Lassa Virus Glycoprotein Signal Peptide Displays a Novel Topology with an Extended Endoplasmic Reticulum Luminal Region*

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Lassa virus glycoprotein C (GP-C) is translated as a precursor (preGP-C) into the lumen of the endoplasmic reticulum (ER) and cotranslationally cleaved into the signal peptide and immature GP-C before GP-C is proteolytically processed into its subunits, GP-1 and GP-2, which form the mature virion spikes. The signal peptide of preGP-C comprises 58 amino acids and contains two distinct hydrophobic domains. Here, we show that each hydrophobic domain alone can insert preGP-C into the ER membrane. Furthermore, we demonstrate that the signal peptide only uses the N-terminal hydrophobic domain for membrane insertion, exhibiting a novel type of a topology for signal peptides with an extended ER luminal part, which is essential for proteolytic processing of GP-C into GP-1 and GP-2.

SECRETORY PROTEINS USE N-TERMINAL SIGNAL PEPTIDES TO INTERACT WITH THE TRANSLOCON AT THE ER M (1–3). SIGNAL PEPTIDES ARE ESSENTIAL FOR THE TRANSLATION AND MEMBRANE INSERTION OF SECRETORY AND MEMBRANE RESIDENT PROTEINS. THEY ARE CLEAVED BY ER RESIDENT SIGNAL PEPTIDASES OR REMAIN UNCLEAVED AND SERVE AS MEMBRANE ANCHORS. SIGNAL PEPTIDES DISPLAY A TRIPARTITE STRUCTURE AS FOLLOWS: (I) AN N-TERMINAL REGION OF VARIOUS LENGTHS, USUALLY COMPRISING POSITIVELY CHARGED AMINO ACIDS; (II) A HYDROPHOBIC REGION WITH 7–15 AMINO ACID RESIDUES; AND (III) A SHORT POLAR C-TERMINAL STRETCH WITH SMALL UNCHARGED RESIDUES IN POSITION –3 AND –1, DETERMINING THE SIGNAL PEPTIDE CLEAVAGE SITE (4).

THE MECHANISM BY WHICH A SIGNAL SEQUENCEadopts a PARTICULAR TOPOLOGY IN THE ER MEMBRANE STILL REMAINS LARGELY UNKNOWN. USUALLY, SIGNAL PEPTIDES HAVE AN NENVIRONMENTAL ORIENTATION TO THE ER MEMBRANE (5), BUT IT IS ALSO POSSIBLE THAT THE N TERMINUS OF THE SIGNAL PEPTIDE IS TRANSLATED INTO THE LUMEN OF THE ER (6). ANALYSIS OF MANY TRANSMEMBRANE DOMAINS SUGGESTED THAT THE RESIDUES FLANKING THE HYDROPHOBIC REGION OF THE N-TERMINAL SIGNAL ANCHOR DETERMINE WHICH TOPOLOGY IS REALIZED (6–8). IT WAS PROPOSED THAT THE NET CHARGES OF THE SEGMENTS FLANKING THE TRANSMEMBRANE SEQUENCE DICTATE ITS ORIENTATION, LEAVING THE MORE POSITIVE SEGMENT CYTOPLASMICALLY ORIENTED (9).

This "positive inside rule" is consistent with the topology of most proteins integrated into bacterial membranes, which, in part, explainable by the negative inside electrical charge across the bacterial cytoplasmic membrane (10). However, this rule has not been successful in determining the topology of eukaryotic secretory proteins (11–13). Based on statistical analysis, Hartmann et al. (7) proposed the "charge difference hypothesis," stating that the orientation of the signal anchor at the N terminus is defined by the net charge in the 15 amino acids on either side of the hydrophobic core of the sequence; this hypothesis remains controversial, however (14).

The current view is that it is not only the net charges flanking the hydrophobic anchor that control the orientation in which a hydrophobic region is inserted into the ER membrane. Rather, the balance of sequence and flanking region properties such as hydrophobicity, charge or conformation of the hydrophobic region, and adjacent sequences dictate the orientation of the polypeptide at the translocon (13, 15, 16).

