Biogenesis of the hepatitis B viral middle (M) surface protein in a human hepatoma cell line: demonstration of an alternative secretion pathway

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In the serum of hepatitis B virus (HBV)-infected patients, two different types of particles, a 42 nm virion and a 22 nm subviral particle, were identified. The envelope of both particles is composed of three proteins, the large (L), middle (M), and major/small (S) surface proteins but the ratio between these components varies in each. The M protein appears in a lesser amount than the S protein in both virion and subviral particles, although it is translated from the same subgenomic RNA, and this is due to its poor initiation context of translation. In addition, only the glycosylated form of M protein is secreted in contrast to both glycosylated and unglycosylated forms of L and S proteins that are secreted. To investigate the biogenesis of M protein, human hepatoma cells transfected with plasmids containing a mutated HBV DNA were used to produce a high amount of M protein. Electron microscopic observation revealed that despite a higher proportion of the M protein being found in the transfected cells, the secreted surface antigen particles possess similar size and density to 22 nm subviral particles. Detailed biochemical analyses showed the following. (1) The unglycosylated M protein was predominantly present in the microsomal fraction but not present in any other subcellular fractions. (2) The M protein formed 22-nm-like particles in the endoplasmic reticulum (ER) and was retained in the post-ER or pre-Golgi regions. (3) In addition to the complex glycosylated form of M protein, a high-mannose form of M protein could be secreted. (4) Normally, no unglycosylated M protein was secreted. However, glycosylation was not essential for M protein secretion since M protein deprived of glycosylation by tunicamycin treatment was detected in the medium. These findings suggest that (i) the M protein was probably translated and co-translocated into the ER and at least one site was glycosylated before leaving the ER resulting in no secretion of unglycosylated M protein, and (ii) the M protein had two secretion pathways, one through the conventional pathway and the other probably directly through the ER.

Introduction

The human hepatitis B virus (HBV) is an enveloped DNA virus. In the blood of HBV-infected patients, besides the 42 nm virion, non-infectious 22 nm spherical or filamentous particles are also present (Pontisso et al., 1989). The envelope of virions and subviral particles is composed of proteins of three species of surface antigens (HBsAgs) in varying amounts (Stibbe & Gerlich, 1982; Heermann et al., 1984). They are translated from a single open reading frame by means of three different in-frame start codons resulting in common carboxyl termini. According to their sizes and amounts present, the HBsAgs are designated as the large (L), middle (M), and major/small (S) surface proteins, each with two forms that differ in the extent of glycosylation. The L protein, containing 389 or 400 amino acids (encoded by the pre-S1/pre-S2/S region) depending on the serotype of the virion, is present in unglycosylated (p39) or glycosylated (gp42) forms. The M protein containing 281 amino acids (encoded by the pre-S2/S region), appears in either a one-chain-glycosylated (gp33) or a two-chain-glycosylated (gp36) form. No unglycosylated M protein (p30) is observed in either virions or subviral particles. The S protein, containing 226 amino acids (encoded by the S region), exists also in either unglycosylated (p24) or glycosylated (gp27) forms (Tiollais et al., 1985; Ganem & Varmus, 1987; also see Fig. 1a). Two glycosylation sites of M protein have been identified, one near the carboxyl end of S region (designated as 'C' site herein) and the other in the pre-S2 domain (designated as N-site herein) (Tiollais et al., 1981; also see Fig. 1a). Glycosylation of the C-site is common in L, M, and S proteins but glycosylation of the N-site is only present in M proteins although this site is also present in L proteins (Peterson et al., 1982; Heermann et al., 1984). The biological significance of glycosylation at the N-site of M proteins and the...
importance of M proteins are unclear, since the avian HBV does not contain this corresponding sequence (Mandart et al., 1984; Sprengel et al., 1988). Findings from recent studies are at variance with each other as to the role of M protein in virion formation (Ueda et al., 1991; Bruss & Ganem, 1991; Fernholz et al., 1993). However, the high conservation of the glycosylation signal in the N-site in all serotypes of HBV (Lo et al., 1986) might reflect its importance. Experiments have also demonstrated that the amino acids in the pre-S2 region of the M protein are highly immunogenic (Mandart et al., 1984; Bruss & Ganem, 1991; Fernholz et al., 1993).

