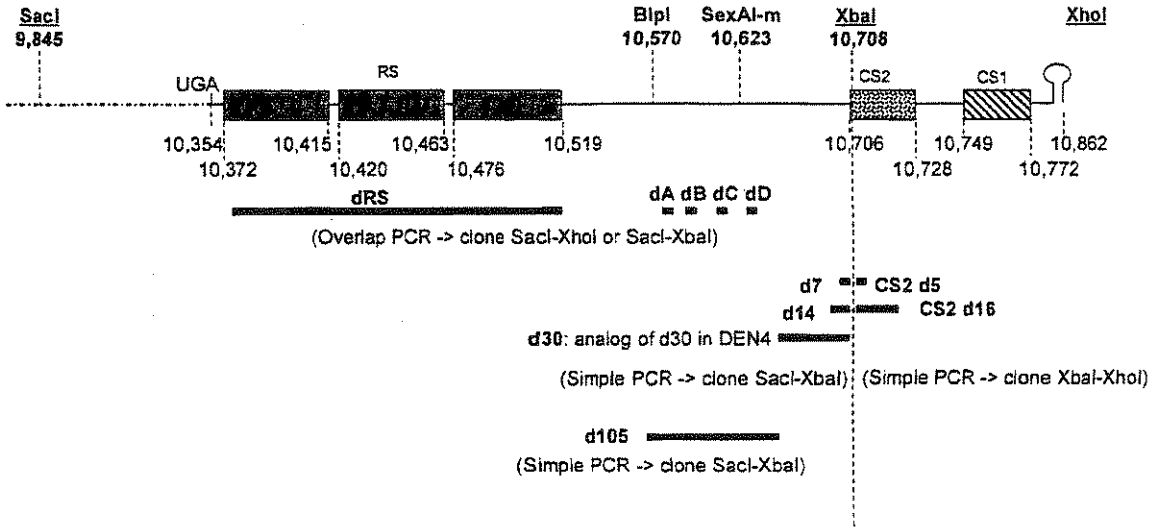
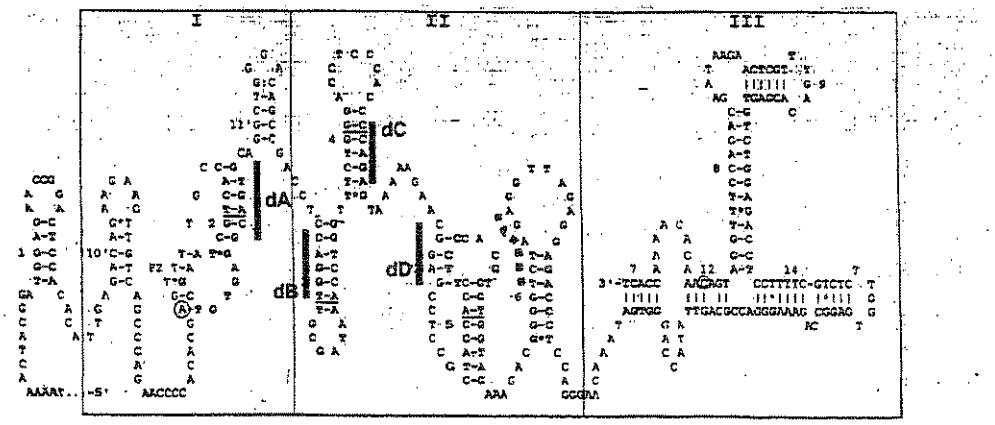
Fig. 1. Deletions in the 3'UTR's of ChimeriVax™-WN04-3'UTR candidates. Panel A: 3'UTR organization and introduced deletions. Panel B: predicted stem-loop structures (Proutski et al., J. Gen. Virol. 78:1543-1549, 1999) including ones that can be destabilized by small deletions dA – dD. Panels C and D, predictions of YF17D and dC mutant 3'UTR structures, respectively, by Zuker's algorithm.

A.



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B.



Note: Dotted line in 2B designates XbaI restriction site at nucleotide 10,708.

Fig. 1. ...continued

C.

