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Intestinal Brush Border Glycohydrolases: Structure, Function, and Development

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ABSTRACT: The hydrolytic enzymes of the intestinal brush border membrane are essential for the degradation of nutrients to absorbable units. Particularly, the brush border glycohydrolases are responsible for the degradation of di- and oligosaccharides into monosaccharides, and are thus crucial for the energy-intake of humans and other mammals. This review will critically discuss all that is known in the literature about intestinal brush border glycohydrolases. First, we will assess the importance of these enzymes in degradation of dietary carbohydrates. Then, we will closely examine the relevant features of the intestinal epithelium which harbors these glycohydrolases. Each of the glycohydrolytic brush border enzymes will be reviewed with respect to structure, biosynthesis, substrate specificity, hydrolytic mechanism, gene regulation and developmental expression. Finally, intestinal disorders will be discussed that affect the expression of the brush border glycohydrolases. The clinical consequences of these enzyme deficiency disorders will be discussed. Concomitantly, these disorders may provide us with important details regarding the functions and gene expression of these enzymes under specific (pathogenic) circumstances.

KEY WORDS: lactase, sucrase-isomaltase, maltase-glucoamylase, trehalase, digestion, nutrition, malabsorption, brush border.

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I. INTRODUCTION

This review will describe the mammalian intestinal brush border glycohydrolases, which are essential in the carbohydrate digestion in the human and most other mammals. To be able to fully appreciate the biochemical, molecular biological, and cell biological aspects of these enzymes, we will discuss such data in a physiological context. We will describe the general role of these enzymes in carbohydrate digestion, in relation to the other glycohydrolases of the gastrointestinal tract. The intestinal brush border glycohydrolases work in concert with salivary and pancreatic enzymes, and are essential to the digestion of complex dietary carbohydrates to absorbable monosaccharides. This hydrolysis is crucial, as di-, oligo-, and polysaccharides cannot be transported across the plasma membrane of cells. Monosaccharides are transported across the intestinal epithelium via specific transporters and are released into the circulation to be used in numerous metabolic pathways. We shall present relevant aspects of enterocyte function responsible for the expres-

Abbreviations: bp, basepairs; CBE, conduritol-β-epoxide; EGF, epidermal growth factor; GPI, glycosyl-phosphatidylinositol; kb, kilobases; kDa, kilodalton; ONP-glc, *ortho*-nitrophenyl-β(l-4)-glucopyranoside; ONP-gal, *ortho*-nitrophenyl-β(l-4)-galactopyranoside; RER, roughendoplasmic reticulum; SCFA, short-chain fatty acids; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; [^{125}I]TID, 3-(trifluoromethyl)-3-(m[^{125}I]-iodophenyl)diazirine; tris, tris(hydroxymethyl) aminomethane.

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sion of the brush border membrane and its glycohydrolases. We will focus on data in humans and a few other relatively well-studied mammals from various classes, as data on comparable-intestinal enzymes from non-mammalian species are very scarce. However, substantial literature on bacterial and fungal glycohydrolases shows some interesting parallels to the mammalian enzymes with respect to catalytic mechanism, structure, and evolutionary descent of these enzymes. Finally, we will delineate the gene expression of the enzymes during mammalian development as well as in (human) disease.

The importance of these enzymes for carbohydrate digestion, and nutrition in general, indicates that enzyme deficiencies, either of genetic origin or caused by tissue damage, will have an important impact on health. Primary glycohydrolase deficiency, caused by genetic defects, will be discussed, as well as secondary enzyme deficiencies caused by intestinal epithelial damage. It is well known that the absence or decline of the glycohydrolases leads to diminished carbohydrate digestion and absorption, and may lead to malnutrition or symptoms of osmotic or fermentative diarrhea. These disorders also provide us with details of the functions and genetic expression of these enzymes under specific circumstances.

II. CARBOHYDRATE DIGESTION AND ABSORPTION IN THE MAMMALIAN GASTROINTESTINAL TRACT

A. Molecular Forms and Nutritional Importance of Dietary Carbohydrates

1. Polysaccharides

The most abundant forms of carbohydrates in nature are large polymers of plant origin. In fact, the most abundant biomolecule on earth is cellulose, present as structural moiety in probably all plants. Cellulose, however, has only very limited, if any, nutritional value for humans. On the other hand, mammals like ruminants are specialized in cellulose digestion, by employing commensal bacteria to degrade this substance. Biochemically, cellulose consists of very large polymers of glucose residues, which are linked by β(1-4) glucosidic bonds. The β-glucosidic bonds in cellulose cannot be hydrolyzed efficiently by humans. Interestingly, lactase is capable of hydrolyzing cellulose, as discussed in Section V, but may not significantly contribute to its digestion because cellulose only exists as an "unsoluble fiber". In contrast, the polysaccharide storage molecules used by plants are particularly important in human nutrition and in nutrition of most other mammals. Starch, which constitutes a mixture of amylose and amylopectin, is the major carbohydrate source in the human diet, and is found in potatoes, rice, and wheat and consequently in plant-based daily products like bread and pasta. Humans and all other mammals are capable of digesting starch, which consists of very large glucose polymers. The glucose residues are linked by α(l-4) glucosidic bonds in amylose as well as in amylopectin, but amylopectin also contains branch points formed by α(1-6) glucosidic bonds between glucose residues. Both types of α-glucosidic bonds can be hydrolyzed by enzymes in all mammals, and most other animals. Thus, starch is an important nutrient.

Animal polysaccharides are of much less importance to human nutrition: only glycogen is present in sufficient amounts to contribute to the human carbohydrate intake. Glycogen is a glucose polymer, which has a similar branched structure as amylopectin, although the number of branch points is usually much higher than in plant. As the biochemical structure of glycogen is identical to amylopectin, it can be digested by the same enzymes.

2. Disaccharides

Another important source of carbohydrates in the human diet is disaccharides. The only important disaccharide of animal origin is lactose, which is found exclusively in mammalian milk. Lactose is the most important carbohydrate source for the newborn mammal, and is exclusively hydrolyzed by small intestinal lactase. Lactose consists of a galactose and a glucose residue, joined by a β(1-4) glycosidic bond. Lactose is normally not ingested by adult mammals and thus lactase is essential mainly in the suckling period of life. As

might be expected, lactase has a specific ontogenetic pattern of regulation (Section VIII). In contrast to lactose, the other important disaccharide in the human diet is plant-derived. Sucrose, found in many fruits and some vegetables, consists of a glucose and a fructose residue, joined by an α(1-4) glucosidic bond, and is hydrolyzed exclusively by small intestinal sucrase (Section VII). A nutritionally unimportant, but biochemically very interesting disaccharide is trehalose, which is found mainly in mushrooms (and in insects). Trehalose consists of a non-reducing dimer of glucose bound α(1-1)α′. This curious disaccharide is the only known natural substrate for the small intestinal trehalase (Section VI). Three monosaccharides occur also in the diet, glucose, galactose, and fructose, principally derived from fruits, but also from honey. These monosaccharides are directly absorbed in the small intestine without further processing.

3. Other Glycoconjugates

Other substances that contain carbohydrate constituents, are glycolipids and glycoproteins. However, these compounds are normally only a small quantity in the food and are probably not digested. The *O*- and *N*-linked glycans of glycoproteins are usually terminated at their reducing ends by α-glycosidically linked galactose, sialic acid, sulfate, *N*-acetyl-galactosamine, or fucose, none of which are substrates for the mammalian brush border glycohydrolases. Thus, the glycons of glycoproteins do not play an important role in nutrition, unless they are fermented by commensal bacteria (Section II.C). The most common glycolipids in the diet, i.e., glucosyl-, galactosyl-, and lactosylceramide are components of plasma membranes and only scarcely present in food of animal origin. The carbohydrate constituents of these glycolipids are bound β-glycosidically to the ceramide backbone, making them susceptible

TABLE 1
Molecular Forms and Processing of Dietary Poly- and Disaccharides

Saccharide	Molecular form of substrate	Organ	Enzyme (-complex)
Amylose	Linear glucose-polymer, α(1-4)-bonds	Mouth	α-Amylase
		Pancreas	α-Amylase
		Small-intestine	Sucrase-isomaltase, maltase-glucoamylase
Glycogen	Branched glucose-polymer, linear, α(1-4) bonds with α(l-6)-branch points	Mouth	α-Amylase
		Pancreas	α-Amylase
		Small-intestine	Sucrase-isomaltase, maltase-glucoamylase
Amylopectin	Branched glucose-polymer, linear, α(1-4) bonds with α(l-6)-branch points	Mouth	α-Amylase
		Pancreas	α-Amylase
		Small-intestine	Sucrase-isomaltase, maltase-glucoamylase
Cellulose	Linear glucose-polymer, β(1-4)-bonds	Small-intestine	Lactase
		Colon	Bacteria
Lactose	Glucosyl-β(l-4)galactose	Small-intestine	Lactase
Sucrose	Glucosyl-α(1-4)fructose	Small-intestine	Sucrase-isomaltase[a]
Trehalose	Glucosyl-α,α′-glucose	Small-intestine	Trehalase
Maltose	Glucosyl-α(1-4)glucose	Small-intestine	Maltase-glucoamylase sucrase-isomaltase

[a] Only the sucrase subunit of sucrase isomaltase has sucrase activity.

to degradation by the only α-glycosidase in the lumen of the gastrointestinal tract: lactase (Section VI).

Food technology has developed many "artificial" saccharides either by modification of foods or by addition of chemically engineered compounds, like sweeteners. Most of these compounds have no nutritional value, but some can be digested by the intestinal glycohydrolases, and thus contribute to the absorbable pool of carbohydrates. Moreover, some saccharides, undigestible by intestinal glycohydrolases, are efficiently fermented by commensal bacteria, thus contributing to the energy requirements of the body (Section II.C). Maltose, which in free form exists only as a starch degradation product, and is not present in the regular diet in significant amounts, is used as syrup for sweetening. Free maltose is further present in malted products like beer. In contrast, pectin, a compound of all plant cell walls, is a common ingredient in food because it is often used to gelatinate fluid foods like jam. This polymer of glucuronic acid and rhamnose cannot be digested by the gastrointestinal glycohydrolases, but may be successfully fermented by colonic bacteria.

B. Digestion of Dietary Poly- and Disaccharides by Salivary, Pancreatic, and Small Intestinal Glycohydrolases

1. Digestion of Starch

The digestion of starch begins rapidly, in the most proximal part of the gastrointestinal tract by the action of α-amylase in salivary fluid. This enzyme acts only during chewing of the food, when it is still in the mouth, because the low pH in the stomach rapidly inactivates the enzyme. The stomach does not produce any glycohydrolase. Thus, as saccharides are generally resistant to low pH, they leave the stomach in very much the same form as they entered. Starch digestion is continued in the duodenum by α-amylase released by the exocrine pancreas, which hydrolyses only the α(1-4) glucosidic bonds in starch. As the α-amylases are not true exoglycosidases, and liberate glucose in units of two or three, free glucose is virtually absent after starch hydrolysis by these enzymes. The combined action of salivary and pancreatic α-amylases leads to production of large amounts of maltose and maltotriose, and to relatively small amounts of the so-called "limit dextrins". Limit dextrins are comprised of a complex mixture containing at least one α(1-6) glucosidic branch point. Thus, the linear glucose polymer amylose is degraded by α-amylases into maltose and maltotriose, while the branched amylopectin (and glycogen) is degraded into glucose (very small amounts), maltose, maltotriose, and small branched α-limit dextrins, containing α(1-6) glucosidic bonds. The resulting luminal mixture of di-, tri- and oligosaccharides is still not absorbed by the intestine; additional action of small intestinal brush border hydrolases is required to liberate the absorbable glucose.

In vivo, the catalytic reactions by the pancreatic α-amylases and the intestinal membrane bound glycohydrolases take place concomitantly. In general, amylopectin and amylose are progressively degraded making them increasingly poor substrates for this pancreatic α-amylase, while these small degradation products become increasingly better substrates for the intestinal glycohydrolases. The digestion of starch cannot be characterized by a clear compartmentalization of the various steps in the different regions of the small intestine. However, in a proximal to distal gradient, there is a gradual decline in concentrations of larger glucose polymers and oligomers, and a concomitant increase of maltose, maltotriose, and glucose, of which the latter is efficiently absorbed in the small intestine. When the capacity of the involved enzymes is too low relative to the ingested starch, undegraded or partially degraded starch reaches the colon, where it is salvaged by fermentation to short-chain fatty acids (SCFA), which can be absorbed by the colon (Section II.C). It is estimated that in normal individuals about 5 to 20% of consumed starch reaches the colon undegraded. The extent of degradation further depends on the source and the preparation of the starch; the concomitant presence of proteins (gluten e.g. in wheat) impairs starch digestion. Thus, rice flour is degraded much more efficiently than wheat flour. Amylose is digested far more efficiently than the branched amylopectin because of its simpler chemical structure.

2. The Brush Border Glycohydrolases

The small intestine harbors 4 membrane-bound glycohydrolases: trehalase, maltase-glucoamylase, lactase, and sucrase-isomaltase (Table 2). Here, we will concentrate on their respective roles in digestion, as the aspects of the structure/function relationships of the individual enzymes will be discussed in Sections IV to VII. The starch-derived products of α-amylase digestion are further degraded by combined actions of the maltase-glucoamylase and sucrase-isomaltase complex, each containing two different active sites. Each of the subunits in these two complexes has maltase and maltotriase activity, hydrolyzing the α(1-4) glucosidic bonds of the non-reducing glucose units (Table 3). The sucrase-isomaltase and the maltase-glucoamylase enzyme complexes are true exoglucosidases, as they liberate free monomeric glucose from their di- and oligomeric substrates. In man, the sucrase-isomaltase is far more abundant than the maltase-glucoamylase, and therefore responsible for about 80% of the maltase (and maltotriase) activity in the small intestine (Semenza, 1986). The α(1-6) glucosidic bonds in limit dextrins are almost exclusively hydrolyzed by the isomaltase subunit of sucrase-isomaltase, thereby liberating non-reducing α(1-6) glucosidically bound glucose residues from these dextrins. Both subunits of maltase-glucoamylase also have this isomaltase activity, but are generally thought to play only marginal roles in man in *in vivo* hydrolysis of these bonds.

3. Brush Border Glycohydrolases Are Primarily Disaccharidases

All small intestinal glycohydrolases have disaccharidase activity, which is generally their main function. The maltase activity of sucrase-isomaltase and maltase-glucoamylase have been mentioned above in relation to the intestinal digestion of starch. Free dietary maltose mainly occurs as an additive in food. Very important for the survival of young mammals is the hydrolysis of lactose which is present in milk. Lactase is the only enzyme in the small intestine possessing the necessary β(1-4) galactosidase activity, and is thus a crucial enzyme during early post-natal mammalian development. In contrast, sucrose is part of the human diet after weaning, and during the remainder of adult life. Sucrose is exclusively hydrolyzed by the sucrase subunit of the sucrase-isomaltase heterodimer. Small intestinal trehalase is the sole enzyme responsible for hydrolysis of trehalose. The significance of trehalose for human nutrition, however, is rather obscure, as this disaccharide is confined to yeast, mushrooms and insects.

4. Absorption of Monosaccharides Involves Three Transporters

Monosaccharides are absorbed by the gastrointestinal tract, either by transcellular transport through specific transporters expressed in the

TABLE 2
Nomenclature of Mammalian Gastrointestinal Glycohydrolases

Preferred name	EC number(s)	Synonym(s)	Source
α-Amylase	3.2.1.1	—	Saliva
α-Amylase	3.2.1.1	—	Pancreas
Trehalase	3.2.1.28	α,α′-Trehalose-1-D-glucohydrolase	Small intestine
Maltase-glucoamylase	3.2.1.20	α-Limit dextrinase glucoamylase complex γ-amylase	Small intestine
Lactase	3.2.1.23, –45, –46, –62	actase-phlorizin hydrolase Lactase-glycosylceramidase β-glycosidase complex	Small intestine
Sucrase-isomaltase	3.2.1.10, –48	Sucrase-α-dextrinase	Small intestine

TABLE 3
Enzymatic Specificities and Natural Substrates of Intestinal Brush Border Glycohydrolases

Enzyme and constituting subunits		General specificity	Natural substrates
Trehalase		α,α′-Trehalose	α,α′-Trehalose
Maltase-glucoamylase	Maltase	α(1-4)Glucosyl bonds	Maltose (-hexose, -octanose), amylopectin, glycogen, starch, amylose, low on isomaltose
	Glucoamylase	α(1-4)Glucosyl bonds	Maltose (-hexose, -octanose), amylopectin, glycogen, starch, amylose, low on isomaltose
Lactase		β(1-4)Glycosyl bonds	Lactose, phlorizin, cellobiose (-triose, -tetrose), cellulose, glucosyl-, galactosyl-, lactosylceramide.
Sucrase-isomaltase	Sucrase	α(1-4)Glucosyl bonds	Sucrose, maltose (-triose)
	Isomaltase	α(1-6)Glucosyl bonds	Isomaltose, maltose (-triose)

Note: The substrate specificites are given per subunit. Lactase contains two active sites, but is present in the brush border as an unsplit transmembrane enzyme, therefore its enzymatic specificities are given for the molecule as an entity. The maltase-glucoamylase and sucrase-isomaltase are both composed of two proteolytically cleaved subunits. As the individual enzymatic specificities of these subunits could be determined individually, these are listed separately.

plasma membrane of the epithelial cells lining the gastrointestinal lumen or by paracellular transport via the tight junctions between these epithelial cells. As we only intend to cover this subject briefly, we refer to the excellent recent reviews by Wright et al. (1994) and Pappenheimer (1993).

Several monosaccharide transporters have been cloned and characterized which are responsible for the active Na^+-coupled, absorption of glucose, galactose, and fructose into the enterocyte. In the apical brush border membrane of the enterocyte, the sodium/glucose (identical to the sodium/galactose, Turk et al., 1991) cotransporter (SGLT1) and the fructose transporter (GLUT5) account for the energy-dependent uptake of D-glucose/galactose and fructose, respectively (Wright et al., 1994). The monosaccharide transporter present in the basolateral membrane of the enterocytes (GLUT2) has a broad specificity for pentoses and hexoses, and is responsible for the energy-independent facilitated diffusion of glucose, galactose and fructose into the blood. These sugar transporters are glycosylated transmembrane proteins, which span the plasma membrane 12 times. SGLT1 and GLUT5 are co-transporters, which transport luminal glucose, galactose, and fructose concomitant with Na^+-ions. Thus, the Na^+-gradient over the plasma membrane, generated by the exchange of Na^+ and K^+ by the basolateral Na^+/K^+ ATPase, is responsible for the efficient uptake of glucose, galactose, and fructose. Because only small amounts of monosaccharides are consumed by the epithelium itself, most absorbed sugars are released from the basolateral membrane of the enterocytes into the circulation. This transport is facilitated by GLUT2.

The absorption of glucose starts in the stomach. However, only low concentrations of free glucose may be present in the stomach, as glucose liberated from di- or polysaccharide-digestion will not appear until the small intestine is reached. The small intestine is the major site for monosaccharide absorption since both SGLT1, GLUT2 and GLUT5 are specifically expressed in the villus enterocytes of the duodenum, jejunum, and ileum. The mammalian colon has only a low capac-

ity to absorb glucose/galactose as it expresses only low levels of SGLT1. However, as indicated in the next section, the colon is involved in a salvage mechanism, which absorbs SCFA derived from luminal fermentation of saccharides by bacteria. SGLT1 or SGLT1-like transporter sequences seem particularly conserved during evolution, as highly homologous forms of these genes as well as proteins are detected in many vertebrates, such as amphibians, alligators, birds, and mammals (Wright et al., 1994).

A second mechanism for absorption of nutrients is via the paracellular route (reviewed by Pappenheimer, 1993). In many experimental conditions it was observed that small solutes like amino acids and monosaccharides were absorbed independent of the above described transporters. And in contrast to transporter-mediated transport, this absorption was non-saturable, suggesting the involvement of pores. The paracellular nutrient absorption is proportional to the fluid flow, the so-called solvent drag, through pores in the tight junctions, resulting form the hyperosmolarity of the extracellular fluid at the basolateral space between the enterocytes (Pappenheimer, 1993). This hyperosmolarity is primarily caused by the release of glucose from the enterocytes into this space, and causes a solvent drag via pores in the tight junctions from the intestinal lumen into the tissue fluid, which results in passive absorption of small solutes. The involvement of pores within the tight junctions is most convincingly implicated by changes in the permeability via these pores, concomitant to changes in the cytoskeletal components, which are known to regulate the conformation of the tight junctions. The paracellular transport mechanism operates particularly efficiently in absorbing high concentrations of small nutrients, whereas the active transcellular transport route works more efficiently in the presence of low concentrations of these solutes.

C. The Role of Commensal Bacteria in Carbohydrate Digestion and Absorption of Short-Chain Fatty Acids

The colon of humans harbors up to 10^{11} bacteria/ml which are capable of fermenting undigested carbohydrate compounds reaching the colon. In the small intestine, the degradation of the di-, oligo-and polysaccharides is the rate-limiting step, as all liberated monosaccharides are readily absorbed by the intestinal epithelium. Therefore, non-digested saccharides in the colon result either from insufficient hydrolytic capacity of the glycohydrolyses due to disease or from a carbohydrate-rich meal that exceeded the disaccharidase capacity in the small intestine of healthy individuals. Pathological situations, involving, e.g., the pancreas or the small intestine, may affect either the glycohydrolytic or the absorptive capacity towards saccharides, and then the amount of starch, starch degradation products, or disaccharides reaching the colon may increase drastically (Section IX). Fermentation of relatively small amounts of these saccharides by colonic bacteria leads to the production of H_2, CO_2, and CH_4 and SCFA: primarily acetate, propionate and butyrate, which are taken up and/or metabolized by the colonocytes. However, this salvage mechanism for uptake of saccharide-based nutrients may have adverse effects if the load of saccharides becomes to high, as the high concentrations of fermentation products often leads to gastrointestinal complaints and osmotic diarrhea. Thus, a deficiency in glycohydrolytic capacity often leads to diarrhea, which in turn leads to decreased absorption of nutrients. As the lack of absorbed nutrients may impair the recovery of the epithelium from the glycohydrolase deficiency, this may result in severe malnutrition (Section IX).

In ruminants, the stomach contains different compartments, each of which houses different types of bacteria. The most important polysaccharide in the ruminant diet is cellulose, which is digested by the bacteria in the rumen to absorbable compounds. Thus, in ruminants the commensal bacteria have become essential for survival. Likewise, a large number of eubacteria and fungi also use cellulose as their primary energy source, and the cellulase family of enzymes is particularly widespread among these phyla.

Absorption of SCFA is in principle possible in the whole intestine, but is predominantly a colonic function, as recently reviewed by Binder and Sandle, 1994. The amount of SCFA is not correlated with carbohydrate-induced (malabsorption driven) diarrhea. The absorption of SCFA

also stimulates the colonic absorption of other electrolytes (Na^+ and Cl^-) and water. Models describing mechanisms for the transport of the SCFA by the colonocytes distinguish passive diffusion of protonated uncharged SCFA through the apical and basolateral membranes of the colonocyte as well as a transporter-mediated transport (Binder and Sandle, 1994). Deprotonated SCFA could be transported through anion carriers in the apical and the basolateral plasma membrane, which exchange a SCFA for HCO_3^- as was demonstrated in isolated plasma membrane vesicles (Binder and Sandle, 1994). However, the same authors, based on other studies in intact mucosa under voltage-clamp conditions, predict that most SCFA are absorbed in the protonated form. Intracellularly, the SCFA could dissociate into H^+ and $SCFA^+$, the H^+ is then exchanged against luminal Na^+, and the SCFA′ is exchanged against luminal Cl^-. Thus, apart from their nutritional value, the presence of SCFA in the colon stimulate the electro-neutral absorption of NaCl, and concomitantly of water.

III. THE SMALL INTESTINAL EPITHELIUM

The gastrointestinal tract is covered by a virtually uninterrupted epithelium. This single-layer epithelium serves as a selective barrier to luminal contents, and is the location of proteins regulating the degradation and selective uptake of nutrients. In this review we will concentrate primarily on the structure and functions of the villus enterocyte as this major cell-type expresses the brush border glycohydrolases.

A. Cell Types

The small intestine harbors four different cell types: enterocytes, goblet cells, enteroendocrine cells, and Paneth cells (Madara and Trier, 1994; Figure 1; Table 4). The enterocytes possess an apical plasma membrane containing closely packed microvilli, generally designated brush border, which contains the glycohydrolases and a large number of other enzymes and transporters.

The enteroendocrine cells constitute a small number of cells, but they produce at least 15 different gastrointestinal hormones (Walsh, 1994). These cells are found throughout the gastrointestinal tract and are a very heterogeneous group, i.e., each cell produces more than one hormone, but often in different combinations. The goblet cells produce large amounts of secretory mucins which are the major component of mucus gel-layers (reviewed by Strous and Dekker, 1992). The number and localization of these cells varies along the gastrointestinal tract. Moreover, the quantity of goblet cells can adapt to specific diets or to stress situations. In the gastric epithelium, this cell type forms a continuous lining of the lumen, while in the small intestine they constitute only about 5% of the epithelial cells, and in the colon about 50%. The secretory mucins produced by these cells form a mucus layer that represents an important protective mechanism. The mucus forms a gel, producing an "unstirred layer", which functions as a molecular sieve (cutoff at about 10 kDa). This mucus gel traps defensive molecules such as lysozyme and IgA, but allows access of small molecules to the apical membrane of enterocytes. The mucus gel also offers resistance to physical erosion, and to pathogens. The Paneth cells are predominantly found in the small intestinal crypts. These cells produce antimicrobial polypeptides such as lysozyme and defensins. Although relatively small in number they apparently are very effective in eliminating bacteria and viruses, and they may be essential to inhibit bacterial overgrowth of the small intestine.

