

Approaches for the estimation of individual human susceptibility to methylated PAH need to be developed. With the basic metabolic patterns of the more tumorigenic members of the class having been established, it should be possible to develop sensitive assays to detect key metabolites or adducts in blood, urine, or exfoliated cells. Such assays are already available for benzo[a]pyrene-DNA adducts.<sup>66</sup> The biological sig-

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nificance of particular measurable adducts or metabolites, with respect to tumor formation, needs to be established in animal studies. These results can possibly be used to provide an index of human susceptibility to methylated PAH tumorigenesis.

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## Nuclease Activity of 1,10-Phenanthroline-Copper Ion

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DNA exhibits conformational variability that is dependent on base composition and environmental conditions. Linear DNA can adopt at least three distinct double-stranded helical forms which retain the fundamental Watson-Crick A-T and G-C base pairings. These include the A, B, and Z structures. Humidity affects the relative stability of the A and B structures in DNA fibers while salt concentration, counterions, heat, and organic solvents can influence the relative stabilities of single-stranded and the three double-stranded structures in soluble DNA. In addition, closed circular DNAs assume superhelical conformations which alter the density of base pairs in a given linear sequence.<sup>1</sup>

Crystallographic analysis of the self complementary dodecamer 5'-CGCGAATTCGCG-3' has enhanced the awareness of the possible sequence dependent variation of DNA even within a prevailing B format. Although the averaged helical parameters of the B helix correspond to those of the Watson-Crick structure, values of the helical twist, base plane roll, propeller twist, and torsion angle vary within the sequence.<sup>2</sup> Steric arguments emphasizing repulsive interactions across the major and minor groove of DNA have been proposed to account for these structural variations.<sup>3,4</sup>

What experimental methods can detect conformational variability in DNA? Does this conformational variability, if it exists, play a functionally significant role in modulating the expression and/or organization of DNA within a cell? Do proteins which decipher the genetic message alter the structure of DNA? The purposes of this Account are to review the chemistry of the oxidative nuclease activity of the 2:1 1,10-phenanthroline-cuprous complex, (OP)<sub>2</sub>Cu<sup>+</sup>, and its

coreactant, hydrogen peroxide, and to present recent results which suggest this nuclease activity provides one useful avenue to approach the issues raised above.

### Discovery of Nuclease Activity

The nuclease activity of (OP)<sub>2</sub>Cu<sup>+</sup> was discovered by our research group in 1979 while investigating the inhibition by 1,10-phenanthroline of the poly[d(A-T)] directed polymerization catalyzed by *E. coli* DNA polymerase I.<sup>5</sup> These studies were initiated on the premise that OP inhibition reflected an essential role for tightly bound zinc ion. Instead, we found that the inhibition required cupric ion and thiol.<sup>6,7</sup> Since preincubation of poly[d(A-T)] with OP, Cu<sup>2+</sup>, and mercaptopropionic acid under aerobic conditions greatly enhanced the inhibition of the polymerization activity, a reaction with DNA was strongly suggested. Electrophoretic analysis of the incubation mixture revealed extensive depolymerization of the poly[d(A-T)] with complete correspondence between the reaction conditions which inhibited enzymatic activity and those which caused extensive degradation of the DNA. Ironically, subsequent work demonstrated that *E. coli* DNA polymerase I was not a zinc metalloenzyme.<sup>8</sup>

### (OP)<sub>2</sub>Cu<sup>+</sup> and H<sub>2</sub>O<sub>2</sub> Are Coreactants

The efficiency of the nuclease reaction was striking since submicromolar levels of copper were sufficient to

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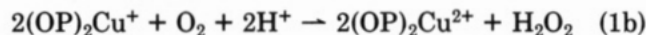
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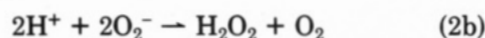
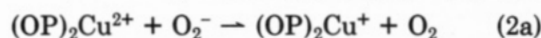
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abolish polymerization activity. The first goal in our investigation of the properties of this reaction was to identify the reactants directly responsible for scission. The blocking of the reaction by catalase provided the first clue of the importance of hydrogen peroxide in the reaction.<sup>5</sup> H<sub>2</sub>O<sub>2</sub> can be generated in a reaction mixture containing OP, Cu<sup>2+</sup>, thiol, and oxygen by the reactions summarized in eq 1a and 1b.<sup>9</sup> However, hydrogen

