Structural Organization of a Hypermethylated Nuclear DNA Component in *Physarum polycephalum*

By OLIVER P. PEOPLES, PAUL A. WHITTAKER, DOUGLAS PEARSTON
AND NORMAN HARDMAN*

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS,
Scotland, UK

(Received 29 June 1984; revised 13 December 1984)

Digestion of *Physarum polycephalum* nuclear DNA using the restriction endonuclease *HpaII* generates two components, distinguishable on the basis of their molecular size. The high-molecular-weight, *HpaII*-resistant component, which accounts for 20% of the DNA, contains a fivefold greater concentration of 5-methylcytosine residues than the low-molecular-weight *HpaII*-digested fraction. Segments of hypermethylated (M^+) DNA are largely composed of a single, long, highly repeated sequence, and this major element is sometimes associated with other less highly repetitive sequences in the M^+ DNA fraction. Restriction mapping of cloned *Physarum* M^+ DNA segments, and Southern blot analysis of genomic DNA using subcloned segments of M^+ DNA as a probe, provide evidence for sequence variation within different copies of the dominant highly repeated element, and possibly the other associated repeats in M^+ DNA, and additionally that almost complete tandemly repeated copies of the major repeat are found in some M^+ DNA segments.

INTRODUCTION

5-Methylcytosine (5-meC) is the only known naturally-occurring modified base in DNA from higher eukaryotes. 6-Methyladenine is additionally found in the DNA of some, but not all, lower eukaryotic species. Using traditional methods of analysis, 5-meC was shown to be the only modified base in DNA from *Chlorella* (Bayen & Dalmon, 1976), *Saccharomyces* (Hattman *et al.*, 1978) and *Physarum* (Evans *et al.*, 1973). Methylation has been implicated in various aspects of eukaryotic DNA function, but most recently attention has been focused on its possible role in the regulation of genetic expression (Mavilio *et al.*, 1983). Studies of the distribution of 5-meC in eukaryotic DNA and the nature of methylated sequences might be expected to provide clues as to its possible functions.

Nuclear DNA in sea urchin (Bird *et al.*, 1979) and *Physarum polycephalum* (Whittaker & Hardman, 1980) contains a clearly distinguishable 'compartment' which resists digestion using the methylation-sensitive restriction endonuclease *HpaII*. In *Physarum*, this component represents 20% of the nuclear DNA, and it comprises DNA segments 20–40 kb long which contain contiguously methylated *MspI* restriction sites (Whittaker & Hardman, 1980). The *HpaII*-resistant DNA fraction can be isolated by sucrose gradient centrifugation (Whittaker *et al.*, 1981). This offers a means to analyse the component biochemically in order to determine the content of 5-meC in *HpaII*-resistant DNA and to identify specific nucleotide sequences associated with it. Previous studies led to the identification of a major repetitive DNA sequence which is confined to the *HpaII*-resistant fraction of the *Physarum* genome (Whittaker *et al.*, 1981; Peoples & Hardman, 1983). The present work was concerned with the base composition analysis of the *HpaII*-resistant compartment of *Physarum* DNA, and the identification of further repetitive sequence families which are associated with this DNA fraction.

Abbreviation: 5-meC, 5-methylcytosine.

METHODS

Sources of DNA. Nuclear DNA was isolated as described previously (Hardman *et al.*, 1979) from *Physarum polycephalum* strain LU648 × LU688 (Cooke & Dee, 1975) a generous gift from Dr Jennifer Dee, Department of Genetics, University of Leicester, UK.

Physarum recombinant plasmid DNA clones pPH29 and pPH53 (McLachlan & Hardman, 1982) contain *Bam*HI/*Hind*III segments of *Physarum* nuclear DNA inserted into the vector pBR322. Subcloning of pPH53 generated pPH53a and pPH53b (Peoples *et al.*, 1983) referred to in this study. The PL series of *Physarum* genomic DNA clones were constructed as described previously (Whittaker, 1982; Peoples *et al.*, 1983).

