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The preS1 Protein of Hepatitis B Virus Is Acylated at Its Amino Terminus with Myristic Acid

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The preS/S coding region of hepatitis B virus encodes two polypeptides (preS1 and preS2) that are larger in size but less abundant than the major viral surface antigen (S) protein. Unlike the preS2 and S proteins, the preS1 protein is preferentially localized on circulating virus particles but is not efficiently secreted from mammalian cells in culture. To search for differences in protein processing that might relate to these properties, we determined whether any of the hepatitis B virus surface proteins are acylated with long-chain fatty acids. Transfected COS cells expressing all three proteins were incubated with ^{3}H-palmitate or ^{3}H-myristate, and the cell extracts were examined by immunoprecipitation. While none of these proteins was labeled with ^{3}H-palmitate, the preS1 protein but not the preS2 or S protein incorporated ^{3}H-myristate via a hydroxylamine-resistant amide linkage. Comparison of the N-terminal amino acid sequences of hepadnaviral preS1 proteins with those of known myristylated proteins suggests that this unusual modification may be a common feature of all hepadnaviral preS1 proteins.

Recent studies of hepatitis B surface antigen (HBsAg) purified from the serum of infected patients or from mammalian cells transfected with viral DNA have identified two HBsAg-related species, designated the preS1 and preS2 proteins, in addition to the more abundant surface antigen (S) protein (12, 14, 23, 25). The coding region for the preS and S proteins comprises a single 1,182-base-pair open reading frame with three translation initiation codons that directs the production of three proteins with a common carboxy terminus: the major viral S protein (226 amino acid residues), the preS2 protein (281 amino acids), and the 380-amino-acid preS1 protein that is the product of the entire open reading frame (Fig. 1). The distribution of these polypeptides is not equivalent among the circulating forms of HBsAg; the preS1 protein is found in higher abundance in viral particles and filaments than in the more numerous 22-nm subviral particles, while the converse is true of the more abundant preS2 and S proteins (12). These findings suggest that preS1 determinants may be important participants in virus assembly or infectivity or both.

In a previous report, we examined the expression of the S and preS1 proteins by transfection of viral DNA into cultured mammalian cells and injection of synthetic mRNAs into *Xenopus* oocytes (25). The findings indicated that the preS1 proteins, unlike their preS2 and S protein counterparts, are not secreted into the culture medium despite the presence of the secretory information contained in the S-specific domains. Furthermore, when the S and preS1 proteins are synthesized together, secretion of the S proteins is strongly and specifically inhibited. This suggests that some element of the preS1 proteins, whether alone or in a mixed aggregate of S and preS protomers, is inhibitory to the secretion of HBsAg polypeptides.

In seeking differences in protein processing that might explain the unexpected secretory properties of the preS1 proteins, we examined the preS and S proteins for the presence of long-chain fatty acids, a known posttranslational modification of viral envelope glycoproteins. To obtain expression of the S and preS proteins in mammalian cells, we constructed plasmid pSV45H (25), which contains the contiguous preS/S coding region downstream of the simian virus 40 (SV40) early promoter (Fig. 1A). This plasmid was then used to transfect COS7 cells. At 48 h following transfection, the medium was removed from the transfected cells and replaced with medium containing ^{3}H-palmitate, ^{3}H-myristate, or ^{35}S-methionine. ^{35}S-methionine labeling was performed in accordance with a previously described procedure (25). For ^{3}H-myristate and ^{3}H-palmitate labeling, the cells were labeled with 1 mCi of the appropriate fatty acid per ml for 20 h (2), and cell extracts were then examined by immunoprecipitation with antibody to HBsAg (25). As expected, ^{35}S-methionine-labeled cells transfected with plasmid pSV45H produced, in addition to the S proteins of 24 and 27 kilodaltons (kDa), both sets of preS-encoded polypeptides: the preS1 proteins of 39 and 42 kDa and the preS2 proteins of 31, 33, and 36 kDa (Fig. 1B, lane M). (In each case, the most rapidly migrating band corresponds to the unglycosylated form, and the slower species corresponds to the glycosylated form(s) of the indicated protein.) None of the S protein species incorporated ^{3}H-palmitic acid; labeled extracts from a duplicate set of transfected cells (Fig. 1B, lanes 5 and 6) as well as cell extracts from untransfected cells (Fig. 1B, lane 4) yielded no immunoprecipitable species. However, when ^{3}H-myristate was used for labeling, a doublet migrating in the position of the preS1 proteins was observed (Fig. 1B, lanes 2 and 3). Despite the contemporaneous production of the preS2 and S proteins in these cells, only the preS1 proteins became labeled in the presence of ^{3}H-myristate.