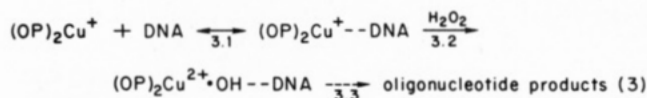


peroxide alone cannot account for the observed rapid rates of DNA cleavage. Strand scission requires the presence of the 2:1 1,10-phenanthroline-cuprous complex as demonstrated by the dramatic synergism observed in the rate of poly[d(A-T)] cleavage when equimolar concentrations (7 mM) of hydrogen peroxide and mercaptopropionic acid were added to a solution of 10 μM OP and 1 μM cupric ion.<sup>10</sup> Superoxide anion can also sustain the cleavage chemistry in the presence of OP and cupric ion by reducing (OP)<sub>2</sub>Cu<sup>2+</sup> (2a) and



spontaneously dismutating (2b) to form the two essential coreactants, (OP)<sub>2</sub>Cu<sup>+</sup> and hydrogen peroxide.<sup>11-13</sup>

The reaction scheme proposed for the oxidative nuclease activity includes the binding of the cuprous complex to DNA (3.1) followed by the generation of a metal ion associated hydroxyl radical-like species by the one-electron oxidation of the cuprous complex by hydrogen peroxide (3.2). This oxidant would be directly responsible for the scission of the phosphodiester backbone (3.3).



Two roles for 1,10-phenanthroline in the reaction can be envisioned. First, it modulates the redox potential of the Cu<sup>+</sup>/Cu<sup>2+</sup> couple. Second, it binds to DNA allowing the oxidative chemistry of the cuprous complex to proceed at the surface of the nucleic acid. Inhibitors of the reaction act either by decreasing the concentration of (OP)<sub>2</sub>Cu<sup>+</sup> (e.g. competitive ligands which sequester copper ion) or hydrogen peroxide (e.g. catalase) or by blocking the association of the coordination complex to the DNA.<sup>10</sup>

### Products of the Scission Reaction

Initial attempts at product analysis were directed at the identification of the potent inhibitors of *E. coli* DNA polymerase I produced in the cleavage of poly[d(A-T)]. Since hydrolysis of digests of poly[d(A-T)] with alkaline phosphatase and exonuclease III abolished the inhibition and restored their competence as a primer-template,<sup>14</sup> the potent inhibitors of polymerase

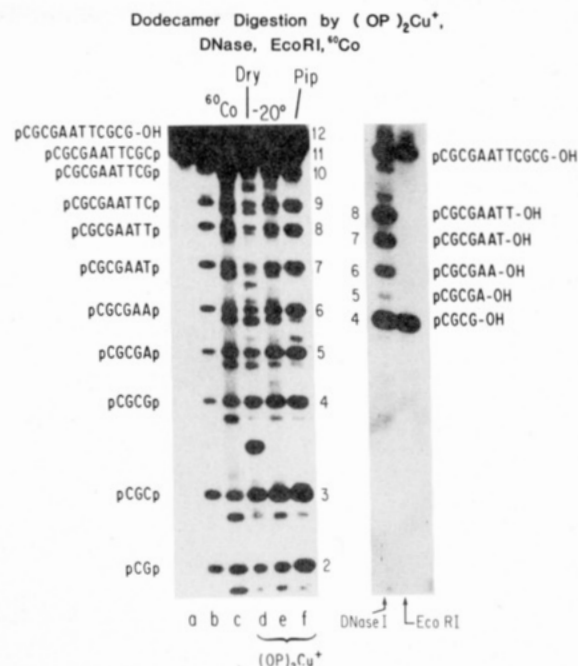
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**Figure 1.** Scission of the dodecamer p-CGCGAATTCGCG by  $\gamma$  radiation, (OP)<sub>2</sub>Cu<sup>+</sup>, DNase I, and Eco RI. Lane a, untreated labeled dodecamer. Lane b, heated with 1 M piperidine. Lane c, irradiated with cobalt-60 (10,000 rads) in water. Lanes d-f cleaved with 170 μM 1,10-phenanthroline, 40 μM cupric sulfate, 4.8 mM mercaptopropionic acid for 10 min at 37 °C, quenched with 2,9-dimethyl-1,10-phenanthroline (2 mM), and then treated as follows: lane d, dried immediately; lane e, frozen and stored for 3 days; lane f, dried immediately and then heated with 1 M piperidine. All samples were dissolved in loading buffer prior to electrophoresis. Additional lanes represent the hydrolysis products produced by DNase I and Eco RI as indicated.

activity were 3'-phosphomonoester termini—long known to be effective dead-end inhibitors of *E. coli* DNA polymerase I.<sup>15</sup> The artificial nuclease also generated 5'-phosphomonoester termini and released the free bases adenine and thymine from poly[d(A-T)] without the detectable formation of any precursor analogous to the base propenal which has been observed with DNAs degraded by bleomycin.<sup>16</sup>

Additional insight into the chemistry of strand scission was provided by studying the (OP)<sub>2</sub>Cu<sup>+</sup> digestion of the self-complementing dodecamer <sup>32</sup>P-CGCGAATTCGCG, an oligonucleotide containing the Eco RI restriction site. Its crystal structure analysis by Dickerson and colleagues has provided the first glimpse of one complete turn of DNA and presented a unique opportunity to study the reaction of a substrate of known structure. Electrophoretic analysis of the digestion products on a 20% sequencing gel confirmed the formation of 3'-phosphomonoester termini and demonstrated, for the first time, the formation of 3'-phosphoglycolate by the coordination complex (Figure 1).

