The high mobility group proteins I and Y (HMGI/Y) are abundant components of chromatin. They are thought to derepress chromatin, affect the assembly and activity of the transcriptional machinery, and associate with constitutive heterochromatin during mitosis. HMGI/Y protein molecules contain three potential DNA-binding motifs (AT-hooks), but the extent of contacts between DNA and the entire protein has not been determined. We have used a protein-footprinting procedure to map regions of the Chironomus HMGI protein molecule that are involved in contacts with DNA. We find that in the presence of double-stranded DNA all AT-hook motifs are protected against hydroxyl radical proteolysis. In contrast, only two motifs were protected in the presence of four-way junction DNA. Large regions that flank the AT-hook motifs were found to be strongly protected against proteolysis in complexes with interferon-β promoter DNA, suggesting amino acid residues outside the AT-hooks considerably contribute to DNA binding.

The family of high mobility group proteins I/Y (HMGI/Y) comprises four structurally related proteins, the mammalian HMGI, HMGY (1), HMGI-C (2), and the Chironomus HMGI protein (3). Moreover, proteins with some extent of similarity to HMGI have been found in plants (for review see Ref. 4), bacteria (5), and Drosophila (6).

The properties of the insect cHMGI protein resemble the major structural features of the mammalian HMGI and Y proteins. It contains three DNA-binding motifs (AT-hooks) (7) and a negatively charged COOH-terminal domain (3) and have similar charge distribution in the regions flanking the AT-hooks (8). Both human and Chironomus HMGI/Y proteins bind preferentially to four-way junction DNA (3) and to AT-tracts of double-stranded DNA (3, 9). Their binding alters the DNA conformation (10–12) and unbinds intrinsically bent DNA (13). Moreover, these proteins from evolutionarily distant organisms are substrates of Cdc-2 kinase (8, 14), mitogen-activated protein (3). Moreover, proteins with some extent of similarity to HMGI have been found in plants (for review see Ref. 4), bacteria (5), and Drosophila (6).

Diverse biological functions for the mammalian proteins have been suggested. Initially the HMGI/Y proteins were considered as specific components of constitutive chromatin (17, 18). Further studies revealed that they are involved in the modulation of transcription of specific genes (for review see Ref. 19). Studies on the human interferon-β (IFN-β) gene and the gene encoding the α-subunit of the interleukin 2 receptor showed transcriptional activation upon binding of HMGI/Y proteins to positive regulatory domains (PRD), which facilitate binding of transcription factors (20–22). More recently, HMGI/Y proteins were identified as components of a repressor complex that inactivates the promoter of the T cell receptor α-chain gene (23) and as crucial host proteins in the HIV-1 preintegration complex (24). Cytological studies of insect polytene chromosomes have demonstrated that the cHMGI protein is present in many transcriptionally active loci and in nucleoli, suggesting that HMGI/Y proteins are involved in polymerase I and II transcription (25). They are highly abundant in undifferentiated and rapidly dividing cells (25, 26). Elevated levels of HMGI/Y in differentiated tissues have been found to be correlated with progressive and neoplastic transformations (27, 28). Disruption or rearrangements of their genes lead to tumorigenesis (29, 30).

Whereas different biological effects of HMGI/Y proteins have been described, the molecular and biochemical mechanism of affecting DNA and chromatin structure is not well defined. The spatial organization of HMGI/Y-DNA complex remains only partially understood. NMR data of a complex of a truncated form of the protein and a DNA dodecamer show that the central part of the AT-hook domain, the Arg-Gly-Arg motif, interacts with the bases and the sugar within the minor groove of the DNA double helix (31). In addition, several residues flanking this motif interact with the sugar-phosphate backbone and are responsible for the strength of the overall protein DNA binding (31). Moreover, the central AT-hook motif mediates specific DNA binding and cooperates the other two AT-hooks (32). Here we report the mapping of the regions of the cHMGI protein involved in contacts with various types of DNA, including linear synthetic poly(dA-dT)poly(dA-dT), four-way junction DNA, and a region of the promoter of the IFN-β gene. The mapping was performed by means of protein hydroxyl radical-footprinting technique (33). The data presented show that the interaction of cHMGI protein with DNA involves residues of two or three AT-hook motifs dependent on the DNA type and/or the protein to DNA ratio. Large regions flanking these motifs also contribute to the binding of cHMGI to DNA.