B. Tissue Homeostasis

The morphology of the small intestine is comprised of characteristic villi and crypts (of Lieberkühn) (Figure 1). All cell types of the epithelium arise from pluripotent stem cells, located near the bottom of the crypts of the mucosa (Ponder et al., 1985). After cell division, the daughter cells either migrate upwards to the villus tip or to the bottom of the crypt. Moving deeper into the crypt, they differentiate into Paneth cells, while moving towards the tip of the villus they differen-

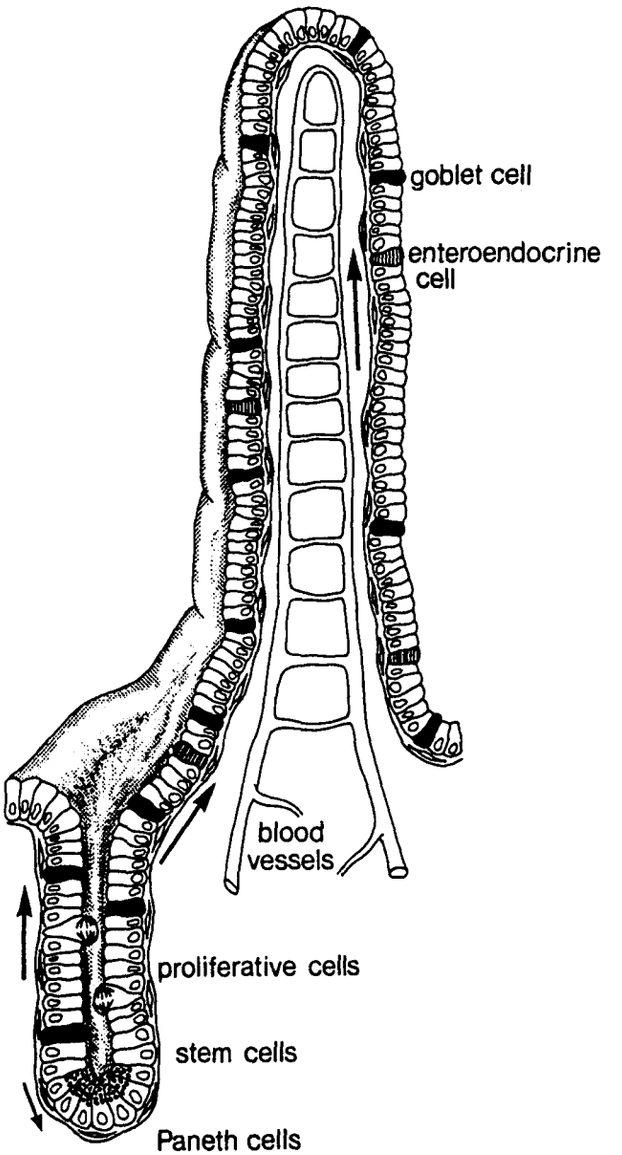

FIGURE 1. Schematic presentation of small intestinal epithelium (After Potten and Loeffler, 1990). Indicated are the 4 differentiated cell types: black, goblet cells; cross-hatched, enteroendocrine cells; granular, Paneth cells; all other cells are enterocytes. A crypt contains about 250 cells. The stem cells (less than 16 per crypt) are located close to the bottom of the crypt. By cell division (taking about 24 h per cell cycle) these stem cells give rise to a class of proliferative cells (about 150 per crypt), which undergo several cell cycles (about 4, taking 12 h per cycle) before differentiating into one of the 4 above mentioned cell types. Concomitant with the divisions of these proliferative cells, virtually all these cells move towards the villus. Only a very small number migrates deeper into the crypt, and gives rise to only one cell type: the Paneth cell. During the migration of the proliferative cells to the basis of the villi all these cells stop dividing and are committed to develop into enterocytes, goblet-, or enteroendocrine cells, thereby confining all proliferative activity to the crypts. The cells produced by each crypt migrate upwards to contribute to several villi. Each villus contains about 3500 epithelial cells. The cells from each villus originate from about 6 to 10 different crypts. The migration rate of the epithelial cells on the villus is about 1 to 2 cell-positions per h, resulting in their removal by either extrusion or apoptosis (Discussed in Section II) at the villus tips at a rate of approximately 1400 cells per day. The arrows indicate the migration direction of the cells in the epithelium.

TABLE 4
The Roles of the Epithelial Cell Types in the Small Intestine in Mucosal Defense and Nutrient Processing

	Lumen		Epithelium			
			Protection mechanism		Nutrient processing	
	Protection mechanism (secretion)	Nutrient processing	Brush border membrane	Intracellular	Brush border membrane	Intracellular
Crypt enterocytes	IgA, Cl^- in diarrhea		Glycoproteins, glycolipids, tight junctions			
Villus enterocytes		Brush border hydrolases	Glycoproteins, glycolipids, tight junctions	Endocytosis and lysosomal degradation of macromolecules and microorganisms	Nutrient transporters	Nutrient-modification (e.g., of lipids)
Goblet cells	Mucins		Tight junctions			
Enteroendocrine cells			Tight junctions			
Paneth cells	Defensins, lysozyme		Tight junctions			

Note: The functions of each of the cell types of the intestinal epithelium can be divided into functions exerted in lumen and in the epithelial cells itself. Each of these functions can be subdivided into protection or nutrient processing tasks. Moreover, these tasks of the epithelial cells can be further divided into functions of the brush border

tiate into a mixture of mostly enterocytes, about 5% goblet cells, and about 1% enteroendocrine cells (Figure 1). The stem cells in the crypt continuously generate new epithelial cells at a high pace. The speed of proliferation is closely matched with removal of cells from the epithelium. The migration of the epithelial cells, from the moment of cell division to the villus tip takes about 3 d in rodents, and about 5 d in humans. These processes of proliferation and differentiation of intestinal epithelium are excellently described by Gordon (1989 and 1993).

Cell death may play a prominent role in tissue homeostasis in two distinct locations. Apoptosis (or regulated cell death) is recently recognized as a potent and general mechanism to regulate cell turnover, and this could also play a prominent role in the intestinal epithelium (Hockenbery et al., 1991; Hall et al., 1994). First, apoptosis functions to restrict the number of stem cells in the crypt. When intestinal tissue is damaged by e.g. radiation, the proliferative activity of the stem cells is enhanced to repopulate the damaged tissue (Potten et al., 1990). However, due to the high activity of the stem cells in combination with the inflicted genetic damage, the number of stem cells with damaged genetic information increases. As it is undesirable to propagate this DNA-damage in the descendants of these stem cells, these stem cells are selectively removed by apoptosis in the crypt (Potten et al., 1990; Potten and Loeffler, 1990). Second, cell death may occur at the top of the villus to remove cells from the epithelium. It is generally excepted that the removal of epithelial cells by either shedding or apoptosis does not leave gaps in the epithelial layer. Thus, the epithelium manages to remove cells without disruption of the occluding functions of the tight junctions, which is very important to maintain an effective barrier function (Table 4). The mechanism by which the cells are removed from the epithelial monolayer is still uncertain. It was long thought, that entire cells were sloughed off into the intestinal lumen (Madara and Trier, 1994). However, there are recent indications that apoptosis is also involved in the removal of cells at the villus tip (Hall et al., 1994; Hockenbery et al., 1991).

Hypothetically, shedding of epithelial cells would be an efficient defensive mechanism of the

intestine against luminal influences. Due to the continuous flow of toxic and noxious substances, and pathogenic organisms (viruses, bacteria, and parasites), cells may be damaged easily. These events may lead to cell damage and loss of protective functions. In the case of infection by viruses or bacteria, it is desirable to shed the damaged cells into the lumen. Similar theoretical arguments for shedding can be found for the accumulation of xenobiotics in the epithelial cells. Apoptosis of infected cells, could lead to resorption of these cells into the organism, which could result in transmission of the infectious agents into the body. Likewise, accumulated xenobiotics could also be involuntarily imported into the body by apoptosis. Removal of cells by apoptosis, on the other hand, would imply that the cell compounds would not be lost, and could be efficiently recycled within the body. Whatever mechanism is responsible for the removal of surplus cells, the high proliferative capacity of the intestinal epithelium is very important in the repopulation of the epithelium after damage (Especially relevant in conjunction with secondary glycohydrolase deficiency; Section XI). A very suggestive and intriguing finding in this respect was done in the guinea pig small intestine. An electron microscopical study showed that effete cells in this species were removed from the mucosal side of the epithelium, leaving only a "cap", consisting of the apical brush border still attached to the tight junctions. Then, this "cap" is presumably disposed into the lumen, by an active "pinching off" mechanism (Iwanaga et al., 1993). These pinched off brush border "caps" could be recovered in the lumen, while nuclei were never found luminally. Curiously, and yet unexplained, the microvilli of these caps, during and after the pinching off, acquire an elongated and branched appearance.

It may well be, that the epithelium is able to switch from one mechanism of removing surplus cells to another mechanism. In normal, non-threatening situations apoptosis could prevail, saving energy by retrieval of materials, whereas under more hostile conditions, the epithelium may switch to shedding of effete cells, to reduce the risk of importing potential damaging agents or organisms.

C. Structure of the Enterocyte

Intestinal villus enterocytes participate in degradation and subsequent absorption of nutrients, which can be subdivided into three phases. The first phase takes place at the brush border of the enterocytes. Here nutrients, especially disaccharides and small peptides, are degraded into absorbable monosaccharides and amino acids, respectively. The uptake of these nutrients by specific transporters (Section II.C) leads to the second phase: the intracellular phase. Here some nutrients, especially fatty acids, are converted into larger complexes, i.e., triglycerides and chylomicrons. Only a minute quantity of the nutrients are used within the enterocytes. Most of these nutrients pass into the blood, and thus enter the third phase of absorption, i.e., export via the portal vene to the liver and the rest of the body.

Enterocytes are highly specialized and polarized cells. Generally, polarized cells express cell type specific proteins at their apical membrane, while non-cell type specific proteins are expressed basolaterally. These two plasma membrane domains are separated by tight junctions, which form diffusion barriers for the membrane components of both domains (Citi, 1993). This also accounts for enterocytes: all specialized functions associated with degradation and uptake of nutrients are located in the apical plasma membrane, which lines the intestinal lumen (Rindler and Hoops, 1994). Apart from a barrier between the apical and the basolateral plasma membrane domains, the tight junctions form very important barriers between the lumen of the gut and the interior of the body (Madara and Trier, 1994; Citi, 1993).

Mature enterocytes develop an intricate and specialized apical microvillar membrane, essential in the degradation and uptake of nutrients: the brush border membrane. This very closely packed array of microvilli is further found in renal tubule epithelium. The biogenesis of the brush border membrane involves the intricate interplay of many cytoskeletal and membrane compounds, as was excellently delineated in the enterocyte-like Caco-2 cell line by Pinto et al., 1983; Peterson and Mooseker (Peterson and Mooseker, 1992; Peterson and Mooseker, 1993; Peterson et al., 1993). Several other features of the enterocytes also play important roles in either mucosal defense

or nutrient processing. Although basically representing the same cell type, enterocytes in either the crypt or villus epithelium have distinct functions (Table 4). In the crypts, the secretory functions of the enterocytes predominate, while the enterocytes of the villi have mainly hydrolytic and absorptive functions. Thus, crypt enterocytes are, e.g., responsible for the secretion of water and chloride-ions in diarrhea via the CFTR-chloride transporter (i.e., the secretory response in many diseases), and IgA-secretion as part of the mucosal defense (Rindler and Hoops, 1994), while villus enterocytes are responsible for the degradation, e.g., by glycohydrolases, and the uptake of nutrients (Table 4). As the enterocytes, after their primary differentiation, migrate progressively towards the villus tip, they undergo a functional switch at the crypt-villus junction. A number of crypt specific genes (such as the CFTR chloride-transporter; Trezise and Buchwald, 1991) are silenced, while villus specific genes are switched on (such as the brush border glycohydrolases; Sections IV to VII).

The differences in enterocyte function between crypt and villus is reflected in the morphology along the crypt-villus axis; for example, crypt enterocytes have less and shorter microvilli than the villus enterocytes. Moreover, crypt enterocytes have an intracellular morphology appropriate for a more secretory function: secretory granules are apparent, and the apical cytoskeleton associated with the microvilli (terminal web) is far less developed (Louvard et al., 1992; Rindler and Hoops, 1994; Madara and Trier, 1994). Similarly to the crypt-villus axis, different regions along the length of the intestine also showed variation in enterocyte function and morphology; for example, the microvilli of colonic enterocytes are far shorter than of jejunal enterocytes (Louvard et al., 1992). The basolateral membrane of the enterocyte is involved in complicated epithelial-stromal interactions. The underlying mesenchymal tissue is very important in establishing the typical crypt-villus architecture of the intestine. The extracellular matrix, partly produced by the epithelial cells and partly by the connective tissue, appears to be a very important determinant of enterocyte development. This important, emerging field of research is beyond this review, and the reader is referred to the excellent review of Louvard et al. (1992).

To date, the most relevant enterocyte-model is the human colonic adenocarcinoma cell line Caco-2. This transformed colon cell line displays virtually all characteristics of small intestinal villus enterocytes. It forms a fully functional brush border, including two brush border glycohydrolases (lactase and sucrase-isomaltase), and displays a large number of characteristics of absorptive enterocytes (Pinto et al., 1983; Hauri et al., 1985; LeBivic et al., 1990; Gilbert et al., 1991; Peterson and Mooseker, 1992; Peterson and Mooseker, 1993; Peterson et al., 1993).

The brush border membranes are characterized by a glycocalyx: a densely packed glycoconjugate complex, consisting of glycolipids and glycoproteins, protruding about 50 nm from the luminal side of the apical membrane (Madara and Trier, 1994). The glycocalyx is thought to take part in the defense of the enterocyte (Table 4). Among these glycoproteins, the presence of (membrane-bound) mucins may be of great importance with respect to protection of the epithelial cells. Importantly, also the brush border glycohydrolases are part of the glycocalyx, as they are 10 to 30 nm projected from the apical membrane (Sections IV to VII). The glycocalyx carries a highly negative charge due to the presence of sialic acid and sulfate residues on the carbohydrate moieties within this structure. Technically, the brush border can be isolated in very pure form. This technique, originally designed by Schmitz et al. (1973) and modified and validated by many other authors, involves mechanical disruption of the tissue or isolated epithelial cells by high speed sheer forces, which vesiculates the brush border membrane. $CaCl_2$ (10 m*M*) is added, and the mixture is placed on ice. The negatively charged brush border vesicles form aggregates with the Ca^{2+}, which can be recovered by differential centrifugation: intracellular membranes (and debris) are collected by low speed centrifugation, brush border vesicles are recovered at higher speed. This preparation is the usual source for further isolation of the brush border glycohydrolases, and is very useful in discriminating intra- from extracellular forms of the enzymes (Sections IV to VII).

D. The Developmental Pattern of the Epithelium is Imprinted

The ability of intestinal stem cells to produce the full repertoire of daughter cells is already present within the epithelium of the embryonic gut. This was initially inferred from the work of Ferguson et al. (1973), who performed isografting experiments of mouse fetal intestinal tissue. It appeared that the development of an isograft from a specific region of fetal intestine, placed under the skin of a mature host, was not affected by the isografting. In other words, the stem cells within the fetal intestine, which at that stage of development is only a hollow mesenchymal tube lined by endodermal epithelium without a crypt-villus morphology, contain all the information for the development of the normal crypt-villus architecture as well as positional information to develop proximal or distal intestinal epithelial characteristics (Ferguson et al., 1973). These studies, also performed in rats, showed that the glycohydrolase expression in the intestine is likewise imprinted during fetal development (Ferguson et al., 1973; Kendall et al., 1979; Montgomery et al., 1981b; Yeh and Moog, 1986; Yeh et al., 1991b). As the isografts in these studies were implanted into mature animals, effects of hormones on the developmental expression of brush border glycohydrolases were indicated to play only a minor role. More recently, these studies were extended to isografting of fetal intestine of transgenic mice (Rubin et al., 1991; Rubin et al., 1992). 600 Bases of promoter sequence of rat fatty acid-binding protein coupled to a human growth hormone reporter gene, was specifically expressed in the small intestine and part of the colon of transgenic mice (Rubin et al., 1992). Moreover, it was also expressed, in similar patterns as in the intact mice, in isografts of 15 to 16 d embryonic transgenic mice placed subcutaneously in adult nude mice. Thus, this elegantly confirms that positional information is imprinted in the stem cells during very early intestinal development.

Recently, the xenograft model was used to specifically address the effect on the expression of brush border glycohydrolases (Duluc et al., 1994). Segments of fetal rat intestine, prepared from 14-d-old rat fetuses and xenografted into nude mice or chicken embryos, showed normal development with respect to their morphology within 2 to 4 weeks. Lactase and sucrase-isomaltase expression in these developing xenografts, determined at both mRNA and protein level, was very similar to the expression of these enzymes in the *in situ* intestine in control animals. Thus, the fetal endoderm holds the temporal and positional information required for mammalian intestinal development (Duluc et al., 1994). However, when fetal endoderm from the ileum was xenografted in heterologous association with mesenchyme from the jejunum, the jejunal mesenchyme induced lactase expression in the developing ileum, where it is not normally expressed. Likewise, small intestinal mesenchyme induced ectopic sucrase-isomaltase expression in the colon. Intestinal mesenchyme was not able to elicit lactase or sucrase-isomaltase expression in other epithelia such as stomach or lung epithelium. Thus, the small intestinal mesenchyme is able to deliver instructive information to the epithelium, but this appears only to have a minor influence on the imprinted information.

Although the fundamental abilities of the stem cells is apparently genetically imprinted during early development, the epithelium displays a surprising flexibility under specific conditions. For example, when the intestine is exposed to food containing high amounts of fiber, the number of goblet cells is greatly increased (Enss et al., 1994; Lundin et al., 1993). Apparently, the differentiation of the progeny of the stem cells is shifted towards a larger number of goblet cells, as an adaptive response. The flexibility of the epithelium and its stem cells is best displayed under conditions of villus atrophy. When the intestinal mucosa is severely damaged, due to toxic or noxious substances or infectious disease, the stem cells, which lie in the crypts, appear to be spared. When the cause of the insult disappears, the stem cells are able to restore the epithelial architecture within a remarkably short time (discussed in relation with secondary glycohydrolase deficiency in Section IX).

IV. TREHALASE

A. Structure and Biosynthesis of Trehalase

Trehalase is present in mammalian small intestinal and renal tubular epithelium (Galand, 1984; Yoneyama and Lever, 1984; Nakano and Sacktor, 1985; Lembcke et al., 1985), while there is also an indication of its expression in rabbit liver (Ruf et al., 1990). Its natural substrate specificity seems limited to trehalose, which serves as a storage disaccharide in many fungi (especially young mushrooms) including yeast. It is the most important sugar for energy transport in insect hemolymph, and it is further found in seaweed (Robinson and Goldsworthy, 1977; Birch, 1963). Consequently, most known trehalases are found in fungi, insects, and bacteria. In the small intestinal brush border, trehalase is a relatively low abundant enzyme, constituting less than 0.1 % of total brush border protein (Galand, 1984; Galand, 1989). Curiously, trehalase is also found in the brush border of the renal epithelium of mammals, while its substrate, trehalose, is absent from plasma. Trehalase has thus far not attracted much attention, as the nutritional importance of trehalose seems limited, mainly because of very limited intake. Therefore, the information acquired from different species is relatively scarce.

1. Isolation

Trehalase was isolated from intestinal as well as renal brush border membranes from human, rat, rabbit, and pig (Galand, 1984; Riby and Galand, 1985; Ruf et al., 1990; Morin and Potier, 1987; Chen et al., 1987; Yoneyama, 1987; Yokota et al., 1986). Contrary to the other brush border glycohydrolases, trehalase is not released upon papain digestion. Trehalase was found to be tightly associated with the brush border membrane fraction in all studies, and could be extracted by Triton X-100. The isolation of trehalase requires several steps, starting with the isolation of brush border membrane vesicles, followed by DEAE-cellulose ion-exchange, Sepharose G-200 gel filtration, and hydroxyapatite chromatography. Usually, trehalase can be purified to homogeneity, as revealed by SDS-PAGE, by a factor of 3,000 to 10,000, with a specific activity of 20 to 400 units/mg of protein as assayed according to Dahlqvist (1964) with trehalose as substrate. Recently, it appeared that trehalase interacts with the apical plasma membrane via a glycosylphosphatidylinositol (GPI)-anchor (Ruf et al., 1990). Trehalase, purified from brush border, displayed pI values of 4.1 to 4.9. Molecular masses, estimated by SDS-PAGE, gel filtration, or density gradient centrifugation, ranged from 65 to 75 kDa. Some studies reported higher molecular masses of 105 to 330 kDa under non-denaturing conditions by gel filtration or density gradient centrifugation (Galand, 1984; Yokata et al., 1986; Morin and Potier, 1987). These over-estimations of the molecular mass were likely due to the binding of a detergent micelle (Triton X-100) to the enzymes, although the existence of di- or oligomers of trehalase in the brush border membrane cannot be excluded.

2. Trehalase Has a Unique Structure Among Brush Border Glycohydrolases

Most structural data is available for rabbit small intestinal trehalase, which was cloned (Ruf et al., 1990). Rabbit trehalase has no homology to any other known mammalian protein. The primary translation product consists of a relatively small enzyme of 65.5 kDa, containing four potential *N*-glycosylation sites, which is much smaller than the other brush border glycohydrolases (Sections V to VII). There is no internal homology within the polypeptide, as found in lactase and sucrase-isomaltase, indicating that there is most likely only one active site, as elaborated in the next section. The rabbit trehalase gene is rather compact, comprising about 20 kb, and containing probably only two introns (Ruf et al., 1990). Interestingly, other known trehalase sequences showed significant homology to this rabbit trehalase. The 60 kDa *E. coli* periplasmic trehalase *treA*, showed a 35% homology at the amino acid level with the rabbit trehalase (Gutierrez et al., 1989). A second 60 kDa trehalase from *E. coli* (Sofia et al., 1994), which is confined to the cytoplasm, shows only 41% homology with its periplasmic counterpart, suggesting a fundamen-

tal difference in the functions and/or descent of cytoplasmic and secreted trehalases. Insect trehalases from *Tenebrio molitor* and *Bombyx mori*, comprise proteins of 65 and 70 kDa, respectively, each containing 5 potential *N*-glycosylation sites and a signal sequence for targeting to the rough endoplasmic reticulum (RER), but no indications for membrane-anchoring via GPI (Takiguchi et al., 1992; Su et al., 1993; Su et al., 1994). In fact, the *C*-terminal transient membrane-anchor of rabbit trehalase is not conserved in any of the known trehalase sequences (Figure 2). The overall amino acid sequence homology of the insect trehalases compared with rabbit trehalase is 43% and 44% for the *T. molitor* and the *B. mori* enzyme, respectively. Furthermore, two very closely related trehalases from yeast (*S. cerevisiae*) were cloned, showing a 77% identity at the amino acid level. They were significantly larger than the rabbit enzyme, about 80 kDa, containing an "extra" *C*-terminal sequence. The yeast enzymes constitute cytosolic proteins, without a signal sequence for RER targeting (Kopp et al., 1993; Kopp et al., 1994; Wolfe and Lohan, 1994). The homology between all seven trehalase sequences was found along the whole length of the trehalase proteins, strongly suggesting that these genes have evolved from a common ancestral gene. This homology is clustered in several regions of the proteins as indicated in Figure 2, suggesting that the active site as well as the major determinants of tertiary structure are confined to these regions. Generally, the nucleophile in the active site of glycosidases is formed by a carboxyl-group of an aspartate or glutamate residue (Semenza, 1987). There are 6 such residues conserved in all 7 trehalase sequences, while only 2 are part of larger stretches (10 to 15 amino acid residues) of homology between the trehalases (Figure 2). Presently, we can only suggest that one or both of these two conserved residues, i.e., E171 or D463 of the rabbit sequence, is/are critical for the catalytic mechanism of trehalase. The tertiary structure of rabbit trehalase may not be very well conserved among

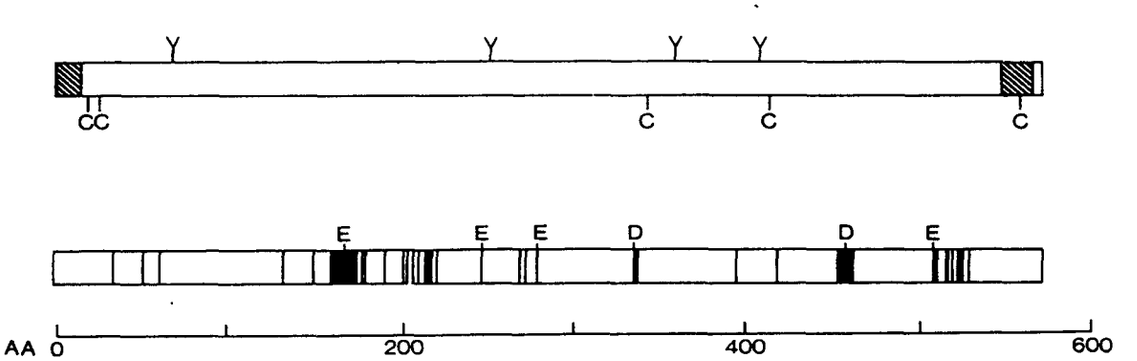

FIGURE 2. Primary structure of rabbit brush border trehalase. The upper bar depicts the sequence of rabbit trehalase (Ruf et al., 1990). Shown are the cysteine residues (C), the *N*-glycosylation sites (Y), and the hydrophobic sequences (cross-hatched). The *N*-terminal hybrophobic sequence functions as a cleavable signal-sequence for translocation into the RER, while the *C*-terminal sequence functions as a transient transmembrane sequence, which is replaced by a GPI-anchor after the translation of the polypeptide is complete (Discussed in a previous section; compare Figure 3). The middle bar indicates the conservation between the known trehalase sequences. Seven trehalase sequences were used in this comparison: the rabbit trehalase (Ruf et al., 1990), *E. coli* periplasmic trehalase *treA* (Gutierrez et al., 1989), cytoplasmic trehalase from *E. coli* (Sofia et al., 1994), insect trehalases from *Tenebrio molitor and Bombyx mori* (Takiguchi et al., 1992; Su et al., 1993; Su et al., 1994), and two trehalases from *S. cerevisiae* (Kopp et al., 1993; Kopp et al., 1994; Wolfe and Lohan, 1994). The sequences were extracted from GenBank, release 85.0 (1994), aligned and compared according to the algorithm of Feng and Doolittle (1987). Each black line indicates an absolutely conserved amino acid residue, and the conserved carboxylated residues, which are potentially important in the active site of the enzyme, are specifically indicated (D, E). The lower bar represents the scale of the picture in number of amino acid residues (AA).

trehalases. Two of the *N*-glycosylation sites are conserved, one in the *T. molitor* and one in the *B. mori* sequence, and none of the sites is conserved in both sequences. Two of the 5 *C*-residues are conserved in both the insect sequences (C28 and C34 of the rabbit sequence), while C354 of the rabbit sequence is only conserved in the *T. molitor* trehalase.

