Two DNA methyltransferases from murine erythroleukemia cells: Purification, sequence specificity, and mode of interaction with DNA

(facilitated transfer)

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ABSTRACT Dye-ligand chromatography on Cibacron blue F3GA-agarose has been used to resolve two species of DNA (cytosine-5-)-methyltransferase from nuclear extracts of uninduced Friend murine erythroleukemia cells. Each species has been highly purified; the activities in the first and second peaks were associated with polypeptides of M_r 150,000 and 175,000, respectively. Analysis of substrate specificity with synthetic DNAs and restriction fragments of ϕ X174 replicative form DNA and pBR322 DNA showed that neither enzyme had dependence on the sequence context of CpG dinucleotides; poly(dG-dC) had the greatest methyl-accepting activity of any unmethylated DNA substrate tested. *De novo* methylation by both enzymes was inefficient relative to methylation of hemimethylated sites. Methyl-accepting activity was strongly dependent on DNA chain length. This observation suggests that binding to DNA, followed by one-dimensional diffusion of enzyme along the DNA molecule, is important in the mechanism by which DNA methyltransferase locates its recognition sites.

DNA methylation in cells from vertebrates is a post-replication process involving the transfer of methyl groups from S-adenosyl-L-methionine (AdoMet) to the 5 position of cytosine residues through the action of DNA methyltransferase [DNA MeTase; DNA (cytosine-5-)-methyltransferase, EC 2.1.1.37]. Methylated cytosine residues are located primarily within the dinucleotide 5' CpG 3' (1) and are found in tissue-specific amounts and positions (2). Sano and Sager (3) reported that, in bovine satellite I DNA, 5-methylcytosine (m^5C) residues are located exclusively 5' to G residues, occur in clusters, and tend to be located within short self-complementary sequences; the distribution of m^5C residues within this DNA showed striking tissue-specific variation.

Changes in amounts and locations of m^5C residues in DNA appear to be a part of the developmental program in higher cells (reviewed in ref. 4), and it has been demonstrated for many different genes that the loss of methyl groups from specific sites is correlated with the transcriptional activation of adjacent sequences (5–9). Also, *in vitro* enzymatic methylation of CpG dinucleotides by the bacterial restriction methyltransferase *M-Hpa* II inhibits expression of viral (10) and cellular (11) genes after introduction of the methylated DNA into cells. The pattern of methylation is maintained during subsequent cell divisions (12). Treatment of cultured cells with inhibitors of DNA methylation leads to the induction of certain genes with a concomitant reduction in the number of associated methylated CpG sites (13).

Despite the apparent biological importance of DNA meth-

ylation it is not at all clear how patterns of methylation are established and maintained within the genome. DNA MeTases prepared from a number of rodent (14–17) and human (18) sources have been shown to require CpG-containing DNA, although additional sequence requirements have not been identified. A crude preparation of DNA MeTase from mouse ascites cells showed a strong preference for hemimethylated sites (19), and a “processive” mode of action has been postulated on the basis of the sensitivity of the methylation reaction to increasing ionic strength (14) and to 5-azacytosine-substituted DNA (20). DNA MeTase has not been purified to a state approaching homogeneity, and as a result not a great deal is known of the physical properties of the enzyme. Very recently, DNA MeTase was reported to form a specific complex with several other enzymes involved in DNA synthesis (21). The isolation of more than one form of DNA MeTase from one cell type has not been reported.

We describe here the characterization of two species of DNA MeTase from nuclei of uninduced Friend murine erythroleukemia cells. The recognition sequence for both species is the CpG dinucleotide, and the methyl-accepting activity of a given segment of phage or plasmid DNA can be largely predicted from its content of CpG sites. One-dimensional diffusion of bound DNA MeTase along the DNA chain (as manifested by an increased rate of methylation of longer DNA chains) is apparently involved in the pathway by which DNA MeTase locates its recognition site and could be of importance to the *in vivo* functioning of the enzyme.

