A simplified method for *in vivo* footprinting using DMS

Alison C.Brewer, Philip J.Marsh and Roger K.Patient
King's College Division of Biomolecular Sciences, Biophysics Section, 26-29 Drury Lane, London WC2B 5RL, UK

Submitted August 6, 1990

Important in understanding the regulation of gene transcription is the elucidation of specific protein-DNA interactions that occur *in vivo*. One strategy for such *in vivo* footprinting involves the treatment of whole cells with dimethyl sulphate (DMS), which leads to methylation of guanine residues in DNA at the N7 position (1, 2). This N7 atom lies in the major groove and its susceptibility to methylation in chromatin is affected by its proximity to bound non-histone proteins (3). Subsequent treatment with piperidine breaks the DNA backbone at methylated sites that can be mapped by various methods, including a sequence specific, radioactive primer extension assay, using a DNA polymerase (1, 4, 5). We and others have used Taq DNA polymerase, since it is very thermostable, allowing for both greater specificity of priming and multiple extension cycles to increase signal. Here we demonstrate that DMS treatment is alone sufficient to terminate Taq polymerisation in our assay, thus obviating the need for the piperidine treatment step (compare lanes 1 and 2 in Fig. 1). This is probably because the incubation at 95°C for 5 minutes that precedes the Taq polymerase reaction is sufficient to break the unstable glycosidic bonds of methylated purines (6), and leads to termination by Taq polymerase one nucleotide before a methylated purine base (Fig. 1).

Piperidine is known to inhibit Taq polymerase (4), and so great care has had to be taken in ensuring its complete removal by workers in the past. The omission of the piperidine step thus makes *in vivo* footprinting with DMS as simple and fast as footprinting with UV light, where damaged bases terminate DNA polymerases directly, without the need for a strand breakage step (7, 8). Presumably other DNA polymerases (eg Klenow, T7 and reverse transcriptase) would also terminate at DMS-methylated purine residues under similar conditions, i.e, if the samples were heated to ensure base removal prior to primer extension.

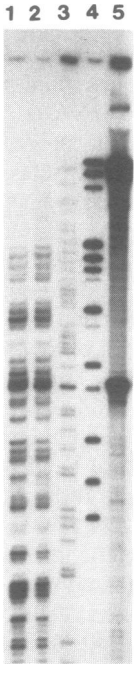

Figure 1. Autoradiogram of a 6% denaturing sequencing gel, showing the effects of methylation and piperidine treatment on DNA revealed by repeated primer extension. The DNA was a Xenopus β globin gene plasmid, digested with EcoRI. The sequence-specific radioactive primer was a 5′ end-labelled 17mer that hybridises to the sense strand of the β globin gene in the first exon. Lane 1:100 ng of DNA that was partially cleaved at G residues by treatment with DMS and piperidine (9) and repeatedly primer extended with 0.5 pmols end-labelled oligonucleotide. Lane 2: 100 ng of DNA that was patially methylated at G residues by treatment with DMS, then primer extended. Lane 3: G>A sequencing ladder on end-labelled DNA, produced by repeated primer extension of 100 ng of plasmid DNA with 0.5 pmols of end-labelled 17mer. In aligning the bands in lanes 1 and 2 with those in lane 3, allowance was made for the fact that piperidine-cleaved DNA molecules contain a 3′ phosphate and run effectively 1.5 nucleotides faster than primer-extended products of identical sequence (10). Futhermore, the G residues mapped in lane 3 are on the opposite strand to those revealed in lanes 1 and 2. Lane 4:5′ end-labelled pAT153/HpaII marker. Lane 5: 100 ng DNA, primer-extended with end-labelled oligo. All primer extension reactions were carried out in a volume of 20 μl, containing 100 ng of DNA, 0.5 pmols end-labelled primer, 2 μl of 10×buffer (100 mM Tris-HCl pH 8.3 at 42°C, 500 mM KCl, 20 mM $MgCl_2$, 0.2% w/v gelatin), 2 μl 5% Tween 20, and a final concentration of 200 μM of each dNTP. After gentle mixing the samples were overlayed with paraffin oil and incubated at 95°C for 5 minutes, in a thermal cycler. They were then held at the annealing temperature (63°C), while 1 unit of Amplitaq (Cetus) was added, and then incubated for 2 minutes at 75°C. A programme of 15 cycles, each consisting of 30 seconds of denaturation at 94°C, 10 minutes of annealing at 63°C, and 2 minutes of chain elongation at 75°C, was used for the amplification procedure. After the last cycle the samples were held at the annealing temperature before adding an equal volume of formamide dyes and loading an aliquot onto the sequencing gel.

REFERENCES

1. Ephrussi,A., Church,G.M., Tonegawa,S. and Gilbert,W. (1985) *Science* **227**, 134-140.
2. Giniger,E., Varnum,S.M. and Ptashne,M. (1985) *Cell* **40**, 767-774.
3. Gilbert,M., Maxam,A. and Mirzabekov,A.D. (Munksgaard, Copenhagen, 1976) in *Control of Ribosome Synthesis*, The Alfred Benzon Symposium IX, N.O.,Kjelgaard and O.Maaloe, eds, pp. 139-148.
4. Saluz,H. and Jost,J.-P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2602-2606.
5. Mueller,P.R. and Wold,B. (1989) *Science* **246**, 780-786.
6. Maxam,A.M. and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560-564.
7. Becker,M.M., Wang,Z., Grossman,G. and Becherer,K.A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5315-5319.
8. Axelrod,J.D. and Majors,J. (1989) *Nucl. Acids Res.* **17**, 171-183.
9. Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.* **65**, 497-560.
10. Sollner-Webb,B. and Reeder,R.H. (1979) *Cell* **18**, 485-499.