Different methods of quenching the reaction mixture led to the trapping of an intermediate which converted

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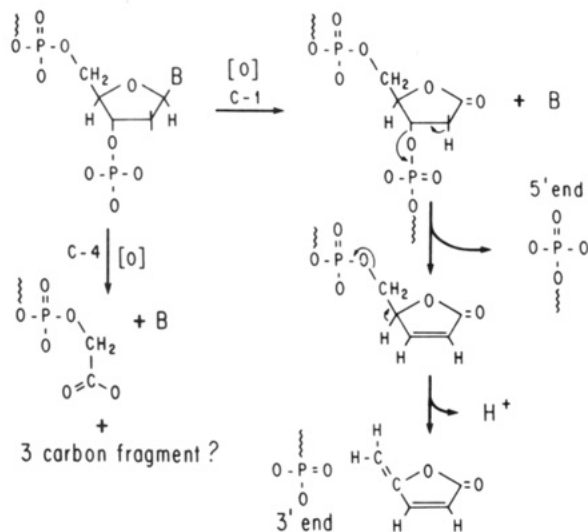


Figure 2. Postulated reaction mechanism.

to 3'-monophosphate ester upon heating with 1 M piperidine for 30 min. The migration of this intermediate does not correspond to the 3'-phosphoglycolate produced by cobalt-60  $\gamma$  irradiation or 3'-hydroxyl products produced by DNase I and Eco RI (Figure 1).<sup>17,18</sup> No comparable precursor of the 5'-phosphomonoester termini was observed when a 3'-labeled restriction fragment (which allows visualization of the 5' end) was used as a substrate. Our postulated reaction mechanism, which is consistent with the product analysis, is summarized in Figure 2. The initial site of attack of the hydroxyl radical-like species, formed by the one-electron oxidation of  $(\text{OP})_2\text{Cu}^+$  by hydrogen peroxide (eq 3) is the C-1' of deoxyribose. A minor site of attack is the C-4' hydrogen, which leads to the production of the 3'-phosphoglycolate apparent in Figure 1. In contrast, hydroxyl radicals produced by  $\gamma$  radiation produce 3'-monophosphate esters and 3'-phosphoglycolates in equivalent yields.<sup>18</sup> They are therefore less restricted in their reactivity than hydroxyl radicals generated by  $(\text{OP})_2\text{Cu}^+$  and hydrogen peroxide.

#### $(\text{OP})_2\text{Cu}^+$ Attacks from the Minor Groove

If the reaction mechanism presented in Figure 2 is correct,  $(\text{OP})_2\text{Cu}^+$  most likely attacks the B helix from a binding site within the minor groove where the hydrogen atoms of C-1' and C-4' of the deoxyriboses are located. Reaction from within the minor groove had previously been proposed by Drew and Travers<sup>19</sup> and is strongly supported by "footprinting" studies in which the dodecamer complexed with the antibiotic netropsin and with the restriction enzyme Eco RI is digested by the coordination complex. The structure of the dodecamer complex with netropsin has been also solved by the Dickerson group who have found that this ligand binds within the minor groove at the central AATT tetranucleotide.<sup>20,21</sup> When  $(\text{OP})_2\text{Cu}^+$  attacks the net-

