Sugars in Nutrition, edited by M. Gracey, N. Kretchmer, and E. Rossi, Nestlé Nutrition Workshop Series, Vol. 25. Nestec Ltd., Vevey/Raven Press, Ltd., New York © 1991.

The Lactase Story: From Physiopathology to Biochemistry, Molecular and Cell Biology—and Back?

Giorgio Semenza and *Salvatore Auricchio

*Department of Biochemistry, ETH Zentrum, 8092 Zürich, Switzerland; *Department of Pediatrics II, University of Naples, 80131 Naples, Italy*

It is unusual that one can recount first-hand the early beginnings and developments of a section of biochemistry, physiology, physiopathology, cell biology, and molecular biology. Although neither of us devoted all his scientific efforts in the last 30 years to intestinal disaccharidases—indeed each of us looked into and solved a number of problems in other areas also—we find it gratifying to think back to the very early 1960s, when our interest in these enzymes was triggered by a patient in Fanconi's Childrens' Hospital at the University of Zürich, who had sucrose-isomaltose malabsorption (1). This condition, which was soon found to be transmitted genetically (2), led to the search by one of us (SA) for *human* normal small intestinal pieces, which were meant to serve as controls, since Arne Dahlqvist has mapped the disaccharidases in the hog (3).

One of us (GS) has recounted elsewhere (4) how serendipity in the course of this search revealed in 1962 the existence of a hitherto unknown condition, adult-type lactose intolerance (adult-type hypolactasia) (5). The report was very soon confirmed (6) and followed by an immense literature (reviewed in refs. 7–12). Adult-type hypolactasia was found to be the most common genetic condition in humans, affecting one third to one half of mankind, mostly those populations without dairy culture. It is discussed in the article by Auricchio and Semenza in this volume (13).

The developments in basic science in this area have been covered throughout the years by a number of reviews (4,12,14–18). Suffice to say that the first, biochemical phase led to the isolation and characterization of intestinal lactase (19–22), which, like the other glycosidases of the brush border, is an intrinsic membrane protein; that is, it can be solubilized only by partial proteolysis (19) or by treatment with detergents (21).

Homogeneous lactase was soon found to be endowed with two catalytic sites, one splitting lactose, cellobiose, and similar β-glycosides (19–21) and the other splitting aryl- and alkyl-β-glycosides, including phlorizin (21,23,24). The natural substrates of the latter site are glycosylceramides (25). In SDS-PAGE, homogeneous lactase-

phlorizin-hydrolase complex (or lactase-glycosylceramidase or β-glycosidase complex) yielded a single, sharp band of approximately 160 kDa. For many years, it was unclear whether the two active sites of this complex are located on a single polypeptide chain, such as in prosucrase-isomaltase) (26), or in two different but similar polypeptides, such as in the final sucrase-isomaltase complex (27,28).

At about the same time as the early biochemical phase, the early biological phase was begun. The pioneer work of Kretchmer, Auricchio, and their associates showed that most of the lactase activity develops fairly late in pregnancy, stays at a high plateau from shortly before birth till the time of weaning, and then, within a week or so, declines to the very low levels of adulthood (29–31). The situation in humans is more complex (13). Interestingly, phlorizin-hydrolase, the other activity of the β-glycosidase complex, also declines after birth (24). The two activities, as also other observations showed, must be subject to the same biological control.

When organ cultures of small intestine became possible, the Danish group (32, 33), later confirmed by those in Berne (34) and in Boston (35), demonstrated that the lactase-phlorizin-hydrolase complex (LPH) is synthesized as a single-chain 220 kDa precursor, which is split intracellularly into the final 160 kDa complex at a stage when it is nearly completely insensitive to endo-H. A very much simplified scheme of events is, therefore

pre-pro-LPH → pro-LPH →→→ pro-LPH →→ LPH →→→ LPH

(in the endoplasmic reticulum and early Golgi membranes)

(in the brush-border membranes)

A couple of years ago, considering the new possibilities that the unprecedented developments in molecular biology now offered, we decided to reenter the field. Time seemed to be ripe for tackling at least the following problems: (a) Are the two active sites in LPH located in one or two polypeptide chains? (b) How is LPH anchored to the brush-border membrane? (c) What are the amino acid sequences of LPH and of pro-LPH, and what relationships are there between the two? (d) At what level is the decline of LPH at weaning controlled?

