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Alimentary tract

Intestinal brush border revisited

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The intestinal brush border is situated at the luminal pole of the enterocyte where it constitutes a functional organelle subserving terminal digestion and absorption of the end products of ingested food. Its component microvillus membrane forms a digestive-absorptive surface the functions of which are complementary to and integrated with those of the basolateral membrane of the enterocyte, ensuring effective transfer of food products across the enterocyte from the intestinal lumen to the interstitial fluid and blood.

Since a previous progress report[1] there has been a virtual explosion of information concerning the intestinal brush border, with an almost exponential increase in the number of publications appearing annually. In 1960 Crane and coworkers isolated brush borders from hamster small intestine and showed in two classic papers that it was the location of the enzymes sucrase and maltase,[2] and also of the sodium dependent transport of glucose and galactose.[3] Twenty five years later, over 22 enzymes and 19 transport or binding functions had been localised to the brush border. Much of this work was carried out using intestinal preparations from small animals, but since 1972 human tissue obtained at operation or by peroral biopsy has been increasingly used.[4,5] Subcellular fractionation techniques for isolating brush borders and microvillus membranes have been refined[4-6] and microvillus membrane vesicles are now used almost routinely in the investigation of intestinal enzyme and transport functions,[7] while definitive information on the structure and function of the microvillus membrane has been obtained through the use of electrophoresis,[8-11] monoclonal antibodies,[11-13] gene sequencing,[14-17] and the study of colon cancer cell lines *in vitro*.[11]

This review is focused on newer, molecular aspects of the structure, biosynthesis and function of the human brush border and its cytoskeleton rather than the traditional, though no less important, areas of digestive and absorptive function.

Brush border structure and composition

From a morphological viewpoint, the intact brush border is composed of numerous fingerlike apical microvilli overlying a transverse fibrillar meshwork, the terminal web. Ultrastructurally and functionally, however, the brush border is better considered as comprising two different substructures, the surface (microvillus) membrane and the underlying brush border cytoskeleton, each with its own distinctive molecular composition (Figure, Table 1).

MICROVILLUS MEMBRANE

The microvillus membrane consists of a lipid bilayer, the external (luminal) face of which is lined with a carbohydrate-rich 'fuzzy coat' or glycocalyx.[18] The membrane possesses a large complement of integral membrane proteins, many of which are glycoproteins and have been identified with specific brush border enzymes (Table 1).[5,8-10] As most, if not all, of the latter have also been localised to the external face of the microvillus membrane,[19] it is possible that they account for at least part of the glycocalyx.

The brush border enzymes which have been investigated in detail share a number of common features (Table 1). All are relatively large (70–320 kDa) glycoproteins and most are apparently composed of two or more subunits. They appear to be essentially globular structures which are attached to the external face of the membrane by a small (2–5 kDa) anchoring segment embedded in the lipid bilayer.[19] In negatively stained preparations, the membrane surface appears to be covered with numerous particles of approximately 50 nm diameter.[46] These can be largely removed by proteolytic treatment with papain or elastase, with the concomitant release of several of the glycosidases and peptidases from the membrane in a soluble form.[47] Careful study of renal microvilli has indicated that before papain treatment, the membrane is coated with particles attached by 'stalks' of between 2·5 and 9 nm in length whereas after treatment only those particles with stalks 2–3 nm in length remain attached.[48] This suggests that brush border enzyme release by papain is largely a function of whether the stalk is long enough to allow access of the papain molecule between the globular portion of the protein and the membrane surface. For most enzymes studied (sucrase-isomaltase, aminopeptidase N, dipeptidyl aminopeptidase IV, γ-glutamyl transferase) the anchoring segment is characterised by a

