acts mainly with the hydrophobic belt delimited by two rings of aromatic residues, consistent with the hypothesis proposed by Cowan and co-workers (*4*). In addition, the close fit of intercalating extracellular domains documented in Fig. 3C is responsible for the ordered interlayer interaction of double-layered 2D crystals.

We observed that the extracellular porin surface may exist in two conformations. This finding is rare but reproducible, consisting of a 5Å shift of the extracellular domains toward the center of the trimer (compare Fig. 4A with Fig. 4B). Although energy requirements cannot be estimated from a static atomic structure (*4*), the displacement observed appears sterically possible and would constrict the channel entrances. This may explain the two open-channel configurations of *E. coli* porins (*11*, *12*).

As demonstrated here, the comparison of the structural data from x-ray crystallography with those from AFM contributes a new insight into the structure of lipids in biological membranes. Also, because 2D crystals of membrane proteins are more easily produced than 3D crystals, the combination of cryoelectron microscopy and AFM may be an attractive alternative. Finally, the two conformations of the extracellular surface of OmpF porin (Fig. 4) and the recent report on conformational fluctuations of active lysozyme (*13*) suggest that the atomic force microscope may become an essential tool in monitoring function-related conformational changes.

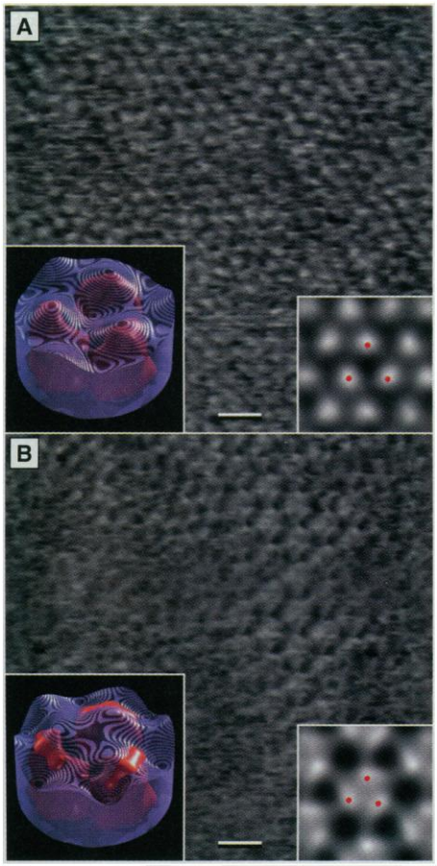

Fig. 4. Two conformations of the OmpF porin as identified by AFM are compared to the model derived from the atomic structure. (**A**) Raw data of a trigonal 2D crystal that exhibits the characteristic morphology of the extracellular porin trimer surface. The overlay shown in the lower left corner of AFM data (transparent, in dark and light blue) and the trimer model (opaque, red) were generated from the threefold symmetrized average (inset, lower right corner). (**B**) A different morphology recorded with the same stylus on the same trigonal crystal as in (A). Trace and retrace scans were merged. Small patches with prominent trimeric protrusions arranged on an undistorted lattice were found embedded within a topography corresponding to that displayed in (A). The comparison of the threefold symmetrized average (inset, right) with the model (inset, left; colors as above) shows that the extracellular domains are shifted by 5 Å toward the threefold axis of the porin trimer. The centers of the protrusions are indicated with red dots. Scale bars in (A) and (B), 10 nm.

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A Morbillivirus That Caused Fatal Disease in Horses and Humans

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A morbillivirus has been isolated and added to an increasing list of emerging viral diseases. This virus caused an outbreak of fatal respiratory disease in horses and humans. Genetic analyses show it to be only distantly related to the classic morbilliviruses rinderpest, measles, and canine distemper. When seen by electron microscopy, viruses had 10- and 18-nanometer surface projections that gave them a "double-fringed" appearance. The virus induced syncytia that developed in the endothelium of blood vessels, particularly the lungs.

The emergence of new viruses may result in previously unrecognized or new diseases (*1*–*3*). Although emerging viruses may contain novel mutations or represent gradual evolution (*3*), more often they emerge because of changes in behavior or the environment whereby they are introduced to a new host (*3*–*5*). Viruses that are pathogenic in novel human and nonhuman hosts include Marburg and Ebola viruses; hantaviruses; human immunodeficiency viruses; Lassa virus; dolphin, porpoise, and phocine morbilliviruses; feline immunodeficiency virus; and bovine spongiform encephalopathy agent (*3*).