MATERIALS AND METHODS

Materials. Cell culture products were from GIBCO, DEAE-Sepharcel and native calf thymus DNA-cellulose were from Sigma, and blue agarose (AffiGel blue) and hydroxyapatite (Bio-Gel HTP) were from Bio-Rad. Synthetic polynucleotides were from P-L Biochemicals, S-adenosyl-L-[methyl- 3H]methionine (10–15 Ci/mmol; 1 Ci = 37 GBq) was from ICN East, and restriction endonucleases and ϕ X174 replicative form DNA were from New England BioLabs or Bethesda Research Laboratories. RNA polymerase was from Boehringer Mannheim; other protein M_r standards were from Bio-Rad. Plasmid pBR322 DNA was propagated in *Escherichia coli* strain HB 101 and purified as described (22). Plasmid pLP32 DNA was the gift of Paul Tesser (Massachusetts Institute of Technology).

Cell Culture. Friend murine erythroleukemia cells were the PC4 subclone of line 745a (23) and were obtained from David Housman (Massachusetts Institute of Technology). They were

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Abbreviations: m^5C , 5-methylcytosine; DNA MeTase, DNA (cytosine-5-)-methyltransferase; AdoMet, S-adenosyl-L-methionine.

maintained in spinner culture at densities between 10^4 and 5×10^5 cells per ml in Dulbecco modified minimal essential medium supplemented with 10% fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100 μ g/ml.

Enzyme Isolation and Assay. Cells were grown in 10 liters of medium to a density of 1×10^6 /ml and washed twice with 0.15 M NaCl/20 mM NaPO₄, pH 7.4, by centrifugation at $500 \times g$ for 5 min. Nuclei were isolated by washing cells three times by centrifugation at $1,500 \times g$ for 5 min in 10 vol of 1 M sucrose/3 mM MgCl₂/0.3% Triton X-100/20 mM Tris-HCl, pH 7.4/0.2 mM phenylmethylsulfonyl fluoride/0.2 mM dithiothreitol (18). Nuclear extracts were prepared from the final pellets by adding 5 M NaCl to a final concentration of 0.3 M to nuclei suspended in 10 vol of the buffer used for isolation of nuclei. The extract was centrifuged at $100,000 \times g$ for 1 hr and the supernatant was dialyzed for 4 hr against 100 vol of 20 mM Tris-HCl, pH 7.4/20 mM NaCl. The dialyzed extract was centrifuged at $10,000 \times g$ for 20 min and the supernatant was chromatographed on a 1.5×15 cm column of DEAE-Sephacel developed with a 200-ml gradient of 0.02–0.4 M NaCl in the buffer used for dialysis.

DNA MeTase activity was recovered as a single symmetrical peak at 0.15 M NaCl. Peak fractions were pooled and made 5 mM in Na₃EDTA and 2.7 M in glycerol. The pooled fractions were chromatographed on a 1.5×10 cm column of blue agarose equilibrated with buffer M (20 mM Tris-HCl, pH 7.4/2.7 M glycerol/5 mM Na₃EDTA/0.2 mM phenylmethylsulfonyl fluoride/0.2 mM dithiothreitol). Protein was eluted with a 200-ml gradient of 0.1–1.0 M NaCl in buffer M. Active fractions were pooled, dialyzed against 5.2 M glycerol in buffer M, and stored at -40°C . The preparation was free of DNA-independent methyltransferase activity but after DEAE-Sephacel chromatography it contained appreciable DNase activity which was removed by blue agarose chromatography. Most experiments described here used enzyme purified through the blue agarose step. Further purification was performed on 0.7×1 cm beds of hydroxyapatite or double-stranded calf thymus DNA-cellulose in columns eluted in a stepwise fashion with 1-ml volumes of buffer M containing additions as described in the figure legends.

