Analogous cleavage of DNA by micrococcal nuclease and a 1,10-phenanthroline-cuprous complex

Bret Jessee, Giuseppe Gargiulo, Farzana Razvi and Abraham Worcel

Department of Biology, Hutchison Hall, University of Rochester, Rochester, NY 14627, USA

Received 21 June 1982; Revised and Accepted 9 September 1982

ABSTRACT

We have examined the DNA cleavage specificity of a 1,10-phenanthroline-cuprous complex and find this agent to recognize the same sites and cleave with the same relative preferences as micrococcal nuclease. In contrast, DNase I and bleomycin-ferrous complex cleave the same 5000 bp *D. melanogaster* histone gene DNA with different specificities, although some of the sites appear to be recognized and cleaved by all four reagents. Our results suggest that the reagents used probably detect discrete conformational perturbations along the DNA double helix.

INTRODUCTION

Micrococcal nuclease and DNase I are two enzymes which have been extensively used to probe chromatin structure. Micrococcal nuclease has long been used to generate the basic subunit of chromatin, the nucleosome (1) and has been more recently employed to demonstrate the phasing of nucleosomes along chromatin DNA sequences (2-6). DNase I has found use in attempts to resolve the linking number paradox, questioning whether the helical periodicity of DNA changes as it traverses the internucleosomal linker onto the nucleosome itself (7) and has also been shown to preferentially digest transcriptionally active chromatin in general (8) and at particularly hypersensitive sites (9).

In spite of their widespread use as probes for chromatin, the mechanism of action of these nucleases is not known. While both enzymes have been studied intensively at the levels of protein structure and enzymatic properties (10-12), the nature of the enzyme-DNA interactions which result in a site-specific cleavage is unknown. Micrococcal nuclease exhibits a marked specificity in the digestion of DNA, as has been recently demonstrated by the use of end-labeled DNA substrates and high resolution sequencing gels (13,14). DNase I has also been reported to cleave DNA in a sequence specific manner, preferring the same sites as micrococcal nuclease but cleaving with a wider range of sensitivity.

The substrate specificities of both micrococcal nuclease and DNase I have been investigated. Micrococcal nuclease displays an "A-T" preference, cleaving most frequently between T and A residues, possibly reflecting the poor base overlap of the 5'T-A 3' stack (15). While the enzyme hydrolyzes single-stranded polynucleotides more rapidly than it does double-stranded ones (16), the supposition that a single-stranded character of AT rich duplexes confers susceptibility to cleavage is challenged by the observation that S1 nuclease recognizes sequences dissimilar to those recognized by micrococcal nuclease (14). Micrococcal nuclease does, however, cleave supercoiled pBR322 preferentially at the location of an inverted repeat recognized by S1 nuclease (14,17,18), implying that micrococcal nuclease recognizes, not so much the primary sequence of DNA per se, but a particular structural conformation imposed upon the DNA by its sequence.

Our laboratories' continued interest in agents used to probe chromatin structure lead us to experiment with a 1,10-phenanthroline-cuprous complex $[(OP)_2\cdot Cu(I)]^1$ which has been shown to cleave poly (dA-dT) in an oxygen-dependent reaction (19). The 1,10-phenanthroline molecule shares the same tricyclic phenanthridinium ring system as ethidium bromide (EB)¹, a known intercalating agent (20); in fact, EB is a potent inhibitor of $(OP)_2\cdot Cu(I)$ -mediated DNA cleavage (21). These observations, as well as the strong, if not absolute preference for double-stranded DNA as compared to single-stranded DNA as a substrate for cleavage (21), imply that $(OP)_2\cdot Cu(I)$ intercalates into DNA during the course of reaction.

Extending previous studies, which had suggested that $(OP)_2\cdot Cu(I)$ cleaves DNA with little sequence specificity (21), we find that this complex cleaves DNA with a specificity remarkably similar to that of micrococcal nuclease. Furthermore, our experiments show that the degree of cleavage preference at any individual site on DNA by micrococcal nuclease is, to a great extent, mirrored by $(OP)_2\cdot Cu(I)$. Additionally, we find that although DNase I preferentially cleaves DNA at many of the same sites as micrococcal nuclease, it also cleaves at many more sites--the micrococcal nuclease sites representing only a subset of the DNase I sites.