To test whether the inability to detect a palmitate-labeled species was due to inefficient labeling with this fatty acid, we examined the unprecipitated cell extracts from palmitate- and myristate-labeled cells. In the presence of ^{3}H-palmitate, many labeled protein species were observed (Fig. 1C, lanes 4 to 6). By contrast, ^{3}H-myristate efficiently labeled only a few cellular proteins (Fig. 1C, lanes 1 to 3), in keeping with observations in other systems (15).

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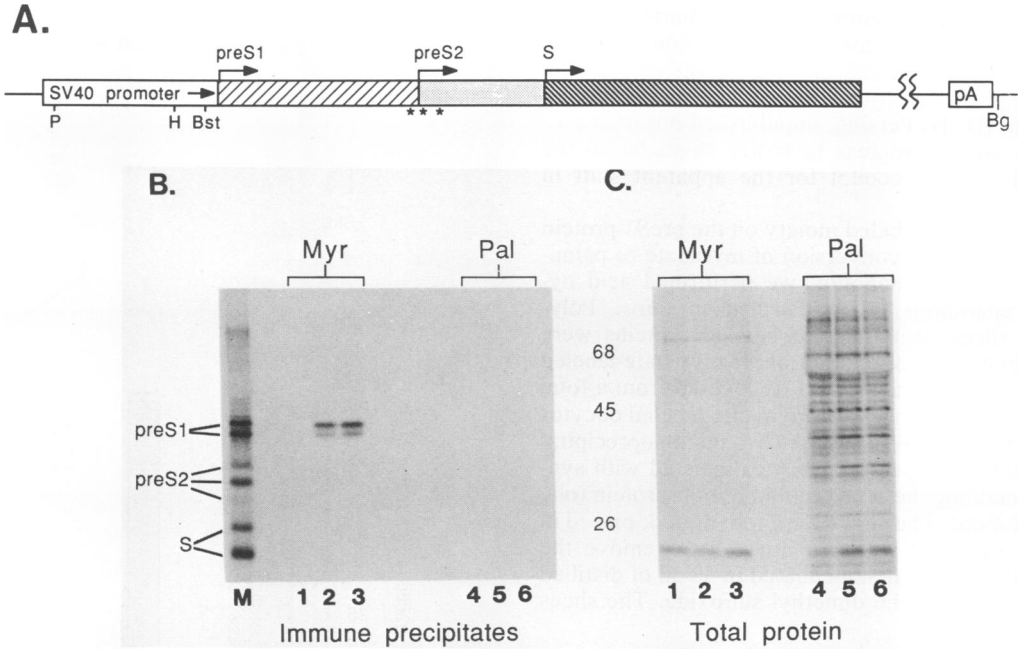

FIG. 1. (A) Expression of the HBV preS and S proteins from an SV40 vector. The construction of plasmid pSV45H has been described elsewhere (25). Shown is the 342-base-pair *Pvu*II (P)-*Hin*dIII (H) fragment of SV40, containing the SV40 replication origin and the SV40 early promoter inserted upstream of the HBV preS/S coding region. The asterisks indicate the approximate transcription initiation sites of the HBV preS2/S promoter. The arrows indicate the probable sites of translation initiation for the preS1, preS2, and S gene products. (B) Immunoprecipitation of ^{35}S-methionine-, ^{3}H-myristate-, and ^{3}H-palmitate-labeled cell extracts from HBsAg-producing COS cells. Plates (60 mm) of COS7 cells were transfected with pSV45H in the presence of DEAE dextran by a procedure previously described (8) and labeled with ^{35}S-methionine, ^{3}H-myristate, or ^{3}H-palmitate. Cell lysates were prepared and immunoprecipitated as described elsewhere (25). Immunoprecipitated samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 12% polyacrylamide gel. The gel was then fixed, fluorographed, dried, and exposed to film for 6 days. Shown are immunoprecipitations of ^{35}S-methionine-labeled transfected cells (lane M), transfected (lanes 2 and 3) and untransfected (lane 1) cells labeled with ^{3}H-myristate, and transfected (lanes 5 and 6) and untransfected (lane 4) cells labeled with ^{3}H-palmitate. (C) SDS-PAGE of HBsAg-producing COS cell extracts labeled with ^{3}H-myristate or ^{3}H-palmitate. A 4-μl quantity of each of the ^{3}H-labeled cell extracts used in the above-described immunoprecipitations (0.8% of the immunoprecipitated volume) was subjected to SDS-PAGE on a 12% polyacrylamide gel. The gel was then fixed, fluorographed, dried, and exposed to film for 3 days. The sources of the unprecipitated cell extracts shown in lanes 1 to 6 are as in panel B. The numbers between panels B and C represent kilodaltons.

