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A versatile *in vivo* footprinting technique using 1,10-phenanthroline–copper complex to study important cellular processes

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ABSTRACT

A number of reagents have been used to define the sequence-specific protein–DNA contacts by footprinting analysis. We report a new *in vivo* technique using the complex of 1,10-phenanthroline and copper [$(OP_2)Cu$] as a probe to study various intracellular DNA–protein interactions in whole cells. The versatility of the protocol is demonstrated by applying the technique to address various processes. The protocol is applied to (i) detect structural alterations in DNA as a result of single base substitution, (ii) footprint site-specific DNA-binding proteins, (iii) analyze promoter occupancy by RNA polymerase and (iv) analyze molecular interactions during transcription initiation. The results demonstrate that *in vivo* $(OP)_2Cu$ probing is a useful tool in studying important cellular processes involving DNA–protein interactions and has potential applications in post-genomic research.

INTRODUCTION

Understanding protein–DNA interactions is necessary to get an insight into various cellular processes such as transcription, replication, DNA repair and recombination. Among the various techniques developed to study molecular interactions, footprinting is most commonly used as it provides valuable information on how a protein recognizes DNA. A number of enzymatic and chemical probes are used that cleave or modify DNA differentially as a result of protein binding to its site. Enzymatic probes include DNase I (1,2), exonuclease III (3,4), P1 nuclease (5) and micrococcal nuclease (6). Dimethylsulfate (DMS) (7,8), ethyl nitrosourea (9), potassium permanganate (10), bis(1,10-phenanthroline)–copper (I) (11), methidiumpropyl-EDTA.iron (II) [MPE.Fe(II)] (12), diethyl pyrocarbonate (13,14), peroxonitrous acid (15) and osmium tetroxide (16) are examples of chemical probes used. While the list of reagents that are used for *in vitro* footprinting reactions is extensive, the agents that are applied to *in vivo* probing reactions are limited. DMS (7,8) and potassium permanganate (10) have proved useful in studying DNA–protein interactions *in vivo*. In addition to these two chemicals, DNase I has been used in ethanol permeabilized *Escherichia coli* cells *in situ* (17). In the post-genomic scenario of functional genomics, it has become important to develop new tools for *in vivo* probing of molecular interactions.

The chemical nuclease activity of 1,10-phenanthroline–copper [$(OP)_2Cu$] was demonstrated in 1978 while studying the mechanism of inhibition of *E.coli* DNA polymerase by 1,10-phenanthroline (OP). The nucleolytic activity of $(OP)_2Cu$ has many advantages as a footprinting reagent when compared to other commonly used reagents: (i) the $(OP)_2Cu$ chelate is a small molecule that gives a high resolution footprint; (ii) $(OP)_2Cu$ cuts at almost every sequence position, though the intensity of cutting may depend on local primary sequence (18,19); (iii) since $(OP)_2Cu$ binds to the minor groove of DNA, it reveals minor groove interactions. Any alterations in the major groove width due to protein binding and distortion will also be reflected as a change in the reactivity pattern of $(OP)_2Cu$ in the minor groove. (iv) $(OP)_2Cu$ has the ability to detect protein induced changes in DNA (20,21). The chemistry of the DNA cleavage reaction of $(OP)_2Cu$ has not previously been developed as an *in vivo* footprinting technique to study DNA–protein interactions. In this study, the potential of $(OP)_2Cu$ as a tool for *in vivo* footprinting assays is considered. The procedure we have developed using the reagent is applied successfully to probe DNA–protein interactions *in vivo*.

MATERIALS AND METHODS

Strains, plasmids, enzymes and chemicals

Escherichia coli DH10B was used for propagating the various plasmids. Plasmids pLW4, pVN184 and p*tin7* (22), pBM_2 (B.D.Paul and V.Nagaraja, unpublished), pACMK (23) and pVR7 (24) were used for different footprinting reactions. Klenow fragment of *E.coli* DNA polymerase and T4 polynucleotide kinase were from New England Biolabs. [γ-^{32}P]ATP was purchased from NEN. The chemicals used for the footprinting reactions, OP, neocuproine, 3-mercaptopropionic acid (3-MPA) and copper sulfate, were purchased from Sigma. DNA manipulation techniques (plasmid isolation, end-labeling of primers with [γ-^{32}P]ATP etc.) were carried out as described (25).