Base composition analysis of DNA. Samples of DNA (50 µg) were dissolved in 0.1 ml 1 mM-MgCl₂/10 mM-Tris-HCl pH 8.0, and digested using 0.15 mg DNAase I for 1 h at 37 °C. Then 0.02 ml 1 M-Tris/HCl pH 9.5 was added followed by 0.05 units of *Crotalus durissus* phosphodiesterase, and the incubation was continued for 1 h. Finally, 2 units of calf intestinal alkaline phosphatase were added and incubation continued for a further 4 h. Enzymes were removed by overnight precipitation at -20 °C in the presence of 2 vols ethanol, followed by centrifugation for 15 min in an Eppendorf microcentrifuge. Nucleosides were recovered from supernatants by evaporating to dryness.

For HPLC analysis, approximately 10 µg nucleosides were dissolved in 50 µl distilled water and injected into a chromatography column containing micro-Bondapak C18 reversed phase silica gel charged with octadecyl groups. The column was equilibrated with a solution of 0.1% phosphoric acid, previously filtered through a 0.45 µm Millipore filter and degassed with helium immediately before use. Nucleosides from test samples were identified by comparison with elution profiles of authentic compounds. The relative abundance of nucleosides was determined from areas under the peaks in the elution profile.

DNA blotting, hybridization and restriction mapping procedures. Routine experimental methods, including plasmid DNA isolation, gel electrophoresis, nick-translation of DNA probes and sub-cloning of DNA fragments, were based on procedures described previously (Hardman *et al.*, 1979; Whittaker *et al.*, 1981; Peoples & Hardman, 1983).

The 'sandwich blot' method (Smith & Summers, 1980), based on the Southern blot technique (Southern, 1975), was used to generate replicate filters for hybridization with different DNA probes. Hybridization was done under conditions described previously (Peoples *et al.*, 1983). Autoradiograms were developed using Fuji-Rx X-ray film.

RESULTS

Isolated HpaII-resistant Physarum DNA is enriched in 5-meC residues

Physarum nuclear DNA, uniformly labelled by growth of microplasmodia in the presence of deoxy[5-³H]adenosine, was terminally-digested using the restriction nucleases *Hpa*II and *Hha*I, and subsequently fractionated by sucrose gradient centrifugation, as described previously (Whittaker *et al.*, 1981). Digestion with a combination of *Hpa*II/*Hha*I increases the resolution between the digested and undigested fractions, which are referred to as 'M⁻' and 'M⁺', respectively (Whittaker & Hardman, 1980). Purified DNA from each fraction was subjected to base composition analysis by HPLC after complete enzymic digestion to nucleosides. Results of the analysis are shown in Table 1. The results show that the mean mol % G + C content of the M⁺ fraction is similar to that of M⁻ or total DNA, whereas the proportion of 5-meC residues in

Table 1. *Base composition analysis of Physarum DNA*

Total *Physarum* nuclear DNA (10 µg) was digested to nucleosides using DNase I/snake venom phosphodiesterase/alkaline phosphatase, and subjected to HPLC analysis as described in the text. Peak assignments were made with reference to elution times of authentic compounds. Recoveries were estimated from peak areas using both the method of internal standards and the method of external standards. The data are means ± SD of five determinations on two different samples of each DNA fraction.

Sample	Mean percentage composition					meC C + meC × 100
	C	meC	A	G	T	
Total DNA	20.92 ± 0.33	1.57 ± 0.48	28.94 ± 1.92	20.52 ± 0.49	27.27 ± 0.58	6.98
M ⁺ DNA	20.61 ± 0.16	4.16 ± 0.41	27.37 ± 0.70	20.41 ± 0.30	27.44 ± 0.42	16.79
M ⁻ DNA	20.84 ± 0.22	0.69 ± 0.15	30.88 ± 1.26	20.40 ± 0.40	27.18 ± 0.68	3.20

Fig. 1. Hybridization of recombinant phage DNAs to restriction digests of *Physarum* nuclear DNA. Samples of *Physarum* nuclear DNA, digested using (1) *Hpa*II, (2) *Hpa*II + *Msp*I, or (3) *Msp*I, were electrophoresed on 0.75% agarose gels, blotted on to nitrocellulose and probed with nick-translated probes of: (a) PL-3; (b) PL-5; (c) PL-12; (d) PL-13; (e) PL-15; and (f) PL-16. Specific activities of probes were 1×10^7 – 5×10^7 c.p.m. (μ g DNA) $^{-1}$. Filters were exposed to X-ray film overnight at -50°C . Arrowheads in (a) indicate the positions of faint high-molecular-weight DNA bands showing homology with each probe. B1 and B2 indicate positions of 2.0 kb and 2.2 kb bands respectively [faint in (d) and (f)].