3. Biosynthesis

The biosynthesis of trehalase has not been described directly, but many aspects can be deduced from biochemical data (Figure 3). The rabbit trehalase sequence indicates that it contains a cleavable hydrophobic signal sequence for translocation into the RER (Ruf et al., 1990). Also the extreme *C*-terminus contains a hydrophobic sequence, which could function in membrane-anchoring. However, the available data indicate that the enzyme is linked to the membrane by a GPI-anchor (Figure 3). Rabbit trehalase could be released from brush border membranes as well as from *X. leavis* oocytes plasma membrane (after transfection of rabbit trehalase cDNA) by digestion with phosphatidylinositol-specific phospholipase C (Takesue et al., 1986; Ruf et al., 1990). As the apically expressed form of the enzyme is GPI-anchored to the brush border membrane, the association by the *C*-terminal hydrophobic region is most likely transient, and is replaced in the RER by the GPI-anchor (Ruf et al., 1990). The GPI-anchor is known to function as a very specific targeting signal for apical transport (Lisanti and Rodriguez-Boulan, 1989). Therefore, it may be assumed that trehalase is efficiently and vectorially transported to the apical brush border membrane, as has been demonstrated for the transport of the brush border enzyme, alkaline phosphatase in Caco-2 cells and enterocytes (LeBivic et al., 1990; Lisanti and Rodriguez-Boulan, 1989). The trehalases from yeast and insects do not possess a GPI-anchor: the two known yeast trehalases are cytosolic proteins (Kopp et al., 1993; Kopp et al., 1994; Wolfe and Lohan, 1994), and the insect enzyme isolated from *T. molitor* is most likely a soluble intestinal enzyme, while the *B. mori* trehalase is most likely localized at the basolateral membrane of many epithelial cells (Takiguchi et al., 1992; Su et al., 1993; Su et al., 1994). The mode of anchoring of the *B. mori* trehalase into the plasma membrane is presently unclear, as no transmembrane sequence was found in its sequence (Su et al., 1993; Su et al., 1994).

Rabbit trehalase has been shown to contain four potential *N*-glycosylation sites (Ruf et al., 1990). High recovery of trehalase on concanavalin A-Sepharose chromatography indicated that the *N*-linked glycans of pig kidney and rabbit small intestinal and renal trehalases were at least in part in the high mannose conformation (Morin and Potier, 1987; Yoneyama, 1987; Nakano and Sacktor, 1985; Yokota et al., 1986). Thus, the *N*-linked glycans of trehalase escape, to some extent complex glycosylation. Nothing is known about possible *O*-glycosylation of trehalase. In the rabbit trehalase sequence there is no evidence for an *O*-glycosylated "stalk", as was previously found for sucrase-isomaltase (Ruf et al., 1990; Section VII). The overall amount of glycosylation may be low, as only 2 mol glucosamine were found per mol of pig trehalase (Yoneyama, 1987). Apparently, trehalase does not form covalent oligomers, as the enzyme was isolated from all sources in monomeric form without reduction. As described above, non-covalent oligomerization, leading to homo-oligomers, may occur, as under some non-denaturing conditions larger aggregates, up to 330 kDa, can be isolated. Yokota and Takesue (1988) were able to identify brush border-bound hydrophobic trehalase from rabbit renal epithelium as well as soluble hydrophilic trehalase. They conclude that the latter may be due to limited autolysis in the brush border membrane; this may also be the source of free soluble trehalase in urine and in amniotic fluid, as described below in Section IV.C.

Trehalase differs from the other brush border glycohydrolases in a number of ways: 1. there is no internal homology within the enzyme, 2. it is a relatively small enzyme, 3. it contains a GPI- anchor instead of a permanent transmembrane sequence, 4. it displays a unique catalytic mechanism. Each of these features may have impact on its enzymatic functioning in the brush border, as discussed below.

B. Substrate Specificity and Hydrolytic Mechanism of Trehalase

Trehalase belongs to the group of intestinal brush border α-glycohydrolases. The only natural substrate for trehalase known today is trehalose. Trehalose is a non-reducing disaccharide, consisting of two glucose moieties (α-D-glucopyranosyl-α-D-glucopyranoside). This rather curious substrate requires an unusual enzyme with a reaction mechanism which is unique among the brush border α-glycohydrolases.

1. Trehalase Has One Catalytic Site

What is known about the catalytic properties of trehalase (from mammals as well as insects) indicates that there is one active site, which specifically hydrolyzes non-reducing α,α′-glycosidic bonds. Apart from trehalose only a few other substrates, none of which occur naturally, were found to be hydrolyzed by human as well as porcine trehalase. Among these substrates were: 1. two epimers of trehalose: α-D-allopyranosyl-α-D-glucopyranoside and α-D-galactopyranosyl-α–

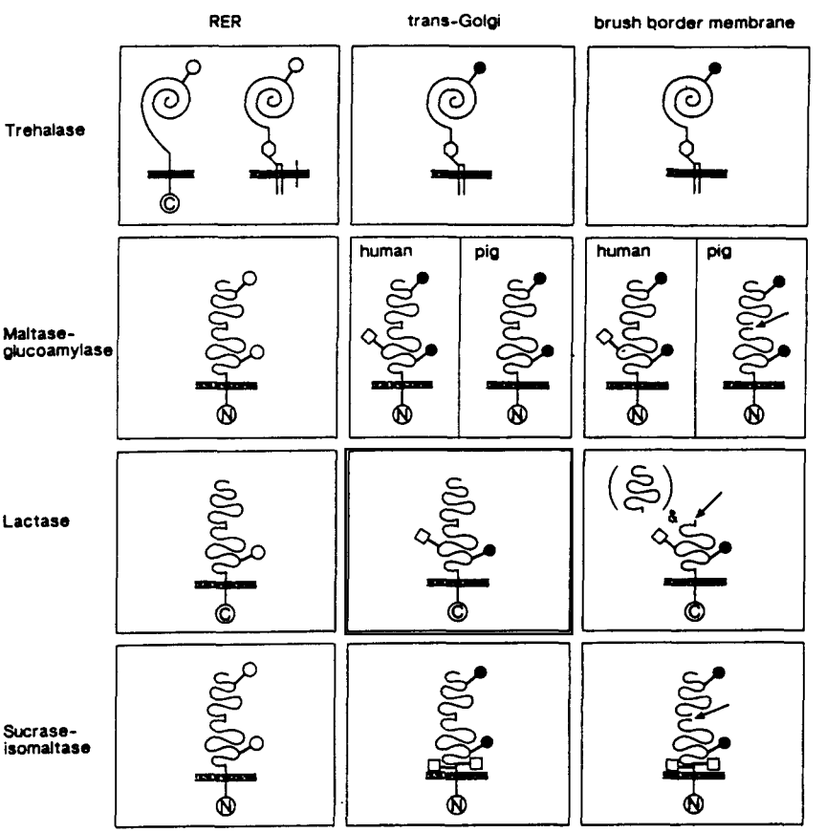

FIGURE 3. Biosynthesis of brush border glycohydrolases. This figure schematically summarizes the biosynthesis of the four known brush border glycohydrolases; more details can be found in Sections IV.A, V.A, VI.A, and VII.A. Indicated are the position of the *N*- or the *C*-terminus of the polypeptide (N/C), the GPI-anchor of trehalase (♀), high mannose (○) and complex *N*-glycosylation (●), *O*-glycosylation (□), proteolytic cleavage (↓), and membrane (=). The configuration of trehalase is deliberately depicted differently from the other three enzymes, to indicate the absence of structural analogy. Notice that maltase-glucoamylase, lactase, and sucrase-isomaltase are all proteolytically cleaved. After this cleavage, the subunits of maltase-glucoamylase and sucrase-isomaltase remain associated, while the *N*-terminal domain of lactase is intracellularly degraded, as indicated by the parentheses. The biosynthesis of maltase-glucoamylase shows two alternatives, as the human intestinal enzyme is not proteolytically cleaved, in contrast to the pig intestinal enzyme (Section V.A). Moreover, although not directly assessed, the biosynthesis of kidney maltase-glucoamylase may proceed very similar to human intestinal maltase-glucoamylase.

D-glucopyranoside, 2. one substrate in which the carbon-group at the 6′ position was omitted: α-D-glucopyranosyl-α-D-xylopyranoside, and 3. two deoxy-compounds: 6′-deoxy-α,α-trehalose and 6,6′-dideoxy-α,α-trehalose (Labat-Robert et al., 1979). Each of these compounds was hydrolyzed less efficiently than trehalose: K_m for trehalose = 5.88 m*M*, and for the other compounds K_m = 5.88 – 57 m*M*, while V_{max} of each compound was only 2 to 23% of the V_{max} for trehalose. The data show that the hydroxyl-groups of trehalase investigated (i.e., the OH-groups at carbon positions; 3, 4, 6, and 6′) as well as the HCOH-group at 6′ have a role in the interaction with the enzyme, but that none of these groups is essential for the hydrolytic mechanism.

The catalytic properties of mammalian trehalase are very similar to non-mammalian trehalases (Clifford, 1980; Hehre et al., 1982; Defaye et al., 1983). The most remarkable feature of the hydrolytic mechanism of trehalase is the inversion at the carbonyl carbon of the liberated glucose moiety: the α-glucose residue is liberated as β-D-glucose. This property is shared by insect as well as mammalian trehalase (Clifford, 1980). When either α- or β-*F*-D-glucopyranoside are offered as substrates, the glucone moiety is liberated as β- or α-D-glucose, respectively (Hehre et al., 1982; Nakano et al., 1989). The hydrolysis of α,α′-trehalose results in equimolar release of α- and β-glucose (Hehre et al., 1982). The other α- and β-glycohydrolases in the brush border liberate the glucone moieties without this conversion (Section VII.B).

2. Structure of the Catalytic Site

The active site of the 67 kDa rat intestinal trehalase was investigated in detail with kinetic studies, which showed that the active site of this enzyme can be functionally divided into a binding site and a catalytic site (Chen et al., 1987). Nakano and Sacktor (1984), working on rabbit renal trehalase, also defined two subsites at the substrate binding site. According to these authors, one site most likely contains a thiol-group, which can be non-competitively inhibited by *N*-ethylmaleimide, Hg^{2+}, and iodoacetate, while the other site involves a carboxyl-group which can be competitively inhibited by tris. Apart from thiol-blocking reagents and tris, trehalase can be fully and competitively inhibited by sucrose, phloretin, and phlorizin (Galand, 1984; Nakano and Sacktor, 1984; Chen et al., 1987). The mutually competitive nature of pairs of these inhibitors suggests that trehalase has two binding sites (Nakano and Sacktor, 1984; Chen et al., 1987). Tris, in non-protonated form, is known to reversibly inhibit all brush border glycohydrolases (Sections V.B, VI.B, and VII.B). Conversely, tris only inhibits trehalase in protonated form (Chen et al., 1987). The seeming involvement of a thiol-group at the catalytic site seems highly unlikely since no conserved cysteine residues were found in the sequences of rabbit trehalase and the other sequenced trehalases (Section IV.A). Thus, it remains uncertain whether a thiol-group actually participates in the catalysis. Moreover, conduritol-β-epoxide (CBE) a transition-state analogue of the other glycosidases (Sections V.B, VI.B, and VII.B), was found not to bind to trehalase, further suggesting a fundamental difference between trehalase and these other enzymes (Ruf et al., 1990). Consequently, CBE cannot be used as reagent to label the active site of trehalase, as was successfully performed on lactase (Section VI.B). Despite differences in the isoelectric point of intestinal and renal rabbit trehalase, there are no differences in kinetic properties, suggesting that the trehalase from these organs is very similar or identical and that the isoelectric point, inferred by different glycosylation, is irrelevant to its catalytic function (Morin and Potier, 1987).

C. Functions and Developmental Expression of Trehalase

1. Trehalase is Widespread Among Mammals

The developmental expression of trehalase in the brush border of enterocytes among various mammals is quite diverse, as reviewed by Galand (1989). In primates (i.e., man and baboon) trehalase seems to be present throughout life. In the mammal orders: Rodentia (e.g., rat), Lagomorpha (e.g., rabbit), Perissodactyla (e.g., horse), and Marsupials,

some trehalase is found neonatally, but the enzyme is strongly up-regulated in adulthood, while in the orders Pinnipedia (e.g., seals), and Fissipedia (e.g., cats) trehalase seems to be absent. These findings may direct our reflections on both the function and regulation of trehalase. The presence of the enzyme indicates trehalose ingestion, or at least the probability of trehalose ingestion. Thus, obligate carnivores (i.e., cats and seals) which customarily do not ingest trehalose, lack trehalase. Other mammals such as rat, rabbit and horse are likely to be exposed to trehalose only as adults, while primates are equipped to digest trehalose all their lives. There is no evidence that the expression of trehalase can be induced by its substrate. Thus, the three different patterns of expression of trehalase in these species (i.e., 1. life-long trehalase deficient, 2. up-regulation of trehalase after weaning, and 3. up-regulation of trehalase before birth) reflect basic differences in trehalase gene regulation.

2. Trehalase is Present in Intestine and in Kidney

The tissue distribution of trehalase in mammals is principally confined to the small intestinal enterocytes and the renal tubular epithelium. Ruf and co-workers (1990) demonstrated the expression of the rabbit trehalase mRNA in liver, but this still remains an unconfirmed observation. This tissue specific expression pattern was also observed for a number of other brush border enzymes, e.g., aminopeptidase N, aminopeptidase A, dipeptidylpeptidase IV, and γ–glutamyltransferase (reviewed by Semenza, 1986), and for maltase-glucoamylase (Section V). In elegant experiments, Riby and co-workers (1990) demonstrated that renal trehalase was enzymatically active against trehalose. They injected trehalose in the blood of rabbits and rats, and determined the amount of trehalose excreted in the urine. The clearance rate of trehalose from the plasma was similar in rabbit and rat. Rats, which do not express trehalase in the kidney, accumulated trehalose in the kidney within 10 min after injection. Rabbits, due to high levels of renal trehalase, show very low trehalose concentrations in urine, indicating that renal trehalase is very efficient in metabolizing trehalose in the glomerular filtrate. However, the physiological role of trehalase in renal brush border remains unclear as trehalose is not normally present in blood.

3. Genetic Regulation of Trehalase is Virtually Unexplored

Gene expression can be predicted to involve tissue specific transcription factors and promoter elements, as the enzyme is restricted to the epithelia of only two specific organs. The pig kidney epithelial cell line LLC-PK_1, showed a specific increase in trehalase expression, when cultured in the absence of glucose. This was not due to a metabolic effect, as glucose is not used as an energy source by these cells. Moreover, other brush border hydrolases were unaffected by glucose deprivation (Yoneyama and Lever, 1988). Trehalase expression in these cells could be strongly increased by incubation with the differentiation inducer, *N,N'*-hexamethylene-bis-acetamide. However, the effect of glucose deprivation was independent of the effect of this differentiation inducer, strongly suggesting that this form of gene regulation is specific for trehalase and is independent from differentiation (Yoneyama and Lever, 1988).

The spatiotemporal pattern of trehalase activity is genetically determined during ontogenesis (Section III). Hormones, particularly glucocorticoids, are able to trigger premature trehalase expression in rat, mouse and rabbit (Galand, 1989). However, glucocorticoids are not essential to reach adult trehalase levels, as the potent anti-glucocorticoid RU 38386 failed to inhibit the rise of α-glycosidases, including trehalase, in the developing rat (Galand, 1988).

4. Trehalase as a Disease Marker

Interestingly, trehalase is also found in amniotic fluid of rabbit and human. In fetal rabbits, this soluble form of trehalase was similar to detergent-solubilized trehalase from kidney as well as intestine, with respect to kinetic properties and molecular mass (Morin and Potier, 1987). As the

isoelectric point of the renal and intestinal enzymes differ, the origin of the rabbit amniotic fluid trehalase could be determined. The appearance and amount of trehalase in the amniotic fluid correlated strictly with the development of trehalase in the fetal kidney and intestine. Trehalase expression in kidney was detected much earlier in gestation than that in the intestine, and consequently trehalase of renal origin was detected earlier in the amniotic fluid than that from intestine (Morin and Potier, 1987). The trehalase in the amniotic fluid originates from the normal effluent of the intestine and the kidney into this fluid, via the urethra of the rabbit fetus. Thus, a lowered trehalase activity in the amniotic fluid could indicate fetal obstructions in the intestine or urethra, or other pathological situations affecting kidney or intestine. Trehalase was found to be a useful marker for cystic fibrosis, a disease leading to the obstruction of lumina of gastrointestinal, urogenital, and tracheobronchial organs, by aviscous mucus secretion. Amniotic fluid samples of human pregnancies, taken at 16 to18 weeks of gestation, contained about I U/mg amniotic fluid protein, while the trehalase level was very significantly lowered to about 0.1 U/mg amniotic fluid protein in fetuses, which were subsequently diagnosed to be affected by cystic fibrosis (Szabó et al., 1984; Szabó et al., 1985). Likewise, lowered trehalase activity in human amniotic fluid was also of high diagnostic value for congenital nephrotic syndrome (Morin et al., 1984).

5. Primary Trehalase Deficiency

Congenital trehalase deficiency is an autosomal recessive disorder that is found very rarely among adult white Americans and adult Danes, whereas it is prevalent in about 10% of Greenland Inuits (Welsh et al., 1978; Gudmand-Hoyeret al., 1988). Another study among normal, healthy adult Swiss revealed two cases of trehalase deficiency in intestinal biopsies (Bergoz et al., 1982). As these two individuals displayed otherwise normal levels of the other brush border glycohydrolases, it is most likely that they expressed a congenital defect. Trehalase deficiency may be far more frequent than it appears from the literature, as very few laboratories carry out routine trehalase assays. Moreover, the resulting trehalose intolerance symptoms may remain subclinical as trehalose-containing foods (mushrooms) are normally only a minor part of the diet. It is presently unclear whether congenital trehalase deficiency is due to lowered gene expression, or to the expression of an inactive enzyme.

V. MALTASE-GLUCOAMYLASE

A. Structure and Biosynthesis of Maltase-Glucoamylase

1. Maltase is a Confusing Term with Respect to Intestinal Brush Border Hydrolases

The small intestinal brush border is known to accommodate at least four separate maltase activities. Two of these maltase activities can be assigned to the sucrase-isomaltase enzyme complex, as will be described in Section VII. The remaining two activities can be attributed to a second enzyme complex: maltase-glucoamylase, which constitutes about 2% of brush border membrane proteins (Galand, 1989). The name "maltase" is thus somewhat misleading, as there are four intestinal maltases. Moreover, the double name "maltase-glucoamylase" suggests that these are two different enzymes, while it will appear from this section that the two subunits of this enzyme complex display nearly identical enzyme activities. Unfortunately, this hampers the interpretation of many papers which have measured "maltase" activity in tissue. By using maltose as a substrate, results refer to the sum of maltase activities, and it can often not be deduced from the measurements which "maltase" is actually assayed. Conversely, the glucoamylase activity, i.e., the liberation of molecular glucose from amylose and/or amylopectin, is unique to this enzyme complex (Section V.B). Thus, *in vitro* studies measuring glucose production from any form of starch, in absence of salivary and pancreatic α-amylase, are a good measure of maltase-glucoamylase activity. Interestingly, in addition to its presence in the intestine, this enzyme com-

plex has also been detected in the renal tubular brush border membrane, as described for trehalase.

2. Maltase-Glucoamylase is Similar in Structure to Sucrase-Isomaltase

Maltase-glucoamylase has been isolated from the intestinal and/or renal brush border of human, horse, rabbit, pig, rat, mouse, and chicken (Norén et al., 1986; Kerjaschki et al., 1984; Skovbjerg and Krasilnikoff, 1986; Simon et al., 1979; Naim et al., 1988b; Quezada-Calvillo et al., 1993; Pereira and Sivakami, 1991; Hu et al., 1987; Hauri et al., 1985; Giudicelli et al., 1985). Like most other brush border glycohydrolases it can be released by mild papain digestion from isolated brush border membranes. Moreover, it can be isolated by detergent solubilization, indicating that this is a membrane-bound complex. This membrane association was further demonstrated for chicken intestinal maltase-glucoamylase by labeling with the hydrophobic labeling reagent 3-(trifluoromethyl)-3-(m[^{125}I]-iodophenyl) diazirine ([^{125}I]TID) Hu et al., 1987). Native maltase-glucoamylase is most often purified under non-denaturing conditions from brush border preparations followed by papain digestion or Triton X-100 solubilization. Most protocols use affinity chromatography on Sephadex G-200 or tris-coupled Sepharose C1-6B (Periera and Sivakami, 1991; Hu et al., 1987; Galand, 1986; Kerjaschki et al., 1984). It is generally thought that this α-D-glucan-hydrolyzing enzyme has affinity for agarose, the main constituent of Sepharose and Sephadex. Also, immunoadsorbent chromatography has been developed to isolate native maltase-glucoamylase complex from pig and man (Sorensen et al., 1982; Naim et al., 1988b). There are persistent discrepancies in the molecular masses between maltase-glucoamylase isolated from different species and organs. Denatured enzyme preparations from pig, rat, rabbit, and chicken intestine contain three forms of the glycoprotein: a minor form of about 245 kDa, and two more abundant forms of 125 to 140 kDa (Sorensen et al., 1982; Quaroni and Isselbacher, 1985; Hu et al., 1987). The two smaller glycoproteins form the subunits of an approximately 250 kDa heterodimer, while the larger glycoprotein is most likely the biosynthetic precursor of these two subunits, as is described below. In contrast, human small intestinal maltase-glucoamylase is not only far larger, i.e., 335 to 355 kDa, than the enzyme for the other species, but also this brush border enzyme exists solely as a single molecular form (Hauri et al., 1985; Naim et al., 1988b). Interestingly, renal maltase-glucoamylase isolated from horse, rat, and human were found to have molecular masses of about 335 kDa, similar to the human intestinal enzyme complex (Reiss and Sacktor, 1981; Giudicelli et al., 1985; Kerjaschki et al., 1984; De Burlet et al., 1979).

By virtue of its papain susceptibility, maltase-glucoamylase can be classified as a "stalked" enzyme, implying that there is an elongated part in the structure of the enzyme, which is proteinase-sensitive and projects the bulk of the enzyme, containing the active site, above the luminal surface of the apical brush border membrane (Kenny et al., 1983). This stalked structure was demonstrated for pig maltase-glucoamylase by electron microscopy, visualizing the monomeric enzyme as a dumbbell-shaped structure of about 18 × 6 nm, attached to the membrane by a thin filamentous region of about 2.5 nm (Norén et al., 1986). The enzyme complex was found to be associated to the apical plasma membrane by an *N*-terminal hydrophobic transmembrane sequence in the proteolytically unprocessed (245 kDa) form (Norén et al., 1986). As was demonstrated for sucrase-isomaltase (Section VII.A), this likely constitutes an uncleaved RER targeting signal sequence, which in addition serves as an *N*-terminal membrane-anchor, rendering a type II transmembrane protein. The *N*-terminus of pig intestinal maltase-glucoamylase is homologous to the *N*-terminus of pig, rabbit, as well as human sucrase-isomaltase (45, 50, and 50% identical amino acids, respectively, of the first 25 amino acids of these polypeptides). However, this sequence, which serves both as RER targeting signal and membrane-anchor, is conserved in a large number of transmembrane proteins from all phyla (Polypeptide sequence database search, GenBank release 85.0, 1994). Therefore, this amino acid sequence likely constitutes a "transmembrane-box" adopted by many proteins during evolution. Nevertheless, all evi-

dence suggests that maltase-glucoamylase has one membrane-spanning domain at the *N*-terminus of the polypeptide.

3. Biosynthesis Differs Among Mammals

The biosynthesis of pig and human intestinal maltase-glucoamylase was assessed directly in metabolic pulse-chase experiments (Danielsen et al., 1984a; Danielsen and Cowell, 1984; Naim et al., 1988b). Pig maltase-glucoamylase is synthesized as a 225 kDa high mannose *N*-glycosylated precursor, which over time is converted to a 245 kDa complexly *N*-glycosylated precursor (Figure 3). Enzymatic deglycosylation of these precursor forms leaves a 200 kDa polypeptide, which likely represents the primary translation product. The complexly *N*-glycosylated maltase glucoamylase precursor was found the be sulfated on tyrosine residues, as demonstrated in metabolic [^{35}S]sulfate labeling of pig small intestinal explants (Danielsen, 1987). The 245 kDa form arrives at the apical brush border membrane of the pig small intestine, and is subsequently proteolytically cleaved into a 125 and a 135 kDa subunit by pancreatic enzymes (Sorensen et al., 1982, Danielsen et al., 1982). In contrast, Naim et al. (1988b) convincingly showed that human small intestinal maltase-glucoamylase is synthesized as a 285 kDa, high mannose *N*-glycan containing precursor, which is converted into a 335 kDa complexly *N*-glycosylated brush border form. Enzymatic removal of *N*-linked glycans from either form of the enzyme reveals a polypeptide chain of 255 kDa. The 335 kDa form, which contains about 35% *O*-linked oligosaccharides, is not cleaved by either endogenous enterocyte — or exogenous pancreatic proteases, as are lactase or sucrase-isomaltase, respectively (Naim et al., 1988b, Sections VI.A. and VII.A). In non-human species, the 125 and 135 kDa subunits of intestinal maltase-glucoamylase remain non-covalently attached, as these can be isolated together under non-denaturing conditions (Galand, 1986; Sorensen et al., 1982; Hu et al., 1987). It has been demonstrated by electron microscopy of the pig intestinal enzyme, that these heterodimers dimerize further to form homohetero-tetramers of the $\alpha_2\beta_2$-type (Norén et al., 1986). These homodimers of pig intestinal maltase-glucoamylase are formed in the RER during biosynthesis, and these dimers can only be isolated by low salt condition, indicating that the interactions between the monomers is generally weak (Danielsen, 1994). In further contrast, Naim and co-workers (1988b), using a bifunctional cross-linking reagent, were unable to show dimerization of the human 335 kDa brush border form of the intestinal enzyme complex. In the kidney, in the absence of soluble proteinases, the pro-maltase-glucoamylase is not cleaved in any species studied, although the enzyme complex is seemingly fully active, as indicated in the next section.