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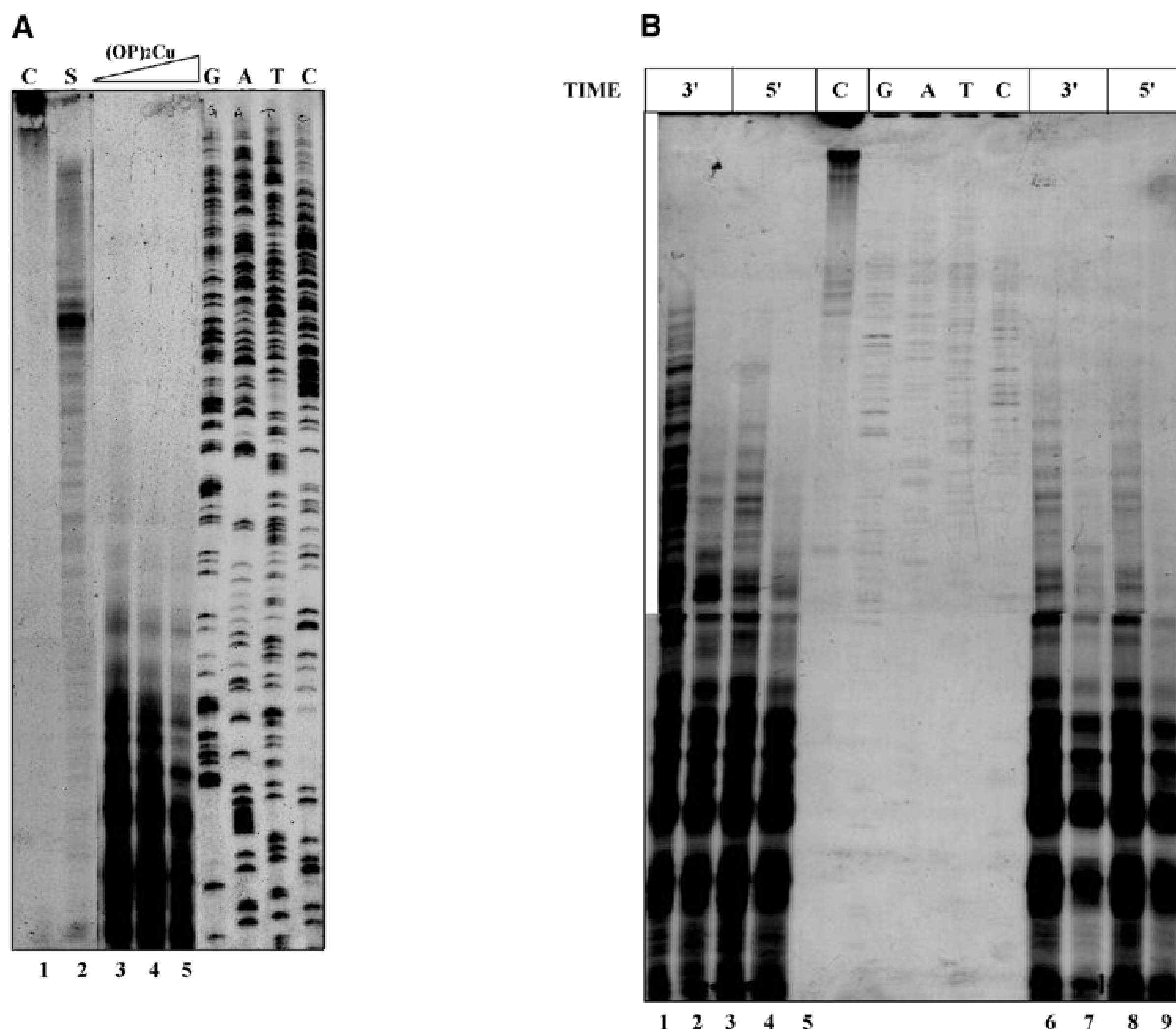

Figure 1. Standardization of *in vivo* $(OP)_2Cu$ probing. (**A**) Single hit conditions. Aliquots of 4.0 ml of culture of *E.coli* DH10B cells harboring plasmid pLW4 were subjected to treatment with 200 µl of increasing concentrations of mixture of OP and copper sulfate (lane 2, 5 mM OP/0.375 mM $CuSO_4$; lane 3, 80 mM OP/6 mM $CuSO_4$; lane 4, 160 mM OP/12 mM $CuSO_4$; lane 5, 400 mM OP/30 mM $CuSO_4$) and 200 µl of 580 mM 3-MPA. The reaction was terminated after 1 min by the addition of 200 µl of 0.1 M neocuproine to the reaction mix as described in Materials and Methods. Primer extension products of the plasmid from untreated culture (C) and near single hit conditions (S) are shown in lanes 1 and 2, respectively. G, A, T and C refer to sequencing lanes with the same primer. (**B**) Time and concentration dependence. *In vivo* $(OP)_2Cu$ reaction was carried out with cells containing plasmid pLW4 alone (lanes 1, 3, 6 and 8) or in combination with pVN184 (lanes 2, 4, 7 and 9) for different time points as indicated in the figure. Lanes 1–4 and 6–9 were treated with 200 µl of a mixture of 20 mM OP/1.5 mM $CuSO_4$ and 40 mM OP/3 mM $CuSO_4$, respectively, and 200 µl of 580 mM 3-MPA as described in Materials and Methods. Primer extension product of plasmid DNA isolated from cells treated with $CuSO_4$ alone is shown in lane 5 (C).