Enzyme assay mixtures contained 1 μ g of DNA, 2 μ Ci (0.2–0.3 μ M) of [*methyl*-³H]AdoMet, and 10 μ l of enzyme solution plus buffer M to a total volume of 100 μ l. Native *E. coli* DNA was used as methyl acceptor during preliminary characterization. Unless stated otherwise, poly(dG-dC)·poly(dC-dG) served as methyl acceptor in the experiments described here. After incubation at 37°C for 2 hr, incorporation of radioactivity was determined by scintillation counting of 10% trichloroacetic acid precipitates collected on glass fiber filters (Enzo Biochemicals, New York). Incorporation of radioactivity into specific DNA fragments was determined after electrophoresis in 2.0% agarose gels by staining in ethidium bromide (0.4 μ g/ml), excising stained bands with a scalpel, autoclaving for 10 min in 1 ml of distilled water, and scintillation counting after addition of 10 ml of Liquiscint (National Diagnostics). *Hae* III fragments of pLP32 were separated by electrophoresis through 8% polyacrylamide gels, and radioactivity in excised bands was determined by scintillation counting after digestion with 20% hydrogen peroxide at 50°C . TLC of formic acid-hydrolyzed DNA was performed as described (15). Plates were fluorographed with 0.4% diphenyloxazole in 2-methylnaphthalene (24).

NaDodSO₄/Polyacrylamide Gel Electrophoresis. Gels were prepared by using the buffer system of Laemmli (25) and were 7% acrylamide/0.2% *N,N'*-methylenebisacrylamide. The $120 \times 150 \times 0.7$ mm gels were run at 20 mA, stained in 0.1% Coomassie blue R-250 in 50% methanol/7% acetic acid, and

destained in the same solution without dye. Protein in dilute solution was concentrated by addition of 0.1 vol of trichloroacetic acid (1 g/ml); after 1 hr at 0°C , precipitates were collected by centrifugation at $12,000 \times g$ for 15 min, washed twice in acetone at 0°C , and heated to 100°C in sample buffer containing 5% 2-mercaptoethanol and 2% NaDodSO₄.

RESULTS

Purification of MEL Cell DNA MeTase. Chromatography of a 0.3 M NaCl extract of MEL cell nuclei on DEAE-Sephacel removes endogenous nucleic acids and brings about a 13-fold increase in the specific activity of DNA MeTase. Chromatography of the single symmetrical peak of activity from DEAE-Sephacel on Cibachron blue F3GA-agarose resulted in the resolution of two activities (Fig. 1). The first activity (DNA MeTase I), which eluted at approximately 0.35 M NaCl, appeared to be correlated with a polypeptide with an apparent M_r between that of β -galactosidase (116,000) and that of myosin heavy chain (200,000). The second activity (DNA MeTase II), which eluted at approximately 0.55 M NaCl, could be correlated with a polypeptide of slightly higher M_r .

Further purification of the two separated activities was achieved by chromatography on hydroxyapatite and double-stranded DNA-cellulose, leading to an essentially homogeneous preparation of DNA MeTase I (Fig. 2). DNA MeTase II, however, still contained some smaller polypeptides after purification by this procedure, but the activity correlated with a single high molecular weight polypeptide (Fig. 3). NaDodSO₄/polyacrylamide gel electrophoresis of the reduced and denatured proteins indicated M_r s of 150,000 for DNA MeTase I and 175,000 for DNA MeTase II by reference to the β' (M_r 165,000) and β (M_r 155,000) subunits of *E. coli* RNA polymerase. Purification of each species was about 600-fold with respect to the specific activity of the extract applied to the DEAE-Sephacel column. The M_r 150,000 and 175,000 proteins were the only ones invariably associated with DNA MeTase activity, although a stoichiometric amount of a much smaller subunit would not have been detected. Large losses of both enzymes during purification [largely due to the formation of insoluble aggregates (14)] mean that only small amounts of purified material can be

FIG. 1. Separation of two DNA MeTase activities by chromatography on Cibachron blue-agarose. Pooled active fractions from a DEAE-Sephacel column were passed over a column of Cibachron blue-agarose and eluted with a 0.1–1.0 M NaCl gradient. A portion of alternate fractions was tested for DNA MeTase activity and a second portion was analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. Distinct peaks of activity eluted at 0.35 and 0.55 M NaCl; > indicate stained bands whose intensities correlate with the amounts of enzymatic activity in corresponding fractions. Protein standards along left ordinate are (in order of decreasing M_r): myosin heavy chain, β -galactosidase, phosphorylase B, bovine serum albumin, and ovalbumin.

