Novel insights into intestinal and renal folate transport. Focus on “Apical membrane targeting and trafficking of the human proton-coupled folate transporter in polarized epithelia”

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FOLATE IS AN ESSENTIAL MICRONUTRIENT that functions as a coenzyme in DNA and RNA synthesis and in the metabolism of several amino acids, including homocysteine (11). Folate deficiency can occur in humans because of poor dietary intake, malabsorption, metabolic blocks, or increased requirements as seen during pregnancy and lactation (3). Symptoms of deficiency are severe and include megaloblastic anemia and neural tube defects in children. Mammals must absorb folate from the diet, because they do not have the capability to synthesize folate. Intestinal absorption occurs by both passive and carrier-mediated mechanisms, with the second process predominating in the proximal small intestine at normal intake levels (3). Intestinal transport is critical for overall body folate homeostasis, as exemplified by the fact that individuals with hereditary folate malabsorption exhibit signs of folate deficiency (5, 6). Dietary folate exists in the polyglutamate form, which is converted to the monoglutamate form before absorption. Transport across the intestinal epithelium is a two-step process; the putative brush-border membrane transporter is pH dependent (i.e., higher activity at low pH) (12), and movement across the basolateral surface is also a carrier-mediated process that has been previously described (13). Recently, three folate transport systems have been identified in mammals: the reduced folate carrier (RFC) (4, 8), the folate receptor (FR) (7), and the newly described proton-coupled folate transporter (PCFT; also called SLC46A1) (9). The relative role of each of these transport systems in intestinal and renal folate absorption is currently not completely understood (although the FR, which was described in renal cells, has not been identified in the intestine).

The recent studies by Subramanian et al. (17) have provided novel evidence that makes a major contribution to our understanding of folate homeostasis. These authors have clearly demonstrated, for the first time, that the human PCFT is a brush-border membrane protein in polarized epithelia; they show that it is functionally expressed on the apical membrane in widely accepted models of the intestinal and renal epithelia. Moreover, in polarized MDCK cells, they noted that the PCFT and the RFC showed differential localization, with the RFC present on the basolateral domain and the PCFT in the apical membrane. Additional experiments were aimed at determining the trafficking motifs of the PCFT and the effect of clinically relevant mutations; results showed that the COOH terminus was not important for apical targeting and that mutation of a region (DXXGR113R) associated with hereditary folate malabsorption (the R113S mutation has been identified in a human patient) (20) abolished a β-turn between membrane-spanning domains 2 and 3 and led to protein retention in the endoplasmic reticulum. Further analyses found that cell surface delivery of the PCFT is dependent on an intact microtubular network. The findings presented in this article reveal new aspects of folate transport that can only be appreciated in the context of previous studies in this field.

Prior investigations suggested that the RFC is important in intestinal and renal folate absorption. Some studies found RFC expressed in the intestinal brush-border membrane in mouse (18) and rat (1) intestine, where it is presumably involved in folate absorption, while others described expression at the basolateral surface of renal epithelial cells (2) and at the plasma membrane when expressed in *Xenopus* oocytes (16). Moreover, the RFC was found to be ontogenically regulated during rat development and was reflective of overall intestinal folate transport (1). Despite these intriguing observations, certain inconsistencies exist between the known properties of intestinal folate transport and those ascribed to the RFC, including the higher activity of the RFC at neutral as opposed to acidic pH (15).

The recent identification of the PCFT brings another potential transporter into the mix. Although originally identified as an intestinal heme transporter [called heme carrier protein 1 (HCP-1)] (14), current evidence suggests that the PCFT is a high-affinity folate transporter (apparent $K_{m}$: 1.3 μM) (9), as evidenced by the severe folate deficiency that results in humans with mutations in the gene encoding the PCFT (20). While it is not unprecedented for one protein to have more than one function, the relatively low affinity of HCP-1 for heme (apparent $K_{m}$: 125 μM) suggests that it may not be a physiologically relevant heme transporter. Importantly, additional evidence suggests that the PCFT is a major folate transporter because it has similar properties to the putative intestinal folate transporter, including an acidic pH optimum and upregulated expression during folate deficiency (10, 19). Despite these recent observations, it is not clear the precise role that the PCFT plays in intestinal folate transport, and furthermore, whether it is involved in renal folate reabsorption is unknown.

The current findings of Subramanian et al. (17) thus provide important clues regarding the physiological role of the PCFT in folate transport. For their studies, they utilized fluorescent-tagged proteins and state-of-the-art live cell imaging techniques to characterize membrane targeting and functionality of expressed human PCFT. The fact that the transporter is expressed on the apical membrane in intestinal and renal epithelial cells and that it functions as a pH-driven folate transporter suggests that this transporter plays a prominent role in intestinal folate absorption and that it may also be involved in renal reabsorption. Qiu et al. (10) have also recently reported that the PCFT is apically expressed in rat and mouse intestinal epithe-
lium, which strengthens and confirms the current observations by Subramanian et al. Overall, these elegant studies from Dr. Said’s group provide another important piece of the puzzle that has been investigated intensely over the past 2 decades regarding the molecular mechanisms of intestinal and renal folate transport.

REFERENCES