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Biochemistry

Regulated expression of Sindbis and vesicular stomatitis virus glycoproteins in *Saccharomyces cerevisiae*

(virus cDNA/yeast vector/recombinant DNA/membranes/glycosylation)

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ABSTRACT **cDNAs encoding either the structural proteins (capsid and glycoproteins E1 and E2) of Sindbis virus or the glycoprotein of vesicular stomatitis virus (VSV) were fused to the *Saccharomyces cerevisiae* galactokinase gene (*GAL1*) promoter and inserted into a yeast shuttle vector. After addition of galactose to yeast transformed with this vector, 2.5–3% of total yeast protein synthesis was detected as virus proteins by specific anti-virus protein antibodies. In cells containing the Sindbis virus structural genes, the virus capsid protein was effectively released from the nascent polypeptide and two endoglycosidase H-sensitive glycoproteins were produced. One of these was identical in its gel mobility to E1 and the other appeared to be p62, a precursor to E2. A low level of E1 protein was detected on the cell's surface membranes. A single molecular weight species of glycosylated VSV glycoprotein was produced and half of the total protein could be detected at the surface membranes of yeast. Addition of long mannose chains and acylation of the virus proteins with fatty acids were not observed. Formation of virus proteins was also examined in yeast secretory mutants; one of these (*sec53*) failed to glycosylate the virus proteins.**

Virus transmembrane glycoproteins have proved to be excellent models for studying a variety of co- and post-translational modifications that occur to membrane-bound polypeptides that traverse the intracellular organelles designed to traffic proteins among various cell compartments and membranes (1–3). We have used the glycoproteins of two RNA enveloped animal viruses—Sindbis virus and vesicular stomatitis virus (VSV)—to study three kinds of alterations to these proteins: proteolytic processing, glycosylation, and fatty acid acylation.

Sindbis virus encodes two major transmembrane glycoproteins (E1 and E2) in its genome, but these proteins are formed initially from a polyprotein precursor translated from a subgenomic virus mRNA (26S RNA) (reviewed in ref. 4). The individual polypeptides are formed through proteolytic cleavages of the nascent peptide chain. The capsid protein is synthesized first and released from the nascent polypeptide, whereas the P62 and the E1 proteins are translocated across the membrane of the rough endoplasmic reticulum to become integral membrane glycoproteins. They are rapidly transported through the Golgi complex to the plasma membrane of the host cell. The VSV glycoprotein is translated from a membrane-bound monocistronic virus mRNA (5), inserted into the lumen of the endoplasmic reticulum, glycosylated, and transported through Golgi to the plasma membrane of the cell.

The three kinds of modifications noted above alter the structure of both virus glycoproteins as they move through the intracellular organelles, and some of these can profoundly affect the protein's conformation and function (6–8). To analyze effects of the alterations on protein structure, it would be advantageous to block transport at specific stages and thereby allow accumulation of intermediate forms of the proteins. This is possible in the yeast *Saccharomyces cerevisiae* as a result of the isolation and characterization of an array of temperature-sensitive "sec" mutants, which are blocked at many stages in the secretory pathway used by yeast to transport proteins to the various cell organelles (reviewed in ref. 9).

In an attempt to exploit the yeast *sec* mutants for studies of animal virus glycoproteins, we took advantage of the recently developed ability to efficiently express foreign genes in yeast (10–14). Plasmids with virus genes fused to the yeast galactokinase (*GAL1*) promoter (15) were constructed, and we report here that wild-type and *sec* mutants of *S. cerevisiae* carrying these plasmids produce glycosylated forms of the virus proteins when cells are grown in galactose.