**EXPERIMENTAL PROCEDURES**

**Preparation of Bacterially Expressed cHMGI and Protein Determination**—The cHMGI protein was overexpressed in *Escherichia coli* (12) and purified on high performance liquid chromatography columns as described previously (34, 35). The eluted protein was vacuum concentrated and lyophilized. The dried cHMGI was quantified gravimetrically.

**23P-Labeling of cHMGI on Ser**—100 μg (9.64 nmol) of the cHMGI
protein were phosphorylated at Ser3 (8) at 30 °C with 8 units of recombinant human Cdc2-kinase (New England Biolabs Inc.) for 4 h in the presence of 3.5 mM ATP and 100–150 μCi [γ-32P]ATP in 8 μl Cdc-2 kinase buffer containing 50 mM Tris/HCl, 10 mM MgCl2, 1 mM dithiothreitol, 1 mM EDTA, pH 7.5. The reaction was stopped by precipitation of the protein by addition of 50% (v/v) CCl3COOH for 30 min at 0 °C. The pellet was washed with 30% CCl3COOH, 0.2% HCl in acetone, twice with pure acetone and dried.

DNA and Oligonucleotides—The synthetic linear poly(dA-dT)poly(dA-dT) DNA was from Amersham Pharmacia Biotech. The approximate average length of this DNA was 5000 bp. The 34-bp fragment of the promoter of the IFN-γ gene containing the PRDII/NRDI sites was prepared from synthetic oligonucleotides. The sequence of the top strand was 5’-GAATGTAAGTGGAAAAATTTCCCTGGAATAGAGAG-3’ (PRDII site is underlined). Four-division junction c was prepared according to Bianchi (36).

Hydroxyl Radical Footprinting—100 pmol of the radioactively end-labeled protein (15,000–30,000 cpm) were incubated in the presence or absence of DNA in 257 mM NaCl and 14.3 mM MOPS, pH 7.2, buffer at room temperature for 15 min. The chemical digestions were started by sequential addition of 1 μl each of the following freshly prepared solutions: (i) 20 mM EDTA and 10 mM (NH4)2Fe(SO4)2; (ii) 0.2 mM sodium ascorbate; and (iii) 0.375% H2O2. If not specified otherwise, the reactions were stopped after 40 min by addition of 3.3 mM of 4-fold SDS sample buffer (4% SDS, 16% glycerol, 25 mM Tris/HCl, pH 6.8, 6% β-mercaptoethanol, and 0.01% bromphenol blue). The reaction products were separated on 16.5% polyacrylamide gels using the Tricine-glycine-SDS buffer system (37). The gels were dried and scanned by a PhosphorImager (Molecular Dynamics).

Size Markers and Assignment of the Hydroxyl Radical Cleavage Sites—Size markers were obtained by limited digestions of the end-labeled CML32 protein with trypsin or protease Glu-C (V8). 100 pmol of end-labeled CML32 were digested with 17 ng of trypsin in 10 μl of 180 mM NaCl, 20 mM Tris/HCl, pH 7.5, at 0 °C for 5 min. Reactions were stopped by addition of 1 μl of 0.14 mM Nε-p-tosyl-L-lysine chloromethyl ketone (TLCK). The cleavage with protease Glu-C (V8) was carried out in the presence of 50 ng of enzyme in 25 mM sodium phosphate, pH 7.8, and 190 mM NaCl at 0 °C for 2 min. Reactions were stopped by addition of SDS sample buffer and immediate boiling of the probe. The end-labeled peptides 1–48 and 1–6 were obtained by cleavage of the CML32 protein with hydroxylamine (38) and trypsin (8), respectively. The peptide 1–32 was synthesized as described previously (8). The assignment of the hydroxyl radical fragments was accomplished using a standard curve.