THE LASSA VIRUS IS A MEMBER OF THE ARENAVIRIDAE, A FAMILY INCLUDING THE LESS PATHOGENIC LYMPHOYCTIC CHORIOMENINGITIS VIRUS (LCMV) AS WELL AS HIGHLY PATHOGENIC MEMBERS LIKE THE JUNIN VIRUS. LASSA VIRUS IS ENDEMIC IN WEST AFRICA, WITH ~100,000–500,000 INFECTIONS OCCurring ANNUALLY OF WHICH AROUND ONE-THIRD RESULT IN ILLNESSES RANGING FROM FLU-LIKE SYMPTOMS TO FULMINANT HEMORRHAGIC FEVER WITH MORTALITY RATES OF UP TO 30% (17). TO DATE, NO VACCINE EXISTS, AND ONLY AN INSUFFICIENT RIBAVIRIN THERAPY IS AVAILABLE FOR TREATMENT.

Lassa virions consist of a nucleocapsid surrounded by a lipid envelope in which viral glycoprotein spikes are embedded. The glycoprotein is synthesized as a 76-kDa precursor (preGP-C). The N-terminal portion of the Lassa virus preGP-C contains a signal peptide of highly extended length comprising 58 residues (18). We have shown recently that the signal peptide is essential for further proteolytic processing of the glycoprotein (19). After cotranslational cleavage of the signal peptide, GP-C is posttranslationally cleaved after non-basic residues into the distal N-terminal subunit GP-1 and the C-terminal membrane-anchored GP-2 by the subtilase SKI-1/S1P (20, 21) (Fig. 1A).

In this study, we demonstrate that the GP-C signal peptide contains two independent hydrophobic domains that can be used for ER translocation of preGP-C. However, topological studies show that only the N-terminal hydrophobic domain of the signal peptide of preGP-C is inserted in the ER membrane, whereas the extended C region is essential for the maturation cleavage of GP-C into GP-1 and GP-2.
FIG. 1. A, schematic overview of the Lassa virus glycoprotein. The primary translation product preGP-C (aa 1–491), the signal peptide SP (aa 1–58), the precursor glycoprotein GP-C (aa 59–491), the distal subunit GP-1 (aa 59–259), and GP-2 (aa 260–491) containing the membrane anchor (aa 427–450; stripes) are shown. The antisera bindings sites, Rb-α-SP (aa 2–18) and Rb-α-GP2-N (aa 259–277), the signal peptidase (SPase) cleavage site between threonine residues 58 and 59 (arrow), the SKI-1/S1P cleavage site C-terminal of leucine 259 (arrow), and putative N-glycosylation sites (Y-like symbols) are indicated. B, the total SP sequence is shown in one-letter amino acid code. Hydrophobic domains (h1) and (h2) within the signal peptide are underlined. Net charges and hydrophobicity of signal peptide domains are illustrated. C, deletion of hydrophobic domains within the Lassa virus GP-C signal peptide. Vero cells were transfected with mutant Δ18–32, mutant Δ43–52, and mutant Δ18–32/Δ43–52 of recombinant Lassa virus glycoprotein preGP-C and the vector pCAGGS for mock transfection (M). WT, wild type. Protein samples were treated with PNGase F if indicated, separated on 10% (upper panel) or 12% (lower panel) acrylamide gels by electrophoresis, blotted onto polyvinylidene difluoride membrane, and immunostained using the antiserum Rb-α-GP2-N. The deglycosylated form of GP-C is marked with an asterisk (GPC*).
Cell Cultures—Vero cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum, 100 units/ml penicillin and 0.1 mg/ml streptomycin.

Vectorial Expression of Lassa Virus Glycoprotein and Mutagenesis—
The genes of the full-length glycoprotein (wild type/preGP-C) and of the signal peptide of GP-C (Lassa virus strain Josiah) were expressed using the β-actin promoter-drug pCAGGS vector (18–22). Lassa virus preGP-C mutants and the signal peptide, or the mutants thereof, were generated by recombinant PCR techniques (23). A list of the respective oligonucleotides will be made available on request. Sequences were confirmed by DNA sequencing. Vero cells were transfected with wild type and mutated recombinant DNA using LipofectAMINE 2000 (Invitrogen).

