Physical Mapping of Temperature-sensitive Mutations of Herpes Simplex Virus Type 1 Using Cloned Restriction Endonuclease Fragments

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(Accepted 30 June 1983)

SUMMARY

Sequences from the whole of the HSV-1 strain 17 genome were cloned into bacterial plasmid vectors, with the exception of part of BamHI v which was deleted in all cloned DNAs spanning this region of the virus DNA. The cloned DNAs were used in intratypic marker rescue experiments to map temperature-sensitive (ts) mutations on to the virus genome. Since the sequences of these DNAs overlapped, any mutation could be rapidly assigned a physical map position. This approach is particularly useful for mapping spontaneous mutations and lesions induced by mutagenesis of whole virus DNA. In this study, we mapped ten ts mutations comprising eight different complementation groups. Five lesions, representing three different cistrons, were located within BglII k (map units 0.098 to 0.166), and three mapped within EcoRIf (map units 0.321 to 0.414), two of which were in previously unidentified cistrons of HSV-1 strain 17. One mutation analysed had a defect within the short repeat region and another had a mutation within EcoRIf (map units 0.632 to 0.720).

INTRODUCTION

Temperature-sensitive (ts) mutants are extremely useful in identifying the functions of essential genes of herpes simplex virus (HSV) (Aron et al., 1975; Francke et al., 1978; Watson & Clements, 1978; Preston, 1979). The marker rescue technique has been successfully used to map HSV-1 and HSV-2 mutations on to the virus genome (Stow et al., 1978; Chu et al., 1979; Parris et al., 1980; Chartrand et al., 1981). The resolution of this method has been greatly improved by the use of cloned restriction endonuclease fragments from wild-type virus DNA (Post et al., 1980; Goldin et al., 1981; Preston, 1981) and has enabled mutations to be located within very small regions of the genome. With the development of DNA sequencing and mRNA selection techniques, it is now possible to determine the precise base pair change causing the defect and to identify the gene in which the mutation maps.

In this study, we have cloned overlapping restriction endonuclease fragments of HSV-1 strain 17 in order to map rapidly any ts mutation on to the virus genome. This approach is of particular value in mapping lesions generated spontaneously or induced by mutagenesis of the virus DNA. We have used sets of cloned fragments to determine the physical map positions of ten ts mutations, information which is essential for the assignment of these lesions to specific genes. Preliminary genetic characterization of the mutants indicates that at least two of the mutations lie in previously unidentified essential genes of HSV-1 strain 17.

METHODS

Cells. Baby hamster kidney cells (BHK-21 clone 13) were grown in Eagle’s minimal essential medium containing twice the standard concentration of vitamins and amino acids, 10% (v/v) tryptose phosphate broth and 10% (v/v) calf serum.

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Table 1. Properties of mutants

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Mutagen</th>
<th>DNA phenotype at the NPT</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts N</td>
<td>BUdR</td>
<td>+</td>
<td>(1)</td>
</tr>
<tr>
<td>ts O</td>
<td>BUdR</td>
<td>-</td>
<td>(2)</td>
</tr>
<tr>
<td>ts R</td>
<td>BUdR</td>
<td>-</td>
<td>(2)</td>
</tr>
<tr>
<td>ts S</td>
<td>BUdR</td>
<td>-</td>
<td>(1)</td>
</tr>
<tr>
<td>ts X</td>
<td>BUdR</td>
<td>-</td>
<td>(3)</td>
</tr>
<tr>
<td>17tsJC113</td>
<td>Ultraviolet light</td>
<td>-</td>
<td>(4)</td>
</tr>
<tr>
<td>17tsVP1203</td>
<td>None</td>
<td>+</td>
<td>(5)</td>
</tr>
<tr>
<td>17tsVP1204</td>
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<td>(5)</td>
</tr>
<tr>
<td>17tsVP1205</td>
<td>None</td>
<td>-</td>
<td>(5)</td>
</tr>
<tr>
<td>17tsVP1206</td>
<td>None</td>
<td>-</td>
<td>(5)</td>
</tr>
</tbody>
</table>