MATERIALS AND METHODS

Materials. Restriction endonucleases, T4 DNA ligase, polynucleotide kinase, and T4 DNA polymerase were from Bethesda Research Laboratories or New England Biolabs. *Escherichia coli* DNA polymerase large fragment (Klenow) and *Bam*HI linkers were from Boehringer Mannheim. ^{32}P-labeled nucleotides, [^{35}S]methionine, and Na^{125}I were from Amersham. Monoclonal antibody specific to Sindbis E1 protein was from J. Roehrig (CDC, Ft. Collins, CO). Monoclonal antibody specific to Sindbis capsid protein was from A. Schmaljohn (University of Maryland). Rabbit anti-Sindbis E1 or E2 monospecific antiserum was from C. Rice (California Institute of Technology). Antibodies to VSV glycoprotein have been described (16).

Bacterial and Yeast Strains. *E. coli* strain MC1061 (17) was used for plasmid transformation and isolation. *S. cerevisiae* YM722 (**a**, *ura3-52, ade2-101, his3Δ200, lys2-801, leu2-3, leu2-112*) was from M. Johnston (Washington University, St. Louis, MO). The secretory mutants of *S. cerevisiae: sec1, sec7, sec18*, and *sec53* (α, *leu2-3, leu2-112, ura3-52, suc2-Δ9*) were from R. Schekman (University of California, Berkeley).

Plasmid Vectors and Recombinant DNA Methodology. A plasmid that carries the cDNA encoding the complete Sindbis virus genome was provided by H. Huang (Washington University). Plasmid pSVGL containing the cDNA for VSV glycoprotein was from J. Rose (Salk Institute). Yeast plasmid pBM258 (15) and pSI-4 (18) were from M. Johnston (Washington University). Plasmid pYEGF-2 (19) was from P. Valenzuela (Chiron Corporation). DNA restriction fragments were isolated from low melting temperature agarose after gel electrophoresis (20). *E. coli* MC1061 was transformed by the $CaCl_2$ procedure (21). Yeast transformation was performed by the spheroplast method (22). Plasmid purification, labeling

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Abbreviations: VSV, vesicular stomatitis virus; endo H and F, endoglycosidases H and F.

of nucleic acid, Southern hybridization, and other manipulations of nucleic acids were performed by standard methods (23).

Labeling and Immunoprecipitation of Virus Proteins. Leu$^+$ yeast transformants were grown at 30°C in minimal synthetic medium supplemented with uracil (20 μg/ml), adenine (20 μg/ml), histidine (30 μg/ml), lysine (30 μg/ml), and 2% glucose. Logarithmic-phase cells were centrifuged and resuspended in the same minimal medium with 5% (vol/vol) glycerol replacing glucose, 1% potassium acetate, and 0.5% ethanol. After overnight growth, galactose was added (final concentration, 5%). After 15 min, [^{35}S]methionine (10 μCi/ml; 1 Ci = 37 GBq) was added for 4 hr and cells were centrifuged and washed with cold 10 mM NaN_3. For labeling of the *sec* mutants, cells were induced in 5% galactose at room temperature for 30 min, shifted to 37°C for 10 min, and [^{35}S]methionine (10 μCi/ml) or [^{3}H]palmitate (15 μCi/ml) was added for 3 hr. Cells were broken with glass beads in 1% $NaDodSO_4$ and extracts were diluted 1:4 for precipitation with antibodies (24).

Other Procedures. Endoglycosidase H (endo H) (Boehringer Mannheim) and F (endo F) (from J. Baenziger) digestions were done according to refs. 25 and 26, respectively. Iodination of cell surfaces was done as described (27, 28) and in cell extracts according to the manufacturer (Pierce).

