Histol Histopathol (2001) 16: 553-561

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Review

Molecular and cellular aspects and regulation of intestinal lactase-phlorizin hydrolase

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Summary. Carbohydrates are hydrolyzed in the intestinal lumen by specific enzymes to monosaccharides before transport across the brush border membrane of epithelial cells into the cell interior. The enzymes implicated in the digestion of carbohydrates in the intestinal lumen are membrane-bound glycoproteins that are expressed at the apical domain of the enterocytes. Absent or reduced activity of one of these enzymes is the cause of disaccharide intolerance and malabsorption, the symptoms of which are abdominal pain, cramps or distention, flatulence, nausea and osmotic diarrhea. Lactose intolerance is the most common intestinal disorder that is associated with an absence or drastically reduced levels of an intestinal enzyme, in this case lactase-phlorizin hydrolase (LPH). The pattern of reduction of activity has been termed late onset of lactase deficiency or adult type hypolactasia. It was thought that the regulation of LPH was post-translational and was associated with altered structural features of the enzyme. Recent studies, however, suggest that the major mechanism of regulation of LPH is transcriptional. Other forms of lactose intolerance include the rare congenital lactase deficiency and secondary forms, such as those caused by mucosal injury, due to infectious gastroenteritis, celiac disease, parasitic infection, drug-induced enteritis and Crohn's disease. This review will shed light on important strucural and biosynthetic aspects of LPH, the role played by particular regions of the LPH protein in its transport, polarized sorting, and function, as well as on the gene expession and regulation of the activity of the enzyme.

Key words: Lactase-phlorizin hydrolase, Biosynthesis, Sorting, Gene expression, Deficiency

Abbreviations: LPH: lactase-phlorizin hydrolase (all forms including pro-LPH); LDC: lactose digesting capacity; L/S: lactase/sucrase ratio; ER: endoplasmic reticulum; TGN: trans-Golgi network

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Introduction

Carbohydrates are essential constituents of mammalian diet which occur as oligosaccharides, such as starch and cellulose (plant origin), glycogen (animal origin) and as free disaccharides, such as sucrose (plant) and lactose (animal). The hydrolysis of these components in the intestinal lumen by specific enzymes to monosaccharides precedes further transport across the brush border membrane of epithelial cells into the cell interior. The enzyme specificity is determined by the chemical structure of the carbohydrates. Most of the known disaccharides are composed of monosaccharide units that are linked to each other in an α-orientation. Examples of this type are starch, glycogen, sucrose and maltose. ß-glycosidic linkages are minor, but are present in one of the most essential carbohydrates in mammalian milk, lactose, which is the primary diet source in the early stages after birth.

The digestion of carbohydrates in the intestinal lumen is achieved by a number of membrane-bound glycoproteins that are present at the apical or microvillus membrane of the enterocytes (Hauri et al., 1985; Naim et al., 1987, 1988). In this review the structural and biosynthetic features of the most important disaccharidases, sucrase-isomaltase and lactase-phlorizin hydrolase and the molecular basis of sugar malabsorption, i.e enzyme deficiencies will be discussed.

Structure, biosynthesis and polarized sorting of intestinal lactase-phlorizin hydrolase

Lactase-phlorizin hydrolase (LPH) (EC 3.2.1.23/62) is an integral membrane glycoprotein of the intestinal brush border membrane. Two major hydrolytic activities are realizd by this enzyme (Schlegel-Haueter et al., 1972; Colombo et al., 1973; Skovbjerg et al., 1981): lactase that hydrolyses the milk sugar lactose, the main carbohydrate in mammalian milk; and phlorizin

hydrolase that digests ß-glycosylceramides, which are found in the diet of most vertebrates. Marked reduction in the lactase activity is associated with diarrhea and accompanying symptoms upon milk drinking in newborns and adults (Phillips et al., 1978; Digeon and Walker-Smith, 1986; Buller and Grand, 1990); a physiological role of phlorizin hydrolase activity is still unknown (Schlegel-Haueter et al., 1972; Colombo et al., 1973; Skovbjerg et al., 1981).