**Virus.** All the ts mutants used in physical mapping experiments were derived from HSV-1 strain 17 and formed non-syncytial plaques on BHK cells. The DNA phenotype at the non-permissive temperature (NPT) and information on the isolation of each mutant are given in Table 1. The ts mutants derived from non-mutagenized HSV-1 wild-type virus were isolated from experiments in which in vitro mutagenized cloned fragments of HSV-1 were recombined into the wild-type virus genome. Since the ts lesions in these mutants did not map in the mutagenized fragment, they were considered to have arisen spontaneously. Additional HSV-1 mutants, ts A (Brown et al., 1973), ts H (Crombie, 1975), 17tsVP1201 (Preston et al., 1983), ts E6, ts F18, ts G8, ts K13, M19 (Schaffer et al., 1973) and ts 656 (Hughes & Munyon, 1975), were used in complementation assays. The mutants derived from HSV-1 strain KOS (ts E6, ts F18, ts G8, ts K13, M19 and ts 656) were kindly provided by P. A. Schaffer. Virus stocks were prepared as described by Brown et al. (1973).

**Virus DNA.** Virus DNA was prepared as described by Wilkie (1973).

**Enzymes.** Restriction endonucleases and T4 DNA ligase were purchased either from Bethesda Research Laboratories or Boehringer Corp. Enzyme reactions were carried out as recommended by the suppliers. The enzyme HindIII was prepared by A. J. Davison.

**Construction of recombinant plasmids.** Unseparated EcoRI, HindIII, BglII and XhoI fragments of HSV-1 DNA were ligated to the EcoRI site of pACYC184 (Chang & Cohen, 1978), the HindIII site of pAT153 (Twigg & Sherratt, 1980), the BglII site of pKC7 (Rao & Rogers, 1979) or the XhoI site of pMK16 (Kahn et al., 1979) respectively. The ligation mixtures contained 20 μg DNA per ml, with a 10-fold molar excess of HSV-1 restriction endonuclease fragments over linearized vector DNA; 1 unit T4 DNA ligase per ml in 50 mM-Tris-HCl pH 7.6, 10 mM-MgCl₂, 10 mM-dithiothreitol, 0.5 mM-ATP. The reactions were incubated at 4 °C overnight and stopped by heating to 70 °C for 5 min.

**Escherichia coli** strain HB101 (Boyer & Roulland-Dussoix, 1969) or DH1 were transformed with the ligated DNA by the calcium shock method (Cohen et al., 1972). Bacterial colonies carrying recombinant plasmids were identified by their antibiotic sensitivity caused by insertional inactivation of a plasmid drug resistance gene, by colony hybridization (Grunstein & Hogness, 1975) and/or by rapid screening of DNA isolated from 'mini' cultures of bacteria (Birnboim & Doly, 1979). Plasmid stocks were prepared by the method of Birnboim & Doly (1979) with the exception that the samples were treated with RNase during dialysis against 10 mM-Tris-HCl pH 8.0, 1 mM-sodium EDTA. The DNA was subsequently extracted with phenol and chloroform before ethanol precipitation. The cloned DNAs were analysed for the presence or absence of known restriction endonuclease sites. Other cloned HSV DNA fragments used in the study either as hybridization probes or in marker rescue experiments were HSV-1 BamHI g, k, r and EcoRI h (supplied by F. J. Rixon); HSV-2 HindIII h and n (supplied by A. J. Davison), HSV-1 BamHI p (Wilkie et al., 1979) and HSV-1 BamHI a and c. Manipulations with bacteria harbouring recombinant plasmids were carried out in accordance with the safety guidelines recommended by G.M.A.G. (U.K.).