FIG. 2. Purification of DNA MeTase I (eluted from Cibacron blue-agarose at 0.35 M NaCl) by sequential chromatography on hydroxyapatite and double-stranded (ds) DNA-cellulose. Conditions for analysis of polypeptide composition and enzyme activity were as in Fig. 1. >, Polypeptide invariably associated with DNA MeTase I activity.

obtained; in addition, DNA MeTase II was inactive after storage at -40°C for 2–3 weeks.

Sequence Specificity. Incubation of MEL cell DNA MeTase I and II with *E. coli* DNA and [*methyl*- ^3H]AdoMet produced exclusively m^5C , as established by TLC of formic acid-hydrolyzed DNA (data not shown). Methylation of cytosine residues in mammals has been shown to be dependent on the presence of a 3' guanosine residue both *in vivo* (1) and *in vitro* (19). Synthetic DNAs that contained cytosine but not guanosine could not serve as substrates for either MEL cell DNA MeTase (Table 1).

The influence of the nucleotides 3' and 5' to CpG doublets on methyl-accepting activity was estimated by correlating sequence and methyl-accepting activity of 28 DNA fragments of known sequence derived from *Hae* III cleavage of ϕX174 replicative form DNA, *Hae* III cleavage of pLP32 DNA, and *Alu* I cleavage of pBR322 DNA. Endonuclease cleavage was performed after methylation of superhelical DNA in the presence of [*methyl*- ^3H]AdoMet, and only fragments that could be clearly resolved by gel electrophoresis were used in the calculations. Contents of CpG doublets and their neighboring sequences were obtained by inspection of the published sequences of ϕX174 (26) and pBR322 (27). Plasmid pLP32 is pBR322 with (dG-dC)₁₆ cloned into the *Bam*HI site (28).

FIG. 3. Purification of DNA MeTase II (eluted from Cibacron blue-agarose at 0.55 M NaCl). All conditions were as in Fig. 2. >, Polypeptide invariably associated with DNA MeTase activity in this material. In this and the preceding figure, the proteins in the DNA-cellulose flow-through volume were present at a concentration too low to be detected by Coomassie blue staining.

Table 1. Activity of DNA MeTase I and II on synthetic polydeoxyribonucleotides

Substrate	Activity	
	DNA MeTase I	DNA MeTase II
Poly(dG-dC)	2.1	2.4
Poly(dG-dC, dm ⁵ C)*	70.1	105.4
Poly(dC)	0.003	0.001
Poly(dC, dA)	0.003	0.001

Activities are expressed as pmol of CH₃ transferred per hr per nmol of cytosine.

* Contained 80 mol % m^5C .

Fig. 4 shows that most of the methyl-accepting activity of the 28 restriction fragments can be explained by their content of CpG dinucleotides alone ($r = 0.86$; $P < 0.01$). The least-squares line fitted to the data passes through a point near the origin; this indicates that sites other than CpG are not efficient substrates for DNA MeTase. The *Hae* III fragment of pLP32 containing the (dG-dC)₁₆ insert was not used in fitting the least-squares line to the data of Fig. 4 but its point fell close to the line.

It has been demonstrated that methylated CpG sites in bovine satellite I DNA tend to be located within short self-complementary sequences (3). DNA MeTase I did not show a preference for such sites when acting on unmethylated DNA *in vitro*;

FIG. 4. Rate of methylation of 28 DNA fragments of defined sequence as a function of CpG content. Intact superhelical phage or plasmid DNA was labeled by incubation with [*methyl*- ^3H]AdoMet and DNA MeTase I to a specific activity of about 5×10^4 cpm/ μg (less than 6% of saturation) and then cleaved with restriction endonuclease. Incorporation of radioactivity into individual DNA fragments was measured after separation by gel electrophoresis. The line was fitted to the data by the method of least squares; the correlation coefficient r for these data is 0.86. The median length of the fragments shown was 267 base pairs and median CpG content was 16 per fragment. ●, *Hae* III fragments of pLP32 DNA; ○, *Alu* I fragments of pBR322 DNA; +, *Hae* III fragments of ϕX174 replicative form DNA. DNA MeTase II gave the same results with *Alu* I-cleaved pBR322 and with *Hae* III-cleaved ϕX174 replicative form DNA; it was not tested on pLP32 DNA. The methyl-accepting activity of the *Hae* III fragment of pLP32 that contains the (dG-dC)₁₆ insert is predicted by the least-squares line (this fragment was not used in the calculations).