Structural features of LPH

The cDNA encoding the LPH protein consists of 6274 bp contained in 17 exons of the LPH gene, which is located on chromosome 2 (Kruse et al., 1988; Mantei et al., 1988; Boll et al., 1991). The amino acid sequence deduced from the LPH cDNA consists of 1927 amino acids. LPH is a type I membrane-bound glycoprotein. It is composed of an NH_2-terminal extracellular domain followed by a transmembrane domain comprising 19 hydrophobic amino acids and a COOH-terminal cytoplasmic domain of 26 amino acids (Mantei et al., 1988) (refer to Fig. 1 for a schematic respresentation of the structure of LPH). The ectodomain consists of an NH_2-terminal cleavable signal peptide (19 amino acids) that is required for translocation of LPH into the ER (Von Heijne, 1986; Mantei et al., 1988). LPH is highly glycosylated; the human enzyme contains 15 potential N-glycosylation sites (the rabbit and rat enzymes possess 14 and 15 respectively). LPH consists of 4 highly conserved structural and functional regions, denoted I-IV, which reveal 38%-55% identity to one another (Mantei et al., 1988). The lactase activity is localized to glutamine1273 in the homologous region III and that of phlorizin hydrolase to glutamine1749 in the homologous region IV (Wacker et al., 1992; Neele et al., 1995; Zecca et al., 1998). By virtue of the striking homologies between the 4 regions LPH may have arisen from two subsequent duplications of one ancestral gene (Mantei et al., 1988). An evolutionary and developmental example that lends support to this notion is given by the sequence similarities of LPH and each of its homologous regions with ß-glycosidases from archaebacteria, eubacteria and fungi. These similarities suggest that LPH is a member of a superfamily of ß-glucosidases and ß-galactosidases. The procaryotic ß-glycosidases are on average about 50-kDa in size which corresponds to approximately a fourth of the size of full length LPH or roughly to the size of one homologous region (I-IV).

Biosynthesis of LPH

The biosynthesis of LPH has been investigated in many species (Danielsen et al., 1984; Buller et al., 1987; Naim et al., 1987). Here, common posttranslational and processing pathways could be unravelled and, in what follows a summary of the biosynthesis and processing of the human enzyme is provided as a representative for other species (Figure 2 depicts the main biosynthetic steps of LPH). LPH is synthesized in small intestinal epitehlial cells as a single chain 215-kDa pro-LPH precursor which is mannose-rich N-glycosylated. After attainment of the monomeric mannose-rich pro-LPH to a correct folding pattern two similar subunits assemble to