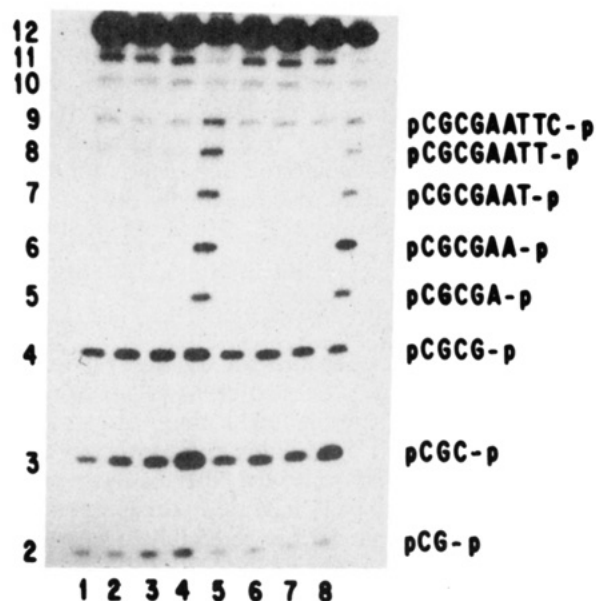


Figure 3. Footprinting of the binding of the minor groove ligand, netropsin, to the dodecamer. Netropsin is added to 2.2  $\mu\text{M}$  of  $^{32}\text{P}$ -dodecamer for 15 min at 37  $^\circ\text{C}$  (lanes 1-4) and 1 h at 0  $^\circ\text{C}$  (lanes 5-8) in 50 mM tris-HCl pH 8.0. Digestion was carried out with 170  $\mu\text{M}$  OP, 38  $\mu\text{M}$   $\text{CuSO}_4$  for 10 min at 37  $^\circ\text{C}$ . Prior to loading on a sequencing gel, the products were treated with piperidine. Concentration of netropsin ( $\mu\text{M}$ ): lane 1, 20; lane 2, 6; lane 3, 2; lane 4, 0; lane 5, 20; lane 6, 6; lane 7, 2; lane 8, 0.

ropsin-dodecamer complex, oxidative degradation at C-9, T-8, T-7, and A-6 is blocked providing an impression or "footprint" of the antibiotic binding site. The only other change in the digestion pattern of the dodecamer is a decrease in the intensity of the trinucleotide pCGC-p band corresponding to the oxidation of the deoxyribose of guanosine at position 4 (Figure 3).<sup>17</sup> The simplest interpretation of netropsin's inhibition of  $(\text{OP})_2\text{Cu}^+$  digestion is the competition between the coordination complex and the drug for binding sites within the narrow groove. The displacement of the protection pattern one nucleotide in the direction of the 3' end (i.e., oxidation at the A-5 deoxyribose) is consistent with groove binding but is difficult to rationalize if the phenanthroline ligand intercalates into the DNA. Viscometric studies with the inactive but geometric equivalent 2,9-dimethyl-1,10-phenanthroline-cuprous complex also do not support an intercalation of the complex during the course of cleavage.<sup>8c</sup>

The crystalline complex of Eco RI with the oligonucleotide TCGCGAATTCGCG (the dodecamer with a 5'-thymidylate residue) has recently been studied by Rosenberg and his colleagues to a resolution of 3.0  $\text{\AA}$ .<sup>22</sup> This pioneering study of a DNA binding protein with its specific recognition sequence has demonstrated that the interaction sites are localized within the major groove of the B-form helix. The minor groove exhibits no obvious interaction sites with the enzyme and is accessible to solvent. Since Eco RI did not inhibit the cleavage of the dodecamer by  $(\text{OP})_2\text{Cu}^+$ , the digestion of this DNA-protein complex by  $(\text{OP})_2\text{Cu}^+$  provides an additional test for the proposed minor groove site of action of the coordination complex.<sup>17</sup>

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### Secondary Structure Specificity

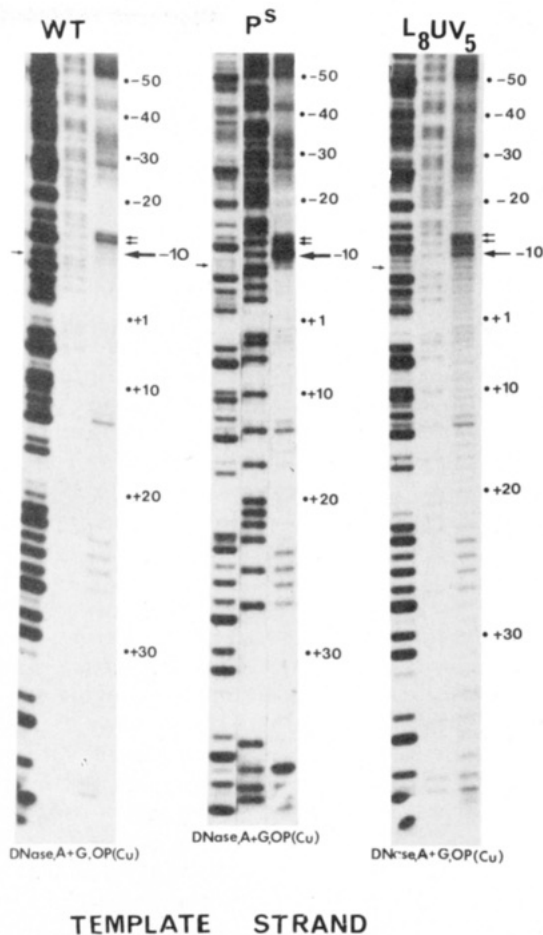
If the essential, noncovalent intermediate between (OP)<sub>2</sub>Cu<sup>+</sup> and B DNA involves the binding of the coordination complex within the minor groove, how efficient is the artificial nuclease activity when the dimensions of the minor groove are changed dramatically (as in the A and Z structure) or lost entirely (as in single-stranded DNA)? Z DNA with its left-handed helix and deep narrow minor groove is not a substrate for the cleavage when it is induced in poly(dG-dC) by high salt. Any synthetic single-stranded polynucleotide incapable of forming a stable secondary structure is also not cleaved.<sup>23</sup> Single-stranded M-13 which can form hairpin turns, presumably in a B DNA format, can be cleaved by (OP)<sub>2</sub>Cu<sup>+</sup> but not if the possibility of local structure is blocked by prior reaction with formaldehyde.<sup>24</sup>