The intracellular targeting of maltase-glucoamylase has not been assessed directly. However, all studies of the biosynthesis and localization of the enzyme complex suggest that virtually all mature enzyme is present in the brush border membrane of the intestine as well as in the kidney (Naim et al., 1988b; Norén et al., 1986; Kerjaschki et al., 1984; Hauri et al., 1985; Hu et al., 1987). There are no indications that maltase-glucoamylase is a GPI-anchored enzyme like trehalase. The presumed cytoplasmic (*N*-terminal) segment of the polypeptide is very small, i.e., 12 amino acids (Norén et al., 1986). It seems unlikely that the signal for apical sorting is confined to this region. Like in sucrase-isomaltase, the signal for apical targeting may be found in the luminal bulk of the protein: speculations are that the sulfated tyrosine could serve as an intracellular targeting signal.

4. Thus Far No Maltase-Glucoamylase Sequences

A meticulous search of all available databases has revealed that no mammalian maltase-glucoamylase has been cloned and sequenced, which severely hampers conclusions about the supposed uniformity in this group of enzymes. In analogy with sucrase-isomaltase, which has been sequenced completely in human, rat and rabbit, and shows a biosynthetic pattern similar to pig intestinal maltase-glucoamylase (Section VII.A), it is generally assumed that each of the individual

intestinal 125 and 135 kDa subunits of maltase-glucoamylase contains an active site. If the renal forms of this enzyme, which are not cleaved, are products of the same gene as expressed in the intestine (that are proteolytically cleaved), then the assumption would be that this approximately 335 kDa form also contains these two active sites. To our knowledge, there are no studies performed to illustrate the identity of both intestinal and renal forms of maltase-glucoamylase from the same species. Therefore, it remains to be demonstrated if the renal and intestinal forms are indeed products of the same gene. Biochemical evidence, i.e., molecular masses and enzymatic characteristics, thus far suggests that intestinal and renal maltase-glucoamylase from rabbit are identical (Pereira and Sivakami, 1991; Galand, 1986). The human intestinal maltase-glucoamylase, which displays a different molecular mass and is not proteolytically cleaved, may be divergent from, or not homologous to, the enzyme of the other species. The intestinal maltase-glucoamylases of other species, such as pig, rat, rabbit, and chicken, appear to form a more homogeneous group, which may reflect a higher homology at the amino acid level. Particularly, the close resemblance between mammalian and avian forms of the enzyme complex with respect to molecular mass, enzymatic properties, and biosynthetic processing suggests a common ancestral gene.

B. Substrate Specificity and Hydrolytic Mechanism of Maltase-Glucoamylase

The maltase-glucoamylase complex accounts for about 20% of the maltase activity in the small intestine, it displays a minor isomaltase activity, and exhibits all of the intestinal glucoamylase activity (i.e., it liberates α-D-glucose from starch) (Semenza, 1986; Semenza and Auricchio, 1989). Maltase-glucoamylase in the renal epithelium is also responsible for all of the maltase, isomaltase, and glucoamylase activities in the kidney (Kerjaschki et al., 1984, Galand, 1986; Reiss and Sacktor, 1981). The maltase-glucoamylase most likely contains two active sites with similar hydrolytic characteristics. The activity of this enzyme complex can be distinguished from the activities of the sucrase-isomaltase complex by the higher stability towards heat and by its somewhat different enzymatic properties (Quezada-Calvillo et al., 1993; Semenza, 1986). Studies of substrate specificities performed by Sorensen et al. (1982) and Taravel et al. (1983) on the intact enzyme complex isolated from pig intestine, without further distinction of the two active sites, can be summarized as follows: 1. α(1-4) glucosidic bonds are most efficiently hydrolyzed: maltose, and longer α(1-4) glucosidically bound glucans up to maltohexose, are the most favored substrates, 2. α(1-6) glucosidic bonds are hydrolyzed to some extent, isomaltose is hydrolyzed at about 2% of the rate of maltose, while indications are that these bonds are more readily hydrolyzed when they are part of larger substrates, 3. there are some activities towards non-natural dietary sugars such as kojibiose, nigerose, and turanose, which contain α(1-2) and α(1-3) glycosidic bonds, 4. the enzyme will not hydrolyze: sucrose (the hydrolysis of which is characteristic for sucrase-isomaltase; Section VII.B), aryl-α-glucopyranosides, and β(1-4) and β(1-6) glycosydic bonds. Most distinctively, maltase-glucoamylase hydrolyzes far larger and complex glucan structures than sucrase-isomaltase, including amylopectin, glycogen, amylose, and α-limit dextrins. The latter, together with vast amounts of maltose and maltotriose, arise from the exhaustive digestion of amylopectin (or glycogen) by pancreatic α-amylase, and consist of a group of glucans containing α(1-4) glucosidically bound D-glucose units with at least one α(1-6) glucosidic branch-point. These α-limit dextrins are far better substrates for maltase-glucoamylase than for sucrase-isomaltase.

1. Proteolytic Processing Not Important for Function

The proteolytical processing of maltase-glucoamylase seems inconsequential to its enzymatic activity. In the kidney of several species studied, soluble proteinases are absent and therefore pro-maltase-glucoamylase exists in an uncleaved form which is nevertheless fully active (Pereira and Sivakami, 1991; Reiss and Sacktor,

1981; De Burlet et al., 1979; Giudicelli et al., 1985; Kerjaschki et al., 1984). The human intestinal maltase-glucoamylase is fully functional, i.e., displays maltase as well as glucoamylase activities, in absence of proteolytic cleavage (Naim et al., 1988b; Hauri et al., 1985). Norén and coworkers (1986) isolated from pig intestine both the single chain promaltase-glucoamylase (245 kDa) as well as the proteolytically processed two chain (125 and 135 kDa) forms, and demonstrated that these forms had identical catalytic properties, with respect to the K_m for maltose and amylopectin, inactivation by CBE, and pH optimum. Moreover, the active sites of rabbit renal and intestinal maltase-glucoamylase were shown to be very similar biochemically, suggesting that they constitute the same enzyme expressed in these two different tissues (Pereira and Sivakami, 1991).

2. Active Site Architecture

The two active sites present in the maltase-glucoamylase are very difficult to distinguish experimentally. Both sites appear equally sensitive to inactivation by CBE, as inactivation of the pig intestinal enzyme complex follows a first order reaction (Norén et al., 1986). However, the same study showed that heat-inactivation at 55°C rapidly inactivated one active site, while a second site, responsible for 50% of the maltase and 30% of the glucoamylase activity, was not inactivated. As for most brush border glycohydrolases, tris is an effective inhibitor (Galand, 1986). The K_m for maltose hydrolysis is in the order of 1 to 5 m*M* for the pig, mouse, and rabbit intestinal enzyme, with a broad pH optimum of 6 to 7, and the hydrolysis follows Michaelis-Menten kinetics (Galand, 1986; Galand, 1989; Sorensen et al., 1982; Quezada-Calvillo et al., 1993; Taravel et al., 1983). Recently, Heymann and Gunther (1994) confirmed these data, and showed that the substrates maltose and maltose-oligomers up to maltoheptose were hydrolyzed following simple Michaelis-Menten kinetics, with K_m values between 0.40 and 2.10 m*M*. Simple Michaelis-Menten kinetics for this supposedly two-active site enzyme complex indicates that these sites are very similar. Thus, the sites would work completely parallel, as was first suggested by Norén et al. (1986) instead of sequential.

With respect to catalytic mechanism and architecture of the active site, maltase-glucoamylase may be very similar to sucrase-isomaltase (Semenza, 1987; Semenza and Auricchio, 1989). As maltase-glucoamylase hydrolyses linear arrays of α(1-4)-linked glucose residues, it should have multiple recognition subsites for glucose residues. Heymann and Gunther (1994) calculated the subsite affinities for glucose moieties in subsequently longer substates (maltose up to maltoheptose). The affinities (kJ/mol) of subsites 1-7 of maltase-glucoamylase were: 7.12, 18.30, 4.19, 0.94, –0.55, –1.17, and –0.58, respectively. As the active sites hydrolyze the bond between the reducing and the penultimate glucose residue, the affinities for the various subsites indicate that maltose, maltotriose, and maltotetrose are increasingly better substrates, while longer substrates become increasingly poor substrates. Among these substrates only maltotriose showed profound substrate inhibition of the enzyme complex, indicating that the binding of this substrate induced a conformational change in the enzyme (Heymann and Gunther, 1994). Little is known about the reaction mechanism of maltase-glucoamylase. Section VII.B contains a general description of the catalytic mechanisms of the glycohydrolases.

C. Functions and Developmental Expression of Maltase-Glucoamylase

1. Maltase-Glucoamylase May Be Present in All Vertebrates

Maltase-glucoamylase, as trehalase, seems specifically expressed in the brush border of small intestinal and renal epithelium; however very little is known about its tissue-specific gene regulation. A number of developmental studies have measured the maltase content of tissue, without a clear distinction between the contribution of sucrase-isomaltase and maltase-glucoamylase to this enzyme activity. The data compiled by Galand (1989) on intestinal glycohydrolases, comprising 18 species from 8 orders of mammals, show that

about half of these species do not express sucrase as newborns or adults. By assuming that the sucrase-isomaltase complex is absent in these species, we can deduce the activity of maltase-glucoamylase. In mouse, rat, and rabbit the maltase-glucoamylase accounts for about 70% of the maltase activity, in contrast with other mammals (Quezada-Calvillo et al., 1993; Galand, 1989). Thus, in these mammals the maltase activity correlates rather well with the maltase-glucoamylase complex, rather than sucrase-isomaltase. The glucoamylase activity, which is the proper measure for the maltase-glucoamylase complex (i.e., the ability to liberate glucose from starch in absence of pancreatic α-amylase) was not tested separately in other mammals. The ruminants among the Artiodactyla (cow and sheep), Marsupials, and Pinnipedia (seals) have no sucrase activity, but have some maltase-glucoamylase activity. Rodents (rat and mouse), Lagomorphs (rabbit), and Perissodactyla (horse) have no sucrase-isomaltase at birth, but already possess high levels of maltase-glucoamylase. Primates and Fissipeda (dog and cat) possess high levels of sucrase and isomaltase, as well as maltase activity throughout life. Interestingly, maltase activity was detected in all mammals examined as newborns as well as adults. Moreover, the maltase-glucoamylase complex was also demonstrated in chicken, pigeon, and in the frog *Rana esculenta* (Galand, 1989). Thus, maltase-glycoamylase could be regarded as an ancient enzyme in the phylogenetic sense, as it is the only glycohydrolytic enzyme present in all vertebrates examined.

2. Gene Regulation Is Different From The Other Brush Border Glycohydrolases

In many respects, among which are its enzymatic properties, maltase-glucoamylase is very similar to sucrase-isomaltase. However, the maltase-glucoamylase tissue specific gene regulation is different from sucrase-isomaltase as well as from lactase and trehalase. In most mammals, maltase-glucoamylase is present much earlier in development than sucrase-isomaltase (Galand, 1989), clearly indicating different regulation of these genes with respect to timing of expression. In human colon, sucrase-isomaltase is frequently expressed in normal as well as in malignant epithelium, while maltase-glucoamylase is virtually absent from the colonic epithelium at all times (Real et al., 1992). Furthermore, in the Caco-2 cell line, sucrase-isomaltase and lactase were expressed, while maltase-glucoamylase is undetectable (Hauri et al., 1985), indicating that the genes encoding these enzyme complexes are not regulated by identical mechanisms. The expression of maltase-glucoamylase in the kidney and intestine further suggests that the tissue specific gene regulation is fundamentally different from that of sucrase-isomaltase and lactase, which are confined to the intestine. The gene regulation of maltase-glucoamylase may resemble the regulation of trehalase, which shows the same tissue distribution.

Seven kidney cell lines of four species were shown to express both trehalase and maltase-glucoamylase (Robinson and Goochee, 1991). Oncogene transfection generated several of the human kidney cell lines without loss of trehalase or maltase-glucoamylase expression. These different kidney cell lines all responded to one or more differentiation inducing agents, such as adenosine, *n*-butyrate, *N,N'*-dimethylformamide,and hexamethylene bisacetamide, with specific up- or down-regulation of kidney-specific gene products (Robinson and Goochee, 1991). Interestingly, trehalase and maltase-glucoamylase responded differently to these differentiation inducing agents. Only hexamethylene bisacetamide was capable of evoking elevated levels of both the enzymes in LLC-PK1 porcine kidney cells. All other agents had effects on maltase-glucoamylase expression, without affecting trehalase levels. This strongly indicates that the maltase-glucoamylase and trehalase genes in these cell lines, originating from 4 different species, are regulated by different mechanisms in response to *in vitro* differentiation stimuli.

Although the basal expression pattern of maltase-glucoamylase is genetically imprinted (Section II), decreased starch intake has been shown to lead to a rapid decrease in maltase-glucoamylase activity, along with decreases in

other brush border glycohydrolase activities, but not in peptidase activities (Goda et al., 1983). Conversely, increased carbohydrate intake evokes increases in rat intestinal sucrase and glucoamylase activities and a modest rise in lactase activity (Bustamente et al., 1986). This indicates a general regulation mechanism, affecting the gene regulation of maltase-glucoamylase as well as of lactase and sucrase-isomaltase. Whether this regulation operates at the transcriptional level remains to be elucidated.

Like the other brush border glycohydrolases, maltase-glucoamylase can be upregulated during early development by glucocorticoids. Hydrocortisone markedly increases expression of the enzyme complex in 14 d old rabbits, while the renal enzyme complex is unaffected by this treatment. Adult levels of intestinal maltase-glucoamylase are not affected by glucocorticoids (Galand, 1986). The pattern of activity is genetically determined during ontogenesis, and hormones are only able to trigger premature maltase-glucohydrolase expression in rat, mouse, and rabbit (Galand, 1989). However, glucocorticoids are not essential for producing the adult enzyme levels, as the potent anti-glucocorticoid RU 38386 failed to inhibit the spontaneous rise of all brush border α-glycosidases, including maltase-glucoamylase, in the developing rat (Galand, 1988). During development of the mouse, maltase-glucoamylase expression was upregulated concomitantly with the development of intestinal microvilli, and is detected earlier than lactase: 16 d versus 18 d of gestation (Calvert et al., 1981). Like lactase in the rat (Rings et al., 1992b), the enzyme complex appears in a proximal-to-distal abundancy gradient in the intestine. In all studies, intestinal maltase-glucoamylase is confined to the mature villus enterocytes (Quaroni and Isselbacher, 1985; Hauri et al., 1985).

3. Primary Maltase-Glucoamylase Deficiency

Recently, the first indications were found for primary maltase-glucoamylase deficiency in humans (Lebenthal et al., 1994). Screening of 511 children with chronic diarrhea revealed that 15 individuals had a glucoamylase deficiency. Several patients were defined as secondary deficiencies, as they showed significant mucosal damage as well as decreased activity of the other brush border glycohydrolases. Seven of these patients had a normal intestinal morphology (including normal lactase, sucrase, maltase, and isomaltase activities) as well as a normal pancreatic α-amylase output. Four of them showed low blood concentrations of glucose after ingestion of starch or short glucose polymers, directly indicating the absence of glucoamylase activity in the gut. And all these seven patients responded to a starch elimination diet, further suggesting the specific absence of maltase-glucoamylase. From this study it was not possible to calculate the prevalence of this deficiency. Most cases of congenital maltase-glucoamylase deficiency could pass unnoticed as the role of maltase-glucoamylase may be subsumed by sucrase-isomaltase which is more abundant. Furthermore, the deficiency might not create symptoms. Maltase-glucoamylase deficiency seems to be very rare as revealed by studies of Skovbjerg and co-workers of large numbers of biopsies of healthy Danes and Greenlanders (Skovbjerg and Krasilnikov, 1986). In isolated congenital sucrase-isomaltase-deficient patients, abnormally low maltase-glucoamylase was measured; however, it would be far too speculative to suggest a relationship between congenital sucrase-isomaltase deficiency and maltase-glucoamylase deficiency.

VI. LACTASE

A. Structure and Biosynthesis Of Lactase

In the mammalian intestine, lactase (EC 3.2.1.23/45/46/62) is the most important glycohydrolase during early post-natal life. In fact, milk is the sole nutrient during the suckling period of all mammals, and lactose is the predominant carbohydrate ingested. The enzymatic hydrolysis of ingested lactose into equimolar amounts of glucose and galactose prior to absorption is mainly attributed to the action of small intestinal lactase. Absence, or low levels, of expression is

associated with symptoms of lactose intolerance (Section IX). Apart from its enzymatic specificity toward lactose (β-D-galactoside galactohydrolase, EC 3.2.1.23), lactase has also catalytic activities towards hydrophobic substrates, such as glycosylceramides (EC 3.2.1.45-46) and phlorizin (EC 3.2.1.62). Classically, this enzyme complex carried a double name, lactase-phlorizin hydrolase, suggesting that there would be a lactose as well as a phlorizin hydrolyzing subunit. In fact, the mature lactase has two active sites but consists of only one polypeptide. Recently, we demonstrated that there is most likely one major active site, which is capable of hydrolyzing both lactose as well as hydrophobic substrates, and a minor active site with residual activity towards hydrophobic substrates (Section VI.B). We will refer to the enzyme as lactase.

1. Isolation

Lactase can be isolated from intestinal brush border vesicles from most mammals of any age, but the enzyme is particularly abundant in the intestine of suckling animals. Classically, lactase was isolated chromatographically, analogous to other glycohydrolases (Sections IV.A and V.A). However, since development of specific antibodies, immunochemical isolation has been general practice. Thus, lactase has been isolated from rat, rabbit, human, and pig (Büller et al., 1987; Naim et al., 1987; Skovbjerg et al., 1981; Wacker et al., 1992). These preparations invariably consist of at least two components: a minor (sometimes two) polypeptides of 200 to 240 kDa, and a predominant polypeptide of 130 to 160 kDa. Both these forms are tightly membrane-associated, and can only be liberated by detergents or mild proteinase treatment (Colombo et al., 1973; Tsuboi et al., 1979; Skovbjerg et al., 1981). From biosynthetic studies, as described below, it was inferred that the larger form represents a precursor of lactase, while the smaller form constitutes mature lactase. Hydrophobic photolabeling of rabbit lactase with [^{125}I]TID showed that there was one membrane spanning domain in the *C*-terminus of the enzyme, and that the orientation was of a type I transmembrane glycoprotein (Wacker et al., 1992). There are indications that mature rat lactase exists as a non-covalent dimer in the brush border membrane (Danielsen, 1990; Danielsen, 1994; Naim et al. 1987; Dudley et al., 1993).

2. Biosynthesis

Lactase biosynthesis has been described using intestinal explants from pig, rat, rabbit, and human (Skovbjerg et al., 1984; Danielsen et al., 1984b; Büller et al., 1987; Rossi et al., 1992; Rossi et al., 1993a; Rossi et al., 1993b; Villa et al., 1993; Naim et al., 1987; Sterchi et al., 1990; Villa et al., 1992; summarized in Figure 3). Moreover, endogenous lactase biosynthesis was described in human Caco-2 cells, and in COS and MDCK cells after transfection of the full-length human lactase cDNA (Naim et al., 1991a; Hauri et al. 1985; Van Beers et al., 1995; Grünberg et al., 1992; Jacob et al., 1994). In all studies, metabolically labeled lactase occurs as a 200 to 220 kDa precursor containing high mannose *N*-glycosylation. This pro-lactase is folded into its three-dimensional configuration within the RER and subsequently transported to the Golgi apparatus, where the *N*-linked glycans are converted to complex forms. Commonly, this complexly *N*-glycosylated prolactase becomes also *O*-glycosylated to some extent, leading within 1 h to a pro-lactase form with a slightly greater molecular mass on SDS-PAGE of 225 to 240 kDa. During, or shortly after, the transport of the pro-lactase to the brush border membrane, the precursor is proteolytically cleaved into its mature form of 130 to 160 kDa and a propeptide, respectively. Proteolytic processing of lactase occurs after passage through the Golgi, but primarily before insertion into the plasma membrane. Proteolytic processing of lactase in explants from pig, human, and rabbit, as well as human lactase expressed in MDCK cells, occurs after passage through the Golgi, but primarily before insertion into the plasma membrane (Skovbjerg et al., 1984; Danielsen et al., 1984; Lottaz et al., 1992; Villa et al., 1993; Grünberg et al., 1992). An intrinsic proteinase of the enterocyte is responsible for this processing, as it occurs *in vivo* in complete absence of luminal proteinases (after ligation of the pancreatic duct),

or in absence of proteinases in the media of tissue or cell cultures (Hauri et al., 1985; Danielsen et al., 1981; Büller et al., 1987; Rossi et al., 1992; Naim et al., 1987; Sterchi et al., 1990; Villa et al., 1992). The responsible proteinase seems to be specific for epithelial cells, as the processing occurs after transfection of lactase cDNA to the epithelial cell line MDCK, but not after transfection to non-epithelial COS cells (Naim et al., 1991a; Jacob et al., 1994; Grünberg et al., 1992; Neele et al., 1994). Nevertheless, some complexly *N*-glycosylated precursor can be found in the brush border membranes of intestinal enterocytes, which has escaped intracellular processing. As eventually all pro-lactase is cleaved, the processing into mature lactase may occur on the brush border membrane, through the action of luminal proteinases or by the presence of intrinsic brush border proteinases. However, it appeared that proteolytical processing is not essential for brush border expression, nor for specific activity (Naim et al., 1991a; Grünberg et al., 1992; Neele et al., 1994).

3. The Function of the Pro-Peptide

In rat, the processing of the 220 kDa complexly *N*-glycosylated lactase precursor proceeds via a two-step mechanism, first into a 180 kDa form and subsequently into a 130 kDa mature form. All three forms (i.e., 220, 180, and 130 kDa) of rat lactase can be found in the brush border membrane in metabolic pulse-chase experiments, but the predominance of the mature form and the absence of proteinases in the culture medium strongly suggests that both proteolytic processing steps proceed via intrinsic proteinases (Büller et al., 1987). The existence of the 180 kDa intermediate form of rat lactase was more recently confirmed by Dudley et al. (1992). This two-step processing was also found for rabbit lactase, which is synthesized as a high mannose pro-lactase of 200 kDa. After conversion into a 215 to 245 kDa form due to complex *N*-glycosylation, proteolytic processing occurred via an intermediary 180 kDa form, before final processing into the 150 kDa mature brush border enzyme (Rossi et al., 1992; Rossi et al. 1993a; Rossi et al., 1993b; Villa et al., 1993).

The function of the approximately 100 kDa pro-peptide, cleaved from the lactase precursor in either one or two steps, has recently been elucidated. Until recently, this proteolytic product could not be detected in any preparation of lactase or in any pulse-chase experiment, indicating that this pro-peptide had no strong interaction with the mature polypeptide. Naim and co-workers (1994) demonstrated, in elegant metabolic labeling experiments, that this pro-peptide plays a crucial role in the initial folding of human pro-lactase in the RER. Using a polyclonal antiserum recognizing the *N*-terminus of human lactase, these authors were able to demonstrate that the pro-peptide: 1. has an apparent molecular mass of 100 kDa, 2. is not associated with mature (160 kDa) lactase after cleavage, 3. is not transported to the cell surface or secreted into the medium, 4. despite the presence of 5 potential *N*-glycosylation sites is neither *N*- nor *O*-glycosylated, and 5. is rapidly intracellularly degraded. Transfection of a truncated lactase sequence, representing the mature lactase, into COS cells led to an improperly folded and rapidly degraded form of lactase, suggesting that the pro-peptide functions as an intramolecular chaperone during folding of lactase (Naim et al., 1994; Oberholzer et al., 1993).

4. Functions of N- and O-Glycosylation

Lactase contains about 10% *N*-glycans, and addition and presence of these glycans on pro-lactase appears important for proper folding and exit from the RER. Inhibition of *N*-glycan modification in metabolically labeled human biopsies, using deoxymannojirimycin, deoxynojirimycin, or swainsonine showed that *N*-glycan processing is important for transport of lactase from the RER to the Golgi complex, but not from the Golgi complex to the plasma membrane (Naim, 1994). Moreover, in absence of *N*-glycosylation, inhibited by tunicamycin, human intestinal biopsies failed to produced any mature lactase (Naim et al., 1987). Differences in mobility on SDS-PAGE of lactase, during development and between different regions of the intestine, were attributed to small differences in structure of the glycans (Srivastava et al., 1987). Terminal fucose is only detected in rat lactase after 20 d, while there is a gradual decrease in sialic acid residues towards adulthood (Büller et al., 1990b). Small differences in mobility between intestinal and colonic lactase

in newborn rats were due to differences in processing of the complexly *N*-linked glycans (Büller et al., 1989a; Büller et al., 1989b; Büller et al., 1990b). This different *N*-glycosylation was, however, without any effect on the catalytic properties of rat lactase. Although processing of the *N*-linked glycans to complex forms occurs before the proteolytic cleavage of the pro-lactase, complex glycosylation is not a prerequisite for this cleavage (Lottaz et al., 1992; Rossi et al., 1993a; Naim, 1992; Naim, 1994).

Lactase contains some *O*-glycosylation in most species studied so far (Büller et al., 1989a; Büller et al., 1989b; Büller et al., 1990b; Naim and Lentze, 1992). A clear *O*-glycosylated "stalk" region as identified in sucrase-isomaltase (Section VII.A) was not identified in the cDNA sequences of lactase. As indicated above, Naim and co-workers found that the *O*-glycosylation was found exclusively on the mature, membrane-bound, part of the human lactase polypeptide; thus the propeptide is neither *N*- nor *O*-glycosylated. Most likely the pro-peptide is folded in such a way that *O*-glycans cannot be attached (Naim et al., 1994). In human intestine, lactase is heterogeneous with respect to *O*-glycosylation: *O*-glycosylated and non-*O*-glycosylated mature lactase can be isolated from the same human tissue samples (Naim and Lentze, 1992). These *O*-glycans, consisting of (sialyl-) T-antigens, conferred a clear effect on the catalytic properties on human lactase. Although the K_m of the two isoforms towards lactose was identical (14 m*M*), the presence of *O*-glycans on the mature enzyme increased its V_{max} fourfold over the enzyme that contained no *O*-glycans. Presently, it is unclear if both isoforms are synthesized within the same enterocyte or whether the existance of these lactase isoforms (with and without *O*-linked glycans) is a reflection of differences in enterocyte differentiation-state. Either way, it accounts for some posttranscriptional regulation of lactase activity in the intestine.

