Synthesis of Virus DNA and Polypeptides by Temperature-sensitive Mutants of Rabbitpox Virus

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SUMMARY

Eighteen temperature-sensitive (ts) mutants of rabbitpox virus were examined for defects in synthesis of DNA and protein. Two mutants (ts-3 and ts-16) were defective in DNA synthesis (DNA−), since both incorporated significantly less than wild-type amounts of labelled thymidine into acid-precipitable material when infected cells were incubated at the restrictive temperature. Both these mutants gave only the ‘early’ class of virus polypeptides when infected cell extracts were examined by SDS-polyacrylamide slab gel electrophoresis following incubation at 40 °C. Nine of the remaining sixteen DNA+ ts mutants (ts-1, ts-2, ts-6, ts-12, ts-15, ts-17, ts-31, ts-32 and ts-33) synthesized wild-type levels of most virus polypeptides at 40 °C; six DNA+ ts mutants (ts-7, ts-8, ts-9, ts-11, ts-23 and ts-24) were defective in the post-translational cleavage of the polypeptides involved in membrane stabilization and particle assembly; one DNA+ ts mutant (ts-14) synthesized only the ‘early’ class of virus polypeptides, implying that either replicated DNA was not fully functional or that a specific early function was required for late transcription.

INTRODUCTION

Poxviruses replicate by an intricately co-ordinated series of molecular events that suggests an intriguing variety of control processes in a eukaryotic context. Despite their large size, certain poxviruses are quite amenable to genetic and biochemical analysis and a substantial amount is becoming known about them. An outline of their genome strategy is taking shape (Moss, 1974). However, their content of genetic information is so large that most of it remains unknown.

Although vaccinia virus has been the most studied, the closely related rabbitpox virus presents a model system that has some advantages for genetic analysis. Exploitation of this system began about 20 years ago (Fenner, 1958), with the acquisition of a recombination linkage matrix for a group of non-ulcerated or white pock (u) mutants of the parental u+ rabbitpox strain RP+ (Gemmell & Fenner, 1960), followed by the examination of one particular class of u mutants that failed to grow in pig kidney cells (p mutants: McClain, 1965; McClain & Greenland, 1965; Sambrook et al. 1965, 1966; Fenner & Sambrook, 1966).

In addition, Sambrook et al. (1966), and Padgett & Tomkins (1968) isolated and characterized a set of 18 temperature-sensitive (ts) mutants from this parent strain. Preliminary studies showed high rates of genetic recombination and complementation between most of

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the mutants, although all synthesized DNA and produced the same range of soluble antigens at the restrictive temperature.

This paper reports the re-examination of the defects of a number of these ts mutants, together with five isolates generated from a more recent isolation programme that is still in progress.

METHODS

Cells and viruses. PK-15 cells (adult pig-kidney) were obtained from Commonwealth Serum Laboratories, Melbourne, Australia and were propagated as monolayer cultures in PK basic medium supplemented with 5% foetal calf serum and 1.4 mg/ml NaHCO$_3$ (sealed vessels) or 0.21 mg/ml NaHCO$_3$ (covered dishes in an air incubator). In the latter case the atmosphere was not gassed. PK basic medium comprised equal volumes of lactalbumin enzymic hydrolysate (5 mg/ml) in Hanks’ salt solution and medium 199, supplemented with 0.01 M-HEPES buffer, pH 7.4, 1 mg/ml bovine serum albumin and 0.18 mg/ml arginine. Confluent PK monolayers were maintained in PK basic medium supplemented with 0.5% foetal calf serum. All sera were heated at 48°C for 30 min before use.

Wild-type rabbitpox virus, strain RP-Utrecht (Fenner, 1958), designated RP+, and the set of ts mutants derived from it have been described previously (Sambrook et al. 1966).

The dermal strain of vaccinia virus, Lederle-7N (Fenner, 1958), was included for certain comparisons.

