BIOCHEMICAL JOURNAL LETTERS

Membrane insertion of the microvillar 110-kDa protein of the enterocyte

Two articles have recently been published concerning the 110-kDa protein of the microvillar cytoskeleton. Glenney & Glenney (1984), studying the structural properties of the protein from chicken small intestine, presented four independent lines of evidence, indicating that the 110-kDa protein is directly bound to the lipid bilayer and, contrary to earlier beliefs, in fact can be described as an integral membrane protein. The criteria for this assertion were the following: (1) requirement for detergent for solubilization, and aggregation upon removal of detergent; (2) partitioning into the detergent phase in Triton X-114 solutions; (3) selective incorporation into liposomes; (4) specific labelling with a hydrophobic probe ($[^{14}\text{C}]$ phenylisothiocyanate). On the basis of these findings, it was suggested that the 110-kDa protein may represent the simplest system of membrane to filament attachment in which an integral membrane protein is also a cytoskeletal protein.

At the same time, we published a paper dealing with the biosynthesis of microvillar cytoskeletal proteins (Cowell & Danielsen, 1984). Here, the kinetics of microvillar expression of these proteins was studied by pulse-chase labelling of pig small intestinal mucosal explants in organ culture, and it was found that newly synthesized cytoskeletal proteins (including the 110-kDa protein, villin and actin) appear in the microvillus within 10 min. In contrast, the microvillar enzymes require about 60 min of chase before they are detected in microvilli. For this latter type of membrane protein, it is known that they are synthesized by membrane-bound ribosomes, and that the membrane-bound transport via the Golgi complex (where processing occurs) delays their microvillar expression (Danielsen *et al.*, 1984). It was therefore concluded that the rapid expression of the cytoskeletal components is indicative of a synthesis taking place in the cytosol by free ribosomes.

The findings of the two reports taken together suggest that the 110-kDa protein becomes post-translationally inserted into the microvillar membrane, a disposition not previously reported for

proteins of this organelle. The problem that arises is how the newly synthesized 110-kDa protein specifically recognizes the microvillar membrane as its proper site for membrane insertion in a way that permits the molecule to perform its function as attachment site for the microvillar filaments? Glenney & Glenney (1984) did not perform experiments to decide the membrane topology of the 110-kDa protein, i.e. whether it spans the lipid bilayer (bi- or polytopic configuration) or is inserted only at the cytoplasmic surface of the lipid bilayer (monotopic configuration). The first possibility would be indicative of a membrane translocation of the 110-kDa protein and thus suggest that the microvillar membrane is translocation competent (Blobel, 1980). However, in Cowell & Danielsen (1984) we observed that washing of a microvillar vesicle preparation with alkaline buffer (pH 11) partially released the 110-kDa protein, whereas the membrane-bound enzymes which span the membrane by a small segment at the *N*-terminus (Kenny & Maroux, 1982) were quantitatively retained in the membrane. This favours a monotopic mode of membrane insertion for the 110-kDa protein, which therefore may resemble two post-translationally inserted microsomal membrane proteins, cytochrome *b₅* and NADH:cytochrome *b₅* oxidoreductase (Borgese & Gaetani, 1980; Okada *et al.*, 1982). These appeared to lack a specificity for endoplasmic membranes in incorporation experiments *in vitro*, which may account for the fact that they can be found in various subcellular membranes (mitochondrial, endoplasmic and Golgi) soon after labelling *in vivo* (Okada & Omura, 1978). The higher concentration of these proteins in the endoplasmic reticulum probably reflects an affinity for other, more specifically inserted, membrane components (Blobel, 1980; Okada *et al.*, 1982). For the 110-kDa protein, such microvillus-specific components could hypothetically be residing in either the membrane or the cytoskeleton. The first possibility seems unlikely, however, since the microvillar architecture is formed before the expression of the enzymes during the differentiation from crypt cell to enterocyte (Shiner, 1983; Danielsen, 1984). The cytoskeleton might therefore act as director for newly synthesized 110-kDa protein to its membrane attachment site. If correct,

the mechanism whereby this occurs could be the one whereby the microvillus as an organelle is formed.

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