Clearly, sequencing a polypeptide chain of some 220 kDa, that is, of around 2,000 amino acids (e.g., pro-LPH), would not be a trivial task, even using modern cDNA technology. We had to secure the collaboration of a first-class molecular biologist, and we were lucky in finding one in Dr. N. Mantei. He, together with some very enthusiastic and capable students and biochemists, could accomplish the heroic feat of sequencing the 1,927 and 1,926 amino acids-long pre-pro-LPH of humans and rabbits (36), respectively. The wealth of biochemical knowledge deduced from this major work was, however, commensurate to it.

First, the N-terminal amino acid of final LPH was identified with pos. 867 (in rabbit) pre-pro-LPH, and all the peptides derived from final LPH as isolated from the brush borders could be unequivocally localized, without exception, in the polypeptide chain of pre-pro-LPH after this position (Fig. 1). Clearly, final LPH derives

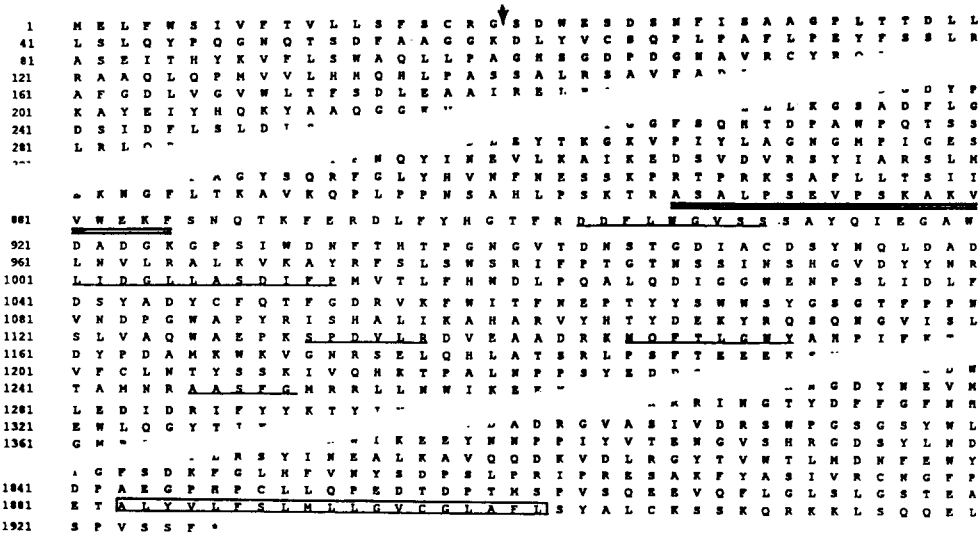

FIG. 1. Parts of the amino acid sequence of rabbit pre-pro-lactase-phlorizin-hydrolase. ↓, putative cleavage site. Doubly underlined sequence, the N-terminal sequence of the final lactase-phlorizin-hydrolase, as isolated from the brush-border membrane. Singly underlined sequences, tryptic peptides obtained from isolated lactase-phlorizin-hydrolase. All peptides from isolated lactase-phlorizin-hydrolase could be localized unambiguously in the cDNA-deduced sequence of pre-pro-lactase-phlorizin-hydrolase from pos. 867 onward. The hydrophobic segment in the C-terminal region is boxed (from res. 1,883 to res. 1,901). Data adapted from Mantei N, *et al.* (36).

solely from the C-terminal portion of pre-pro-LPH after pos. 867. It follows that LPH is composed of a single type of polypeptide, which must, therefore, carry both active sites. This observation by itself answered in a straightforward manner the question of why lactase activity and phlorizin hydrolase activity are subject to the same biological control—because they belong to the same translational unit, and, indeed, they are located in the same polypeptide. The N-terminal portion of pre-pro-LPH prior to pos. 867 does not appear in the brush-border membrane. Its cellular location, its possible enzymatic activity, and its physiological role still are unknown.