Data Analysis—The phosphorimages were essentially analyzed according to Heyduk et al. (39) and Baichoo and Heyduk (40). Briefly, phosphorimages of the full lanes width were scanned and the intensities were plotted versus mobility (ImageQuant Software, Molecular Dynamics). The intensity plots were aligned to correct distortions between different lanes using ALIGN software (gift from Dr. T. Heyduk, Chironopharm). The hydroxyl radical footprinting patterns exhibited relatively small changes after binding to DNA and were therefore difficult to analyze by visual comparison (Fig. 1A, hydroxyl radical). A quantitative analysis of the data showed that defined regions were protected from chemical proteolysis, whereas others were not. A comparison of the mean intensities (radioactivity) measured in the lanes (from Fig. 2A) is shown in Fig. 2C. The plot of normalized differences measured between corresponding lanes with and without poly(dA-dT)poly(dA-dT) DNA shows the protected and exposed regions of the DNA-bound protein (Fig. 2D). The protected and the exposed regions found by this procedure matched those found in the enzymatic approach (compare Fig. 1C and Fig. 2D). The sizes and positions of the DNA-protected areas were constant over a wide range of digestion time (Fig. 2, C and D). For further experiments, 40 min of digestion were selected since under these conditions the ratio of small to long fragments is well balanced (Fig. 2C, 40 min), and 55–70% of the protein remained uncleaved (Fig. 2B), suggesting conditions of single cleavage (42). Furthermore, simultaneous DNA digestion by hydroxyl radicals was low under these conditions (not shown).

In the Presence of poly(dA-dT)poly(dA-dT) DNA, Three AT-hook Domains Are Protected from Digestion—Earlier work has revealed that mammalian HMG1 protein binds strongly to sequence specific proteinases or hydroxyl radicals in the presence or absence of DNA, and (iii) the digestion products were separated on polyacrylamide gels. (iv) Finally the gels were subjected to quantitative scanning and objective data analysis, i.e. corrections for gel loading, cleavage efficiency, and the transformation of the electrophoretic mobility of the bands into residue numbers (39, 40).

Cleavage Conditions and Assignment of the Bands—Phosphorylation of the CML32 protein at Ser3 could be used as an end-labeling procedure because this is a unique target of Cdc-2 kinase, and because its modification does not change the DNA binding properties of the protein (8). Limited digestion of the labeled CML32 protein Glu-C or trypsin followed by electrophoresis yielded patterns in which the individual bands could be assigned to peptides of defined lengths (Fig. 1, A, Glu-C and trypsin, and B). Application of end-labeled peptides 1–48, 1–32, and 1–6 facilitated this assignment. Nonlinear regression enabled transformation of the relative mobilities of the cleavage products into residue sites within the protein (Fig. 1B).

In the absence of DNA, trypsin cut the CML32 protein preferentially at five positions, amino acid residues 27/28, 31/32, 53/54, 76, and 84/85 (two adjacent lysine residues are present in four of the sites, and therefore the precise position of cleavage is uncertain). In the presence of poly(dA-dT)poly(dA-dT) DNA, some bands nearly disappeared, whereas the intensity of others was only slightly reduced. (Fig. 1A, lanes marked trypsin). In particular, the cutting frequencies at the tryptic cleavage sites 53/54 and 76 were strongly reduced, whereas the cutting frequencies at position 27/28 and 85/85 were increased (Fig. 1C). The percentage of cleavage at sites 31/32 remained almost unchanged. These results show that regions corresponding to the second and the third AT-hook motif in the protein were protected after DNA binding, whereas other regions were more exposed. Since trypsin (like other proteinases), because of relatively large size, does not gain access to all cleavage sites of a protein and is able to cleave the peptide backbone at certain positions, these data provide only preliminary information on the structure of the CML32/DNA complex. To obtain more precise information on regions of the protein that contact the DNA, hydroxyl radicals were used as a chemical probe (33, 39, 41).