In contrast, right-handed A DNA, which corresponds to the structure formed by DNA-RNA hybrids and double-stranded RNA, has a shallow minor groove and is cleaved by (OP)<sub>2</sub>Cu<sup>+</sup> at one third the rate of B DNA. The A DNA used in these studies was poly(T) annealed to poly(rA), which allowed its rate of scission to be directly compared to poly(T) annealed to poly(dA). The reduced reactivity is therefore associated with the different conformations of the poly(T) and not any intrinsic chemical difference (e.g., inductive effects). The susceptibility of the A DNA to cleavage also indicates that a rigid geometry is not essential for attack at the deoxyribose. (OP)<sub>2</sub>Cu<sup>+</sup> can bind to a polynucleotide in more than one site and oxidize the deoxyribose.

In addition to permitting a direct test of the influence of conformation on the reactivity of a polydeoxyribonucleotide strand, the synthetic DNA-RNA hybrids permit evaluation of the relative reactivity of polydeoxyribonucleotides and polyribonucleotides to (OP)<sub>2</sub>Cu<sup>+</sup> when both strands are constrained to identical conformations. Insertion of label both in the deoxyribose strand (poly(T)) and in the ribose strand (poly(rA)) of poly(T)-poly(rA) demonstrated that both strands were solubilized at the same rate albeit at one third the rate of a polydeoxyribonucleotide in the B form. Therefore, the chemical sensitivity of both the deoxyribose and ribose to the oxidative degradation of (OP)<sub>2</sub>Cu<sup>+</sup> and H<sub>2</sub>O<sub>2</sub> are the same; the coordination complex with its coreactant is an RNase as well as a DNase.

### Conformational Variability in the Lac Control Region

The lac control region of *E. coli* provides a convenient sequence to investigate the conformational variability of DNA in a region of clear biochemical function. It contains the binding sites of three proteins—the cyclic AMP receptor protein, RNA polymerase, and the lac repressor.<sup>25,26</sup> Comparison of the digestion patterns generated by DNase I and (OP)<sub>2</sub>Cu<sup>+</sup> of the 5'-labeled template strands of three variants of the lac control region provided interesting insights not only into the



**Figure 4.** Comparison of the DNase I and (OP)<sub>2</sub>Cu<sup>+</sup> digestion patterns for the wild-type, Ps and UV-5 promoters. See ref 27 for experimental details.

#### Template Strand Sequences:

	3'	-13	-12	-11	-10	-9	-8	5'
WT		C	A	T	A	C	A	
Ps		C	A	T	A	T	A	
UV5		C	A	T	A	T	T	

mechanisms of action of these nucleolytic activities but also into DNA structure as well.<sup>27</sup> The three control regions contained promoters of varying strength (legend to Figure 4 for comparative sequence). The wild type promoter is relatively weak and requires the cyclic-AMP binding protein to serve as a positive effector for transcription by RNA polymerase.<sup>25,26</sup> The Ps variant contains a promoter that is different from the wild type by a single base change and, although dependent on the positive effector protein, nevertheless supports weak transcription by RNA polymerase in the absence of the CRP protein. Finally, the UV-5 promoter is a strong promoter that efficiently supports transcription by RNA polymerase in the absence of any additional cofactor and differs from the Ps mutant by one further base change.

The efficiency of cutting by the coordination complex varies throughout the 186 base-pair sequence of the three DNA fragments studied (Figure 4).<sup>27</sup> The pattern of variation with (OP)<sub>2</sub>Cu<sup>+</sup>, however, is different from that observed with DNase I. The enzyme exhibits discrete preferred sites of attack distributed along the length of the sequence. In contrast, the hyperreactive

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sites with  $(OP)_2Cu^+$  are clustered in different regions. Although secondary binding sites can explain the non-uniform cutting pattern by DNase I,  $(OP)_2Cu^+$  is compact and lacks potential for extensive interaction with the DNA. We conclude that the kinetic differences in reaction with  $(OP)_2Cu^+$  at different phosphodiester bonds reflects sequence-dependent variation of minor groove geometry.