RESULTS

Construction of Recombinant Plasmid. A complete cDNA of the 11-kilobase Sindbis virus genome (29) has been constructed (C. Rice and H. Huang, personal communication) and the plasmid (noted Toto II in Fig. 1*A*) contains, in addition to the seven virus genes, pBR322 sequences and the phage SP6 promoter at the 5′ end of the Sindbis cDNA. To construct a plasmid containing only the virus structural genes—formally equivalent to the subgenomic 26S RNA found in Sindbis virus-infected cells (4)—the Toto II DNA was digested with *Bam*HI and *Cla* I, followed by fill-in with Klenow fragment and blunt-end ligation to regenerate the *Bam*HI site at the 5′ end of the cDNA. This plasmid (pMS-1) has the entire sequence of the 26S cDNA plus 263 extra nucleotides at the 5′ end. To remove several potential ATG initiation sites within this extra sequence, plasmid DNA was linearized with *Bam*HI and partially digested with *Ava* II. The fragment containing an intact 26S cDNA sequence with

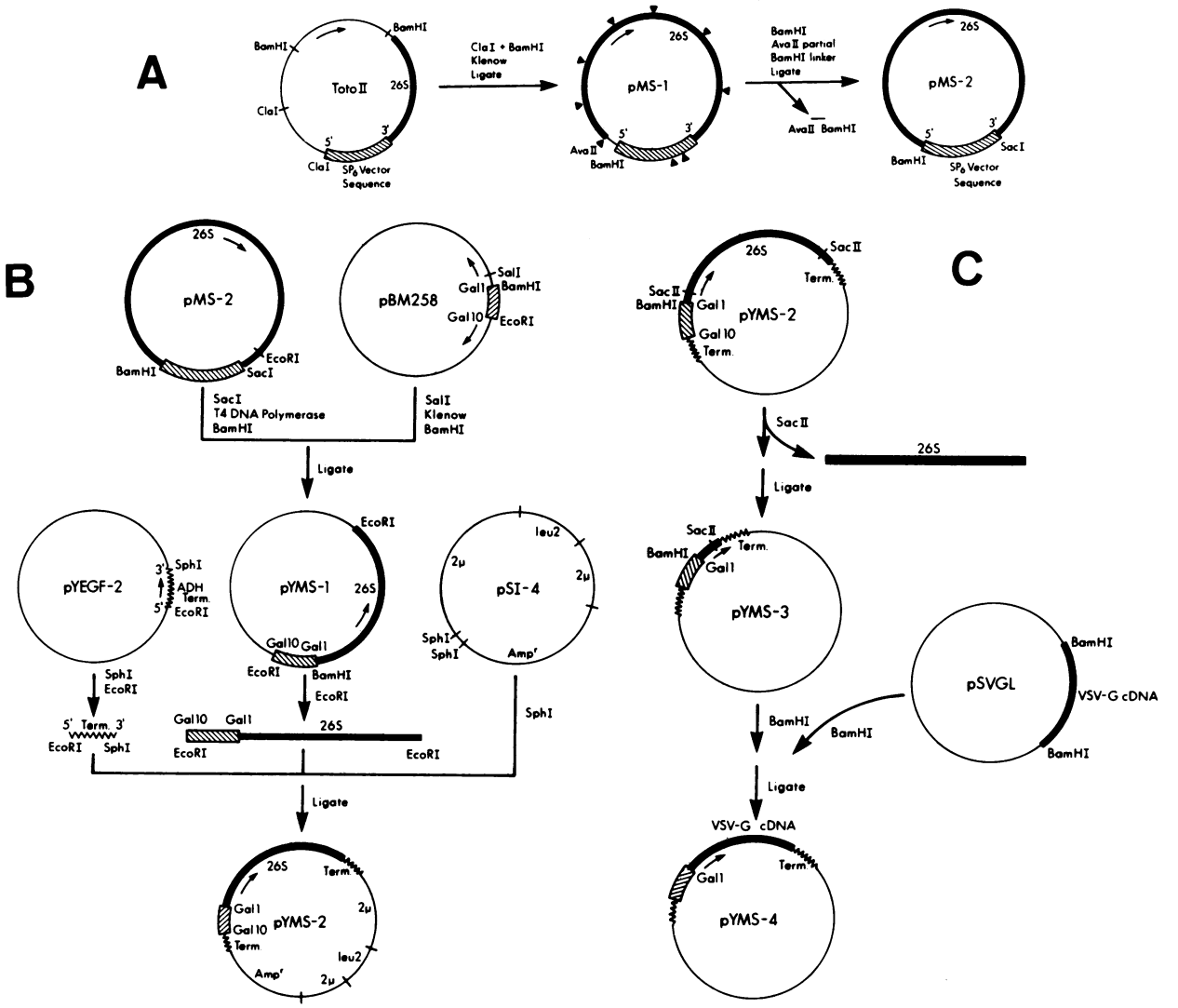

FIG. 1. Construction of the yeast expression vector. (*A*) Construction of the Sindbis cDNA. Plasmid Toto II carries the entire sequence for the Sindbis genome; the virus structural proteins (26S cDNA) are encoded by the 3′ end of the genome. The 5′ portion of the cDNA was removed to form plasmid pMS-2. (*B*) Construction of the Sindbis/yeast vector. The 26S cDNA was ligated to the yeast *GAL1* gene promoter and *ADH1* gene terminator and inserted into the yeast shuttle vector pSI-4 to form pYMS-2. (*C*) Construction of the VSV glycoprotein/yeast expression vector. The Sindbis cDNA was removed and the VSV glycoprotein cDNA was inserted (refer to text for details).

no extra ATGs was blunt-end ligated to *Bam*HI linkers. After *Bam*HI digestion, the ends were rejoined to yield plasmid pMS-2. The 26S cDNA was placed into a yeast *E. coli* shuttle vector by the following set of reactions (Fig. 1*B*). pMS-2 was digested with *Sac* I, blunt-ended, and cut with *Bam*HI to isolate the 26S cDNA sequences. pBM258, a plasmid carrying the yeast *GAL* promoters 1 and 10, was digested with *Sal* I; the ends were filled in with Klenow and cut with *Bam*HI. The 26S cDNA fragment was ligated