ISOLATION OF REPETITIVE DNA FROM TWO MAMMALIAN SPECIES USING S_1 NUCLEASE

Myles L. Mace, Jr., Roger W. Clark and Frances E. Arrighi

Section of Cell Biology
The University of Texas System Cancer Center
M. D. Anderson Hospital and Tumor Institute and
The University of Texas at Houston
Graduate School of Biomedical Sciences

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SUMMARY: DNA isolated from Mus musculus and Peromyscus eremicus was examined by analytical ultracentrifugation after treatments which allow reassociation of repeated sequences. Regions containing unique copies were removed with a single-strand specific nuclease (S_1), resulting in a simple effective isolation of regions of repeated sequences.

INTRODUCTION: There are two common methods for isolating repetitive DNA fractions, cesium salt density gradient centrifugation, and elution from hydroxylapatite columns. The former is tedious and dependent on natural or inducible differences in density between repetitive DNA and the remainder of the genome. The latter method may require shearing of DNA to speed column elution, resulting in material which is too low in molecular weight to band effectively during density gradient centrifugation in cesium salts. Furthermore, hydroxylapatite columns do not provide an absolute separation between double and single stranded DNA (1,2). This report describes s simple, rapid method for isolating repetitive DNA using a single-strand specific nuclease (S_1) prepared from Aspergillus oryzae (2,4).

MATERIALS AND METHODS: Two rodent species were used in this study, the laboratory mouse Mus musculus, and the cactus mouse Peromyscus eremicus. DNA was isolated from M. musculus liver by a modified Marmur procedure (3). P. eremicus DNA was isolated from a tissue culture cell line as follows. Cells were lysed with a solution containing 0.15M NaCl, 0.05 M Tris, 0.015 M EDTA, and 0.3% Sarcosyl NL-97 (Geigy Chemical Co.) at pH 8.5. The lysate was pronase digested (100 μg/ml of lysate) for 18 hours at 37°C, and deproteinized by the Marmur procedure, omitting the ethanol

precipitation. Phenol was removed by dialysis against 1,000 volumes of SSC (0.15 M NaCl, 0.015 M Na-citrate, pH 7.0) at 25°C, and the solution was digested with pancreatic ribonuclease (50 μg/ml of lysate) for 4 hours at 25°C. The pronase digestion, phenol extraction and dialysis were repeated as before.

DNA samples in SSC were heated at 100°C for 10 minutes for denaturation, and either fast cooled or reassociated to the desired $C_o t$ value. M. musculus DNA was reassociated to a $C_o t$ of 5 or 10 at 65°C. P. eremicus DNA was reassociated to a $C_o t$ of 5, at 70°C. Reassociated samples were split into two fractions, one was retained for centrifugation without further treatment, and the other was prepared for S_1 nuclease digestion.

S_1 nuclease was prepared by the method of Sutton and contained 5000 units/ml as defined by him (2). For the digestion procedure, a reassociated DNA sample was dialysed against 0.1 X SSC, and a 0.6 ml aliquot containing 25-50 μg of DNA was added to 0.3 ml of S_1 buffer (equal volumes of 0.1 M NaCl, 0.3 M Na-acetate, 10^{-4} M $ZnSO_4$, pH 4.5). To the buffered sample, 0.1 ml of S_1 was added and the solution incubated at 37°C for 1 hour. Prior to centrifugation, the sample was dialysed 24 hours at 4°C against SSC to remove free nucleotides and short pieces of DNA.

All centrifugations were performed in a Beckman Model E analytical ultracentrifuge (An-F rotor, 25°C, 42,040 rpm, 24 hours) in neutral CsCl (n_D = 1.4015). Cells contained 1-3 μg of sample DNA and 1 μg of reference DNA isolated from the Bacillus subtilus bacteriophage 2C which has a buoyant density of 1.742 g/ml. Tracings were prepared from ultraviolet (265 nm) photographs with a Joyce-Lobel microdensitometer, and a uniform baseline obtained with a Dupont curve resolver.