The inhibitory property of preS1 protein expression on S protein secretion has recently been documented in a variety of experimental systems (4, 5, 25, 32). Since we and others have observed the inhibitory effects of preS1 protein expression on S protein secretion in *Xenopus* oocytes, we were interested in whether myristylation of the preS1 protein occurs in this system (25, 32). Plasmids containing the S or preS/S coding region downstream from the SP6 promoter were constructed, and synthetic mRNAs were synthesized from the appropriate template by in vitro transcription with SP6 polymerase. Injection of *Xenopus* oocytes was carried out as previously described (24, 25; K. Simon, E. Perrara, and V. Lingappa, submitted for publication). Briefly, a 40-nl volume containing 60 to 120 ng of transcript in transcription buffer was injected into each of 15 freshly dissected oocytes, after which the transcript was allowed to equilibrate for 12 h. The medium was then removed and replaced with medium containing 5 mCi of ^{35}S-methionine or 2 mCi of ^{3}H-myristate per ml, followed by incubation at 18°C for 24 h. Cell homogenates were prepared as described previously (25) and spun in an Eppendorf centrifuge for 10 min, and the resulting supernatants were removed for immunoprecipitation with antibody to HBsAg (Calbiochem-Behring). As in mammalian cells, the preS1 proteins, but not the S proteins, were labeled with ^{3}H-myristic acid, whether or not S mRNA was coinjected (Fig. 2, lanes 1 and 3, and data not shown).

The failure of S and preS2 proteins to be labeled with ^{3}H-myristate suggested that the preS1 domain itself was the site of fatty acid addition. To further examine this possibility, we constructed a fusion gene encoding a hybrid protein consisting of the first 110 amino acids of the preS1 protein fused to the N terminus of chimpanzee α-globin, a nonmyristylated cytoplasmic protein (22). In vitro transcripts encoding this protein were injected into *Xenopus* oocytes in the presence of ^{3}H-myristate, and the labeled translation products were immunoprecipitated with anti-globin antiserum (Calbiochem). As predicted, the hybrid protein was labeled efficiently with ^{3}H-myristate (Fig. 2, lane 4). This result indicates that the C-terminal 278-amino-acid residues of the preS1 protein, including S and nearly all of the preS2 domains, are dispensable for acylation.

It is interesting to note that the myristate-labeled hybrid protein appeared as a doublet of 22 and 26 kDa. The reason for the presence of the multiple species of the fusion protein is unclear. The preS1 region of the ayw hepatitis B virus (HBV) subtype used in these experiments contains two in-phase ATG codons separated by 10 codons; independent initiation at each ATG is thus unlikely to account for the observed 3-kDa difference between the two species. Alternatively, the bands could result from posttranslational processing events (e.g., proteolysis, glycosylation, or other modifications). The shift in molecular weight is probably not due

to the presence of the high-mannose oligosaccharide moiety; under conditions of endoglycosidase H digestion, in which the glycosylated forms of the S and preS proteins are fully sensitive, the slower-migrating form of the hybrid protein remains resistant (D. H. Persing, unpublished observation). Further studies are in progress to better characterize the modifications that may account for the apparent shift in mobility.

To be certain that the labeled moiety on the preS1 protein was not derived from the conversion of myristate to palmitate or another novel metabolite, we performed acid hydrolysis on preparations of gel-purified proteins. Polyacrylamide gel slices containing ^{3}H-labeled proteins were obtained from immunoprecipitation of ^{3}H-myristate-labeled COS cells transfected with pSP45H (recovered from a total of four immunoprecipitations), ^{3}H-myristate-labeled oocytes injected with synthetic preS1 mRNA (two immunoprecipitations), and ^{3}H-myristate-labeled oocytes injected with synthetic mRNA encoding the preS1-globin hybrid protein (one immunoprecipitation). The slices were rehydrated, placed in 50 ml of 100% dimethyl sulfoxide for 2 h to remove the diphenoxazole fluor, and then incubated in 50 ml of distilled water for 1 h to remove the dimethyl sulfoxide. The slices were then incubated in 0.3 ml of 6 N HCl at 110°C for 24 h. Both the acid hydrolysate and the polyacrylamide pellet were extracted three times with 0.5 ml of toluene, and reversed-phase thin-layer chromatography was performed as described by Buss and Sefton (2).