This study also examines the specificity of DNA cleavage by a bleomycin-ferrous complex $[BLM\cdot Fe(II)]^1$. The antitumor drug bleomycin bears a bithiazole-containing moiety and a cationic terminus thought to be responsible for the binding of this molecule to DNA in an intercalative or partially intercalative fashion, as evidenced utilizing NMR and fluorescence spectroscopy (22). Reports indicate preferential attack on purine-pyrimidine

(3'---5') sequences, and pyrimidine-pyrimidine (3'---5') sequences with cleavage of all (5'---3') GT and GC sequences (23,24). In our hands BLM•Fe(II) cleaves DNA at sites bearing only a minor relationship to those cleaved by micrococcal nuclease.

MATERIALS AND METHODS

The mapping of enzyme and chemical cleavage sites on DNA was performed as described in the legends of Figures 1 to 5 using ^{32}P end-labeled 5.0 kb histone gene repeat of *D. melanogaster* which was prepared as follows: cDm506 DNA (25), which contains slightly over one copy of the major 5.0 kb histone gene repeat inserted into the ColEI plasmid, was digested with either Hind III or Bam HI and the DNA was electrophoresed in a 1% agarose slab gel. The 5.0 kb histone gene DNA was recovered from the agarose by electroelution (26) and purified by DEAE-cellulose chromatography (27). The 5' phosphate groups were removed with calf intestine alkaline phosphatase and replaced with ^{32}P using γ - ^{32}P -ATP and T4 polynucleotide kinase (28). End-labeled DNA was purified by phenol/chloroform extraction and ethanol precipitation, and passed over a Sephadex G-50 column to remove ATP. DNA at a concentration of 5 $\mu\text{g}/\text{ml}$ was ligated at 4°C with T4 DNA ligase, concentrated by butanol extraction (29), and purified by phenol/chloroform extraction and ethanol precipitation.

For sequencing gel analysis, a short fragment of the 5.0 kb histone gene repeat was prepared by cleaving this DNA with Ava I to yield three fragments. A 1056 bp fragment was separated by agarose electrophoresis, purified and end-labeled with γ - ^{32}P -ATP, as described previously. This DNA was then digested with Hinf I to yield radiolabeled fragments 381 bp and 252 bp in length which were purified from agarose as before. The 252 bp fragment extending from the Ava I site depicted in Figure 1 toward the H4 gene was sequenced by the Maxam and Gilbert methods (28) and the same DNA digested with micrococcal nuclease or $(\text{OP})_2\text{Cu(I)}$, as described in Figure 5, was electrophoresed along side.

Micrococcal nuclease was obtained from Worthington and DNase I from Sigma. Aldrich supplied 1,10-phenanthroline, which was prepared in an active nucleolytic form $[(\text{OP})_2\text{Cu(I)}]$ by dissolving 1,10-phenanthroline, CuSO_4 and 3-mercaptopropionic acid to final concentrations of 40 μM , 4 μM and 14 mM in 50 mM $\text{Tris}(\text{OAc})_2$ -5 mM $\text{Mg}(\text{OAc})_2$ -1 mM $\text{Ca}(\text{OAc})_2$ pH 8.5. Activated bleomycin [BLM•Fe(II)] was prepared by dissolving Bleomoxane, the sulfates of a number of bleomycin analogues obtained from Bristol Laboratories, in the above buffer containing, in addition, 10 μM $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$.

RESULTS

(OP)₂•Cu(I) Cleaves DNA Non-randomly

To determine whether (OP)₂•Cu(I) cleaves DNA at any site preferentially, we have utilized a closed circular 5.0 kb DNA molecule carrying the histone gene repeat of *D. melanogaster* labeled with ³²P at the Hind III site (see Materials and Methods) as a substrate. Figure 1 shows the autoradiogram of an agarose gel electrophoretic separation of the DNA fragments produced by (OP)₂•Cu(I) cleavage compared to the known micrococcal nuclease cleavage pattern of this substrate DNA (4). The fragments have been digested with Bam HI, which cleaves at a unique site 68 bp away from the labeled Hind III site (25), to yield molecules with a common Bam HI end. The molecular weight markers depicted in all autoradiograms in this report are internal restriction fragments of the same end-labeled 5.0 kb DNA used in cleavage assays which allows unambiguous location of cleavage sites on this substrate.

Immediately obvious is the fact that (OP)₂•Cu(I) cleaves the substrate with definite site specificity. Even more striking is the appearance that (OP)₂•Cu(I) cleaves this DNA at locations remarkably similar to the sites of micrococcal nuclease cleavage. To more clearly compare the sites of micrococcal nuclease and (OP)₂•Cu(I) cleavage, samples of DNA cleaved with each agent were electrophoresed side-by-side.