M^+ DNA is approximately fivefold greater than the M^- fraction. It is concluded that the M^+ fraction is hypermethylated; although the compartment contains only 20% of the DNA (Whittaker & Hardman, 1980), it contains about two-thirds of all the 5-mC in the genome.

Identification of sequences located in cloned M^+ DNA segments

The construction of a clone bank of *Physarum* nuclear DNA, using the bacteriophage vector λ -1059, has been described previously (Whittaker, 1982; Peoples & Hardman, 1983). The library contains *Physarum* DNA inserts about 10–15 kb in length. Cloned segments of M^+ DNA were identified by Benton–Davis hybridization screening (Benton & Davis, 1977) using non-homologous cloned *Physarum* DNA probes specific for the M^+ fraction (pPH29 and pPH53; Whittaker *et al.*, 1981). It was thus concluded that the repetitive elements present in the clones pPH29 and pPH53 were closely linked in virtually all cloned segments of M^+ DNA, and a collection of six cloned M^+ -specific DNA segments was selected for further study (PL-5, 6, 12, 13, 15 and 16). A long, major repetitive element, the *Hpa*II-repeat, has already been identified in these DNA clones (Peoples & Hardman, 1983).

In order to identify repeated DNA sequences in these segments other than those displaying homology with pPH29 and pPH53, DNA from each recombinant phage was isolated, nick-translated and used to probe Southern blots of *Physarum* genomic DNA digested using *Hpa*II, *Msp*I or *Hpa*II + *Msp*I (Fig. 1). In all cases, the probes detected high-molecular-weight fragments in *Hpa*II digests, typical of M^+ -specific DNA sequences, thus confirming their origin (lanes 1). In contrast the sequences were digested with the isoschizomer *Msp*I (lanes 2 and 3),

Fig. 2. Hybridization of pPH29 and pPH53 probes to *Sau3AI* digests of *Physarum* genomic DNA. Various DNA samples were restricted using *Sau3AI*, separated on 1.5% agarose gels, and duplicate nitrocellulose blots prepared using the 'sandwich blot' method. Filters were probed using (a) pPH29 DNA, or (b) pPH53 DNA. All lanes contained 1 µg DNA with the exception of lanes A and L which contained 0.1 µg of isolated pPH29 and pPH53 *Physarum* DNA inserts, respectively. Lanes C and J contained *HindIII* restriction fragments of ³²P-labelled PM2 as size markers. Hybridization conditions and autoradiography were as described previously (Peoples & Hardman, 1983). A, pPH29 insert DNA; B, K, *Physarum* genomic DNA; C, J, marker DNA fragments; D, PL-3 DNA; E, PL-5 DNA; F, PL-12 DNA; G, PL-13 DNA; H, PL-15 DNA; I, PL-16 DNA; L, pPH53 insert DNA.

which cleaves both the unmethylated sequence CpCpGpGp and the methylated analogue CpmeCpGpGp. Only one major band of hybridization was detected in all the *MspI* digests, corresponding to DNA fragments in genomic DNA with a uniform length of 4.3 kb. This signal, and the faint bands of hybridization of high-molecular-weight (arrows, Fig. 1), probably arise from the presence of highly-repeated pPH29/pPH53-homologous sequences with a uniform arrangement of *MspI* (*HpaII*) restriction sites, referred to as the *HpaII*-repeat (Peoples & Hardman, 1983); similar DNA bands can be seen in ethidium bromide-stained agarose gels of *MspI*-digested *Physarum* DNA (Whittaker & Hardman, 1980), supporting the idea that they are derived from highly repetitive elements in *Physarum* DNA. Additional, fainter bands of hybridization were detected at 2.0 kb using PL-5 and PL-13 (B1), and at 2.2 kb using PL-16 (B2). These signals result from the presence in the clones of additional sequences unrelated to the *HpaII*-repeat, since the bands are not detected when similar restriction digests of genomic DNA are probed using pPH29 or pPH53 DNA (Peoples & Hardman, 1983).