The pre-S2 domain has been shown to bind to polymerized human albumin (Machida et al., 1983; Yu et al., 1985; Cheng & Moss, 1987), and may be associated with the infectivity of HBV and a defective virus enveloped by HBsAg. Unlike the secreted protein which contains a leader sequence for guiding a nascent peptide to the endoplasmic reticulum (ER) to complete its translation and eventual secretion (Walter & Lingappa, 1986), the HBsAg has no leader sequence but contains three stretches of hydrophobic domains in the S region. It has been demonstrated that the S protein is synthesized initially as a transmembrane protein using the first stretch of hydrophobic amino acids as a signal (Eble et al., 1986, 1987). A recent in vitro study has demonstrated that the M protein translocation across the ER is also mediated by downstream signals of hydrophobic amino acids within the S domain (Eble et al., 1990). Although the in vitro-translated M protein has been shown to be transported into the ER, the question as to whether the M protein is synthesized in the cytoplasm prior to being transported into the ER in vivo still remains unanswered. Studies of expression and secretion of the M protein have already been reported in various transfected cell systems (Cheng & Moss, 1987; McLachlan et al., 1987; Molnar-Kimber et al., 1988) but full information on glycosylation and secretion pathway is lacking. In this study, we used transfected human hepatoma cells that produce higher amount of M proteins (Sheu & Lo, 1992) to address the biogenesis of M protein in more detail.

Methods

Plasmid constructions. The plasmid pMTMS was derived from a partial deletion of pMH3/3097 plasmid (Junker et al., 1987) as described previously (Sheu & Lo, 1992). Briefly, the 1.6 kb HindIII–MstII fragment of pMH3/3097 was deleted followed by Klenow fragment fill-in and blunt-end ligation. After transfection into human hepatoma cells, the pMTMS plasmid under the human metallothionine (hMT) promoter control can be transcribed into a 2.1 kb mRNA fragment of pMTMMS was substituted by the sequence 5’ ACCATGG 3’ resulting from mutation in the flanking initiation sequence of the pre-S2 region. The difference between pMTMS and pMTMMS plasmids is indicated by ACCATGG present in the pre-S2 region.

Cell transfection. A standard calcium–phosphate method (Graham & van der Eb, 1973) was employed to transfect a well-differentiated human hepatoma cell line, HuH-7 (Nakabayashi et al., 1982), with either pMTS or pMTMMS plasmid DNA. Three days after transfection, cells were washed with PBS and then incubated with 5 ml of methionine-free Dulbecco’s modified Eagle’s medium (DMEM) for

Fig. 1. Organization of the HBV genome and physical maps of plasmids containing HBV DNA. (a) The open boxes with an arrow represent the four open reading frames of the HBV genome and the direction of gene expression. Wavy lines indicate the 2-4- and 2-1-kb mRNA that are transcripts for the synthesis of HBsAg. Heavy lines indicated by the letters L, M, and S, respectively, correspond to distinct sizes of co-C-terminal HBsAg. The circles marked on the heavy lines are the locations of the glycosylation sites of the HBsAg. The numerals below the heavy lines are the total amino acid residues (aa) of each HBsAg. (b) The three plasmids containing HBV DNA are indicate by their corresponding lengths on the linearized HBV genome in the panel (a). Plasmid pMH3/3097 contains a hMT promoter and the 3.5-kb HBV DNA fragment. Plasmid pMTMMS is derived by partial deletion of pMH3/3097 plasmid from the HindIII (Hd) to MstII (Mt) restriction sites. Plasmid pMTMMS resulted from mutation in the flanking initiation sequence of the pre-S2 region in the pMTMS plasmid. The difference between pMTMS and pMTMMS plasmids is indicated by ACCATGG present in the pre-S2 region.
1 h at 37 °C. Subsequently, the transfected cells were labelled with 100 μCi/ml of [35S]methionine (> 1000 mCi/mmol). After 30, 60 min or 6 h (most cases), the culture medium was collected and the cells were washed with PBS buffer and detached from plates with 0.125% trypsin solution containing 0.05% EDTA and 0.05% glucose. The harvested cell pellet was mixed with 500 μl of NTE buffer (150 mM-NaCl, 50 mM-Tris–HCl pH 7.5, and 0.5 mM-EDTA) containing 0.5% Nonidet P-40 and 1 mM-PMSF, and then clarified by centrifugation at 12000 r.p.m. in an Eppendorf microfuge for 15 min. For chase experiments, cells were labelled for 6 h, washed by PBS, and chased for various periods by incubation in 5 ml of fresh DfMEM supplemented with complete or methionine-free serum at 37 °C.