All the viruses were recovered from chorioallantoic membrane (CAM) stocks stored at -70°C since their original isolation. Stocks were initially amplified by passage on CAMS before two further passages in PK cells at 36.5°C. When 95% of cells in PK monolayers were showing c.p.e. the cells were resuspended and concentrated by centrifugation. Most of the progeny virus remained in the cell pellet, which was resuspended in a small volume of PK basic medium, subjected to sonic vibration with a Branson probe and used as stock virus. Mutants ts-1 to ts-17 were isolated by Sambrook et al. (1966) after mutagenesis with 5-bromodeoxyuridine. Mutants ts-23 and ts-24 were isolated after irradiation to approx. 10$^{-3}$ survival with u.v. light (D. Boyle, personal communication) and ts-31, ts-32 and ts-33 after mutagenization with 5-bromodeoxyuridine.

Infectivity assays. Plaque assays of RP+ virus and the ts mutants were made on confluent PK monolayers formed overnight at 40°C in 24-well plastic Linbro trays under PK basic medium containing 5% foetal calf serum and 0.21 mg/ml NaHCO$_3$. Each well (16 mm diam.) contained approx. 2 x 10$^6$ cells/ml. The medium was decanted and the monolayers infected with 0.1 ml of serial 10-fold virus dilutions in phosphate-buffered saline (PBS). The inoculum was allowed to adsorb for 1 h at 36.5°C before the cultures were overlaid with 1 ml of maintenance medium. The trays were incubated for 40 to 42 h in air incubators (permissive temperature of 36.5°C) or in plastic boxes immersed in water baths (restrictive temperature of 40°C). The medium was removed and the cells stained with 1% crystal violet solution. There was no evidence of secondary plaque formation under these conditions.

Virus purification. Radioactively labelled purified virus was prepared from PK monolayers which had been infected at 0.1 p.f.u./cell and incubated with $^{35}$S-methionine (2 μCi/ml) in Eagle’s medium containing 0.5% foetal calf serum, 0.7 mg/ml NaHCO$_3$ and one-sixth the normal concentration of methionine. The culture was harvested 30 h p.i. and the cell pellet sonicated. Virus was purified by the method of Joklik (1962) by sedimentation in 25 to 40% sucrose gradients followed by an additional centrifugation on 20 to 50% potassium tartrate gradients, both in 0.01 M-tris-HCl buffer, pH 9.0.

Isotopic labelling of virus DNA. Confluent PK monolayers grown in 24-well plastic Linbro plates were maintained in low serum (0.5% foetal calf serum) medium for 3 days
at 36.5 °C. The monolayers were infected at 10 p.f.u./cell, and the virus allowed to adsorb for 1 h at 36.5 or 40 °C depending on the labelling conditions. Unadsorbed virus was removed with repeated washing, the cultures overlaid with 1 ml of PK basic medium containing 0.5% foetal calf serum and incubated in water baths at the two temperatures. At intervals post-infection, the medium was removed and the cells pulse labelled with 0.1 ml of 3H-thymidine (10 μCi/ml) for 30 min. The cells were then washed with PBS and lysed with 1% sodium dodecyl sulphate (SDS). The solubilized cells were precipitated with 10% trichloroacetic acid (TCA), washed with 5% TCA and 90% ethanol, collected on glass fibre discs (Whatman GF/A) and counted for radioactivity.

**Labelling and electrophoresis of infected cell extracts.** Confluent PK monolayers established in 24-well plastic Linbro plates were infected at 10 p.f.u. cell. The virus was allowed to adsorb for 1 h at 36.5 or 40 °C depending on the labelling conditions. Unadsorbed virus was removed with repeated washing and the cultures overlaid with 1 ml of PK maintenance medium and incubated at the required temperature.

At designated times post-infection, the cells were radioactively labelled for 1 h with 0.1 ml 35S-methionine (120 μCi/ml) in methionine-free Eagle's medium. The medium was then removed and replaced with 1 ml of medium containing five times normal amounts of methionine. All cultures were harvested at 24 h p.i. The cells were gently scraped from the plastic, pelleted by low speed centrifugation, washed in ice-cold PBS and lysed in 0.1 ml of sample buffer [0.0625 M-tris-HCl, pH 6.8, 2% (W/V) SDS, 5% 2-mercaptoethanol, and 10% glycerol]. The samples were sonicated and boiled for 2 min before analysis on SDS-polyacrylamide slab gels.

