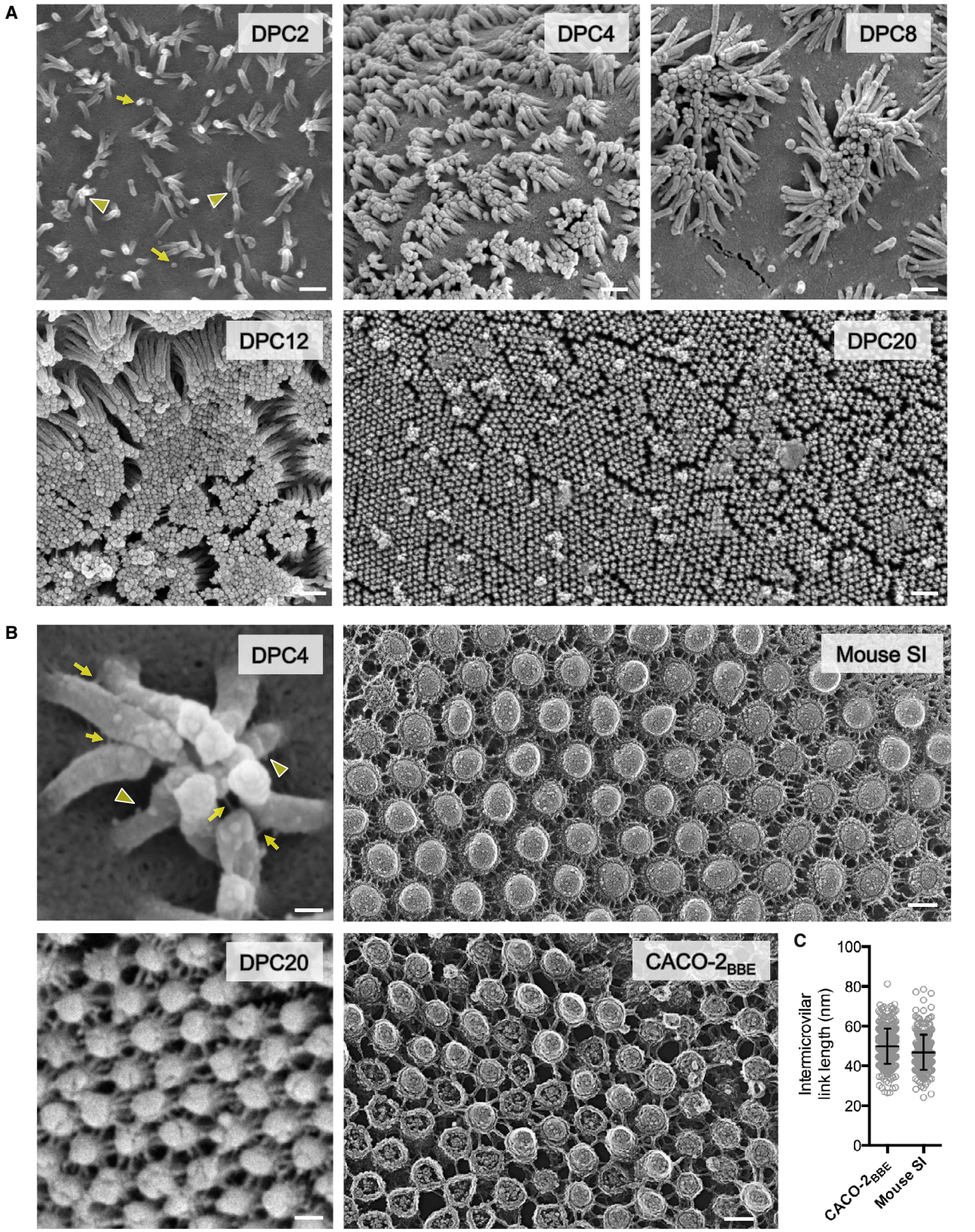
INTRODUCTION

Enterocytes are transporting epithelial cells that line the luminal surface of the intestinal tract where they function in nutrient processing, absorption, and host defense. One of the defining features of terminally differentiated enterocytes is the densely packed array of microvilli known as the brush border (BB), which extends from the apical surface into the lumen. BB microvilli are actin bundle-supported membrane protrusions that exhibit striking uniformity in length and are maximally packed in a hexagonal pattern such that a single cell presents up to ~1,000 of these protrusions (Mooseker, 1985). This unique morphology allows enterocytes to maintain a vast reservoir of membrane enriched in nutrient processing and transport machinery on their apical surface (Maroux et al., 1988). In addition to functioning as the sole site of nutrient absorption, the BB also plays an active role in defending host tissues against luminal microbes and microbial toxins (Mukherjee et al., 2008; Selsted and Ouellette, 2005; Shifrin and Tyska, 2012).

Due to the constant regenerative renewal of the gut epithelium, enterocyte differentiation and BB assembly are processes that continue throughout our lifetime. Differentiation occurs as enterocytes migrate along the crypt-villus axis, during which time they exhibit a marked upregulation of cytoskeletal genes including actin, villin, and espin (Chang et al., 2008; Mariadason et al., 2005), changes that likely contribute to BB assembly. Despite the development and characterization of numerous animal models lacking key BB components (Revenu et al., 2012; Saotome et al., 2004; Tyska et al., 2005), little is known about how the BB is assembled during enterocyte differentiation. Fundamental questions on how microvillar actin filaments are nucleated, how microvillar length is regulated, and how these protrusions achieve dense packing during BB assembly remain unanswered.

Adhesion molecules play essential roles in numerous biological processes including the organization of cells during tissue morphogenesis (Guillot and Lecuit, 2013), and in the development and function of stereocilia, specialized mechanosensory protrusions on the surface of inner ear sensory cells (Kazmierczak et al., 2007). Usher syndrome is a major form of inherited deaf-blindness that is caused by loss-of-function mutations in components of the stereocilia Ca^{2+}-dependent adhesion complex, which includes protocadherins and the scaffolding protein, harmonin (Pan and Zhang, 2012). Although primarily viewed as a sensory disorder, patients with type 1 Usher syndrome with mutations in harmonin present with enteropathy and perturbations in villus morphology (Bitner-Glindzicz et al., 2000). However, the cellular basis for this pathology and, more generally, the role of adhesion molecules in the assembly and organization of the BB have not been investigated.

Here, we report that cadherin family adhesion proteins play a critical role in the assembly and organization of the BB. We show that microvilli on the surface of native and cultured intestinal epithelial cells are organized by an extensive matrix of ~50 nm



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