Final LPH shows only one hydrophobic sequence of sufficient length, some 20 amino acids, to act as the membrane anchor (Fig. 2). This is clearly the *permanent* anchor and is not exchanged for a phosphatidyl inositol anchor (36), as is the case for several other brush-border proteins. This C-terminal location of the anchor is very uncommon, if not unique, among intrinsic brush-border membrane proteins.

The sequence of pre-pro-LPH begins with a typical signal for which Heijne's algorithm gives a very high splitting score between pos. 19 and 20. Between this position and the beginning of the hydrophobic anchor at the C-terminal (or rather the beginning of the putative stalk), the sequence shows a fourfold inner homology (Fig. 3), which is strongly indicative of two cycles of gene duplication. Of the four homologous regions, only two occur in final LPH. Each of these four encompasses a segment that clearly is a part of a catalytic site.

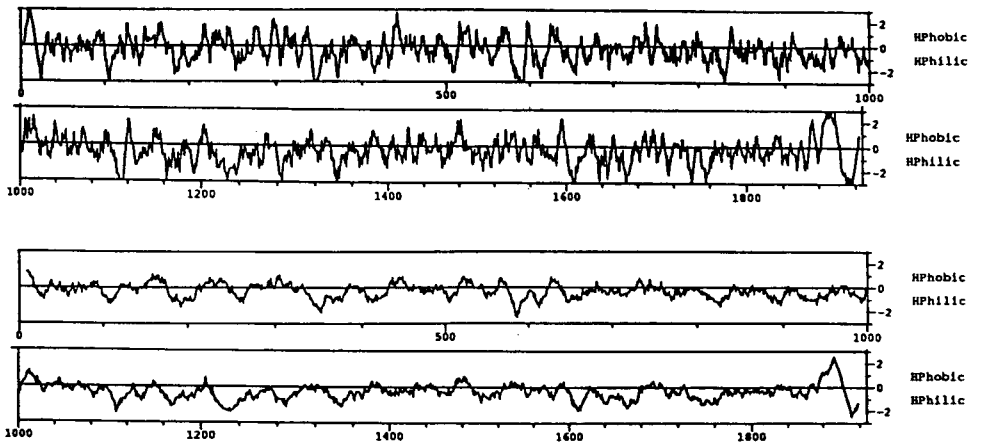

FIG. 2. Kyte-Doolittle plots of human pre-pro-lactase-phlorizin-hydrolase. Windows: 7 residues (top) or 19 (bottom). The top panel clearly shows the hydrophobic sequence within the (cleavable) signal, and the much longer one in the C-terminal region, which serves as anchor. The bottom panel shows clearly the latter only.

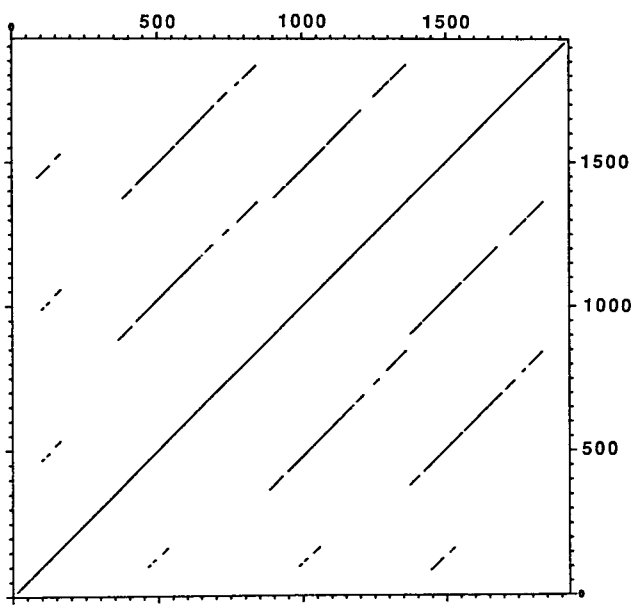

FIG. 3. Dot plot of pre-pro-LPH versus pre-pro-LPH.