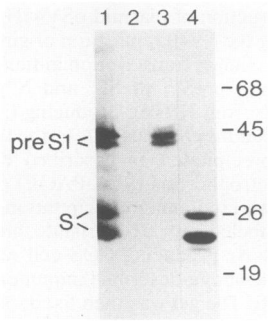

FIG. 2. Production of ^{3}H-myristate-labeled preS1 proteins and preS1-globin fusion proteins by injection of synthetic mRNAs into *Xenopus* oocytes. The construction of plasmids pSP24H and pSP45H (not shown) has been described elsewhere (25). To construct plasmid pSP45GLO (also not shown), we first inserted the 2.8-kilobase *Bgl*II fragment of HBV into the *Bam*HI site of pSP125E (a plasmid containing the chimpanzee α-globin coding region downstream of the *Salmonella* phage SP6 promoter) and then screened resultant clones for orientation by restriction analysis. A clone bearing the preS1 region in the correct transcriptional orientation was digested with *Nco*I and treated briefly with Bal31 to provide blunt ends in all three possible reading frames near the 5′ end of the α-globin coding region. Next, the exonuclease-treated clone was digested with *Eco*RI to remove most of the HBV sequences upstream of the α-globin coding region, with the exception of the coding regions for preS1 and the first few nucleotides of preS2. The preS1 region was then fused to globin sequences by the addition of T4 DNA ligase. preS and S proteins were produced by injection of synthetic mRNAs of each polypeptide into *Xenopus* oocytes (25; Simon et al., submitted) in the presence of ^{35}S-methionine or ^{3}H-myristate and detected by immunoprecipitation. Shown are immunoprecipitates of ^{35}S-methionine-labeled oocytes coinjected with equal amounts of S- and preS1-specific mRNAs (lane 1), uninjected oocytes labeled with ^{3}H-myristate (lane 2), ^{3}H-myristate-labeled oocytes coinjected with equal amounts of S- and preS1-specific mRNAs (lane 3), and an anti-globin immunoprecipitate of ^{3}H-myristate-labeled oocytes injected with RNA encoding a preS1-globin hybrid protein (lane 4). Samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 12% polyacrylamide gel, which was then fixed, fluorographed, dried, and exposed to film for 4 days. The numbers at the right represent kilodaltons.