(OP)₂•Cu(I) and Micrococcal Nuclease Cleave DNA at Similar Sites

Shown in Figure 2 is the autoradiogram of a side-by-side analysis of (OP)₂•Cu(I) and micrococcal nuclease on DNA labeled, in this case, at the Bam HI site and aligned by cleavage at the Hind III site. No bands appear in the micrococcal nuclease cleavage pattern which do not appear in the (OP)₂•Cu(I) cleavage pattern. Some variation in the frequency of cleavage by the two agents at particular locations on the DNA can be discriminated but on the whole micrococcal nuclease and (OP)₂•Cu(I) seem to cleave at a given site with equivalent frequencies. An equitable comparison in terms of extent of DNA cleavage can be made between the 2 min. micrococcal nuclease sample and the 15 min. (OP)₂•Cu(I) sample in Figure 2. Major sites of cleavage for both agents map to the H1-H3 intergenic spacer, the 5' ends of H3 and H4 histone genes and the H4-H2A intergenic spacer, as well as to other sites as shown.

The almost identical cleavage pattern by micrococcal nuclease and (OP)₂•Cu(I) lead us to question whether other agents cleave the substrate DNA in a similar manner and, indeed, whether any agent could be found which cleaves the DNA with a different specificity. As shall next be discussed, we found two agents, DNase I and a bleomycin-ferrous complex, which cleaved our

Figure 1. Mapping of micrococcal nuclease and $(OP)_2 \cdot Cu(I)$ cleavage sites on ^{32}P -Hind III end-labeled 5 kb histone gene repeat of *D. melanogaster*.

Naked DNA with a label at the single Hind III site in the circularized molecule (see Materials and Methods) was cleaved with either micrococcal nuclease or $(OP)_2 \cdot Cu(I)$. Micrococcal nuclease reactions containing 20 ng ^{32}P -DNA, 50 ug carrier DNA, 100 mM Tris-Acetate 5 mM Mg-Acetate pH 7.4, 1 mM $CaCl_2$ and 0.04 U micrococcal nuclease in volumes of 20 ul were incubated at 25°C for 0, 2, 5 and 10 min., and terminated by adding EGTA to 10 mM. The $(OP)_2 \cdot Cu(I)$ reactions containing 20 ng ^{32}P -DNA, 50 ug carrier DNA, 20 uM 1, 10-phenanthroline, 2 uM $CuSO_4$, 7 mM 3-mercaptopropionic acid (see Materials and Methods) and 100 mM Tris-Acetate 5 mM Mg-Acetate pH 7.4 in volumes of 8 ul were incubated at 25°C for 0, 20, 30, 45 and 60 min., and terminated by adding 2 ul of a 1.5 mM neocuproine 25 mM EDTA solution. After ethanol precipitation the DNA fragments were cleaved with Bam HI, which also cleaves at a single site in the circularized molecule to yield fragments with a common Bam HI end 68 bp upstream from the ^{32}P -Hind III site. Electrophoresis on 1% agarose slab gels and autoradiography reveals the sites of DNA cleavage. Molecular weight markers were obtained by cleaving the same ^{32}P -DNA with the following restriction enzymes listed in order of decreasing molecular weight: Bam HI, Xho I+Bam HI, Ava I+Bam HI, Sst I+Bam HI, Hpa I+Bam HI, Hae II, Bgl II+Hind III and Pst I+Hae II.

DNA with varying degrees of dissimilarity from micrococcal nuclease and $(OP)_2 \cdot Cu(I)$.

DNase I and BLM·Fe(II)-mediated Cleavage of DNA

The autoradiogram shown in Figure 3 illustrates the non-random double-strand scission mediated by DNase I and BLM·Fe(II). The variation in

Figure 2. Comparison of micrococcal nuclease and $(OP)_2Cu(I)$ cleavage sites on ^{32}P -Bam HI end-labeled 5 kb histone gene repeat of *D. melanogaster*.