HpaII-repeats located in cloned *M*⁺ DNA segments have a common internal structure

In order to demonstrate that cloned copies of the repeated elements derived from *M*⁺ DNA have a structure similar to those in *Physarum* genomic DNA, duplicate filters of *Sau3AI*-digested DNA samples were probed using nick-translated insert DNA fragments recovered from pPH29 or pPH53. The lack of cross-homology of pPH29 and pPH53 insert DNA sequences (Fig. 2a, lane L, and Fig. 2b, lane A) confirms the purity of the probes, and is in accord with previous work suggesting that they are derived from different portions of the *HpaII*-repeat (Peoples & Hardman, 1983). Hybridization signals to genomic DNA tracks (lanes B and K) were very similar to the 'average' pattern deduced for the cloned *M*⁺ DNA sequences PL-3, 5, 12, 13, 15 and 16 (lanes D-I), indicating that the cloned copies of these repeated elements are

Fig. 3. Restriction mapping of PL-5 and PL-15 subcloned DNA fragments. (a) Restriction maps of PL-5 and PL-15 showing the *HpaII*-restriction fragments subcloned into the *Clal* restriction site of pBR322, and a schematic representation of the arrangement of the major repetitive elements in PL-5 and PL-15, indicated by the letters A, B, C and D. This was deduced from the restriction analysis summarized in (b) and in the text. The right (R) and left (L) arms of the λ -1059 cloning vector are shown as hatched areas. Restriction sites are indicated by symbols that are defined in (b).

(b) Detailed restriction maps of the subcloned *HpaII* restriction fragments of PL-5 and PL-15. The maps are aligned to show regions of similarity with each other and with the pPH29, pPH53a and pPH53b probe sequences. Only insert DNA segments are shown in all cases. The data described in the text suggest that the repeated element is defined by a ~6.3 kb segment with internal restriction cuts at an *EcoRI* site (A) and a *HpaII* site (B). V1 and V2 denote the positions *HpaII* (*MspI*) restriction sites which are absent in some copies of the repeated element (see text for details). Horizontal dotted arrows indicate the positions of inverted repeat elements (foldback sequences) in pPH29 and pPH53a (Peoples *et al.*, 1983). A simplified summary map is included to indicate the positions of internal *BamHI*, *EcoRI*, *HindIII* and *HpaII* (*MspI*) restriction sites which define the structure of the repeated element; the broken line indicates undefined segments bordering the repeat. Restriction sites are indicated by symbols: □, *BamHI*; ○, *EcoRI*; ▲, *HhaI*; △, *HindIII*; ●, *HpaII*; ■, *Sau3AI*.

similar in structure to those in genomic DNA. Some differences were observed, and these were investigated further by the subcloning and restriction mapping of two clones, PL-5 and PL-15. The results are summarized in Fig. 3.

The maps of the entire cloned *Physarum* DNA segments in PL-5 and PL-15 are shown in Fig. 3(a), and more detailed restriction data are presented for plasmid-derived subclones in Fig. 3(b), together with maps of the pPH29, pPH53a and pPH53b probes (Peoples *et al.*, 1983) for comparison. Analysis of the data shows that PL-15 probably contains an incomplete copy of a long repeated element, >5.8 kb, which comprises a 4.3 kb *BamHI/HindIII* segment corresponding to the pPH29 element, a contiguous *HindIII/HpaII* (*MspI*) 1.5 kb segment which hybridizes to pPH53b, and a contiguous *HpaII* (*MspI*)/*BamHI* DNA segment which anneals to pPH53a (Fig. 3b). One *HpaII*-fragment subclone of PL-15, S3, has a similar structure and hybridization properties to the PL-15 subclone S4, indicating that PL-15 contains two, incomplete tandem head-to-tail copies of the same long repeated sequence, as indicated diagrammatically in Fig. 3(a). The incomplete copies of the repeats in PL-5 and PL-15 are joined to vector arms at the predicted positions of *Sau3AI* restriction sites; this is expected since