Subcellular fractionation. The transfected cells were suspended in 0.3 M-sucrose–TM buffer (10 mM-Tris–HCl pH 8.0, 5 mM-MgCl2 and 5 mM-DTT) at a density of 2 × 10^7 cells/ml and homogenized by applying 50 strokes of a Dounce homogenizer. The nuclei and unbroken cells were spun down by centrifugation at 700 g for 10 min, and the supernatant was then centrifuged at 24000 g for another 10 min. The pellet containing mitochondria and lysosomes was removed and the supernatant was centrifuged at 20000 g for another 30 min in order to yield a pellet of microsome and supernatant of cytosol fraction. The [35S]methionine labelled microsomes were directly resuspended in NTE buffer containing 0.5% Nonidet P-40 and 1 mM-PMSF. Both microsomal and cytosol fractions were immunoprecipitated prior to conducting SDS–PAGE analysis.

CsCl density gradient centrifugation analysis. To measure the buoyant density of the intracellular HBV surface antigen particles, the isolated microsomal fraction was dissolved with NTE buffer in a total volume of 12 ml in the presence of 22% (w/w) CsCl, and centrifuged in a SW41 rotor at 38000 r.p.m. for 36 h. Twenty-four fractions were collected from the top to the bottom of the tube and analysed by an HBsAg EIA kit (Ever New Biotech Co.) after 50-fold dilution. The densities of CsCl density gradient fractions were measured using a refractometer.

Partial purification of surface antigen particles. Surface antigen particles were prepared from both the culture medium and cell lysate. Culture medium from the transfected cells was collected and clarified at 12000 r.p.m. in a JA20 rotor for 30 min. The particulate material in the supernatant was concentrated by centrifugation at 45000 r.p.m. in a Ti55.2 rotor for 2.5 h. Likewise, surface antigen particles in the high HBsAg positive peaks of CsCl density gradient fractions from cell lysate described above were harvested by centrifugation.

Electron microscopy. Appropriate fractions from CsCl gradients or the concentrated particulate materials from the culture medium were applied to Formvar resin-coated specimen grids and stained with 1% uranyl acetate. The samples were examined and photographed using a JOEL electron microscope (Lee et al., 1988).

Radioimmunoprecipitation (RIP) and gel electrophoresis. The cell lysate and the collected medium were treated with protein A–Sepharose-bound monoclonal antibody M27 which recognizes an epitope located in the pre-S2 region (Lo et al., 1990). RIP by anti-albumin (Sigma) was carried out in parallel in experiments using brefeldin A (BFA). The immunoprecipitated proteins were dissolved in sample buffer and fractionated by SDS–PAGE following the method of Laemmli (1970). The gels were fixed, dried and fluorographed using a Fuji X-ray film at -70 °C.

Glycosidase digestion. For endoglycosidase H (endo H) digestion study, the immunoprecipitated products from the intracellular and extracellular fractions of pMTMS- or pMTMMS-transfected cells were digested for 18 h at 37 °C with 3 μU of endo H in a total volume of 30 μl 100 mM-sodium phosphate buffer, pH 5.5 containing 0.5 M-EDTA, 0.1% Triton X-100 and 0.05% SDS. For endoglycosidase F (endo F) cleavage study, the extracellular products after immunoprecipitation were incubated at 37 °C for 1 or 24 h in a total volume of 30 μl with 0.2 U of enzyme in the presence of 0.25 M-sodium acetate pH 6.0, 20 mM-EDTA, 10 mM-2-mercaptoethanol, 0.1% Triton X-100 and 0.2% SDS.

Tunicamycin and BFA treatment. At day 3 after DNA transfection, the cells were first incubated for 1 h with 5 ml of methionine-free DMEM in the presence of tunicamycin or BFA (final concentrations 25 μg/ml and 2 to 10 μg/ml, respectively). Cells were then labelled in the presence of these drugs. Following labelling for 6 h, cell lysate and cell culture medium were prepared and immunoprecipitated as described above. To test cell lysis caused by drugs, cell culture medium were collected for an AST (aspartate aminotransferase) assay (EIA kit, from Abbot Laboratory).

Results

Assembly and secretion of 22-nm-like surface antigen particles in the M protein high production cells

Our previous experiments have demonstrated that the HuH-7 human hepatoma cells transfected by the pMTMMS plasmid which contains an HBV sequence with an optimal translational initiation context of pre-S2 region can produce a higher amount of M protein, and that the ratio of M to S protein differs inside and outside of cells (Sheu & Lo, 1992). We then examined whether the surface antigens in the pMTMMS-transfected cells, which consist of more M than S proteins in contrast to more S than M proteins in the wild-type, could form particles. For comparison, the intracellular and extracellular products from both the pMTMS- (containing a wild-type HB sequence) and pMTMMS-transfected cells were therefore analysed by CsCl isopycnic centrifugation and electron microscopy. The pMTMS-transfected cells producing the 22-nm-like particles served as a positive control.