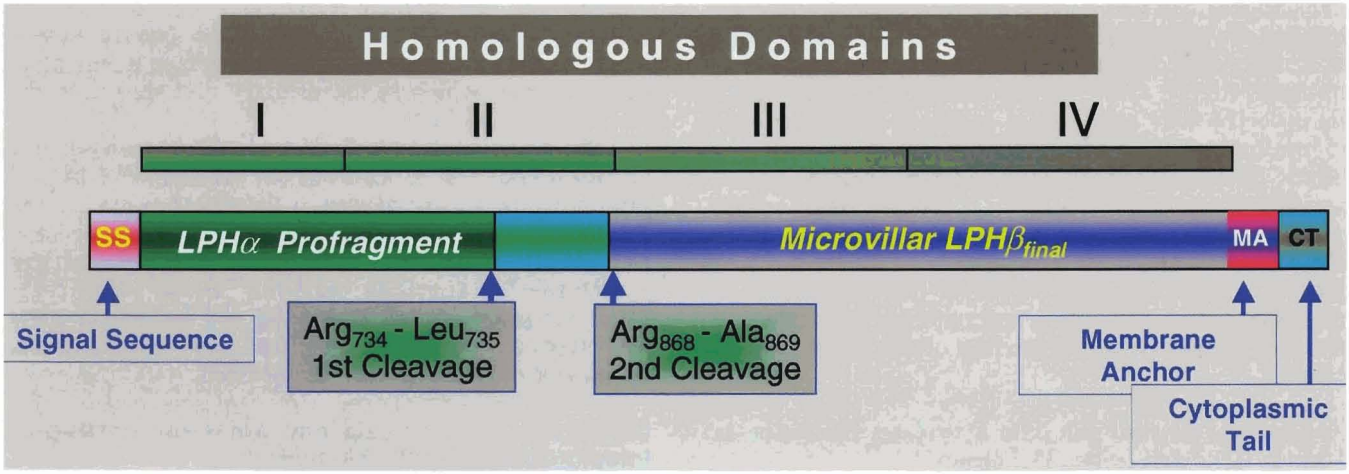

Fig. 1. Schematic representation of the structure of pro-LPH in human small intestinal cells. Some important structural features of pro-LPH in human small intestinal cells compiled from data which employed biosynthetic studies in human small intestinal explants (Hauri et al., 1985; Naim et al., 1987; Naim and Lentze, 1992), cDNA cloning (Mantei et al., 1988), and recombinant expression in COS-1 (Naim et al., 1991; Jacob et al., 1996; Wuthrich et al., 1996) or MDCK (Grunberg et al., 1992; Jacob et al., 1994) cells. The N-terminus starts with a cleavable signal sequence (SS) (Met_1-Gly_{19}) for cotranslational translocation into the ER. The ectodomain (Ser_{20} to Thr_{1882}) can be divided into four homologous domains as indicated. The membrane anchoring domain (MA) extends from Ala_{1883} to Leu_{1902} and the cytosplasmic tail (CT) from Ser_{1903} to Phe_{1927}. Pro-LPH is subject to two proteolytic cleavage steps. An intracellular one between Arg_{734} and Leu_{735} to generate $LPHß_{initial}$ and a second extracellular cleavage between Arg_{868} and Ala_{869} in the intestinal lumen by pancreatic trypsin that generates the microvillar mature enzyme, $LPHß_{final}$ (Jacob et al., 1996; Wuthrich et al., 1996). The LPHα profragment (Ser_{20} to Arg_{734}) plays a role as an intramolecular chaperone in the folding of pro-LPH.

a homodimer prior to egress from the ER and transport to the Golgi apparatus. The transmembrane domain of pro-LPH is directly implicated in the dimerisation process (Naim and Naim, 1996). Deletion mutants lacking this region do not dimerise, are blocked in the ER and ultimately undergo intracellular degradation. Additional to its decisive role in generating transport-competent pro-LPH forms, dimerisation is also crucial for the acquisition of lactase enzymatic activity (Naim and Naim, 1996). During transport through the Golgi apparatus to the cell surface, processing of the mannose-rich N-linked sugar chains by mannosidases of the cis-Golgi takes place and several types of sugars are added, resulting in a complex glycosylated protein of an