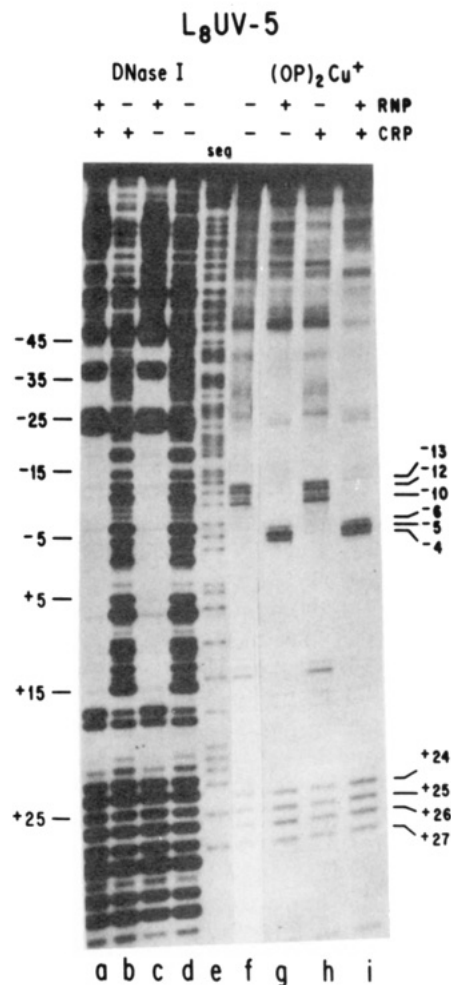
Does this structural variability have functional significance? The promoter conserved sequence<sup>28</sup> constitutes one region of hyperreactivity.<sup>27</sup> Since other reactive sites are apparent, minor groove width conferring  $(OP)_2Cu^+$  sensitivity is necessary but clearly not sufficient to facilitate recognition of a sequence by RNA polymerase. For the case of RNA polymerase, the sequence in the -35 region and the spacing of this sequence from the downstream polymerase recognition sequence are also important for enzyme binding.<sup>29</sup> Evidence that a characteristic DNA geometry influences polymerase binding is demonstrated by the dramatic changes of reactivity to the reagent associated with single base changes in the promoter-conserved region which enhance transcription. The single base change in the template strand at position -9, changing a cytosine in the wild-type sequence to a thymidine in the Ps, enhances effector-independent transcription and changes the  $(OP)_2Cu^+$  cleavage pattern in the promoter-conserved sequence from a pattern of two bands at -13 and -12 to a quartet of bands at positions -13, -12, -11, and -10. Base substitution at -9, therefore, alters the oxidation of deoxyriboses at positions -10, -11, -12, and -13. Local perturbation in DNA conformation which influences the binding of the coordination complex, is the most plausible explanation for the change in reactivity. The minor inductive changes associated with the transition of a C to a T at position -9 are insulated from the deoxyribose at -10; in addition, all the present chemical evidence indicates that the scission reaction of  $(OP)_2Cu^+$  is independent of the structure of base bound to the deoxyribose residue oxidized. The readjustment of the relative intensities of the cleavage products at positions -10, -11, -12, and -13, evident when the Ps promoter is converted to the UV-5, provides further evidence of the conformational origin of these reactivity changes. In this case, the mutation at position -8 replaces an adenosine in Ps with a thymidine in the UV-5.

These experiments demonstrate for the first time that mutations can alter biochemical function by modifying the conformation of DNA. The increased affinity of RNA polymerase for the lac UV-5 promoter is associated with a new DNA conformation in which perturbation of the minor groove geometry enhances reactivity towards  $(OP)_2Cu^+$ . If the DNA conformation of the UV-5 promoter more closely corresponds to that of the enzyme-bound DNA than that of the wild type, then less binding energy of the enzyme will be required to induce a conformation transition in the DNA and the apparent  $K_m$  of RNA polymerase for this promoter will be reduced. This explanation has roots in previous discussions of the role of strain in catalysis.<sup>30</sup>

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**Figure 5.** Footprinting the binding *E. coli* RNA polymerase to the L8-UV-5 control region. Lanes as indicated: CRP, cyclic AMP binding protein; RNP, RNA polymerase. See ref 27 for experimental details.

### Footprinting the Binding of RNA Polymerase to Lac Promoters

Using  $(OP)_2Cu^+$  and DNase I as footprinting reagents to study the binding of RNA polymerase to the lac promoter illustrates the advantages of  $(OP)_2Cu^+$  in the analysis of the binding of ligands to proteins. Addition of RNA polymerase to the strong promoter L8UV-5 forms an "open" enzyme-DNA complex kinetically competent to initiate transcription.<sup>26</sup> The binding of the enzyme to the DNA fragment has been visualized using DNase I as a footprinting reagent. The data presented in the Figure 5, which reproduces earlier work,<sup>31</sup> demonstrates strand scission in the 5'-labeled template strand is blocked from sequence positions 20 to -25. An additional region of protection is from -25 to -34 which includes the upstream promoter-conserved sequence.

