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Cell-free synthesis of high-molecular-weight small intestinal polypeptides

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An intrinsic membrane glycoprotein, M_r 131 000, is a major developmentally specific component of the neonatal guinea-pig small intestinal microvillar membrane. Such high-molecular-weight proteins are often difficult to translate in vitro. In this study we report a successful strategy for the identification of the primary translation product of this glycoprotein, a high-molecular-weight precursor polypeptide of approximate M_r 225 000.

The small intestinal microvillar membrane is a highly specialised plasmamembrane domain containing differentiation-specific hydrolases, many of which are high-molecular-weight transmembrane glycoproteins with active sites directed towards the lumen of the gut [1]. In previous studies we have reported the systematic mapping of the polypeptides of the developing guinea-pig small intestinal microvillar membrane, from the foetal to the adult stage, using 2-dimensional isoelectric focussing/SDS-polyacrylamide gel electrophoresis techniques [2,3]. A polypeptide of approximate M_r 131 000 was identified as a major component of the neonatal microvillar membrane (Fig. 1A). Here we investigate its cell-free synthesis.

The M_r 131 000 microvillar protein is also the major concanavalin A-binding component of the membrane (Fig. 1B). Detergent treatment extracts all the concanavalin A-reactive glycoproteins from the membrane, including the component of M_r 131 000 (Fig. 1B). The latter glycoprotein, purified to homogeneity from such detergent extracts by sequential gel filtration and ion-exchange chromatography [4–6], is highly enriched in lactase activity (intestinal homogenates show a lactase activity of approx. 1.3 nmol galactose/h per μg which increases to 200 nmol galactose/h per μg (154-fold) in the final preparation of the M_r 131 000 glycoprotein).

Two antisera were raised in rabbits, one against the purified glycoprotein M_r 131 000, the other against SDS-denatured neonatal microvillar membrane vesicles. The first of these antibodies, used in immunoblotting [7], showed the M_r 131 000 glycoprotein to be neonatally enriched (Fig. 1, panel C).

Cell-free translation of mRNAs encoding high-molecular-weight membrane-bound proteins is often very difficult. This proved to be the case with neonatal guinea-pig small intestinal mRNA, isolated by sequential guanidinium thiocyanate extraction [8] and oligo-dT cellulose chromatography [9]. Translation of such mRNA in a rabbit reticulocyte lysate system [10] supplemented with [^{35}S]methionine efficiently translated polypeptides of M_r 25 000–95 000, but failed to produce high-molecular-weight (M_r 100 000–300 000) translation products (Fig. 2A). However, mRNA size-

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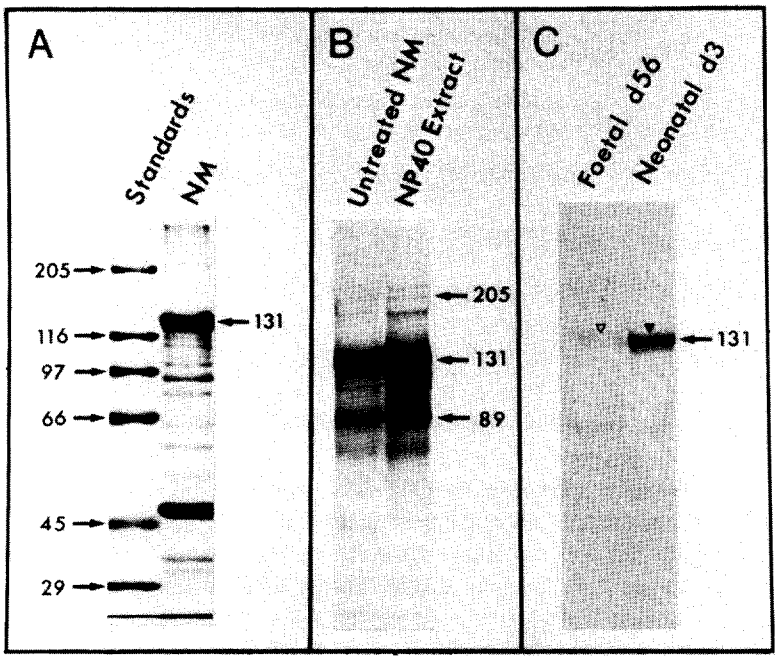

Fig. 1. (A) Major polypeptides of the neonatal guinea-pig small intestinal microvillar membrane. Microvillar membranes were prepared by cation precipitation [2] and analysed by SDS polyacrylamide gel electrophoresis with subsequent Coomassie blue staining. The migration of standard proteins (Sigma, St. Louis, U.S.A.) is also shown. (B) Detergent solubilisation of the major glycoproteins of the neonatal microvillar membrane. Purified microvillar membranes were treated with 1% (w/v) Nonidet P40 at 4°C for 30 min, followed by centrifugation (100000 × *g*, 30 min). Supernatant polypeptides extracted by the detergent were analysed by SDS-polyacrylamide gel electrophoresis [20] with subsequent nitrocellulose transfer and Concanavalin A overlaying [21]. The molecular weights of the major Concanavalin A-reactive polypeptides solubilised from the neonatal membrane (NM) are indicated. (C) Immunoblotting with antibody raised against the purified microvillar glycoprotein of M_r 131000. Microvillar membranes prepared from the two stages of development indicated [2] were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose before immuno-overlay with anti-131000 antibody [7]. The M_r 131000 component showed high levels of expression at the neonatal stage.

selected on sucrose density gradients by the method of Buell [11] and shown to consist of mRNA species greater in size than 20 S by agarose gel electrophoresis (Fig. 2B) after glyoxylation [12], very efficiently directed the synthesis of an extended size spectrum (M_r 60000–250000) of translated proteins (Fig. 2C).