Vero N-Antiserum—Rabbit anti-GP2-N was raised by immunization of a rabbit with a chemically synthesized peptide homologous to the N terminus of GP-2 (amino acid positions 259–279), which was covalently cross-linked to keyhole limpet hemocyanin (KLH; Pierce) as a carrier protein, as described previously for antibodies Rab-α-SP and Rab-α-GP2 (18–20). A monoclonal (FLAG M2) and a polyclonal antiserum directed against the FLAG epitope were purchased from Sigma. A polyclonal calnexin antiserum was purchased from StressGen Bioreagents.

Acrylamide Gel Electrophoresis, Immunoblotting, and Glycosidase Treatment—Proteins were separated by SDS-PAGE using 10% acrylamide gels (24) or 16.5% polyacrylamide gel and Tricine buffer (25), as described previously (18, 19). Immunoblotting was performed as described earlier (20). If indicated, samples were incubated overnight with PNGase F (New England Biolabs).

Pulse-Chase Experiments and Immunoprecipitation—Plasmid-transfected Vero cells were starved 20 h post transfection for 1 h with Dulbecco’s modified Eagle’s medium lacking methionine and cysteine before cells were labeled with 10 μCi [35S]methionine and [35S]cysteine Premix (Amersham Biosciences) for 30 min. The radioactive medium was then replaced by Dulbecco’s modified Eagle’s medium during a 2-h chase or various chase times as indicated. The labeled cells were lysed in radioimmune precipitation assay buffer containing 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 5% Trasylol, 150 mM sodium chloride, 20 mM Tris, and 10 mM EDTA, pH 8.5, and sonicated (40 watt; Branson sonifier). Non-soluble material was removed by centrifugation (20,000 × g for 30 min), and supernatants of the cell lysates were incubated overnight with protein A-Sepharose coupled to the desired antibodies. Immunoprecipitated proteins were analyzed by SDS-PAGE followed by autoradiography on BioMax films (Kodak) (18, 19).

Selective Membrane Permeabilization and Immunocytochemistry—Vero cells were grown on coverslips and LipofectAMINE-transfected with appropriate plasmid constructs for protein expression. Twenty hours after transfection, cells were washed and either incubated for 5 min at 4°C with intracellular buffer (20 mM HEPES buffer, 110 mM potassium acetate, 5 mM sodium acetate, 2 mM magnesium acetate, and 5 mM EDTA, pH 7.3) containing digitonin (2 μg/ml) for selective permeabilization of plasma membrane or incubated for 5 min at room temperature with aceton/ethanol (1:1) (v/v) for total permeabilization of membranes. Cells were subsequently washed and incubated for 1 h with 1:200 diluted primary rabbit antibodies followed by incubation for 45 min with 1:400 diluted anti-rabbit antibody from goat coupled to rhodamine (Dianova, Hamburg, Germany). Protein expression of cells was examined using an immunofluorescence microscope (Axioskop, Zeiss).

RESULTS

Topology of the Signal Peptide—The hydrophobic plot program by Kyte and Doolittle (26) predicts two hydrophobic transmembrane domains, designated h1 and h2, within the signal peptide ranging from positions 18 to 32 and 43 to 52, respectively (Fig. 1B). To determine which of the hydrophobic domains has an ER translocation capacity, both were deleted alternatively and together (Fig. 1C). Deletion of only one hydrophobic domain, mutant Δ18–32 or Δ43–52, still led to the N-glycosylated viral glycoprotein GP-C as confirmed by deglycosylation (Fig. 1C, lanes 3, 4, 6, and 7), whereas deletion of both domains (mutant Δ18–32/Δ43–52) resulted in the non-glycosylated form of preGP-C (Fig. 1C, lane 5). The absence of N-glycans indicates that the mutated preGP-C, which lacks both hydrophobic domains, is not translocated into the ER lumen, whereas deletion of only one hydrophobic domain did not prevent translocation. According to this, mutant Δ18–32/Δ43–52 migrates slower than mutant Δ18–32 and wild type preGP-C, because signal peptide cleavage does not occur within this mutant (Fig. 1C, lanes 5, 6, and 8). Mutant Δ43–52 shows incomplete signal peptide cleavage (lane 7), most likely because a hydrophobic domain is missing in proximity to the signal peptide cleavage site for correct processing (27).