Table 1 *Brush border enzymes and related proteins of the microvillus membrane*

Function	*Protein*	*Molecular mass (kDa)*	*Carbohydrate content*	*DNA sequence*	*Primary deficiency*	*Abundance (%)*	*References*
Glycosidase	Maltase-glucoamylase	330/125+135§	+				9 11 20 21
	Sucrase-isomaltase	145+151	+	+	+	10	9 11 14 20 120
	Lactase-phlorizin hydrolase	160 (×2)	+	+	+		9 11 15 22-24 120
	Trehalase	80 (×2)	+		+	0·2	25 26 120
Peptidase	Aminopeptidase A	170 (×2)	+				27-29 60
	Aminopeptidase N	162 (×2)	+			4	9 11 28 29
	Aminopeptidase W	130	+			1	13
	Carboxypeptidase P	130	+				30 60
	Dipeptidyl aminopeptidase IV	136 (×2)	+				9 11 29 30
	Peptidyl dipeptidase*	180					11 29 31
	Pteroyl polyglutamate hydrolase†	91					32
	Enteropeptidase	300§	+		+	<0·1	33-36 120
	Endopeptidase-24.11	96 (×2)	+			0·1-1§	37 87
	Endopeptidase-2‡	100 (×2)	+				38 39
	γ-Glutamyl transferase	62+21	+	+	+		16 40 41
Phosphatase	Alkaline phosphatase	86 (×2)	+	+		0·1	17 42 43
	Phosphodiesterase-I						51
Unknown	'140kDa Glycoprotein'	140	+			1	53 54
Regulatory	Guanylate cyclase						44
	Phospholipase A_2						45

*Angiotensin converting enzyme; †'folate conjugase'; ‡'PABA peptidase'; §variable, dependent on species. Where possible reference has been made to human data.

single hydrophobic sequence of about 20 amino acids which spans the membrane only once in an α-helical conformation.[19] This hydrophobic domain is located adjacent to a short, hydrophilic N-terminal sequence which is exposed at the cytoplasmic face of the membrane.

A second mode of anchorage of brush border enzymes was recently discovered in which the protein is anchored to the membrane not by an N-terminal hydrophobic peptide but through the diacylglycerol moiety of a phosphatidylinositol glycan attached covalently to the C-terminal amino acid.[49,50] Proteins anchored in this way are released only slowly or not at all by papain, but are readily released from the membrane by treatment with phospholipase. Brush border alkaline phosphatase,[50] phosphodiesterase-I[51] and trehalase[52] have so far been shown to have this mode of anchorage.

These structural features are not unique to the microvillar enzymes. Thus Gorvel *et al* have identified a 140 kDa microvillar glycoprotein which is apparently devoid of enzyme activity but is, like the brush border enzymes, attached externally to the membrane surface.[53,54] The function of this glycoprotein remains to be elucidated but, unlike the brush border enzymes, it is present in immature crypt cells as well as in mature enterocytes.

Although the lipid composition of intestinal brush borders was investigated over 20 years ago, much less is known about the lipids of the brush border membrane than about the proteins. The microvillus membrane has, however, been reported to contain cholesterol, phospholipids and various neutral lipids, together with an assortment of glycolipids, and to differ from the basolateral membrane in its lipid

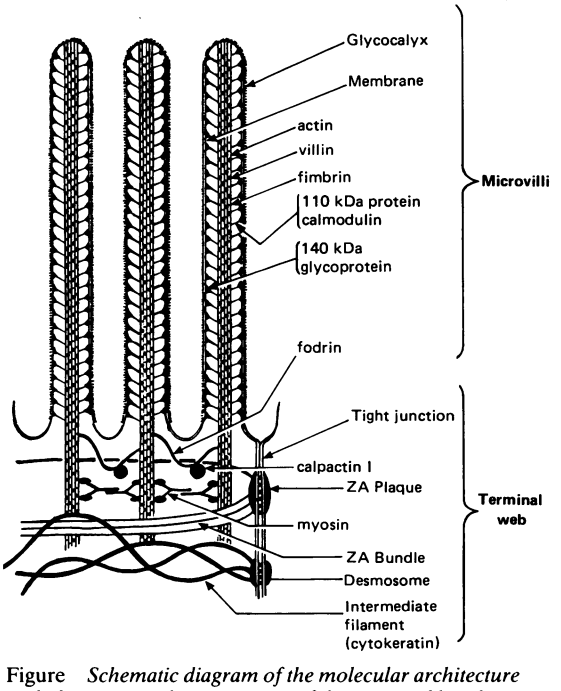

Figure *Schematic diagram of the molecular architecture and ultrastructural organisation of the intestinal brush border. Adapted from references 63 64 65.*

