Proteus mirabilis Phage 5006M: a Physical Characterization

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SUMMARY

This report deals with physical characterization of the generalized transducing Proteus mirabilis phage 5006M. The morphology of the phage is presented, the buoyant density was determined (1.491 g/ml) and the G + C content of the phage DNA was found to be 44%. The phage genome has a length of 14.8 μm and mol. wt. of 3.07 x 10^6. Denaturation mapping revealed non-random circular permutation of the phage DNA. The genome exhibits 3.6% terminal redundancy as shown by homoduplex analysis. The existence of concatemeric precursors of phage 5006M DNA is inferred and the results are interpreted in terms of a sequential headful packaging mechanism.

INTRODUCTION

Proteus mirabilis phage 5006M is a generalized transducing phage. It has given rise to variants which are capable of high frequency transduction of the kanamycin and ampicillin resistance markers of the R394 plasmid (Coetzee, 1974, 1975), the streptomycin and sulphonamide markers of R904 (Coetzee, 1976), the ampicillin marker of RP4 (Coetzee, 1977) and the leucine marker of the host strain, P. mirabilis PM5006 (Krizsanovich-Williams, 1975). The host strain is cryptically lysogenic for phage 5006M (Krizsanovich, 1973) and the cryptic prophage, which is non-inducible, is occasionally liberated. Upon re-lysogenization of strain PM5006, prophage 5006M is inducible.

Phage 5006M resembles Proteus phages 13M and 34 in host range, neutralization by antiserum, receptor site and electron microscopic appearance. These phages are responsible for lysogenic conversion which results in inability to re-adsorb phage. P. mirabilis strains 13, 5006, 34 and 12 lysogenized with any of these are resistant to all three phages (Krizsanovich, 1973).

Proteus phages have not been well characterized physically. Phage 5006M was chosen for such characterization. We report here on our findings.

METHODS

Bacteria and phages. Proteus mirabilis PM5006, Escherichia coli W3110 (ColE1) and phage 5006M were from the laboratory stocks of this Unit and E. coli C1 and phage φX174, from Dr W. D. Taylor, The Pennsylvania State University.

Media. Media, phage cultivation and general techniques were as described by Krizsanovich (1973) and Adams (1959).

Purification of phage 5006M. A crude lysate containing 10^9 particles/ml was centrifuged at 6000 g for 10 min. The supernatant was subsequently centrifuged at 35000 g and room temperature for 2 h in a Sorval SS-34 centrifuge, using an SS-34 rotor. The pellets were resuspended overnight in phosphate buffer (50 mM-Na_2HPO_4-20 mM-KH_2PO_4, pH 7.2, 70 mM-
NaCl) at 4 °C. Six ml of a CsCl solution, density 1.55 g/ml, was introduced underneath the pooled material in a centrifuge tube and centrifugation was for 3 h at 42000 g and 15 °C, using a Spinco SW27 rotor. The well-defined band which formed in the CsCl was extracted and dialysed against phosphate buffer.

Buoyant density. Phages 5006M and φX174 at 10^7 particles/ml were centrifuged to equilibrium in a CsCl gradient, initial density 1.44 g/ml. Centrifugation was for 48 h at 90000 g in a Spinco SW65 rotor at 4 °C. The gradient was fractionated, refractive indices of all fractions were measured and the corresponding densities were calculated, using ρ = 10.8601γ - 13.4974, where ρ = density and γ = refractive index (Bruner & Vinograd, 1965). All fractions were diluted and spot-titrations were followed by duplicate plating of appropriate fractions.

Phage electron microscopy. This was performed as described by Coetzee et al. (1979) using negative staining with 3 % phosphotungstic acid.

Nucleic acid isolation. E. coli DNA was extracted from strain CI by means of the method of Mandell & Hershey (1960). Col E1 DNA was extracted from E. coli W3110 (Col E1) as described by Clewell & Helinsky (1969). Phage 5006M DNA was phenol-extracted from purified phage preparations.