5. Sorting and Dimerization

The apical sorting of lactase occurs very efficiently, and there are some indications that the sorting signal may be located in the pro-peptide. Lactase is sorted apically with high efficiency after transfection into MDCK cells (Grünberg et al., 1992; Jacob et al., 1994). These studies independently showed that complexly *N*-glycosylated uncleaved pro-lactase was predominantly sorted to the apical membrane, which implied that proteolytic processing was not a prerequisite for correct sorting. Grossly mutating lactase by complete removal of the pro-peptide resulted in degradation of lactase early in biosynthesis (Naim et al., 1994). Thus, the putative role of the propeptide in sorting could not be studied further. Perhaps more subtle mutations of the pro-peptide region will elucidate its possible functions in sorting without distortion of its obviously important role in lactase folding and egress from the RER.

An unexpected finding, in our laboratories, was localization of the lactase mRNA in rat enterocytes in the apical part of the cell (Rings et al., 1992a), wherein another mRNA encoding a mitochondrial protein was sorted close to the mitochondria within the same cells and an mRNA encoding a cytosolic polypeptide was present throughout the entire cytoplasm. Thus, the lactase mRNA appeared to be sorted specifically toward the apical part of the enterocytes (Rings et al., 1992a). The apical localization of lactase mRNA was also observed in human enterocytes with non-radioactive *in situ* hybridization (Maiuri et al., 1994). Very recently, more examples of mRNA sorting were found, as reviewed in Rings et al. (1994a). The concept is, although still controversial, that mRNA delivery close to the final destination of the encoded polypeptide would help to minimize chaos in the cell as well as minimize energy required for delivery of proteins.

Studies by Danielsen have indicated that lactase likely forms dimers as a late step in biosynthesis. Pig lactase could be cross-linked exclusively in the mature form, strongly suggesting non-covalent dimerization (Danielsen, 1990). This was confirmed by isolation of metabolically labeled pig lactase dimers by sucrose density gradient centrifugation: monomeric lactase was high mannose *N*-glycosylated, whereas dimeric lactase was cleaved to the mature molecular weight as well as complexly *N*-glycosylated (Danielsen, 1994). Also electron micrographs of purified pig lactase suggest that these molecules form dimers (Skovbjerg et al., 1981).

The mean residence time of lactase in the brush border of enterocytes was estimated 7.8 h in *in vivo* experiments in adult rat (Dudley et al., 1993). While, *in vitro* measurements showed a long half life of 11 h in adults rats (Castillo et al., 1990). In Caco-2 cells, the brush border lactase has a relatively long half-life, compared to sucrase-isomaltase, as the amount of lactase activity is not coupled to its rate of biosynthesis, and activity remains high in confluent Caco-2 cultures for at least two weeks in absence of significant levels of biosynthesis (Van Beers et al., 1995).

6. Structure Revealed By cDNA Sequences

The complete lactase cDNAs from human, rabbit and rat small intestine have been cloned and sequenced (Mantel et al., 1988: Duluc et al., 1991). The deduced translation products were 1926, 1927, and 1928 amino acids long for rabbit, human and rat, respectively. The homology between these sequences is 75 to 78%, and this homology is equally spread along the entire length of the sequences (Figure 4). As anticipated from the biosynthetic studies, it was deduced that these cDNAs give rise to a single chain high molecular weight precursor. The known *N*-termini of the mature lactase from rat (Dudley et al., 1993), human (Montgomery et al., 1991), and rabbit (Mantei et al., 1988) were found within these sequences, at about residue 867 as part of a "trypsin-like" cleavage sequence, which is not particularly well conserved (Figure 4). A single hydrophobic domain was identified at the extreme *C*-termini of all cDNAs which function as a transmembrane domain, as shown by hydrophobic labeling with [^{125}I]TID (Mantei et al., 1988; Wacker et al., 1992). The resulting type I membrane-bound enzyme has a very short cytoplasmic tail of about 25 residues. The primary sequence of lactase from human, rabbit and rat revealed a fourfold internal homology (designated domain I-IV, Figure 4), most likely due to two independent duplication events during evolution. The domains II-IV of all lactase cDNA sequences show homologies of 38 to 55%. Of the four domains, domain I has diverged most: only over a stretch of about 90 residues does high homology exist with domains II-IV. However, high homology between the domains I of the various species suggests an analogous function such as in folding of the pro-lactase in the RER.

Apart from lactase homology between mammalian species, significant homology was found with bacterial β-glycosidases (Gräbnitzet al., 1991; Henrissat, 1991; Henrissat and Bairoch, 1993; Trimbur et al., 1992). The bacterial β-glycosidases possess molecular masses around 50 kDa, further suggesting that an ancestral gene, coding a protein of about 50 kDa was duplicated twice to generate the mammalian lactase. On the contrary, there is no sequence homology between human lactase and the lysosomal β-galactosidase (Oshima et al., 1988).

The amino acid positions for the structurally important *N*-glycosylation and cysteine residues are remarkably conserved among the three lactase sequences known to date (Figure 4). The various lactase sequences contain 17 to 18 cysteine residues, of which 15 are conserved, while 11 of the 14 to 15 potential *N*-glycosylation sites are conserved. As is clear from the biosynthetic studies no disulfide bonds are formed between the propeptide (domains I and II) and the mature part of the polypeptide (domains III and IV). From labeling studies with CBE, it was found that mature lactase contained two active sites (carboxyl-groups), whereas it was initially not ruled out that the highly conserved pro-peptide could also contain an active site (Colombo et al., 1973; Mantei et al., 1988). As will be discussed in the next section, active site glutamate residues were only identified in domains III and IV, while the homologous positions in domains I and II were not conserved.

B. Substrate Specificity And Hydrolytic Mechanism Of Lactase

1. Two Active Sites

Early studies indicated that lactase isolated from brush border preparations contained two active sites. Colombo et al. (1973) could isolate one protein, which displayed both lactase and

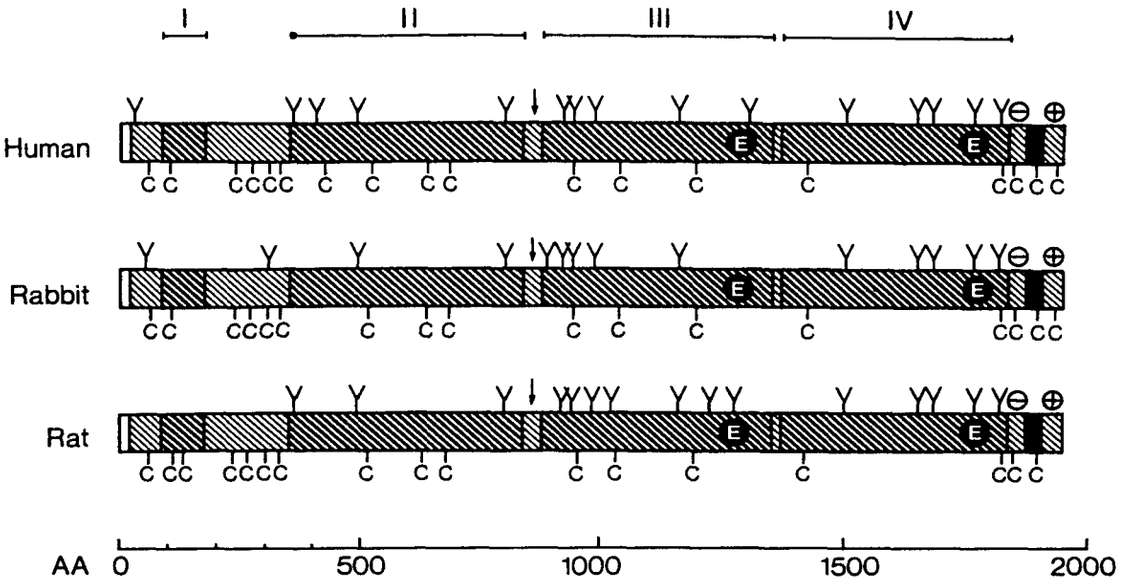

FIGURE 4. Primary structure of lactase. The sequences of human, rabbit, and rat lactase are shown, as these were deduced from the respective cDNAs (Mantei et al., 1988; Duluc et al., 1991). The sequences were extracted from GenBank, release 85.0 (1994), aligned and compared according to the algorithm of Feng and Doolittle (1987). The domains I-IV indicate internal homology within each lactase sequence. The white *N*-terminal box indicates the cleavable signal sequence. The black box indicates the *C*-terminal transmembrane sequence. The lightly and heavily shaded areas indicate regions of low and high homology, respectively, between the corresponding domains of lactase from these species. (Y), *N*-glycosylation site; (C), cysteine residue; (E), glutamate residue implicated as active site residue; (+), heavily positively charged domain; (–), heavily negatively charged domain; AA, amino acid residues. (↓), indicates the proteolytic cleavage site with the consensus sequence: S(K or R)(V,T, or A)R↓(A or V). Reproduced with permission from Rings et al. (1994d).

phlorizin hydrolase activity. Heat inactivation suggested that there were two different enzymatic sites, one for the hydrolysis of hydrophilic compounds such as lactose and cellulose, and one site for the hydrolysis of hydrophobic substrates such as phlorizin and β-glycosylceramides: The putative lactase site was more heat sensitive (Colombo et al., 1973; Skovbjerg et al., 1981; Leese and Semenza, 1973). These studies further showed different sensitivity of lactose and phlorizin hydrolysis to various inhibitors such as tris. Tris inhibited lactose hydrolysis at concentrations not affecting phlorizin hydrolysis. This indicated that there were two sites, both β-glycosidases, capable of degrading β-galactosidic as well as β-glucosidic compounds. Purified pig lactase has a pH optimum for lactose hydrolysis of 6, while pH 5 as well as 7 still give half maximal activities; the K_m for lactose and phlorizin were 21 m*M* and 0.44 m*M*, respectively (Skovbjerg et al., 1981). These K_m values and pH optima were well within range of those for lactase from rat, human, and hamster (Colombo et al., 1973; Büller et al., 1989c; Naim and Lentze, 1992). Isolated lactase hydrolyzes many β-glycosidic compounds, which can be summarized as follows: 1. lactose, which is hydrolyzed at low K_m, but at high speed, 2. hydrophilic compounds such as cellobiose and cellodextrins, 3. polymeric cellulose although hydrolyzed poorly (Skovbjerg et al., 1982), 4. naturally occurring hydrophobic substances such as phlorizin, β-glucosyl- and β–galactosylceramide, 5. a large number of (hydrophobic) test substrates, such as ortho-nitrophenyl-β(1-4)-galactopyranoside (ONP-gal), and -glucopyranoside (ONP-glc). There is no activity to any

α-glycosidic compound tested. Generally, lactase displays lower K_m values for β-glucopyranosides than for β-galactopyranosides, indicating that the listing of lactase as only a β-galactosidase is only half of the story; it would be better to refer to it as a β-glycosidase.

Substrate specificity has been studied using a series of chemically defined substrates resembling lactose. Most of the hydroxyl-groups in the molecule were changed independently into either hydrogen- or methyl-groups (Rivera-Sagredo et al., 1992). The OH-groups at positions 2 and 3 of the galactose moiety are essential for hydrolysis, while absence of the hydroxyl-group at position 6 rendered a better substrate. The OH-groups at position 3 or 6 of the glucose moiety, proved important but not essential for hydrolysis (Rivera-Sagredo et al., 1992). These findings also explain why the same active site of lactase is able to exert β-glucosidase as well as β-galactosidase activity. The conformation of the two hydroxyl-groups at the 2 and 3 position, which were shown to be essential for catalysis, are identical in glucose and galactose. Only the conformation of the OH-group at position 4 is different, but this group is irrelevant for catalysis. Thus, lactase is able to hydrolyze β-gluco- and β-galactopyranosides, and the substrate specificity is further determined by the capability of the α-glycan moiety to fit into the catalytic cleft.

Interestingly, the cleavage of pro-lactase has no effect on the catalytic properties of lactase. After transfection of either human or rat lactase cDNA into monkey COS cells, a fully active lactase was produced in the absence of proteolytic cleavage (Naim et al., 1991a; Neele et al., 1994). This strongly suggests that cleavage of the propeptide does not result in an "activated" enzyme, as in many proteinases.

2. Only One Main Active Site with b-Gluco- as well as b-Galactosidase Activity

The complete and covalent inhibition of the enzymatic activities of lactase by the binding of CBE (Wacker et al., 1992), demonstrated that the reaction mechanism of lactase resembled that of sucrase-isomaltase (Section VI.B). The inhibition by CBE indicates that both critical active site nucleophiles were carboxyl-groups, and that lactase belongs to the configuration-retaining glycosidases, such as maltase-glucoamylase and sucrase-isomaltase, but unlike trehalase (Sections IV, V, and VII). The study of Wacker et al. (1992) demonstrated that there were two active sites in rabbit lactase, both inactivated by CBE, but with different kinetics of inactivation. Both sites contained a critical glutamate residue: while both nucleophiles in sucrase-isomaltase are aspartate residues (Section VI.B). Sequencing of the CBE-labeled peptides and comparison with the known rabbit lactase cDNA sequence revealed that E^{1271} and E^{1747} were the active site residues in domains III and IV, respectively. From the inactivation kinetics of the two active sites by CBE, it was concluded that domains III and IV of the rabbit lactase likely contained the active site for hydrolysis of lactose and phlorizin, respectively (Wacker et al., 1992). Recently, this was explored more directly in our laboratories by site directed mutagenesis of the deduced active site residues (Neele et al., 1994). The corresponding active site residues of rat lactase E^{1274} and E^{1750} were substituted (Table 5). Mutated rat lactase cDNAs were transfected into COS cells, which were shown to express fully active "wild-type" rat lactase after transfection of "wild-type" lactase cDNA, i.e., the K_m values of the transfected rat lactase towards lactose and ONP-glc were very similar to the values for "wild-type" lactase isolated from rat brush border. It appeared that the mutations, changing the E^{1274} into other residues, hardly affected the activity of lactase towards hydrophilic (lactose and cellulose) or hydrophobic (ONP-glc, ONP-gal, and phlorizin) substrates (Table 5; Neele et al., 1994). Conversely, mutating E^{1750} in domain IV abolished all activity towards hydrophilic substrates, as well as about 75% of the activity towards hydrophobic substrates. Thus, we concluded that all the lactase activity as well as most of the activity towards hydrophobic substrates is confined to domain IV, while domain Ill only displays minor activity towards the hydrophobic substrates.

TABLE 5
Identification of the Active Sites of Rat Lactase by Site-Directed Mutagenesis

Enzyme/Substrate	Lactose	Cellobiose	ONP-glc
Control	< 1%	< 1%	<1%
Wild-type	100%	100%	100%
E1274G	98%	95%	91%
E1274D	94%	102%	86%
E175OG	< 1%	< 1%	24%
E1750D	< 1%	< 1%	26%
"Double"	< 1%	< 1%	< 1%

Note: Full-length rat lactase cDNA was transiently transfected into COS cells (Neele et al., 1994). Lactase expressed in transfected cells had similar catalytic activity as wild-type lactase, isolated from rat intestinal brush border membranes: The K_m values of wild-type (brush border) lactase towards lactose and ONP-glc were 52 and 2.3 m*M*, respectively, while K_m values of the transfected rat lactase were 48 and 3.1 m*M*, respectively. The control values of COS cells for the hydrolysis of lactose, cellobiose, and ONP-glc were negiligible . The values of the wild-type lactase, transfected to COS cells, were defined as 100%. The activities of the mutants were expressed relative to the hydrolytic activity of the wild-type transfectant. The "double' mutant contains two mutated active site residues, i.e., E1274G and E1750G. The constructs were transfected to COS cells and allowed to produce lactase for 48 h, then cells were lysed, the lactase was immunoprecipitated, and the enzymatic activities were measured during 1 h at saturating substrate concentrations. The molecular weight and amount of lactase molecule produced in each transfection was analyzed on SDS-PAGE, and was found to be very similar in every case (Data not shown).

The amino acid sequences surrounding the active site residues were found to be highly conserved (Figure 4). All three domains III contain the active site sequence PIYITE^{1274}NG,while all domains IV contain PIYVTE^{1750}NG (Mantei et al., 1988; Duluc et al., 1991). The only difference in the above sequences is I^{1272}, which corresponds to V^{1748} when compared with domain IV, the primary active site. A point mutation changing I^{1272} into V^{1272}, thereby making the least active domain (III) identical to the major active site within domain (IV) over a stretch of eight amino acid residues, failed to convert the activity of domain III into that of domain IV, indicating that other, more distant structures determine the substrate specificities (Neele et al., 1994). Comparison of all reported bacterial b-glycosidase sequences revealed a PIY(I/V)TENG consensus sequence around the active site nucleophile, of which the **ENG** sequence was absolutely conserved in all β-glycosidases examined (Trimbur et al., 1992; Wacker et al., 1992). Interestingly, the **ENG** sequence within domain II of all three lactase cDNAs has been changed during evolution into **GNG**. Furthermore, the domains I of lactase do not contain **ENG** sequences, strongly arguing against a possible β-glycosidase activity of the pro-peptide of lactase.

VII. SUCRASE-ISOMALTASE

A. Structure and Biosynthesis of Sucrase-Isomaltase

Two of the four maltase activities of the small intestinal brush border involved in digestion of starch are assigned to sucrase-isomaltase, an enzyme complex containing two separate polypeptides (Skovbjerg et al., 1979). This enzyme complex, which repesents quantitatively the most important maltase activity in humans, comprises about 10% of brush border membrane proteins. It further contains all intestinal sucrase activity, which is essential for the digestion of sucrose. Classically, sucrase-isomaltase was isolated from brush border membranes, by solubilization of the enzyme complex with papain, affinity chromatography on Sephadex G-200, and ion-exchange chromatography on DEAE-Sephadex G-25 (Alvarado and Mahmood, 1979). With the availability of versatile anti-sucrase-isomaltase monoclonal antibodies, it has become standard to immunoisolate sucrase-isomaltase from various species (e.g., Sjöström et al., 1982; Danielsen and Cowell, 1984; Hauri et al., 1985; Naim et al., 1988a). The enzyme complex is most specifically detected by its sucrase activity, which is unique to this complex, and distinguishes it from the maltase-glucoamylase complex.

1. Isolation and Structure

The sucrase-isomaltase complex is tightly associated with the brush border membrane, as it can only be solubilized by detergents such as Triton X-100 or by mild papain digestion. The molecular masses of the isolated enzyme complexes vary slightly between the various species examined. Preparations of the mammalian enzyme complex from human, rat, and pig consist of two noncovalently associated subunits, of which the smaller one (120 to 140 kDa) is sucrase, while the larger one (140 to 151 kDa) represents isomaltase (Hauri et al., 1980; Sjöström et al., 1982; Danielsen and Cowell, 1984; Hauri et al., 1985; Naim et al., 1988a; Gorvel et al., 1991; Hoffman and Chang, 1991). In chicken sucrase-isomaltase, the sucrase subunit appeares to be the larger subunit (Hu et al., 1987). Often a third band of 230 to 260 kDa is observed in preparations of sucrase-isomaltase, which has been shown to represent a single polypeptide precursor form of the complex. Only the isomaltase subunit is directly associated with the membrane, while the sucrase subunit interacts non-covalently with the isomaltase subunit, but not with the membrane (Cowell et al., 1986). This interaction of the isomaltase with the brush border membrane is mediated by a hydrophobic sequence in the extreme *N*-terminus of human, rabbit, pig, and rat sucrase-isomaltase as revealed by *N*-terminal sequencing of the intact isomaltase subunit (Spiess et al., 1982; Wacker et al., 1981; Sjöström et al., 1982). Also the chicken sucrase-isomaltase was found, by hydrophobic labeling with [^{125}I]TID, to be linked to the brush border membrane by its isomaltase subunit, indicating phylogenetic conservation of the membrane insertion mode and the subunit organization of the enzyme complex (Hu et al., 1987). The visualization by electron microscopy of the intact pig sucrase-isomaltase complex revealed that the complex is projected from the brush border membrane by a 3.5 nm-long "stalk", while the banana-shaped bulk of the enzyme protrudes maximally 17 nm from the membrane (Cowell et al., 1986). This study also revealed that sucrase-isomaltase forms dimers of the $\alpha_2\beta_2$-type. Metabolic studies also indicated that pig sucrase-isomaltase homodimerized in the RER (Danielsen, 1994). Dimerization of human sucrase-isomaltase could not be demonstrated in Caco-2 cells by chemical cross-linking experiments under a wide variety of conditions, while the brush border enzyme, dipeptidylpeptidase IV, was readily shown to oligomerize as result of a Golgi located process (Jaskur et al., 1991). Beaulieu et al. (1989) also found that all biosynthetic intermediates of Caco-2 cell sucrase-isomaltase had identical sedimentation characteristics, further suggesting that dimerization does not occur. As the overall structure, biosynthesis and functions are very similar among sucrase-isomaltase complexes from different species, species differences may be less likely, which leads to speculation that technical factors may have accounted for failure to detect dimers in Caco-2 cells.

2. Biosynthesis and Intracellular Transport

The study of the biosynthesis of sucrase-isomaltase has revealed that the sucrase and the isomaltase subunit are synthesized as part of the same polypeptide precursor: pro-sucrase-isomaltase (Figure 3). Cell-free synthesis of sucrase-isomaltase from rabbit intestinal RNA, in the presence of RER membrane vesicles, produced a 270 kDa polypeptide (Wacker et al., 1981). Likewise, *in vitro* metabolic labeling studies performed on pig, rat, human, and rabbit explants and in human cell lines all showed the synthesis of a pro-sucrase-isomaltase (Danielsen et al., 1981; Danielsen and Cowell, 1984; Hauri et al., 1985; Naim et al., 1988a; Hoffman and Chang, 1991). The precursor, located in the RER, was found to be extensively high mannose *N*-glycosylated. These *N*-glycans were demonstrated to be essential for the proper folding and subsequent egress of pro-sucrase-isomaltase from the RER: non-*N*-glycosylated, malfolded precursors were rapidly proteolytically degraded (Danielsen, 1992). The exit of this pro-sucrase-isomaltase from the RER is relatively slow compared to other brush border enzymes, such as aminopeptidase N and peptidylpeptidase IV. This is most likely due to the relatively slow conformational maturation of the precursor (Matter and Hauri, 1991; Danielsen, 1992). The asynchronous transport of brush border enzymes to the cell surface is not due to timing differences of trimming of *N*-linked oligosaccharides (Matter et al., 1989). Both the rate limiting steps of transport to the Golgi complex and the degradation of the malfolded pro-sucrase-isomaltase was further confirmed in heat-shocked Caco-2 cells. At 42.5°C, pro-sucrase-isomaltase was no longer transported to the brush border, but accumulated instead in a malfolded form in the RER where it was subsequently degraded. In contrast, the biosynthesis and transport of aminopeptidase N and dipeptidylpeptidase IV were unaffected at this temperature in Caco-2 cells (Quaroni et al., 1993).

After proper folding in the RER, the pro-sucrase-isomaltase is transported to the Golgi complex to be complexly *N*- and *O*-glycosylated, leading to a precursor with an approximately 20 kDa higher molecular mass than the high mannose form (Danielsen and Cowell, 1984; Hauri et al., 1985; Naim et al., 1988a; Hoffman and Chang, 1991). Like maltase-glucoamylase, sucrase-isomaltase is tyrosine-sulfated as a result of a trans-Golgi event (Danielsen, 1987). Then, the pro-sucrase-isomaltase is transported to the apical brush border membrane. Naturally occurring mutations in human sucrase-isomaltase often lead to transport incompetent enzyme molecules (Naim et al., 1988a). Cell-biological analysis of this transport incompetence revealed that the intracellular sorting signal for apical transport resided in the luminal domain of the membrane-bound isomaltase subunit (Fransen et al., 1991). Danielsen (1994) found that the homodimerization of sucrase-isomaltase was not an absolute requirement for transport, as some complexly *N*-glycosylated sucrase-isomaltase was found in the monomeric form in the brush border membrane.

Sucrase-isomaltase is efficiently transported to the apical membrane of Caco-2 cells by vectorial transport (>90%), while missorted sucrase-isomaltase is rapidly transcytosed to the apical membrane in Caco-2 (LeBivic et al., 1990). This apical transport in Caco-2 cells was demonstrated to be highly facilitated by association of the transport vesicles to microtubules. Sucrase-isomaltase was still primarily delivered to the apical brush border membrane in absence of microtubules, but the missorting to the basolateral membrane increased markedly, and the rate of transport to the apical membrane decreased drastically (Gilbert et al., 1991). Interestingly, when the brush border is not assembled, sucrase-isomaltase is no longer transported to the plasma membrane in contrast with other brush border enzymes, such as dipeptidylpeptidase IV, neutral aminopeptidase, and neutral endopeptidase. This was demonstrated in Caco-2 cells in which brush border assembly was perturbed by expression of villin antisense RNA (Costa de Beauregard et al., 1995). Caco-2 cells, transfected with the villin antisense DNA, fail to sort sucrase-isomaltase to the apical membrane, although significant amounts of the protein were made. As the cells become fully polarized with respect to most other specific basolateral and apical membrane compounds, the direct route to the apical membrane apparently demands villin and/or a developed brush border.