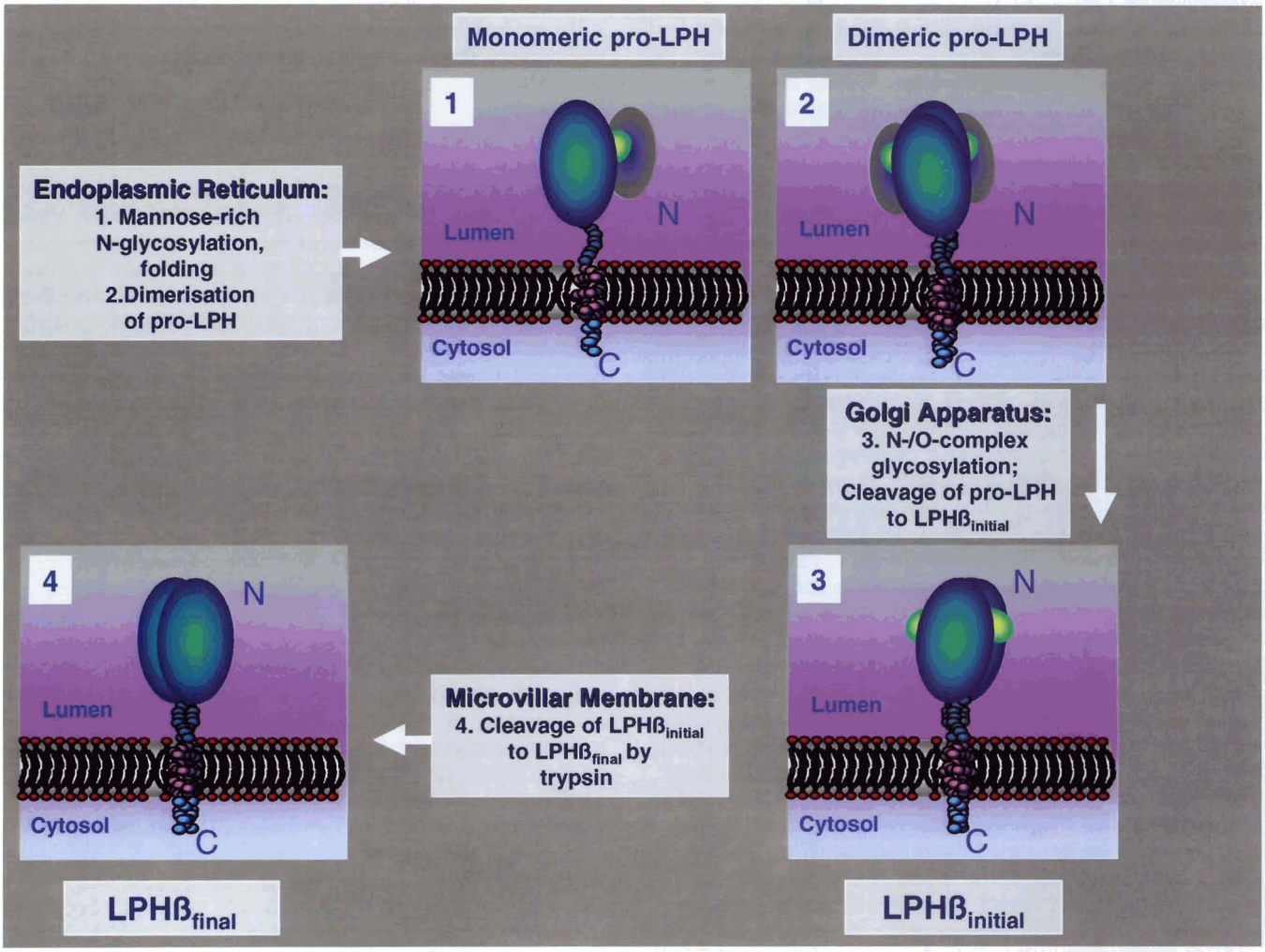

Fig. 2. Biosynthesis and processing of LPH. **Panel 1.** LPH is synthesized as a pro-lactase-phlorizin hydrolase precursor (pro-LPH). This form is cotranslationally modified in the endoplasmic reticulum by mannose-rich N-linked core glycosylation at the potential 15 asparagine residues. Correct folding takes place and the form generated at this stage is monomeric mannose-rich pro-LPH. The ectodomain or the luminal domain of pro-LPH comprises three important regions: the LPHα profragment (shown as a gray-dark blue ellipsoid) (Ser_{20}-Arg_{734}), the LPHß$_{final}$ domain (Ala_{869}-Thr_{1882}) (shown as a blue-green ellipsoid) and an intermediate polypeptide (Leu_{735}-Arg_{868}) (shown as a small light green-yellow ellipsoid) that belongs to LPHß$_{initial}$ (see panel 3) and is cleaved extracellularly in the brush border membrane by trypsin. **Panel 2.** Another essential event along the secretory pathway of pro-LPH is its assembly into dimers in the ER. **Panel 3.** In the Golgi apparatus modification of the N-linked mannose-rich glycans to the complex type as well as O-glycosylation takes place. The mature dimeric pro-LPH form undergoes a first intracellular cleavage process at Arg_{734}-Leu_{735} by which the LPHα profragment (gray-blue) is eliminated. The resulting form, LPHß$_{initial}$, is sorted with high fidelity to the apical or microvillar membrane. **Panel 4.** In the microvillar membrane the intermediate polypeptide Leu_{735}-Arg_{868} (light green-yellow) in LPHß$_{initial}$ is eliminated by trypsin to generate the final mature microvillar form LPHß$_{final}$ (blue-green).

approximate apparent molecular weight of 230-kDa (Hauri et al., 1985; Naim et al., 1991). Pro-LPH also undergoes O-glycosylation at particular Ser or Thr residues. This event commences in the cis-Golgi and ends in the trans-Golgi network leading to a 4-fold increase in the enzymatic activity of LPH (Naim and Lentze, 1992).

