Complete nucleotide sequence of the non-structural gene of the human influenza virus strain A/WS/33

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A/WS/33 is an important strain of influenza A virus because it represents both the first isolate of human influenza virus (1) and the only strain, with the possible exception of A/Mel/35, to yield stable neurotropic variants, A/NWS/33 (2) and A/WSN/33 (3). Reassortant experiments with WSN have indicated three influenza genes, including the non-structural (NS) gene, are necessary to express full neurovirulence (4). The NS gene encodes two proteins: NS_1, translated from a full-length mRNA transcript, and NS_2, encoded by a spliced mRNA species (5). The synthesis of the NS_1 protein is essential for the normal replication of vRNA (6) and directly interacts with vRNA (7), while a mutation in NS_2 has been shown to facilitate abherrent replication of the polymerase gene (8). During infection of mouse brain with A/WSN/33 the synthesis of NS_2 protein is reduced relative to the parental A/WS/33 strain while production of NS_1 protein remains high (9). To examine the molecular basis of this, the complete NS gene sequence of A/WS/33 was determined from independent cDNA clones of the gene.

There are only 6 nucleotide changes (99.3% homology) between A/WS/33 and its neurovirulent derivative A/WSN/33 and only two of these produce an amino acid change: a conservative change in NS_1 and a non-conservative change in NS_2 (Table 1). However, five of the changes are in the intron with two of these at its 3′ end, a region implicated in the control of splicing of this gene (10). The latter two changes would serve to increase the stability of the mRNA secondary structure in this region relative to the WS strain (Figure 1). The decreased levels of NS_2 protein may thus be due to reduced splicing caused by this more stable structure, which may serve to mask the splice site from the splicing enzymes.

The sequence of the NS gene A/WS/33 is 97.1% homologous to that of the strain A/PR/8/34 (11), isolated only one year later. Interestingly, it is slightly more homologous (97.5%) to A/Bel/42 (12), isolated a further 8 years later. This suggests that A/PR/8/34 is unusually removed from the slender evolutionary tree of the NS genes, rather than having acquired mutations caused by repeated passage, as previously argued (12).

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REFERENCES

1. Smith,W.C., *et al.* (1933) *Lancet* **i**, 66–68.
2. Stuart-Harris,C.H. (1939) *Lancet* **i**, 497–499.
3. Francis,T. and Moore,A.E. (1940) *J. Exp. Med.* **72**, 717–728.
4. Sugiura,A. and Ueda,M. (1980) *Virology* **101**, 440–449.
5. Lamb,R.A. and Lai,C.-J. (1980) *Cell* **21**, 475–485.
6. Hatada,E., *et al.* (1990) *J. Gen. Virol.* **71**, 1283–1292.
7. Hatada,E., *et al.* (1992) *J. Gen. Virol.* **73**, 17–25.
8. Odagiri,T. and Tobita,K. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5988–5992.
9. Bradshaw,G.L., *et al.* (1990) *Virology* **176**, 390–402.
10. Plotch,S.J. and Krug,R.M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5444–5448.
11. Baez,M., *et al.* (1980) *Nucleic Acids Res.* **8**, 5845–5858.
12. Buonagurio,D.A., *et al.* (1986) *Science* **232**, 980–982.
13. Tinoco,I., *et al.* (1973) *Nature* **246**, 40–41.

Table 1. Sequence changes between the NS gene of strain A/WS/33 and its neurotropic variant A/WSN/33 (12) [EMBL Accession no. M12597].

Nucleotide (mRNA sense)	A/WS/33	A/WSN/33	Amino acid change (A/WS/33→A/WSN/33)
114	T	G	Ser^{30}→Ala^{30} NS_1
245	C	T	None
257	T	C	None
488	A	G	None
518	T	C	None
686	A	G	Glu^{63}→Gly^{63} NS_2

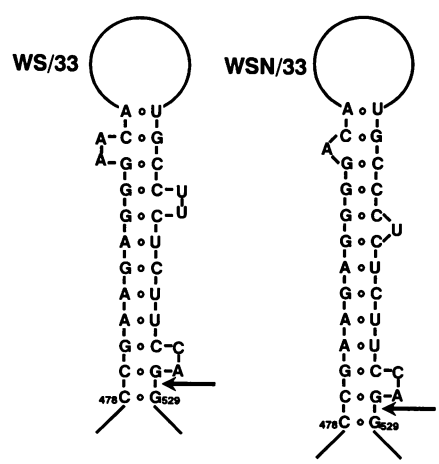

Figure 1. Potential RNA secondary structure around the 3′ splice site (which is indicated by the arrow). The increase in free energy resulting from the changes in WSN relative to WS is ca. −7.0 kcal/mol (13).

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