As a result, the following three models are conceivable for showing how both hydrophobic domains could interact with the ER membrane (Fig. 2): (i) a double spanning transmembrane model in which the N and C termini of the signal peptide are present in the ER lumen, and both hydrophobic domains (h1 and h2) serve as membrane anchors; (ii) a model with a long N-terminal cytoplasmic peptide segment that includes the N-terminal hydrophobic domain, h1, with the hydrophobic domain h2 as membrane anchor; and (iii) a model with a short N-terminal region in which the hydrophobic domain h1 serves as transmembrane anchor and the second hydrophobic domain is present within the ER lumen.

Several different experimental approaches were performed to determine the topology of the signal peptide in the ER membrane. First, we applied an immunocytochemical method that was successfully used previously for determination of the topology of the hepatitis C envelope protein (28). We inserted an HA tag (YPYDVPDYA) as an antigenic epitope at different sites of the Lassa virus glycoprotein signal peptide (Fig. 3A). Cell cultures were transfected with the three different HA-tagged signal peptide constructs. All signal peptide mutants were detected after permeabilization of all cellular membranes by acetone-methanol treatment using immunofluorescence. In contrast, treatment of the cells with digitonin, which selectively permeabilizes plasma membranes but not ER membranes, resulted in fluorescence of only the N-terminally HA-tagged construct (Fig. 3A). This method allows us to distinguish between the HA epitopes of the Lassa preGP-C signal peptide, which are present on the cytosolic or the luminal side of the ER membrane. Mutant SP-HA-N indicates that the HA-epitope is oriented toward the cytoplasm, suggesting that only one hydrophobic domain is membrane-anchored in the native signal peptide. The other HA-tagged mutants, mutant SP-HA-M, carrying the HA-epitope between both hydrophobic domains, and mutant SP-HA-C, with the HA tag after the second hydrophobic region, indicate that their antigenic epitopes are not accessible for immunostaining in digitonin-permeabilized cells and, therefore, face the ER lumen. Using another antigenic epitope (FLAG tag) instead of the HA tag, we obtained the same results (data not shown). As controls for correct permeabilization, cells expressing the ER marker calnexin show, as expected, no fluorescence signal in digitonin-
FIG. 3. A, determination of Lassa virus glycoprotein signal peptide topology by selective immunocytochemistry. Vero cells grown on coverslips were transfected with pCAGGS vector containing the signal peptide mutants SP-HA-N, SP-HA-M, or SP-HA-C, respectively. 20 h post transfection, cells were either incubated with acetone/methanol for complete membrane permeabilization or incubated with digitonin for selective permeabilization. Cells were then incubated with antisera against the HA-Tag (αHA) or, as control, against the ER luminal part of calnexin (αCalnexin). Immunoreaction was visualized by rhodamine stain. B, signal peptide topology determined by insertion of N-glycosylation sites. Mutants SP-Glyc-M, SP-Glyc-M2, SP-Glyc-N, and SP-Glyc-N2, with additional N-glycosylation sites, were expressed in Vero cells using a radioactively labeled pCAGGS expression vector system. Wild type and mutated signal peptides were precipitated with Rb-α-SP coupled to protein A-Sepharose from solubilized transfected cells. As indicated, an aliquot of each sample was treated with PNGase F overnight. Untreated and PNGase F-treated precipitated proteins were subjected to SDS-PAGE on 16.5% acrylamide gels, followed by autoradiography. M, mock transfection; WT, wild type. Protein was separated under non-reducing conditions by Tricine/SDS-PAGE (16.5%) and immunoblotted. Monomeric and dimeric forms of the signal peptide were immunostained using FLAG-antiserum.
permeabilized cells, but a fluorescence signal is shown in acetone/methanol-treated cells using antiserum directed against the ER luminal part of calnexin.