to the linearized pBM258 and the mixture was used to transform *E. coli*. The resulting plasmid with the proper size insert is noted as pYMS-1. For the final construction, the fragment carrying the *GAL* promoters and Sindbis cDNA from pYMS-1 was isolated from an *Eco*RI digestion. pYEGF-2 was digested with *Sph* I and *Eco*RI, and the small 380-base-pair alcohol dehydrogenase gene (*ADH1*) terminator sequence was isolated. The 26S cDNA–*GAL* promoter fragment (900 ng) and the *ADH1* terminator fragment (300 ng) were ligated and cut with *Sph* I. The *GAL*–26S fragment, which was flanked with *ADH1* terminator sequence, was isolated from agarose gel and ligated to 50 ng of *Sph* I-digested plasmid pSI-4. *E. coli* was transformed with the ligation mixture and ampicillin-resistant clones were screened by colony hybridization with nick-translated ^{32}P-labeled 26S cDNA. From a selected clone, the pYMS-2 DNA was prepared and used to transform yeast strain YM722. Transformants were selected by their leucine-independent phenotype. A yeast vector carrying the VSV glycoprotein cDNA sequences was prepared as follows (Fig. 1*C*). The pYMS-2 was digested with *Sac* II and vector sequences were isolated and ends rejoined by T4 DNA ligase. This vector is referred to as pYMS-3. pSVGL (30) was cut with *Bam*HI and the DNA fragment encoding the glycoprotein was isolated. pYMS-3 was digested with *Bam*HI and ligated to the glycoprotein fragment. Plasmids prepared from *E. coli* transformants were tested for the correct orientation of the glycoprotein sequence insertion by analysis of restriction endonuclease digestions.

Synthesis of Sindbis Virus Proteins in Yeast. Yeast cells harboring plasmid pYMS-2 were induced by galactose and proteins labeled with [^{35}S]methionine. The selective induction of virus capsid and glycoproteins is seen in Fig. 2 (lane 2). Very low amounts of these proteins were made in cells grown only in glycerol (lane 3) and none were in cells carrying a vector lacking the virus genes (lane 4). The radioactive virus bands were not detected when 50 μg of unlabeled Sindbis virus proteins was added prior to immunoprecipitation or when a heterologous antisera was used (lanes 5 and 6).