Fig. 4. Hybridization of PL-5 DNA probes to restriction digests of *Physarum* genomic DNA. Samples (1 μ g) of *Physarum* nuclear DNA were digested with (1) *Hpa*II, (2) *Msp*I, (3) *Hpa*II/*Msp*I, (4) *Bam*HI/*Msp*I, (5) *Hind*III/*Msp*I, or (6) *Bam*HI/*Hind*III, and separated on 1% agarose gels. Replicate filters were probed using: (a) total *Physarum* DNA, (b) PL-5/S5 subclone DNA, or (c) a 7 kb *Hpa*II fragment isolated from PL-5. Probes had a specific activity of 0.8×10^8 – 10^8 c.p.m. (μ g DNA) $^{-1}$. Autoradiographs were exposed overnight at -50°C . The arrows labelled B1 and B2 in (a) point to faint hybridization bands of similar size to those detected by cloned *Physarum* DNA segments PL-5, PL-13 and PL-16 (see text and Fig. 1).

the clones are derived from a collection of partial *Sau*3AI-digested fragments of *Physarum* genomic DNA. By inspection of the restriction maps of these cloned sequences it can be concluded that the variation in hybridization properties of the different cloned copies of the *Hpa*II-repeat in PL-15 and PL-5 (Fig. 2) can probably be explained by addition or deletion of internal *Sau*3AI restriction sites in repeated elements of uniform length. The absence of part of the repeat at the junction between the two tandem copies of the sequence in PL-15 in a region showing homology with pPH53a (Fig. 3) accounts for the lack of the small, pPH53-hybridizing *Sau*3AI restriction fragments in this clone.

Characterization of additional repeated sequences in PL-5

Highly repetitive sequences in *Physarum* DNA would be expected to generate the most prominent hybridization signals with a nick-translated, high-complexity total DNA probe (Wensink *et al.*, 1979; Peoples *et al.*, 1983). Fig. 4(a) shows the result obtained when various restriction digests of *Physarum* genomic DNA were hybridized to total *Physarum* DNA. The probe can be seen to hybridize to M⁺ DNA segments in *Hpa*II digested DNA (lane 1). These sequences are cleaved by *Msp*I generating a heterogeneous collection of DNA fragments and a single, predominant 4.3 kb DNA band (lanes 2 and 3). These major bands of hybridization correspond to the highly abundant *Hpa*II-repeat sequence, since a nearly identical result was obtained using a *Hpa*II-repeat subclone (PL-5/S5) as probe (Fig. 4b), except that in this case the background hybridization signals in each track were considerably reduced. The hybridization properties of this probe can be predicted from the known restriction properties of the cloned PL-5/S5 sequence. No major bands of hybridization were detected using a total *Physarum* DNA

probe except those associated with the *HpaII*-repeat sequence. It is concluded that *HpaII*-repeats are probably the only major family of long, highly repetitive elements in the *Physarum* genome.

High-molecular-weight fragments in *MspI* digests showing homology with PL-5/S5 (Fig. 4*b*, lanes 2 and 3) were digested by *Bam*HI, generating a predominant hybridization signal to fragments 5.8 kb long (lane 4). This almost certainly arises due to cleavage of additional *HpaII*-repeats which have lost an internal *MspI/HpaII* restriction site (V1 variable site, Fig. 3*b*). A similar conclusion was reached previously on examining the properties of the pPH53b sequence (V2 variable site, Fig. 3*b*) (see Peoples & Hardman, 1983). As shown below, variation in the position of *MspI/HpaII* sites may also occur within the additional repeated element located in the PL-5 sequence.