Secondly, N-glycosylation of a potential N-glycosylation site inserted into a predicted ER luminal amino acid sequence was investigated to substantiate the topology of the signal peptide. As N-glycosylation only occurs in a hydrophilic environment but not in the proximity of hydrophobic regions, attachment sites for N-glycosylation were inserted between the hydrophobic domains h1 and h2 (mutant SP-Glyc-M) flanked by an HA tag and a FLAG tag as spacers. Indeed, the signal peptide of mutant SP-Glyc-M shifted to a band near 14 kDa, which disappears after PNGase F treatment, indicating that the region between both hydrophobic domains is exposed to the ER lumen and can thus be glycosylated (Fig. 3B, lanes 2 and 3). To exclude the possibility that the insertion of the charged residues of the HA- or the FLAG-tagged epitope causes a switch in the topology, the charge of the wild type signal peptide was maintained by amino acid exchange in mutant SP-Glyc-M2. This mutant shows the same glycosylation pattern as mutant SP-Glyc-M, indicating that the topology of the signal peptide was not affected by the insertion of charges in this region (Fig. 3B, lanes 4 and 6). As negative controls, two mutants were generated with an N-glycosylation site inserted at the N terminus flanked by a FLAG tag (mutant SP-Glyc-N) or simply with an N-glycosylation site near the N terminus of the signal peptide (mutant SP-Glyc-N2), respectively (Fig. 3B). Both control mutants, SP-Glyc-N and SP-Glyc-N2, do not show any band shift in SDS-PAGE before or after PNGase F-treatment, confirming that the N-terminal N-glycosylation site is cytoplasmically orientated and, therefore, not accessible for the N-glycan attachment (Fig. 3B, lanes 6–9). The differences in electrophoretic mobility between all mutants (Fig. 3B, lanes 3, 5, 7, and 9) are due to the differing lengths of their respective insertions. Interestingly, insertion of the charged epitopes did not alter the topology of any of the signal peptide mutants. Alternative attempts to control the topology of the signal peptide by exchanging the charges surrounding the first hydrophobic region did not result in a switch of the topology either (data not shown).

The Lassa virus signal peptide forms dimers when expressed in eucaryotic cells. It contains three cysteine residues, which could play a role in dimer formation. Two are located at the C terminus of the signal peptide (residues Cys-53 and Cys-57), whereas one is located in between the h1 and h2 domain (residue Cys-41). To prove, by using a third method, that the signal peptide is inserted via the h1 domain leaving a long C-terminal part facing the ER lumen, we analyzed the role of cysteine 41 for dimerization. For this purpose, a signal peptide mutant was constructed in which the cysteine residues Cys-53 and Cys-57 were mutated to serine (SP C53S, C57S-FLAG-N). Fig. 3C shows that this mutant still forms covalently linked dimers under non-reducing conditions, indicating that the region between both hydrophobic regions is ER luminal and, thus, accessible for disulfide linkage. To avoid disulfide formation caused by artificial oxidation of free sulfhydryl groups by air, N-ethylmaleimide was added to the lysis buffer at a final concentration of 20 mM. Furthermore, a mutant with all cysteine residues changed to serine (SP C41S/C53S/C57S-FLAG-N) does not form covalently linked dimers, indicating that dimerization is strictly disulfide-dependent. Finally, a mutant with a cysteine residue introduced at the N terminus and all other cysteine residues changed to serine (SP G2C/C41S/C53S/C57S-FLAG-N) also shows no disulfide formation.

Taking our topological data together, we show that the Lassa virus glycoprotein signal peptide possesses a novel topology for signal peptides, as depicted in Fig. 2C, consisting of an N-terminal cytoplasmic peptide segment (17 amino acid residues) followed by a transmembrane domain (h1) comprising the amino acids from 18 to 32 and an exceptionally extended C-terminal, ER luminal region comprising amino acids 33 to 58, including the second hydrophobic domain (h2).