SDS-discontinuous gradient slab gels (9 to 18% acrylamide) were prepared using the Laemmli buffer system (Laemmli, 1970). Modifications adopted were a 4% stacking gel and the inclusion of 10% glycerol in the 18% acrylamide solution to form a glycerol gradient that stabilized the resolving gel during pouring. The gels were then run overnight at 10 mA. Following electrophoresis, the gels were fixed for 30 min in 50% methanol and 7% acetic acid before being treated for fluorography by the method of Bonner & Laskey (1974). The treated gels were exposed to Kodak RP X-Omat film at -70 °C.

**Standard proteins.** Marker proteins for mol. wt. determination were rabbit skeletal muscle myosin (212000), phosphorylase A (94000), bovine serum albumin (69000), catalase (60000), fumase (49000), carboxypeptidase (34000), soybean trypsin inhibitor (22700), tobacco mosaic virus protein (17650), ribonuclease (13700) and cytochrome c (11500). These ten proteins were co-run with purified RP+ virus labelled with 35S-methionine. Their migration profile was detected by staining the gel with 0.2% Coomassie brilliant blue. Excess stain was removed and the gel dried and subjected to autoradiography to determine the pattern of virus polypeptides.

**Chemicals and radioisotopes.** All standard chemicals used were Analar grade. Methyl-3H-thymidine (48-1 Ci/nmol) and 35S-methionine (1040 Ci/nmol) were both purchased from the Radiochemical Centre, Amersham, Bucks.

**RESULTS**

**Polypeptides of purified rabbitpox virus particles**

Electrophoresis on SDS-polyacrylamide slab gels of purified rabbitpox virus strain Utrecht, and vaccinia virus strain Lederle-7N, both labelled with 35S-methionine, resolved some 40 virion structural polypeptides for each virus (Fig. 1). Individual polypeptides were identified by their mol. wt. so that a polypeptide of mol. wt. 43000 was designated p43. Minor differences were consistently detected between the two viruses, for example, polypeptides p95 and p50 absent from rabbitpox virions and p84 and p47 absent from...
Fig. 1. Thirteen % linear slab gel electropherogram (30 cm gel length) of 35S-methionine-labelled virion polypeptides of purified rabbitpox virus, strain Utrecht and vaccinia virus, strain Lederle-7N. The nomenclature (VP1 to VP12) follows that used by Sarov & Joklik (1972) as far as the two gel patterns could be correlated, although all the polypeptides were designated by their mol. wt. in the text. G and P denote those polypeptides labelled when infected cells were incubated with 3H-glucosamine and 32P-orthophosphate, respectively. The bars between the gels indicate the major polypeptide differences between the two viruses.
### Table 1. Synthesis of cytoplasmic DNA in PK cells infected with rabbitpox virus ts mutants