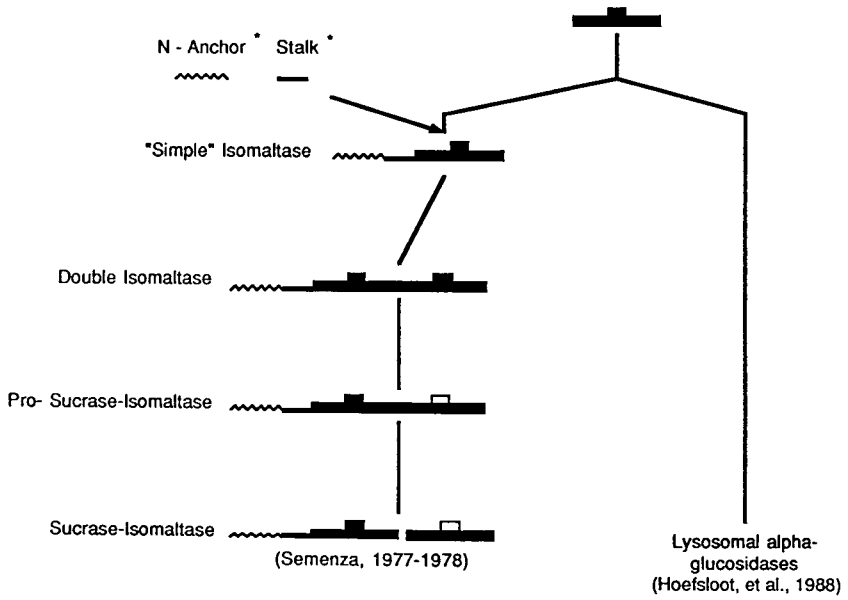

FIG. 4. Phylogenetic pedigree of sucrase-isomaltase as of 1988. The N-anchors and the stalks are homologous to those in maltase-glucoamylase, endopeptidase 24.11 (both brush-border enzymes) and those in the asialoglycoprotein receptors (of the basolateral membrane). From Semenza G (18).

As mentioned previously, little is known about the role of the pro portion (i.e., of homologous regions I and II). However, it is clear that the same translational unit leads to two proteins at different cellular locations, one (LPH) in the brush-border membrane, the other (the pro portion of the precursor) in a cytosolic organelle.

This situation should be compared with the evolutionary pedigree of the sucrase-isomaltase complex and of the related lysosomal α-glucosidase. In this system, an archetypal α-glucosidase gene duplicated into the gene eventually leading to the lysosomal enzyme (37), on the one hand, and to another α-glucosidase (the isomaltase), which, on receiving the exon of the N-terminal anchoring led to an ancient brush border isomaltase. Partial duplication followed by mutation(s) led eventually to prosucrase-isomaltase (Fig. 4) (17,18). In the β-galactosidase system, the exons coding for the brush-border enzyme and those coding for the glycosidase of a different cellular location are still associated in a single translational unit.

Otherwise, brush-border α-glucosidases and brush-border β-glycosidases show very little resemblence in that their mode of anchoring is different, their sequences show no homology, their biological control mechanisms are quite different, and their genes are located in different chromosomes (38,39). The patterns of spontaneous development of lactase and sucrase are quite different. In baby rabbits, lactase appears before birth, reaches a maximum in the early days of life, and declines at the time of weaning (40–46). Sucrase, again in rabbits (but not in humans) appears after birth (at weaning, in fact) and remains high in adulthood (Fig. 5) (31). The events