In vivo 1,10-phenanthroline–copper $[(OP)_2Cu]$ footprinting reaction

Overnight-grown *E.coli* cells harboring various plasmid constructs (described in Results and figure legends) were used as pre-inoculum (1%) and the cultures were grown in 4.0 ml of Luria–Bertani medium to an OD_{600} of 0.6. Various concentrations of OP and $CuSO_4$ were used in order to standardize the conditions of single hit kinetics, and representative data are shown in Figure 1A. A representative autoradiogram for the pattern of cleavage with respect to $(OP)_2Cu$ concentration and time is depicted in Figure 1B. Copper sulfate, OP or 3-MPA alone did not cleave the plasmid DNA. Single hit pattern would depend on the size of the plasmid, copy number and also number of different plasmids present in the cell. Accordingly, different concentrations of the reagents were used for different plasmids after appropriate standardization. For plasmids pLW4, p*tin7* and T2G, 200 µl of a mixture of 5 mM OP and 0.375 mM $CuSO_4$ and 200 µl of 580 mM 3-MPA were added to 4.0 ml of culture and shaken vigorously at 37°C for 1 min. The reaction was quenched by adding 200 µl of 0.1 M neocuproine (2,9-dimethyl-1,10-phenanthroline) to the culture and mixing thoroughly for another 10 s. The culture tubes were chilled in ice and processed immediately for plasmid isolation by the boiling method (25). For the pBM_2 and pVR7 as well as pUC18 and pACMK plasmid systems, 100 µl of 5 mM OP and 0.375 mM $CuSO_4$ and 100 µl of 580 mM 3-MPA were used

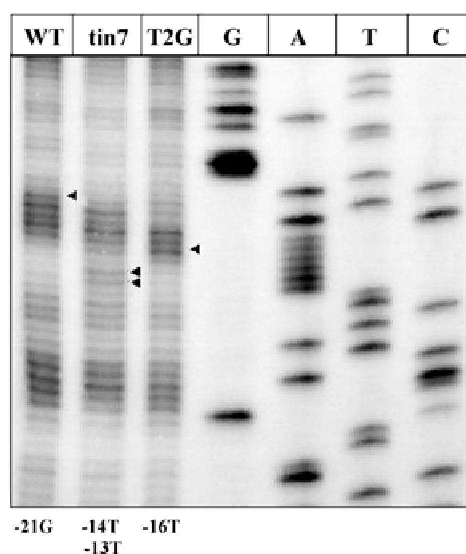

Figure 2. *In vivo* $(OP)_2Cu$ cleavage to detect changes in DNA structure. *Escherichia coli* cells harboring plasmid pLW4 having wild-type sequence in the spacer region of the promoter (WT) or two other substitution mutations (*tin7* and T2G, respectively) in the spacer region of the promoter were used for $(OP)_2Cu$ cleavage reaction as described in Materials and Methods. G, A, T and C refer to sequencing lanes using Sanger's dideoxy method. Arrowheads indicate the hypersensitive residues upon $(OP)_2Cu$ cleavage.

and the reaction was carried out for 30 s to get single hit conditions. Primer extension reactions were carried out using end-labeled primers (5′-GGAATCCGCCTTAAATAACA-3′ and 5′-TGACCGGCAGCAAAATG-3′) as described earlier (7). Samples were electrophoresed in a 6% denaturing PAGE and autoradiographed. Using the same end-labeled primers Sanger's dideoxy sequencing was carried out.