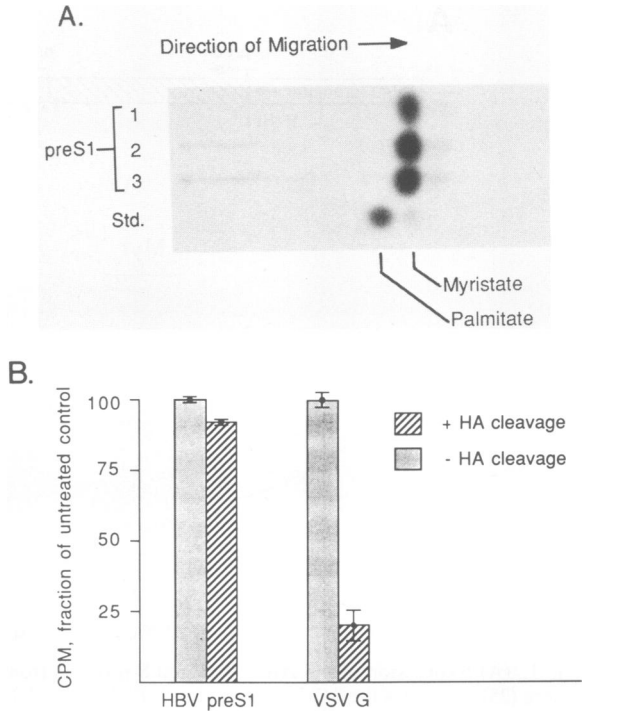

FIG. 3. Confirmation of the labeled moiety as an amide-linked myristic acid residue. (A) Thin-layer chromatographic analysis of acid hydrolysates of preS1 proteins. Gel slices containing the electrophoretically resolved, ^{3}H-myristate-labeled preS1 proteins produced in COS cells and *Xenopus* oocytes were rehydrated and suspended in 6 N HCl. After incubation in 110°C for 24 h, the liberated products were extracted into nonpolar solvents and analyzed by reversed-phase thin-layer chromatography. Shown are the chromatographed acid hydrolysis products of ^{3}H-labeled gel slices from oocytes injected with synthetic preS1 mRNA (lane 1), oocytes injected with RNA encoding the preS1-globin fusion protein (lane 2), and preS1 proteins precipitated from HBsAg-producing COS cells (lane 3). Lane std. contains the authentic palmitate and myristate standards. (B) Hydroxylamine (HA) sensitivity of the preS1-myristate linkage. Two identical polyacrylamide gels containing ^{3}H-myristate-labeled HBV preS1 proteins as well as VSV G protein labeled with ^{3}H-palmitate were run. One gel was fixed and fluorographed immediately after electrophoresis, and the other was soaked for 1 h in 1 M hydroxylamine (pH 8) fixed, and fluorographed. The gels were exposed to preflashed film for 4 days, and the ^{3}H-labeled protein bands were excised from the gel and counted in a liquid scintillation counter (36). The results are shown in the histogram as a percentage of results for the untreated control; 2 standard deviations are indicated at the top of each bar. Actual counts per minute (±2 standard deviations) obtained from the samples were as follows: 7,189 ± 47 for untreated preS1; 6,988 ± 44 for hydroxylamine-treated preS1; 1,102 ± 33 for untreated VSV G protein; and 222 ± 37 for hydroxylamine-treated VSV G protein.

Figure 3 shows the results of this analysis. Authentic myristic acid runs ahead of palmitic acid on thin-layer chromatograms because of its shorter carbon-chain length. The acid hydrolysis products from purified preS1 proteins produced in COS cells or oocytes are shown in Fig. 3A, lanes 1 to 3. All of the acid cleavage products migrated with the mobility expected for myristic acid. Small amounts of a

faster-migrating species may have been an artifact of hydrolysis or a 12-carbon metabolite of the labeled myristic acid.

To assess the nature of the covalent bond between the preS1 protein and ^{3}H-myristic acid, we examined the hydroxylamine sensitivity of the linkage. Whereas ^{3}H-palmitate and other acyl groups are typically bound to polypeptide backbones via an ester linkage, ^{3}H-myristate is usually bound via an amide bond. Because hydroxylamine efficiently cleaves ester and thioester linkages but leaves amide bonds intact, treatment with this agent distinguishes between the two chemically distinct types of linkages. ^{3}H-myristate-labeled preS1 proteins were immunoprecipitated from HBsAg-producing COS cells as described above. For use as a positive control, ^{3}H-palmitate-labeled vesicular stomatitis virus (VSV) G protein was produced by infecting COS cells with 20 PFU of VSV Indiana (a generous gift from Judy White) per cell. The ^{3}H-palmitate-labeled VSV G protein and the ^{3}H-myristate-labeled HBV preS1 proteins were run in adjacent wells of identical 12% polyacrylamide gels. After electrophoresis, one gel was treated for 1 h at 20°C with 1 M hydroxylamine (pH 6.8), fixed, and fluorographed (15); the other was fixed and fluorographed immediately for use as a control (Fig. 3B). Whereas there was no significant decrease in the level of label associated with the preS1 proteins following hydroxylamine treatment, the label bound to the VSV G protein decreased fourfold following such treatment. The relative resistance of the preS1 protein to hydroxylamine treatment suggests that ^{3}H-myristic acid is bound to this protein via a hydroxylamine-resistant amide link, as found for other myristylated proteins (15).

A small number of cellular and viral proteins are known to be modified by myristate addition; modification usually occurs at the N terminus of the protein (1, 2, 3, 13, 17, 27). Comparison of the N-terminal amino acid sequences of these proteins reveals that the N-terminal methionine residue is invariably followed by a glycine (Fig. 4A). Following cleavage of the methionine, an amide linkage is formed between the COOH group of myristic acid and the exposed amino group of the glycine. Examination of the N-terminal residues predicted by the mammalian and avian preS open reading frames reveals a similar sequence motif (Fig. 4B). The conservation of this structure across considerable evolutionary distances suggests that preS1 myristylation is likely to be a common feature of the hepadnaviruses.