<table>
<thead>
<tr>
<th>Virus</th>
<th>37 °C</th>
<th>40 °C</th>
<th>DNA phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP+</td>
<td>4051 ± 166</td>
<td>3050 ± 97</td>
<td>+</td>
</tr>
<tr>
<td>ts-1</td>
<td>2629 ± 684</td>
<td>2512 ± 145</td>
<td>+</td>
</tr>
<tr>
<td>ts-2</td>
<td>3225 ± 1048</td>
<td>3059 ± 138</td>
<td>+</td>
</tr>
<tr>
<td>ts-3</td>
<td>1414 ± 13</td>
<td>622 ± 54</td>
<td>-</td>
</tr>
<tr>
<td>ts-6</td>
<td>792 ± 35</td>
<td>877 ± 69</td>
<td>+</td>
</tr>
<tr>
<td>ts-7</td>
<td>3772 ± 1890</td>
<td>3916 ± 141</td>
<td>+</td>
</tr>
<tr>
<td>ts-8</td>
<td>1007 ± 161</td>
<td>885 ± 82</td>
<td>+</td>
</tr>
<tr>
<td>ts-9</td>
<td>1092 ± 290</td>
<td>1181 ± 194</td>
<td>+</td>
</tr>
<tr>
<td>ts-11</td>
<td>659 ± 465</td>
<td>1039 ± 35</td>
<td>+</td>
</tr>
<tr>
<td>ts-12</td>
<td>855 ± 12</td>
<td>1329 ± 135</td>
<td>+</td>
</tr>
<tr>
<td>ts-15</td>
<td>1630 ± 449</td>
<td>1391 ± 349</td>
<td>+</td>
</tr>
<tr>
<td>ts-16</td>
<td>1672 ± 191</td>
<td>449 ± 37</td>
<td>-</td>
</tr>
<tr>
<td>ts-17</td>
<td>3756 ± 120</td>
<td>5701 ± 506</td>
<td>+</td>
</tr>
<tr>
<td>ts-23</td>
<td>4447 ± 187</td>
<td>3995 ± 300</td>
<td>+</td>
</tr>
<tr>
<td>ts-24</td>
<td>1892 ± 172</td>
<td>2555 ± 93</td>
<td>+</td>
</tr>
<tr>
<td>ts-31</td>
<td>4378 ± 983</td>
<td>4697 ± 604</td>
<td>+</td>
</tr>
<tr>
<td>ts-32</td>
<td>2759 ± 530</td>
<td>3037 ± 274</td>
<td>+</td>
</tr>
<tr>
<td>ts-33</td>
<td>1115 ± 30</td>
<td>1582 ± 319</td>
<td>+</td>
</tr>
<tr>
<td>Mock-infected</td>
<td>774 ± 199</td>
<td>285 ± 48</td>
<td></td>
</tr>
</tbody>
</table>

* Infected cells were labelled with 1 μCi/ml methyl-3H-thymidine for the period 2:25 to 4 h p.i. and the cytoplasmic fractions assayed for acid-insoluble radioactivity. Results given are the average of two cytoplasmic fractions ± the variation from the average.

Poxvirus proteins and DNA of ts mutants

Poxvirus proteins and DNA of ts mutants

vaccinia virions. Whether these represent qualitative differences in polypeptides or differences in migration rate of the same functional polypeptides could not be determined. Migration differences were also detected with the major polypeptides P43 and P37 of rabbitpox virus (p41 and P35, respectively in vaccinia virus). These results agree closely with the polypeptide patterns previously reported for vaccinia virus (Sarov & Joklik, 1972; Stern & Dales, 1976).

Further analysis of purified rabbitpox virions showed that P43 was the only polypeptide labelled if infected cells were incubated in the presence of 3H-glucosamine·HCl, and p11·5 was the major polypeptide labelled if infected cells were incubated in the presence of 32P-orthophosphate [the polypeptides p58, p43, p37, p35 and p20·5 were also phosphorylated to a lesser extent (unpublished observations)]. These results agree with the glycosylation and phosphorylation protein patterns for purified vaccinia virus (Sarov & Joklik, 1972).

### Synthesis of virus DNA

Padgett & Tomkins (1968) previously reported that the 18 ts mutants they examined (ts-1 to ts-18) all synthesized virus DNA at 39.5 °C when measured by autoradiography.

In the present study, virus DNA synthesis was assayed by the comparison of 3H-thymidine incorporation into serum-starved infected cells at 36·5 and 40 °C. The deprivation of serum severely restricted host cell DNA synthesis, so that any incorporation into virus infected cells was a reliable estimate of the cytoplasmic virus DNA synthesis. The kinetic data for the mutants resulted in the re-classification of ts-3 and ts-16 as DNA-defective mutants since 3H-thymidine was incorporated into acid-insoluble material to a much lower extent at 40 than at 36·5 °C (Fig. 2). The remaining ts mutants incorporated 3H-thymidine to a similar level at both temperatures, and in most infections, as with the wild-type virus, the incorporation was greater at 40 °C.
Fig. 2. Kinetics of \(^3\)H-thymidine incorporation into serum-starved PK cells infected with wild-type virus (RP\(^+\)), selected \(ts\) mutants or mock-infected (MI). Infected cells were incubated at 36.5 \(^\circ\)C (□) or 40 \(^\circ\)C (○) and were pulsed for 30 min with 10 \(\mu\)Ci/ml \(^3\)H-thymidine at the indicated times post-infection.