The hydroxyl radical footprinting patterns exhibited relatively small changes after binding to DNA and were therefore difficult to analyze by visual comparison (Fig. 1A, hydroxyl radical). A quantitative analysis of the data showed that defined regions were protected from chemical proteolysis, whereas others were not. A comparison of the mean intensities (radioactivity) measured in the lanes (from Fig. 2A) is shown in Fig. 2C. The plot of normalized differences measured between corresponding lanes with and without poly(dA-dT)poly(dA-dT) DNA shows the protected and exposed regions of the DNA-bound protein (Fig. 2D). The protected and the exposed regions found by this procedure matched those found in the enzymatic approach (compare Fig. 1C and Fig. 2D). The sizes and positions of the DNA-protected areas were constant over a wide range of digestion time (Fig. 2, C and D). For further experiments, 40 min of digestion were selected since under these conditions the ratio of small to long fragments is well balanced (Fig. 2C, 40 min), and 55–70% of the protein remained uncleaved (Fig. 2B), suggesting conditions of single cleavage (42). Furthermore, simultaneous DNA digestion by hydroxyl radicals was low under these conditions (not shown).

In the Presence of poly(dA-dT)poly(dA-dT) DNA, Three AT-hook Domains Are Protected from Digestion—Earlier work has revealed that mammalian HMG1 protein binds strongly to
poly(dA-dT) (9). Because this synthetic DNA is also a good ligand of cHMGI, we chose it for our experiments. cHMGI was footprinted in the presence of concentrations of poly(dA-dT) ranging from 16 to 120 bp per protein molecule (Fig. 3, A and B). At lower ratios (16 bp:1 and 32 bp:1) protection was observed at amino acid residues 10–22 and 54–60. In addition, protection at amino acid residues 30–40 was detected. The region between residues 10 and 20 corresponds to part of the first AT-hook sequence motif and adjacent NH2-terminal stretch. The region 53–60 constitutes the second AT-hook motif. At protein to DNA ratios of 60 bp:1 (Fig. 3 B) and 120 bp:1 (not shown) an additional region, amino acid residues 75–81, which comprises the third AT-hook, was protected. Under these conditions, protection of region 30–40 was no longer observed, and in contrast to lower DNA to protein ratios, a large portion of the protein (residues 29 to 50) was found to be highly susceptible to digestion and should therefore be exposed to the solvent. At all concentrations of DNA used an enhanced
susceptibility to digestion was observed for the negatively charged COOH terminus of the protein.

Binding of cHMGI to Oligonucleotide with Binding Site of Human HMGI Involves the Three AT-hooks and Regions Flanking AT-hooks Two and Three—In vivo binding sites of the insect HMGI on the DNA are not known. Because most properties of this protein are identical or similar to those of mammalian HMGI, we analyzed the binding determinants of cHMGI in complexes with a DNA sequence comprising a mammalian ligand. A 34-bp DNA carrying PRDII/NRDI elements of the promoter region of the human IFN-β gene was selected for the experiments. The stoichiometry of cHMGI binding to this DNA is 1:2.1. Footprinting revealed protection of residues 46–82 and 8–10 (Fig. 3, C and D). The NH₂-terminal located region corresponds to part of the first AT-hook motif, whereas the large protected region comprises the other two AT-hooks and areas flanking these motifs. Regions of cHMGI rich in negatively charged residues (20–44 and 85–90), as in the complex with poly(dA-dT)poly(dA-dT) were found to be cleaved more frequently than without DNA and therefore probably exposed to solvent in complex with the DNA.