RESULTS

Detection of differences in the DNA conformation

$(OP)_2Cu$ cleaves the phosphodiester backbone of nucleic acids at physiological pH and temperatures. Though the reagent cuts at every base, the intensity of the cleavage is dependent on the structure of DNA due to a particular base sequence (18,19). We have probed the variation in DNA structure by assessing the sensitivity pattern to single hit cleavage by $(OP)_2Cu$ for three different DNA sequences that differ from each other by a single base in the promoter spacer region (Fig. 2). In the top strand, –21G was hypersensitive in the wild-type (WT) promoter spacer region whereas in T4G (*tin*7), –14T and –13T were cleaved more often. T2G mutant showed a hypersensitive band at position –16T, while –19T and –20A were less susceptible to cleavage with $(OP)_2Cu$ as compared to WT and T4G. We have observed this difference in the cleavage pattern in DNA sequences in *in vitro* $(OP)_2Cu$ analysis and correlated to promoter strength of WT and mutant spacer sequences (26). The results presented in Figure 2 show the utility of the technique for *in vivo* probing to assess structural changes in DNA arising as a result of base substitutions.

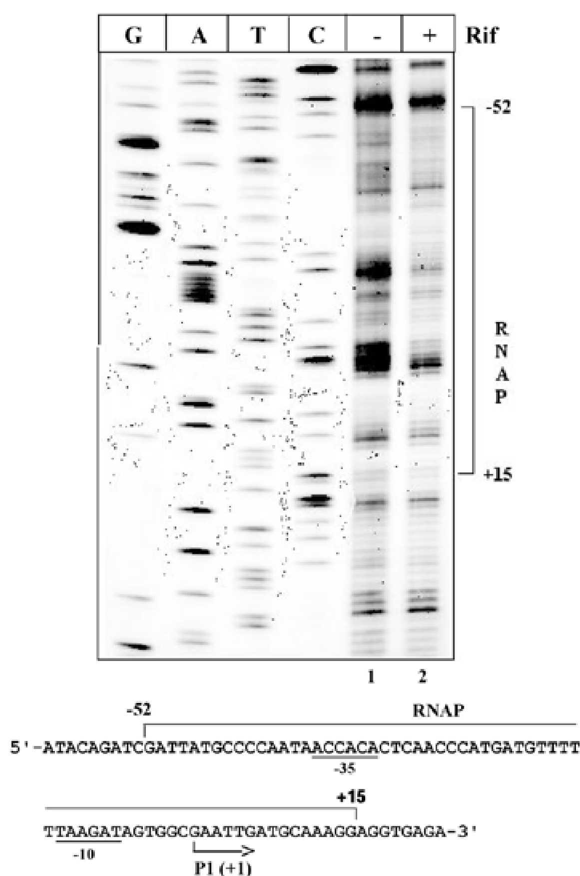

Figure 3. RNAP binding to the promoter. Absence (–) or presence (+) of rifampicin is indicated. G, A, T and C refers to sequencing lanes. The sequence below shows the Mu *mom* regulatory region. The protected region is indicated with a bracket. The –10 and –35 elements are underlined.

Probing DNA–protein interactions involved in different cellular events

Binding of RNA polymerase (RNAP) to promoter sequences. Binding of RNAP at a promoter is the first step in the transcription initiation process. Expression of a large number of genes is regulated at this step. Consequently, RNAP contacts at different promoters have been studied extensively *in vitro* to delineate the features of promoter–polymerase interactions. Other than $KMnO_4$ probing, which assesses open complex formation at the promoter, there are no other convenient tools to evaluate *in vivo* promoter occupancy by RNAP. Use of $(OP)_2Cu$ as an *in vivo* probe for RNAP binding to a promoter is demonstrated in Figure 3. *Escherichia coli* DH10B cells harboring the p*tin*7 plasmid were treated with rifampicin (200 µg/ml) to arrest RNAP at the promoter, followed by treatment with $(OP)_2Cu$ as described in Materials and Methods. A region of 67 bp was protected upon rifampicin treatment of the cells from –52 to +15 with respect to the +1 transcription start site of the promoter (Fig. 3). DNase I footprinting with the promoter of the *tin7* mutant revealed a protected region from –56 to +21 (22). The larger footprint obtained by DNase I could be because of steric hindrance between RNAP and