Surface proteins in the isolated microsomal fraction from the pMTMS- or pMTMMS-transfected cells were found by using isopycnic CsCl gradient centrifugation to have a buoyant density of about 1.19 g/ml (Fig. 2a and b), this value being similar to that of the 22-nm particle (Kaplan et al., 1976). It was noted that the surface proteins from the pMTMMS-transfected cells appeared in a broader HBsAg-positive peak than those from the pMTMMS-transfected cells. Electron microscopic examination indicated that those particles present in the microsomal fraction of the pMTMMS cells were similar in size to surface antigen particles found in HBV-infected patients (see inset, Fig. 2b). Furthermore, 22 nm-like particles were also detected in the concentrated medium of the pMTMMS-transfected cells (data not shown). Taken together, we concluded that 22-nm-like subviral particles could be assembled in the ER and then secreted by M protein-high producing cells.
Fig. 2. Determination of buoyant density of surface proteins and electron microscopic observation in microsomal fraction of pMTMS- or pMTMMS-transfected human hepatoma cells. The isolated microsomal fraction from pMTMS- (a) or pMTMMS- (b) transfected cells was dissolved and centrifuged into an isopycnic CsCl gradient. Fractions were collected, divided into 24 samples, and then analysed by a HBsAg EIA kit after 50-fold dilution. Results obtained using a spectrophotometer represent the relative amount of HBsAg, and are shown as (---). The CsCl densities of gradient fractions were measured by a refractometer and are shown as (•--). The inset of (b) shows a HBsAg-positive peak from CsCl gradients of (b) that was applied directly to Formvar resin-coated copper specimen grids and stained with 1% uranyl acetate. The grids were then examined and photographed. The bar marker represents 100 nm.

Glycosylation patterns of M protein in secreted and non-secreted forms

Our previous study has also revealed that the secreted M proteins gp33 and gp36 are more complex than those of intracellular counterparts, resulting in their slower migration in SDS-PAGE (Sheu & Lo, 1992). In this study, the superscript "*" is used to designate the complex form of glycosylation, i.e. gp33* and gp36* for complex forms versus gp33 and gp36 for simple forms. In order to closely examine the dynamic change of the glycosylation process of M protein, the pMTMMS-transfected cells were metabolically labelled with [35S]methionine for 30, 60 min, or 6 h (most of cases) and then chased for another 30, 60 min or 24 h. The collected culture medium and the cell lysate were analysed by immunoprecipitation and gel electrophoresis.

The results indicated little/no difference in the SDS–PAGE pattern when the intracellular products were analysed at different chase times (Fig. 3, lanes 2 and 3). Two dominant forms of gp33 and gp36 were present together with a lesser amount of p30 and a trace amount of complex form of M protein. However, the SDS–PAGE pattern of secreted M proteins differed between the pulse and chase experiments (Fig. 3, lanes 4 to 6). Four bands of gp33, gp36, gp33* and gp36* in similar amounts were clearly observed in the extracellular products after 30 or 60 min chase in contrast to the presence of two dominant bands of gp33* and gp36* in a pulse experiment (Fig. 3, lanes 5 and 6 as compared with lane 4). This pattern of four bands persisted in a 24 h chase experiment (data not shown). As in previous studies, unglycosylated p30 protein was undetectable in the medium fraction during all chase and pulse experiments (Fig. 3, lanes 4, 5 and 6).

Glycosylation processes of M proteins

It is well known that endo H cleaves asparagine-linked carbohydrate between the most proximal N-acetylglucosamine residues in simple glycosyl side-chains (high mannose) but does not cleave complex glycosyl side-chains derived from processing of simple high-mannose side-chains. These latter complex side-chains can however be cleaved by endo F. Thus endo H and F are useful aids for distinguishing between high-mannose and complex sugar modified glycoproteins (Elder & Alex-
Fig. 4. Characterization of the nature of carbohydrate on the surface proteins synthesized in pMTMS- or pMTMMS-transfected human hepatoma cells by the digestion of endoglycosidase H (a) and endoglycosidase F (b). (a) Immunoprecipitated intracellular and extracellular products of pMTMS- (lanes 1 and 2) or pMTMMS- (lanes 3 to 6) transfected human hepatoma cells are treated with (marked by ‘+’) or without (marked by ‘−’) endo H. The experimental conditions in lanes 7 and 8 were similar to those of lanes 5 and 6 except that the transfected cells had been chased for another 60 min. (b) Immunoprecipitated extracellular products of pMTMMS-transfected cells are treated with (marked by ‘+’) or without (marked by ‘−’) endo F. Lanes 2 and 3 show glycoproteins digested with 0.2 U of endo F for 1 or 24 h, respectively. L, M and S and ‘*’ are the same as in Fig. 3.