The further processing of mature pro-LPH is characterized by two proteolytic cleavage steps (Jacob et al., 1996; Wuthrich et al., 1996) (Fig. 2). The first cleavage takes place intracellulary and constitutes the removal of the large profragment LPHα at Arg_{734}/Leu_{735} leaving the membrane-bound $LPHß_{initial}$ which extends from Leu_{735} to Tyr_{1927}. This form is targeted to the brush border membrane where it is cleaved by pancreatic trypsin at Arg_{868}/Ala_{869} to $LPHß_{final}$ (Ala_{869}-Tyr_{1927}) (Danielsen et al., 1984; Buller et al., 1987; Naim et al., 1987). This form, known also as the 160-kDa mature brush border LPH, comprises the functional domains of the enzyme. The implication of the intracellular proteolytic cleavage steps on the transport, sorting and function of pro-LPH as well as the possible role of the large profragment LPHα in this regard have been investigated by recombinant expression of pro-LPH in heterologous cell systems. Stable Madin-Darby canine-kidney cell line (MDCK) expressing pro-LPH has helped localize the initial cleavage step (pro-LPH to $LPHß_{initial}$) to the trans-Golgi network (Jacob et al., 1994). Interestingly, in another mammalian cell line, the simian virus-transformed COS cells, intracellular proteolytic cleavage does not take place suggesting that this event is probably not an essential modification along the secretory pathway of pro-LPH (Naim et al., 1991). In fact, recombinant expression of pro-LPH in COS-1 cells revealed that this molecule is efficiently transported to the cell surface and is as an enzymatically active protein as the native intestinal mature brush border LPH (Naim et al., 1991). It became clear therefore that the intracellular proteolytic processing of pro-LPH in intestinal epithelial cells is not a prerequisite for acquisition of transport competence and biological function. The intriguing question arising in this respect is that related to the fate of the large profragment, which we named later LPHα. In biosynthetic labeling experiments of intestinal biopsy samples and immunoprecipitation with an epitope-specific antibody directed against a 12 amino acids peptide immediately downstream the signal sequence of pro-LPH, a 100-kDa polypeptide was isolated that appears concomitantly with the appearance of LPHß (Naim et al., 1994). This polypeptide is neither N-glycosylated, despite the presence of 5 potential N-glycosylation sites, nor O-glycosylated. However, it possesses a high content of hydrophobic amino acids and has a tendency to form a compact, rigid and trypsin-resistant structure immediately after translation. Along this it seems that the N- and O-glycosylation sites are embedded in the interior space of LPHα offering a possible explanation for the inaccessibility of the N- and O-glycosylation sites to glycosyltransferases. LPHα therefore has characteristics of an intramolecular chaperone that is directly involved in the folding of the LPHß domain. In fact, individual expression of a cDNA encoding LPHß generates a protein that was not as transport-competent as the wild type species and furthermore the enzymatic activity of this protein in the absence of LPHα domain was below detection limit. Finally, LPHß alone is readily degraded by the protease, pointing to its malfolded structure. Despite the structural homologies between regions I, II, contained in LPHα, and III and IV, encompassing LPHß and therefore the enzymatic centers, lactase and phlorizin hydrolase, no activity could be detected in LPHα (Naim et al., 1994). Altogether, the proregion of pro-LPH, LPHα, is exclusively required in the context of correct folding of pro-LPH.