Dolphin, porpoise, and phocine morbilliviruses, rinderpest virus, and measles virus belong to the genus *Morbillivirus* within the family Paramyxoviridae. Although nonhuman morbilliviruses have been associated

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with new and emerging animal diseases, no new human morbillivirus has been reported since the 10th century, when measles was described (6). In this paper, we describe a new species of morbillivirus that fatally affected 14 horses and 1 human.

In late September 1994, an outbreak of severe respiratory disease occurred in horses. Within 2 weeks, 14 of 21 sick horses, which were mainly on the 1 Brisbane (Queensland) property, died or were killed after an acute illness characterized by high fevers (up to 41°C) and severe respiratory difficulty. These deaths followed that of a similarly affected pregnant mare 2 weeks previously on the same property. The 49-year-old-trainer of the horses and a 40-year-old stablehand who had close contact with the dying mare also became ill with a severe influenzalike illness. The stablehand recovered, but the trainer died after 6 days in intensive care and was found at postmortem to have had severe interstitial pneumonia. Although the trainer had a prior history of Ross River and hepatitis A virus infections, he was well immediately before this illness.

Because a virus etiology was possible (7), homogenates of spleen and lung from two of the horses were inoculated into two recipient horses intravenously and by intranasal aerosol as well as into a range of cell culture monolayers (8). On day 3 after inoculation, examination of the Vero cell cultures inoculated with lung homogenate showed a cytopathic effect with focal syncytia formation, which subsequently spread throughout the entire monolayer. Postmortem lung, liver, kidney, and spleen samples from the deceased human were also inoculated into cell cultures. After 12 days, prominent syncytia were seen in LLC-MK2 and MRC5 cultures inoculated with kidney material. Syncytia formation in cell culture is characteristic of a number of viruses, including morbilliviruses (9).

After inoculation, the horses showed little sign of infection for 6 and 10 days, respectively; they then developed a disease of 2 days' duration, with high fever and signs of respiratory distress, and were killed (10) on days 8 and 12 after inoculation. Supernatant from Vero cells containing 2×10^7 median tissue culture infectious dose ($TCID_{50}$) per milliliter of virus was administered to another two horses intravenously (5 ml) and by exposure to about 10 ml of nebulized aerosol. These horses became similarly affected, although the time course to terminal disease was only 4 days in one and 5 in the other. Viral nucleocapsids were identified by electron microscopy in lung homogenates from all four recipient horses, and virus containing similar nucleocapsids was reisolated from the lungs, liver, kidney, and lymph nodes of all experimental horses.

There was histological evidence in 13 field horses and 4 experimental horses of interstitial pneumonia with proteinaceous alveolar edema associated with hemorrhage, dilated lymphatics, alveolar thrombosis and necrosis, alveolar macrophages that may be necrotic, and necrosis of the walls of small blood vessels. The outstanding gross lesion in the lung was edema, which in the field but not the experimental cases was accompanied by hemorrhage and froth in airways. On the other hand, the experimental cases had more advanced dilation of ventral lymphatics. Vascular lesions were remarkable in that there were syncytial giant cells in blood vessel walls, especially in the endothelium (Fig. 1A). These were common in lung capillaries and arterioles, and in experimental cases were also seen in blood vessels associated with parenchymal lesions, in lymph nodes, the spleen, brain, stomach, heart, and kidney. Endothelial and syncytial cells as well as other tissues, including occasional foci of cells in bronchial epithelium, reacted strongly in indirect immunofluorescence and immunoperoxidase tests with high-titer serum from the human cases (Fig. 1B). Syncytial