The preS1 proteins differ from previously described myristylated proteins in one important respect. Other myristylated proteins are thought to be synthesized on free polyribosomes in the cytoplasm; in one well-studied case, myristate addition to the N terminus of the viral transforming protein pp60^{src} has been shown to be important in the subsequent affiliation of the protein with the inner (cytoplasmic) face of the plasma membrane (6, 7). By contrast, the preS1 proteins, like their S protein counterparts, are initially synthesized as transmembrane glycoproteins spanning the membrane of the endoplasmic reticulum (B. Eble, V. Lingappa, and D. Ganem, unpublished data). We presume that, as in other cases, myristylation occurs while the growing polypeptide is in the cytoplasm. The presence of N-terminal myristate does not appear to prevent the membrane translocation of downstream protein domains, since glycosylation of the S domain of the preS1 protein (12; K. Simon, V. Lingappa, and D. Ganem, manuscript in preparation) proceeds with normal efficiency.

What are the biological roles of the preS1 proteins, and how might myristylation influence them? Recent findings from this (23, 24; Simon et al., in preparation) and other (12, 19, 20) laboratories have provided new insights into the structure of the preS1 proteins and their disposition in HBsAg particles. Studies by Heerman et al. (12) indicate that preS1 polypeptides are preferentially localized on virions. At least some preS1-specific epitopes are immunogenic (19, 24) and are exposed on the virion surface (12). In addition, a role for some of these determinants in host cell binding has recently been suggested (20). Interestingly, however, the extreme N terminus of the protein is not reactive with specific antipeptide antisera (20; D. Persing, unpublished data), a finding that could result either from interference by the acyl group itself or from a resulting insertion of the N terminus into the lipid of the envelope. Such insertion could be important for the correct disposition of the exposed regions of the molecule.

Like the surface glycoproteins of most enveloped viruses but unlike the S proteins, the preS1 proteins are not secreted from the cell (4, 5, 25, 32). Like other envelope proteins, they are only exported from cells as part of a virion (although, unlike these proteins, their intracellular locale is primarily the endoplasmic reticulum rather than the plasma membrane). These observations are consistent with a role for the preS1 proteins in virion assembly. For most budding viruses, interactions between the cytoplasmic domains of envelope proteins and their nucleocapsid or matrix proteins are believed to play a role in the targeting of virion components to the appropriate membrane for morphogenesis and perhaps in the triggering of the budding event itself (26, 31, 37). Membrane interactions mediated by N-terminal myristic acid could be important in anchoring the N terminus of the preS1 protein in the membrane of the endoplasmic reticulum

A. Known myristylated proteins

MLV p15^{gag}	MGQTVTTPLSLTLGHW
pp60$^{v\text{-}src}$	MGSSKSKPKDPSQRRR
PK-A	MGNAAAKKGSEQSVK
pp56^{lck}	MGCVCSSNPEDDWME
CALCINEURIN B	MGNEASYPLE

B. Hepadnavirus preS1 proteins

GSHV	MGNNIKVTFDPNK
WHV	MGNNIKVTFNPDK
DHBV	MGQHPALSMDVR
HBV (ayw)	MGQNLSTSNPLG
HBV (adw2)	MGGTSSLPALGMG TNL

FIG. 4. N-terminal amino acid sequence comparisons of known myristylated proteins (A) and hepadnavirus preS1 proteins (B). The N-terminal methionine-glycine motif, thought to be a myristylation target sequence, is boxed. Abbreviations in panel A: MLV p15^{gag}, the 15-kDa *gag* protein of murine leukemia virus (13, 29); pp60$^{v\text{-}src}$, the transforming protein of RSV (Schmidt-Ruppin A) (2, 22); PK-A, the catalytic subunit of cyclic AMP protein kinase (3, 30); pp56^{lck}, the 56-kDa tyrosine kinase found in LSTRA T-cell lymphoma cells (17, 18); and calcineurin B, the calcium-binding B subunit of calcineurin (1). Abbreviations in panel B: GSHV, ground squirrel hepatitis virus (28); WHV, woodchuck hepatitis virus (10); DHBV, duck HBV (16); HBV (ayw), the ayw subtype of human HBV (11); and HBV (adw2), the adw2 subtype of human HBV (35).

(thereby preventing its spontaneous secretion) or in orienting the preS1 domain to allow interactions with other viral components. Further studies will be necessary to explore the role of this modification in the structure, assembly, and infectivity of hepadnaviruses.