**Marker rescue assay.** Marker rescue was performed essentially as described by Stow et al. (1978) and Preston (1981). The KpnI fragments, used in marker rescue experiments, were derived from KpnI-digested cloned EcoRI f and purified by hydroxylapatite chromatography as described by Wilkie & Cortini (1976). All other plasmid DNAs were cleaved with the appropriate restriction endonuclease before being used in the marker rescue assay.

**Complementation test.** The complementation yield test was carried out as described by Brown et al. (1973) except that cell monolayers (1·5 × 10⁶ cells/dish) were used instead of cells in suspension.

**Alkaline nuclease assay.** BHK cell monolayers (approx. 3 × 10⁶ cells/dish) were infected with virus at an m.o.i. of 5. Virus-infected cells were harvested at 15 h post-infection and crude extracts were prepared as described by Francke et al. (1978). The enzyme assay mixture (100 μl) contained crude extract (equivalent to 10⁴ cells), 13-5 μg
Mapping of HSV mutations

Fig. 1. Physical map locations of HSV-1 ts mutations determined by marker rescue experiments. The genome positions of the restriction endonuclease fragments used in these studies are also given.

RESULTS

Cloning of HSV-1 restriction endonuclease fragments

The genomic position of each of the cloned HSV-1 restriction endonuclease fragments used initially to map mutations is shown in Fig. 1. With the exception of EcoRI f, each of the cloned HSV-1 DNA fragments contained the expected restriction endonuclease sites present in the corresponding fragment in virion DNA. The cloned EcoRI f contained a deletion of about 100 to 200 base pairs within the region common to BamHI v and KpnI v. This deletion was present in all the independently isolated HSV-1 strain 17 EcoRI f clones analysed so far and has also been found in cloned HSV-1 strain 17 KpnI v fragments (A. J. Davison, personal communication).

Physical mapping of ts mutations

The map locations of the lesions of ten ts mutants of HSV-1 were determined by marker rescue using cloned DNA fragments from wild-type HSV-1 DNA. The 16 fragments shown in Table 2 were used in the initial mapping experiments. In some cases cells were co-infected with mutant DNA and more than one DNA fragment in order to reduce the number of samples in an experiment (Table 2). One example of the use of pooled fragments to map mutations is shown in Table 3. The mutant 17tsJC113 was rescued by sets of fragments 1, 4, 5 and 7. The only sequences common to all four sets of fragments lie within the short and long repeats, within the sequences shared by XhoI c and EcoRI c (map units 0.81 to 0.87) (Fig. 1 and Table 2). This was confirmed in a subsequent experiment in which cells were co-infected with the mutant and EcoRI c or XhoI c alone (Table 4). Since 17tsJC113 fails to complement ts T and ts K (V. G. Preston, unpublished results), this mutant must have a defect within the short repeat sequence in the gene encoding Vmw 175. The map positions of most of the mutations were refined by marker rescue of mutants with smaller DNA fragments (Table 4). Three of the ten mutants analysed contained lesions within EcoRI f and five had defects within BglII k. The mapping data on ts S
Table 2. Sets of HSV-1 restriction endonuclease fragments used in marker rescue experiments

<table>
<thead>
<tr>
<th>Set</th>
<th>EcoRI $(j+k)$</th>
<th>XhoI $m$</th>
<th>EcoRI $d$</th>
<th>BgII $o$</th>
<th>EcoRI $g$</th>
<th>XhoI $f$</th>
<th>EcoRI $f$</th>
<th>BamHI $r$</th>
<th>EcoRI $m$</th>
<th>XhoI $h$</th>
<th>EcoRI $a$</th>
<th>HindIII $l$</th>
<th>EcoRI $i$</th>
<th>XhoI $b$</th>
<th>XhoI $p+i$</th>
<th>EcoRI $h$</th>
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<tbody>
<tr>
<td>1</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>6</td>
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<td>8</td>
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</table>
### Table 3. Rapid mapping of the 17tsJC113 mutation using sets of restriction endonuclease fragments in the marker rescue assay