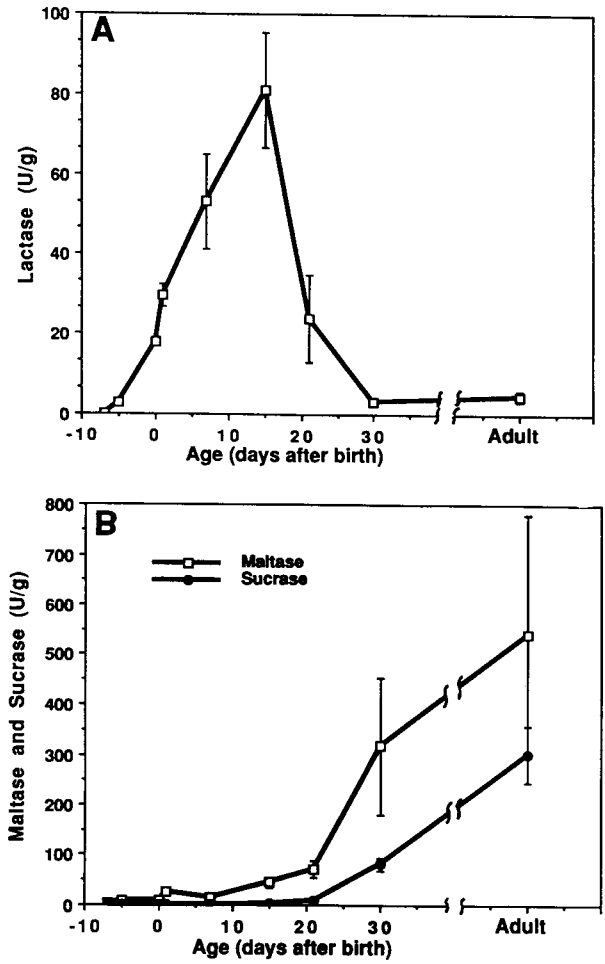

FIG. 5. Jejunal disaccharidase activities (U/g protein) in rabbits at different ages of prenatal and postnatal life. **A.** Lactase activity. **B.** Sucrase and maltase activities. From Sebastio G, *et al.* (31).

involved in the decline of lactase in most mammals at the time of weaning, which are most likely to be similar to or identical with those leading to the adult-type of lactose intolerance in humans, are quite complex and interesting.

It has been suggested that the decline of lactase at the time of weaning takes place by at least two mechanisms acting in sequence (47): first, the accelerated movement of enterocytes along the villous surface makes them shred off before they fully express lactase. Subsequently, lactase synthesis declines, or its processing is disturbed, or its degradation is accelerated.

Our data (31) on the levels of lactase mRNA in rats and rabbits give interesting

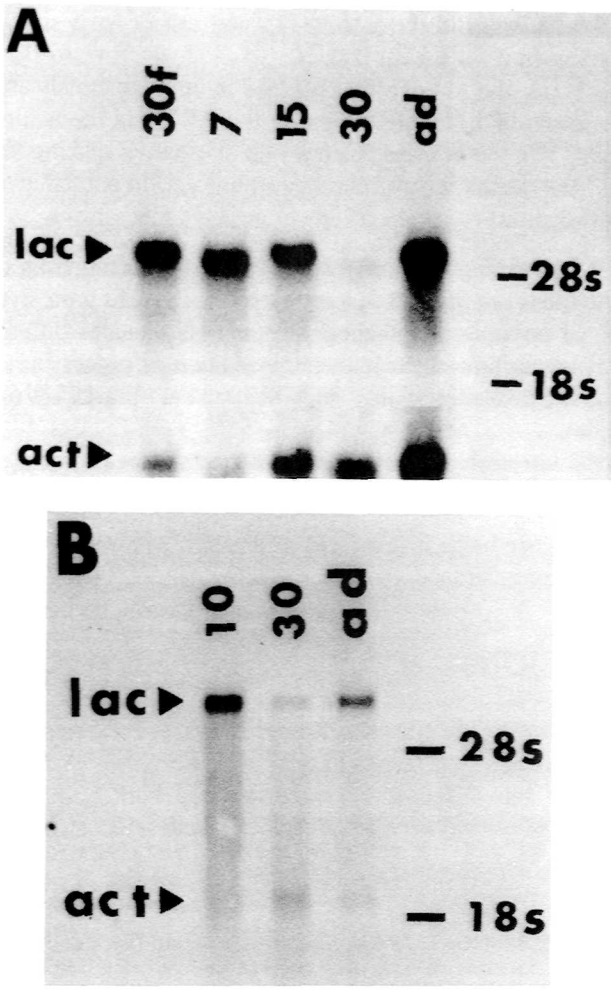

FIG. 6. Northern blot of **(A)** rabbit and **(B)** rat intestinal poly(A)$^+$ RNAs from animal of various ages. The upper bands correspond to lactase mRNA, with an approximate size of 7 kb. The lower bands correspond to β-actin. The migration of 18S and 28S RNA is indicated. From Sebastio G, *et al.* (31).

clues (Fig. 6). Northern blots clearly show that lactase mRNA is high at birth in rabbits and remains at high levels till the verge of weaning. At the end of weaning, that is, at 30 days of extrauterine life, the lactase mRNA is at low levels. This observation is compatible with a transcriptional control and also with the suggested mechanism of the accelerated cell kinetics (48,49).