Fig. 3. Hydroxyl radical footprinting of the cHMGI protein on poly(dA-dT)poly(dA-dT) (A and B), PRDII/NRDI (C and D), and four-way junction DNA (E and F). A and B, 100 pmol of 32P-labeled cHMGI on Ser¹ were digested with hydroxyl radicals in the absence or presence of 1.6, 3.2, 6, and 12 nmol/bp of poly(dA-dT)poly(dA-dT), respectively. C–F, the same amount of protein was footprinted in the presence of 50, 100, and 200 pmol of PRDII/NRDI or four-way junction DNA, respectively. Panels A, C, and E, representative phosphorimages of the gels from single experiments. The schematic primary structure of cHMGI with AT-hooks (gray boxes) is shown at the left of each image. Panels B, D, and F, in each case difference plots show averaged data from comparisons of 12 lanes. Bold lines above the plots indicate regions where the observed protection/exposition was statistically significant according to a Student’s t-test (confidence level of 0.95). Only the statistical analysis for the highest DNA concentration used is shown. Schematic primary structure of cHMGI with AT-hooks (gray boxes) is shown in the lower part of panels B, D, and F.

DISCUSSION

Histones and the HMG proteins are the most abundant components of chromatin (for review see Refs. 43 and 44). In
the structure of the histones and HMGI1/2 proteins, well characterized folded domains are found in central positions. Another characteristic structural feature shared by these proteins is the presence of large structurally undefined regions, *termini, bristles, or tails*. Two groups of the HMG proteins, the families HMGI/Y and HMGI14/17, are thought to be composed mainly of flexible regions of undefined structure. Binding to DNA induces a spatial ordering of regions containing residues involved in contacting DNA. Binding of the HMGI protein to DNA simultaneously leads to specific ordering of the protein structure (31) and induces changes in the conformation of the DNA (10–13). The contacts of the entire HMGI molecule to DNA in protein-DNA complexes have been unknown. Here we have mapped for the first time the regions of an HMGI protein that are involved in the binding to DNAs of various types.

Our protein footprinting experiments revealed some general and some DNA structure-specific features of the cHMGI-DNA complex (Fig. 4). Binding of cHMGI to poly(dA-dT) to poly(dA-dT), to cruciform DNA and to HMGI binding site in interferon-β promoter DNA (PRII/NRDI) involved contacts by the first and second *AT-hook* motifs and led to exposition to the solvent of large parts of the region joining these two motifs and the COOH-terminal acidic 

---

**Fig. 4. Summary of the footprinting experiments.** Upper box schematically represents the cHMGI protein. The gray and checkered areas indicate the positions of the three *AT-hook* and the PKRP-sequence motifs, respectively. Horizontal filled boxes (below) show regions of cHMGI protein protected from hydroxyl radical cleavage in the presence of the three different types of DNA. Regions exposed to digestion upon protein binding to DNA are indicated by hatched boxes. The numbers refer to the residue positions.

---

compared with binding to rigid or intrinsically curved AT-rich DNA (47). The PRIII/NRDI-DNA is intrinsically pre-bent by about 20°, and binding of human HMGI to this DNA induces a partial reversal bending (13). A similar unbending of the PRDII/NRDI-DNA was also exhibited by the insect HMGI protein.3 Our finding that the insect HMGI protein makes extensive contacts with this specific DNA is in accordance with data from recent NMR studies that suggest extensive contacts between HMGI and PRDII-DNA and have revealed that 19 of the 42-residues-long HMGI(2/3) peptide are directly involved in the interaction with a PRDII-dodecamer (31).

In contrast, in complexes with cruciform DNA the third motif of the cHMGI protein remained unprotected, suggesting that this DNA cannot accommodate the entire protein or that the protein has a specific conformation in a complex with the cruciform DNA. Because this type of DNA is able to accommodate up to two HMGI molecules (48), the second possibility appears to be more probable. Interestingly, in this complex the region joining the second and the third motif was protected. It contains another sequence motif, PKRP (Fig. 4), which occurs not only in the HMGI/Y proteins but is also characteristic for proteins of the families HMGI14/17 and HMGI1/2. Peptides carrying this motif interact specifically with the minor groove of the DNA (35). Because all of these proteins exhibit preferential binding to cruciform DNA, it is possible that this motif plays a role in the recognition of this type of DNA.