Naked DNA labeled at the single Bam HI site in the circularized molecule (see Materials and Methods) was cleaved with either micrococcal nuclease or $(OP)_2Cu(I)$, as described in the legend of fig. 1, for the times indicated. Cleavage of the DNA fragments with Hind III, which also cleaves at a single site in the circularized molecule, yields fragments with a common Hind III end 68 bp downstream from the ^{32}P -Bam site. Slab gel electrophoresis in 1% agarose and autoradiography was performed utilizing as molecular weight markers the same ^{32}P -DNA cleaved with the following restriction enzymes listed in order of decreasing molecular weight: Hind III, Hpa I+Hind III, Sst I+Hind III, Ava I+Hind III, Hae II, Bgl II+Hind III, Pst I+Hae II and Mbo II+Hae II.

cleavage frequencies along the DNA is quite broad for $BLM \cdot Fe(II)$. The variation in cleavage frequencies along the DNA is quite broad for $BLM \cdot Fe(II)$, while DNase I appears to cleave preferred regions with roughly the same frequencies. The more frequent cleavage by $BLM \cdot Fe(II)$ in the H2A-H4 spacer region and to the 3' end of the H3 gene are consistently reproducible. At very mild points of digestion, as represented by the first BLM lane in Figure

Figure 3. Mapping of DNase I and BLM·Fe(II) cleavage sites on ^{32}P -Hind III end-labeled 5 kb histone gene repeat of *D. melanogaster*.

DNA labeled at the Hind III site (see fig. 1) was cleaved in 100 μl reaction volumes containing 5 ng ^{32}P -DNA, 5 μg carrier DNA, 25 mM Tris-Acetate 10 mM Mg-Acetate 2 mM Ca-Acetate pH 8.5 and either BLM·Fe(II) (0,10,100 and 200 $\mu\text{g}/\text{ml}$; see Materials and Methods) or DNase I (0.001 and 0.003 U/ml) at 25°C for 10 min. After addition of EDTA to 10 mM to terminate the reaction, the DNA fragments were ethanol precipitated, cleaved with Bam HI and electrophoresed with molecular weight markers, as described in the legend of fig. 1. Autoradiography shows the sites of DNase I and BLM·Fe(II) cleavage as aligned from the Bam HI site.

3, when all bands are likely to be produced by a single cleavage event, the H2A-H4 spacer region cleavages already predominate. More extensive digestion, where bands are likely to be produced by multiple cleavage events, exhausts the supply of higher molecular weight fragments but, as shown in BLM lane 3, the same set of cleavage products is detected as before. Thus qualitative comparisons in cleavage patterns produced by the nucleolytic agents used in this report can be made between all but the most extensive digestions of DNA.

To highlight the similarities and differences in cleavage of DNA by micrococcal nuclease, DNase I and BLM·Fe(II), DNA labeled at the Hind III site was cleaved with these agents and electrophoresed side-by-side (Figure 4). A more extensive digestion by micrococcal nuclease shows the predominant cleavages to the 5' of the H1 gene, which are not detected at any extent of

Figure 4. Comparison of the sequence specificities of micrococcal nuclease, DNase I and BLM·Fe(II).

DNA labeled at the Hind III site (see fig. 1) was cleaved under conditions described in fig. 3 with either 0.05 U/ml micrococcal nuclease, 0.001 U/ml DNase I, or 100 ug/ml BLM·Fe(II), digested with Bam HI to align the cleavage sites, electrophoresed and autoradiographed as described in fig. 1.

digestion by BLM·Fe(II) in Figure 3. Major cleavage sites of micrococcal nuclease as seen in Figures 1 and 4 map to approximately the same locations as DNase I cleavages on the same DNA substrate, although some differences are visible. DNase I and BLM·Fe(II) cleavages map to roughly the same broad regions, excepting the area 5' of gene H1 previously noted. A close examination of Figures 3 and 4, however, shows that particular bands in the DNase I and BLM·Fe(II) patterns do not align precisely, vary in intensity or appear to be missing completely. Given that the limit of resolution in agarose gels may be ± 20 -50 bp, depending on the location of the band on the gel, a minor variation on these gels indicates a major variation when the exact base pair(s) cleaved is considered.

Sequencing of Micrococcal Nuclease and $(OP)_2Cu(I)$ DNA Cleavage Sites

To determine whether the identity of micrococcal nuclease and $(OP)_2Cu(I)$ cleavage sites holds up to the actual phosphodiester bond(s) involved, we electrophoresed the cleaved DNA on high resolution sequencing gels. The

Figure 5. Sequencing of micrococcal nuclease and $(\text{OP})_2\text{Cu(I)}$ cleavage sites.