Immunoprecipitation of such high-molecular-weight translation products with protein A-Sepharose [13] using pre-immune serum from rabbits before exposure to antigen showed several products to be nonspecifically precipitated (Fig. 2C). However, immunoprecipitation carried out with the anti-neonatal microvillar membrane antiserum showed at least two clearly-defined specifically immunoprecipitated polypeptides of approximate M_r 100000 and 225000. The antibody raised against the purified microvillar glycoprotein M_r 131000 specifically immunoprecipitated only one of these components, approximate M_r 225000, corresponding to the primary translation product of this glycoprotein.

This neonatal microvillar glycoprotein may be lactase (EC 3.2.1.23), since the developmental expression of lactase activity and polypeptide M_r 131000 in the guinea-pig microvillar membrane is coincident [2,3]. Both are detectable by 56 days of gestation, reach maximal levels in the early neonate and are undetectable in the adult. Moreover, the purification of this glycoprotein to homogeneity is accompanied by a 154-fold increase in lactase specific activity.

The M_r of the fully glycosylated microvillar membrane form of this protein is only 131000,

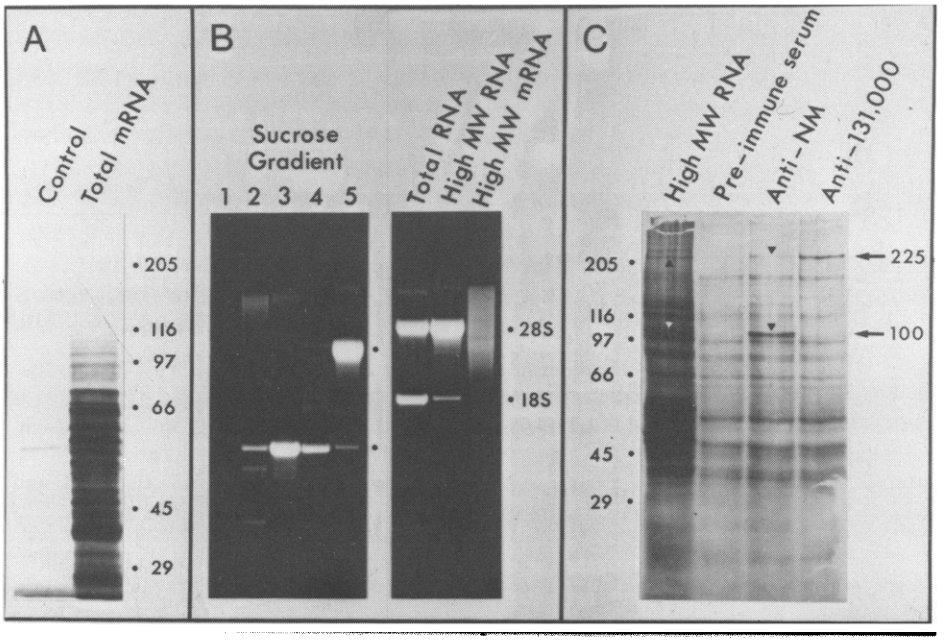

Fig. 2. (A) In vitro translation of total intestinal mRNA. Cell free translation was performed in a rabbit reticulocyte lysate with subsequent SDS-polyacrylamide gel electrophoresis analysis of translation products and fluorography [22]. Total translation products from lysates primed with either no RNA (control) or total unfractionated polyadenylated RNA prepared from the neonatal guinea-pig small intestine are shown. The molecular weights of marker proteins run on the same gel are indicated. (B) Size fractionation of total RNA isolated from the neonatal guinea-pig small intestine. Separations were performed on sucrose gradients [11]. Pooled RNA fractions, numbered from the top of the gradient, were then analysed by agarose gel electrophoresis after glyoxylation [12] with ethidium bromide staining. The positions of the 18 S and 28 S ribosomal RNAs on such gels are indicated by dots. Also shown is the final high-molecular-weight mRNA preparation used for in vitro translation. (C) In vitro translation and immunoprecipitation of unprocessed microvillar glycoprotein, M_r 131 000. Total translation products and products immunoprecipitated with either pre-immune serum, anti-neonatal microvillar membrane or anti-131 000 antisera are shown in lysates primed with high-molecular-weight polyadenylated RNA, size-selected as shown in panel B from total RNA prepared from the neonatal guinea-pig small intestine. The molecular weights of the two major specifically immunoprecipitated components are indicated.

while its unglycosylated primary translation product has a molecular weight of 225 000. This suggests that it is synthesised as a large precursor that is cleaved intracellularly into its two subunits, which is in agreement with lactase biosynthesis in pig mucosal explants [14,15] and human colon adenocarcinoma cells [16].

Only two other high-molecular-weight intestinal microvillar glycoproteins, rabbit sucrase-isomaltase and pig aminopeptidase N, have been successfully translated in cell-free systems. Two polypeptides, M_r 270 000 and 240 000, were identified as the translation products of sucrase-isomaltase [17], although the two enzymes are present in the microvillar membrane as an M_r 140 000/160 000 complex [18]. In contrast, aminopeptidase N has a primary translation product of M_r 115 000 [19], which is lower than its final M_r in the microvillar membrane (166 000). The major guinea-pig protein described here, preliminarily identified as microvillar lactase, joins sucrase-isomaltase in the family of microvillar hydrolases that are synthesised in vitro as high-molecular-weight precursors.

Thus the preparation of size-selected mRNA has enabled the successful in vitro translation of this major high-molecular-weight microvillar glycoprotein. It has also yielded a size-enriched mRNA population from which to initiate the cDNA cloning of this molecule. A knowledge of the minimum size of the mRNA which encodes the glycoprotein (at least 6.6 kb in the guinea-pig),

together with an animal model in which it shows developmental expression, may prove important in such future studies.

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