RESULTS: Figures 1 and 2 show the CsCl density profiles for DNA of M. musculus and P. eremicus respectively. The profile for the native

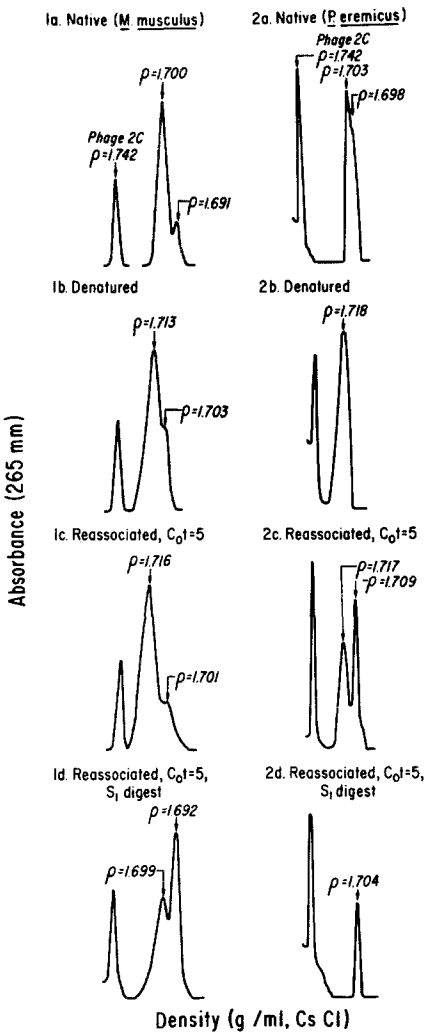

Figure 1: Microdensitometer absorbance profiles of M. musculus DNA samples banded in neutral CsCl in the Model E analytical ultracentrifuge. (a) native; (b) heat denatured, fast cooled; (c) denatured and reassociated to $C_o t$ 5; (d) denatured-reassociated to $C_o t$ 5, then digested with S_1. The reference DNA on the left is from bacteriophage 2C, density 1.742 g/ml.
Figure 2: Treatments as in figure 1, but with DNA isolated from P. eremicus.

mouse DNA is comparable to what is generally known: the main band peak at 1.700 and a satellite peak (ca 10% of the total DNA) at 1.691. After denaturation (figure 1b) the densities of mouse DNA shift to 1.713 and 1.703 respectively, with the satellite not as pronounced as the control sample. Figure 1c illustrates the profile of DNA denatured and reassociated

to a $C_o t$ of 5. When samples reassociated to a $C_o t$ of 5 or 10 were treated with S_1 nuclease, the profile (figure 1d) showed a substantial reduction in main band DNA and enriched satellite DNA, (ρ = 1.692). The S_1 resistant satellite sequences still represent about 10% of the total mouse genome. However, a certain amount, about 20% of the starting DNA, is S_1 resistant in the main band region (ρ = 1.699 g/ml). The amount of DNA forming this peak does not appear to change significantly between $C_o t$ values of 5 and 10.

The native DNA of P. eremicus contains a heavy region (ρ = 1.703 g/ml) adjacent to a light shoulder (ρ = 1.698 g/ml). The heavy region can be distinguished as a highly absorbant and sharply defined band in the UV photographs, but in densitometer tracings, it merges with the lighter shoulder (figure 2a). Figure 2b shows the profile of heat denatured and fast cooled DNA as a single peak with a buoyant density of 1.718. After reassociation to a $C_o t$ of 5 (Figure 2c), two distinct peaks appear, a denatured peak at ρ = 1.717, and a lighter peak at ρ = 1.709. The heavy peak is slightly less dense than total denatured DNA, as would be expected if a heavy fraction were removed. The peak with the lower buoyant density presumably represents the partially reassociated heavy fraction with some single-strand pieces still attached. There is only one detectable peak (ρ = 1.704 g/ml) in the reassociated sample treated with S_1 nuclease (Figure 2d). This density value is extremely close to the buoyant density of the native heavy fraction (ρ = 1.703 g/ml). We conclude therefore, that the heavy region of P. eremicus is similar to the satellite regions of other animals, in that it is composed of repetitive sequences. If there are repeated sequences in the light region, they are too short to band in CsCl under our conditions, too few in number to register as UV absorbing material, or sufficiently mis-matched to be degraded by S_1 nuclease.

DISCUSSION: Our data suggest that the procedure described here

(reassociated DNA treated with S_1 nuclease) may be useful as a first step for the isolation and characterization of repetitive sequences. It is simple and effective. The nuclease is strongly specific for single-stranded DNA in mixtures of both single and double strands (5), and has both endo- and exonuclease activity on single-stranded material. The progress of digestion can be followed by spectrophotometric monitoring, and analytical ultracentrifugation will indicate incomplete digestion through the presence of denatured DNA in the gradient. The DNA samples were almost entirely double-stranded after reassociation and S_1 digestion, as was shown by CsCl buoyant density measurements, which are essentially identical to those of native fractions. The procedure should be applicable to the DNA of any organism, and will aid in demonstrating repetitious DNA fractions which are ordinarily masked by the remainder of the genome, and may resist more complicated means of detection. More detailed studies are currently underway with human and Chinese hamster DNA preparatory to localization, by *in situ* hybridization, of various families of these repetitive sequences.

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