ander, 1982; Tarentino & Maley, 1974). We therefore tried to differentiate various forms of M proteins by digestion of M proteins obtained from cell lysate and culture medium with either endo H or endo F. In endo H digestion experiments, both gp33 and gp36 of intracellular fraction from the pMTMMS cells were converted into p30 (Fig. 4a, compare lanes 3 and 4). Likewise, all gp27 from the pMTMS cells was converted into p24 (Fig. 4a, compare lanes 1 and 2). These results indicated that all M or S proteins present inside of cells are located in the ER or the pre-Golgi region. Most of the extracellular fraction of M protein from the pMTMMS cells appeared to be resistant to endo H treatment, remaining as gp33* and gp36* (Fig. 4a, lane 6). A small amount of p30 was present which might indicate that a small amount of gp33 and gp36 was secreted and suggest that there were two secretion pathways. Such supposition was more evident in the chase experiments; an obvious p30 band was obtained from the endo H digestion of M proteins present in the medium during the chase periods to result in almost an equal intensity of three bands (p30, gp33*, and gp36* in lane 8 of Fig. 4a). Fig. 4(b) shows that the extracellular gp33* and gp36* were indeed sensitive to endo F treatment, indicating they were glycosylated by complex sugars in the Golgi complex before secretion (Fig. 4b compare lanes 1 to 3).

Taken together, the results from the endoglycosidase digestion and pulse and chase experiments led to the following conclusions. First, almost all glycosylated M proteins in the intracellular fraction, possessing a high-mannose side-chain, are resident in either an ER or a pre-Golgi compartment. Secondly, the M proteins are transported to a late Golgi compartment for further oligosaccharide processing, and are rapidly secreted. This process was obviously less time consuming than the synthesis and glycosylation of M proteins in the ER, since no or little gp33* and gp36* was observed intracellularly. Thirdly, those gp33 and gp36 secreted during the chase period are endo H sensitive, suggesting that they either escaped further modification after entering the post-Golgi compartment or bypassed the Golgi region and were secreted directly after glycosylation in the ER.

Two secretion pathways for M proteins

In order to explore the secretion pathway of gp33 and gp36 M proteins with high-mannose side-chains, BFA treatment experiments were carried out. BFA is a fungal antibiotic that blocks the transport of membrane and secretory proteins through the Golgi complex (Misumi et al., 1986). If the gp33 and gp36 were secreted through the Golgi complex while escaping further modification, the BFA-treated pMTMMS cells should show no M protein secretion. In contrast, if gp33 and gp36, but not gp33* or gp36* were detected in the medium, it indicated that the M proteins were likely to have been secreted directly from the ER. Results revealed that little or no differences between gp33 and gp36 in both cell lysates from BFA-treated or control cells were observed but with a lesser amount of p30 and a broader band of gp36 being found in the BFA-treated cells as compared with the control cells (Fig. 5a, lanes 1 and 2). However, only gp33 and a
Glycosylation is not an essential process for M protein secretion

The fact that there was no detectable p30 secretion might indicate that glycosylation is both a signal and a required process for M protein secretion. To examine this possibility, transfected cells were treated with tunicamycin, an N-linked glycosylation inhibitor that can block the first step in the glycosylation process and prevent further glycosylation (Elbein, 1981). Results indicated that with tunicamycin treatment of either pMTMS- or pMTMMS-transfected cells, most of M and S proteins were present in an unglycosylated form intracellularly (Fig. 6, lanes 1 and 2), although a small amount of gp33 and gp36 were present in the pMTMMS cells, presumably caused by incomplete inhibition by tunicamycin. Furthermore, both unglycosylated M protein (p30) and S protein (p24) were present in the extracellular fraction of pMTMMS cells (Fig. 6, lane 4). Since no cell lysis was detected by AST assay, the unglycosylated M protein detected from the medium of tunicamycin-treated cells was indeed secretable. Thus, the glycosylation of M protein is not essential for secretion.
Production is never higher than the S protein in the accomplishment by using a high M protein production system. It needs to be borne in mind that M protein particles (Fig. 2b, inset), contained a higher amount of unglycosylated M protein and little or no gp36 (Fig. 7, inset in Fig. 2b and Fig. 7, lane 3). It is tempting to suggest that the M protein can be glycosylated at the pre-S2 (N-) site but not (or slowly) at the S (C-) site before being packed into particles. Although we do not fully know how the mechanism of particle formation operates, it is clear that there exists an ER fraction containing particles, unglycosylated p30, and little or no gp36 (Fig. 7).