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LITERATURE CITED

1. **Aitken, A., P. Cohen, S. Santikarn, D. H. Williams, A. G. Calder, A. Smith, and C. B. Klee.** 1982. Identification of the NH_2-terminal blocking group of calcineurin B as myristic acid. FEBS Lett. **150**:314–318.
2. **Buss, J. E., and B. M. Sefton.** 1985. Myristic acid, a rare fatty acid, is the lipid attached to the transforming protein of Rous sarcoma virus and its cellular homolog. J. Virol. **53**:7–12.
3. **Carr, S. A., K. Biemann, S. Shoji, D. C. Parmelee, and K. Titani.** 1982. *n*-Tetradecanoyl is the NH_2-terminal blocking group of the catalytic subunit of cyclic AMP-dependent protein kinase from bovine cardiac muscle. Proc. Natl. Acad. Sci. USA **79**:6128–6131.
4. **Cheng, K. C., G. L. Smith, and B. Moss.** 1986. Hepatitis B large surface protein is not secreted but is immunogenic when selectively expressed by recombinant vaccinia virus. J. Virol. **60**:337–344.
5. **Chisari, F. V., P. Filipi, A. MacLachlan, D. Milich, M. Riggs, S. Lee, R. D. Palmiter, C. A. Pinkert, and R. L. Brinster.** 1986. Expression of hepatitis B virus large envelope polypeptide inhibits hepatitis B surface antigen secretion in transgenic mice. J. Virol. **60**:880–887.
6. **Courtneidge, S. A., A. D. Levinson, and J. M. Bishop.** 1980. The protein encoded by the transforming gene of avian sarcoma virus ($pp60^{src}$) and a homologous protein in normal cells ($pp60^{proto\text{-}src}$) are associated with the plasma membrane. Proc. Natl. Acad. Sci. USA **77**:3783–3787.
7. **Cross, F. R., E. A. Garber, D. Pellman, and H. Hanafusa.** 1984. A short sequence in the $pp60^{src}$ N terminus is required for $pp60^{src}$ myristylation and membrane association and for cell transformation. Mol. Cell. Biol. **4**:1834–1842.
8. **Crowley, C. W., C.-C. Liu, and A. D. Levinson.** 1983. Plasmid-directed synthesis of hepatitis B surface antigen in monkey cells. Mol. Cell. Biol. **3**:44–55.
9. **Eble, B. E., V. R. Lingappa, and D. Ganem.** 1986. Hepatitis B surface antigen: an unusual secreted protein initially synthesized as a transmembrane polypeptide. Mol. Cell. Biol. **6**:1454–1463.
10. **Galibert, F., T. N. Chen, and E. Mandart.** 1982. Nucleotide sequence of a cloned woodchuck hepatitis virus genome: comparison with the hepatitis B virus sequence. J. Virol. **41**:51–65.
11. **Galibert, F., E. Mandart, F. Fitoussi, P. Tiollais, and P. Charnay.** 1979. Nucleotide sequence of hepatitis B virus genome (subtype *ayw*) cloned in E. coli. Nature (London) **281**:646–650.
12. **Heermann, K. H., U. Goldmann, W. Schwartz, T. Seyffarth, H. Baumgarten, and W. H. Gerlich.** 1984. Large surface proteins of hepatitis B virus containing the pre-S sequence. J. Virol. **52**:396–402.
13. **Henderson, L. E., H. C. Krutzsch, and S. Oroszlan.** 1983. Myristyl amino terminal acylation of murine retroviral proteins: an unusual post-translational protein modification. Proc. Natl. Acad. Sci. USA **80**:339–343.
14. **Machida, A., S. Kishimoto, H. Ohumura, H. Miyamoto, K. Baba, K. Oda, T. Nakamura, and Y. Miyakawa.** 1983. A hepatitis B surface antigen polypeptide (P31) with the receptor for polymerized human as well as chimpanzee albumins. Gastroenterology **85**:268–274.
15. **Magee, A. I., and S. A. Courtneidge.** 1985. Two classes of fatty acylated proteins exist in eukaryotic cells. EMBO J. **4**:1137–1144.
16. **Mandart, E., A. Kay, and F. Galibert.** 1984. Nucleotide sequence of a cloned duck hepatitis B virus genome: comparison with woodchuck and human hepatitis B virus sequences. J. Virol. **49**:782–792.
17. **Marchidon, G. A., J. E. Casnellie, K. A. Walsh, and E. G. Krebs.** 1984. Covalently bound myristic acid in a lymphoma tyrosine protein kinase. Proc. Natl. Acad. Sci. USA **81**:7679–7682.
18. **Marth, J. D., R. Peet, E. G. Krebs, and R. M. Pearlmutter.** 1985. A lymphocyte-specific tyrosine kinase gene is rearranged and overexpressed in the murine T cell lymphoma LSTRA. Cell **43**:393–404.