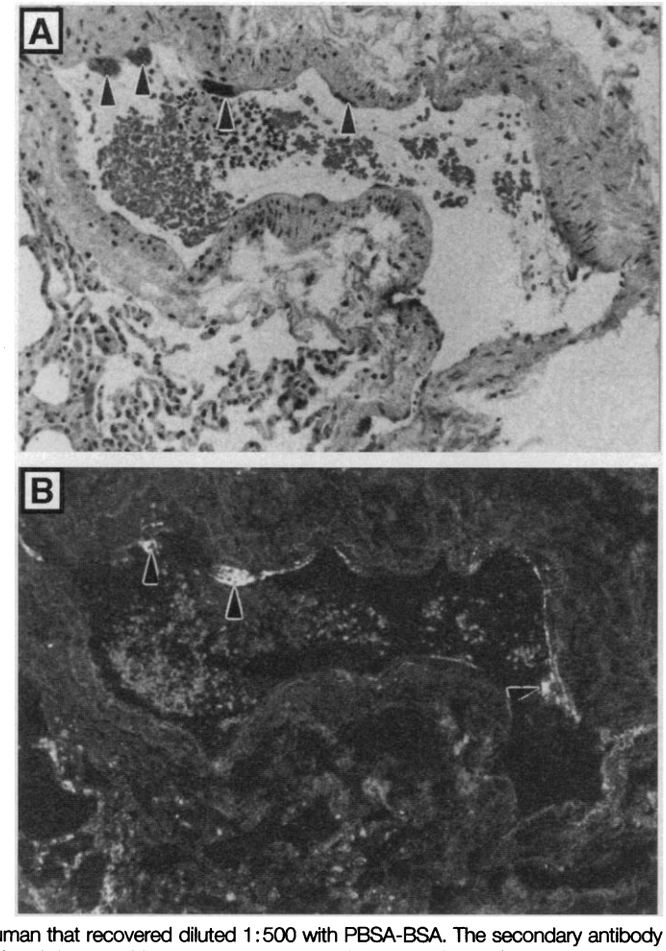

Fig. 1. Blood vessel in the lung of a horse killed because of severe respiratory disease 5 days after intravenous and intranasal inoculation with tissue culture supernatant containing 2×10^7 $TCID_{50}$ of virus. (**A**) A hematoxylin and eosin–stained section of horse lung (×400) showing a number of multinucleate giant cells (arrowheads) in the endothelium. (**B**) Virus identified in the same cells (arrowheads) by an indirect immunofluorescence test (×400). Hematoxylin and eosin–stained sections were prepared from paraffin-embedded formalin-fixed tissues. The immunofluorescence test was done on duplicate tissues. These were digested with 0.05% protease (Pronase E, Sigma P-5147) in phosphate-buffered saline A (PBSA) for 20 min at 37°C. Nonspecific binding was blocked with 5% normal goat serum in PBSA and 0.1% bovine serum albumin (BSA) for 20 min, and samples were then reacted for 60 min with serum from the human that recovered diluted 1:500 with PBSA-BSA. The secondary antibody was a 1:30 dilution of biotinylated sheep anti-human immunoglobulin (Amersham) in PBSA-BSA, incubated for 60 min. A 1:100 dilution of streptavidin-fluorescein (Amersham) diluted with 0.1% BSA, 1.0 ml of 0.04% Evans blue, and 9.0 ml of PBSA was then reacted with antigen in the tissues for 1 hour at 37°C and examined by an ultraviolet light microscope. Controls for the test were duplicates of the tissues, except that the primary antibody was replaced with normal human serum.

Table 1. Titers of human and equine sera tested by a serum neutralization (SN) test with virus isolated from the horses grown on Vero cells and immunofluorescence reactions when tested at a 1:10 dilution on acetone-fixed virus-infected Vero cells.

Name	Date	SN antibody titer	Immunofluorescence
Trainer	5/7/94	<2	Negative
	9/26/94	64	Positive
Stablehand	9/24/94	64	Positive
	10/5/94	64	Positive
Horse no. 1	9/24/94	<2	Negative
	10/1/94	256	Positive
Horse no. 2	9/24/94	1024	Positive
	11/5/94	1024	Positive
Horse no. 3	9/24/94	16	Positive
	11/5/94	>2048	Positive
Horse no. 4	9/24/94	128	Positive
	11/5/94	>2048	Positive

giant cells are common in morbillivirus infections (*11*), and their presence in the blood vessels indicated the vascular tropism of this virus. Within these giant cells, cytoplasmic inclusion bodies consistent with the ultrastructure of nucleocapsids described for the family Paramyxoviridae (*12*) were observed by electron microscopy. These structures were identical to those seen within tissue culture cells inoculated with horse lung homogenates and were gold-labeled by immunoelectron microscopy after reaction with sera from the human cases (Fig. 2A). Similarly, free-lying nucleocapsids, which were observed within the horse lung homogenates, were gold-labeled (*13*).