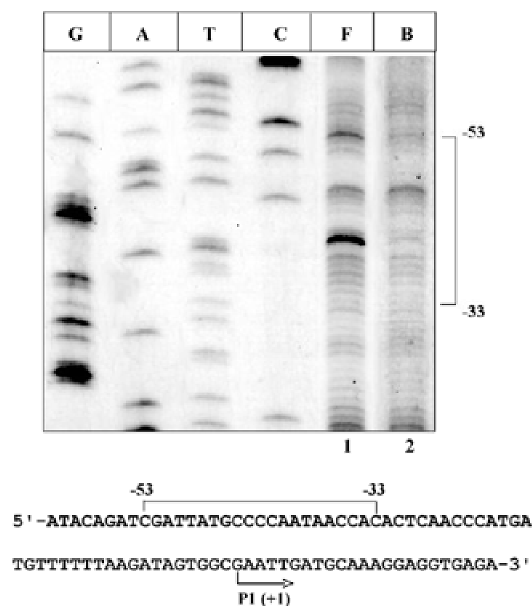

Figure 4. Footprinting of site-specific DNA-binding protein. 'F' indicates free DNA where cells containing p*tin7* plasmid have been treated with $(OP)_2Cu$ as described in Materials and Methods. 'B' indicates C protein bound to its site in the regulatory region of the *mom* operon (as shown by a bracket in the sequence below). The transcription start site is indicated with an arrow. Sequencing lanes are marked as G, A, T and C.

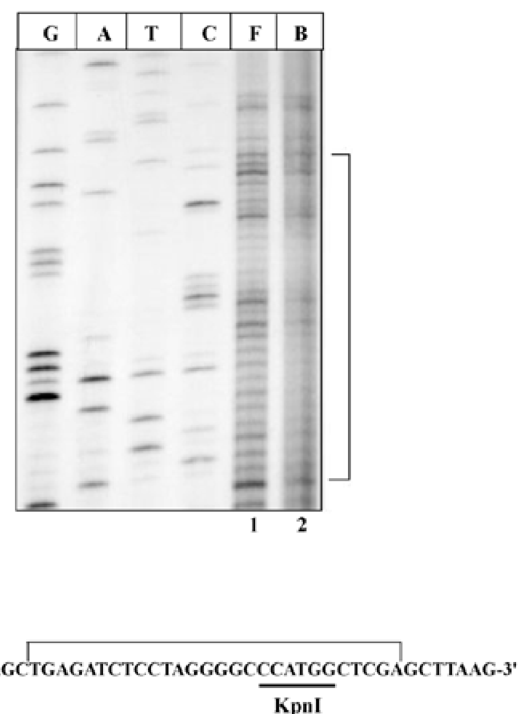

Figure 5. Footprinting of *Kpn*I methyltransferase. Free (F) and bound (B) lanes contain pUC18 and pACMK plasmids, respectively, after treatment with $(OP)_2Cu$ and extension with DNA polymerase I (Klenow) as described in Materials and Methods. The bracket indicates the protected region both in the figure and in the sequence of the multiple cloning site of pUC18 plasmid. The recognition sequence for *Kpn*I methyltransferase is underlined. G, A, T and C are the sequencing lanes.

DNase I. $(OP)_2Cu$ being a small molecule, a better resolved RNAP footprint on the same promoter was obtained.

Footprinting of cis elements with trans factors. Site-specific interactions of a large number of DNA-binding proteins at their respective *cis* site serve as determinants of diverse molecular events. A large number of regulatory proteins influence gene expression by binding to their recognition sequence. A wealth of information is available on *in vitro* footprinting with various *trans* factors at their binding sites near or distant from the promoter region. The utility of $(OP)_2Cu$ as an *in vivo* tool to probe the specific binding is addressed by using regulatory protein C from bacteriophage Mu. The C binding site has been identified by *in vitro* analysis using a variety of footprinting reagents (27). Plasmids p*tin7* (harboring the C binding sequence) and pVN184 (C protein producing plasmid) were used to map the C protein binding site. A footprint from –53 to –33 (with respect to the transcription start site of the *mom* gene) was obtained in the presence of C protein (Fig. 4, lanes 1 and 2). The location of the binding site lies within the region of *in vitro* DNase I footprint (–57 to –28) with purified C protein (28).

In order to assess the versatility of the technique, we extended the footprinting analysis to map the DNA binding by other types of DNA recognition proteins. DNA methyltransferases and their corresponding restriction enzymes recognize specific DNA sequences in a site-specific fashion. Application of the *in vivo* $(OP)_2Cu$ footprinting technique to assess the interaction of *Kpn*I methyltransferase with its recognition site present in pUC18 plasmid is shown in Figure 5. The plasmid pACMK (23) was used for the expression of *Kpn*I methyltransferase. An overnight-grown (16 h) culture (4.0 ml) of both pUC18 and pACMK were subjected to $(OP)_2Cu$ probing as described in Materials and Methods. A region of 28 bp was protected encompassing the *Kpn*I recognition sequence (Fig. 5, lane 2). These results demonstrate the usefulness of the technique to probe diverse site-specific interactions.