A 252 bp fragment of the 5.0 kb histone gene repeat was isolated and labeled at the 5' end provided by *Ava* I digestion (see Materials and Methods). Digestions of DNA were carried out in 100 μl volumes of 25 mM Tris-Ac 5 mM Mg-Ac 1 mM CaAc pH 8.5 for 10, 15 or 20 min (left to right) containing 5 μg sheared calf thymus DNA and 5 ng γ ^{32}P -ATP-labeled DNA and either 0.005 U micrococcal nuclease or 100 μM 1,10-phenanthroline, 10 μM CuSO_4 and 10 mM 3-mercaptopropionic acid. Reactions were terminated with 10 mM EDTA and ethanol precipitation. After extraction of the resuspended DNA with *n*-butanol the samples were precipitated three times with ethanol before electrophoresis. The Maxam and Gilbert chemical degradation is shown in the G, G+A, T+C and C channels. M, micrococcal nuclease generated fragments; P, phenanthroline generated fragments. The numbers on the left of the figure represents the length of the DNA fragments in bases from the ^{32}P labeled *Ava* I site. Sequences on the right shows the most commonly cleaved micrococcal nuclease (arrow pointing down) or $(\text{OP})_2\text{Cu(I)}$ sites (arrow pointing up).

products of degradation of a 5' end labeled 252 bp DNA fragment by micrococcal nuclease and $(\text{OP})_2\text{Cu(I)}$ are shown in Figure 5 along with the Maxam and Gilbert sequencing of this DNA. The DNA is labeled at the *Ava* I site depicted

in Figure 1 and the sequence extends toward the H4 gene. All of the fragments seen in the portion of gel shown in Figure 5 are greater than one half of the length of the complete fragment and arise from mild to moderate digestions; one expects these fragments to be produced by mainly single cleavage events.

Under these mild conditions of digestion, both $(OP)_2Cu(I)$ and micrococcal nuclease produce a discrete set of bands, while at more extensive digestion conditions a large number of lower molecular weight bands are produced (results not shown). As indicated in Figure 5, the most prominent micrococcal nuclease (M) sites (the three TATC sites in this particular fragment) are also cleaved by phenantroline (P). Some of the secondary micrococcal nuclease sites are also cleaved by $(OP)_2Cu(I)$ (TGTG, TAAC) while some are not (CTGC, ATCC). Although more sequences will have to be examined for a meaningful analysis, it is already clear that both reagents can cleave the same phosphodiester bond at some highly preferred sites.

DISCUSSION

The main observation to be gleaned from this study is that the relatively small three-ringed chemical agent $(OP)_2Cu(I)$ seems to cleave DNA at the same sites cleaved by micrococcal nuclease, a 16800 dalton enzyme (30). $(OP)_2Cu(I)$ cleaves an end-labeled substrate with approximately the same pattern of cleavage intensity as micrococcal nuclease, which shows that not only do $(OP)_2Cu(I)$ and micrococcal nuclease cleave at the same sites on DNA, but that they cleave any given site with the same relative degree of frequency. Our findings should serve as a warning to workers in the chromatin field that 1,10-phenantroline cannot be used as a "sequence-neutral" probe for nucleosome phasing. Similar results to ours have been independently obtained by Cartwright and Elgin (31).

The fact that $(OP)_2Cu(I)$, micrococcal nuclease, and to a lesser extent DNase I appear to recognize and cleave the same sites on the 5,000 bp DNA molecule, suggests that those agents may be detecting site specific perturbations along the linear double helix. As recently proposed (32), such discrete DNA conformational changes could also be recognized by the nucleosomal histones and other chromosomal proteins in their interaction with DNA.

What could be the nature of the DNA structural perturbations detected by $(OP)_2Cu(I)$ and micrococcal nuclease? The elucidation of the Z DNA structure of the alternating GC polymer by Rich and coworkers (33) has brought into focus the possibility that a number of different DNA structures may exist (see

Cold Spring Harbor Symp. of Quant. Biol. Vol. 47). Some, like the left handed Z DNA, are extreme departures from the Watson-Crick double helix, while many, no doubt, will be less dramatically different, but different nevertheless. A more extensive sampling of DNA sequences recognized by $(OP)_2\text{Cu(I)}$ and micrococcal nuclease is clearly needed before one could attempt to suggest possible consensus DNA structures recognized by those reagents.

ACKNOWLEDGEMENTS:

We thank Dr. Henry Sobell for his enlightening discussion and criticism and Dr. Thomas Krugh for his helpful suggestions. This work was supported by U.S.P.H.S. grant nos. 30332 and 30339.