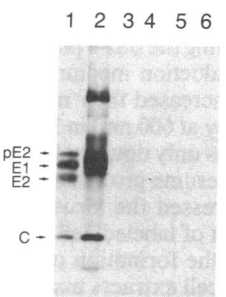

FIG. 2. Synthesis of Sindbis proteins in yeast. Lanes: 1, lysate from Sindbis-infected chicken embryo fibroblasts; 2, extracts of yeast transformed with plasmid pYMS-2 and induced with galactose; 3, same as lane 2 except that cells were grown in 5% glycerol; 4, same as lane 2 except that cells were transformed with plasmid pSI-4; 5, same as lane 2 except that extracts were mixed with 50 μg of unlabeled virion proteins prior to immunoprecipitation; 6, same as lane 2 except that nonspecific rabbit antibodies were used. Materials for lanes 2–6 were from immunoprecipitates with anti-virus antibodies. C, capsid.

The virus glycoproteins were further analyzed with antibodies specific to glycoproteins E1 and E2 and with immunoprecipitated material also treated with endo H and F, which remove asparagine-linked oligosaccharides. Anti-E1 antibodies precipitated a protein whose mobility was significantly increased after endo F treatment (Fig. 3, lane 4), and the pattern resembled that obtained with extracts of Sindbis virus-infected chicken embryo fibroblasts (lanes 1 and 2). Monospecific anti-E2 antisera precipitated the expected precursor p62 and E2 bands from infected chicken cells (lanes 5 and 6) and two major proteins from yeast extracts (lane 7). One of the latter appeared identical to p62; the higher molecular weight species could be a read-through translation product containing E1 sequences as well, since it was precipitated by a monospecific anti-E1 rabbit antiserum (see Fig. 4, lanes 7 and 8). Less than half of p62 increased in mobility after endo F treatment (Fig. 3, lane 8); thus, only part of this protein was glycosylated in yeast. The endo F-resistant form of p62 may be a nonglycosylated read-through protein containing p62 and the M_r 6000 hydrophobic sequences that lie between p62 and E1 on the 26S mRNA. A monoclonal antibody specific to the nucleocapsid confirms the formation of capsid in yeast (lane 10). A small amount of a lower molecular weight form of the capsid detected in yeast extracts may be a degraded form of this protein. Very little E2 was detected.

Synthesis of Sindbis Virus Proteins in *sec* Mutants. *sec* mutants representative of the several complementation groups involved in the secretory pathway of yeast were transformed by the vector and the pattern of virus proteins was examined by gel analysis of immunoprecipitates. Mutants blocked at the endoplasmic reticulum (*sec18*), Golgi (*sec7*), or secretory vesicles (*sec1*) produced patterns indistinguishable from the wild type (Fig. 4), while the *sec53* mutant made an E1 protein with an electrophoretic mobility similar to a nonglycosylated form (lanes 7 and 8). *sec53* has been characterized as a pleiotropic mutant defective in transferring dolichol-linked oligosaccharides to proteins and in other activities associated with the endoplasmic reticulum (31, 32). We recovered little nonglycosylated p62 in *sec53*, possibly indicating that it was unstable, but we found substantial amounts of a higher molecular weight form, which we postulate (see above) is a nonglycosylated p62 that retains the