Virus DNA synthesis was also measured by comparing cytoplasmic \(^3\)H-thymidine incorporated at the two temperatures. These results provided the same classification of function (Table 1).
Polypeptides specified by rabbitpox virus ts mutants at permissive and restrictive temperature

Analysis by SDS-polyacrylamide gel electrophoresis of cells infected with vaccinia virus has shown that host protein synthesis is rapidly turned off (Esteban & Metz, 1973; Opperman & Koch, 1976), and an estimated 80 virus-induced polypeptides can be detected (Pennington, 1974).

In order to establish the polypeptide pattern produced in a normal wild-type infection with rabbitpox virus, the ‘early’ polypeptides (pre-DNA replication) were examined in the presence of 10⁻² M-hydroxyurea (HU) to prevent virus DNA replication (Pogo & Dales, 197x). When infected PK cells were labelled with ³⁵S-methionine in 1 h pulses at times post-infection (Fig. 3), at least 11 polypeptides were detected prior to DNA synthesis (denoted by the bars to the left of the HU 6 h channel) and a further 13 polypeptides were synthesized following DNA synthesis (denoted by the bars to the left of the 9 h channel). Further classification of the polypeptides was enhanced by labelling infected cultures in the presence of 10 µg/ml actinomycin D (AMD). This treatment should only allow the synthesis of the particular sub-set of early proteins which are made before the inoculum virus is completely uncoated (Munyon & Kit, 1966). Only one virus polypeptide (migrating with a mol. wt. of 13K) was evident under these conditions (denoted by the bar to the left of the AMD 4 h channel), although the expression of host cell polypeptides was altered when mock-infected cells were similarly treated. This paradox makes the identification of early virus-specified polypeptides or de-repressed host polypeptides more difficult and may be answered by improving the gel resolution with a two-dimensional system, and analysing the ‘in vitro’ translation products of the early class RNA from poxvirus-infected cells (Cooper & Moss, 1979).

The polypeptide profile for each ts mutant was then analysed following a series of pulse-chase labelling experiments at 36.5°C and 40°C (Fig. 4 to 9). Each gel is a composite of three ts mutant infections and is analysed in an identical manner. The (a) channel shows each mutant infection pulsed and chased under permissive conditions, so that all virus functions were expressed. When these control infections were compared to the wild-type infection (Fig. 3), no mutant was defective at 36.5°C. The (c) channel shows each mutant infection pulsed and chased under the restrictive conditions and is compared directly to the corresponding (a) channel allowing the detection of any aberrant polypeptide synthesis at the higher temperature denoted by the bars to the left of each (c) channel. The (b) channel shows a temperature shift-down experiment, wherein each infection is labelled at the restrictive temperature, but chased at the permissive temperature, so that polypeptides modified by post-translational cleavage may be detected. The 18 ts mutants were accordingly grouped into three major categories.

Early mutants

This group comprises ts-3, ts-14 and ts-16. Each of these mutants gave a pattern of polypeptide synthesis that resembled the early pre-DNA pattern shown by wild-type infections treated with hydroxyurea. However, whereas infections with ts-3 and ts-16 failed to incorporate ³H-thymidine at 40°C, the infection with ts-14 demonstrated wild-type levels of incorporation (Fig. 2), suggesting that the virus DNA synthesized was not fully functional or that an early function which is required for late transcription was defective.

