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RESEARCH NOTE

Sequence and structure relatedness of matrix protein of human respiratory syncytial virus with matrix proteins of other negative-sense RNA viruses

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ABSTRACT

Matrix proteins of viruses within the order Mononegavirales have similar functions and play important roles in virus assembly. Protein sequence alignment, phylogenetic tree derivation, hydropathy profiles and secondary structure prediction were performed on selected matrix protein sequences, using human respiratory syncytial virus matrix protein as the reference. No general conservation of primary, secondary or tertiary structure was found, except for a broad similarity in the hydropathy pattern correlating with the fact that all the proteins studied are membrane-associated. Interestingly, the matrix proteins of Ebola virus and human respiratory syncytial virus shared secondary structure homology.

Keywords Ebola virus, matrix protein, Mononegavirales, respiratory syncytial virus, RNA viruses

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Matrix proteins of negative-sense single-strand RNA viruses play an important role in virus

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Table 1. Negative-sense ssRNA viruses included in the study

Family	Genus	Type species
Bornaviridae	Borna virus	Borna disease virus
Filoviridae	'Marburg-like viruses'	Marburg virus Ebola virus
Paramyxoviridae	Respirovirus	Human parainfluenza virus 1 Measles virus Rinderpest virus Canine distemper virus
	Morbillivirus	Mumps virus
	Rubulavirus	Newcastle disease virus
	Pneumovirus	Human RSV (hRSV) Bovine RSV (bRSV)
	Metapneumovirus	Turkey rhinotracheitis (TRTV)
	Paramyxovirus	Sendai virus
Rhabdoviridae	Vesiculovirus	Vesicular stomatitis virus (VSV)
Orthomyxoviridae	Influenza virus A	Influenza A virus

assembly and budding. They interact with envelope proteins, cell membranes and viral nucleocapsids, and also self-oligomerise [1–3]. Matrix proteins inhibit cellular transcription early in infection, and subsequently interact with viral nucleocapsids to inhibit viral transcriptase activity [4–8]. As matrix proteins of viruses belonging to the Mononegavirales have similar functions, they may also share structural features. This study analysed the matrix protein sequences of representative viruses (Table 1) for relatedness, with human respiratory syncytial virus (hRSV) matrix (M) protein as the reference.

The matrix proteins studied ranged in length from 142 amino-acids (16 kDa) for Borna virus to 375 amino-acids (41 kDa) for mumps virus, with pI values of 7.6–9.5. The hRSV M protein sequence was aligned with matrix proteins of other negative-sense RNA viruses, and a distance matrix was calculated with the PileUp and Eprotdist programs [8]. Only one amino-acid (V94 in hRSV) was conserved in all viruses (except Borna disease virus). A sequence alignment of the viruses within the Paramyxoviridae

family was then carried out to determine whether viruses in the same family had greater sequence similarity, but only six amino-acids were conserved (L48, I/V57, I/V112, T118, L202, G211 in hRSV). V94, the single conserved amino-acid in the mononegaviruses, was not conserved when a different grouping (paramyxoviruses) was used, probably because of the way in which PileUp first performs a pairwise alignment that scores the similarity between every possible pair of sequences. These similarity scores are used by PileUp to align the two sequences most related to each other in order to produce the first cluster. A series of such pairwise alignments produces the final alignment. Hence, in the two alignments generated, vesicular stomatitis virus (VSV), measles virus and mumps viruses had amino-acids 169, 157 and 172 consistently aligned together. Turkey rhinotracheitis virus (TRTV) and hRSV had amino-acid V94 aligned in the first alignment (negative-sense RNA virus alignment) and had amino-acid 112 aligned in the second alignment (Paramyxoviridae virus alignment). Thus, in both cases, two similar residues, V and I, were aligned.

The two sets of alignment data were used to calculate robust phylogenetic trees with the ESeq-Boot and Econsense programs. The groupings for viruses within the order Mononegavirales (Fig. 1A) did not have high significance values (values < 50), indicating low sequence homology among the sequences. Mumps, measles and parainfluenza 1 viruses were grouped together, separate from the rest of the viruses. Significant values (> 50) were obtained more often for viruses belonging to the Paramyxoviridae family (Fig. 1B). The groupings derived were broadly similar to those of the International Committee on Taxonomy of Viruses (Table 1), but Sendai virus

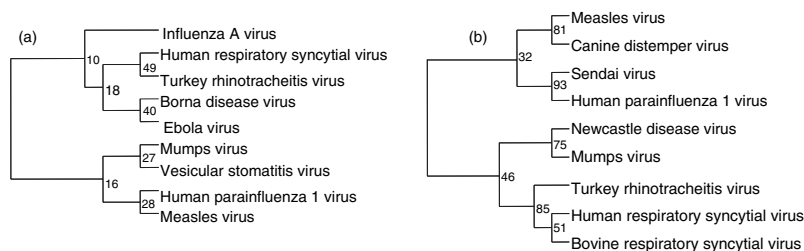


Fig. 1. Phylogenetic relationships based on matrix protein sequences among (a) the negative-sense viruses and (b) viruses of the family Paramyxoviridae. A rooted phylogram was generated by distance measure for protein sequences, using maximum likelihood estimates based on the Dayhoff PAM matrix following alignment of matrix protein amino-acid sequences. The significance levels are indicated on the tree.

grouped with parainfluenza virus 1, and rubulaviruses grouped with Pneumovirinae rather than Paramyxovirinae. These results suggested that phylogenetic trees derived from matrix protein amino-acid sequences may be used as a basis for classification of viruses.