Forskolin, a drug which diminishes cellular cAMP levels, completely inhibited the apical transport of sucrase-isomaltase in Caco-2 cells, by a presently unknown mechanism (Rousset et al., 1989). The pro-sucrase-isomaltase is proteolytically cleaved into its mature form by pancreatic proteinases in the gut lumen. In isolated loops of the small intestine, the precursor is not hydrolyzed (Montgomery et al., 1981a). Disconnection of the pancreatic duct also prohibits the conversion to mature sucrase-isomaltase (Cowell et al., 1986). In fetal rat, which has not yet developed a functional pancreas, only pro-sucrase-isomaltase is produced in the intestine (Hauri et al., 1980). Moreover, in numerous *in vitro* studies on intestinal explants or on cell lines, the pro-sucrase-isomaltase was not proteolytically split unless exogenous proteinases were added to the culture medium (e.g., Danielsen et al., 1982; Hauri et al., 1985; Naim et al., 1988a; Fransen et al., 1991; Shapiro et al., 1991). In Caco-2 cells, the brush border sucrase-isomaltase has only a short half-life, as the amount of enzyme activity is closely coupled to its rate of biosynthesis (Van Beers et al., 1995). Likewise, the mean residence time *in vivo* of rat sucrase-isomaltase in small intestinal brush border membrane is rather short: 5.8 h. To maintain a steady state level of the enzyme complex, the cells have to synthesize about 4 times the amount of active enzyme present in the brush border membrane each day (Dudley et al., 1993). Klumperman et al. (1991), studying endocytosis of brush border membranes in Caco-2 cells, found that sucrase-isomaltase as well as dipeptidylpeptidase IV are most likely recycled to the apical membrane after endocytosis, and not transported to the lysosomal compartment, indicating that the degradation of brush border enzymes may proceed by other mechanisms.

3. Primary Amino Acid Sequences

The cDNAs of sucrase-isomaltase from rabbit, human, and rat have been completely sequenced (Hunziker et al., 1986; Chantret et al., 1992; Chandrasena et al., 1994). The deduced polypeptide sequences of the rabbit, human, and rat sucrase-isomaltase consist of 1827, 1827, and 1841 amino acids, respectively (Figure 5). The human sequence is 84% identical with the rabbit sequence, while the rat sequence is somewhat less conserved (about 75%) with respect to both the rabbit and human sequence, most likely reflecting the phylogenetic distance between these three species. All three sequences show twofold internal homology in their sucrase and isomaltase regions. The identical amino acids between the sucrase and isomaltase subunits in each of these species comprise 38 to 41%, while another 34 to 40% of the amino acids appeared to be conservatively changed. The homologous residues between the complete polypeptides are scattered along the lengths of these sequences, and thus there are no indications of major insertions or deletions in the sequence. Each isomaltase subunit contains a sequence of about 65 residues which has no homologous counterpart in the sucrase sequence. This sequence in the extreme *N*-terminus of pro-sucrase-isomaltase contains the only hydrophobic region. Starting at amino acid 13 of the sequence of all three species and comprising 20 residues, this sequence functions as a signal sequence for translocation into the RER and concomitantly as a transmembrane anchor, rendering a class II transmembrane protein, with a very short cytoplasmic tail of 12 residues (Hunziker et al., 1986; Chantret et al., 1992; Chandrasena et al., 1994). It was demonstrated that the non-cleavable signal anchor sequence of rabbit sucrase-isomaltase could be converted to a cleavable signal sequence by single amino acid substitutions, or small deletions (Hegner et al., 1992). Introduction of a proline residue at position 28 or 29, i.e., at about two thirds of the hydrophobic sequence was very effective in yielding a signal sequence, which mediated translocation, and was subsequently cleaved. Shortening of the hydrophobic sequence from 20 to 12 amino acids yielded a fully functional, cleavable signal sequence. The consequence of these mutations expressed in an *in vitro* translation/translocation system was a soluble sucrase-isomaltase. The effects of these mutations were not tested by transfection into eukaryotic cells, and thus it remains uncertain what the consequences are of these mutations for folding in the RER, intracellular transport, and polar expression.

The isomaltase sequences show a 24 to 32 amino acids threonine/serine-rich region, follow-

ing the transmembrane region, and are presumably *O*-glycosylated. Heavily *O*-glycosylated regions, known as mucin-like regions, adapt extended filamentous configurations (Strous and Dekker, 1992). This region likely represents the "stalk" of the isomaltase subunit observed by electron microscopy.

The human, rabbit, and rat sequences contain 18, 18, and 16 *N*-glycosylation sites, respectively, of which 9 are found at homologous positions (Figure 5). This most likely reflects physical constraints with respect to the tertiary structure of the enzyme complex. Also some *N*-glycosylation sites are found in similar positions, i.e., within five amino acid stretches, when the sequences of the sucrase and isomaltase subunits are aligned. Eight of the structurally important C-residues are conserved between the sucrase and isomaltase subunits of all three sequences. Both subunits of rabbit, rat, and human sucrase-isomaltase contain the conserved sequence, DGLWID*MNE, of which the *C*-terminal D-residue (indicated by an asterisk) has been assigned as the active site residue (Section VII.B). The proteolytic cleavage which results in the two subunits occurs between arginine 1007 and isoleucine 1008 in all three sequences, as was already inferred from *N*-terminal sequencing of the isolated sucrase subunits of various species (Semenza, 1986).

4. Sequence Similarity to Other α-Glucosidases

The sequences of sucrase-isomaltase show remarkable similarities to human lysosomal α-glucosidase. The human lysosomal (acid) α-glucosidase (EC 3.2.1.20) is able to hydrolyze lysosomal glycogen and demonstrates homology of about 26% to the sucrase as well as the

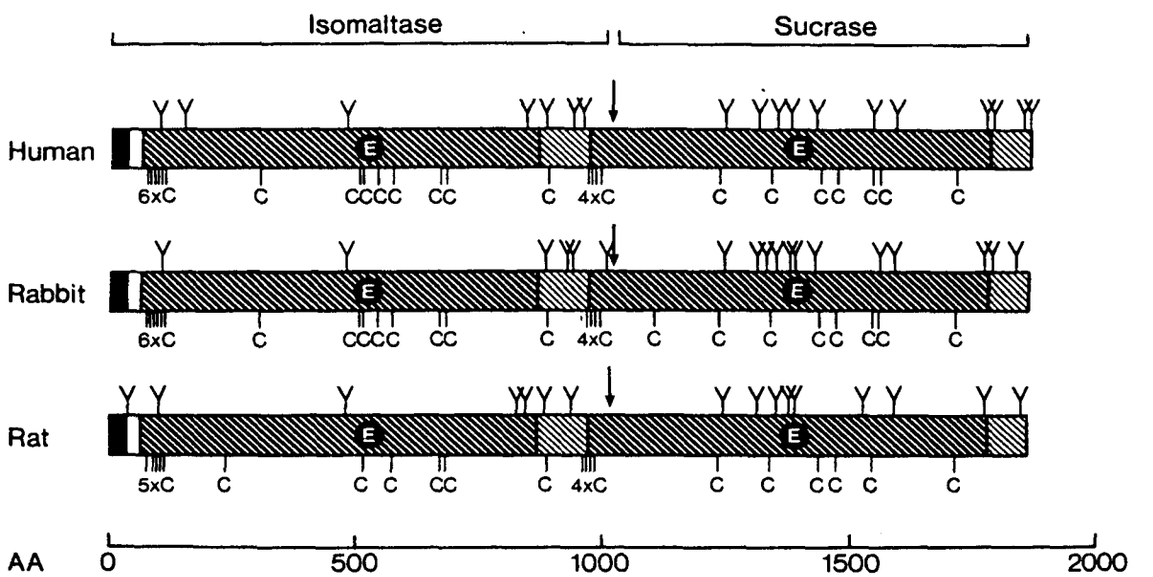

FIGURE 5. Primary structure of sucrase-isomaltase. The sequences of human, rabbit, and rat sucrase-isomaltase are shown, as these were deduced from the respective cDNAs (Hunziker et al., 1986; Chantret et al., 1992; Chandrasena et al., 1994). The sequences were extracted from GenBank, release 85.0 (1994), and aligned and compared according to the algorithm of Feng and Doolittle (1987). The isomaltase region of each enzyme complex shows high homology to the sucrase region. The black box indicates the *N*-terminal non-cleavable signal sequence, which in addition serves as transmembrane sequence. The white *N*-terminal box indicates the highly *O*-glycosylated region within each isomaltase sequence. The lightly and heavily shaded areas indicate regions of low and high homology, respectively, between the correspondi ng regions of sucrase-isomaltase from these species. (Y), *N*-glycosylation site; (C), cysteine residue; (↓), proteolytic cleavage site; (E), glutamate residue implicated as active site residue; AA, amino acid residues.

isomaltase subunits of rabbit, rat, and human sucrase-isomaltase (Hoefsloot et al., 1988). Seven of the 11 cysteine residues of lysosomal α-glucosidase are conserved in each of the sucrase and isomaltase subunits of the three species. Also, 3 of the 8 *N*-glycosylation sites occur at homologous positions in the lysosomal enzyme when compared with each of the sucrase and isomaltase subunits. As originally suggested by Hunzinker and co-workers (1986), the sucrase-isomaltase gene likely resulted from a duplication of an ancestral α-glucosidase. As the human lysosomal α-glucosidase is about half the size of sucrase-isomaltase, and presumably contains only one active site sequence (the DGLWID*MNE sequence is also present in this sequence, and was assigned independently as active site (Dinur et al., 1986), it seems very likely that during evolution this enzyme derived from the same ancestral gene as sucrase-isomaltase. If this is true, the gene duplication event leading to separate lysosomal and brush border α-glucosidases was a much earlier event in evolution than the duplication leading to the two-active site polypeptide of sucrase-isomaltase. This is indicated by the lower homology of the lysosomal enzyme with each of the sucrase and isomaltase subunits (about 26%) versus the homology between the sucrase and isomaltase subunits (over 40%), and also by the two-fold internal homology of sucrase-isomaltase, which does not occur in lysosomal α-glucosidase. The sucrase-isomaltase complex also contains homology with several yeast α-glucosidases (EC 3.2.1.20), further suggesting the evolutionary existence of an ancestral α-glucosidase gene. For example, an α-glucosidase of *Candida tsukubaensis* has 35% amino acid homology with the isomaltase, and 36% homology with sucrase as well as 34% homology with human lysosomal α-glucosidase (Kinsella et al., 1991). The highly conserved region GIWDLDMNE was distinguished in this sequence, possibly representing the active site of this yeast α-glucosidase. Furthermore, a glucoamylase cDNA cloned from *Schwanniomyces occidentalis* showed an amino acid homology of about 35% compared to human acid α-glucosidase, rabbit sucrase, and rabbit isomaltase. The homology is scattered along the whole length of the sequence, and includes a presumed active site sequence very similar to sucrase-isomaltase (DGIWADMNE, Naim et al., 1991b).

B. Substrate Specificity and Hydrolytic Mechanism of Sucrase-Isomaltase

Sucrase-isomaltase accounts for about 80% of the maltase activity in the small intestine. Both sites of sucrase-isomaltase possess maltase activity, but the enzyme complex exhibits only very limited activity towards larger glucans (Semenza, 1987). The two active sites within the complex have overlapping substrate specificities, and also overlap in specificity with the two sites of the maltase-glucoamylase complex (Section V.B). However, the sucrase subunit is responsible for all of the sucrase activity in the small intestine. The activity of the isomaltase subunit can be distinguished from the activity of the sucrase subunit by the fact that it does not display any sucrase activity, but instead displays isomaltase activity, which is not exhibited by the sucrase subunit. The activity of sucrase-isomaltase can further be distinguished from the activities of the maltase-glucoamylase complex by its relatively low heat-stability (Quezada-Calvillo et al., 1993; Semenza, 1986).

1. Substrates

Studies on the substrate specificities performed by Gray et al., (1979) and Cogoli and Semenza (1975) on the intact enzyme complex, and its individual subunits from rat and rabbit, respectively, can be summarized as follows: 1. the sucrase subunit hydrolyzes sucrose, but not α(1-6) glucosidic bonds, 2. the isomaltase subunit hydrolyzes α(1-6) glucosidic bonds but not sucrose, 3. both subunits hydrolyze maltose and maltotriose, 4. both subunits hydrolyze hydrophobic aryl-α-glucopyranosides to some extent, and 5. the complex shows no activity towards polymeric glucans, such as starch, and both subunits show only minor activity towards small α-limit dextrins. Compared to sucrase-isomaltase, the specific activity of maltase-glucoamylase

towards degradation of α-limit dextrins is much higher (Section V.B).

Proteolytic processing is of little consequence to the enzymatic activities of the sucrase-isomaltase enzyme complex. The pro-sucrase-isomaltase, produced by tissue explants of pig, rat, and human in the absence of proteolytic cleavage, is as active as the mature, cleaved form (Sjöström et al., 1980; Montgomery et al., 1981a; Hauri et al., 1982). Rabbit sucrase activity shows a broad pH optimum with maximum at pH 7.0, and with half-maximal activities at pH 5.5 and 8.5, respectively (Alvarado and Mahmood, 1979). Both sites appear equally sensitive to inactivation by CBE, and, as for most brush border glycohydrolases, tris is an effective inhibitor (Cogoli and Semenza, 1975). The K_m for sucrose, isomaltose, and maltose hydrolysis is in the order of 4 to 20 m*M* for the rabbit, mouse, and rabbit intestinal enzyme, and the hydrolysis follows Michaelis-Menten kinetics. Simple Michaelis-Menten kinetics for this two-active site enzyme complex indicates that these sites are quite similar, and the sites should work in parallel rather than sequentially.

2. Sucrase-Isomaltase. A Model for the Catalytic Mechanism

With respect to catalytic mechanism and architecture of the active site most glycohydrolases may function by very similar reaction mechanisms. For instance, sucrase-isomaltase may resemble maltase-glucoamylase, and (to a lesser extent) lactase. The reaction mechanism of trehalase may be fundamentally different, as best indicated by the stereo-chemical conversion at the Cl-atom (Section IV.B). Most work on the reaction mechanism has been performed on sucrase-isomaltase (Cogoli and Semenza, 1975). As all of the reaction mechanisms of glycosidases studied thus far seem to follow the rules originally suggested by these authors (reviewed by Semenza, 1987; Semenza and Auricchio, 1989), this subject is summarized here only in the form of Figure 6.

A central feature of the catalysis by glycohydrolases is the stabilization of an oxocarbonium ion by a carboxyl-group (Figure 6). In sucrase-isomaltase this carboxyl-group was found to be an aspartate residue in both subunits of the complex, identified by affinity labeling with CBE (Quaroni and Semenza, 1976). After the complete sequencing of the cDNAs of rabbit, rat, and human sucrase-isomaltase, this active site aspartate was identified as part of an absolutely conserved nonpeptide sequence (DGLWID*MNE) in all sucrase and isomaltase subunits (Figure 5). The other residue participating in the catalysis, the proton donor, was thus far not identified in the sucrase-isomaltase sequences.

VIII. GENE REGULATION OF LACTASE AND SUCRASE-ISOMALTASE

A. Gene Structure and Tissue Specific Gene Expression Of Lactase and Sucrase-Isomaltase

In recent years, the structure of the lactase and sucrase-isomaltase genes has been analyzed, enabling detailed studies of the spatial and temporal expression of these enzymes. Expression of lactase as well as sucrase-isomaltase is exclusively found on the microvillar membranes of intestinal enterocytes. Both enzymes display specific, but very different, developmental expression patterns, which will be discussed in the next section. Here, we will concentrate on the molecular biological data to identify elements regulating tissue specific gene expression.

1. Lactase Gene

The human lactase gene is located on chromosome 2 (Kruse et al., 1988). The expression of lactase exhibits essentially two phenotypes, as discussed at length in Section VIII.B: in about 75% of humans the level of lactase activity steeply declines after weaning (often referred to as adult-type hypolactasia), while in a minority (mostly Caucasians) lactase levels persist throughout life. Therefore, the human lactase gene was isolated to determine if these phenotypic differences in lactase expression could be attributed to allelic varia-

tion. The entire human lactase gene, comprising about 70 kb, has been isolated, including all 16 introns and 1100 bp of its 5′ flanking region (Boll et al., 1991). The complete lactase coding sequence of six individuals was elucidated, including two subjects with adult-type hypolactasia (ratio sucrase-isomaltase/lactase activities >>4). Although mutations were found, none of the mutation-bearing alleles was concordant with either lactase persistence or lactase decline in adult life (Boll et al., 1991). Thus, it was concluded that the persistence of or decline in lactase expression in adult humans is not due to qualitative differences in the enzyme, but related to the amount of active enzyme. However, the lactase phenotype is an inheritable trait, and therefore the phenotype is most likely inherited independently from the lactase structural gene. The double duplication within the lactase structural gene as indicated by the lactase cDNA sequences (Figure 4; Section VI.A), was not found in the pattern of intron locations in the human lactase gene. Only one intron was located at a homologous position in domains II, III, and IV, while most introns were clustered in domains I and IV (Boll et al., 1991), suggesting that the addition of introns is more recent than the gene duplication.

A recent report, which diverges from the general one-gene concept for lactase expression, describes the presence of multiple (three) independent lactase genes in rabbit (Villa et al., 1993). These genes give rise to three individual lactase mRNAs, encoding polypeptides which are about 94% homologous. Interestingly, the sequences diverge most in domain IV, which was shown in rat lactase to carry the principal catalytic site of lactase (Section VI.B). All three lactase mRNAs were collectively expressed in the same individual rabbit, but at different positions along the length of the intestine. Thus far, data do not suggest that more than one lactase gene exists in other species.

2. Lactase Gene Promoter

In the promoter sequence of human lactase several potential binding sites for transcription factors were identified (approximate position relative to the transcription initiation site in bp): a TATA-box (-30), Sp1 (-210), SRF (-250), AP-2 (-410), CTF/NF-1 (-535), CREB (-635), and Oct 1/Oct 2 (-980) (Boll et al., 1991). Moreover, two *Alu* sequences were found in this promoter sequence. To date, functional analysis of these promoter elements has not been performed. It should be noted that there are no potential binding sites for hormone receptors such as those for glucocorticoid, steroid, or thyroid hormones, which suggests that these hormones do not exert a direct effect on lactase gene expression. The above-mentioned transcription factors are more or less "general" factors known to regulate many genes. As lactase displays a unique expression pattern both during development and in tissue, it seems logical that one or more novel factors should govern lactase expression. Such a factor may have been identified by Troelsen and co-workers (1992). They identified a *trans*-acting factor which interacts with the pig lactase promoter and co-varies with enzymatic activity of lactase during postnatal life. The available 980-bp of the pig lactase promoter was 74% identical with that of the human, while no *Alu* sequences (generally specific for the human genome) were found. A 125 bp and a 980 bp fragment of the pig lactase promoter were placed in front of a β-globin reporter gene, and transfected into Caco-2 cells, which are known to display endogenous (human) lactase expression. The expression of the β-globin reporter gene was shown to mimic the expression of the endogenous lactase in Caco-2 cells. Incubation of the promoter sequence with nuclear extracts from pig intestine led to the identification of a protected DNA fragment, corresponding to bp –40 to –54 of the promoter sequence. This *cis*-acting element, designated CE-LPH1, had a unique sequence, and was bound by a factor, designated NF-LPH1. NF-LPH 1 was absent from liver, while in intestine it was 15 times more abundant in newborn than in adult pig intestine, which show high and low lactase activity, respectively. Thus, the presence of the *trans*-acting factor NF-LPHI correlates well with the expression of lactase, suggesting that it plays a role in determining the level of lactase expression and perhaps its tissue specific distribution. The 1 kb fragment of the pig lactase promoter was further shown to direct the cell-specific expression of the β-globin reporter gene in transgenic mice, and also the developmental expression, which mimicked that of lactase in nor-

mal mice (Troelsen et al., 1994a). This strongly indicates that 1 kb of the pig lactase promoter contains all *cis*-acting elements to direct the correct cell type and temporal expression of lactase. However, elements regulating correct expression in the proximal-to-distal axis of the intestine are largely absent in this construct. Information within this pig lactase promoter does direct expression in Caco-2 cells as well as in the transgenic mice, indicating that the signals and mechanisms of transcriptional regulation, governing lactase expression, may be well conserved among these species.

One kb sequence of the rat 5′ flanking region of the lactase gene has been sequenced and compared to that of the human gene. It was found that the first 155 bases upstream from the transcriptional start site showed 72% homology, while the more distant sequences were only 50% homologous (Boukamel and Freund, 1992). As in the human sequence, potential CTF/NF and AP2 sites were found in rat, while CREB, Octl/Oct2, SRF, and SP1 sites, identified in the human sequence, were not present in the rat sequence (Boukamel and Freund, 1992). On the other hand, the rat lactase promoter showed a potential calcium response element and a C/EBP binding site, which were not present in the human sequence. Three C/EBP consensus binding sites were also recently identified in the promoter of human sucrase-isomaltase (Wu et al., 1992; see below). In our laboratories, we were able to show that developmental expression of the three C/EBP isoforms in rat intestinal tissue were identical to the expression of rat lactase, suggesting that C/EBP may in part be responsible for the tissue specific expression of lactase (Montgomery et al., 1995). This coordinate expression of lactase and C/EBP in the rat was also found independently by other authors (Van den Hoff et al., 1994). Various constructs were made by cloning promoter fragments of rat lactase to a human growth hormone reporter gene. Subsequently, these constructs were transfected into Caco-2 cells and HepG2 cells (Verhave et al., 1994). By progressively shortening the promoter it was found that positive and negative regulatory elements are present upstream of the transcriptional start site, between nucleotide positions 1580-2040 and 74-821, respectively. Very little promoter activity was found in HepG2 cells, indicating that the 1 kb lactase promoter fragment was able to direct tissue specific gene expression (Verhave et al., 1994).

3. Sucrase-Isomaltase Gene and its Promoter

The human sucrase-isomaltase gene is located on chromosome 3 and 12.2 kb of its promoter has been cloned (Green et al., 1987; Wu et al., 1992). When 3.6 kb of the promoter region were sequenced, only very few consensus sites for binding of transcription factors were found: a TATA sequence was located at –27 bp, and three C/EBP binding sites were identified at –808, –991, and 2524 bp, while *Alu* sequences were absent (Wu et al., 1992). The presence of putative C/EBP binding sites was very interesting as C/EBP is expressed in the rat intestinal epithelium, making this *trans*-acting factor a candidate to direct tissue specific expression of sucrase-isomaltase.

Sucrase-isomaltase promoter constructs of various lengths were cloned upstream of a reporter gene and transfected into various human cell lines to study tissue specific gene expression (Wu et al., 1992). Constructs containing only 303 or 324 bp of the sucrase-isomaltase promoter were capable to direct high expression in Caco-2 cells, while the cell lines HeLa and HepG2, which lacked endogenous sucrase-isomaltase expression, showed only background activity, even with a promoter fragment up to 3424 bp. The human sucrase-isomaltase promoter was shown to direct nearly appropriate tissue specific expression of a reporter gene in transgenic mice (Markowitz et al., 1993). A reporter gene driven by 3424 bp of this promoter was expressed in the jejunum, ileum, and to some extent in the colon. This was slightly different from the endogenous mouse sucrase-isomaltase, which was confined only to the small intestine. However, the expression was restricted to enterocytes. The crypt-villus distribution of the expressed reporter gene in the small intestinal epithelium was also identical to the endogenous mouse sucrase-isomaltase. This indicates that this portion of the promoter contains virtually all information for tissue specific expression of sucrase-isomaltase. Moreover, this promoter seems evolutionarily conserved since the human pro-

moter directed correct expression of the reporter gene in the mouse.

In analyzing the human sucrase-isomaltase promoter further, Traber and co-workers (1992b) identified three novel *cis*-acting elements and the *trans*-acting factors binding to these respective elements. First, these authors cloned 3.4 kb of the mouse sucrase-isomaltase promoter, and demonstrated that it and the human promoter were homologous between —183 bp and the transcription initiation site. As was inferred from the expression of the human promoter in transgenic mice, the mouse and human promoter should contain common elements, functional in both species. Three common elements were found, designated SIF1, SIF2, and SIF3, along with a highly conserved TATA region. Constructs containing a reporter gene and various portions of the human 183 bp promoter fragment were transfected to Caco-2 cells and non-sucrase-isomaltase expressing cell lines. SIF1 was demonstrated to be essential for proper tissue specific expression, while SIF2 and SIF3 were positive regulatory elements, enhancing sucrase-isomaltase expression. DNase I footprinting demonstrated that the protein, binding to SIF1 (designated SIF1-BP), was only present in enterocyte-like cells. SIF2 and SIF3 binding sites were probably bound by similar proteins, which were not exclusively found in enterocytes. Thus, the SIF1 sequence and SIF1-BP are strong candidates for directing the tissue specific sucrase-isomaltase expression in human and mouse. None of the conserved binding sites bound C/EBP, although a potential C/EBP site was present in the SIF1 region, indicating that if C/EBP were to play a role, it binds outside this 183 bp region (Traber et al., 1992b).

Very recently, Troelsen et al. (1994b) found an interesting link between the tissue specific expression of lactase and sucrase-isomaltase. They analyzed the binding of nuclear proteins, extracted from Caco-2 cells, to DNA fragments representing the CE-LPH1 sequence (as identified in the pig lactase promoter) and the SIF1 sequence (as identified in the mouse and human sucrase-isomaltase promoters). Both of these DNA sequences competed for the same nuclear factors, which were present only in Caco-2 extracts, and the binding factors, NF-LPH1 and SIF1-BP, were found to be of a similar size: 50 kDa. Thus, it was concluded that the longest homologous sequence within SIF1 and CE-LPH1, i.e., ATTTT, could bind a similar 50-kDa factor, which is able to confer tissue specificity to the expression of both lactase and sucrase-isomaltase. However, now that the tissue specificity of the expression of these genes may be at least partly resolved, the very different developmental expression of these genes (discussed in the next section) still awaits elucidation.

B. Regulation of Developmental Expression of Lactase and Sucrase-Isomaltase

The tissue specific gene expression patterns of lactase and sucrase-isomaltase is very similar: both enzyme complexes are confined to the enterocytes of the small intestine. However, the developmental expression of lactase and sucrase-isomaltase are very different, and in most aspects completely opposite: lactase is strongly up-regulated before birth, and declines during weaning (only in some Caucasian humans does lactase remain high), remaining low during adulthood, while sucrase-isomaltase is not present until weaning, but thereafter remains at high levels during adult life. Instead of describing these well-known phenomena (reviewed elsewhere, Semenza and Auricchio, 1989), we will concentrate on the identification of the mechanisms underlying the developmental expression patterns of lactase and sucrase-isomaltase.