<table>
<thead>
<tr>
<th>Set</th>
<th>Virus titre at PT (p.f.u. per 10⁶ cells)</th>
<th>Virus titre at NPT (p.f.u. per 10⁶ cells)</th>
<th>Percentage of ts⁺ recombinants amongst progeny virus</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>7.1 × 10⁷</td>
<td>3.0 × 10⁶</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>4.7 × 10⁷</td>
<td>&lt;10²</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>7.8 × 10⁷</td>
<td>&lt;10²</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>6.2 × 10⁷</td>
<td>2.0 × 10⁴</td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td>2.7 × 10⁶</td>
<td>4.6 × 10⁶</td>
<td>17.0</td>
</tr>
<tr>
<td>6</td>
<td>3.1 × 10⁷</td>
<td>&lt;10²</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>7</td>
<td>4.7 × 10⁷</td>
<td>2.5 × 10⁷</td>
<td>53.2</td>
</tr>
<tr>
<td>8</td>
<td>3.5 × 10⁵</td>
<td>&lt;10²</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1+2+3+4</td>
<td>3.0 × 10⁷</td>
<td>1.8 × 10⁶</td>
<td>6.0</td>
</tr>
<tr>
<td>None</td>
<td>6.6 × 10⁷</td>
<td>&lt;10²</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Marker rescue assay was performed as described in Methods. After 3 or 4 days incubation at 31 °C the progeny virus was titrated at 31 °C and 39 °C. The DNA fragments present in each set are shown in Table 2.

### Table 4. Summary of marker rescue results

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Cloned fragments which rescued the mutant</th>
<th>Physical map position of the fragment</th>
<th>Percentage ts⁺ recombinants amongst progeny virus</th>
<th>Map position of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts O</td>
<td>EcoRI d</td>
<td>0.080-0.192</td>
<td>17.0</td>
<td>0.098-0.103</td>
</tr>
<tr>
<td>ts O</td>
<td>BglII k</td>
<td>0.098-0.166</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>ts O</td>
<td>HindIII o</td>
<td>0.081-0.104</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>ts O</td>
<td>XhoI m</td>
<td>0.078-0.103</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>ts N</td>
<td>EcoRI d</td>
<td>0.080-0.192</td>
<td>0.4</td>
<td>0.098-0.145</td>
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<tr>
<td>ts N</td>
<td>BglII k</td>
<td>0.098-0.166</td>
<td>3.8</td>
<td></td>
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<tr>
<td>ts N</td>
<td>BamH1 a</td>
<td>0.078-0.145</td>
<td>0.2</td>
<td></td>
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<tr>
<td>ts R</td>
<td>EcoRI d</td>
<td>0.080-0.192</td>
<td>27.7</td>
<td>0.145-0.166</td>
</tr>
<tr>
<td>ts R</td>
<td>BglII k</td>
<td>0.098-0.166</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>ts R</td>
<td>XhoI g</td>
<td>0.126-0.172</td>
<td>25.0</td>
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<tr>
<td>ts R</td>
<td>BamH1 a</td>
<td>0.145-0.224</td>
<td>1.3</td>
<td></td>
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<tr>
<td>ts S</td>
<td>BglII k</td>
<td>0.098-0.166</td>
<td>5.2</td>
<td>0.145-0.166</td>
</tr>
<tr>
<td>ts S</td>
<td>XhoI g</td>
<td>0.126-0.172</td>
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<td>ts S</td>
<td>BamH1 a</td>
<td>0.145-0.224</td>
<td>2.5</td>
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<td>EcoRI d</td>
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<td>88.2</td>
<td>0.145-0.166</td>
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<td>ts X</td>
<td>BglII k</td>
<td>0.098-0.166</td>
<td>73.5</td>
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<td>ts X</td>
<td>XhoI g</td>
<td>0.126-0.172</td>
<td>21.8</td>
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<tr>
<td>ts X</td>
<td>BamH1 a</td>
<td>0.145-0.224</td>
<td>16.0</td>
<td></td>
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<tr>
<td>17tsVP1204</td>
<td>EcoRI f</td>
<td>0.312-0.414</td>
<td>1.0</td>
<td>0.322-0.344</td>
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<tr>
<td>17tsVP1204</td>
<td>KpnI i</td>
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<tr>
<td>17tsVP1203</td>
<td>EcoRI f</td>
<td>0.312-0.414</td>
<td>21.2</td>
<td>0.374-0.392</td>
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<tr>
<td>17tsVP1203</td>
<td>BamH1 g</td>
<td>0.342-0.392</td>
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<td>0.312-0.414</td>
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<td>0.408-0.414</td>
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<td>17tsVP1205</td>
<td>BamH1 r</td>
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<tr>
<td>17tsVP1206</td>
<td>XhoI b</td>
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<td>0.708-0.720</td>
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<tr>
<td>17tsVP1206</td>
<td>EcoRI i</td>
<td>0.632-0.720</td>
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<tr>
<td>17tsJC113</td>
<td>EcoRI c</td>
<td>0.000-0.082</td>
<td>6.9</td>
<td>0.808-0.886</td>
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<tr>
<td>17tsJC113</td>
<td>XhoI c</td>
<td>0.000-0.022</td>
<td>6.6</td>
<td>0.960-1.000</td>
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</table>