The ~7 kb *HpaII* restriction fragment of PL-5 was isolated from agarose gels, nick-translated and used as a probe to a Southern blot filter of *Physarum* genomic DNA similar to those shown in Fig. 4(*a, b*). The results (Fig. 4*c*) show that this fragment contains sequences belonging to the 2.7 kb *HindIII/MspI* segment of the *HpaII*-repeat, and the repeated element responsible for the 2.0 kb hybridization band detected in *MspI* digests of genomic DNA using a PL-5 probe (Fig. 1*b*). This result confirms that the 2.0 kb band originates from an additional repetitive element in the 7 kb *HpaII* (*MspI*) fragment of PL-5 which is distinct from the *HpaII*-repeat, and that this cloned repeated element has a different distribution of surrounding *MspI* sites from the majority of the genomic copies of the sequence. As in the case of the *HpaII*-repeats, this probably resulted from deletion of a *HpaII* (*MspI*) restriction site. Close inspection of Fig. 4(*a*) shows that a faint hybridization signal corresponding to the position of these repeated elements is generated using a total *Physarum* DNA probe (B1, Fig. 4*a*). Likewise a signal of similar intensity can be seen at 2.2 kb (B2, Fig. 4*a*), at a position expected for the additional repetitive element located in PL-16 (Fig. 1). The results are in accord with the hypothesis that these additional repeated elements are less abundant than the *HpaII*-repeats with which they are associated in M⁺ DNA.

DISCUSSION

Although previous work in this laboratory has led to the identification of a *HpaII*-resistant compartment in *Physarum* DNA we had not presented evidence that M⁺ DNA is enriched in 5-meC. In the present study we demonstrate that the *HpaII*-resistant, M⁺ fraction of the *Physarum* genome is indeed hypermethylated; it is enriched approximately fivefold in 5-meC compared with M⁻ sequences. No 6-methyladenine was detected, in accord with previous observations using different methods of analysis (Evans *et al.*, 1973). Evidence is presented that 5-meC is located in CpG doublets of *HpaII* and *HhaI* restriction sites in M⁺ DNA, but many additional sites of methylation are required to account for the observed levels of 5-meC. Nearest neighbour analysis of total *Physarum* DNA gives a CpG doublet frequency of 2 mol % (Whittaker, 1982). HPLC analysis shows that the M⁺ DNA fraction contains 4.16 mol % 5-meC. If CpG doublets were equally frequent in M⁺ and M⁻ DNA then only half of the 5-meC in the M⁺ fraction can be accommodated in CpG sequences. This figure is even less if CpG doublets occur less frequently in M⁺ DNA, as indicated by the lack of *MspI* and *HhaI* restriction sites in cloned DNA from these regions (see below). It is therefore likely that 5-meC residues may be present in sequences other than CpG. In plants, where as much as 30% of the cytosine residues may be methylated (Shapiro, 1976), 5-meC occurs in CpA, CpC and CpT as well as CpG, in sequences with the general formula CpXpG (Gruenbaum *et al.*, 1981). Additional sites of methylation such as these conceivably could affect restriction of M⁺ DNA sequences by enzymes other than *HpaII* or *HhaI*.

MspI restriction sites occur much less frequently in hypermethylated, M⁺ segments of *Physarum* DNA than in the remainder of the genome (Whittaker & Hardman, 1980; Whittaker *et al.*, 1981). This observation is consistent with the suggestion that methylated sequences may be subject to an enhanced rate of mutation (Bird, 1980), since these restriction sites are preferentially methylated in M⁺ DNA. Point mutations in the recognition sequence would lead to elimination of the restriction site, and evidence presented here indicates that such changes

have occurred at *MspI* restriction sites in the *HpaII*-repeats which dominate this DNA compartment, and possibly also in other less highly repeated elements in M⁺ DNA, such as the '2.0 kb' repeats in PL-5.