Polarized sorting of pro-LPH

Pro-LPH is transported to the luminal surface of epithelial cells, i.e. to the brush border membrane where it exerts its physiological function in hydrolysing lactose. Polarized sorting of membrane and secretory proteins in epithelial cells is synergetically achieved by sorting signals in the protein itself and cellular components that interact with these signals. Proteins destined to the basolateral surface are in most cases tyrosine-based cryptic signals in the cytoplasmic tail of the protein (Casanova et al., 1991; Hunziker et al., 1991). Besides their function in polarized sorting, these signals also act as endocytic signals and it is thought that basolateral sorting uses a mechanism similar to that of endocytosis through clathrin-coated pits (Matter et al., 1992; Monlauzeur et al., 1995). The sorting to the apical membrane of proteins such as pro-LPH is not as unique and is mediated by diverse signals. For example, Glycolipid (glycophosphatidyl inositol, GPI) anchors direct proteins to the apical surface of several types of epithelial cells (Lisanti et al., 1990; Dotti et al., 1991) by associating in the trans-Golgi network (TGN) with membrane microdomains enriched in glycosphingolipids and cholesterol (Brown and Rose, 1992; Danielsen, 1995; Simons and Ikonen, 1997). N- and O-linked glycans on some secreted and membrane-bound proteins constitute the apical transport signal (Fiedler and Simons, 1995; Scheiffele et al., 1995), although this mechanism does not apply to all secreted proteins (Marzolo et al., 1997; Yeaman et al., 1997). These sorting signals do not apply to pro-LPH, since pro-LPH is not associated with microdomains and processing of N- or O-linked glycans remains without effect on the sorting pattern of LPH (Naim, 1994; Danielsen, 1995; Alfalah et al., 1999; Jacob et al., 1999). Additionally, neither is the proteolytic cleavage process implicated in the sorting event of LPH by, for example, exposing masked sorting elements, nor does the large profragment LPHα that is generated upon proteolytic cleavage of pro-LPH contain apical sorting signals (Grunberg et al.,

1992; Jacob et al., 1994). On the other hand, recent observations have demonstrated that putative sorting signals of pro-LPH are exclusively located in the domain corresponding to the brush border-associated LPHß (Jacob et al., 1997). The importance of the ectodomain in the intracellular transport and polarized sorting of LPH has been analyzed in deletion-mutants of homologous region IV contiguous to the transmembrane domain (Panzer et al., 1998). It could be demonstrated that a region that reveals features of a "stalk-region" similar to that found in intestinal sucrase-isomaltase and aminopeptidase N is not required for the polarized targetting of pro-LPH to the apical membrane. Deletion of 236 amino acids containing more than one third of phlorizin-hydrolase (homologous region IV) including the catalytic domain has almost no influence on LPH dimerization and transport to the plasma membrane, whereas further deletion of 87 amino acids upstream in the phlorizin-hydrolase domain affected the dimerization and intracellular transport of the protein, but had no gross structural alterations on the tertiary protein structure. These anaylses could narrow down the domain implicated in the polarized sorting of pro-LPH to that encompassing Ala_{869} to Glu_{558}.

Gene expression of lactase-phlorizin hydrolase

Cell differentiation is characterized by substantial alterations in the pattern of expression of many genes either due to activation or repression of gene transcription. The gene regulation in these phases is controlled by tissue-specific transcription factors or proteins. Transcription factors can activate multiple genes in the same cell and the expression of these genes generates novel cellular phenotypic markers that are often associated with dramatic morphological and functional alterations of the differentiated cell. This pattern of gene expression could be followed best during the differentiation of intestinal crypt cells to columnar epithelial cells which reveal two structurally and functionally different domains, the apical and basolateral domains (Cohn et al., 1992; DuBois et al., 1995; Simon et al., 1995; Traber and Silberg, 1996). This event is accompanied by the expression of the gene encoding the disaccharidase pro-LPH (Olsen et al., 1996). A number of studies have established the meanwhile widely accepted pattern of expression of pro-LPH. Functional, immunohistochemical and in situ hybridization studies have demonstrated that the LPH protein is barely detectable in the crypts, but its pattern of expression reaches maximum levels between the lower and mid-villus and decreases at the villus tip (Hauri et al., 1985; Rings et al., 1992). The LPH gene is approximately 55 kb and comprizes 17 exons (Boll et al., 1991). The gene contains binding sites for common transcription factors such as CTF/NF-1 and AP2 that were identified with 1 kb of the 5'-flanking region of the rat and human genes (Troelsen et al., 1994). Glucocorticoids enhance the lactase activity in rats during the first weeks in life and have also been shown to regulate human lactase (Freund et al., 1991; Yeh et al., 1991). However, glucocorticoid-responsive elements in the 5'-flanking region of LPH have not been identified. Analysis of 5'-flanking sequences fused to human growth hormone (hGH) as a reporter gene in transfected Caco-2 cells or the non-intestinal cell line HepG2 have demonstrated the exclusive and specific function of these sequences in cells of intestinal lineage (Markowitz et al., 1995). Footprint analysis of the promoter region of LPH has led to the identification of a nuclear protein (NF-LPH1) that binds a 15 bp region just upstream from the transcription site (between – 54 an – 40), which is functional in the adenocarcinoma cell line Caco-2 and is probably involved in regulation of lactase activity within LPH (Troelsen et al., 1992, 1994).