* The progeny virus from cells transfected with only mutant DNA contained <0.001% ts⁺ virus.
Table 5. Complementation between ts mutants with lesions in BglII k

<table>
<thead>
<tr>
<th>Mutant</th>
<th>ts N</th>
<th>ts O</th>
<th>ts R</th>
<th>ts S</th>
<th>ts X</th>
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<tbody>
<tr>
<td>ts N</td>
<td>1*</td>
<td>177</td>
<td>247</td>
<td>151</td>
<td>83</td>
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<td>ts O</td>
<td>1</td>
<td>523</td>
<td>87</td>
<td>696</td>
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<tr>
<td>ts R</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
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<td>1</td>
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</tbody>
</table>

* Values represent complementation indices calculated from the formula given in Brown et al. (1973) for the yield complementation test.

Table 6. Complementation of ts O, N and R with mutants from defined complementation groups

<table>
<thead>
<tr>
<th>Complementation group</th>
<th>Prototype mutant</th>
<th>ts N</th>
<th>ts O</th>
<th>ts R</th>
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<tr>
<td>1-5</td>
<td>ts E6</td>
<td>11*</td>
<td>50</td>
<td>223</td>
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<td>ts F18</td>
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<td>393</td>
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<td>123</td>
<td>394</td>
</tr>
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<td>1-10</td>
<td>ts K13</td>
<td>21</td>
<td>1</td>
<td>139</td>
</tr>
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<td>1-12</td>
<td>ts M19</td>
<td>164</td>
<td>1</td>
<td>612</td>
</tr>
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</table>

* Values represent complementation indices calculated from the formula given in Brown et al. (1973) for the yield complementation test.

confirm and refine the map location of this lesion given in Stow & Wilkie (1978). The two groups of mutant viruses were further characterized in order to determine the number of essential genes with ts defects in EcoRI f and BglII k regions.

Analysis of mutants with lesions in BglII k

Complementation studies

On the basis of the complementation yield test (Brown et al., 1973) the mutants ts O, R, S, X and N were placed into three different complementation groups (Table 5). Mutants ts R, S and X all gave low complementation indices in crosses with each other and were therefore considered to be in the same cistron. On the other hand, ts O and N complemented each other well and also complemented ts R, S and X. This suggests that ts O and ts N lie in different genes. Therefore, three cistrons, one defined by ts R, S and X, another by ts O and the third by ts N, were identified in BglII k.