Role of the Topology for GP-C Cleavage—We have shown recently that the signal peptide of the Lassa virus glycoprotein GP-C is essential for the maturation cleavage of the glycoprotein as a trans-acting maturation factor (19). The signal peptide topology suggests that its C-terminal portion facing the ER lumen is responsible for the interaction with GP-C. Therefore, the N-terminal region of the signal peptide up to the transmembrane segment, which is cytoplasmically orientated, may well be dispensable for the cleavage of GP-C into GP-1 and GP-2. To prove this assumption, three preGP-C deletion mutants (mutant Δ2–17, truncated from the N terminus to the transmembrane domain; mutant Δ2–30; and mutant Δ2–41, truncated until position 41, leaving the second hydrophobic domain intact) were constructed and vectorially expressed in Vero cells (Fig. 4). Western blot analysis of wild type and expressed truncated preGP-C mutants shows that deletion of the N terminus of the signal peptide up to the transmembrane segment, h1, has no effect on GP-C maturation (Fig. 4, lanes 2 and 3). Further truncations of up to 41 residues abolished cleavage of GP-C by SKI-1/S1P, whereas translocation was not affected (Fig. 4, lanes 4 and 5). These results strengthen the topology model and further demonstrate that the interaction between the signal peptide and GP-C occurs within the ER lumen. The N-terminal cytoplasmic region of the signal peptide is dispensable for GP-C cleavage into GP-1 and GP-2.

**DISCUSSION**

The data presented in this study show that the signal peptide of the Lassa virus glycoprotein possesses two independent hydrophobic domains, h1 and h2. Both have the potential to mediate membrane insertion and translocate GP-C into the ER.
The cysteine residues within the signal peptides of the glycoproteins of arenaviruses are indicated by arrows. The thick arrow at position 57 marks the only conserved cystein residue. Asterisk (*), conserved; double dot (:), partially conserved; single dot (.), not conserved.

**Table I**

| Glycoprotein | Amino Acid Sequence |
|--------------|---------------------|
| Lassa        | MGQIVTFFQEVPHVLIEEVMIVSLVLSAILIKGIVYNTCCGIGLTLFILGCRSCS--S |
| Mopeia       | MGQIVTFFQEVPHVLIEEVMIVLTSILAILIKGIVYNTCCGIGLTLFILGCRSCS-- |
| LCMV         | MGQIVTFFMFAEDPHIDEVNIVIIVLIIITSSKAVYNTCCGILSVLFILAGS--- |
| Whitewater   | MGQISFFGEIPSSIIEAMIALIAVSLISIEAVINWGSQGPFFVFLLAGRCSYK |
| Sabia        | MGQISFFEGEEPYNMEAMIALIAVLSLALACGMINLWKMQGQLIFFFILAGSFR |
| Tacaribe     | MGQISFMQEIPFVQLAEAMIALAVSILICVGLYNYRCEGQLMVFILAGRSCSEE |
| Junin        | MGQISFMQEIPFVQLAEAMIALAVSLAIIIKGVNYNLKGYQGPFFVFLILAGRSCSEE |
| Pichinde     | MGQIVTFISFVQVNLALVSTLCIGKGVNMLRCQGQLSVLLIALAGRSCDM |

The cysteine residues within the signal peptides of the glycoproteins of arenaviruses are indicated by arrows. The thick arrow at position 57 marks the only conserved cystein residue. Asterisk (*), conserved; double dot (:), partially conserved; single dot (.), not conserved.
To determine further functions after proteolytic activation of the glycoprotein into GP-1 and GP-2. Because of its unusual topology, it cannot be excluded that the signal peptide might even engage in additional functions after proteolytic activation of the glycoprotein. Therefore, it will be interesting to determine further potential functional aspects in the life cycle of this unusual signal peptide.