Secondly, it is not known exactly why/how there is little gp36 in the fraction of ER mentioned above. It is possible that this simply reflects the delay in the second glycosylation reaction occurring until particles are formed. However, we favour the hypothesis that this second glycosylation occurs in a different compartment. In other words, the p30, gp33 and particles must be transported to a post-ER/pre-Golgi region for the formation of gp36. Since gp33 and gp36 are the dominant forms of M protein in the cell, it appears that most of the particles are retained in the post-ER or pre-Golgi regions and, similar to S protein-forming particles (Huovila et al., 1992), the process is unlike the retention of L proteins in the ER (Kuroki et al., 1989; Prange et al., 1991).

Finally, our results suggest two secretion pathways for M protein, one possibly through the ER to the plasma membrane and the other through the Golgi complex. The secretion of gp33 and gp36, a possible unconventional secretion pathway, was not inhibited by BFA treatment and was not due to cell lysis as proven by light microscopic observation, AST assay and the results of absence of p30 and albumin in the medium of BFA treated cells (Fig. 5). To our knowledge, such dual secretion pathways of proteins have not been reported before. Interestingly, two secretion pathways of M proteins were only observed in the cells present in the complete medium during the chase time (Fig. 3, lanes 5 and 6). The secretion pattern varies with incubation time.
conditions, the Golgi complex secretion pathway predominating over the alternative secretion pathway if cells were continuously incubated in methionine-free medium or in complete serum medium with calcium ionophore, A23187, or with constant agitation (S. Y. Sheu and S. J. Lo, unpublished). This phenomenon is similar to the recent finding of immunoglobulin M receptor expression in tolerant B lymphocytes (Bell & Goodnow, 1994) in two respects: (i) there is a regulation step of intracellular transport between ER and Golgi; and (ii) this step can be regulated by external signals.

Based on these results, a general biogenesis pathway for HBV M protein in hepatoma cells is suggested in Fig. 8. Although the exact location for M protein translation is not completely certain, our results support the notion that M protein is translated on and co-translocated into the ER to produce p30 inserted onto the ER membrane since we did not detect any p30 in the cytosol fraction even with a short pulse-labelling time of 30 min (data not shown). After the p30 is inserted onto the ER, it is subjected to three types of modifications: (i) glycosylation, (ii) dimerization (S. Y. Sheu & S. J. Lo, unpublished), and (iii) evagination into the ER lumen to form 22-nm-like particles (Stage I). Thereafter, the 22 nm particles are transported from the ER to the post-ER or pre-Golgi region (transition element), the glycosylation of M proteins is then completed to form gp33 and gp36 (Stage II). This transition region is also a shunt region for directing the 22 nm particle-containing vesicles either to the Golgi complex for further modification and secretion of gp33* and gp36* or directly to the plasma membrane for secretion of gp33 and gp36 (Stage III).

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References


Fig. 8. The scheme of M protein biogenesis. Three stages are depicted: (Stage I) The M protein is translated and co-translocated into the ER and 22-nm-like particles are formed. (Stage II) The 22-nm-like particles are transported into transporting vesicles and retained for a period of time. (Stage III) The transporting vesicles fuse with the Golgi complex for further glycosylation. The 22-nm-like particles are secreted via secretory vesicles or directly through transporting vesicles. The top gels show the glycosylation pattern of M proteins at different stages, indicated by the arrows. The major bands in the gels (from left to right) are: p30 and gp33; p30, gp33 and gp36; and gp33* and gp36*. The horizontal heavy arrows indicate the major pathway of M protein maturation whereas the small arrows indicate the minor pathway. The circular particles present in the lumen of ER, transporting vesicles and extracellular space represent the high-mannose modified M proteins, whereas particles marked by asterisks represent the complex sugar form of M proteins. ER, Endoplasmic reticulum; PM, plasma membrane.
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