composition.[55–57] In the early 1980s, it was reported that the microvillus membranes of the enterocyte were much less fluid (more rigid) than their basolateral counterparts[55] and, since a lower membrane fluidity implies a lower passive membrane permeability and an increased mechanical stability, this may have functional implications.[56,57] The lower fluidity of the apical membrane has been attributed to its distinctive lipid composition and, specifically, to a high cholesterol:phospholipid ratio.[55] A recent analysis of this topic, however, has emphasised that, when due allowance is made for *all* the major classes of membrane lipid, the principal difference between microvillar and basolateral lipids is a replacement in the microvillus membrane of most of the phosphatidylcholine by glycosphingolipids, to the extent that these account for one third of the total microvillar lipid.[56,57] Because glycosphingolipids are more highly ordered than phospholipids because of their extensive hydrogen bonding capacity, and are also invariably located to the external face of cellular membranes, it seems likely that the lower fluidity of the microvillus membrane is essentially a property of these components of the outer leaflet of the lipid bilayer.[56,57]

The protein composition of the microvillus membrane varies both during development[58,59] and along the length of the intestine,[60–62] as might be expected in view of the temporal and regional variations that are known to occur in intestinal function. Thus considerable changes were observed in the electrophoretic profile of microvillar proteins of neonatal rats during intestinal maturation and weaning,[58] some of which could be correlated with functional development – for example, the appearance of sucrase activity and the postweaning decline in lactase. Differences between duodenal and ileal microvillar protein profiles have also been recorded for human[8,60,61] and animal[62] intestine, but correlation with functional differences has been more difficult, possibly owing to a low abundance of the relevant proteins (transport-related proteins such as the Na^+-glucose cotransporter or the ileal vitamin B_{12} intrinsic factor receptor are minor components of the microvillus membrane, each representing 0·2% or less of the total microvillar protein). Differences in glycosylation between proximal and distal microvillus membranes have also been recorded, as shown by altered binding of lectins to isolated brush border membranes and changes in the electrophoretic mobility of such enzymes as lactase, aminopeptidase N and dipeptidyl aminopeptidase IV.[62] These differences could be explained on the basis of less complete glycosylation in the proximal intestine and substantially greater sialic acid incorporation distally, but the physiological significance of this is unclear.

CYTOSKELETON

The brush border cytoskeleton, comprising the microvillus core and terminal web, was long regarded as a relatively simple, inert support for the microvillus membrane. Recent advances in cell biology have stimulated great interest in this structure, which is now known to be a highly organised, dynamic complex of at least three inter-relating filamentous networks, each with its own complement of specialised, filament forming proteins (Figure). This not only serves as a supporting structure to maintain the architecture of the entire apical pole of the enterocyte, but also may play an active role in regulating the uptake of some nutrients into the cell and in the control of paracellular permeability.

In transmission electron micrographs of well prepared specimens, each microvillus can be seen to contain an ordered bundle of 20–30 microfilaments composed predominantly of the β and γ -isoforms of actin and running longitudinally from the microvillus tip into the underlying terminal web (Figure).[63–65] The core is attached laterally to the membrane through spirally arranged bridges containing a complex of an unnamed 110 kDa protein, the 17 kDa Ca^{2+}-binding protein calmodulin, and a 140 kDa membrane binding glycoprotein. The portion of the microvillus core embedded in the terminal web, the core rootlet, lacks the 110 kDa-calmodulin complex but contains instead another actin binding protein, tropomyosin.

The axial core bundle of actin microfilaments is cross linked into a regular hexagonal array through short, cross-bridging filaments which are organised longitudinally in an α-helical arrangement.[63–65] Two actin binding proteins, fimbrin and villin, are involved in the interfilament cross links. Fimbrin is an ubiquitous 68 kDa globular protein which, in the presence of K^+ and divalent cations, cross links actin filaments *in vitro* into highly ordered bundles closely resembling the native microvillus core. Villin is a 95 kDa globular protein of restricted tissue distribution, and may prove to be useful as a tumour marker.[66] It has three Ca^{2+}-binding sites per molecule and interacts with actin in a Ca^{2+}-dependent manner, promoting filament bundling at submicromolar Ca^{2+} concentrations but causing fragmentation at higher Ca^{2+} levels.