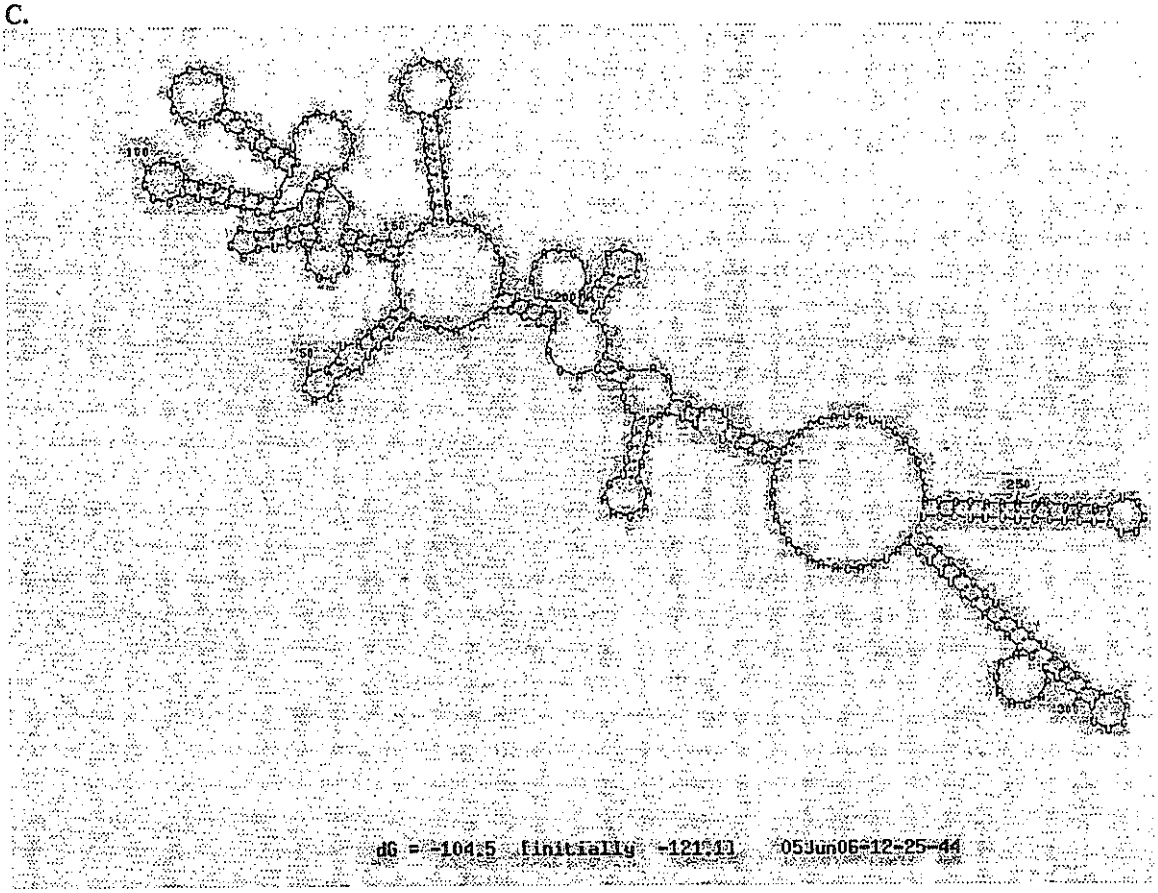


Fig. 1. ...continued

D.

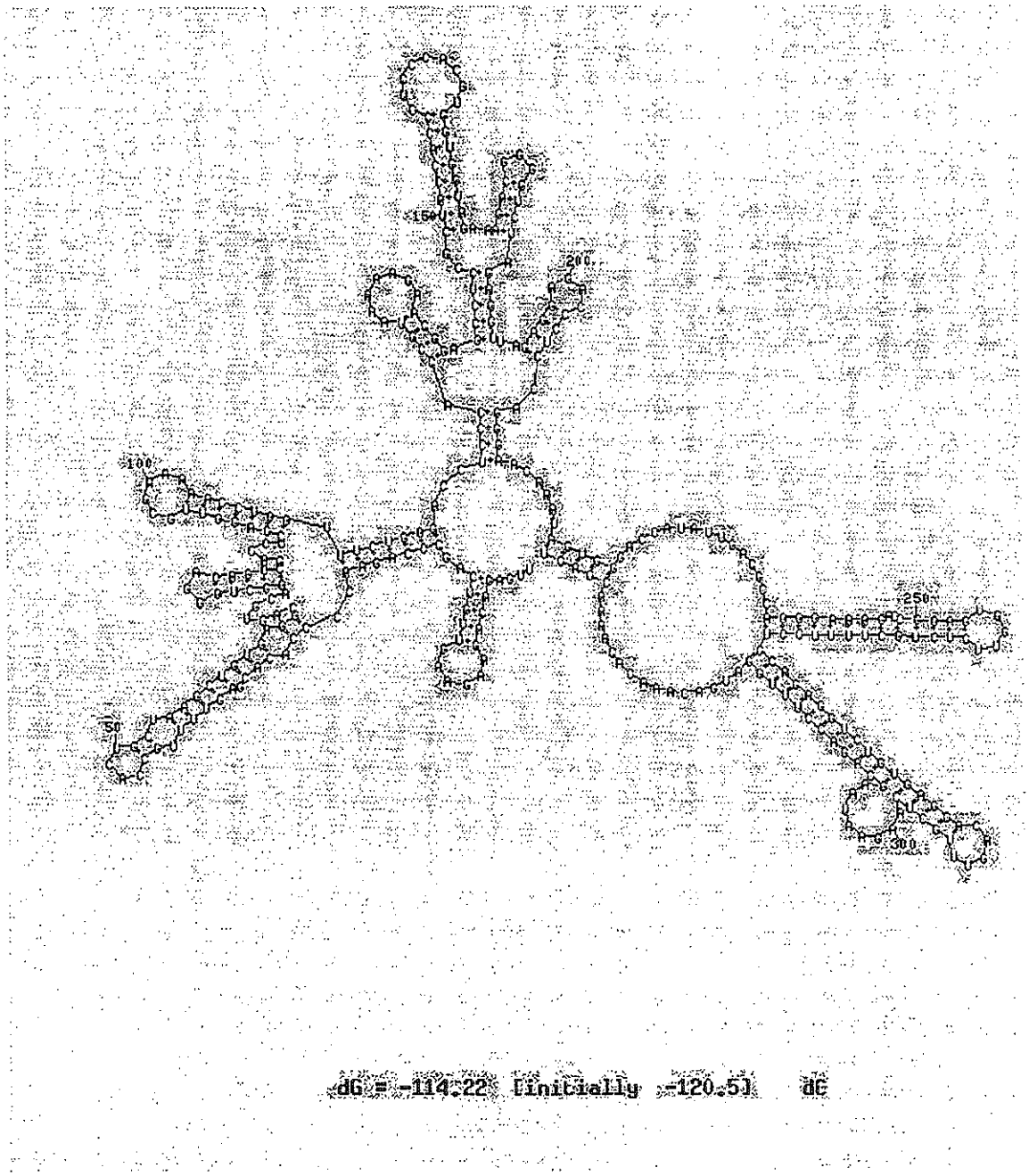
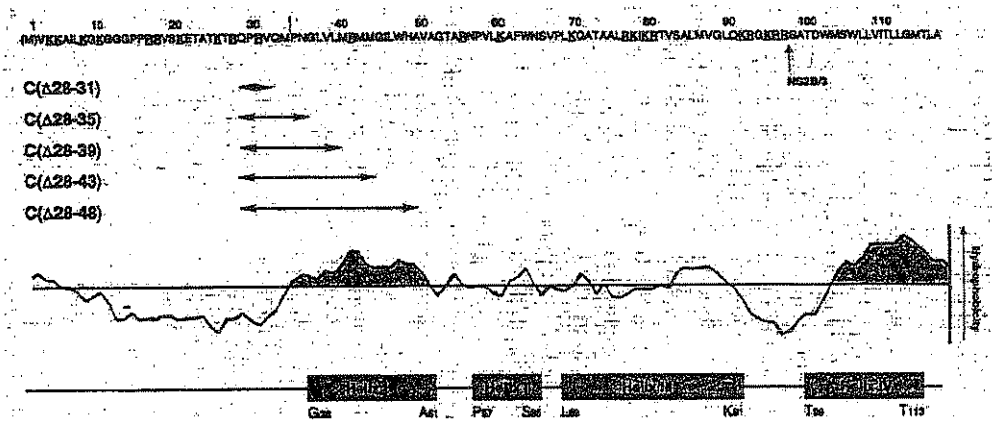