a plot of specific methyl-accepting activity (cpm per CpG) vs. proportion of CpG sites in 4-base-pair palindromic sequences (C-C-G-G, T-C-G-A, A-C-G-T, and G-C-G-C) for the 28 fragments of Fig. 4 indicated a lack of significant correlation ($r = 0.41$; $P = 0.15$), and two fragments with all CpG sites outside of palindromic sequences showed greater than average methyl-accepting activity. Partially purified DNA MeTase from HeLa cells has been reported to methylate preferentially the sequence C/GpCpG in HeLa cell DNA (18). There was no correlation ($r = -0.12$) between content of this sequence in ϕ X174, pBR322, or pLP32 DNA fragments and methyl-accepting activity for DNA MeTase I. The (dG-dC)₁₆ insert in pLP32 is present in the left-handed or Z form at high levels of negative superhelicity (28). However, incorporation of methyl groups into the fragment bearing the (dG-dC)₁₆ insert relative to the remainder of the molecule was not dependent on superhelicity (unpublished data).

Double-stranded poly(dG-dC, dm⁵C) containing 80 mol % m⁵C was the kind gift of Barry Rosen and Alexander Rich (Massachusetts Institute of Technology). The polymer was calculated to contain 4% unmethylated CpG sites, 64% doubly methylated sites, and 32% hemimethylated CpG sites (assuming random incorporation of C and m⁵C during synthesis). Unmethylated cytosine residues in poly(dG-dC, dm⁵C) were methylated 30- to 50-fold more efficiently (Table 1) than unmethylated cytosines in poly(dG-dC). Saturation of CpG sites *in vitro* within microgram amounts of unmethylated DNA does require prolonged incubation times with repeated additions of enzyme and AdoMet (data not shown).

Preferential Methylation of Longer DNA Chains. The initial rate of methylation of *Hae* III fragments of ϕ X174 replicative form DNA is dependent on fragment size, CpG sites within longer fragments being methylated more readily than those within shorter fragments (Fig. 5). This is a result of differences in chain length and not of intrinsic differences in methyl-accepting activity among regions of the DNA because cleavage after methylation revealed only a 2-fold or smaller variation in extent of labeling among the fragments. The same influence of DNA chain length on methyl-accepting activity was seen when *Alu* I fragments of pBR322 were analyzed in the same fashion (data not shown).

Mammalian DNA MeTases are inhibited by NaCl concentrations >0.05 M (14) but become resistant if enzyme and DNA are preincubated in the absence of salt (20). This indicates a two-step reaction mechanism in which the first step (the association of DNA and enzyme) is salt-sensitive but the action of the complex is relatively salt-resistant (20). Fig. 5 shows that the length dependence of the methyltransferase reaction is not abolished by increasing the NaCl concentration to 0.15 M (although total incorporation of radioactivity is decreased by a factor of 1/3); this indicates that length dependence is conferred during the second or salt-resistant step of the reaction. The unusually high methyl-accepting activity of the 1,307-base-pair fragment (more pronounced with DNA MeTase II) is unexplained but does not seem to be a result of special sequence features because it is not heavily labeled when methylation is performed prior to endonuclease digestion (dashed lines in Fig. 5).

DISCUSSION

The two species of DNA MeTase described here are probably closely related in view of their similar physical and enzymatic properties, although it is not known that they play the same role in the cell. Hypomethylation of DNA occurs during the erythroid differentiation of MEL cells (29), and it will be of interest to determine if there are associated changes in the number or

FIG. 5. Length-dependent methylation of *Hae* III-cleaved ϕ X174 replicative form DNA. DNA fragments were labeled by incubation with [*methyl*-³H]AdoMet and DNA MeTase I (A) or DNA MeTase II (B) at different NaCl concentrations. Incorporation of radioactivity was determined after gel electrophoresis. The shape of the curves was not altered by changing AdoMet concentrations over the range 0.2–20 μ M. $\circ$, No added NaCl; $\bullet$, 50 mM NaCl; $\times$, 150 mM NaCl.

relative amounts of DNA MeTase.