Within the terminal web two distinct regions can be distinguished (Figure). The inter-rootlet zone consists of a dense meshwork of fine, non-actin filaments which appear to attach the core rootlets of adjacent microvilli.[63–65] These filaments contain non-muscle myosin (200 kDa) and fodrin, a calmodulin binding, spectrin-related protein comprising 240 and 235 kDa subunits. At the level of the zonula adherens (ZA), the junctional complex at the lateral margin of the cell consists of a circumferential bundle of actin

filaments, beneath which lies a network of thicker, intermediate filaments containing cytokeratin. The ZA bundle filaments, which resemble the stress fibres of cultured fibroblasts, contain myosin, tropomyosin and α-actinin and appear to be closely associated with the ZA tight junctions through adhesion plaque like structures containing α-actinin and vinculin.[63–65] Like stress fibres, the ZA bundle is contractile through an ATP-dependent process,[67] and this provides an attractive candidate mechanism to control the opening of the tight junctions between adjacent enterocytes and thereby regulate paracellular permeability.[68,69]

Two other proteins of the brush border cytoskeleton are of interest, although their structural and functional roles are unclear. One, a relatively minor 80 kDa component of the microvillus core, appears to be immunologically related to a substrate for the tyrosine kinase of the epidermal growth factor receptor.[70] The other, a 36 kDa protein named calpactin I, is confined to the terminal web.[71] Calpactin-I is a calmodulin-, phospholipid-, and actin-binding protein which is also a major substrate of the oncogene-related pp60 *src* tyrosine kinase.[71,72] The presence of these proteins raises the intriguing possibility of functional regulation of the brush border cytoskeleton through hormone or growth factor receptor-induced protein phosphorylation.

Biosynthesis of brush border proteins

The past decade has seen considerable progress in understanding the biosynthesis of brush border proteins and it now appears that most, if not all, microvillar enzymes are produced in a fundamentally similar fashion.[11,19,29,39,73] Translation of the appropriate mRNA takes place in the rough endoplasmic reticulum, beginning with an N-terminal, hydrophobic 'signal sequence' whose function is to provide anchorage to the endoplasmic reticular membrane while the nascent polypeptide chain passes across the membrane into the cisternal space. The first and apparently co-translational phase of *N*-linked glycosylation of the molecule takes place with the addition *en bloc* of oligomannosyl glycans at asparagine residues. The resulting 'high mannose' product is a transient form which undergoes further processing in the Golgi apparatus. There, the linear, *N*-linked high-mannose oligosaccharide chains are converted into the complex, branched structures of the mature enzyme and *O*-linked glycosylation at serine and threonine residues also takes place. In several mammalian species, including man,[74] rabbit[75] and dog,[76] the brush border of secretors is rich in blood group determinants and the carbohydrate antigens responsible are expressed on the microvillar enzymes, notably sucrase-isomaltase[20] and aminopeptidase N,[75] as well as on microvillar glycolipids. Finally the mature enzyme is transported by an undefined mechanism to the brush border and incorporated into the microvillus membrane, where it remains anchored by the N-terminal hydrophobic signal sequence (see above). There is some evidence that transfer of at least aminopeptidase N to the brush border from the Golgi may be indirect and involve transient passage through the basolateral membrane,[65] but at present this interpretation is controversial.

Superimposed on this basic biosynthetic theme are a number of variations. So far as is known, all brush border enzymes are synthesised as single polypeptide chains, each having a single anchoring peptide. Many, especially peptidases, appear to dimerise at some point after synthesis and there is dual anchorage of the dimer to the microvillus membrane.[19,73] Other enzymes, notably sucrase-isomaltase, are again synthesised as large, single chain polypeptides but, after insertion into the microvillus membrane, are cleaved into two subunits through the action of luminal proteases, particularly elastase.[19,73] The resulting dual enzyme is thus attached to the membrane *via* a single anchor which, in the case of sucrase-isomaltase, resides in the sucrase subunit while the isomaltase subunit is anchored indirectly *via* the sucrase moiety. Essentially the same basic biosynthetic scheme applies to maltase-glucoamylase and lactase-phlorizin hydrolase, both of which are double enzymes with a single anchor,[19] and also to γ-glutamyl transferase which differs from the disaccharidases only in that the larger of its two subunits is enzymatically inactive.[41]

A slightly different biosynthetic route must be postulated for alkaline phosphatase, phosphodiesterase-I and trehalase which possess a phospholipid anchor instead of a hydrophobic anchoring peptide. In these cases, the original N-terminal signal sequence required for membrane attachment during translation is presumed to be cleaved intracellularly and replaced by covalently attached phospholipid at the C-terminus.[49,50] The subsequent processing and membrane insertion of these enzymes is likely to resemble that of the aminopeptidases.