The lesions in the HSV-1 strain KOS mutants ts E6, F18, G8, K13 and M19 have been located in the far left-hand end of the long unique region (Morse et al., 1977; Parris et al., 1980; S. K. Weller, W. R. Sacks, D. T. Aschman, D. Coen & P. A. Schaffer, personal communication. The mutants ts N, O and R were therefore complemented with these mutants to determine whether any of the strain 17 mutants represented a new complementation group. The results in Table 6 show that ts N is a member of the complementation group 1-6 since this mutant failed to complement ts F18. On the other hand, ts R clearly belongs to a different cistron from those represented by the HSV-1 KOS mutants used in this study. Although ts O gave negative complementation values with both ts K13 and ts M19, it is unlikely that this mutant contains two defects since these HSV strain KOS mutants are now thought to belong to a single complementation group (S. Weller, W. R. Sacks, D. T. Aschman, D. Coen & P. A. Schaffer, personal communication).

Induction of alkaline nuclease activity by the mutants

Following infection of susceptible cells with HSV, there is an increase in the activity of a number of enzymes, including an alkaline nuclease (Morrison & Keir, 1968). This enzyme is at least partially virus-coded since the activity induced by the HSV-2 mutant ts 13 is thermolabile both in vivo and in vitro (Francke et al., 1978). The nuclease lesion in ts 13 maps between coordinates 0.12 and 0.21 on the virus genome (Moss et al., 1979). Preston & Cordingley (1982)
Mapping of HSV mutations

Table 7. Complementation between ts mutants with lesions in EcoRI f

<table>
<thead>
<tr>
<th>Mutant</th>
<th>17tsVP1205</th>
<th>17tsVP1203</th>
<th>17tsVP1204</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts H</td>
<td>1*</td>
<td>487</td>
<td>69</td>
</tr>
<tr>
<td>ts 656</td>
<td>79</td>
<td>29</td>
<td>50</td>
</tr>
<tr>
<td>ts A</td>
<td>39</td>
<td>65</td>
<td>18</td>
</tr>
<tr>
<td>17tsVP1201</td>
<td>139</td>
<td>151</td>
<td>52</td>
</tr>
<tr>
<td>17tsVP1205</td>
<td>1</td>
<td>138</td>
<td>18</td>
</tr>
<tr>
<td>17tsVP1203</td>
<td>1</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>17tsVP1204</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Values represent complementation indices calculated from the formula given in Brown et al. (1973) for the yield complementation test.

subsequently showed that the gene encoding the HSV-1 alkaline nuclease lies within the region of the virus DNA common to EcoRI d and BamHI a (approx. map position 0-145 to 0-185). Since BglII k of HSV-1 maps within this part of the genome and the virus-induced enzyme activity appears to be essential for virus growth (H. Moss, personal communication), the HSV-1 mutants ts O, R, S, X and N were screened for their ability to induce alkaline nuclease activity at the permissive and non-permissive temperatures. Since all the mutants induced amounts of this enzyme activity similar to that induced by wild-type virus at both temperatures (data not shown), it is unlikely that the temperature-sensitivity of any of these mutants results from a defect in the alkaline nuclease gene.

Analysis of mutants with lesions in EcoRI f

Three of the ten mutants, 17tsVP1203, 17tsVP1204 and 17tsVP1205, each contained a defect within EcoRI f, a region known to contain several essential genes. These viruses were complemented with each other and with mutants which contained defects in EcoRI f within the DNA polymerase gene (ts H) (Stow et al., 1978; Stow & Wilkie, 1978; Chartrand et al., 1980), the major DNA-binding protein (ts 656) (Weller et al., 1983), the B glycoprotein (ts A) (Stow et al., 1978; Schaffer et al., 1978) or the nucleocapsid polypeptide p40 (17tsVP1201) (Preston et al., 1983). The results of the complementation yield test are given in Table 7. We conclude that the mutant 17tsVP1205 is in the same complementation group as ts H, whereas 17tsVP1203 and 17tsVP1204 lie in previously unidentified cistrons of HSV-1 strain 17.