Fig. 2. Attenuating deletions for ChimeriVax™-WN04-C variants (U.S. Patent Application Nos. 60/674,546 and 60/674,415). Panel A: previously described deletions introduced in the C protein of TBE virus (Kofler et al., J. Virol. 76:3534-3543, 2002). Panel B: computer-predicted structure of the YF 17D-specific protein C and proposed deletions C1-C5 (boxed) to be introduced in ChimeriVax™-WN02.

A.



B.

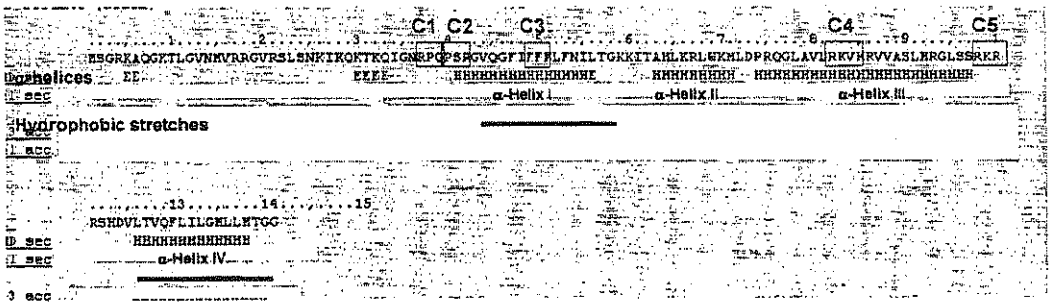


Fig. 3. Replication in HepG2 cells.

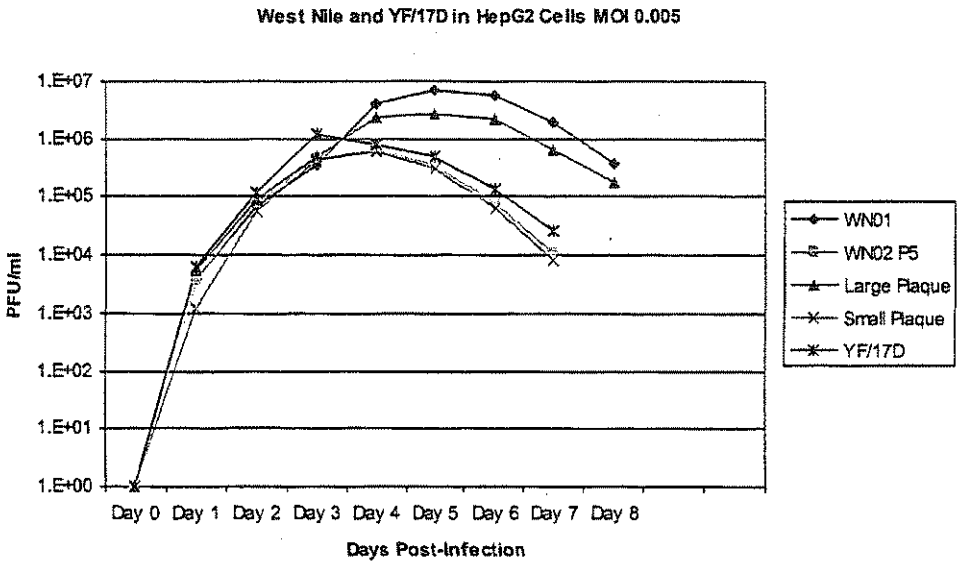


Fig. 4. Replication in THLE-3 cells.