The action of luminal proteases on newly synthesised microvillar enzymes can be regarded either as a late biosynthetic event, as described above for sucrase-isomaltase, or as an early event in the enzyme's degradation, as for example in the case of porcine aminopeptidase N.[73] Both organ culture experiments and studies in animals with pancreatic duct diversion have clearly shown that the mature, brush border form of this enzyme is a 160 kDa glycoprotein, whereas porcine microvillus membranes which have been exposed to normal levels of pancreatic enzymes *in vivo* contain substantial

amounts of 120 and 60 kDa breakdown products.[73]

Pancreatic proteases also appear to be involved in brush border catabolism through their action in solubilising microvillar enzymes and releasing them from the membrane into the intestinal lumen.[47 77] This release mechanism has been demonstrated *in vivo* for disaccharidases and alkaline phosphatase and, most notably, for enteropeptidase.[78] For disaccharidases, this appears to be a major factor in regulating their turnover and in determining their mucosal activity, since subtotal pancreatectomy or pancreatic bypass causes a 50% increase in disaccharidase activity and a decrease in the rate of turnover in rats.[77 79] Turnover rates of brush border proteins are known to be rapid (half-life of approximately 18 h) when compared with the whole mucosal homogenate ($t_{1/2}$=31 h) and turnover of disaccharidases is even more rapid ($t_{1/2}$=11·5 h), as larger brush border proteins generally have shorter half-lives than the smaller components.[77 80 81]

Compared with the microvillus membrane, relatively little is known about the biosynthesis of the brush border cytoskeleton although its assembly has been extensively investigated and reviewed. That the cytoskeleton is not a static structure is indicated by the transient microvillus shortening which occurs, for example, during fasting.[82] *In vivo* pulse chase experiments have indicated that turnover of actin and other components of the cytoskeleton takes place at relatively low rates,[83] but *in vitro* studies have shown that villin and the 110 kDa-glycoprotein are synthesised very rapidly, approximately five times faster than for brush border membrane proteins.[84]

Functions of the brush border

The primary functions of the brush border undoubtedly relate to the terminal digestion and absorption of nutrients. While these have, for the most part, been well documented over many years, newly discovered enzymes and transport functions have to be integrated into the overall picture. In addition there are newly recognised functions, mainly of a regulatory nature, such as the possible modulation of paracellular permeability through the brush border cytoskeleton, and of ion transport by receptors and other regulatory proteins located in the brush border.

DIGESTIVE/ENZYMATIC FUNCTIONS

The importance of the disaccharidases in carbohydrate digestion has long been recognised.[85] It is widely assumed that the brush border peptidases have an analogous function in protein digestion but it has proved difficult to define the role of individual peptidases, possibly because of their overlapping specificities and the existence of intact peptide absorption.[85] Recent studies have clearly shown, however, instances where two or more brush border peptidases may function in concert in the degradation of oligopeptides. Thus the degradation of gliadin peptides was shown to be dependent on the concerted action of angiotensin converting enzyme and aminopeptidase N,[86] and the combined action of endopeptidase-2 and aminopeptidase N[87] on various substrates has also been demonstrated. The vexed question of the function of intestinal alkaline phosphatase has also been partly resolved: a recent investigation of its role in phosphate absorption concluded that the enzyme had no direct role in the absorption of phosphate but was involved indirectly through the vectorial release of inorganic phosphate from dietary organic phosphates,[88] a role analogous to that of sucrase in glucose absorption from sucrose.

TRANSPORT FUNCTIONS

In 1961 Crane and coworkers made the definitive proposal that monosaccharides such as glucose and galactose were transported across the brush border membrane by a carrier mechanism which cotransports Na^+.[89] The energy required for sugar transport is derived from the flux of Na^+ at high concentration