What differences are observed when  $(OP)_2Cu^+$  replaces DNase I as the footprinting reagent? Although the DNA is also protected from scission by the binding of RNA polymerase in the region including the promoter-conserved sequence, a dramatic difference is the appearance of a family of hypersensitive bands at positions -6, -5, -4, and -3. Hyperreactivity towards

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(OP)<sub>2</sub>Cu<sup>+</sup> at this sequence is only observed when the template strand is labeled. If the scission of the non-template strand is visualized by appropriate labeling, protection rather than scission is observed at these positions. Experiments with the wild-type and Ps variants indicate that these bands are the signature of catalytically competent enzyme-promoter complexes; they are not observed with the wild-type promoter unless the cyclic AMP binding protein is added. Conversely, the positive effector does not generate these bands in the absence of polymerase for any of the promoters nor does it transform the wild-type pattern of two bands into the Ps and UV-5 quartet. Reflecting the intermediate affinity of the enzyme for Ps, the new hyperreactive sites can be observed in the absence of the cyclic AMP binding protein but their intensity is greatly increased upon the addition of the effector protein. However, the pattern of bands susceptible to (OP)<sub>2</sub>Cu<sup>+</sup> is the same for each promoter when the catalytically competent open complex is formed.

The pattern of hypersensitive bands reveals an interesting new feature of the (OP)<sub>2</sub>Cu<sup>+</sup> nuclease activity. Sites of cutting include and/or are adjacent to cytosine residues which methylation experiments with dimethyl sulfate have demonstrated to be single-stranded in the enzyme-DNA complex.<sup>32</sup> If (OP)<sub>2</sub>Cu<sup>+</sup> reacts sluggishly with single-stranded DNA free in solution, why does it show hyperreactivity to single-stranded DNA at the active site of an enzyme? This apparent inconsistency can be resolved by considering that the cleavage chemistry only requires that the binding of reagent occur near the site of scission. Nicking of A DNA, albeit at a slower rate, emphasizes that no fixed orientation is required for the chemistry to proceed. No complementarity of the transition state to a binding surface as required by true enzymic catalysis is essential for the (OP)<sub>2</sub>Cu<sup>+</sup> cleavage reaction to proceed. Possibly, the binding of the coordination complex near the template is stabilized by the interaction with the protein surface, allowing the coordination complex to bind with high affinity. Exclusive attack on the template strand clearly demonstrates that specificity in the binding; (OP)<sub>2</sub>Cu<sup>+</sup> does not cut the nontemplate strand even though it must also be single-stranded and proximal to the binding site. These experiments confirm that (OP)<sub>2</sub>Cu<sup>+</sup> detects protein- or ligand-induced conformation changes in the DNA. These structural transitions can include perturbations of the minor groove geometry, as observed with the case of the Eco RI dodecamer complex, or the formation of protein single-stranded DNA complexes, as in the case of RNA polymerase-promoter complexes.

### Nuclease Activity of Cuprous Complexes of 1,10-Phenanthroline Derivatives

The nuclease activity of cuprous complexes prepared with substituted 1,10-phenanthroline derivatives was examined to determine if altered and useful specificities could be achieved with the addition of simple substituents. Substitution ortho to the chelating nitrogens at position 2 or 2 and 9 blocks the nuclease activity, possibly by hindering formation of square-planar (OP)<sub>2</sub>Cu<sup>2+</sup> (eq 3). In contrast, the cuprous complexes

of 1,10-phenanthrolines substituted at position 5 with Br, NO<sub>2</sub>, methyl, phenyl, amidoacetyl, and amidoglycyl exhibit the same digestion pattern of the lac operon restriction fragment as that seen with the parent compound, although rate differences are observed.<sup>33</sup> The only inactive 5-substituted derivative possesses the negatively charged amidosuccinylate group; its binding to the polyanionic DNA must be blocked by electrostatic repulsion. Phenyl substitution at the 4 and 7 positions (bathophenanthroline) however gave a dramatically different result. The cuprous complex of this phenanthroline exhibited a greatly reduced sequence preference. No preferential cutting at the promoter-conserved region was observed. Computer-assisted model building demonstrated that 5 substitution does not hinder the lodging of the complexes in the minor groove but that substitution at the 4 and 7 positions does interfere. The cuprous complex of 4,7-diphenyl-1,10-phenanthroline therefore must bind in a fundamentally different geometry than the other phenanthroline derivatives.