Ultrastructural studies were made of virus isolated from the Queensland horses, the experimental horses, and from the kidney of the deceased human. The virus was pleomorphic, possessed an envelope, and ranged in size from approximately 38 nm to more than 600 nm. The envelope was covered with 10- and 18-nm surface projections giving the particle a "double-fringed" appearance (Fig. 2, B and C). The supernatant of the cell cultures contained free-lying herringbone nucleocapsids that were 18 nm wide and had a periodicity of 5 nm (Fig. 2D). These observations are consistent with those described for the family Paramyxoviridae (*14*) and the genera *Paramyxovirus* and *Morbillivirus*. However, the presence of double-fringed surface projections is unique and may represent two viral surface glycoproteins that differ in length more than is usual (*15*). Viruses were gold-labeled with sera from convalescent horses and from both human cases (Fig. 2E), which suggests that horses and humans were infected with the same virus. The human and horse sera also neutralized the horse virus, whereas preexposure sera from the deceased trainer and experimental horses did not. Sera from the surviving horses reacted strongly with, and had high neutralizing antibody titers to, the virus isolated from the dead horses (Table 1).

The virus was tested against antisera to a range of paramyxoviruses, morbilliviruses, and pneumoviruses by immunofluorescence and protein immunoblot analyses (*16*). Rinderpest virus antisera gave very weak reactions by immunofluorescence and to a protein of approximately 1.8×10^5 kD by protein immunoblot, whereas all other sera were negative. None of these antisera neutralized the virus. The virus did not agglutinate erythrocytes from a range of species (*17–18*) or possess detectable neuraminidase activity (*19*), indicating that the virus was not a paramyxovirus.

From the above, the virus appeared to be a member of the genus *Morbillivirus*. This was confirmed by comparative sequence analyses of a portion of the matrix protein gene. A suite of polymerase chain reaction