Multi-protein complex formation: activation of transcription initiation. The majority of molecular events are triggered as a result of the interaction of a number of proteins with DNA. In most cases, complexes of several proteins establish a molecular communication network. The potential of *in vivo* footprinting with $(OP)_2Cu$ to study the processes involving interplay of factors is assessed in Figure 6 by analyzing the transcription activation process. Binding of the transactivator protein C is a prerequisite step for the recruitment of RNAP to the *mom*P1 promoter of bacteriophage Mu. When *E.coli* cells harboring the pBM_2 plasmid (containing the *mom*P1 promoter) are arrested with rifampicin, RNAP fails to show any detectable footprint (Fig. 6, lane 3). However, in the presence of C protein, there is an extended region of protection from –59 to +16 (top strand) indicative of binding of RNAP to the promoter. These results essentially reproduce the earlier *in vitro* footprinting experiments with purified C protein and RNAP (28).

DISCUSSION

Here, we demonstrate the use of $(OP)_2Cu$ as a versatile probe to assess the *in vivo* interaction of protein(s) with DNA. The

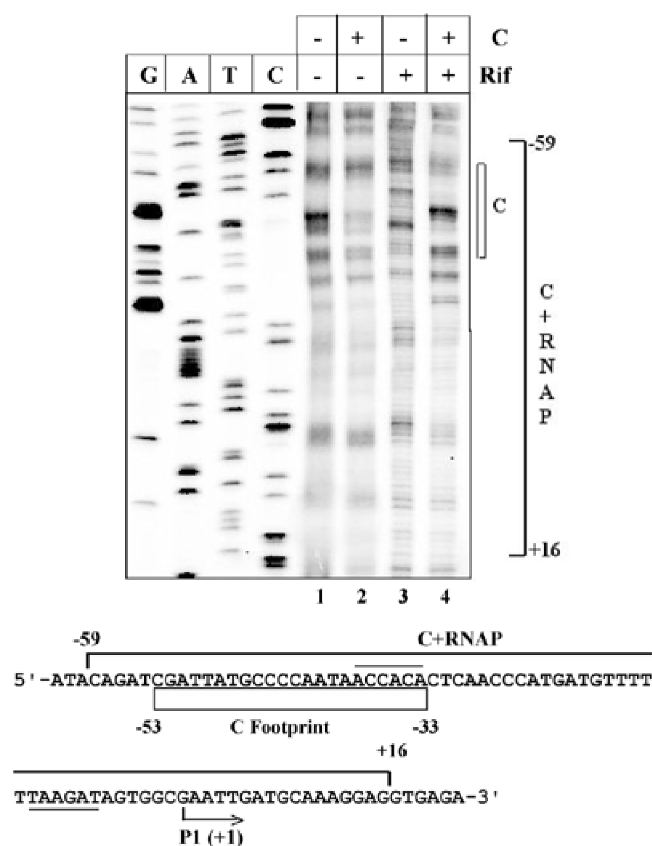

Figure 6. Activator-mediated RNAP binding to weak promoter. Absence (–) or presence (+) of C protein (C) or rifampicin (Rif) are indicated. C footprint is shown as an open rectangle in the sequence of the *mom* regulatory region below the figure. The –10 and –35 hexamers are underlined and overlined, respectively. The transcription start site is indicated with an arrow. The bracket indicates the extended footprint obtained due to binding of both C protein and RNAP. G, A, T and C are sequencing lanes.

method is successfully applied to study (i) structural differences in DNA, (ii) interaction of RNAP with promoter, (iii) interaction of specific DNA-binding proteins with their cognate sites and (iv) recruitment of transcription machinery.

Amongst the wide range of probes available to define DNA–protein interactions *in vitro*, very few reagents have been used to analyze *in vivo* interactions. The small size of the molecule, sequence-independent recognition, backbone cleavage chemistry and the ability to bind to minor groove, are the features of $(OP)_2Cu$ that makes it an attractive *in vivo* probe. In contrast, both DMS and $KMnO_4$ are specific base modifiers revealing A, G or T contacts (29,30). Thus, $(OP)_2Cu$ footprinting provides complementary information to the data obtained with DMS and $KMnO_4$. Though DNase I has been used for *in vivo* footprinting (17), the cells require pre-treatment with ethanol for the entry of DNase I into the cells. The $(OP)_2Cu$ footprinting technique, on the other hand, does not require permeabilization and gives better resolution of interaction of the protein with its specific site as compared to the DNase I footprinting method. In our protocol of *in vivo* footprinting with $(OP)_2Cu$ there is no need for any pre-treatment of cells. Also, no post-treatment of the DNA is required as opposed to the piperidine cleavage reaction required for DMS and $KMnO_4$ footprinting reactions. Sequence-dependent structural variability can also be detected using $(OP)_2Cu$. Although the scission reaction of $(OP)_2Cu$ is not specific for any particular base, the rate of cutting the DNA backbone does rely on the nucleotide sequence (18,19). We have utilized this property of $(OP)_2Cu$ for comparing *in vivo* scission pattern to reveal differences in DNA conformation of mutant promoters. Another important feature is that arduous protein purification steps are eliminated. Thus, the technique is readily applied to study the interactions of single protein or multi-protein–DNA complexes as there is no need to purify and reconstitute all the protein components to study their effect.