Because synthetic duplex poly(dG-dC) is methylated at a high rate by both MEL cell DNA MeTases, it is unlikely that either enzyme has nearly absolute sequence specificity of the type exhibited by bacterial restriction MeTases. This is confirmed by the observation that the relative methyl-accepting activity of restriction fragments of ϕ X174 replicative form DNA and pBR322 DNA can be predicted from CpG content (Fig. 4). The correlation is not perfect, however, and it is likely that initial rates of methylation are dependent on sequence to some extent. Hemimethylated sites in duplex poly(dG-dC, dm⁵C) are methylated at a rate about 30–100 times greater than unmethylated sites in poly(dG-dC) (Table 1); mouse ascites cell DNA MeTase has been reported to be about 100-fold more active on hemimethylated ϕ X174 replicative form DNA (19). It should be pointed out that the observed residual activity (1–3%) exhibited toward unmethylated CpG sites would be sufficient to alter methylation patterns with cycles of DNA replication if no other constraints are operative within the cell; therefore, separate "maintenance" and "de novo" MeTases need not be postulated.

Regulatory proteins that must locate a specific sequence element in a large background of nonspecific DNA often show a search efficiency that is much higher than the predicted diffusion-limited rate. This can be accounted for by postulating a

two-step reaction mechanism in which the first step is the diffusion-limited association of DNA and protein and the second is the one-dimensional diffusion of protein along the DNA chain in a process termed "facilitated transfer" (30). *EcoRI* (31) and *lac* repressor (30) have been calculated to scan about 10^3 base pairs per second per molecule by this mechanism, increasing the kinetic efficiency of site location by at least 2 orders of magnitude over the diffusion-limited rate. *E. coli* RNA polymerase shows similar properties (32).

Facilitated transfer typically leads to an increasing efficiency of site location with increasing chain length, contradicting the expectation for a diffusion-limited reaction (30–32). This results primarily because of the increased frequency with which bound protein slides off the ends of short chains and because (in highly dilute solution) the dissociation of bound protein from long chains is more likely to lead to prompt reassociation due to the higher microscopic concentration of DNA when the protein is in the vicinity of long rather than short DNA chains (30).

As demonstrated here, MEL cell DNA MeTases show just such a dependence on DNA chain length. In addition, the mammalian DNA MeTase reaction occurs in two steps, as shown by the resistance of the methylation reaction to moderate ionic strengths when salt is added to reactions in progress, even when the final ionic strength is high enough to inhibit methylation if present at the beginning (20, 33). This has been interpreted to indicate a salt-sensitive association of protein and DNA to form a salt-resistant complex. Bound DNA MeTase would then travel along the DNA chain in what was termed a "processive" mode (20), which usually connotes an energy-requiring unidirectional movement of protein along the DNA chain (30). Facilitated transfer, in which the protein slides along the DNA in a one-dimensional random walk driven by thermal motion, is a more plausible explanation.

Mammalian DNA MeTase does not appear to have an intrinsic dependence on the sequence context of CpG sites, yet specific methylation patterns are established *in vivo*. Chromatin structure may play a major role in this process, although direct interaction of DNA MeTase with sequence elements characteristic of the cell of origin are possible; because the enzyme locates its recognition sequence by a mechanism involving facilitated transfer, these regulatory features could be anywhere in the vicinity of the affected CpG sites. One way in which specific methylation patterns might be maintained is through specific features in chromatin that would impede the translocation of DNA MeTase along the DNA chain, either by inducing dissociation of the DNA-protein complex or by hindering the progress of the enzyme. CpG sites in the vicinity of such features would be relatively resistant to methylation. The DNA of herpesvirus and simian virus 40 has been reported to be refractory to methylation *in vitro* (14, 34) and perhaps contains DNA sequences that have this effect. Alternatively, certain sequence features or chromatin proteins might enhance rates of *de novo* methylation of CpG sites in their vicinity. Either possibility could account for the clustered arrangement of methylated CpG sites in bovine satellite I DNA (3). Testing these

conjectures requires more cases in which the detailed distribution of m^5C residues along specific DNA segments is known and requires a test of the ability of DNA MeTase from the homologous source to reproduce the pattern on the unmethylated (cloned) DNA.

