A Map of the Restriction Targets in Yeast 2 Micron Plasmid DNA Cloned on Bacteriophage Lambda

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Summary. The 2 micron circular DNA from *S. cerevisiae* has been cloned on bacteriophage \( \lambda \). The two forms of circular DNA which exist in equilibrium due to recombination between inverted repeat sequences were separated as stable clones, and a map of the targets for restriction endonucleases *EcoRI*, *HindIII* and *HpaI* was constructed. The circular DNAs isolated from a particular oligomycin resistant strain and its parent oligomycin sensitive strain were compared by restriction endonuclease analysis, and no difference was detected. The potential uses of cloned 2 micron DNA in determining the possible biological role of these plasmids are considered.

The presence of plasmid DNA is widespread in bacteria but infrequent among eucaryotes. A homogeneous population of small circular DNA molecules has been described in *Saccharomyces cerevisiae* (Sinclair et al., 1967; Bilheimer and Avers, 1969; Hollenberg et al., 1970; Guerineau et al., 1971; Stevens and Moustacchi, 1971). There are 50 to 80 such molecules per haploid genome and these constitute 3% of the mass of the DNA (Guerineau et al., 1971; Stevens and Moustacchi, 1971; Bak et al., 1972; Clark-Walker, 1973), and larger molecules, which are probably dimers and trimers, have also been observed.

Recently Guerineau et al. (1976) have shown that the 2 micron circular DNA has a 600 base inverted repeat sequence, separated by non-repeated DNA. The existence of inverted repeat sequences, and the previous report (Guerineau et al., 1974) that the presence of the 2 micron circular DNA is correlated with an oligomycin resistance determinant, makes possible analogies to bacterial insertion sequences attractive.

The 2 micron circular DNA has the same density as main band nuclear DNA, which is distinct from that of mitochondrial DNA, and is not enriched in the heavy nuclear satellite fraction (Guerineau et al., 1971; Clark-Walker, 1972). The location of the small circular DNA remains to be determined, though opposing claims for its intracellular position have already been advanced (Stevens and Moustacchi, 1971; Clark-Walker, 1972; Clark-Walker and Miklos, 1974; Griffiths et al., 1975). Replication of the small circular DNA does not proceed at the restrictive temperature in two different temperature sensitive mutants defective in the propagation of nuclear DNA synthesis, but is unaffected in an analogous initiation mutant (Petes and Williamson, 1975).

This paper describes the cloning of the 2 micron DNA on bacteriophage lambda receptors and the subsequent restriction enzyme and electron microscopic analysis.

Materials and Methods

Media

The rich medium was L broth (Lennox, 1955) containing (in g/l): Difco Bacto-Tryptone, 10; Difco Bacto yeast extract, 5; NaCl, 5; Glucose, 1; adjusted to pH 7.2.

Phage stocks were prepared on L broth agar solidified by Difco agar (10 g/l).

Phage assays and transfections were made on Baltimore Biological Laboratories Trypticase (BBL) agar, containing (in g/l): Trypticase, 10; NaCl, 5; Difco agar, 10 for plates and 6.5 for top layers (Parkinson, 1968).

Phages were diluted and resuspended in phage buffer containing (in g/l): Na2HPO4, 7; KH2PO4, 3; NaCl, 5; MgSO4·7 H2O, 0.25; CaCl2, 0.15; gelatin, 0.01.

The low phosphate medium for preparing \( ^{32}P \)-labelled phage was that of J. Abelson (Murray and Murray, 1975).

Enzymes and Chemicals

Pancreatic DNAase and RNAase were purchased from Worthington Biochemical Corporation, Freehold, New Jersey; restriction endonuclease *EcoRI* was prepared essentially as described by Yoshimori (1971), and some preparations were kindly provided by V. Tanyashin and P. Southern.
Preparations of phage were made by infection of exponentially growing cultures of QR48 or ED8641 (unless stated otherwise) in L broth containing 10^{-3} M MgSO_4. Growth was followed spectrophotometrically and when the A_650nm reached a minimum (2.5 to 4.5 h after infection) lysis was completed by addition of CHCl_3 (1 ml/l) and 10 min later the lysate was clarified by centrifugation.