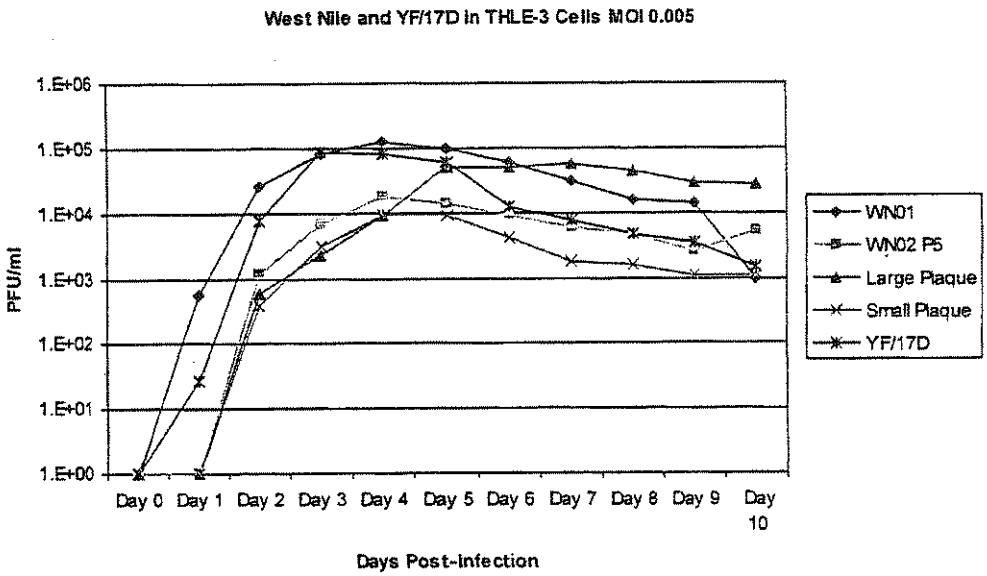
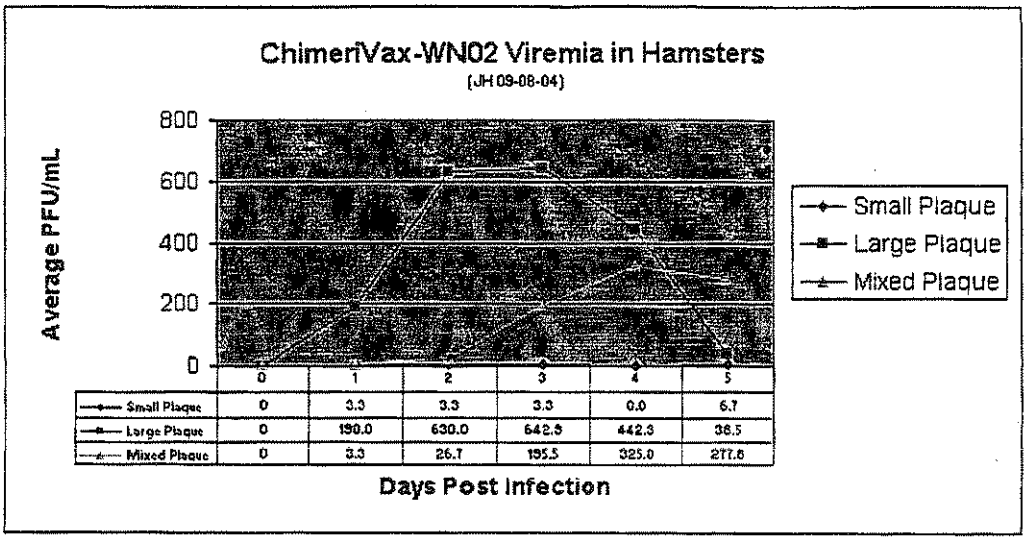


Fig. 5. Viremia in hamsters inoculated with ChimeriVax™-WN02 P5 (mixed plaque), S plaque (PMS, P10), or L plaque (PMS, P10) viruses.



	0	1	2	3	4	5
Small Plaque	0	3.3	3.3	3.3	0.0	6.7
Large Plaque	0	190.0	630.0	642.5	442.3	36.5
Mixed Plaque	0	3.3	26.7	195.5	325.0	277.6

Fig. 6. Small-scale genetic stability passages (g.s.) from the uncloned P2 PMS virus in SF Vero cells.