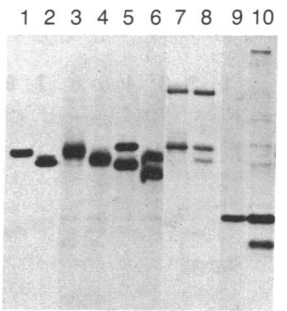

FIG. 3. Endo F treatment of Sindbis proteins synthesized in yeast. The virus proteins were precipitated with monospecific anti-E_1, E_2, or capsid antibodies and treated with endo F at 37°C for 18 hr. Endo H digestion gave the same results as the endo F digestion. Lanes: 1, lysate from Sindbis-infected chicken embryo fibroblasts precipitated by anti-E1 antibody; 2, lysate as in lane 1, but treated with endo F; 3, yeast extract precipitated by anti-E1 antibody; 4, yeast extract precipitated by anti-E1 antibody and treated with endo F; 5, lysate as in lane 1, but anti-E2 antibody was used; 6, lysate as in lane 1, but anti-E2 antibody was used and treated with endo F; 7, yeast extract precipitated by anti-E2 antibody; 8, yeast extract precipitated by anti-E2 antibody and treated with endo F; 9, lysate as in lane 1, but anti-capsid antibody was used; 10, yeast extract precipitated by anti-capsid antibody.

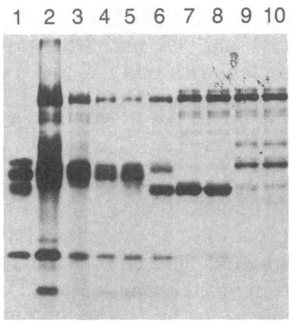

FIG. 4. Expression of Sindbis proteins in *sec* mutants. Lanes: 1, lysate from Sindbis-infected chicken embryo fibroblast; 2, wild-type yeast extracts; 3, *sec1* extracts; 4, *sec7* extracts; 5, *sec18* extracts; 6, *sec53* extracts; 7, *sec53* extracts with anti-E1 antibody; 8, same as lane 7, but endo H treated; 9, *sec53* extracts, with anti-E2 antibody; 10, same as lane 9, but endo H treated. Samples in lanes 1–6 were immunoprecipitated with anti-virus antibodies.

M_r 6000 linker sequences between p62 and E1 (lanes 9 and 10).

Formation of VSV Glycoprotein in Wild-Type and *sec* Mutants of Yeast. Expression of the VSV glycoprotein gene resulted in readily detectable amounts of a single protein species precipitable by an anti-glycoprotein antibody (Fig. 5, lane 3). After treatment with endo H, a faster moving form of glycoprotein appeared that was identical in mobility to a G_o species found in short pulse-labeled VSV-infected cells after endo H digestion. This pattern of glycoprotein was found also in the *sec18* mutant defective in transport beyond the endoplasmic reticulum, but only a nonglycosylated glycoprotein form accumulated in *sec53* (lane 8). The mobilities of glycoproteins made in *sec1* and *sec7* were identical to that made in wild-type yeast (data not shown).

Detection of Virus Glycoproteins at Yeast Surface Membrane. To determine if the virus glycoproteins were transported to the yeast cell surface, we radioiodinated the surface-membrane proteins of intact yeast cells that were grown in galactose medium for 4 hr. The total amount of virus glycoprotein was measured by iodinating extracts prepared by disrupting cells with glass beads in 1% $NaDodSO_4$. Iodinated virus glycoproteins were precipitated with specific antibodies, separated by $NaDodSO_4$/PAGE, and analyzed by densitometric scanning of autoradiograms (Table 1). The iodinated VSV glycoprotein appeared as a single sharp band in the gel with a mobility identical to that shown in Fig. 5, lane 3. Of the total iodinatable VSV glycoprotein synthesized in wild-type yeast, 68% was accessible to surface labeling. To determine the validity of this measurement and obtain a "background" level for the procedure, we repeated the analysis with the *sec18* mutant grown at a nonpermissive temperature (37°C) so that transport of secretory proteins was blocked at the endoplasmic