The expression of LPH during development follows a similar pattern at the protein and mRNA levels (Hecht et al., 1997).

LPH deficiency and its phenotyes

The absence of an enzymatic activity that is able to digest carbohydrates often leads to abdominal pain, cramps or distention, flatulence, nausea and osmotic diarrhea. The reason is that the intestinal epithelium does not have mechanisms that enable the uptake of disaccharides or higher order carbohydrates through cellular membranes to the cell interior. The most common intestinal disorder that is associated with reduced or absent levels of an intestinal enzyme is lactose intolerance in which lactase activity is affected. Lactase deficiency has been described for adults as well as children and could be of a primary or a secondary cause (for a review see (Kretchmer, 1989)). In almost all mammals, lactase activity is high at birth and during nursing, when milk is the exclusive nutrient. Around weaning and before adulthood an almost 80-90% decline in lactase activity to low adult levels takes place in most mammals. In the human population this decrease happens at around age 5. The exceptions to this expression profile are North Europeans and their descendents and some other isolated minorities mainly in Africa. 95% of whites show autosomal dominant inheritance of a lifelong high lactose digesting capacity (LDC). The age-related reduced lactose-digesting activity has been termed late onset of lactase deficiency or adult type hypolactasia. Genetic analysis of homozygotes and heterozygotes of lactase-persistent and lactase non-persistent families supported the initial idea that lactase activity is inherited as a single autosomal dominant gene. There are currently several ideas that attempt to explain the late onset of lactase activity. Initially it was proposed that the regulation of lactase activity in hypolactasics is associated with altered posttranslational processing of LPH as compared to individuals with LDC (Witte et al., 1990; Lorenzsonn et al., 1993). These alterations may generate an enzymatically inactive LPH or may lead to its

intracellular degradation. This hypothesis was later discussed when mRNA levels of lactase in the intestine of adult rats were found to be almost similar to mRNA levels in fetal rats (Buller et al., 1990). Another study used biopsy material to demonstrate that appreciable levels of lactase mRNA were detected in the intestines of hypolactasic individuals in Southern Italy (Rossi et al., 1997). These observations have led to the belief that the lactase protein is synthesized in adult type hypolactasia, but undergoes posttranslational modifications that end up with a malfolded or an enzymatically inactive protein that is degraded at a relatively high turnover.