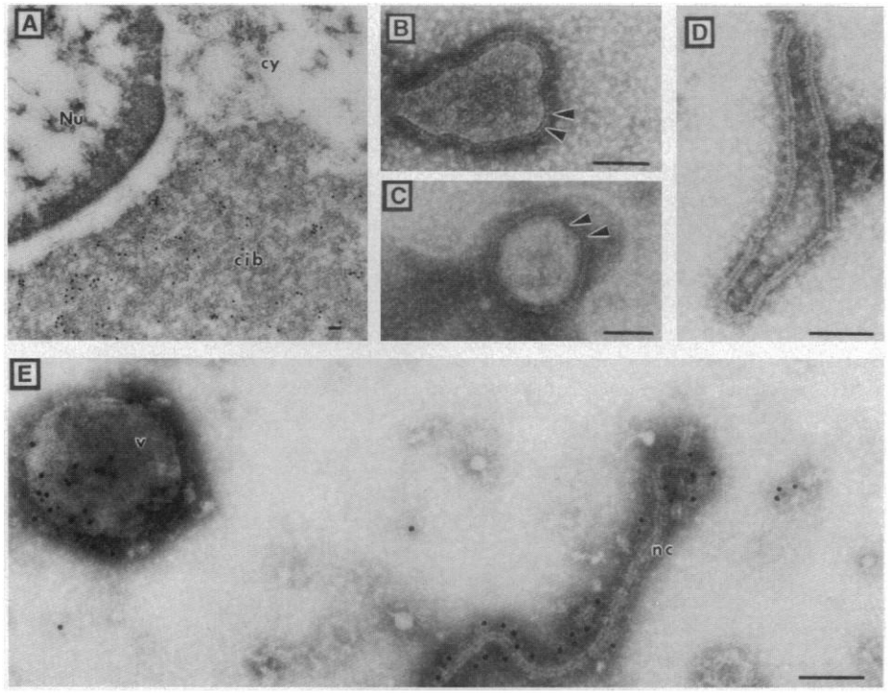

Fig. 2. (**A**) Electron micrograph of an ultrathin section containing an infected endothelial cell within the lung of an affected horse. The image shows a nucleus (Nu), cytoplasm (cy), and a cytoplasmic inclusion body (cib) that had been gold-labeled with protein A–gold and the serum from the deceased trainer. (**B** and **C**) Ultrastructure of viruses isolated from affected horses and the kidney of the deceased trainer. Arrowheads indicate surface projections of two distinct lengths. (**D**) A virus nucleocapsid displaying a herringbone pattern was observed after negative staining of tissue culture supernatant from infected cells. Other nucleocapsids were observed in association with intact and disrupted viruses. (**E**) Intact virus (v) and a free-lying nucleocapsid (nc) that were isolated from the lung of an affected horse were gold-labeled with protein A–gold and the serum from the deceased trainer. All magnification bars represent 100 nm. Lung tissue from affected horses was fixed in 0.1% (v/v) glutaraldehyde and 2% (w/v) paraformaldehyde in 0.1M cacodylate buffer (pH 7.2; 300 mosmol) for 60 min. Samples were processed through to L. R. White resin as described by Hyatt (*27*). Ultrathin sections on gold grids were labeled as described by Hyatt (*27*) with the use of sera from the deceased trainer and 9-nm protein A–gold. Supernatant from infected cell cultures was adsorbed to carbon-coated parlodion-filmed grids and stained with 2% phosphotungstic acid adjusted to pH 6.5 with 1 M KOH. Supernatants from infected culture cells and horse lung homogenates were adsorbed onto carbon-coated parlodion-filmed grids and labeled as described by Hyatt (*27*). The primary antibodies were either serum from convalescent horses or serum from the deceased trainer. Reactions with human serum were labeled with protein A–gold and those using horse serum were labeled with protein G–gold (Amersham). All preparations were specifically gold-labeled; only one is shown.

(PCR) primers (*20–24*) to amplify specific sequences from morbilliviruses (5′-ATGTTTATGATCACAGCGGT and 5′-ATTGGGTTGCACCACTTGTC), paramyxoviruses (5′-ACATACAGTGGGATAAGAACC and 5′-CAACCATGAAGCCTCATCAGG), and pneumoviruses (5′-AATGGAAAAGAAATGAAATTTG and 5′-CAATCACTTCATAGAAGCT) were tested for their capacity to initiate DNA synthesis in PCRs with RNA derived from viruses isolated from affected horses and humans (Fig. 3A). All of these primer pairs were negative in PCRs. Due to the weak but consistent serological cross-reactions against rinderpest virus, other primer pairs were designed to amplify regions from the matrix protein (5′-TTCTTAATGGTATAATAGAAG and 5′-TGAAATTGCCGATATGTACCAT), the fusion protein (5′-GTTCAGGGAGTCCAGGACTACGTC and 5′-TCCAGTCTGTGGACTATAACCCCCTT), or the L protein (5′-GCATGGAGGGAGTTGGCCACC and 5′-TGACACTGTCATTTTGTAAGTCA) of morbilliviruses by initiation of PCRs at possible conserved nucleotide regions of these proteins. Only the matrix protein primer pair amplified a product of approximately 400 base pairs (bp) after PCR. This was sequenced, and another pair of primers was designed to be specific for the amplified equine viral sequence. These primers (5′-CATGTAGATGCCGGAGTCAT and 5′-TTGTGTTCGGGTCCTCTGGC) gave a specific product of approximately 200 bp when the equine viral RNA was used. The virus isolated from a kidney of the horse trainer also gave a positive reaction, and sequence analysis revealed that both PCR products were identical (Fig. 3A). Translation of this sequence and comparisons with