Our studies of the reactivities of the phenanthroline derivatives have led to two significant results. First, they strongly support our conclusion that the reagent reacts from within the minor groove of B DNA. Secondly, they suggest that the 5-position would be the preferred locus to attach ligands for targeting of the reagent to specific nucleotide sequences. Recently, we have demonstrated that the attachment of 5-amidoglycyl OP to the 5'-phosphorylated end of an oligonucleotide via a phosphoimidazole coupling procedure<sup>34</sup> accomplishes the sequence-specific cutting of complementary DNA following hybridization.<sup>35</sup>

### Coordination Chemistry and DNA Structure

In addition to (OP)<sub>2</sub>Cu<sup>+</sup>, other coordination complexes are proving useful as drugs active at the DNA level and in the analysis of DNA structure. The metal complexes which have proven to be of significant pharmacological importance include bleomycin and *cis*-platin. Bleomycin, as the iron complex, has an *in vitro* DNA cleavage reaction with parallels to that of (OP)<sub>2</sub>Cu<sup>+</sup>;<sup>16</sup> *cis*-platin binds to the N(7) atoms of adjacent guanines on a DNA strand.<sup>36</sup>

In 1982, Dervan and colleagues demonstrated that EDTA attached to an ethidium derivative degrades DNA in the presence of ferrous ion, dithiothreitol, and O<sub>2</sub>.<sup>37</sup> The reagent binds to DNA via intercalation and cleaves DNA by generating diffusible hydroxyl radicals as suggested by the equivalent yields of 3-phosphoglycolates and 3'-phosphorylated termini.<sup>38</sup> Attachment of the EDTA moiety to netropsin analogues and oligonucleotides has permitted the targeting of the reagent.<sup>39,40</sup>

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Barton and colleagues have made the interesting observation that octahedral complexes of phenanthroline, particularly of the 4,7-diphenyl derivative, demonstrate enantiomeric specificity.<sup>41</sup> The  $\Delta$  isomer binds preferentially to B DNA; both the  $\Lambda$  and  $\Delta$  isomer bind to Z DNA. Therefore the  $\Lambda$  is potentially a Z-specific probe. In the presence of light, the  $\Lambda$  isomer of the cobalt complex cleaves pBR 322 at a limited number of sites which may correspond to DNA regions in a left-handed Z format. Major groove binding with intercalation of at least one OP into the DNA has been proposed for the binding of the  $\Delta$  isomer to DNA. Possibly, the 4,7-diphenyl-1,10-phenanthroline-cuprous complex, which exhibits anomalous reactivity, also interacts with the major groove.

### Concluding Remarks

The artificial nuclease activity of  $(OP)_2Cu^+$  cleaves DNA by oxidatively degrading the deoxyribose from its binding site within the minor groove of DNA. Since the coordination complex is small and structurally constrained, it detects local structural variability of this feature of the DNA helix. In experiments with the lac operon control elements, the reagent has revealed that the conformation of the promoter-conserved sequence

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is altered by single base changes which increase promoter strength. The coordination complex can also reveal functionally important conformational changes induced by protein binding and be used as a "footprinting" reagent to visualize ligand-DNA interactions.

Further work is proceeding in two additional directions.  $(OP)_2Cu^+$  is active in the Ames mutagenesis test and therefore cleaves DNA intracellularly. Since methods are now available to analyze nucleolytic cuts indirectly without prior labeling of the substrate DNA,<sup>42</sup> structural features of the nucleic acids can be compared both in vivo and in vitro. For complex structures such as nucleosomes, this should prove advantageous. Targeting the reaction to specific nucleic acid sequences, viruses,<sup>43</sup> or specific cell types might have useful practical implications.

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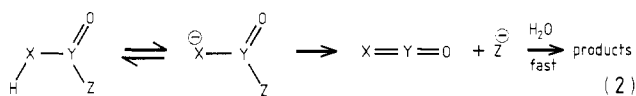
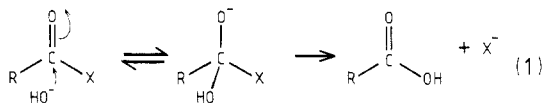
## Elimination-Addition Pathways for Thiol Esters

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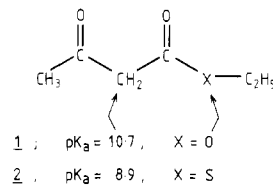
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A central feature of the acyl-transfer mechanism is the common, associative route involving a tetracoordinate intermediate, eq 1.<sup>1,2</sup> During the late sixties and the seventies, awareness increased of a poor relation, the elimination-addition pathway for acyl transfer at carbon, phosphorus-, and sulfur-based acyl sites,<sup>3,4</sup> eq 2.



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Equally established by that time was the acidity at the  $\alpha$ -carbon of thiol esters, a feature used to rationalize much of their behavior in organic synthesis, as well as in biochemical mechanisms.<sup>5</sup> The acidifying effect of a thioalkyl substituent is substantial. Compare the  $pK_a$  of the bridging methylene group in ethyl acetoacetate, 1, with that in *S*-ethyl thioacetoacetate 2. For an



equilibrium, such as acid dissociation,  $\Delta G_{eq}^\circ = -RT \ln K_a$ . Thus, the difference in free energy ( $\Delta\Delta G_{eq}^\circ$ ) is given by  $\Delta\Delta G_{eq}^\circ = 2.303 RT (\Delta pK_a)$ , where  $\Delta pK_a$  is the difference in  $pK_a$  values of the two species. For the

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