Four-way junction DNA has been suggested as a model for DNA in chromatin at the site where it enters and exits the nucleosome (49, 50). The binding of HMGI to this DNA may reflect a constitutive function of this protein in the organization of chromatin, in contrast to specific functions as organization of promoter complexes of particular genes. We have recently shown that phosphorylation at various residues may be involved in the adaptation of members of the HMGI/Y family to fulfill different cellular functions (8). In further studies, this possibility could be checked by investigating the binding of various phosphoforms of HMGI/Y proteins to DNA as well as to nucleosomes. By means of the protein-footprinting technique applied in this study it would also be possible to map contacts of the cHMGI protein within reconstituted chromatin or its selected components.

Acknowledgments—We thank Dr. U. Grossbach for the continuous support and interest in this work, Dr. T. Heyduk (St. Louis University) for providing the ALIGN software for the quantitative analysis of the phosphorimages and stimulating discussion.
REFERENCES

1. Johnson, K. R., Lehn, D. A., and Reeves, R. (1989) *Mol. Cell. Biol.* 9, 2114–2123
2. Manfioletti, G., Giancotti, V., Bandiera, A., Buratti, E., Seuttiere, P., Cary, P., Crane-Robinson, C., Coles, B., and Goodwin, G. H. (1991) *Nucleic Acids Res.* 19, 6793–6797
3. Claus, P., Schulze, E., and Wizniewski, J. R. (1994) *J. Biol. Chem.* 269, 33942–33948
4. Grasser, K. D. (1995) *Plant J.* 7, 185–192
5. Nicolas F. J., Cayuela, M. L., Martinez-Argudo, I. M., Ruiz-Vasquez, R. M., and Murillo F. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 963–976
6. Ashley, T. C., Pendleton, C. G., Jennings, W. W., Saxena, A., and Glover, C. V. (1989) *J. Biol. Chem.* 264, 8394–8401
7. Reeves, R., and Nissen, M. S. (1996) *J. Biol. Chem.* 271, 8573–8582
8. Schwanbeck, R., and Wizniewski, J. R. (1997) *J. Biol. Chem.* 272, 27476–27483
9. Solomon, M. J., Straus, F., and Varshavsky, A. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 1276–1280
10. Lehn, D. A., Elton, T. S., Johnson, K. R., and Reeves, R. (1988) *Biochem. Int.* 16, 963–976
11. Nissen, M. S., and Reeves, R. (1995) *J. Biol. Chem.* 270, 4355–4360
12. Heyduk, T., Heyduk, E., Claus, P., and Wizniewski, J. R. (1997) *J. Biol. Chem.* 272, 19763–19770
13. Falvo, J. V., Thanos, D., and Maniatis, T. (1995) *Cell* 83, 1101–1111
14. Nissen, M. S., Langan, T. A., and Reeves, R. (1991) *J. Biol. Chem.* 266, 19945–19952
15. Xiao, D., and Huang, K. (1997) *Chang-Kuo-I-Hsueh-Ko-Hsueh-Yuan-Ko-Hsueh* 18, 79–83
16. Reeves, R., Langan, T. A., and Nissen, M. S. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 1671–1675
17. Straus, F., and Varshavsky, A. (1984) *Cell* 37, 889–901
18. Disney, J. E., Johnson, K. R., Magnussen, N. S., Sylvester, S. R., and Reeves, R. (1989) *J. Cell Biol.* 109, 1975–1982
19. Bustin, M., and Reeves, R. (1996) *Prog. Nucleic Acids Res. Mol. Biol.* 54, 35–100