Table 2 *Major transport functions of the brush border*

	Substrate(s)	*Na^+-dependence*	*Primary deficiency*	*References*
Monosaccharides	glucose, galactose	+	+	90 91 93
	fructose	−		92
Amino acids	acidic; – glutamic acid	+	+	
	neutral; – alanine	+	Hartnup	85 93
	basic; – lysine	+	Cystinuria	
	imino; – proline	+		
	phenylalanine	+		
Peptides	dipeptides	?		94
Fatty acids	long chain – oleic acid	?		95
Bile salts	such as, taurocholate	+	+	96 97
Vitamins	ascorbic acid	+		
	biotin	+	?	
	folic acid	+	+	
	inositol	+		98
	pantothenic acid	+		
	thiamine	+		
Ions	Ca^{2+}			99
	Fe^{2+}, Fe^{3+}			100
	Na^+/H^+		+	101 102
	Cl^-/HCO_3^-		+	103 104
	phosphate, sulphate	+		105 106

outside to low concentration inside the cell, the gradient being maintained by the Na^+-ATPase pump in the basolateral membrane. As shown in Table 2, Na^+-dependent transport has been demonstrated for a wide variety of substances including sugars, amino acids, bile salts, and some vitamins and ions. Although much of this information was obtained from studies using animal tissues, steady progress has been made in confirming the findings using human preparations, particularly microvillus membrane vesicles.[91 96 99 101 106] Support for the existence of some of these transport pathways comes from the recognition of specific clinical conditions in which a congenital absence or inactivity of the membrane carrier protein leads to inability to absorb the corresponding substrate (Table 2).

The structure and biochemical characteristics of most of the carrier proteins are unknown, but considerable effort has been directed to the isolation and characterisation of the Na^+-glucose co-transporter. This appears to be a large protein comprising three 75 kDa subunits, each with an active site for glucose and Na^+.[107 108] Expression of the transporter mRNA in Xenopus oocytes has been studied and the gene sequenced.[109 110] Work is in progress to determine which biochemical features of the molecule are responsible for its transport properties, and to unravel the nature of the molecular defect in primary glucose-galactose malabsorption. Some progress has also been made in identifying a 100 kDa microvillar polypeptide as a component of the proline carrier.[111]

Transport of some macromolecules is effected by receptor-mediated endocytosis. Thus vitamin B_{12}-intrinsic factor complex binds in a Ca^{2+}-dependent manner to an ileal microvillar receptor protein[112 113] before absorption by endocytosis.[114] A similar mechanism is thought to be responsible for the absorption of immunoglobulin-G in the neonate.[115]

REGULATORY FUNCTIONS

Although a fast growing discipline, investigation of the regulation of brush border function is still in its infancy and accounts of the processes involved are largely hypothetical. Nevertheless the brush border is known to contain at least two Ca^{2+}- and cyclic nucleotide-sensitive transport systems, viz Na-Cl cotransport (by linked Na^+/H^+ and Cl^-/HCO_3^- exchange) and an electrogenic Cl^- channel,[116] while a number of potential regulatory proteins have been found there. These include guanylate cyclase; cyclic AMP-dependent protein kinase; a brush border specific, 86 kDa G-kinase; 25 and 21 kDa phosphoproteins acting as cosubstrates for the A- and G-kinases; several phosphoprotein substrates for a Ca^{2+}- and calmodulin-dependent kinase and the Ca^{2+}- and phospholipid-dependent protein kinase C; several G-proteins including the oncogene-related p21 *ras*; and phospholipase A_2.[44 45 116–118] There is thus the potential for both direct regulation – for example, by the cyclic AMP- or GMP-stimulated phosphorylation of a component of a transporter molecule, and for indirect processes mediated through the release of second messengers such as Ca^{2+} acting, for example, *via* protein kinase C and the phosphatidylinositol cascade system.[116–118] Except in certain enteropathic diarrhoeas, however (see below), it is unclear which of these mechanisms operate *in vivo*.

Brush borders and disease

PRIMARY DISORDERS

Primary alteration in the structural or functional organisation of the brush border membrane due to the congenital or acquired absence or inactivity of a specific functional component provides a rational explanation of certain clinical conditions of impaired digestion and absorption (Tables 1 and 2). With the exception of primary acquired lactase deficiency, which is common and of racial origin,[119] these conditions are rare and most of them manifest by malabsorption of the specific substrate involved. Specific malabsorption syndromes have been associated with absence or inactivity of the following enzyme proteins: sucrase-isomaltase, lactase, trehalase, enteropeptidase, and γ-glutamyl transferase.[120–122] In patients with disaccharidase deficiency – for example, lactase, avoiding the specific substrate (lactose) brings about clinical remission; alternatively this has been achieved by feeding the appropriate enzyme orally – for example, lactase (of microbial origin) in hypolactasia.[123]