A head-to-tail tandem duplication of the *HpaII*-repeat occurs in PL-15 (Fig. 3). Similar organizations in genomic DNA may account for the faint high-molecular-weight bands in *MspI* restriction digests (Fig. 1 and Fig. 4b) probed with these sequences. The 2.7 kb *HindIII/MspI* segment of the *HpaII*-repeat is similarly duplicated in the PL-5/S5 subclone (Fig. 4b) and the 7 kb *HpaII* fragment (Fig. 4c) of PL-5. Previous studies of pPH53a show that it contains a head-to-head tandem repeat of the *BamHI/EcoRI* portion of the *HpaII*-repeat (Fig. 3b) (Peoples *et al.*, 1983). This indicates that local repetition of *HpaII*-repeats may be a general feature of M⁺ DNA segments. These properties raise additional questions regarding the nature of the sequences at the termini of *HpaII*-repeats, which are currently being investigated.

Some interesting comparisons can be made between the properties of the long, abundant *HpaII*-repeat in *Physarum* DNA and similar repeated elements in other eukaryotic organisms. *HpaII*-repeats have a similar length, methylation properties and repetition frequency (Peoples & Hardman, 1983) to the *KpnI* elements in primate DNA (Maio *et al.*, 1981; Grimaldi & Singer, 1982) and the MIF-*BamHI* elements in mouse DNA (Meunier-Rotival *et al.*, 1982; Fanning, 1983). Recent studies have revealed extensive and significant sequence homology between these long, repetitive elements in mouse and primate genomes (Singer *et al.*, 1983) indicating that they have a common origin. Their relatedness to the *HpaII*-repeated element in *Physarum* DNA is not known, but is currently being investigated in this laboratory. Other similarities include the observation that segments of *HpaII*-repeats (Peoples & Hardman, 1983) and *KpnI*-repeats (Lerman *et al.*, 1983) may be scrambled in some DNA segments, possibly similar to highly repetitive sequences in chicken and *Drosophila* DNA (Musti *et al.*, 1981; Wensink *et al.*, 1979).

Apart from the *HpaII*-repeats which dominate the methylated compartment of *Physarum* DNA, the present study provides evidence for the presence of other distinct, less highly repeated sequences in some segments of M⁺ DNA. These additional sequences have yet to be characterized. Additionally, it remains to be determined why the regions of the genome containing clusters of these specific repetitive elements are preferentially methylated, and whether their role in eukaryotic DNA is largely structural or related to gene function.

The authors are grateful to the Science & Engineering Research Council and Medical Research Council for supporting this work, and for the award of an MRC Research Studentship to D. P. We are also indebted to Mairi Gordon for excellent technical assistance and Professor H. M. Keir for provision of research facilities.

REFERENCES

- BAYEN, M. & DALMON, J. (1976). 5-Methylcytosine in *Chlorella pyrenoidosa* DNAs. *Biochimica et biophysica acta* **432**, 273–280.
- BENTON, W. D. & DAVIS, R. W. (1977). Screening of λ -gt recombinant clones by hybridization to single plaques *in situ*. *Science* **196**, 180–182.
- BIRD, A. P. (1980). DNA methylation and the frequency of CpG in animal DNA. *Nucleic Acids Research* **8**, 1499–1504.
- BIRD, A. P., TAGGART, M. H. & SMITH, B. A. (1979). Methylated and unmethylated DNA compartments in the sea urchin genome. *Cell* **17**, 889–901.
- COOKE, D. J. & DEE, J. (1975). Methods for the isolation and analysis of plasmodial mutants in *Physarum polycephalum*. *Genetical Research* **24**, 175–188.
- EVANS, H. H., EVANS, T. E. & LITTMAN, S. (1973). Methylation of parental and progeny DNA strands. *Journal of Molecular Biology* **74**, 563–577.
- FANNING, T. G. (1983). Size and structure of the highly-repetitive *BamHI* element in mice. *Nucleic Acids Research* **11**, 5073–5091.
- GRIMALDI, G. & SINGER, M. F. (1982). A monkey Alu sequence is flanked by 13-base pair direct repeats of an interrupted α -satellite DNA sequence. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 1497–1500.
- GRUENBAUM, Y., NAVEH-MANY, T., CEDAR, H. & RAZIN, A. (1981). Sequence specificity of methylation in higher plant DNA. *Nature London* **292**, 860–862.
- HARDMAN, N., JACK, P. L., BROWN, A. J. P. & MCLACHLAN, A. (1979). Distribution of inverted repeat sequences in nuclear DNA from *Physarum polycephalum*. *European Journal of Biochemistry* **94**, 179–187.
- HATTMAN, S., KEISTER, T. & GOTTEHRER, A. (1978). Sequence specificity of DNA methylases from *Bacillus amyloliquefaciens* and *Bacillus brevis*. *Journal of Molecular Biology* **124**, 701–711.
- LERMAN, M. I., THAYER, R. E. & SINGER, M. F. (1983). *KpnI* family of long interspersed repeated DNA sequences in primates: polymorphism of family members and evidence for transcription. *Proceedings*