However, and perhaps even more interesting, the levels of LPH-mRNA *in the*

adult animal are high again, although their lactase activity is very low. This we have shown for the rat and the rabbit (47), and others have simultaneously and independently shown it for the rat (50).

Clearly, therefore, the low levels of lactase in adult mammals are *not* due to a decline in the levels of LPH-mRNA, but is the mRNA in the adult identical with that in the baby? Are we perhaps dealing with alternative splicing, frame shifts, or other changes? We cannot answer these questions yet. In particular, we cannot yet distinguish between the two main groups of possible mechanisms.

1. Adult lactase may differ in some subtle but decisive details from the baby counterpart, with the result that it is susceptible to intracellular proteolytic degradation (this group of possible hypothetical mechanisms includes differences in chromosomic genes, differences in either transciption or processing of the mRNA, differences in posttranslational modification of pro-LPH or LPH, e.g., glycosylation).
2. In adult small intestine, there may exist a processing or degrading machinery for lactase that does not exist or operate in the baby small intestine.

These questions are being investigated at present, with respect to adult mammal and human lactose intolerance.

ACKNOWLEDGMENTS

The support of the Nestlé Nutrition Research Grants Programme is gratefully acknowledged. We express our thanks to our co-workers also, who made this project possible with their knowledge, enthusiasm, and hard work.

REFERENCES

1. Auricchio S, Prader A, Mürset G, Witt G. Saccharoseintoleranz. Durchfall infolge hereditären Mangels an intestinaler Saccharaseaktivität. *Helv Paediatr Acta* 1961;16:483–505.
2. Auricchio S, Rubino A, Prader A, et al. Intestinal glycosidase activities in congenital malabsorption of disaccharides. *J Pediatr* 1965;66:555–64.
3. Dahlqvist A. The separation of intestinal invertase and three different intestinal maltases on TEAE-cellulose by gradient elution, frontal analysis, and mutual displacement chromatography. *Acta Chem Scand* 1959;13:1817–27.
4. Semenza G. Intestinal oligo- and disaccharidases. In: Randle PJ, Steiner DF, eds. *Carbohydrate metabolism and its disorders*. New York: Academic Press, 1981:425–79.
5. Auricchio S, Rubino A, Landolt M, Semenza G, Prader A. Isolated intestinal lactase deficiency in the adult. *Lancet* 1963;2:324–6.
6. Dahlqvist A, Hammond JD, Crane RK, Dunphy JV, Littman A. Intestinal lactase deficiency and lactose intolerance in adults: preliminary report. *Gastroenterology* 1963;45:488–91.
7. Kretchmer N. Lactose and lactase. A historical perspective. *Gastroenterology* 1971;61:805–13.
8. Haemmerli UP, Kistler HJ, Ammann R, et al. Acquired milk intolerance in the adult caused by lactose malabsorption due to a selective deficiency of intestinal activity. *Am J Med* 1965;38:7–30.
9. Simoons FJ. Primary adult lactose intolerance and the milking habit: a problem in biological and cultural interrelations. *Am J Dig Dis* 1969;14:819–36.
10. Gray GM. Intestinal disaccharidase deficiences and glucose-galactose malabsorption. In: Stanbury