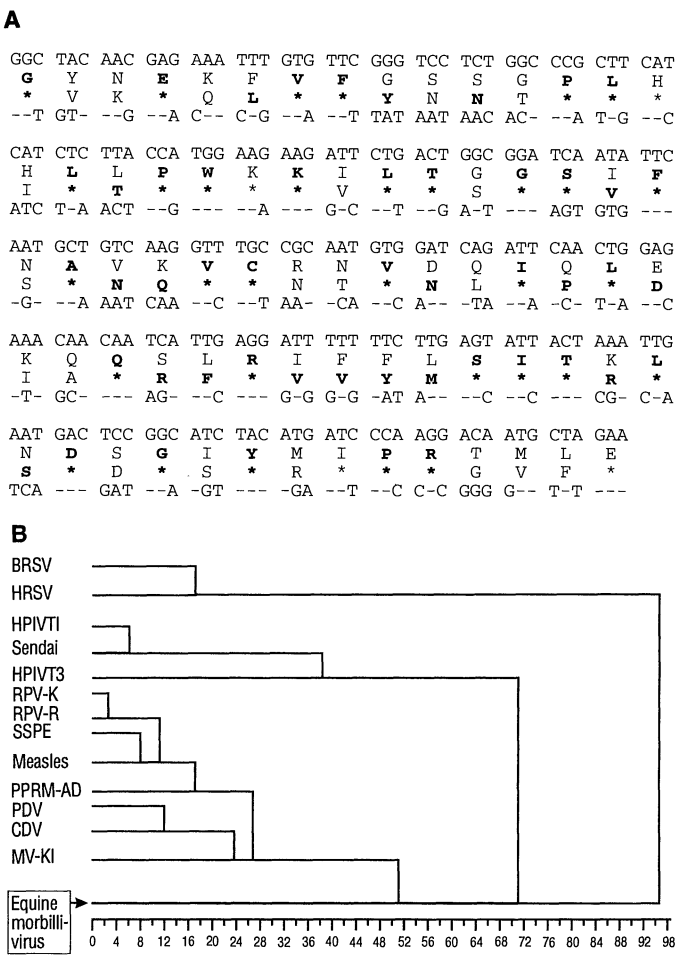

Fig. 3. (**A**) The alignment of the nucleotide sequence (top) coding for the equine morbillivirus matrix protein with the cognate region of canine distemper virus (CDV7; amino acids 106 to 180) shows conserved nucleotide and amino acid sequences as dashes or asterisks, respectively (*28*). The amino acid sequences shown in bold for CDV were those that had previously been found to be conserved among all morbilliviruses (*29*). The amino acids shown in bold for the equine morbillivirus are conserved among all known morbillivirus matrix proteins. (**B**) Phylogenetic analyses of matrix protein sequences of Paramyxoviridae were done on aligned sets of peptide sequences and analyzed by programs of the PHYLIP package (*25*). The relations are presented as an unrooted tree. The sequences for measles, CDV, and phocine distemper virus (PDV) are described (*29*); the peste des petits ruminants sequence (PPRM-AD) is from Diallo and Barrett (*30*). Other sequences are equine morbillivirus (described in this paper); subacute sclerosing panencephalitis virus (SSPE; strain Biken) (*31*); bovine respiratory syncytial virus (BRSV; strain A51908) (*32*); human respiratory syncytial virus (HRSV; strain A2) (*33*); Sendai virus (Sendai; strain Harris) (*34*); bovine morbillivirus (MV-K1) (*35*); human parainfluenza virus type 3 (HPIVT3) (*36*); and human parainfluenza virus type 1 (HPIVT1) (*37*).

known Paramyxoviridae matrix proteins revealed 50% similarity with the morbillivirus matrix proteins and 80% similarity if conservative amino acid substitutions were used. Phylogenetic analyses on matrix protein sequences by means of the DNADIST and PHYLIP programs of Felsenstein (*25*) (Fig. 3B) indicated that this morbillivirus is distantly related to the other known members of the group.

No further cases were seen in horses or humans after the outbreak. Serosurveillance of approximately 1600 horses and 90 people by a serum neutralization test indicates that the disease has not spread. These observations suggest that these new host species had not previously been exposed to the virus.

Before the isolation of this virus, the host range of each virus in the genus *Morbillivirus* was restricted to a single mammalian order (*26*). Phylogenetic analyses suggest that the virus has not resulted from a single mutation or a few key point mutations but most likely is a virus that has emerged from its natural host. Investigations are now under way to identify the original host species and the circumstances under which the virus changed hosts, and to establish whether the virus remains a threat. Physicians and veterinarians will now have the opportunity to investigate whether previous cases of acute respiratory disease syndrome might have any association with this virus.

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28. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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