1. Regulation is Primarily Transcriptional

It is widely accepted that the regulation of lactase and sucrase-isomaltase expression is primarily transcriptional. A large number of studies, in man, rabbit, rat, sheep, and pig have described the mechanism of regulation of lactase gene expression, and found that the lactase expression correlates well with the amount of lactase mRNA (Büller et al., 1990a; Escher et al., 1992; Duluc et al., 1993; Lacey et al., 1994; Rings et al., 1992b; Keller et al., 1992; Rings et al., 1994c; Harvey

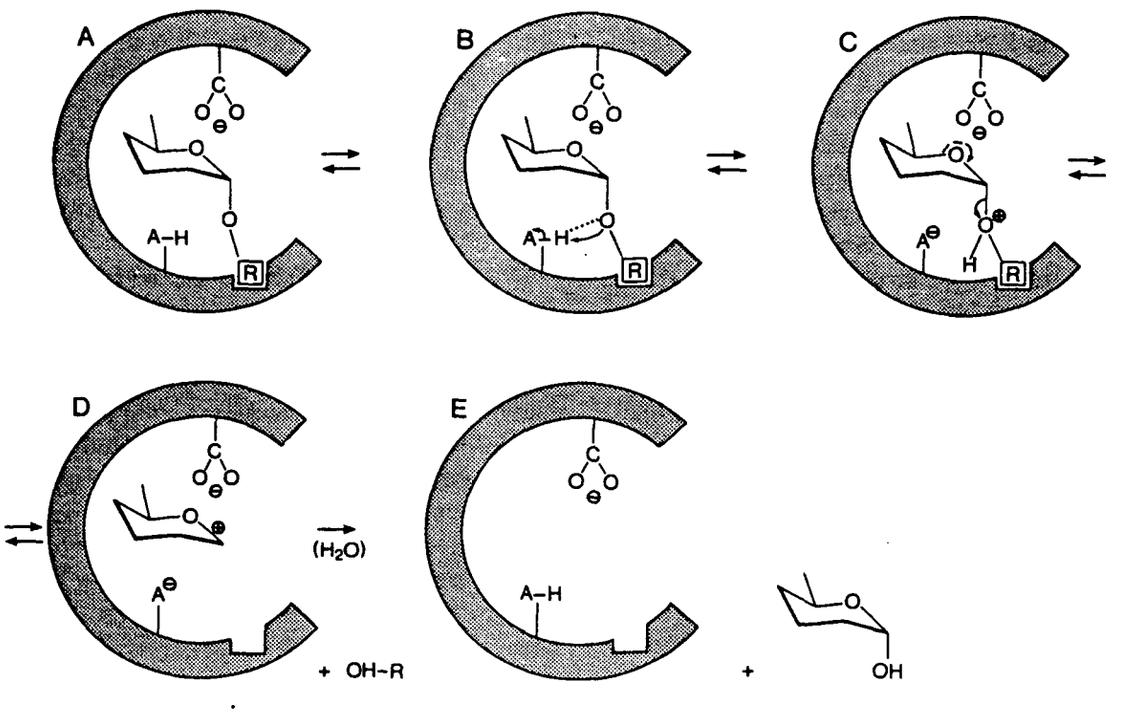

FIGURE 6. The minimal catalytic mechanism of brush border glycohydrolases (After Semenza, 1987). This figure gives the outline of the catalytic mechanism, as demonstrated for sucrase-isomaltase (i.e., α-glucosidase activity). This mechanism likely also applies to lactase and maltase-glucoamylase, but is most likely not applicable to trehalase (For reasons indicated in Section IV.B). Arguments indicating that the catalytic mechanisms are quite similar for brush border glycohydrolases include: 1. similar pH-curves, 2. competitive inhibition by the non-protonated form of tris, nojirimycin, as well as deoxynojirimycin, 3. irreversible inactivation by the substrate analog CBE, and 4. retention of the C1-configuration of the liberated glucan-moiety after hydrolysis. (A) The substrate is bound to the active site of the enzyme. The a-glycan moiety (indicated as (R) is bound to the enzyme, outside the active site of the enzyme. (B) The glucosyl oxygen is protonated by a proton-donor (indicated as -A-H, presumably a glutamyl-γ-COOH). (C) The positive charge on the glucosyl-oxygen is relayed towards the C1-atom of the glucosyl residue, the bond between the glucosyl C1-atom and the oxygen splits, and the a-glycan (R-OH) is liberated from the enzyme as the first product. (D) The resulting glucose oxocarbonium ion is temporarily stabilized by a negative charge (aspartate-β-COO^-). (E) Finally, the oxocarbonium ion is substituted by a nucleophile (OH^-), from the same side, from which the a-glycan left. Thus, α-glucose is liberated, with the same configuration at the C1-atom as in the original substrate.

et al., 1995; Torp et al., 1993; Villa et al., 1992; Krasinski et al., 1994). Similar studies demonstrated that sucrase-isomaltase expression in mouse, human and rat is also primarily transcriptionally controlled (Markowitz et al., 1993; Traber, 1990; Traber et al., 1992a; Krasinski et al., 1994; Rings et al., 1994c). Transcriptional control of lactase and sucrase-isomaltase was also found in the Caco-2 cell line (Beaulieu and Quaroni, 1991; Chantret et al., 1994; Hauri et al., 1994; Van Beers et al., 1995).

Lactase expression in humans is polymorphic: two phenotypes can be distinguished, one expressing lactase at high levels throughout lifetime, and a second expressing lactase only during early childhood, and with low levels during adult life. As recognized by Sahi et al. (1973) and reviewed by Flatz (1987), lactase persistence during adult life is an autosomal dominant trait, primarily found in Caucasians, while the adult-type hypolactasia is the predominant phenotype, identified in about 75% of the human population.

Moreover, the ability to hydrolyze lactose during adult life has been found to be a concordant trait among monozygotic twins (Metneki et al., 1984). It should be noted that lactase is never turned off completely; all adult mammals display low, but significant, lactase activity (Section IX). In fact, the adult-type hypolactasia represents the common phenotype as found in all other mammals studied. As mentioned above, human lactase levels have been shown to be regulated transcriptionally: low lactase activity in humans is strictly correlated with low mRNA levels (Escher et al., 1992; Fajardo et al., 1994; Harvey et af., 1995; Lloyd et al., 1992). However, it was found that there were no sequence differences in 1 kb of the human lactase gene promoter with respect to lactase persistence and adult-type hypolactasia (Lloyd et al., 1992). Thus, neither the structural part of the gene (Boll et al., 1991) nor the promoter of the lactase gene (Lloyd et al., 1992) shows consistent differences between the two lactase phenotypes in humans. Therefore, it is likely that the phenotype of lactase expression is not linked to the lactase gene itself. Instead, it may well be that the inheritance of adult-type hypolactasia is linked to a transcription factor, regulating human lactase expression.

The expression of sucrase-isomaltase and lactase is confined to the villus enterocytes. During epithelial cell migration from the crypt to the villus, sucrase-isomaltase and lactase mRNAs did not appear until at the crypt-villus junction, concomitant with the appearance of both enzyme activities and sucrase-isomaltase and lactase polypeptides (Rings et al., 1992b; Rings et al., 1994c; Hauri et al., 1980; Hoffman and Chang, 1991; Traber, 1990; Traber et al., 1992a; Markowitz et al., 1993). Interestingly, lactase and sucrase-isomaltase mRNA expression is maximal in the lower half of villus, while the level gradually declines towards the villus tip. This was observed in rabbit intestinal epithelium for lactase mRNA, while in the same study a very similar pattern of expression was found for SGLT1 mRNA (Freeman et al., 1993). Furthermore, maximal expression of sucrase-isomaltase mRNA was found in the rat as well as in human small intestine (Traber, 1990; Traber et al., 1992a). Also in studies from our laboratories, it was found that the mRNAs of rat lactase and sucrase-isomaltase were restricted after birth to the basal part of the villi (Rings et al., 1994c). However, the lactase mRNA in embryonic epithelium was found along the entire length of the rudimentary villi (Rings et al., 1992b). Thus, it seems that in fully functional villi, the transcription of the genes encoding brush border proteins are turned on 1 to 2 d before the cells are removed from the epithelium at the villus tip.

Starvation of 12-d-old rats induced the precocious expression of sucrase-isomaltase, while the lactase expression was virtually unaffected (Nsi-Emvo et al., 1994). It appeared that this enhanced sucrase-isomaltase expression was independent of cell proliferation, and was regulated at the transcriptional level. Like in normally fed adult rats, sucrase-isomaltase expression in starved rats was first detected in enterocytes at the crypt-villus junction in starved rats. However, when (14-d-old) rats were refed after 2 d of starvation, the migrating enterocytes at the crypt-villus junctions stopped expressing sucrase-isomaltase, resulting in a band of sucrase-isomaltase positive enterocytes migrating towards the tip of the villus (Nsi-Emvo et al., 1994). Thus, enterocytes passing the crypt-villus junction are apparently not able to down-regulate the gene expression, once committed to sucrase-isomaltase expression. This suggests a "point-of-no-return" principle for the up-regulation of brush border glycohydrolases at the crypt-villus junction.

Thus, in all cases, during developmental expression, in adult human lactase expression, in cell lines, and in crypt-villus expression, the expression of lactase and sucrase-isomaltase appears to be primarily at the transcriptional level. This can be well illustrated by a series of experiments, performed in our laboratories, which described the expression of lactase and sucrase-isomaltase in the developing rat (Rings et al., 1992b; Rings et al., 1994c; Krasinski et al., 1994). These studies were conducted at the histochemical level (enzyme histochemistry, immunohistochemistry, and *in situ* hybridization) and biochemical level (enzyme activity, mRNA and pre-mRNA measurement by RNase protection assay, and polypeptide measurement by rocket electrophoresis). They indicated that in all segments of the intestine, during all stages of development (16 embryonic days to adult), the levels of lactase and sucrase-isomaltase activity were directly correlated with

the levels of the respective mRNAs (Figure 7). Interestingly, a restriction of the lactase expression was noticed after weaning in the proximal-to-distal axis of the intestine (Figure 8). Peak levels of lactase expression were found in the middle part of the jejunum. Thus, apart from the general decline of the lactase activity in the intestine, the functional area of lactase expression also declines dramatically. The combination of the decline and restriction of lactase expression has undoubted consequences for the lactose digesting capacity of the intestine. Furthermore, this implies that the high lactase expression in lactase persistent in adult humans is primarily a failure to switch off the lactase gene.

2. Post-Translational Control Is Secondary

Apart from the primary regulation of lactase and sucrase-isomaltase at the transcriptional level, there are a number of indications for secondary post-transcriptional regulatory mechanisms. In

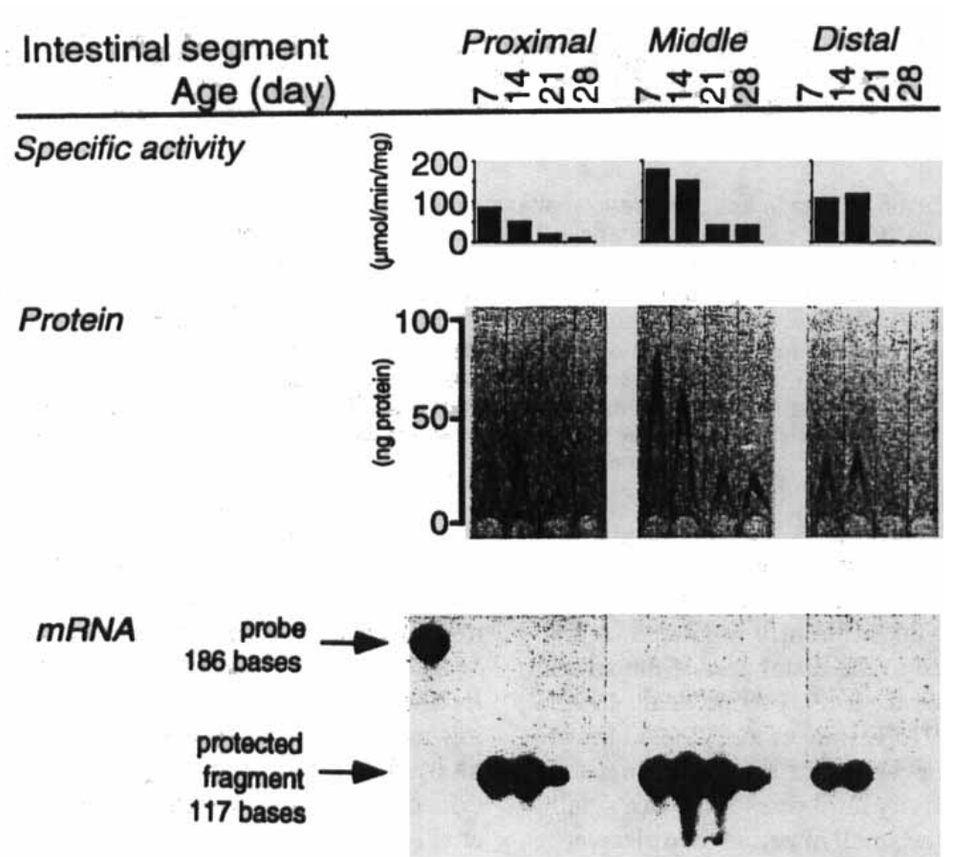

FIGURE 7. Coordinate relationship between lactase specific activity, lactase protein and lactase mRNA abundance during postnatal development in the small intestine of the rat. Expression of lactase was determined as enzyme activity, protein quantity (rocket immunoelectrophoresis), and mRNA abundance (RNase protection assay). Proximal segments were taken 1 to 2 cm distal to the pylorus; middle sections were taken from the geometrical middle of the intestine; distal sections were taken 1 to 2 cm proximal to the ileocecal junction. The amounts of enzyme activity, protein content, and mRNA quantity show closely parallel patterns at any age and at any segment of the small intestine, indicating transcriptional regulation as the major factor determining lactase activity during rat development. Reproduced with permission from Krasinski et al. (1994).

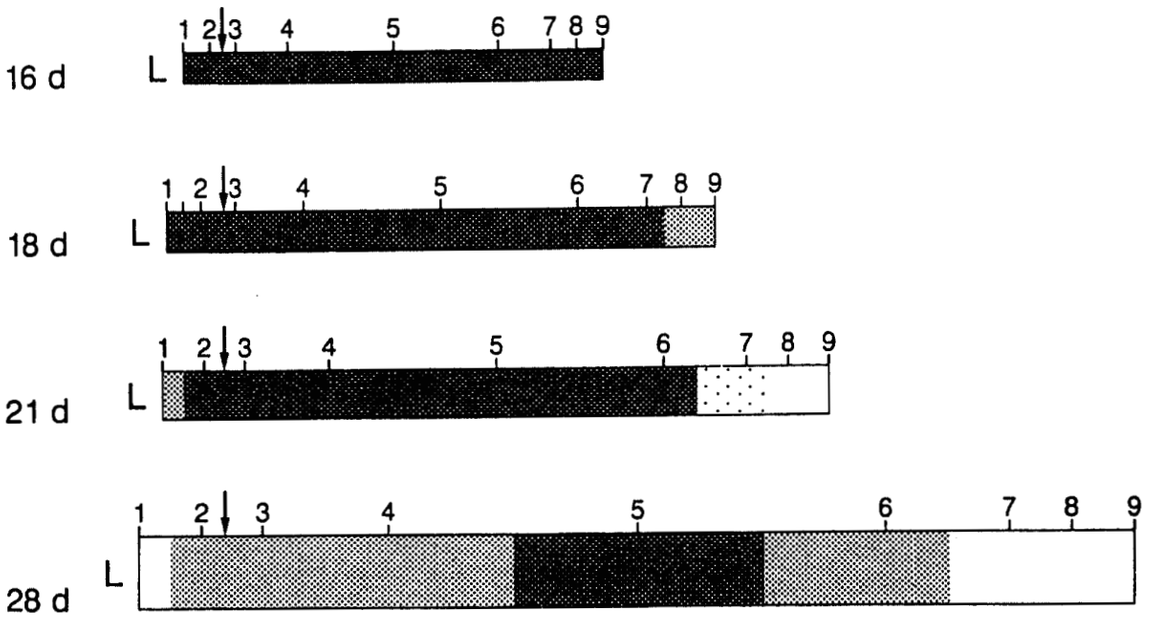

FIGURE 8. Distribution of lactase and sucrase-isomaltase proteins along the length of the rat small intestine. The expression of lactase (L) and sucrase-isomaltase (S) was determined at various postnatal ages (in days) by immunohistochemistry using specific monoclonal antibodies. Numbers 1 to 9 indicate the location of the small intestinal segments examined. The lactase and sucrase-isomaltase distribution along the length of the small intestine is indicated by different shadings. Changes occuring between 2 points are arbitrarily shown at mid-point. The length and width of the bars represent the relative size of the small intestine at these ages. Shadings: white, no expression (as in Figure 9c); light shading, mosaic expression (as in Figure 9d); middle dark shading, expression in about half of the enterocytes (as in Figure 9b); dark shading, expression in all villus enterocytes. The arrow indicates the ligament of Treitz. Reproduced with permission from Rings et al. (1994c).

some studies, lactase mRNA quantity is greater than the respective enzyme activity. For instance, some investigators feel that in the developing rat, lactase appears to be differently regulated in the proximal compared to the distal part of the small intestine (Freund et al., 1989; Freund et al., 1990; Freund et al., 1991; Duluc et al., 1993). From these studies it appeared that after weaning, the lactase mRNA/activity ratio was higher in a small distal segment of the small intestine then elsewere along the intestine. Other studies also indicated that the rat lactase mRNA/activity ratio increased during ageing of the rats (Nudell et al., 1993). In rabbits, the rate of biosynthesis of both lactase and sucrase-isomaltase correlated well with their respective mRNA levels. However, in proximal parts of the small intestine there was an excess of lactase mRNA, relative to the lactase activity, while the converse was true for sucrase-isomaltase (Keller et al., 1992).

The most likely explanation for the relative paucity of enzyme activities of lactase and/or sucrase-isomaltase relative to their respective mRNAs can be found in differences in turnover of the enzymes on the enterocyte. The half-life of lactase and sucrase-isomaltase activities in ageing mammals was generally found to decrease, likely due to enhanced degradation or inactivation of the enzymes (Quan et al., 1990; Rossi et al., 1993b; Tsuboi et al., 1992; Dudley et al., 1992; Castillo et al., 1990). The brush border glycohydrolases have different sensitivities towards luminal contents of the intestine. Sucrase and maltase activities were relatively insensitive to luminal proteinases, while lactase activity was rapidly lost after incubation with proteinases (Young and Das, 1990). Elastase is the most potent in releasing the enzyme from the plasma membrane. However, there was no correlation between the rate of release and the rate of enzyme

inactivation, as the kinetics of release of lactase, maltase, and sucrase activities were similar (Young and Das, 1990). This indicates that released brush border enzyme sucrase-isomaltase, still contributed to the luminal digestion of disaccharides, whereas lactase activity is lost upon release from the membrane. Studies by Seetharam et al. (1980) indicated that the increased turnover of brush border enzymes in the neonatal rat was paralleled by the intracellular increase of lysosomal proteinases, particularly cathepsin B. Thus, apart from luminal factors, such as proteinases, intracellular turnover of brush border enzymes may partly determine the levels of glycohydrolase activity on the brush border. In Caco-2 cells different half-lives were found for sucrase-isomaltase and lactase, indicating that the half-life of these enzymes are independently regulated by enterocytes (Van Beers et al., 1995; Beaulieu and Quaroni, 1991). Thus, regulation of sucrase-isomaltase and lactase activities is most likely primarily at the transcriptional level, and to a minor degree at the post-translational (protein stability) level. From the very different developmental expression patterns in all models studied, it appears that the various mechanisms of regulation of lactase and sucrase-isomaltase operate independently.

3. Effects of Hormones on Expression

Despite the unequivocal finding that sucrase-isomaltase as well as lactase expression patterns are imprinted during embryonic development (Section II), hormones are found to exert influence on the expression of these enzymes, especially during pre-weaning development. Generally, pituitary and adrenal cortical hormones enhance intestinal maturation during post-natal development. Deprivation of thyroxine and growth hormone, by hypophysectomy in 6-d-old rats, virtually stopped intestinal maturation, and lactase activity remained abnormally high, while sucrase isomaltase activity developed only partially during the ensuing 20 d (Castillo et al., 1991). Supplementation with thyroxine and growth hormone restored normal developmental patterns of intestinal development as well as both glycohydrolases. In another study, cortisone coordinately increased levels of both lactase and sucrase-isomaltase activities as well as the corresponding mRNA levels in 6 d old rats (Yeh et al., 1991a; Yeh et al., 1991b). Administration of thyroxine alone had little effect on either enzyme. While thyroxine enhanced the induction of sucrase-isomaltase by cortisone, it antagonized the enhancing effect of cortisone on lactase expression (Yeh et al., 1991a; Yeh et al., 1991b). Administration of triiodothyronine in adult rats led to the development of villus hyperplasia and down-regulation of lactase mRNA, while sucrase-isomaltase mRNA was not affected (Hodin et al., 1992). Gutschmidt and Emde (1981) demonstrated by careful morphometrical studies, that in adult rat thyroxine specifically diminished the amount of lactase, while the crypt-villus architecture was not affected. Similarly, Raul et al. (1984) demonstrated that total parenteral nutrition led to increased lactase levels in adult rats, while supplementation with thyroid hormones was able to restore intestinal lactase levels. In neonatal rats, Liu et al. (1992) demonstrated that in the absence of thyroxine lactase activity remained elevated and failed to decline at weaning, while the half-life of the enzyme was approximately doubled. Injection of thyroxine in rat pups accelerated enterocyte migration, while this migration was reduced in hypothyroid rats (Liu et al., 1992). Thus, thyroid hormones exert their actions on the expression of brush border glycohydrolases by effects on growth as well as on differentiation of the intestinal epithelium.

Glucocorticoids stimulate the expression of sucrase-isomaltase in suckling rats (10 to 15 d old), while the glucocorticoid antagonist RU-38486 impedes sucrase-isomaltase expression in the developing intestine (Hauri et al., 1980; Foltzer-Jourdainne et al., 1993; Galand, 1988; Nsi-Emvo et al., 1994). In adult diabetic rats, the sucrase-isomaltase mRNA as well as activity level are increased, indicating a stimulatory role for insulin in the biosynthesis of sucrase-isomaltase (Hoffman and Chang, 1992).

Receptors for epidermal growth factor (EGF) have been demonstrated on the enterocytes of the human fetal gut (Menard and Pothier, 1991). EGF was shown to enhance intrauterine intestinal maturation and lactase expression in fetal rabbits (Buchmiller et al., 1993a; Buchmiller et al., 1993b). Cross and Quaroni (1991) found that EGF enhanced the proliferation of Caco-2 cells. Inde-

pendent of this growth stimulation, sucrase-isomaltase mRNA and biosynthesis levels were greatly reduced in 12 d confluent Caco-2 cells, while other brush border enzymes were unaffected, indicating a selective down-regulation of sucrase-isomaltase (Cross and Quaroni, 1991). Also in Caco-2 cells grown under serum-free conditions, EGF (as well as triiodothyronine) inhibited sucrase-isomaltase expression (Jumarie and Malo, 1991). In contrast, EGF was able to induce precocious sucrase-isomaltase expression in suckling rats (Foltzer-Jourdainne et al., 1993).

EGF was found to have a very rapid and direct effect on the surface area of the brush border membrane (Hardin et al., 1993). Small intestinal loops of young rabbits were exposed *in vivo* to EGF and the effect on the brush border were measured. Within 10 min the absorptive area of the apical membrane was increased at least two-fold, which was mostly attributed to lengthening of the microvilli. This increase in membrane area was most likely due to redistribution of pre-existing microvillar membrane vesicles, as the composition of the microvillar membrane did not change during the increase of surface area (Hardin et al., 1993).

4. Caco-2 Cells are a Very Valuable Model

Caco-2 cells were originally isolated from a colonic adenocarcinoma; however, these cells display virtually all characteristics of small intestinal enterocytes (Pinto et al., 1983; Section II). Some of the aspects of hormonal control of sucrase-isomaltase and lactase expression have been evaluated in Caco-2 cells. Caco-2 cells, growing generally in the presence of 10 to 20% fetal calf serum, could be adapted to grow on a chemically defined medium, without loss of differentiation characteristics (Jumarie and Malo, 1991). Triiodothyronine increased the expression of sucrase-isomaltase in these cells to some extent, but hydrocortisone had no effect. As these hormones had no major effects on sucrase-isomaltase expression in Caco-2, the effects of these hormones measured *in vivo* may be secondary. Two independent studies in Caco-2 cells found that forskolin (an activator of adenylate cyclase) inhibited sucrase-isomaltase expression, while enhancing the lactase expression. Rousset et al. (1989) found that diminishing the cAMP content of Caco-2 cells affected the sucrase-isomaltase synthesis at three levels: 1. sucrase-isomaltase mRNA decreased, 2. biosynthesis was impaired, and 3. transport to the microvillar membrane was completely inhibited. These effects were reversible, but the experiments indicate that cAMP depletion has independent effects on these three levels of sucrase-isomaltase expression (Rousset et al., 1989). More recently, Hauri et al. (1994) confirmed these results of sucrase-isomaltase expression, and compared it with the effects of forskolin on lactase expression in Caco-2 cells. In contrast to effects on sucrase-isomaltase, lactase expression appeared to be induced by cAMP depletion, and this effect was primarily exerted at the transcriptional level. Moreover, hydrocortisone had opposite effects on sucrase-isomaltase and lactase expression: lactase was inducible by hydrocortisone, while sucrase-isomaltase was inhibited. Hydrocortisone may work via an indirect mechanism, as the effects were only measurable after several days (Hauri et al., 1994). The developmental impact of the opposite effects of cAMP on lactase and sucrase-isomaltase expression can hardly be overestimated. The expression patterns of lactase and sucrase-isomaltase during development in animals are mirror images: lactase is switched off at weaning, while sucrase-isomaltase is turned on. Therefore, it seems plausible that one factor, which would lower the cAMP concentration in enterocytes, could up-regulate lactase as well as down-regulate sucrase-isomaltase during development. Further confirmation on this point is obviously required.

5. Mosaic Expression

An interesting yet unexplained finding in the cellular expression of lactase during development is the mosaic pattern found within the intestinal epithelium. In rat, human and rabbit intestinal epithelia occasionally individual cells can be found with very high lactase expression, while the surrounding enterocytes seem devoid of any lactase (Maiuri et al., 1991; Maiuri et al., 1992; Lorenzsonn et al., 1993; Rings et al., 1994c).