- of the National Academy of Sciences of the United States of America **80**, 3966–3970.
- MCLACHLAN, A. & HARDMAN, N. (1982). Analysis of foldback sequences and repetitive sequences in cloned segments of nuclear DNA from *Physarum polycephalum*. *Biochimica et biophysica acta* **697**, 89–100.
- MAIO, J. J., BROWN, F. L., MCKENNA, G. W. & MUSICH, P. R. (1981). Highly-repetitive component α and related alphoid DNA in man and monkeys. *Chromosoma* **83**, 127–144.
- MAVILIO, F., GIAMPAOLO, A., CARE, A., MIGLIACCIO, G., CALANDRINI, M., RUSSO, G., PAGLIARDI, G. L., MASTROBERARDINO, G., MARINUCCI, M. & PESCHLE, C. (1983). Molecular mechanisms of human haemoglobin switchings: selective undermethylation and expression of globin genes in embryonic, foetal and adult erythroblasts. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 6907–6911.
- MEUNIER-ROTIVAL, M., SORIANO, P., CUNY, G., STRAUSS, F. & BERNARDI, G. (1982). Sequence organization and genomic distribution of the major family of interspersed repeats of mouse DNA. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 355–359.
- MUSTI, A. M., SOBIESKI, D. A., CHEN, B. B. & EDEN, F. C. (1981). Repeated deoxyribonucleic acid clusters in the chicken genome contain homologous sequence elements in scrambled order. *Biochemistry* **20**, 2989–2999.
- PEOPLES, O. P. & HARDMAN, N. (1983). An abundant family of methylated repetitive sequences dominates the genome of *Physarum polycephalum*. *Nucleic Acids Research* **11**, 7777–7788.
- PEOPLES, O. P., ROBINSON, A. C., WHITTAKER, P. A. & HARDMAN, N. (1983). Sequence organisation in nuclear DNA from *Physarum polycephalum*: genomic organisation of DNA segments containing foldback sequences. *Biochimica et biophysica acta* **741**, 204–213.
- SHAPIRO, H. S. (1976). Distribution of purines and pyrimidines in deoxyribonucleic acid. In *Handbook of Biochemistry and Molecular Biology*, vol. II, pp. 241–311. Edited by G. D. Fasman. Cleveland, USA: CRC Press.
- SINGER, M. F., THAYER, R. E., GRIMALDI, G., LERMAN, M. I. & FANNING, T. G. (1983). Homology between the *KpnI* primate and *BamHI* (MIF-1) rodent families of long interspersed repeated sequences. *Nucleic Acids Research* **11**, 5739–5745.
- SMITH, G. E. & SUMMERS, M. D. (1980). The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. *Analytical Biochemistry* **109**, 123–129.
- SOUTHERN, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503–517.
- WENSINK, P. C., TABATA, S. & PACHL, C. (1979). The clustered and scrambled arrangement of moderately-repetitive elements in *Drosophila* DNA. *Cell* **18**, 1231–1246.
- WHITTAKER, P. A. (1982). *Methylation of nuclear DNA in Physarum polycephalum*. PhD. thesis, University of Aberdeen, UK.
- WHITTAKER, P. A. & HARDMAN, N. (1980). Methylation of nuclear DNA in *Physarum polycephalum*. *Biochemical Journal* **191**, 859–862.
- WHITTAKER, P. A., MCLACHLAN, A. & HARDMAN, N. (1981). Sequence organisation in nuclear DNA from *Physarum polycephalum*: methylation of repetitive sequences. *Nucleic Acids Research* **9**, 801–814.