To confirm the previously reported data of Padgett & Tomkins (1968), that each of the lesions of ts-3, ts-14 and ts-16 was in a separate complementing gene function, mixed infection experiments were done. All three mutants readily complemented each other as measured by infectivity, demonstrating a 20-fold increase in the mixed infections com-
Fig. 3. Autoradiogram of electrophoresed polypeptides (SDS discontinuous 9 to 18% acrylamide gradient gel) from mock-infected PK cells (MI) and from PK cells infected with RP + virus. The control and infected cells were labelled with 35S-methionine for 1 h at the times (h) indicated post-infection. Hydroxyurea (HU, 10^{-2} M) or actinomycin D (AMD, 10 \mu g/ml) were added to designated cultures at the time of infection to differentiate the pattern of polypeptide synthesis as indicated in the text. The mol. wt. are given at the side of the gels.
Fig. 4. Autoradiograms of electrophoretically separated polypeptides from PK cells infected with mutants \(ts-1\), \(ts-2\) and \(ts-3\) at 36.5 and 40 °C. The cells were labelled for 1 h with \(^{35}\)S-methionine at 12 h p.i. and chased for a further 11 h. Each mutant infection was subjected to three labelling conditions: (a) pulsed at 37 °C and chased at 37 °C; (b) pulsed at 40 °C and chased at 37 °C; (c) pulsed at 40 °C and chased at 40 °C.
Fig. 5. Autoradiograms of electrophoretically separated polypeptides from PK cells infected with mutants ts-6, ts-7 and ts-8 at 36.5 and 40 °C. For further details see legend to Fig. 4.
Fig. 6. Autoradiograms of electrophoretically separated polypeptides from PK cells infected with mutants ts-9, ts-11 and ts-12 at 36.5 and 40 °C. For further details see legend to Fig. 4.
Fig. 7. Autoradiograms of electrophoretically separated polypeptides from PK cells infected with mutants ts-14, ts-15 and ts-16 at 36·5 and 40 °C. For further details see legend to Fig. 4.
Fig. 8. Autoradiograms of electrophoretically separated polypeptides from PK cells infected with mutants *ts*-17, *ts*-23 and *ts*-24 at 36.5 and 40 °C. For further details see legend to Fig. 4.
Fig. 9. Autoradiograms of electrophoretically separated polypeptides from PK cells infected with mutants *ts*-31, *ts*-32 and *ts*-33 at 36.5 and 40 °C. For further details see legend to Fig. 4.
Table 2. **Complementation of DNA synthesis between ts-3 and ts-16**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Ct/min/cytoplasm</th>
</tr>
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<tbody>
<tr>
<td>ts-3</td>
<td>3136±1331</td>
</tr>
<tr>
<td>ts-16</td>
<td>2670±210</td>
</tr>
<tr>
<td>ts-3×ts-16</td>
<td>5495±1120</td>
</tr>
</tbody>
</table>

* Single and mixed infections were labelled with 1 µCi/ml ³H-thymidine for the period of 2-5 to 5 h p.i. at 40 °C and the cytoplasmic fractions assayed for acid-insoluble radioactivity. Results given are the average of three fractions.

pared to each single infection (J. R. Lake, unpublished observations), and ts-3 and ts-16 also complemented each other when measured by ³H-thymidine incorporated under restrictive conditions (Table 2).

**Cleavage-defective mutants**

The mutant infections ts-7, ts-8, ts-9, ts-11, ts-23 and ts-24 all showed some defect in post-translational cleavage. These defects were alleviated if the infections were shifted down and the label chased under permissive conditions. The polypeptides mainly affected were p94 and p65 and their respective cleaved products the major virion polypeptides p62 and p60 (Katz & Moss, 1970) and p32, p25 and p18, the precursors of which are unknown. All six mutants showed an accumulation of p94 and p65 (except ts-23 which had little p65) with a subsequent reduction in the processing to their products p62 and p60 (except ts-8 which was cleaved as normal and ts-9 which had normal amounts of p60). In addition, p32 was reduced in infections ts-7, ts-9, ts-11 and ts-23; p25 was reduced in infections ts-7 and ts-9, and p18 was reduced in infections ts-7, ts-9 and ts-23.

Similar findings were reported by Lake et al. (1979) for vaccinia virus, where five assembly-defective mutants were studied. The authors concluded that the mutations were in separate complementing gene functions which acted in a co-ordinated fashion so as to allow complete virus morphogenesis. Similar complementation and recombination studies are currently in progress for the six rabbitpox virus cleavage defective mutants.

