DYNAMICS OF BRUSH BORDER CYTOSKELETAL PROTEINS REVEALED BY TWO-PHOTON FRAP: EXPERIMENTAL RESULTS AND THEORETICAL TOOLS

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Intestinal epithelial cells contain a brush-like membrane composed of numerous finger-like protuberances called microvilli. Each microvillus is composed of a core bundle containing actin filaments and an helical array of brush border myosin I (Myo1a, formerly BBMI) cross bridges between the plasma membrane and actin filaments.

Highly dynamic processes occurs during brush border assembly. In particular, the number and the length of microvilli increase during the final step of adult enterocyte differentiation, as well as during the last stage of embryogenesis [1]. The rapid turnover of cytoskeleton and membrane proteins in the mature enterocytes also suggests that dynamic processes take place while the enterocyte maintains a stationary morphology [2].

Microvilli dynamics might be driven by the polymerization of actin that is sufficient for the rocketing motion of bacteria, for example. Alternatively, and non-exclusively, microvilli dynamics might be powered by Myo1a either by generating a force as proposed by M. Sheetz during the extension of growth cones filopodia [3], or by delivering membrane component to the apical domain.

In order to elucidate the dynamics of Myo1a and actin in the enterocyte brush border, we combined two-photon fluorescence imaging of GFP-tagged living cells with fluorescence recovery after photobleaching (FRAP). Experiments were performed on Caco-2 cells expressing GFP-Myo1a or GFP-actin. These cells, that spontaneously differentiate at confluency into cells that exhibit many of the morphological and functional characteristics of mature enterocyte, do represent a useful in vitro model to study normal human intestinal cells.

