Comparison of the nucleotide sequence of the SH gene and flanking regions of mumps vaccine virus (Urabe strain) grown on different substrates and isolated from vaccinees

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The small hydrophobic (SH) protein gene and flanking regions of the Urabe Am9 vaccine strain of mumps virus were amplified by the polymerase chain reaction and sequenced directly by the dideoxynucleotide chain termination method. The 434 bp sequence was identical for the Urabe strain isolated from vaccines produced by three manufacturers and for virus isolated following post-vaccination parotitis. No changes were detected for coding, non-coding or intergenic regions between virus grown on different substrates. The Urabe virus SH coding region differed from the published sequence for strain SBL-1 by 14.4% at the nucleotide level and 24.6% at the amino acid level. The 5' non-coding SH region was strongly conserved between the two strains (2% different), whereas the other non-coding regions were not.

The gene order in the paramyxovirus mumps virus has been shown to be 3' NC-P-M-F-SH-HN-L 5' (Elango et al., 1988; Elliott et al., 1989). The small hydrophobic (SH) protein gene of strain SBL-1 encodes a putative protein of 57 amino acids and Mr 6.7K (Elango et al., 1989; Elliott et al., 1989). Although the SH protein has not yet been detected in infected cells, the SH gene is transcribed into mRNA (Elango et al., 1989; Elliott et al., 1989). The transcription pattern varies between strains, with the monocistronic mRNA translatable in the SBL-1 strain grown on Vero cells whereas, on the same cell type, the Enders strain produces mostly fusion protein (F)-SH bicistronic mRNA transcripts (Elliott et al., 1989). Transcription also varies when virus is grown in different cell types (Afzal et al., 1990). These findings could be due to a number of mechanisms, including the occurrence of mutations in the intergenic regions or the involvement of host factors.

The nucleotide sequence of the SH gene and flanking regions was determined for a number of virus preparations derived from the Urabe Am9 vaccine strain of mumps virus (Yamanishi et al., 1973). Total RNA was extracted (Macadam et al., 1989) from vaccine bulks without prior passage, or from tissue culture fluid following low passage of clinical isolates in Vero cells. Virus RNA was converted into cDNA with reverse transcriptase and primer P1 (mRNA sense 5' GTCGATGATCTCATCAGGTAC 3'), complementary to the genomic sequence encoding the C terminus of the F gene (Elliott et al., 1989). Primer P1 was used in conjunction with primer B63 (vRNA sense, 5' AACGAGAATCCCATGGAAACATA 3'), corresponding to nucleotide positions 744 to 721 of the haemagglutinin-neuraminidase (HN) gene (Waxham et al., 1988), to amplify the SH gene and surrounding regions by the polymerase chain reaction (Saiki et al., 1988). The 1129 bp dsDNA product was purified on a Size Select-400 spin column (Pharmacia) with 100 mM-NaCl, 10 mM-Tris–HCl pH 7.4, 1 mM-EDTA as the buffer. After denaturation by boiling and rapid cooling, the template was sequenced with T7 DNA polymerase (Pharmacia) (Sanger et al., 1977), using conditions based on those recommended by the supplier. The sequencing primers were P2 (vRNA sense, 5' AGTTTCCAGGGCTCCAT 3'), which corresponds to the beginning of the HN gene (Elliott et al., 1989), and P1. The software package of the Genetics Computer Group (University of Wisconsin) was used to process sequence data.

The antigenome sequence of 434 bases obtained (Fig. 1; EMBL accession No. X52816) was identical for the Urabe Am9 vaccine strain from three manufacturers. The virus had been propagated on chick embryo fibroblast cells in two cases and in the amnion of embryonated hens' eggs in the other. Two mumps virus strains (CA1 and CA3) isolated from cases of post-
vaccination parotitis (Forsey et al., 1990) had exactly the same sequence in the SH region as the vaccine preparations of the Urabe Am9 strain, confirming that they originated from the vaccine rather than from infection with wild-type virus (Forsey et al., 1990). The fact that the Urabe Am9 strain maintained the same intergenic sequences on either side of the SH gene, even after passage through a human host, shows that these intergenic regions are relatively stable. Such stability is also evident if these sequences from egg-grown (Elliott et al., 1989) and cell-grown (Elango et al., 1988) SBL-1 strains are compared. This suggests that where host cell-dependent differences in virus transcription are observed (Afzal et al., 1990) they are due to variation in host factors or possibly to alterations in the virus polymerase.

Comparison of the Urabe strain sequence in Fig. 1 with the corresponding region of strain SBL-1 (Elango et al., 1989; Elliott et al., 1989) reveals 13.8% nucleotide differences overall (Table 1). The SH gene coding regions differ by 14.4% at the nucleotide level (Table 1) and 24.6% (14/57) at the amino acid level (Fig. 2). The SH gene appears to be far more variable between strains than other mumps virus genes, such as the F and RNA polymerase (P) genes which differ by 5% between strains (Elliott et al., 1989; Yamada et al., 1989; Forsey et al., 1990).

The SH coding region and flanking non-coding and intergenic regions all show significant (>12%) differences between strains SBL-1 and Urabe Am9, with the exception of the 5' non-coding SH region, which differs by only 2.0% (Table 1). These data support the idea that the 5' non-coding region of the SH gene is involved in the expression of the SH product. One possibility is that the 5' non-coding SH region contains a secondary structure which prevents translation from the AUG codon starting at nucleotide position 53 (Fig. 1). Such translation would produce a peptide of only six amino acids (Elliott et al., 1989; Fig. 1). However, the proposed secondary structure would allow translation from the authentic AUG starting at nucleotide position 59. A candidate palindrome allowing for G-U pairing in RNA is shown in Fig. 1. The single nucleotide difference in the 5' non-coding SH region of the SBL-1 strain compared to that of the Urabe Am9 strain changes a G-U base pair to A-U at the base of the potential stem. In contrast to the SH gene, the 5' non-coding region of HN does not have any additional AUG codons before the authentic start codon, and is widely divergent between the SBL-1 and Urabe strains (Table 1).

Work is in progress to identify the putative SH protein either in vivo or in vitro, which is clearly required to test hypotheses on the function of the SH region.

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### Table 1. Nucleotide differences between the SH regions of the Urabe Am9 and SBL-1 strains of mumps virus

<table>
<thead>
<tr>
<th>Urabe positions</th>
<th>Region</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 41</td>
<td>Part of F 3' non-coding</td>
<td>6/41 14.6</td>
</tr>
<tr>
<td>42 to 49</td>
<td>F-SH intergenic</td>
<td>1/8 12.5</td>
</tr>
<tr>
<td>50 to 98</td>
<td>SH 3' non-coding</td>
<td>1/49 2.0</td>
</tr>
<tr>
<td>99 to 272</td>
<td>SH coding</td>
<td>25/174 14.4</td>
</tr>
<tr>
<td>273 to 364</td>
<td>SH 3' non-coding</td>
<td>14/92 15.2</td>
</tr>
<tr>
<td>365 to 367</td>
<td>SH-HN intergenic</td>
<td>1/3 33.3</td>
</tr>
<tr>
<td>368 to 434</td>
<td>Part of HN 5' non-coding</td>
<td>12/67 17.9</td>
</tr>
<tr>
<td>1 to 434</td>
<td>All above</td>
<td>60/434 13.8</td>
</tr>
</tbody>
</table>

Fig. 2. Comparison of predicted SH proteins from SBL-1 and Urabe Am9 strains of mumps virus. A line between two amino acids indicates identity, two dots indicate a comparison value of >0.5 and one dot indicates a comparison value of >0.1 but <0.5.
References


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