The present protocol could be applied to study the *in vivo* DNA–protein interactions in diverse biological systems. In organisms where permeability to the reagents could pose a problem, pre-treatment of cells with chemicals such as toluene or alcohol would aid the entry of $(OP)_2Cu$ complex. Reagents such as EDTA and SDS could be used to alter the permeability of the cells. We have shown previously that low concentrations of SDS (0.01%), which do not affect growth of cells, also lead to permeabilization of cells (31). $(OP)_2Cu$ could also be used *in vivo* to study DNA–protein interactions at the chromosomal level when combined appropriately with a PCR-based amplification method of primer extension to increase its sensitivity further. It should, however, be mentioned that for each plasmid or chromosomal system (depending on the size of the plasmid/chromosome) the concentration of $(OP)_2Cu$, 3-MPA and neocuproine to be used has to be standardized accordingly to get a single hit pattern.

The *in vivo* footprinting technique presented here appears to be a powerful probe to study the dynamics of various cellular molecular processes involving DNA–protein interactions. For example, interplay of regulatory factors influencing gene regulation at the transcription level, replication initiation, repair pathways, recombination reactions or functional interaction in nucleo–protein complexes could be analyzed. Another obvious application is in *in vivo* promoter mapping to identify novel promoters. As the present protocol does not require purified species-specific components, it has significant application potential for post-genomic research for functional probing of a large number of proteins whose DNA-binding property is unknown. Thus, the method developed in this study using $(OP)_2Cu$ has many potential applications in analysing DNA–protein interactions occurring in intracellular processes.

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REFERENCES

1. Schmitz,A. and Galas,D.J. (1979) The interaction of RNA polymerase and lac repressor with the lac control region. *Nucleic Acids Res.*, **6**, 111–137.
2. Buchanan,R.L. and Gralla,J.D. (1987) Factor interactions at simian virus 40 GC-box promoter elements in intact nuclei. *Mol. Cell Biol.*, **7**, 1554–1558.