DNA melting. The method of Mandel & Marmur (1968) was used, employing a Beckman DK-2 spectrophotometer.

Mol. wt. of phage 5006M genome. This was determined electron microscopically relative to the mol. wt. of the Col E1 plasmid.

DNA microscopy. The methods of Coetzee & Pretorius (1979) involving benzylalkyl-dimethylammonium chloride (BAC), were used.

Data processing. Micrographs of molecules were traced by means of an Electronic Graphics Calculator (Numonics Corporation, Lansdale, Pennsylvania) interfaced with a Hewlett Packard 9825A calculator.

Computer simulations of partially denatured molecules were aligned and plotted, using a two-stage alignment procedure (M. C. Van Dijken & W. F. Coetzee, unpublished data) which consists essentially of the following: each molecule is treated as a circle. In the first stage a reference map is constructed by addition of individual molecules in a pre-determined sequence, utilizing a correlation function and optimizing for denaturation ‘bubble’ overlap. The sum serves as a reference map for the next molecule to be fitted, finally resulting in a reference map representing all the molecules. In the second stage, individual molecules are sequentially subtracted from the reference map, re-aligned to the resultant map and then added, hence producing a refined map. This is repeated and successively obtained refined maps are compared. An iteration procedure is thus followed which terminates after identical maps have been generated. The last map obtained is considered final as are the corresponding displacements of individual molecules.

RESULTS

Morphology of phage 5006M

Negative staining electron microscopy showed that phage 5006M has a hexagonal form, suggesting icosahedral symmetry. This is in agreement with previous observations (Krizsanovich, 1973; Prozesky et al. 1965). The lateral and apex diam. are 44 and 48 nm, respectively. The phage has a short (16 nm) tail with diam. of 16 nm.
Circularly permuted DNA

Assuming a buoyant density of $1.402 \pm 0.001$ g/ml for φX174 (Hurst & Incardona, 1969) it was established by means of co-sedimentation that the buoyant density of phage 5006M is $1.491$ g/ml.

Nucleic acid melting

The melting profiles of E. coli DNA and phage 5006M DNA were determined in $0.1 \times$ SSC ($1 \times$ SSC is $0.15 M$-NaCl, $0.015 M$-sodium citrate, pH 7.0). Assuming $(G + C)\% = 50$ for E. coli DNA (Marmur & Doty, 1962) and using the relationship $(G + C)\% = 2.44 (T_m -$
53.9) (Mandel & Marmur, 1968) it was found that $T_m = 71.9^\circ C$ and $(G+C)_\% = 44$ for phage 5006M DNA.

**Mol. wt. of phage genome**

Phage 5006M DNA was mixed with Col E1 DNA and mounted for electron microscopic investigation. The lengths of 5006M molecules were measured relative to that of Col E1 circles on the same micrographs and a distribution of length ratios was obtained (Fig. 1). Assuming the mol. wt. and length of the Col E1 plasmid to be $4.8 \times 10^6$ and 2.31 $\mu$m respectively (Clowes, 1972) the corresponding values for the 5006M genome were established as $30.7 \times 10^6$ and 14.8 $\mu$m respectively.
Circularly permuted DNA

Fig. 4. Homoduplex circle of 5006M DNA. Denaturation conditions were: 12 µg/ml DNA in 90% formamide, 0.01 mM-EDTA, pH 8.0, at 60 °C for 10 min. Renaturation was accomplished by addition of 0.6 vol. of 5 x SSC, pH 8.0, and incubation at room temperature for 2 h. Other conditions were as in Fig. 2. The arrows mark the sites of single strand protrusions.