The hRSV M protein sequence was most closely related with bovine RSV and TRTV, suggesting that hRSV and TRTV matrix proteins have a close evolutionary relationship. TRTV and hRSV have matrix proteins of a similar size (251 and 256 amino-acids, respectively). The partial gene order of TRTV is 3'-M-F-5', similar to that of the Paramyxovirinae sub-family, compared to the hRSV gene order of 3'-M-SH-G-F-5' in the Pneumovirinae sub-family [3]. This suggests that the divergence in evolution between hRSV and TRTV occurred when there was an insertion between the M and F genes in the virus genome.

A hydropathy plot was derived for hRSV M, and a comparison of probable structure with TRTV, VSV and mumps virus matrix proteins was made. The hydrophobic moment was calculated as described by Eisenberg *et al.* [9], except that the hydrophobic moment was normalised for the local hydrophobicity of the amino-acids in the window where the moment was being determined. Such hydropathy patterns can give an indication of regions of membrane association, as well as solvent-exposed regions of the folded secondary structure of proteins [10]. hRSV M protein contains one hydrophilic region at the N-terminus and one highly hydrophobic region close to the C-terminus. Amino-acid residues 1–50 of TRTV and hRSV showed high hydropathy conservation; both have a hydrophobic region followed by a hydrophilic peak. Amino-acid residues 170–200 had a hydrophobic region in both TRTV and hRSV. The hydropathy pattern of mumps virus and VSV matrix proteins did not have any obvious similarities with that of hRSV M protein, but all three showed a common region of low hydrophobicity at the N-terminus, followed by a gradual increase in hydrophobic value towards the C-terminus. The length of the hydrophobic regions was less than the minimum length required to form a transmembrane domain, but may mediate peripheral attachment to the host cell membrane [11].

The calculated hydrophobic moments for hRSV M protein were also used to derive helical wheel

projections for comparison with similar projections of VSV, TRTV and mumps virus matrix protein sequences. There was no correlation of sequence with predicted exposed surface.

The similarity in hydropathy patterns could indicate a similarity in three-dimensional structure. The FUGUE [12], INGBU [13] and 3DPSSM [14] programs were used to search secondary structure libraries for proteins sharing the predicted secondary structure of hRSV M. The results were submitted to the Pcons [15] neural network. Within mononegaviruses, only the crystal structures of VSV, Ebola and influenza virus matrix proteins have been determined. The N-terminal regions of hRSV M and Ebola virus matrix protein (VP40) had similar secondary structure. The N-terminal region of VP40 has been shown to direct its oligomerisation and the formation of specific conformational states, which may be functionally important, although no function has yet been assigned [16]. VP40 and hRSV matrix N-terminal sequences did not share structural similarity with VSV and influenza matrix proteins.

Matrix proteins bind to cell membranes, although they are not integral proteins (lacking a transmembrane region) and are not true peripheral proteins (unable to be released from membrane or bilayers by ionic means). Matrix proteins bind to membranes largely by hydrophobic interactions [1]. The regions of hRSV M protein with the highest likelihood of interacting with the membrane are the three predicted helices, PADLLIKELA (helix 1; amino-acids 43–53), DLNTLENITTEFKNAITN (helix 2; amino-acids 173–191) and KGAFKY (helix 3; amino-acids 210–216). VP40 secondary structure also has two helices predicted to facilitate membrane interactions in positions corresponding to hRSV helix 2 and 3. A flexible link separates two (possibly functional) domains in the VP40 crystal structure [17]. A similar flexible link is also present in VSV and influenza virus matrix protein structures [18,19] and is predicted to be present in hRSV M protein.

In summary, the similar functions of negative-sense virus matrix proteins were not generally reflected in their primary or secondary structure. Overall, the only similarity observed was in hydropathy profiles, correlating with the fact that all the matrix proteins studied are known to be strongly membrane-associated. Interestingly, there was some similarity between the predicted

tertiary structure of hRSV and the known crystal structure of Ebola virus matrix proteins. This similarity at the level of tertiary structure may be more general, but given the limited availability of crystal structures of matrix proteins, such conclusions cannot be drawn at present.

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RESEARCH NOTE

MOP-UP: an online tool for finding strain-specific primers or motifs in DNA or protein alignments

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ABSTRACT

MOP-UP is a web-based application that enables efficient searching of nucleic acid or amino-acid alignments for sequences or motifs that are unique to a subset of the members represented in the alignment. This has applications in the design of assays that aim to detect particular strains or species. Since molecular-based characterisation of microbes is becoming increasingly important, MOP-UP can aid microbiologists in finding the best loci on which to base such assays. The program is accessible at: <http://www.hpa.org.uk/srmd/bioinformatics/tools/mop-ups.htm>.

Keywords Computer program, identification, MOP-UP, sequence alignment, typing

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