A study of a race- and sex-balanced cohort in which lactose tolerance, levels of jejunal lactase protein, activity, and mRNA were measured clearly demonstrated that black heritage predicts low LDC, and white heritage predicts high LDC (Fajardo et al., 1994). One parameter used in this study was the assessment of the lactase/sucrase ratio (L/S) in jejunal biopsy specimens. All subjects with a high LDC had an L/S ratio >0.5, immunodetectable LPH protein and measurably higher LPH mRNA levels than subjects with low LDC. Furthermore, LPH mRNA levels are highly correlated with lactase-specific activity (r=0.80) and L/S ratio (r=0.88). The direct correlation between LPH mRNA levels and lactase expression argues that the gene responsible for the human lactase polymorphism regulates the level of LPH mRNA. Similarly, studies in the rat small intestine have essentially described a similar coordinate pattern of interrelationship between mRNA and protein levels of LPH (Buller et al., 1990). The lactase activity, although clearly dependent on the presence of LPH mRNA pattern, and the regional distribution of activity did not absolutely correlate with the mRNA and protein levels along the proximal-distal axis. This suggests that additional secondary mechanisms, perhaps posttranslational, influence the lactase activity. O-glycosylation for example, a posttranslational event that commences in the cis-Golgi, increases the activity of LPH by a factor of 4-fold (Naim and Lentze, 1992). The interesting aspect of this observation is that variations in the extent of O-glycans are concomitant with the differentiation state of intestinal cells to polarized enterocytes. These events are associated with dramatic alterations in the gene expression of intestinal proteins and the coordinate synthesis of typical intestinal markers such as sucrase-isomaltase, LPH and also a number of glucosyl-transferases that affect the glycosylation pattern of glycoproteins. Despite this, the major mechanism of regulation of LPH is transcriptional since it has been clearly demonstrated that adequate levels of LPH mRNA must be present to detect LPH activities (Escher et al., 1992; Lloyd et al., 1992; Fajardo et al., 1994; Krasinski et al., 1994; Lacey et al., 1994; Harvey et al., 1995). With this notion are data in which a specific region in the LPH promoter, CE-LPH1, has been identified and shown to interact with an intestinal and LPH-specific trans-acting nuclear factor, NF-LPH1, that is strongly associated with the regulation of gene transcription of LPH (Troelsen et al., 1992, 1997). Nevertheless, clear differences in the promoter region of LPH between individuals with low LDC versus high LDC have until present not been found. Likewise no data are present that describe variations in the absence or presence of transcription factors associated with variations in LDC.

There are also minor forms of lactose intolerance. For example congenital lactase deficiency, a rare autosomal recessive trait which is characterized by the absence of active LPH (Lifshitz, 1966). Here, posttranslational mechanisms may be crucial in the regulation of the activity, as has been shown for congenital sucrase-isomaltase deficiency, in which malfolding, altered processing and targeting of another brush border disaccharidase occur (Lloyd and Olsen, 1987; Naim et al., 1988; Fransen et al., 1991). Immature intestinal development leads to primary lactase deficiency in premature infants. Other forms of lactase deficiency are secondary and are caused by mucosal injury, due to infectious gastroenteritis, celiac disease, parasitic infection, drug-induced enteritis and Crohn's disease.

Future perspectives

A fairly appreciable knowledge has been accummulated meanwhile on various aspects of the structure, biosynthesis, sorting, gene expression and regulation of the function of intestinal disaccharidases. This basic information is of primordial importance for elucidating the mechanisms that control the gene expression of these proteins in carbohydrate absorption disorders, for example in adult type hypolactasia, congenital lactase deficiency and congenital sucrase-isomaltase deficiency. In the latter two cases, posttranslational mechanisms appear to be responsible for the onset of these disorders (Naim et al., 1988; Fransen et al., 1991). For adult type hypolactasia it is still premature to determine with certainty which molecular mechanism(s) are implicated. The consensus has emerged now that LPH activity is controlled at the transcriptional level. Despite ample progress in localisation of upstream regions and identification of transcription factors implicated in the onset of hypolactasia, major pieces of information are still required to solve the puzzle of LPH deficiency. Major attention should be paid to trans-acting elements, which have been almost neglected. For this transgenes should be made that contain constructs of the entire LPHcDNA decorated with randomly selected trans-located introns from individuals with high LDC versus low LDC. Also, the potential role of particular posttranslational events in the regulation of LPH activity should not be excluded (Naim and Lentze, 1992). Our preliminary data show that LPH contains an endocytic signal in its cytoplasmic tail that is respressed in "normal" LPH by the strong apical signal located in the ectodomain. This signal is located in close proximity to the membrane, is hardly

flexible or accessible for interaction with clathrin-coated pits and becomes effective only when the apical signal of LPH is deleted. It would be interesting to screen representative sequences of the cytosolic tails of the LPH gene from individuals with high versus low LDC.

Acknowledgements. The original work from my laboratory cited in this review has been supported by grants from the German Research Foundation (DFG), the Ministry for Education and Research (BMBF), Bonn, Germany and the Swiss National Science Foundation, Bern, Switzerland. I am indebted for Dr. Ralf Jacob for his assistance in the preparation of the manuscript.

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Accepted October 30, 2000