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- Hattman, S. (1981) in *The Enzymes*, ed. Boyer, P. (Academic, New York), 3rd. Ed., Vol. 14, pp. 517–548.
- Wigler, M. (1981) *Cell* **24**, 285–286.
- Sano, H. & Sager, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3584.
- Razin, A. & Friedman, J. (1981) *Prog. Nucleic Acid Res. Mol. Biol.* **25**, 817–828.
- Bird, A. & Southern, E. (1978) *J. Mol. Biol.* **118**, 27–47.
- Mandel, J. & Chambon, P. (1979) *Nucleic Acids Res.* **7**, 2081–2103.
- van der Ploeg, L. & Flavell, R. (1980) *Cell* **19**, 947–958.
- Sheffrey, M., Rifkind, R. & Marks, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1180–1184.
- Haigh, L., Blanchard, B., Hellewell, S. & Ingram, V. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5332–5336.
- McGeady, M., Jhappen, C., Ascione, R. & VandeWoude, G. (1983) *Mol. Cell. Biol.* **3**, 305–314.
- Stein, R., Razin, A. & Cedar, H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3418–3422.
- Wigler, M., Levy, P. & Perucho, M. (1981) *Cell* **24**, 33–40.
- Groudine, M., Eisenman, R. & Weintraub, H. (1981) *Nature (London)* **292**, 311–317.
- Adams, R., McKay, E., Craig, L. & Burdon, R. (1979) *Biochim. Biophys. Acta* **561**, 345–357.
- Gruenbaum, Y., Stein, R., Cedar, H. & Razin, A. (1981) *FEBS Lett.* **142**, 67–71.
- Kalousek, F. & Morris, N. (1968) *J. Biol. Chem.* **243**, 2440–2442.
- Sneider, T., Teague, W. & Rogachevsky, L. (1975) *Nucleic Acids Res.* **2**, 1685–1700.
- Roy, P. & Weissbach, A. (1975) *Nucleic Acids Res.* **2**, 1669–1683.
- Gruenbaum, Y., Cedar, H. & Razin, A. (1982) *Nature (London)* **295**, 620–622.
- Taylor, S. & Jones, P. (1982) *J. Mol. Biol.* **162**, 679–692.
- Noguchi, H., ver Reddy, G. & Pardee, A. (1983) *Cell* **32**, 443–451.
- Clewell, P. (1972) *J. Bacteriol.* **110**, 667–672.
- Marks, P. & Rifkind, R. (1978) *Annu. Rev. Biochem.* **47**, 419–448.
- Bonner, W. & Stedman, J. (1978) *Anal. Biochem.* **89**, 247–256.
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
- Sanger, F., Air, G., Barrell, B., Brown, U., Coulson, A., Fiddes, J., Hutchison, C., Slocumbe, P. & Smith, M. (1977) *Nature (London)* **265**, 687–695.
- Sutcliffe, G. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 77–90.
- Peck, L., Nordheim, A., Rich, A. & Way, J. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4560–4564.
- Christman, J., Weich, N., Schoenbrun, N. & Acs, G. (1980) *J. Cell Biol.* **86**, 366–370.
- Berg, O., Winter, R. & von Hippel, P. (1981) *Biochemistry* **20**, 6929–6948.
- Jack, W., Terry, B. & Modrich, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4010–4014.
- Park, C., Hellel, Z. & Wu, L. (1982) *J. Biol. Chem.* **257**, 6944–6949.
- Drahovsky, D. & Morris, N. (1971) *J. Mol. Biol.* **61**, 343–356.
- Simon, D., Grunert, F., Acken, V., Doring, H. & Kroger, H. (1978) *Nucleic Acids Res.* **5**, 2152–2167.