JB, Wyngaarden DS, Frederickson DS, eds. *The metabolic basis of inherited disease*. New York: McGraw-Hill, 1978:1526–36.
11. Paige DM, Bayless TM, eds. *Lactose digestion. Clinical and nutritional implications*. Baltimore: The Johns Hopkins University Press, 1981.
12. Semenza G, Auricchio S. Small-intestinal disaccharidases. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic basis of inherited disease*. 6th ed, vol 2. New York: McGraw-Hill, 1989:2975–97.
13. Auricchio S, Semenza G. Late-onset hypolactasia and persistent high lactase activities in humans. In: Gracey M, Kretcher N, Rossi E, eds. *Sugars in nutrition*. Nestlé Nutrition Workshop Series, vol 25. Vevey: Nestec Ltd; New York: Raven Press, 1991;241–8.
14. Semenza G. Anchoring and biosynthesis of stalked brush border membrane proteins: glycosidases and peptidases of enterocytes and of renal tubuli. *Annu Rev Cell Biol* 1986;2:255–313.
15. Hauser H, Semenza G. Sucrase-isomaltase: a stalked intrinsic protein of the brush border membrane. *CRC Crit Rev Biochem* 1983;14:319–45.
16. Semenza G. Glycosidases. In: Kenny AJ, Turner AJ, eds. *Ectoenzymes*. Amsterdam: Elsevier, 1987:165–87.
17. Semenza G. The sucrase-isomaltase complex, a large dimeric amphipathic protein from the small intestinal brush border membrane: emerging structure-function relationships. In: Ahlberg P, Sundelöf LO, eds. *Structure and dynamics of chemistry*. Symp 500th Jubilee University of Uppsala, Sweden, 1978:226–40.
18. Semenza G. The insertion of stalked proteins of the brush border membranes: the state of art in 1988 (Purkinj Lecture). *Biochem Int* 1989;18:15–33.
19. Schlegel-Haueter S, Hore P, Kerry KR, Semenza G. The preparation of lactase and glucoamylase of rat small intestine. *Biochim Biophys Acta* 1972;258:506–19.
20. Skovbjerg H, Norén O, Sjöström H, Danielsen EM, Enevoldsen BS. Further characterization of intestinal lactase/phlorizin hydrolase. *Biochim Biophys Acta* 1982;707:89–97.
21. Skovbjerg H, Sjöström H, Norén O. Purification and characterization of amphiphilic lactase/phlorizin hydrolase from human small intestine. *Eur J Biochem* 1981;114:653–61.
22. Potter J, Ho MW, Bolton H, Furth AJ, Swallow D, Griffith B. Human lactase and the molecular basis of lactase persistence. *Biochem Genet* 1985;23:423–39.
23. Kraml J, Kolínská J, Elledérová H Hiršová D. β-glucosidase (phlorizin hydrolase) activity of the lactase fraction isolated from the small intestinal mucosa of infant rat and the relationship between β-glucosidases and β-galactosidases. *Biochim Biophys Acta* 1972;258:520–30.
24. Colombo V, Lorenz-Meyer H, Semenza G. Small intestinal phlorizin hydrolase: the β-glycosidase complex. *Biochim Biophys Acta* 1973;327:412–24.
25. Leese HJ, Semenza G. On the identity between the small-intestinal enzymes phlorizin-hydrolase and glycosylceramidase. *J Biol Chem* 1973;248:8170–3.
26. Hunziker W, Spiess M, Semanza G, Lodish H. The sucrase-isomaltase complex: primary structure, membrane orientation and evolution of a stalked intrinsic brush border protein. *Cell* 1986;46:227–34.
27. Kolinska J, Semenza G. Studies on intestinal sucrase and on intestinal sugar transport. V. Isolation and properties of sucrase-isomaltase from rabbit small intestine. *Biochim Biophys Acta* 1967;146:181–95.
28. Brunner J, Hauser H, Braun H, et al. The mode of association of the enzyme complex sucrase-isomaltase with the intestinal brush border membrane. *J Biol Chem* 1979;254:1821–8.
29. Doell RG, Kretchmer N. Studies of small intestine during development. 1. Distribution and activity of ρ-galactosidase. *Biochim Biophys Acta* 1962;62:353–62.
30. Rubino A, Zimbalatti F, Auricchio S. Intestinal disaccharidase activities in adult and suckling rats. *Biochim Biophys Acta* 1964;92:305–11.
31. Sebastio G, Villa M, Sartorio R, et al. Control of lactase in human adult-type hypolactasia and in weaning rabbits and rats. *Am J Hum Genet* 1989;45:489–97.
32. Danielsen EM, Skovbjerg H, Norén O, Sjöström H. Biosynthesis of intestinal microvillar proteins. Intracellular processing of lactase-phlorizin hydrolase. *Biochem Biophys Res Commun* 1984;122:82–90.
33. Skovbjerg H, Danielsen EM, Norén O, Sjöström H. Evidence for biosynthesis of lactase-phlorizin, hydrolase as a single-chain high-molecular weight precursor. *Biochim Biophys Acta* 1984;789:247–51.
34. Naim HY, Sterchi EE, Lentze MJ. Biosynthesis and maturation of lactase-phlorizin hydrolase in the human small intestinal epithelial cells. *Biochem J* 1987;241:427–34.