**Late mutants**

This group included ts-1, ts-2, ts-6, ts-12, ts-15, ts-17, ts-31, ts-32 and ts-33. Although each mutant had a distinct pattern of polypeptide synthesis, none was defective in DNA synthesis or post-translational cleavage. No differences in polypeptide pattern were seen for ts-17 at 40 °C, while infections with ts-2, ts-6 and ts-15 only showed reduced synthesis of p40. Greater degrees of defectiveness, that is, more polypeptides with reduced synthesis at restrictive temperature, were shown in infections with ts-32 (p40, p18); ts-33 (p75, p44); ts-12 (p44, p25, p20); ts-31 (p75, p30, p14) and ts-1 (p62, p60, p42, p25, p20, p18, p14). Some of these blocks in polypeptide synthesis were released if the infected cultures were chased under permissive conditions.

**Discussion**

We have described a preliminary characterization of 18 ts mutants of rabbitpox virus RP⁺, a parental poxvirus strain chosen for the information already available on its genetic character. A comparison of the virion and cytoplasmic proteins made by rabbit-pox virus with the more extensively studied vaccinia virus showed that the polypeptide patterns and temporal sequences were closely similar for the two viruses, as may be expected from the close antigenic relationship.
The more quantitative analysis of virus DNA synthesis by measurement of the cytoplasmic thymidine incorporation, allowed two of the ts mutants (ts-3 and ts-16) to be re-classified as defective in DNA synthesis at the restrictive temperature (DNA-). They were therefore defective in a function expressed before or during DNA synthesis on which continued DNA synthesis depended. The remaining 16 mutants were classified as DNA+, since their lesion occurred in a function expressed before or after DNA synthesis, but which had no effect on DNA replication. Over 80% of the mutants studied were classified as DNA+. This distribution of DNA+ and DNA- function in a poxvirus ts mutant isolation programme was comparable to that reported by Chernos et al. (1971) where 8 out of 15 mutants were DNA+ and more recently by Dales et al. (1978) where 72 from 78 mutants were DNA+.

The 16 DNA+ ts mutants of our study were grouped into three phenotypic categories according to the polypeptides made at the restrictive temperature. The high levels of 3H-thymidine incorporated in infections with ts-14 at 40 °C classified this mutant as DNA+. However, the absence of a 'late' pattern of polypeptide synthesis contradicted this assumption, but since many virus enzymes are expected to be involved in the discontinuous synthesis and transcription of virus DNA (Holowczak & Diamond, 1976), a defect in any one might prevent the correct functioning of newly-made deoxyribonucleotide sequences.

Six mutants (ts-7, ts-8, ts-9, ts-11, ts-23 and ts-24) were classified as possessing some defect in post-translational cleavage. Their polypeptide patterns resembled those reported for a number of ts mutants isolated from two strains of vaccinia virus (Drillien et al. 1977; Stern et al. 1977; Dales et al. 1978; Lake et al. 1979). These vaccinia mutants had several defects associated with assembly (accumulation of DNA paracrystals, few immature particles and no association of the external spicule layer with sheets of unit membrane), and resembled the effects of the antiviral inhibitor, rifampicin. Preliminary genetic experiments with vaccinia virus established co-variance between these two markers, since the introduction of rifampicin resistance into the genome of two such ts mutants modified the temperature sensitivity of the resultant double mutant (Lake et al. 1979). Such faults in the assembly of virus-specific lipoprotein envelopes were associated with the absence of polypeptides p62, p60, p25 and p18, which are known to be processed by post-translational cleavage of higher mol. wt. precursors. This relatively common ts mutation in poxviruses emphasizes the highly co-ordinated biogenesis, and the different complementation groups exhibiting this phenotype suggest that the mutations are in polypeptides that alter the conformation of immature particles which are then unavailable for further maturation by cleavage.

The remaining nine mutants (ts-1, ts-2, ts-6, ts-12, ts-15, ts-17, ts-31, ts-32 and ts-33) were classified as late mutants. Qualitative and quantitative differences were detected for each mutant, but the importance of these defects could not be determined. Investigation of further parameters such as the structural features within infected cells examined by electron microscopy may complement the reported data and provide a greater insight into the complex biogenesis of poxviruses.

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Poxvirus proteins and DNA of ts mutants


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