DISCUSSION

We have mapped ten ts mutations on to the virus genome using overlapping cloned HSV-1 DNA fragments in marker rescue experiments (Fig. 1). Six of these lesions were generated by mutagenesis of the whole HSV-1 genome and four probably arose spontaneously. By using groups of pooled fragments spanning the whole genome, mutations can be rapidly assigned physical map positions. With the exception of part of BamHI v, which has been deleted in our cloned DNAs spanning that region of the virus genome, we have, to the best of our knowledge, cloned DNA representative of the whole genome. The rearrangement in BamHI v does not appear to be a random event in the cloning procedure since independently isolated EcoRI f clones always contained BamHI v fragments with a similar sized deletion to the initial clone, as assessed from the mobility of the DNAs on agarose gels. Spaete & Frenkel (1982) have found that this region of HSV-1 strain Patton is also deleted in cloned DNA fragments.

We have identified by complementation analysis three essential genes which map within HSV-1 BgII k. Two of these cistrons are required early in infection since the mutants with defects in these genes failed to synthesize DNA at the NPT. ts N, a mutant with a lesion in the third cistron within this region, has a DNA-positive phenotype at the restrictive temperature. None of these viruses appears to have a mutation in the alkaline nuclease gene. It is, nevertheless, possible that this enzyme may have another function separable from the nuclease activity. In this context, it should be noted that the gene encoding the structural protein VP11-12, which has been mapped to the left-hand end of the long unique region, has a similar molecular weight to the alkaline nuclease (Lemaster & Roizman, 1980). Another structural gene, encoding
Whether this mutant has a defect in either of these structural genes. Neither other mutants used in this study have thermolabile virus particles (data not shown). (Lemaster & Roizman, 1980). Further characterization of VP18.8, has also been assigned a map position in the region where the 17tsVP1203 mutation maps close to the major DNA-binding protein of approximate mol. wt. 136000. The mutant, however, has a DNA-positive phenotype at the NPT and is unlikely to have a defect in this gene since all the viruses with ts lesions in this protein, to date, fail to synthesize virus DNA under restrictive conditions (Powell et al., 1981; Weller et al., 1983). The defect in 17tsVP1204 maps within BamHI u, probably within a structural gene (C. Addison & V. G. Preston, personal communication).

From our study and others (Stow et al., 1978; Stow & Wilkie, 1978; Parris et al., 1978, 1980; Weller et al., 1983), it is clear that it is easier to obtain ts mutations affecting virus viability in some regions of the genome than in others. There are several possible explanations for this finding. First, genes, essential for virus growth in tissue culture, could be clustered in certain regions of the virus DNA. EcoRI f of HSV-1, for example, contains at least six such essential genes (Stow et al., 1978; Chartrand et al., 1980; Chu et al., 1979; Parris et al., 1980) and it is very easy to generate mutations in this fragment using a variety of mutagens. Indeed, two of the four spontaneous mutants examined contained lesions in this region. Secondly, the probability of obtaining a ts mutation within a gene is likely to be a function of gene size. Again EcoRI f contains several large genes, for example, the B glycoprotein, the major DNA-binding protein and the DNA polymerase. Thirdly, there may be some essential genes with only a few or no sites which can be altered to produce a ts phenotype. Fourthly, there may be large regions of the HSV genome which contain non-essential genes for growth of virus in tissue culture. In this context, Post & Roizman (1981) have reported that the immediate-early polypeptide ICP22 (Vmw IE 68) is not required for virus growth in Vero cells. Another example is the thymidine kinase gene. This virus enzyme is, however, essential for virus growth in ‘resting’ tissue culture cells (Jamieson et al., 1974) and by altering the selective conditions it has been possible to isolate ts mutations in the thymidine kinase gene. Further investigation is required to determine why is it difficult to isolate ts mutations affecting virus growth in certain regions of the genome, for example, the short unique region.

We are grateful to R. Thompson for providing us with bacterial and plasmid vector stocks required for cloning HSV-1 DNA fragments. B. Matz was the recipient of a research fellowship from the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg.

REFERENCES


(Received 23 February 1983)