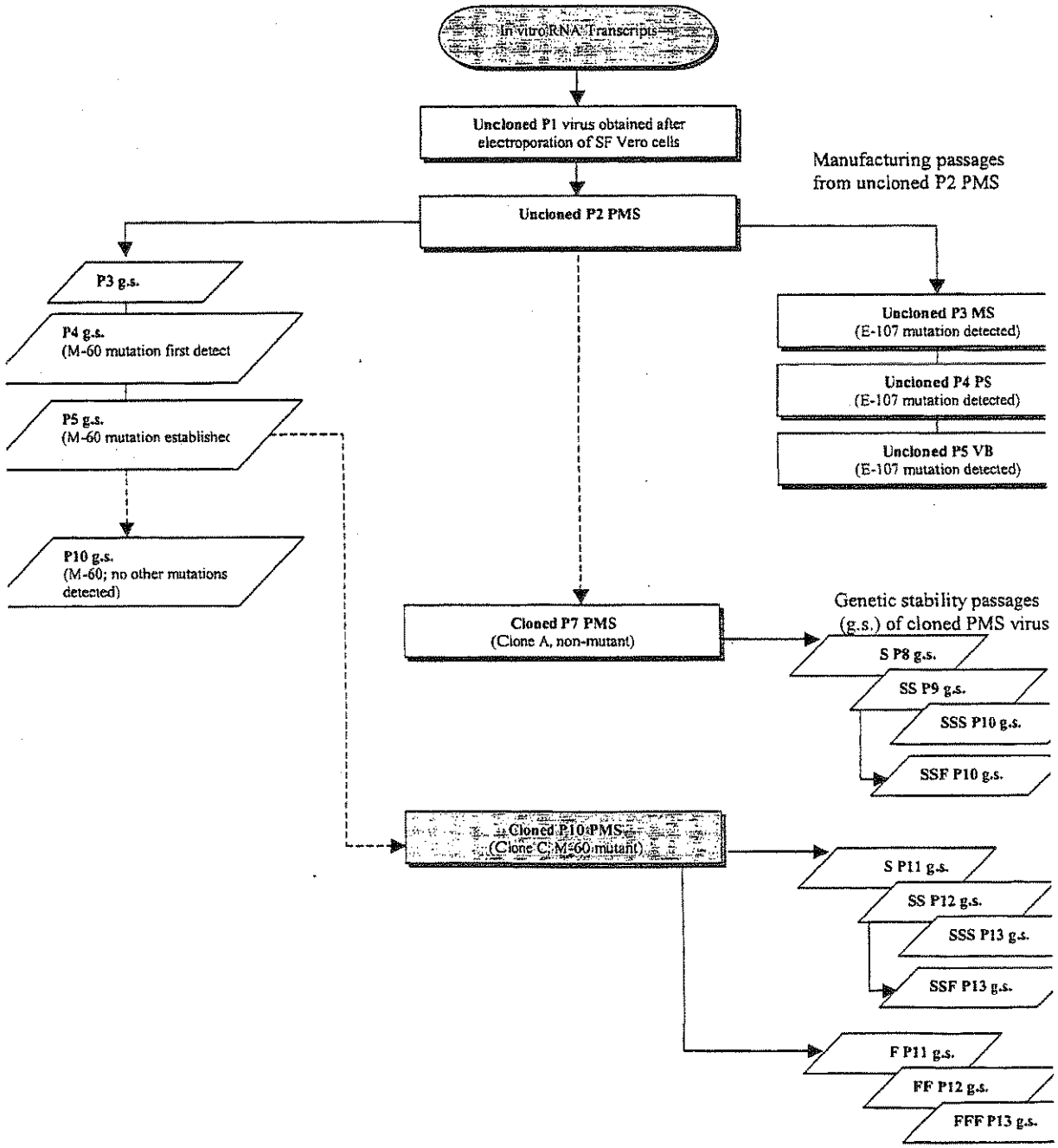


Fig. 7. A graph showing growth curves of SF ChimeriVax™-JE viruses of the invention (uncloned P2, P3 MS (E-107), P4 PS (E-107), P5 g.s. (M-60), and P5 VB (E-107)) at the indicated times post-infection, which shows higher growth rates in SF culture of virus samples containing the M-60 and E-107 mutants as compared to non-mutant virus (P2).

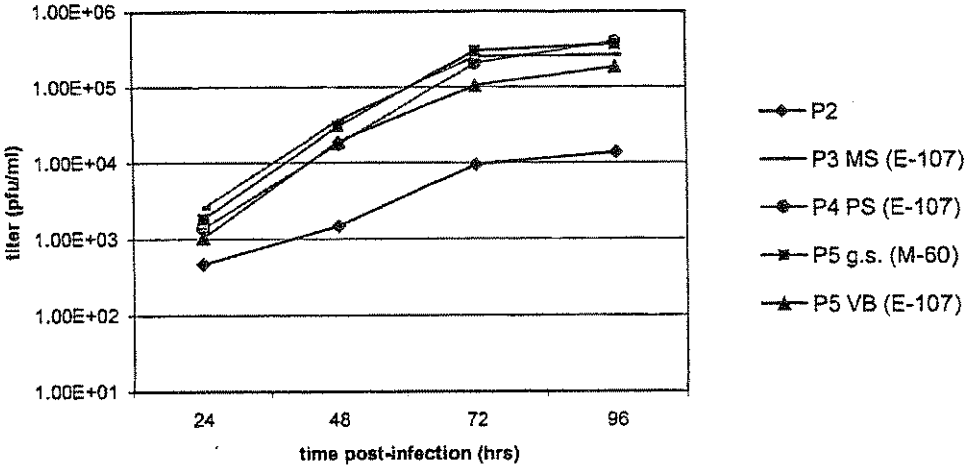


Fig. 8A. pH infectivity threshold analyses.

Fusion assay of
Clone E (M-5 mutant) compared to nonmutant Clone A P7,
Clone C P10 (M-60), and clone I (E-107) of ChimeriVax-JE