19. **Milich, D. R., G. B. Thornton, A. R. Neurath, S. B. Kent, P. Tiollais, and F. V. Chisari.** 1985. Enhanced immunogenicity of the pre-S region of hepatitis B surface antigen. Science **228**:1195–1198.
20. **Neurath, A. R., S. B. H. Kent, N. Strick, and K. Parker.** 1986. Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. Cell **46**:429–436.
21. **Patzer, E. J., G. R. Nakamura, C. C. Simonsen, A. D. Levinson, and R. Brands.** 1986. Intracellular assembly and packaging of hepatitis B surface antigen particles occur in the endoplasmic reticulum. J. Virol. **58**:884–892.
22. **Pellman, C., E. A. Garber, F. R. Cross, and H. Hanafusa.** 1985. An N-terminal peptide from $pp60^{src}$ can direct myristylation and plasma membrane localization when fused to heterologous proteins. Nature (London) **314**:374–376.
23. **Persing, D. H., H. E. Varmus, and D. Ganem.** 1985. A frameshift mutation in the pre-S region of the human hepatitis B virus genome allows production of surface antigen particles but eliminates binding to polymerized albumin. Proc. Natl. Acad. Sci. USA **82**:3440–3444.
24. **Persing, D. H., H. E. Varmus, and D. Ganem.** 1986. Antibodies to pre-S and X determinants arise during natural infection with ground squirrel hepatitis virus. J. Virol. **60**:177–184.
25. **Persing, D. H., H. E. Varmus, and D. Ganem.** 1986. Inhibition of secretion of hepatitis B surface antigen by a related presurface polypeptide. Science **234**:1388–1392.
26. **Rodriguez Boulan, E., and D. D. Sabatini.** 1978. Asymmetric budding of viruses into epithelial monolayers: a model system for study of epithelial polarity. Proc. Natl. Acad. Sci. USA **75**:5071–5075.
27. **Schulz, A. M., L. E. Henderson, S. Oroszlan, E. A. Garber, and H. Hanafusa.** 1985. Amino terminal myristylation of the protein kinase $pp60^{src}$, a retroviral transforming protein. Science **227**:427–429.
28. **Seeger, C., D. Ganem, and H. E. Varmus.** 1984. Nucleotide sequence of an infectious molecularly cloned genome of ground squirrel hepatitis virus. J. Virol. **51**:367–375.
29. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukemia virus. Nature (London) **293**:543–548.
30. **Shoji, S., D. C. Parmelee, R. D. Wade, S. Kumar, L. H. Ericsson, K. A. Walsh, H. Neurath, G. L. Long, J. G. DeMaille, E. H. Fischer, and K. Titani.** 1981. Complete amino acid sequence of the catalytic subunit of bovine cardiac muscle cyclic AMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA **78**:848–851.
31. **Simons, K., H. Garoff, and A. Helenius.** 1982. How an animal virus gets into and out of its host cell. Sci. Am. **246**:50–66.
32. **Standring, D. N., J. S. Ou, and W. J. Rutter.** 1986. Assembly of viral particles in *Xenopus* oocytes: pre-surface-antigens regulate secretion of the hepatitis B viral surface envelope particle. Proc. Natl. Acad. Sci. USA **83**:9338–9342.
33. **Stibbe, W., and W. Gerlich.** 1983. Structural relationships between minor and major proteins of hepatitis B surface antigen. J. Virol. **46**:626–628.
34. **Tiollais, P., A. Dejean, C. Brechot, M. Michel, P. Sonigo, and S. Wain-Hobson.** 1984. Structure of hepatitis B virus DNA, p.

49–65. *In* G. N. Vyas, J. L. Dienstag, and J. H. Hoofnagle (ed.), Viral hepatitis and liver disease. Grune & Stratton, Inc., Orlando, Fla.

35. **Valenzuela, P., P. Gray, M. Quiroga, J. Zaldivar, H. M. Goodman, and W. J. Rutter.** 1979. Nucleotide sequence of the gene coding for the major protein of hepatitis B virus surface antigen. Nature (London) **280**:815–819.

36. **Walter, P. W., R. C. Jackson, M. M. Marcus, V. R. Lingappa, and G. Blobel.** 1979. Tryptic dissection and reconstitution of translocation activity for nascent presecretory proteins across microsomal membranes. Proc. Natl. Acad. Sci. USA **79**:1795–1799.

37. **Wiley, D.** 1985. Viral membranes, p. 45–68. *In* B. N. Fields (ed.), Virology. Raven Press, Publishers, New York.