A Partial Genetic Map of the Baculovirus,
Autographa californica Nuclear Polyhedrosis Virus, based on
Recombination Studies with ts Mutants

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SUMMARY

Recombination experiments involving a series of two-factor crosses have been
done with ts mutants of the baculovirus, Autographa californica nuclear poly-
hedrosis virus (NVP). Six mutants which fail to produce non-occluded virus at
33 °C (NOV mutants) have been ordered on a linear genetic map.

In a previous publication we described the isolation of 27 temperature-sensitive (ts)
mutants of Autographa californica nuclear polyhedrosis virus (NVP) and the assortment of
to of these into five complementation groups (Brown et al. 1979). We now report recombina-
tion experiments, involving a series of two-factor crosses, done with six ts mutants
which fail to produce both non-occluded virus (NOV) and polyhedra at 33 °C (NOV
mutants) and with tsB4, a mutant which produces NOV but is restricted in formation of
polyhedra at 33 °C (a Poly mutant).

The wild-type (wt) virus used was an MP strain of A. californica MNPV plaque-purified
at 33 °C (Brown et al. 1979). Ts mutants were isolated following treatment of wt virus with
N-methyl-N'-nitro-N-nitrosoguanidine (3 μg/ml) and were grown to passage 3 as working
stocks using an input m.o.i. of 0.01 (Brown et al. 1979). The mutants used in these experi-
ments were: MP ts (NOV) A9g, MP ts (NOV) A9g, MP ts (NOV) A12g, MP ts (Poly) B4g,
MP ts (NOV) C7g, MP ts (NOV) D8g, MP ts (NOV) E10g, representing each of the five
complementation groups. Two other mutants, MP m (Poly)-5g and MP m (Poly)-6g were iso-
lated from separate mutagenized stocks. These morphology mutants, which produced single
large crystals in infected cells at 25 °C, were named using the system proposed by Brown
et al. (1979) with ‘m’ representing the morphology phenotype of the mutation.

Spodoptera frugiperda cells, IPLB-SF-21 (Vaughn et al. 1977), were used between passages
335 and 372 and were cultured at 25 and 33 °C in BML-TC/10 medium (Gardiner &
Stockdale, 1975) containing gentamicin (50 μg/ml) and 10% heat-inactivated foetal calf
serum. Cells used in experiments at 33 °C had been grown at 33 °C for 33 to 67 passages
prior to the experiment.

For recombination experiments, 2 × 10^6 viable S. frugiperda cells were seeded in plastic
tissue culture dishes (35 mm diam.) and infected at a total input m.o.i. of 12 with single
mutants or with a mixture containing two mutants in equal proportions. Cultures were
incubated at 25 °C for 36 h. Culture fluid was harvested, clarified by centrifugation at
850 g for 30 min and stored at 4 °C for titration by plaque assay.

Each sample was titrated at 25 and 33 °C. The plaque assay procedure using a solid
overlay containing 1.5% agarose (Miles Laboratories, Elkhart, Ind., U.S.A.) has been
described (Brown & Faulkner, 1978). Plates incubated at 33 °C were seeded with 7 × 10^6
cells; those incubated at 25 °C were seeded with 1 × 10^6 cells grown at 33 °C. Recombina-
tion frequency (RF) was calculated as follows:

\[ RF = \frac{\text{titre of } (a \times b) \text{ at } 33 ^\circ C}{\text{titre of wt at } 25 ^\circ C} \times \frac{\text{titre of wt at } 25 ^\circ C \times 2}{\text{titre of wt at } 33 ^\circ C} \times 100 \% \]
Fig. 1. Progeny from crosses of ts A1 × ts D8 and ts A1 × ts Eto were plated at 33 °C and four well-isolated plaques were picked. The titres of plaque isolates were amplified by growth in S. frugiperda cells at 33 °C and NOV was harvested 5 days p.i. Each preparation was titrated by plaque assay at 33 °C and plaques were counted after staining monolayers with INT 4 days p.i. □—□, ts A1 × ts Eto, recombinant 1; ○—○, ts A1 × ts Eto, recombinant 2; ■—■, ts A1 × ts D8, recombinant 1; ●—●, ts A1 × ts D8, recombinant 2.

Fig. 2. Genetic map of A. californica NPV. Each ts mutation site is marked by the corresponding mutant number above the heavy line. The segment containing the m-5 and m-6 mutations is shown. RF between each pair of mutants (± standard error) is shown on the line connecting the two mutation sites. Crosses were done one to three times. RF for crosses ts A1 × ts A12, ts A12 × ts A9 and ts A1 × ts A9 were 0.4%, 0.4% and 1.2±0.6% respectively.
where $a$ and $b$ are different mutants. The ratio of wt titre at 25 °C to wt titre at 33 °C was included in the calculation of RF as a correction factor for the difference in plaquing efficiency of wt at 25 and 33 °C. Revertants were measured in initial experiments but were found to be negligible with respect to calculation of RF.

To demonstrate that recombinant plaques were in fact initiated by recombinants and were not due to co-infection of a cell with two complementing mutants, four well-isolated plaques were picked from plates incubated at 33 °C for 5 days. Culture fluids were harvested, clarified by centrifugation and titrated at 33 °C. Each of the four isolates gave a linear dose response (Fig. 1) indicating that each plaque was initiated with a single virion (Davis et al. 1973) and therefore was not due to complementation.