Of the primary enzyme deficiencies, only in sucrase-isomaltase deficiency have detailed biochemical investigations been carried out.[120–122] Three distinct phenotypes were recently identified with the aid of monoclonal antibodies.[122] In phenotype I there was overproduction of an intracellular, high-mannose precursor of the enzyme which accumulated in the endoplasmic reticulum and was degraded intracellularly without reaching the brush border; this precursor was poorly converted into the mature, complex glycosylated form, apparently because of its inability to be properly transported in the endoplasmic reticulum. Phenotype II was characterised by apparently normal synthesis of the high-mannose precursor but without conversion to the complex form; the precursor accumulated in the Golgi apparatus, where it was degraded without reaching the brush border. In phenotype III, biosynthesis of both high-mannose and complex precursors appeared normal and the mature enzyme protein was incorporated into the microvillus membrane and cleaved

into separate subunits; the absent sucrase activity in the presence of normal isomaltase indicated a mutation affecting the sucrase active site alone, although the presence of immunoreactive enzyme at the basolateral membrane suggested that the intracellular sorting responsible for directing the enzyme to its correct cell surface destination was also affected.

Patients with primary deficiencies of transport carrier proteins have also been described showing malabsorption of glucose-galactose, glutamic acid, alanine, lysine, bile salts, folic acid, Na^+ and Cl^- (Table 2). In none of these disorders, however, has an abnormal transporter been characterised.

In the rare condition congenital microvillus atrophy the brush border may appear hypoplastic or even invaginated into the cytosol.[124 125] Electrophoretic analysis of microvillar proteins has shown a marked reduction in the myosin band and this disorder may therefore be caused by a defect of the brush border cytoskeleton.[126]

SECONDARY MALABSORPTION

In secondary malabsorption there is structural or functional damage to the brush border membrane as a consequence of other disease. Thus in coeliac disease the microvilli are abnormally short, irregular and sparse and the terminal web is incompletely developed.[127] Brush border enzymes are reduced to around 10% of normal but recover on gluten withdrawal, except for β-glucosidase and lactase which show a slower and less complete response.[128] The microvillus membrane composition is severely deranged in untreated disease but returns virtually to normal after treatment.[129 130] In postinfective tropical malabsorption damage to the microvilli is less severe and microvillar enzyme activities are less affected.[131] In extensive small intestinal Crohn's disease total microvillar membrane function can be severely reduced. In Crohn's disease not involving the jejunum, brush border disaccharidase activities were reduced[132] but, in contrast, subcellular fractionation of diseased ileal mucosa has shown an essentially normal distribution of marker enzymes.[133]

Bacterial overgrowth of the small intestine appears to involve adherence of sugar binding proteins (lectins) of the microorganism to specific oligosaccharides of the microvillus membrane.[134 135] Brush border enzyme activities may then be reduced by the action of bacterial proteases and this may contribute to the malabsorption found.[136 137] Similarly in Giardiasis, there may be a significant reduction in lactase, sucrase and aminopeptidase activities which is reversible with effective treatment.[138] In cholera, the B subunits of the enterotoxin bind with high affinity to specific glycolipid receptors (GM_1-ganglioside) on the microvillus surface. After binding has occurred the A subunit penetrates the membrane and activates adenylate cyclase, thereby initiating the secretory diarrhoea.[116 139] *E coli* and Yersinia heat stable toxins bind to a specific microvillar glycoprotein and provoke secretion by activating guanylate cylase,[116 139 140] although Ca^{2+}, calmodulin and the phosphoinositol cascade appear also to be involved. Binding of Clostridial enterotoxin A to a microvillar glycoprotein has also been shown[141] and recent work suggests that it may induce diarrhoea partly by altering paracellular permeability through an effect on the cytoskeleton.[69]

Conclusion

The immense amount of work carried out over the past 30 years has enabled much of the structure and function of the intestinal brush border to be described in molecular terms. It is now apparent that the microvillus membrane and the subjacent cytoskeleton interact functionally and there can be little doubt that further details of this interaction will be forthcoming. Rapid progress is being made concerning the mode of production, regulation and biological function of individual proteins of the microvillus membrane, and it is anticipated that the isolation and molecular characterisation of additional transport proteins will not be long delayed. The gene sequence of several brush border proteins is already known and others are doubtless being determined. The intestinal brush border has thus truly come of age: it is no longer solely of interest to gastroenterologists and gut physiologists but is now playing a central role in answering fundamental questions at the heart of cell biology itself.

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