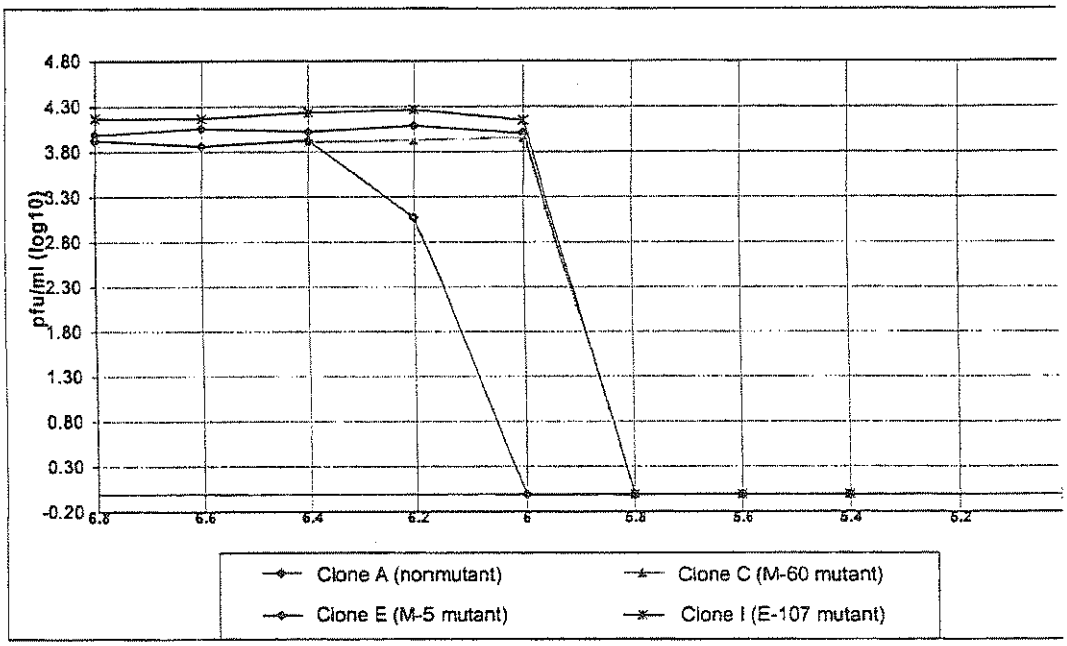
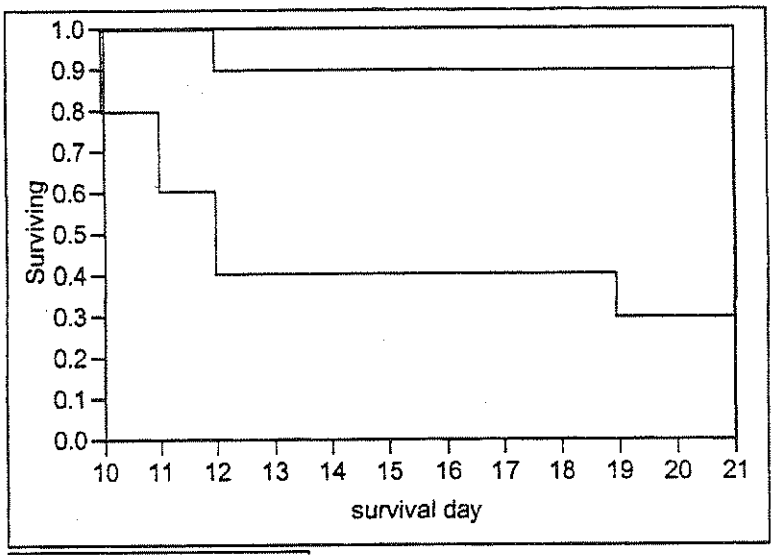
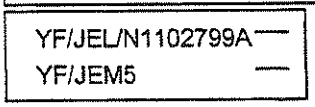


Fig. 8B. Survival Plot of ChimeriVax™-JE vaccine (1.9 log_{10} PFU/dose as determined by back titration of inocula) in comparison to ChimeriVax™-JE M5 mutant (1.4 log_{10} PFU/dose as determined by back titration of inocula) in 3-4 day old suckling mice inoculated by the intracerebral route.





Time to event:
survival day
Grouped by
virus

Summary

Group	N Failed	N Censored	Mean	Std Error
YF/JEL/N1102799A	10	0	20.1	0.9
YF/JEM5	10	0	14.8	1.57621
Combined	20	0	17.45	1.07232

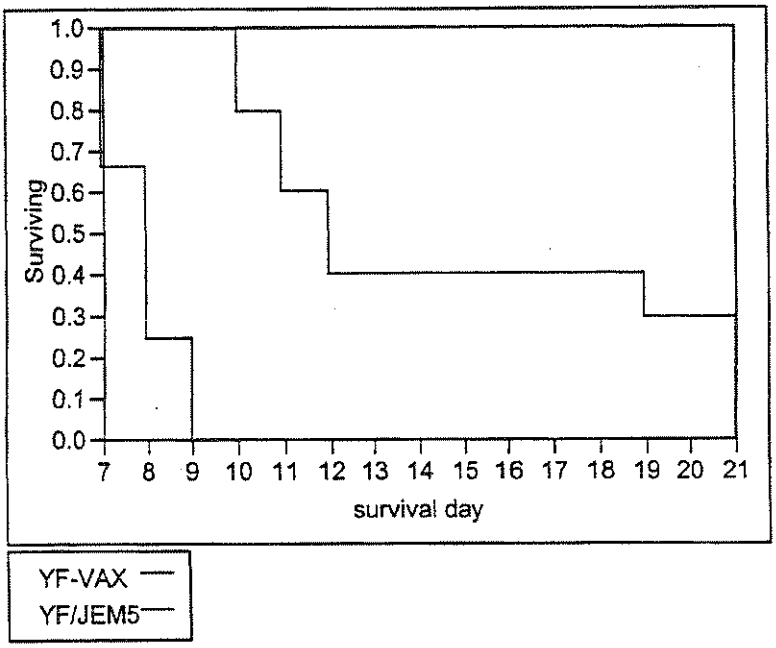
Quantiles

Group	Median Time	Lower 95%	Upper 95%	25% Failures	75% Failures
YF/JEL/N1102799A	21	12	.	21	21
YF/JEM5	12	10	.	11	21
Combined	21	12	.	12	21

Tests Between Groups

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	7.6842	1	0.0056
Wilcoxon	7.4597	1	0.0063

Fig. 8C. Survival Plot of ChimeriVax™-JE M5 mutant virus (1.4 log_{10} PFU/dose as determined by back titration of inocula) in comparison to YF-VAX® (0.9 log_{10} PFU/dose as determined by back titration of inocula) in 3-4 day old suckling mice inoculated by the intracerebral route.