Denaturation mapping

Phage 5006M DNA was partially denatured to 4 to 15%. Sixty-two photographed molecules were traced, mapped and aligned with respect to their denaturation patterns (Fig. 2, 3a) yielding a denaturation map (Fig. 3c). The distribution of ends with respect to the denaturation map (Fig. 3b) indicates that the phage genome is non-randomly circularly permuted and that the permutation extends over 46% of the phage genome.

Homoduplex formation

Denaturation - self-reannealing experiments revealed a high yield of circular molecules with single strand protrusions at two different sites on each molecule (Fig. 4). Branch migration (Lee et al. 1970) was observed on these molecules.

Circle formation is a result of the circularly permuted nature of the molecules (Rhoades et al. 1968). The circumference and lengths of single strand protrusions were measured on 46 molecules and spacing between protrusions could unequivocally be measured on 36 mole-
cules. Since the length of each single strand protruding from the circumference corresponds to the length of terminal repetition, the latter was calculated as $3.6 \pm 0.9 \%$ of the phage genome length. The inter-protrusion distance is a measure of the staggering in permutation between the reannealed strands (Tye et al. 1974). A maximum staggering of $40 \%$ is observed (Fig. 5).

**DISCUSSION**

The results presented above indicate that the genome of phage 5006M is non-randomly circularly permuted. Partial denaturation experiments (Fig. 3) indicate that the permutation extends over $46 \%$ of the genome. This finding is corroborated by the homoduplex experiments (Fig. 5) and closely parallels that of Tye et al. (1974) for phage P22.

Recently, Jackson et al. (1978a, b) employed restriction endonuclease cleaving to study phage P22 DNA packaging. Their conclusions as to the validity of a sequential packaging mechanism are in excellent agreement with those of Tye et al. (1974), which were arrived at by means of electron microscopic techniques. This enhances confidence in the experimental approach of the present study.

Although no direct evidence for the existence of concatemeric precursors of phage 5006M DNA has been reported, the present findings are consistent with such a model. Utilizing a $43 \%$ extent of permutation and the observed $3.6 \pm 0.9 \%$ terminal repetition, a concatemeric length of maximally $12 \pm 3$ headfuls is suggested for phage 5006M precursors. Tye et al. (1974) found 10 headfuls for P22 concatemers via a similar calculation, in agreement with previous direct measurements on the physical size of P22 concatemers (Botstein, 1968; Botstein & Levine, 1968; Botstein et al. 1973).

The distribution of ends (Fig. 3b) suggests that encapsulation of headfuls of DNA initiates at a unique site on each concatemer. This site is located in a relatively stable region of the genome (Fig. 3c) resembling the location of the pac site of P22 (Tye et al. 1974; Jackson et al. 1978b). Since a decrease from left to right is observed in the distribution of ends, a unique direction of sequential encapsulation is inferred. This interpretation relies on the assumption that encapsulation of each sequential headful is more probable than the following (Tye et al. 1974). The present results show that encapsulation of monomers extending to the maximum concatemeric length is a rare event, supporting the view that most mature linear phage genomes are packaged in the first few sequential headfuls, suggesting that several packaging sequences can initiate on one concatemer. The results in Fig. 3(b) indicate that in the present case, $75 \%$ are packaged in the first four headfuls.

It has been suggested that generalized transducing phages Φ1 and 15 may have packaging mechanisms similar to the variation (Tye et al. 1974) of the headful mechanism described by Streisinger et al. (1967), which applies to P22 (Tye et al. 1974; Jackson et al. 1978b). The latter authors have drawn attention to a potential resemblance of packaging of specialized transducing phage Λ and generalized transducing phage P22. Also, the generalized transducing properties of phage T1 have been interpreted in terms of a permuted genome and it has been suggested that packaging of the phage Mu genome, which is unique, may have features in common with the headful packaging model (Bukhari & Taylor, 1975; MacHattie & Gill, 1977).

We conclude that our findings on phage 5006M are consistent with existing models for DNA maturation and processing during encapsulation and that this contributes to a generalization of the sequential packaging mechanism.

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