Phage Lysates for DNA Preparations

Preparations of phage were made by infection of exponentially growing cultures of QR48 or ED8641 (unless stated otherwise) in L broth containing 10^{-3} M MgSO_4. Growth was followed spectrophotometrically and when the A_650nm reached a minimum (2.5 to 4.5 h after infection) lysis was completed by addition of CHCl_3 (1 ml/l) and 10 min later the lysate was clarified by centrifugation (10 min at 10,000 g). Phage were pelleted (3 h at 40,000 g), resuspended and treated with DNAase and RNAase (10 μg/ml for 1 h at room temperature) and banded by equilibrium centrifugation in 41.5% (w/w) caesium chloride solution (Kaiser and Hogness, 1960).

To prepare ³²P-labelled phage ED8612 growing in 150 ml L-broth was infected with phage (m.o.i. = 10) and shaken at 37°C for 20 min to allow adsorption of phage. The cells were then pelleted and resuspended in 100 ml low phosphate medium containing L-methionine (40 μg/ml), L-tryptophan (40 μg/ml) and ³²P (1 μCi). After about 4 h growth the lysate was clarified and phage were pelleted, etc., as described above. The caesium chloride step was repeated.

Preparation of Phage DNA

Phage in caesium chloride were dialysed against 10 mM Tris HCl (pH 8.0), 1 mM EDTA. DNA was extracted by gentle rolling with freshly distilled phenol (Kaiser and Hogness, 1960) followed by dialysis against 10 mM Tris HCl (pH 8.0), 1 mM EDTA (4 x II volumes in 24 h).

Preparation of 2 Micron DNA

The methods of extraction and purification of 2 micron circular DNA from yeast have been described (Guérineau et al., 1974, 1976).

Restriction Endonuclease Digestion

Restriction endonuclease digestion were carried out as described by Borck et al. (1976). As 2 micron DNA had two targets for endo R.HindIII the yeast DNA was only partially digested for the purpose of cloning the entire molecule. Preliminary experiments were carried out before each cloning to determine the conditions of restriction which converted the majority of the circles to full length linear molecules as determined by agarose gel electrophoresis.

Electrophoresis

Electrophoresis on 1% agarose slab gels was carried out as described by Sharp et al. (1973). Gradient acrylamide gels were prepared and run essentially as described by Allet (1973). Before photographing, gels were soaked in water for 1 to 2 h and then transferred to black polythene to minimise the background fluorescence. Negatives were scanned with a Joyce Loebel densitometer.

Ligase Reactions

Restricted 2 micron DNA and vector DNA were mixed in the ratio 2.3 by weight and incubated with T4 polynucleotide ligase under conditions previously described (Borck et al., 1976).

Transfection of E. coli to Recover Recombinant Phages

E. coli ED8641 cells were made competent for transfection by the method of Lederberg and Cohen (1974). DNA (3 μg/ml in cold 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) was mixed with competent cells in the ratio 1:2 (v/v), chilled in an ice-bath for 30 min, heated at 42°C for 2 min and then chilled again before mixing with top layer agar containing 10^{-5} M MgSO_4 and plating on BBL agar (Media). To emphasise the difference between clear and turbid plaques with this host a second layer of top agar (without cells) was added.

Phages

To clone the yeast 2 micron plasmids, lambda vectors (constructed in this laboratory by N.E. Murray) were chosen which had genomes sufficiently smaller than wild-type lambda to allow the stable insertion of a sequence 3.9 million daltons in size without favouring deletions or duplications in the genome. As both phage vectors were red-, generalized phage recombination was not a problem in the maintenance of cloned sequences. The λ vector 641 (Fig. 1a) for fragments of DNA generated by endo R.EcoRI has a chromosome deleted for approximately 19 per cent of the wild-type complement of DNA, but with a single target for endo R.EcoRI. The HindIII receptor phage (λ vector 598 Fig. 1b) has a genome approximately 18 per cent smaller than the wild-type genome, and has only one target for endo R.HindIII (Murray et al., 1975). In both of these receptor phages the site of cleavage is in the imm4 region, and insertion of fragments of donor DNA into these restric-