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REFERENCES

1. Blobel, G., and Dobberstein, B. (1975) J. Cell Biol. 67, 835–851
2. Johnson, A. E., and van Waes, M. A. (1999) Annu. Rev. Cell Dev. Biol. 15, 799–842
3. Schatz, G., and Dobberstein, B. (1996) Science 271, 1519–1526
4. von Heijne, G. (1985) J. Mol. Biol. 184, 99–105
5. Mothes, W., Prohn, S., and Rapoport, T. A. (1994) EMBO J. 13, 3973–3982
6. von Heijne, G. (1989) Nature 341, 456–458
7. Hartmann, E., Rapoport, T. A., and Lodish, H. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5786–5789
8. Parks, G. D., and Lamb, R. A. (1991) Cell 64, 777–787
9. von Heijne, G. (1994) Sabiell. Biochem. 22, 1–19
10. Cao, W., Henry, M. D., Borrow, P., Yamada, H., Elder, J. H., Ravklev, E. V., Nichol, S. T., Compan, R. W., Campbell, K. P., and Oldstone, M. B. (1998) Science 282, 2079–2081
11. Spiess, M. (1995) FEBS Lett. 369, 76–79
12. Sato, M., Hresko, R., and Mueckler, M. (1998) J. Biol. Chem. 273, 25203–25208
13. Geder, V., and Spiess, M. (2003) EMBO J. 22, 3645–3653
14. Andrews, D. W., Young, J. C., Mirels, L. F., and Czarnota, G. J. (1992) J. Biol. Chem. 267, 7761–7769
15. Denzer, A. J., Nahholz, C. E., and Spiess, M. (1995) EMBO J. 14, 6311–6317
16. Wahlberg, J. M., and Spiess, M. (1997) J. Cell Biol. 137, 555–562
17. McCormick, J. B., King, J. J., Webb, P. A., Johnson, R. M., O’Sullivan, R., Smith, E. S., Trippel, S., and Tong, T. C. (1987) J. Infect. Dis. 155, 445–455
18. Eichler, R., Lenz, O., Strecker, T., and Garten, W. (2003) FEBS Lett. 538, 293–296
19. Eichler, R., Lenz, O., Strecker, T., Eickmann, M., Klenk, H. D., and Garten, W. (2003) EMBO Rep. 4, 1084–1088
20. Lenz, O., ter Meulen, J., Feldmann, H., Klenk, H. D., and Garten, W. (2000) J. Virol. 74, 11418–11421
21. Lenz, O., ter Meulen, J., Klenk, H. D., Seidah, N. G., and Garten, W. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12701–12705
22. Beyer, W. R., Popplau, D., Garten, W., von Laer, D., and Lenz, O. (2003) J. Virol. 77, 2866–2872
23. Higuchi, R., Krummel, B., and Saiki, R. K. (1988) Nucleic Acids Res. 16, 7351–7367
24. Laemmli, U. K. (1970) Nature 227, 860–865
25. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
26. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
27. Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. (1997) Int. J. Neural Syst. 8, 581–599
28. Coopquand, L., Op de Beeck, A., Lambot, M., Roussel, J., Delgrange, D., Piliez, A., Wychowski, C., Penin, F., and Dubuisson, J. (2002) EMBO J. 21, 2903–2902
29. Froschke, M., Basler, M., Groettrup, M., and Dobberstein, B. (2003) J. Biol. Chem. 278, 41914–41920
30. Izard, J. W., and Kendall, D. A. (1994) Mol. Microbiol. 13, 765–773
31. Martiglio, B., and Dobberstein, B. (1998) Trends Cell Biol. 8, 410–415
32. Lemberg, M. K., and Martoglio, B. (2002) Mol. Cell 10, 735–744
33. Lindemann, D., Pietschmann, T., Picard-Maureau, M., Berg, A., Heinkelein, M., Thurov, J., Knaus, P., Zentgraf, H., and Rethwilm, A. (2001) J. Virol. 75, 5762–5771
34. Sanz, M. A., Madan, V., Carrasco, L., and Nieva, J. L. (2003) J. Biol. Chem. 278, 2051–2057