Sometimes, the lactase positive cells are found grouped together, and this has been termed "patchy" expression. Generally, this mosaic expression is found at the proximal and distal boundaries of lactase expression, in zones of the intestine with low lactase activity (Maiuri et al., 1992; Rings et al., 1994c; Figure 9). In duodenal biopsies of Caucasians with low lactase expression, mosaic lactase expression has been found (Maiuri et al., 1991; Lorenzsonn et al., 1993; Maiuri et al., 1994). However, as the expression of lactase in the duodenum is generally low, this mosaic expression likely represents the proximal transition zone of lactase expression. Duodenal biopsies are often used clinically to detect lactase deficiency. Obviously, as lactase is generally lower in this region, mosaicism must be very carefully interpreted. Furthermore, mosaic expression of brush border glycohydrolases may be induced by damage to the intestinal epithelium. In some individuals with mild villus atrophy, sucrase-isomaltase, maltase-glucoamylase, as well as lactase were found to be mosaically expressed (Nichols et al., 1992). Maiuri et al. (1993b) compared the surface

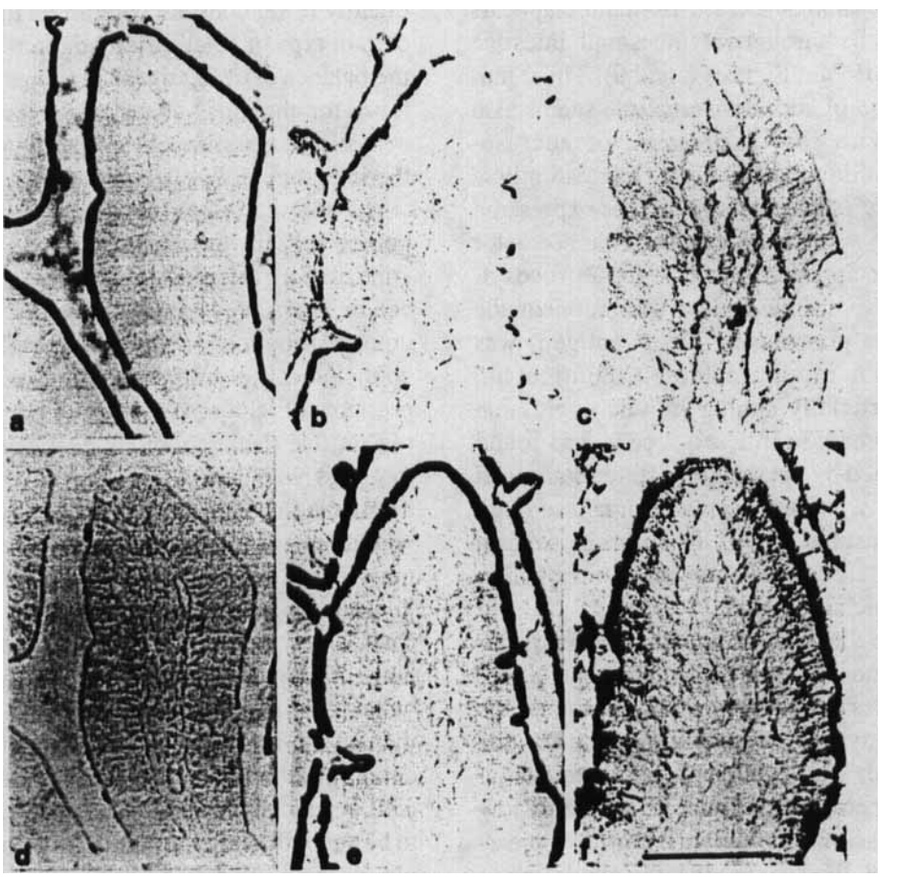

FIGURE 9. Mosaic expression of lactase and sucrase-isomaltase around weaning in the proximal small intestine of the rat. The expression of sucrase-isomaltase and lactase was detected immunohistochemically, using specific monoclonal antibodies: Panel a to c, lactase expression; panel d to f, sucrase-isomaltase expression. Notice that lactase and sucrase-isomaltase expression was determined in adjacent sections. (a) At 16 days, lactase is expressed in all enterocytes along the villus. (b) Mosaic expression of lactase is observed at 21 days. (c) Lactase is no longer detectable at 28 days. (d) Mosaic expression is found for sucrase-isomaltase at 16 days. (e) At 21 days, sucrase-isomaltase is present along the entire villus. (f) At 28 days sucrase-isomaltase is detected at the entire villus, although the cells at the extreme villus-tip are only faintly stained. Reproduced with permission from Rings et al. (1994c).

staining pattern of enterocytes of whole rabbit and human villi. They found that the expression on the human villi was patchy, i.e., discrete patches of lactase positive cells were found, whereas the lactase-positive enterocytes in rabbit were found as ribbons along the villi. Therefore, the regulation of the mosaicism of lactase in human and rabbit may be fundamentally different: in rabbit it likely has a clonal origin (i.e., lactase-positive cells may all be daughter cells of a certain stem cell), while in humans mosaicism may arise as consequence of other mechanisms.

Similar to lactase, mosaic expression was also observed for sucrase-isomaltase in 16-d-old rats: The first expression of sucrase-isomaltase appears in isolated cells throughout the small intestine (Rings et al., 1994c; Figures 8 and 9). Thus, mosaic expression of sucrase-isomaltase seems also associated with low expression of sucrase-isomaltase within the epithelium. Interestingly, a similar mosaic pattern was found for expression of bloodgroup A by enterocytes of A nonsecretor individuals. It appeared that the α(1-2) fucosyltransferase responsible for the formation of the H-antigen (the precursor of the A-antigen) was expressed in a mosaic pattern (Maiuri et al., 1993a). Superficially similar mosaic expression of sucrase-isomaltase in Caco-2 cells was found to be determined by the state of differentiation: all cells eventually express sucrase-isomaltase (e.g., Vachon and Beaulieu, 1992). Thus, this mosaicism in Caco-2 cells is most likely of another nature than the mosaicism *in vivo*.

Presently, there is no unequivocal explanation for the mosaic expression of brush border glycohydrolases in enterocytes. Furthermore, it is very hard to envisage a specific function for this phenomenon. It may well be an epiphenomenon, as it only appears in regions of generally low enzyme expression, in otherwise normal appearing enterocytes. We propose that the regulation of the mosaic glycohydrolase expression may resemble the regulation of the relative amounts of the various cell types in the intestinal epithelium. In fact, the occurrence of goblet cells and enteroendocrine cells in the epithelium is mosaic as well, although nobody refers to this as mosaic expression. These two terminally differentiated cell types occur, seemingly random, throughout the epithelium. As indicated above, the up-regulation of glycohydrolases at the crypt-villus junction represents also a terminal differentiation characteristic, as the enterocytes once expressing these enzymes do so for the rest of their (short) lives. The random differentiation toward a goblet cell, enteroendocrine cell, or glycohydrolase positive enterocyte may be explained by similar hypothetical mechanisms, by what we would like to refer to as the "stimulus/threshold mechanism". The commitment toward a certain final differentiation may be evoked by reaching a threshold level of a hypothetical stimulus. If this stimulus is diminished, increasingly fewer cells are exposed to threshold levels of the stimulus, and consequently fewer cells are committed to undergo this certain type of final differentiation. For instance, the balance between threshold value and stimulus level for the final commitment towards goblet cell differentiation may be such that only 5% of the immature epithelial cells in the crypt make it to the threshold level: thus, there would be 5% goblet cells in the epithelium. As virtually all entities in nature show a stochastic distribution, it can be envisioned that this would result in a random distribution of the goblet cells within the epithelium. Extending this discussion to the expression of glycohydrolases, it is important to realize that their random appearance is confined to zones with low expression. Consequently, if the threshold value for glycohydrolase expression would remain unaltered, a lower level of stimulus may cause random up-regulation of glycohydrolases in only a small number of enterocytes. The same commitment to differentiation could alternatively be initiated by a higher threshold value at an unaltered stimulus. Although the actual stimuli as well as the mechanisms for differentiation into either goblet cell, enteroendocrine cell, or glycohydrolase expressing cell are likely to be very different, the proposed "stimulus/threshold mechanism" may be applicable to any variety of cell type specific differentiation.

6. Effects of Substrates on Enzyme Activities are Minimal

The expression of brush border glycohydrolases is developmentally imprinted. Nevertheless, there still is a role for carbohydrate sub-

strates in the regulation of the level of the individual enzymes. Lactase activity is hardly affected by the presence of its substrate, while only a slight inhibitory effect has been shown for glucose (reviewed in Rings et al., 1994d); administration of lactose does not elevate lactase specific activity. In adult rabbits and rats no adaptation of lactase activity was observed in the presence of milk or lactose in the diet (Plimmer, 1906). However, it has been reported that, irrespective of its source, a high concentration of glucose slightly increases lactase activity in rat (Goda et al., 1985). The level of lactase expression is not influenced by the presence of high amounts of glucose or fructose in the diet, while these monosaccharides were shown to slightly induce sucrase-isomaltase (Collins et al., 1989). The independence of lactase development from substrate supply is emphasized by the fact that lactase gene expression is increased prior to birth, and high levels of activity are present at birth, in the complete absence of lactose. Thus, all evidence suggests that the level of lactase activity is genetically determined; only minor control may be exerted by glucose, but no regulation is exerted by lactose. In humans neither prolonged ingestion of lactose nor elimination of lactose from the diet altered lactase activity (Kogut et al., 1967; Gilat et al., 1972).

Sucrase-isomaltase is more prone to substrate regulation than lactase (reviewed by Henning, 1981; Henning, 1985; Bustamante et al., 1986). A decreased intake of starch led to a decrease in all brush border glycohydrolases, indicating a specific adaptation to low carbohydrate intake, as brush border peptidases were not affected (Goda et al., 1983). Starvation had different effects on jejunal glycohydrolases: lactase activity remained virtually unchanged, while sucrase-isomaltase activity increased, due to enhanced and impaired polypeptide synthesis, respectively (Holt and Yeh, 1992). The up-regulation of sucrase-isomaltase by dietary sucrose was clearly a specific process that increased the amount of active enzymes per cell, as sucrose did not in any way change the cell migration rate, the number of enterocytes, or crypt-villus ratio (Ferraris et al., 1992).

The administration of galactose as sole carbohydrate source to mice led to a state of malnutrition, most likely due to the inability of this species to efficiently metabolize this compound (Smith et al., 1990). The malnutrition led to a decreased rate of enterocyte biogenesis, and these enterocytes showed strongly decreased lactase levels, while the sucrase-isomaltase levels were unaffected. Thus, lactase and sucrase-isomaltase were regulated by different mechanisms in the enterocytes of these malnourished mice.

Fructose has a peculiar effect on the expression of brush border enzymes. In cultured pig small intestinal explants, 10 to 50 m*M* of fructose prevented the expression of aminopeptidase N, and decreased expression of sucrase-isomaltase to about 20%. Fructose induced a very rapid degradation of newly synthesized enzymes, probably due to aberrant *N*-glycosylation (Danielsen, 1989). Apart from its effect on *N*-glycosylation, fructose perturbed the intracellular membrane traffic in the enterocyte. Thus, fructose or sucrose at physiological concentrations may induce dramatic changes in enterocyte function, by some not yet identified mechanism (Danielsen et al., 1991).

7. Expression in Colon in Health and Disease

From the above, it is clear that the expression of brush border glycohydrolases is generally confined to the small intestinal enterocytes, and that the physiologically important functions of these enzymes are exerted in the small intestine. However, expression of these enzymes in the colon is also a common phenomenon in early development or under specific conditions. In suckling rat, expression of lactase is found in the colon, which was biochemically indistinguishable from jejunal lactase. In these young animals, the colonic epithelium of the rat has a crypt-villus architecture, which disappears towards weaning, and expression was confined to these rudimentary colonic villi (Büller et al., 1989b; Colony et al., 1989). The expression of lactase in rat was highest in the proximal colon, while adult rats express low levels of lactase in the colon, but not sucrase-isomaltase (Colony et al., 1989; Foltzer-Jourdainne et al., 1989). Although sucrase-isomaltase is normally not expressed in the rat colon, expression could be evoked in the colon by treatment of 4-d-old rats with thyroxine and

hydrocortisone, suggesting that induction of jejunal characteristics is possible in young rat colon (Foltzer-Jourdainne et al., 1989).

In humans, low levels of sucrase-isomaltase are expressed in the adult colon, while higher amounts are present in the fetal colon (Beaulieu et al., 1990; Gorvel et al., 1991; Real et al., 1992; Andrews et al., 1992). The colonic sucrase-isomaltase is biochemically indistinguishable from the jejunal enzyme (Gorvel et al., 1991). There is a marked over-expression of sucrase-isomaltase in colonic polyps and in adenocarcinomas (Real et al., 1992; Beaulieu et al., 1990). Lactase and maltase-glucoamylase are usually absent from the adult colonic epithelium, and are only seldom expressed in colorectal cancer, but are markedly up-regulated in transitional tissue next to these cancers (Real et al., 1992). There is also an increase in sucrase-isomaltase expression in ulcerative colitis and dysplasia resulting from this chronic inflammatory bowel disease (Andrews et al., 1992).

Under the above-mentioned pathogenic circumstances, the epithelial cells are in a state of hyper-proliferation and concomitant de-differentiation. Also, many human colonic cell lines, such as Caco-2 and HT29, express sucrase-isomaltase and sometimes lactase. These cell lines, usually derived from adenocarcinomas, are known to acquire small intestinal-like characteristics. Fetal colon resembles small intestine in many aspects, e.g., there is a crypt-villus morphology and expression of brush border enzymes. As lactase and sucrase-isomaltase are expressed in the fetal colon, the expression of these enzymes in hyperplastic and carcinoma tissues and in affiliated cell lines, may logically result from this state of de-differentiation. Thus, the expression of lactase and sucrase-isomaltase may be regarded as fetal characteristics of colonocytes.

IX. PRIMARY AND SECONDARY SUCRASE-ISOMALTASE AND LACTASE DEFICIENCIES

By their nature, deficiencies in sucrase-isomaltase or lactase are serious disorders. Substrates to these enzymes, lactose in early childhood, and sucrose and maltose in adult life form very important elements in the diet. From an evolutionary point of view, there should be selection against mutations in these genes, as their inactivation would most probably have been lethal. As a result, primary glycohydrolase deficiencies are very rare today. Complete absence of glycohydrolase activity or ectopic expression in other organs or aberrant cellular domains are hardly encountered. Secondary glycohydrolase deficiencies are caused by diseases affecting the intestinal epithelium, thereby diminishing the number of functional enterocytes and thus, enzyme activities. Secondary deficiencies are much more common than primary deficiencies, as diseases affecting the intestinal epithelium are among the most common human disorders. As the clinical manifestations of either type of deficiency are very similar, the symptomatology of the (very rare) primary sucrase-isomaltase and lactase deficiencies will be discussed together with the secondary deficiencies in this section.

A. Primary Deficiencies Are Rare

Primary deficiencies in sucrase-isomaltase and lactase become only clinically apparent if a subject lacks a functional enzyme. As the sucrase-isomaltase and lactase deficiencies have a recessive phenotype, a patient must possess two defective alleles of a gene to give clinical symptoms. Congenital lactase deficiency is very rare, and only very few families with this disorder were found (Savilahti et al., 1983; Mobassaleh et al., 1985). Lactase activity was completely absent in these individuals, but this defect has not been elucidated at the molecular level. In contrast, primary sucrase-isomaltase deficiency, which is somewhat less rare (2% of white American subjects may be heterozygote; Welsh et al., 1978), was studied at the molecular level. Sucrase-isomaltase deficiency is inherited as an autosomal recessive trait, and at least six different phenotypes were defined: I. Arrest of pro-sucrase-isomaltase in the RER; II. Arrest and degradation of pro-sucrase-isomaltase in the Golgi apparatus; III. Catalytically altered enzyme in the brush border membrane, and partial missorting to the basolateral membrane; IV. Missorting to basolateral membrane, and accumulation in the RER;

V. Intracellular loss of sucrase subunit, and expression of isomaltase subunit at the brush border membrane; VI. No immunoreactive protein detectable (Louvard et al., 1992). Thus, most genetic defects seem to lead to changes in the primary amino acid sequence of sucrase-isomaltase. The resulting mutant proteins display a broad variety of aberrant intracellular sorting and/or premature degradation. Together, these naturally occurring mutations provide us with a number of clues about the locations of the sorting signals within the protein. For example, study of sucrase-isomaltase biosynthesis of patients with phenotype V deficiency led to the conclusion that the apical sorting signal was located in the isomaltase subunit of the sucrase-isomaltase complex (as discussed in Section VII.A; Fransen et al., 1991). It is expected that future elucidation of the molecular defects in the other natural mutations will reveal important information about the intracellular sorting mechanisms of sucrase-isomaltase.

B. Adult Lactase Levels in Humans

A typical human phenomenon is often referred to as adult-type hypolactasia. We will only discuss this topic briefly, and refer to several recent reviews on lactase (Büller and Grand, 1990; Montgomery et al., 1991; Montgomery et al., 1993; Rings et al., 1994b). Subjects of Caucasian extraction and a few other ethnic groups express high levels of lactase as adults (Flatz, 1987). Normally, lactase is down-regulated around weaning in most mammals, when the developing animal changes from milk as the primary carbohydrate source to adult food. This switch in lactase expression is genetically determined, and is governed by a decreased transcriptional activity of the lactase gene (Section VIII); it occurs around the age of 5 years in humans. Thus, when adult humans (or other adult mammals) are tested for the ability to digest lactose, there is very high probability that "lactose deficiency" will be diagnosed. The term deficiency is, however, a misnomer, as low adult lactase activity is a natural condition in adult humans: the lactase persistence is in fact the aberrant phenotype. Therefore, we prefer use of the term "low adult lactase levels", when referring to low levels of lactase in human adults. It should be noted that lactase activity is rarely totally absent in adults, except for the very rare primary lactase-deficient condition. In animals, the total lactase activity in adults may be equal to that in pre-weaned neonates, but the activity is dispersed along a much greater surface area as the surface of the intestine grows substantially during development (Büller and Grand, 1990; Montgomery et al., 1991; Montgomery et al., 1993). Moreover, the region of lactase expression may be more restricted to the central part of the jejunum, as found in adult rats (Rings et al., 1994c).

C. Secondary Deficiencies

Secondary sucrase-isomaltase or lactase deficiency has a very similar symptomatology to primary deficiencies of these enzymes. As secondary deficiencies are due to damage of the intestinal epithelium, the causes and the effects of brush border glycohydrolase deficiencies are very similar, irrespective of the affected enzyme. The main symptom of deficiencies of either origin is diarrhea after intake of substrates for these enzymes. Primary defects in lactase or sucrase-isomaltase can be distinguished from the more general effects of secondary deficiencies, by their substrate specific symptomatology. Primary lactase deficiency will only give clinical symptoms after ingestion of lactose; while sucrose, starch, or maltose will give no symptoms. The converse is true for primary or secondary sucrase-isomaltase deficiency. The complaints of lactose maldigestion can be easily treated by avoiding lactose-containing food, or by hydrolyzing lactose by adding exogenous β-galactosidase (Rosado et al., 1984; Biller et al., 1987; Lami et al., 1988; Medow et al., 1990: Lin et al., 1993).

The common symptom in glycohydrolase deficiency is diarrhea, often resulting in malnutrition and dehydration. First, the loss of brush border hydrolases and transporters may lead to incomplete digestion and uptake of nutrients. These nutrients are fermented by colonic bacteria, which may result in diarrhea (Section II.C). Another type of diarrhea is secretory diarrhea, which is an active defense mechanism of the epithelium. This diarrhea is provoked by the active chloride secretion by crypt enterocytes, resulting in secretion of

water into the lumen, in order to drain toxic and noxious substances. Generally, the diarrhea seen in infectious diseases, such as rotavirus infection, is primarily caused by secretory diarrhea, while chronic diarrhea is coupled to non-infectious causes. Thus, secretory diarrhea is not necessarily coupled to epithelial damage, while chronic diarrhea is often coupled with epithelial damage. Clinically, the colonic fermentation of carbohydrates is used to assess malabsorption of these carbohydrates, which leads to the production of H_2, which can be measured in breath after ingestion of a test meal containing the saccharide in question (Hyams et al., 1980; Büller and Grand, 1990; Montgomery et al., 1991; Parnes et al., 1994; Rings et al., 1994b).

D. Diseases Leading to Secondary Glycohydrolase Deficiency

A large number of disorders affects the gastrointestinal tract, leading to loss of digestive and protective functions (Table 6). Most of these diseases are very common and each has a high incidence. Some of these diseases are chronic, e.g., celiac disease and Crohn's disease. Generally, the brush border glycohydrolases disappear from the epithelium with increasing villus atrophy. Sucrase-isomaltase, lactase and trehalase were severely affected in inflamed duodenal mucosa, and their activities were inversely proportional to the severity of the histological degree of inflammation (Jönsson and Bodemar, 1990). This inverse correlation between villus atrophy and lactase and sucrase-isomaltase activities was also clearly demonstrated in a very extensive survey of 798 small intestinal samples (Heitlinger et al., 1991). However, decreases in brush border glycohydrolase activities are not necessarily related to villus atrophy. In a clinical study, 41% of children with a *Giardia lamblia* infection displayed a generalized disaccharidase deficiency, in all cases affecting more than two brush border glycohydrolases, although the morphology of the small intestine was unaffected (Welsh et al., 1984).

Celiac disease, which is caused by an unexplained hypersensitivity towards α-gliadin from gluten, leads to a progressive villus atrophy in the small intestine. This has long been recognized to affect the ability to digest disaccharides (Arthur et al., 1966). The shortening of the villi in villus atrophy is accompanied by a proportional lengthening of the crypts. The morphological changes in the small intestinal mucosa lead to a progressive loss of brush border enzymes (Mercer et al., 1990; Lojda, 1981). Remarkably, each brush border enzyme is affected to a different extent; trehalase is always present along the entire villus,

TABLE 6
Diseases Affecting Epithelial Integrity of the Intestine

Disease	Cause	Affected organ	Effects on Epithelium	Cure
Celiac Disease	α-gliadin from gluten	Small intestine	Villus atrophy	Gluten-free diet
Gastroenteritis (infectious)	Rotavirus Bacterium Parasites	Gastrointestinal tract	Villus damage, Cl^--secretion	Rest, re-hydration (acute diarrhea), antibiotics (bact. and parasites)
Crohn's Disease	Unknown, may be partially immunological	Small intestine, colon	Villus atrophy, epithelial damage, lesions	Anti-inflammatory drugs, surgery
Ulcerative Colitis	Unknown, may be partially immunological	Colon (rarely in small intestine)	Epithelial damage, lesions	Anti-inflammatory drugs, surgery
Food Allergy	Food compounds	Small intestine	Villus damage	Change diet
Radiation/ chemotherapy	Stem cell damage	Gastrointestinal tract	Epithelial damage, villus Atrophy, Lesions	None

while it is very rapidly lost upon the first signs of subtotal villus atrophy (Mercer et al., 1990). Lactase gene expression also disappears rapidly from the small intestinal brush border upon damage, while sucrase-isomaltase (measured as isomaltase activity) only disappeared in the most severe cases of celiac disease (Mercer et al., 1990). In contrast, the presence of alkaline phosphatase, dipeptidylpeptidase IV, and aminopeptidase N was less affected by the state of villus atrophy or the severity of the disease. In this respect, the gene expression of the glycohydrolases differs fundamentally from the latter brush border enzymes.

Rotavirus infection is a very common cause of diarrhea in humans, both in developing and in Western countries. These viruses infect the villus enterocytes, particularly at the top of the villi. The effects of this infection on brush border glycohydrolases was demonstrated in a model study in mice by Collins and co-workers (1990). Developing mice of 7 to 14 d old were infected with rotavirus, which resulted in severe diarrhea in about a week. This infection was self-limiting, as all animals recovered from the diarrhea, but the animals did not develop significant villus atrophy. During the first 48 h post-infection, the lactase expression was unaffected, and the animals showed no diarrhea. Then, when diarrhea started, lactase expression declined concomitantly, recovering only slowly during the next five days. Alpha-glucosidase activity (sucrase-isomaltase and maltase-glucoamylase) developed first at 14 d in control mice, while precocious α-glucosidase activity was detected in 11-d-old mice, infected with rotavirus at day 8 (Collins et al., 1990). Thus, the rotavirus infection leads to down-regulation of lactase expression in the infected villus enterocytes of these developing mice, while an independent developmental adaptation leads to an accelerated appearance of sucrase-isomaltase and maltase-glucoamylase expression.

Cow's milk allergy offers another example of generalized secondary glycohydrolase deficiency, with clinical manifestations similar to celiac disease. Infants of 5 weeks to 11 months, with documented allergy to cow's milk protein, underwent small intestinal biopsy before and after withdrawal of milk proteins (Poley et al., 1978). When exposed to milk proteins all patients showed villus atrophy as well as depression of all glycohydrolase activities measured. After 3 to 5 weeks on a milk-protein-free diet all patients showed recovery of mucosal and epithelial architecture, while the activities of the brush border glycohydrolases increased concomitantly (Poley et al., 1978).

Villus atrophy can be accompanied by microvillus atrophy, as often observed in young children examined for glycohydrolase deficiency (Phillips et al., 1980). The causes of the disaccharide intolerance of these children was diverse, and the deficiencies were exclusively secondary. Apart from a mild to severe villus atrophy, the microvilli were very significantly shortened. Thus, the secondary glycohydrolase deficiency may in part be attributed to the inability of the enterocytes to produce the proper microvillar membrane. More recently, some forms of microvillus atrophy were established as distinct congenital disorders. These patients failed to form a proper apical brush border membrane, and instead seem to accumulate apical membrane compounds in "inclusion bodies" (Phillips et al., 1993). These patients showed normal biosynthesis of the brush border hydrolases, however very little of these enzymes were present on the apical membrane of the enterocytes, but indeed found in large amounts in these microvillous "inclusion bodies". Control experiments, studying lysosomal enzymes in the same enterocytes, demonstrated that the direct constitutive pathway of vesicle delivery is not disturbed in the affected enterocytes, suggesting that the delivery to the brush border involves another defect (Phillips et al., 1993).

Thus, the primary and secondary glycohydrolase deficiencies provide us with interesting data on the expression of these enzymes under specific circumstances. Moreover, these data may provide us with insight and experimental handles to manipulate the expression of brush border glycohydrolases. This may eventually lead to better clinical management of these deficiencies.

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