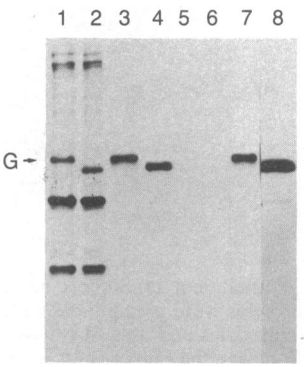

FIG. 5. Synthesis of the VSV glycoprotein (G) in yeast. Lanes: 1, lysate from VSV-infected chicken embryo fibroblasts pulse-labeled with [^{35}S]methionine for 10 min; 2, same as lane 1, but endo H treated; 3, extracts of yeast transformed with pYMS-4; 4, same as lane 3, except endo H treated; 5, same as lane 3, except yeast was grown in 5% glycerol; 6, same as lane 3, except yeast was transformed with plasmid pSI-4; 7, same as lane 3, but extracts were from *sec18*; 8, same as lane 7, but *sec53* cells were used. Anti-glycoprotein antibody was used for all yeast samples.

Table 1. Presence of virus glycoproteins at the yeast surface membrane

Protein	Strain	Amount of protein	Surface/total ratio	wt/*sec18* ratio
G	wt	240 (surface)	68%	52%
G	wt	352 (total)		
G	*sec18*	73 (surface)	16%	
G	*sec18*	442 (total)		
E1	wt	270 (surface)	44%	12%
E1	wt	613 (total)		
E1	*sec18*	74 (surface)	32%	
E1	*sec18*	234 (total)		

^{125}I-labeled proteins were precipitated by antibodies specific to virus glycoproteins and were analyzed by $NaDodSO_4$/PAGE. The amount of glycoprotein was quantitated by densitometric scanning of autoradiograms and was recorded as arbitrary units. G, glycoprotein; wt, wild type.

reticulum. Except for small amounts due to "leakage" of the mutation, there should be no virus glycoproteins transported to the *sec18* cell surface. In *sec18* cells, 16% of VSV glycoprotein was detected on the surface (Table 1); thus, a minimum value for the fraction of VSV glycoprotein on the wild-type yeast surface was 52%. Similar analysis of the E1 protein of Sindbis virus made in wild type and *sec18* showed that 12% of this virus glycoprotein was on the yeast cell-surface membrane. We were unable to obtain clearly resolved distinct iodinated bands of p62 from immunoprecipitates of the surface-labeled material from the two yeast strains.

Fatty Acylation of Virus Glycoproteins Made in Yeast. To detect protein acylation, we analyzed extracts of wild-type and *sec18* strains of *S. cerevisiae* that had been incubated for 3 hr with [^{3}H]palmitic acid after inducing expression of virus protein. Although nonviral yeast proteins were readily labeled with lipid (33) in *sec18*, we failed to detect significant amounts of radioactive virus proteins (data not presented). The yeast acylation enzymes apparently did not recognize the virus glycoproteins, or the receptor sites for acylation on these proteins were masked in the yeast membranes.

Growth of Yeast and Accumulation of Virus Proteins. The amounts of virus proteins were estimated by measuring the fraction of total radioactivity that was immunoprecipitated by the specific antibodies. For VSV glycoprotein expressed in wild-type or *sec53* cells, between 2% and 3% of total protein synthesized was immunoprecipitated. The values for Sindbis virus proteins in the same strains were 2.5%. However, it was not possible to convert these values to mass of protein because the cells almost stopped growing upon induction of the foreign proteins. Yeast carrying the pSI-4 plasmid vector alone doubled every 3 hr in the induction medium, but cultures expressing VSV glycoprotein increased their mass by no more than 50% (based on absorbancy at 600 nm) in 10 hr, and those expressing Sindbis glycoproteins only doubled in mass in the same period. These cultures did resume growth after 48 hr, but at that time they no longer expressed the virus proteins as measured by immunoprecipitation of labeled cell extracts.