Time to event: survival day
Grouped by virus

Summary

Group	N Failed	N Censored	Mean	Std Error
YF-VAX®	12	0	7.91667	0.22891
YF/JEM5	10	0	14.8	1.57621
Combined	22	0	11.0455	1.02876

Quantiles

Group	Median Time	Lower 95%	Upper 95%	25% Failures	75% Failures
YF-VAX®	8	7	.	7	9
YF/JEM5	12	10	.	11	21
Combined	9	8	11	8	12

Tests Between Groups

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	21.2967	1	<.0001
Wilcoxon	18.1747	1	<.0001

Fig. 8D. Indirect Fusion assay: comparison P7 and P10 of ChimeriVax™-DEN1-4 viruses.
The virus output for each experiment was determined by standard plaque assay. A, ChimeriVax™-DEN1 PMS P7 (triangles) and P10 (diamonds); B, ChimeriVax™-DEN2 PMS P7 (triangles) and P10 (diamonds); C, ChimeriVax™-DEN3 PMS P7 (triangles) and P10 (diamonds); D, ChimeriVax™-DEN4 PMS P7 (triangles) and P10 (diamonds)

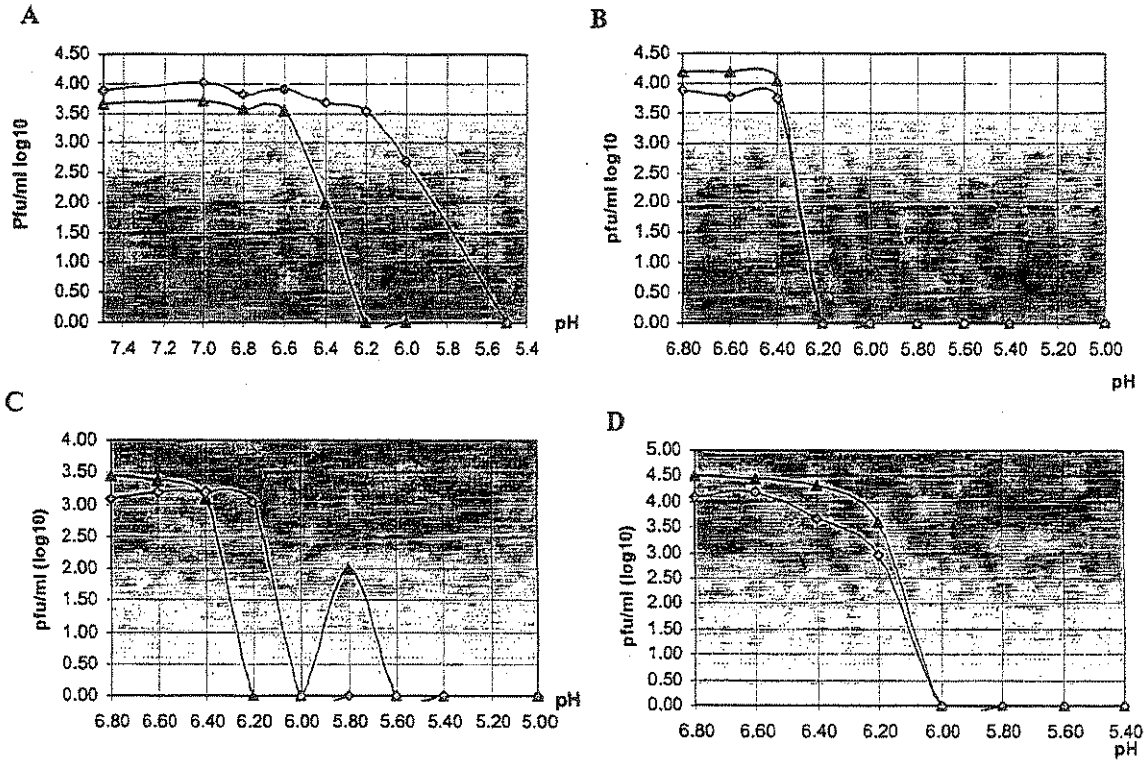


Fig. 8E. Indirect Fusion assay with the ChimeriVax™-DEN3, comparing the PMS (P7) vaccine with the Vaccine lot (P10) and the P15 virus.

The virus output for each experiment was determined by standard plaque assay. ChimeriVax™-DEN3 PMS P7 (triangles), P10 (diamonds), and P15 (squares).

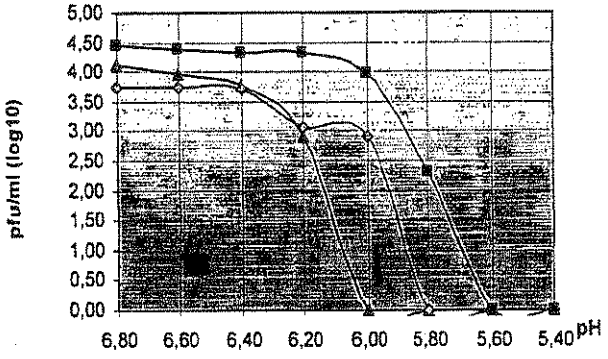


Fig. 8F. Structure of DEN1 E-protein dimmer (aa 1 to 394) of ChimeriVax™-DEN1 virus (Guirakhoo et al., J.Virol. 78:9998-10008, 2004). (A) The position of the positively charged lysine (K) at residue 204 of the P7 (PMS, 204K) virus is shown by CPK (displays spheres sized to van der Waal radii) representation. Three structural domains are shown in medium grey (domain I), light grey (domain II), and dark grey (domain III). (B) Close up of marked area in panel A. (C) The same area as in panel B from the E protein model of the mutant DEN1 virus (P10, 204R shown in red). Selected amino acids in panel B and C are shown in stick representation. Grey, carbon; blue, nitrogen; red, oxygen; yellow, sulfur.