¹Abbreviations: $(OP)_2\text{Cu(I)}$, 2:1 1,10-phenanthroline-cuprous complex; EB, ethidium bromide, BLM-Fe(II), bleomycin-ferrous complex.

REFERENCES

1. Kornberg, R. (1977) *Ann. Rev. Biochem.* 46, 931-954.
2. Wu, C. (1980). *Nature* 295, 854-860.
3. Louis, C., Schedl, P., Samal, B. and Worcel A. (1980). *Cell* 22, 387-392.
4. Samal, B., Worcel, A., Louis, C. and Schedl, P. (1981). *Cell* 23, 401-409.
5. Musich, P.P., Brown, F.L. and Maio, J.J. (1982). *Proc. Nat. Acad. Sci. USA* 79, 118-112.
6. Bryan, P.N., Hofstetter, H. and Birnstiel, M.L. (1982). *Cell* 27, 459-466.
7. Klug, A. and Lutter, L.C. (1981). *Nucl. Acids Res.* 9, 4267-4283.
8. Weintraub, H. and Groudine, M. (1976). *Science* 93, 894-853.
9. Elgin, S.C.R. (1981). *Cell* 27, 413-415.
10. Moore, S. (1981). *The Enzymes* 3rd edition, Vol. 14-A (Boyer, P.D. ed.) pp. 281-295, Academic Press, New York.
11. Cotton, F.A. and Hazen, E.E., Jr. (1971). *The Enzymes* 3rd edition, Vol 4 (Boyer, P.D. ed) pp.153-176, Academic Press, New York.
12. Anfinsen, C.B., Cutarecasas, P. and Taniuchi, H. (1971). *The Enzymes* 3rd edition, Vol. 4 (Boyer, P.D. ed.) pp. 177-204, Academic Press, New York.
13. Horz, W. and Altenburger, W. (1981). *Nucl. Acid Res.* 9, 2643-2658.
14. Dingwall, C., Lomonosoff, G.P. and Laskey, R.A. (1981). *Nucl. Acids Res.* 9, 2659-2673.
15. Klug, A., Jack, A., Viswamitra, M.A., Kennard, O., Shakked, Z. and Steitz, T.A. (1979). *J. Mol. Biol.* 131, 669-680.
16. Kacian, D.L. and Spiegelman, S. (1974). *Analyt. Bioch.* 58, 534-540.
17. Lilley, D.M.J. (1980). *Proc. Natl. Acad. Sci. USA* 77, 6468-6972.
18. Panayotatos, N. and Wells, R.D. (1981). *Nature* 289, 466-470.
19. Sigman, D.S., Graham, D.R., D'Aurora, V.D. and Stern, A.M. (1979). *J. Biol. Chem.* 254, 12269-12272.
20. Fuller, W. and Waring, M.J. (1964). *Ber. Bunsenges. Physik. Chem.* 68, 805.
21. Marshall, L.E., Graham, D.R., Reich, K.A. and Sigman D.S. (1981). *Biochem.* 20, 244-250.
22. Sakai, T.T., Riordan, J.M. and Glickson, J.D. (1982). *Biochem.* 21, 805-816.

23. D'Andrea, A.D. and Haseltine, W.A. (1978). *Proc. Natl. Acad. Sci. USA* 75, 3608-3612.
24. Takeshita, M., Kappen, L.S., Grollman, A.P., Eisenberg, M. and Goldberg, I.H. (1981). *Biochem.* 20, 7599-7606.
25. Goldberg, M.L. (1979). Ph.D. Thesis, Stanford University, Palo Alto, California.
26. McDonnell, M.W., Simon, M.N. and Studier, F.W. (1977). *J. Mol. Biol.* 110, 119.
27. Smith, H.O. and Birnstiel, M.L. (1976). *Nucl. Acids Res.* 3, 2387.
28. Maxam, A.M. and Gilbert, W. (1980). *Meth. Enzymol.* 65, 499.
29. Stafford, D.W. and Bieber, B. (1975). *Biochim. Biophys. Acta* 378, 19.
30. Eisenberg, D. (1970). *The Enzymes* 3rd edition, Vol. 1. (Boyer, P.D. ed) pp. 1-89.
31. Cartwright, I.L. and Elgin, S.C.R. (1981). *Nucl. Acids Res.* (in press).
32. Keene, M.A. and Elgin, S.C.R. (1981). *Cell* 27, 57-64.
33. Wang, A.H.-J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., van Boom, J.H., vander Marel, G. and Rich, A. (1979). *Nature* 282, 680-686.