Six NOV mutants were used for recombination experiments and from the RFs calculated for all possible crosses, a linear genetic map was drawn (Fig. 2). It is not surprising that the ts $At$, ts $A9$ and ts $At2$ mutations map together since these mutants all belong to complementation group A. The order of these mutations within the cluster is not established. Three-factor crosses would be useful for this purpose.

It was not established whether the mutations mapped in Fig. 2 were scattered throughout the full length of the NPV genome or whether they represented only part of the genome, e.g. that section involved in synthesis of NOV. Recombination experiments were done to extend the map using mutants $m-5$ and $m-6$ which yielded NOV at 33 °C but induced formation of large cuboidal crystals rather than typical polyhedra in infected cells at 25 and 33 °C. Progeny from crosses of $m-6$ and the NOV mutants were titrated at 25 and 33 °C and the results indicated that the $m-6$ mutation mapped outside the region represented by the NOV mutations. Since $m-6$ alone formed plaques at 33 °C, it was necessary to examine the plaques microscopically 4 days p.i. to distinguish $m-6$ plaques from recombinant plaques and to determine the proportion of plaques at 33 °C which were initiated by recombinants. NOV mutants alone did not form plaques at 33 °C. Plates were stained with INT [0.2T (w/v) 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride in phosphate-buffered saline: 140 mm-NaCl, 24 mm-KCl, 8 mm-Na$_2$HPO$_4$, 1.5 mm-KH$_2$PO$_4$ (pH 7.3)] for 8 to 12 h at 25 °C and the total number of plaques was counted. The titre of recombinants between $m-6$ and NOV mutants was calculated as the total number of plaques multiplied by the proportion of recombinant plaques as determined by microscopic examination of plaques at 33 °C. The recombinant titre was used in the equation given earlier for calculation of RF. Values for RF ranged from 4.7% in crosses with ts $C7$ to 38.8% in crosses with ts $D8$. It was not possible to map the $m-6$ mutation at a specific site on the genome but the lesion could be mapped within a segment of the genome extending beyond the region containing the NOV mutations (Fig. 2). The two mutations, $m-5$ and $m-6$ are shown together since examination of 272 progeny plaques from an $m-5$ and $m-6$ cross failed to locate one recombinant plaque, i.e. RF < 0.4%. Since these two mutants were isolated from separate stocks, it is unlikely that the mutation sites are identical but they appear to be very close.

A Poly mutant, ts $B4$, was also used in recombination experiments and results indicated that the ts $B4$ mutation is well separated from the others. Since ts $B4$ alone initiated plaque formation at 33 °C, but did not form polyhedra in infected cells, plaques were examined microscopically to determine relative proportions of wt and ts $B4$ mutant plaques in order to calculate the recombinant titre. When progeny from crosses of ts $B4$ with all six NOV mutants were analysed, RFs ranged from 26 to 48%, indicating that the ts $B4$ mutation is far removed from the NOV mutations. This is evidence that the map in Fig. 2 represents only part of the genome. The genome contains closed circular DNA (Schvedchikova et al. 1968; Summers & Anderson, 1972, 1973; Brown et al. 1977) and recently a circular physical
map has been presented (Miller & Dawes, 1979). Confirmation of a circular genetic map will depend on mapping several mutations on either side of the \(ts\) \(AI\)–\(ts\) \(D8\) segment with RF values low enough to be additive (less than 35\%, Foss & Stahl, 1963). In order to map other Poly mutants, an improved method of distinguishing recombinant and mutant plaques at 33 °C, other than microscopic examination of individual plaques, is necessary. Of particular interest will be the mutation sites of \(ts\) \(A2\) and \(ts\) \(A11\) which are included in complementation group \(A\) but are Poly mutants unlike the other mutants in group \(A\) which are NOV mutants.

Microscopic examination of plaques at 33 °C resulting from progeny of crosses involving \(ts\) \(B4\) and \(m-6\) revealed that mixed plaques were sometimes present. These mixed plaques, which accounted for 1 to 10\% of the total, displayed both wt phenotype and that of the parental Poly mutant. A similar observation was reported in recombination studies with herpes simplex virus type 1 (Brown & Ritchie, 1975). Such mixed plaques may be initiated by recombinants with heteroduplex DNA at the \(ts\) mutation site, that is, both wt and mutant alleles are present (Watson, 1976; Radding, 1978). These segregate in replication so that within one plaque, some cells express the wt and others the mutant phenotype. The progeny of such plaques, when re-plated, should yield two plaque types. Selection of isolated NPV plaques with mixed morphology was difficult since they were in a low proportion. One mixed plaque from a \(ts\) \(B4\) × \(ts\) \(D8\) cross was picked and re-plated. Four out of seven plaques displayed wt morphology, indicative of recombinant plaques; the morphology of three plaques was characteristic of the \(ts\) \(B4\) mutant. This is consistent with heteroduplex DNA at the \(ts\) mutation site in the parental virion.

Thus, recombination has been demonstrated between genomes of baculovirus \(ts\) mutants and a primitive genetic map of \(A.\) \(californica\) NPV has been established. Although it is expected that recombination should occur between double-stranded virus DNA genomes, this has not been previously reported for baculoviruses. Much work is still to be done to extend this map and to correlate it with a physical map of the genome using techniques such as marker rescue and heteroduplex mapping.

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