3. Metzger,W., Schickor,P. and Heumann,H. (1989) A cinematographic view of *Escherichia coli* RNA polymerase translocation. *EMBO J.*, **8**, 2745–2754.
4. Pavco,P.A. and Steege,D.A. (1990) Elongation by *Escherichia coli* RNA Polymerase is blocked *in vitro* by a site specific DNA binding protein. *J. Biol. Chem.*, **265**, 9960–9969.
5. Bhaduri,T., Basak,S., Sikder,D. and Nagaraja,V. (2000) Inhibition of *Mycobacterium smegmatis* topoisomerase I by specific oligonucleotides. *FEBS Lett.*, **486**, 126–130.
6. Zhang,L. and Gralla,J.D. (1989) Micrococcal nuclease as a probe for bound and distorted DNA in lac transcription and repression complexes. *Nucleic Acids Res.*, **17**, 5017–5028.
7. Gralla,J.D. (1985) Rapid 'footprinting' on supercoiled DNA. *Proc. Natl Acad. Sci. USA*, **82**, 3078–3081.
8. Borowiec,J.A. and Gralla,J.D. (1986) High-resolution analysis of lac transcription complexes inside cells. *Biochemistry*, **25**, 5051–5057.
9. Siebenlist,U. and Gilbert,W. (1980) Contacts between *Escherichia coli* RNA polymerase and an early promoter of phage T7. *Proc. Natl Acad. Sci. USA*, **77**, 122–126.
10. Sasse-Dwight,S. and Gralla,J.D. (1989) $KMnO_4$ as a probe for lac promoter DNA melting and mechanism *in vivo*. *J. Biol. Chem.*, **264**, 8074–8081.
11. Sigman,D.S., Graham,D.R., D'Aurora,V. and Stern,A.M. (1979) Oxygen-dependent cleavage of DNA by the 1,10-phenanthroline. cuprous complex. Inhibition of *Escherichia coli* DNA polymerase I. *J. Biol. Chem.*, **254**, 12269–12272.
12. van Dyke,M.W. and Dervan,P.B. (1983) Chromomycin, mithramycin, and olivomycin binding sites on heterogeneous deoxyribonucleic acid. Footprinting with (methidiumpropyl-EDTA) iron (II). *Biochemistry*, **22**, 2373–2377.
13. Herr,W. (1985) Diethyl pyrocarbonate: a chemical probe for secondary structure in negatively supercoiled DNA. *Proc. Natl Acad. Sci. USA*, **82**, 8009–8013.
14. Johnston,B.H. and Rich,A. (1985) Chemical probes of DNA conformation: detection of Z-DNA at nucleotide resolution. *Cell*, **42**, 713–724.
15. King,P.A., Jamison,E., Strahs,D., Anderson,V.E. and Brenowitz,M. (1993) 'Footprinting' proteins on DNA with peroxonitrous acid. *Nucleic Acids Res.*, **21**, 2473–2478.
16. Galazka,G., Palecek,E., Wells,R.D. and Klysik,J. (1986) Site-specific OsO_4 modification of the B-Z junctions formed at the $(dA\text{-}dC)_{32}$ region in supercoiled DNA. *J. Biol. Chem.*, **261**, 7093–7098.
17. Cassler,M.R., Grimwade,J.E., McGarry,K.C., Mott,R.T. and Leonard,A.C. (1999) Drunken-cell footprints: nuclease treatment of ethanol-permeabilized bacteria reveals an initiation-like nucleoprotein complex in stationary phase replication origins. *Nucleic Acids Res.*, **27**, 4570–4576.
18. Veal,J.M. and Rill,R.L. (1989) Sequence specificity of DNA cleavage by bis(1,10-phenanthroline)copper(I): effects of single base pair transitions on the cleavage of preferred pyrimidine-purine-pyrimidine triplets. *Biochemistry*, **28**, 3243–3250.
19. Yoon,C., Kuwabara,M.D., Spassky,A. and Sigman,D.S. (1990) Sequence specificity of the deoxyribonuclease activity of 1,10-phenanthroline-copper ion. *Biochemistry*, **29**, 2116–2121.
20. Spassky,A. and Sigman,D.S. (1985) Nuclease activity of 1,10-phenanthroline-copper ion. Conformational analysis and footprinting of the lac operon. *Biochemistry*, **24**, 8050–8056.
21. Spassky,A. (1986) Visualization of the movement of the *Escherichia coli* RNA polymerase along the lac UV5 promoter during the initiation of the transcription. *J. Mol. Biol.*, **188**, 99–103.
22. Balke,V., Nagaraja,V., Gindlesperger,T. and Hattman,S. (1992) Functionally distinct RNA polymerase binding sites in the phage Mu *mom* promoter region. *Nucleic Acids Res.*, **20**, 2777–2784.
23. Chandrashekaran,S., Babu,P. and Nagaraja,V. (1999) Characterization of DNA binding activities of over-expressed KpnI restriction endonuclease and modification methylase. *J. Biosci.*, **24**, 269–277.
24. Ramesh,V., De,A. and Nagaraja,V. (1994) Engineering hyperexpression of bacteriophage Mu C protein by removal of secondary structure at the translational initiation region. *Protein Eng.*, **7**, 1053–1057.
25. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
26. Basak,S., Lars,O., Hattman,S. and Nagaraja,V. (2001) Intrinsic DNA distortion of the bacteriophage Mu momP1 promoter is a negative regulator of its transcription. A novel mode of toxic gene expression. *J. Biol. Chem.*, **276**, 19836–19844.
27. Ramesh,V. and Nagaraja,V. (1996) Sequence-specific DNA binding of the phage Mu C protein: footprinting analysis reveals altered DNA conformation upon protein binding. *J. Mol. Biol.*, **260**, 22–33.
28. Gindlesperger,T.L. and Hattman,S. (1994) *In vitro* transcriptional activation of the phage Mu *mom* promoter by C protein. *J. Bacteriol.*, **176**, 2885–2891.
29. Maxam,A.M. and Gilbert,W. (1980) Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.*, **65**, 499–560.
30. Sasse-Dwight,S. and Gralla,J.D. (1991) Footprinting protein-DNA complexes *in vivo*. *Methods Enzymol.*, **208**, 146–168.
31. Nagaraja,V. (1981) Interaction of Mycobacteriophage I3 with its host *Mycobacterium smegmatis*. Ph.D. Thesis, Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore, India.