35. Büller HA, Montgomery RK, Sasak WV, Grand RJ. Biosynthesis, glycosylation and intracellular transport of intestinal lactase-phlorizin hydrolase in rat. *J Biol Chem* 1987;262:17206–11.
36. Mantei N, Villa M, Enzler T, et al. Complete primary structure of human and rabbit lactase-phlorizin hydrolase: implications for biosynthesis, membrane anchoring and evolution of the enzyme. *EMBO J* 1988;7:2705–13.
37. Hoefsloot LH, Hoogenveen-Westerveld M, Kroos MA, van Beeumen J, Reuser AJJ, Oostra BA. Primary structure and processing of lysosomal α-glucosidase: homology with the intestinal sucrase-isomaltase complex. *EMBO J* 1988;7:1697–704.
38. Kruse TA, Bolund L, Grzeschik K-H, et al. The human lactase-phlorizin hydrolase gene is located on chromosome 2. *FEBS Lett* 1988;240:123–6.
39. Kruse TA, Bolund L, Byskov A, et al. Mapping of the human lactase-phlorizin hydrolase gene to chromosome 2. *Cytogen Cell Genetics* 1989;51:1026 (Human Gene Mapping 10, Tenth International Workshop on Human Gene Mapping).
40. Koldovsky O. Developmental, dietary and hormonal control of intestinal disaccharidases in mammals (including man). In: Randle PD, Steiner DF, Whelan WJ, eds. *Carbohydrate metabolism and its disorders*, vol 3. New York: Academic Press, 1989:418–29.
41. Koldovsky O. Role of nutritional, hormonal and intrinsic factors in expression of normal perinatal developmental pattern on intestinal disaccharidases in mammals. In: Lebenthal E, ed. *Textbook of gastroenterology and nutrition of infancy*. New York: Raven Press, 1981:77–82.
42. Moog F. Perinatal development of the enzymes of brush border membrane. In: Lebenthal E, ed. *Textbook of gastroenterology and nutrition in infancy*. New York: Raven Press, 1981:139–47.
43. Lebenthal E, Lee PC. Interactions of determinants in the ontogeny of the gastrointestinal tract: a unified concept. *Pediatr Res* 1983;17:19–24.
44. Henning SJ. Ontogeny of enzymes in the small intestine. *Annu Rev Physiol* 1985;47:231–54.
45. Kédinger M, Haffen K, Simon-Assmann P. Control mechanism in the ontogenesis of villous cells. In: Desnuelle P, Norén O, Sjöström H, eds. *Molecular and cellular biology of digestion*. Amsterdam: Elsevier, 1986:315–26.
46. Auricchio S, Sebastio G. Development of disaccharidases. In: Lebenthal E, ed. *Testbook of gastroenterology and nutrition in early childhood*. 2nd ed. New York: Raven Press, 1989:451–70.
47. Smith MV, James PS. Cellular origin of lactase decline in postweaned rats. *Biochim Biophys Acta* 1987;905:503–6.
48. Tsuboi KK, Kwong LK, Neu J, Sunshine P. A proposed mechanism of the normal intestinal lactase decline in the postweaned mammal. *Biochem Biophys Res Commun* 1981;101:645–52.
49. Buts JP, De Meyer R. Postnatal proximodistal development of the small bowel mucosal mass in growing rat. *Biol Neonate* 1981;40:62–9.
50. Freund JN, Duluc I, Raul F. Discrepancy between intestinal lactase enzymatic activity and mRNA accumulation in sucklings and adults: effect of starvation and thyroxine treatment. *FEBS Lett* 1989;248:39–42.