We could detect the formation of virus glycoproteins also by immunoblots of cell extracts made after 5–12 hr of growth in galactose medium. Their amounts were estimated by comparison with known levels of the glycoproteins from purified virus. In wild-type yeast, the values for Sindbis and VSV proteins accumulated after 5 hr were $\approx$1 μg per 10^8 cells.

DISCUSSION

The yeast expression vector constructed for these studies functions very effectively in producing viral gene products from inserted cDNAs. It is fortunate that we elected to use a promoter for transcription that tightly regulates expression

of the gene because production of virus glycoproteins inhibited yeast growth. A similar result was found when an intact form of the VSV glycoprotein was expressed in *E. coli* (34), and it was postulated that the large amounts of glycoprotein inserted into the bacterial transmembrane transport system jammed the *E. coli* secretory pathway and inhibited export of essential proteins to the cell periplasmic space and outer membrane. A similar explanation could be offered here, for the evidence is quite clear that viral glycoproteins were inserted into the yeast secretory pathway.

The glycosylation of glycoprotein made in yeast is unambiguously demonstrated by the shift in mobility of glycoprotein after treatment with endo H and this deglycosylated form migrated in $NaDodSO_4$/PAGE identical to that of endo H-treated glycoprotein obtained from virus-infected cells labeled for 10 min. In addition, we detected a single band of the VSV glycoprotein comigrating with virion glycoprotein using [^{3}H]mannose as a label for yeast cells (data not shown). We did not analyze the oligosaccharides, but based on gel mobility of glycoprotein, the large mannose structures found in many yeast glycoproteins (9) were apparently not added to the virus glycoprotein, even though a significant fraction (≈50%) of glycoprotein transited through Golgi to the yeast membranes near the cell surface. Glycosylation of Sindbis virus glycoproteins was also detected and virtually all of the E1 contained the mannose core oligosaccharide. This protein could also be labeled with [^{3}H]mannose (data not shown). Very low amounts of the E2 glycoprotein were found but its glycosylated precursor p62 was formed. The normal expression of Sindbis virus glycoproteins requires several cotranslational proteolytic cleavages and recognition of signal sequences present at the beginning of p62 and in a hydrophobic region between the p62 and E1 genes. These protease activities functioned and signal sequences for p62 and E1 were recognized in yeast. In addition, the postulated autoprotease of the virus nucleocapsid, which releases capsid from nascent polyprotein, occurred during expression in yeast.

Few differences were found in Sindbis virus expression in the *sec* mutants examined, suggesting that those glycoproteins successfully expressed were probably not being transported from the endoplasmic reticulum, or, that the virus glycoproteins may not be accessible to yeast mannosyl transferases in the Golgi compartment. In contrast to VSV glycoprotein, only 12% of total E1 could be detected at the cell surface. This low value may reflect the inability of E1 to be effectively transported when there are lower amounts of normal p62 formed, a situation that occurred in the yeast cell. A temperature-sensitive mutant of Semliki Forest virus, a virus closely related to Sindbis, that was defective in making p62 also produced an E1 glycoprotein that failed to be transported to the infected cell surface (35).

One other animal virus glycoprotein has been expressed in yeast (14). Two forms of the influenza hemagglutinin cDNA, a complete sequence and one lacking the signal sequence, were inserted in vectors such that expression was controlled by the yeast *ADH1* gene promoter. Both proteins were detected in yeast extracts but only the complete hemagglutinin was glycosylated and associated with membranes. Expression of the complete hemagglutinin, but not the signal-minus form, slowed yeast cell growth and decreased stability of the plasmid—a result similar to that described here for Sindbis and VSV glycoprotein expression. We have induced the expression of VSV glycoprotein lacking the membrane anchor region but found that yeast growth was still inhibited (unpublished experiments).

The amounts of virus glycoproteins made after several hours in galactose medium have been estimated from immunoblots to be of the order of 1 μg per 10^8 yeast cells or $\approx 10^5$ molecules per cell. This vector is relatively easy to adapt to new gene insertions and may prove useful for expression of other foreign genes.

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