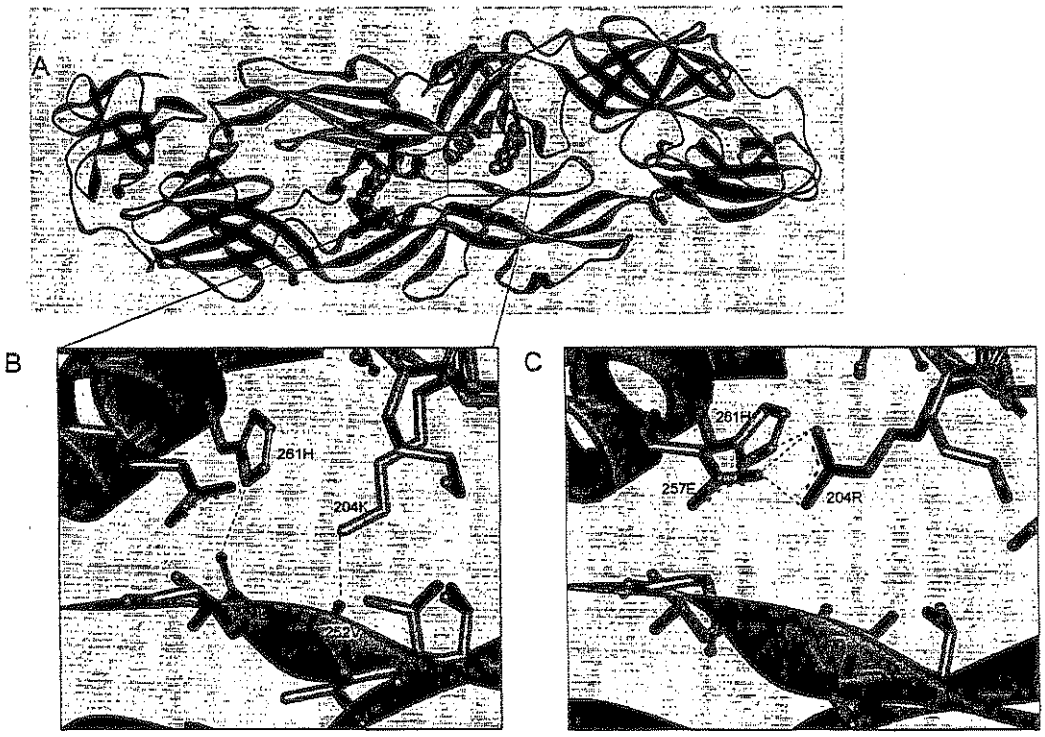


Fig. 9A. A graph showing the penetration efficiency of ChimeriVax™-JE viruses M60 mutant (Clone C), E107 mutant (Clone I), and non-mutant (Clone A) at the indicated times. These results indicate that the M60 mutation facilitates penetration in SF Vero cells apparent at the 5 and 10 minute time points. SF Vero cells were infected with appropriately diluted viruses (Clones A, C, and I in serum free medium) for 5, 10, 20, or 60 minutes, and then were treated for 3 minutes with a solution of 0.1 M glycine, 0.1 M NaCl, pH 3.0, to inactivate extracellular virus. Wells were washed twice with PBS and then monolayers were overlaid with methyl-cellulose followed by staining plaques on day 5 with crystal violet. Efficiency of penetration is shown as percentages of observed plaque numbers after glycine treatment as compared to control infected wells that were treated with PBS instead of glycine.

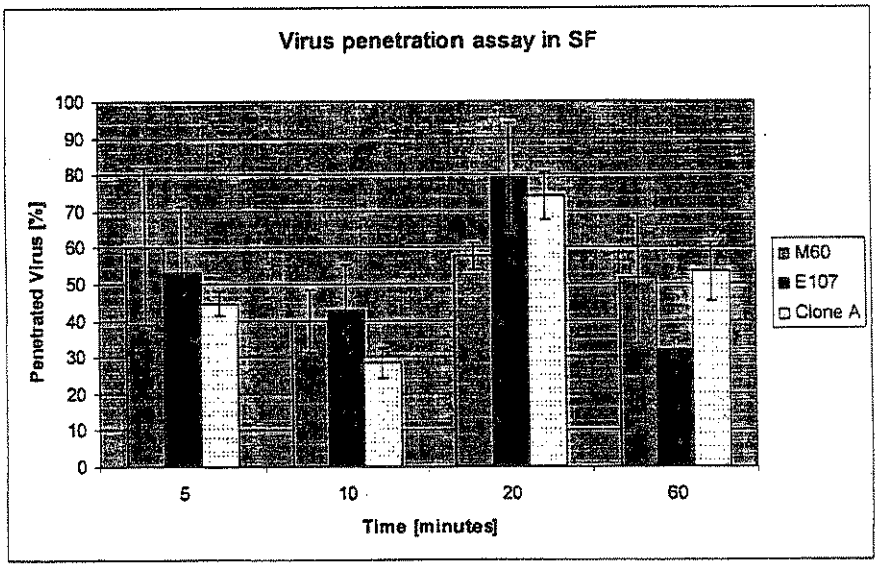


Fig. 9B. Schematic representation of the location of the E-107, M-5, and M-60 amino acid residues in the envelope proteins E and M, illustrating the hypothetical effect of the M-5 residue on fusion. The dashed stretch at the tip of domain II of the E protein containing the E-107 residue represents the fusion peptide (c-d loop), which inserts into the cell membrane (Rey et al., Nature 375:291-298, 1995). The M-5 residue is at the N terminal of the ectodomain part of the M protein. The E protein monomers rearrange into trimeric complexes, which fold to force the cell and virus membranes to fuse (Modis et al., Nature 427(6972):313-319, 2004). M may be a functional component of the complexes, e.g., facilitating fusion of the viral membrane with the cell membrane via its interaction with the E protein. The M-60 residue is between the two C-terminal transmembrane stretches of M and may participate in the interaction of the cell and viral membranes during fusion.