20. Thanos, D., and Maniatis, T. (1992) *Cell* 71, 777–789
21. Thanos, D., and Maniatis, T. (1995) *Cell* 83, 1091–1100
22. John, S., Reeves, R. B., Lin, J.-X., Child, R., Leiden, J. M., Thompson, C. B., and Leonard, W. J. (1993) *Cell.* 74, 1786–1796
23. Bagnoli, R., and Emerson, B. M. (1997) *Genes Dev.* 11, 629–639
24. Farnet, C. M., and Bushman, F. D. (1997) *Cell* 88, 483–492
25. Ghidelli, S., Claus, P., Thies, G., and Wizniewski, J. R. (1997) *Chromosoma* 105, 369–379
26. Johnson, K. R., Disney, J. E., Wyatt, C. R., and Reeves, R. (1990) *Exp. Cell Res.* 207, 60–76
27. Ram, T. G., Reeves, R. B., and Hosick, H. L. (1993) *Cancer Res.* 53, 2655–2660
28. Berlingeri, M. T., Manfioletti, G., Santoro, M., Bandiera, A., Visconti, R., Giancotti, V., Fusco, A. (1995) *Mol. Cell. Biol.* 15, 1545–1553
29. Berner, J. M., Meza-Zapeda, L. A., Kos, P. P. J., Forus, A., Schoenmakers, E. F. P. M., Van de Ven, W. J. M., Fodstad, Ø., and Myklebost, O. (1997) *Oncogene* 14, 2955–2961
30. Tkachuk, A., Ashar, H. R., Meloni, A. M., Sandberg, A. A., and Chada, K. K. (1997) *Cancer Res.* 57, 2276–2280
31. Huth, J. R., Bewley, C. A., Nissen, M. S., Evans, J. N. S., Reeves, R., Gronenborn, A. M., and Clore, G. M. (1997) *Nat. Struct. Biol.* 4, 657–665
32. Yie, J., Liang, S., Merika, M., and Thanos, D. (1997) *Mol. Cell. Biol.* 17, 3649–3662
33. Heyduk, E., and Heyduk, T. (1994) *Biochemistry* 33, 9643–9650
34. Wizniewski, J. R., and Schulze, E. (1994) *J. Biol. Chem.* 269, 10713–10719
35. Wizniewski, J. R., Heffler, K., Claus, P., and Zechel, K. (1997) *Eur. J. Biochem.* 243, 151–159
36. Bianchi, M. I., Beltrame, M., and Pannessa, G. (1989) *Science* 243, 1056–1059
37. Schagger, H., and von Jagow, G. (1987) *Anal. Biochem.* 166, 368–379
38. Bornstein, P., and Balian, G. (1997) *Methods Enzymol.* 367, 132–142
39. Heyduk, T., Heyduk, E., Severinov, K., Tang, H., and Ehrlich, R. H. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 10162–10166
40. Baichoo, N., and Heyduk, T. (1997) *Biochemistry* 36, 10830–10835
41. Greiner, P. D., Hughes, K. A., Gunasekera, A. H., and Mears, C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 71–75
42. Brenowitz, M., Senear, D. P., Shea, M., and Ackers, G. K. (1988) *Methods Enzymol.* 130, 132–181
43. van Holde, K., Zlatanova, J., Arents, G., and Moundrianakis, E. (1994) in *Chromatin Structure and Gene Expression* (Elgin, S. C. R., ed) pp. 1–26, IRL Press, Oxford
44. Wizniewski, J. R., and Grossbach, U. (1996) *Int. J. Dev. Biol.* 40, 177–187
45. Maher, J. F., and Nathans, D. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 6716–6720
46. Drew, H., and Dickerson, R. E. (1981) *J. Mol. Biol.* 151, 535–556
47. Reeves, R., and Wolfe, A. P. (1996) *Biochemistry* 35, 5063–5074
48. Hill, D. A., and Reeves, R. (1997) *Nucleic Acids Res.* 25, 3523–3531
49. Varga-Weisz, P., Zlatanova, J., and van Holde, K. (1997) *J. Biol. Chem.* 266, 20699–20706
50. Varga-Weisz, P., Zlatanova, J., Leuba, S. H., Schroth, G. P., and van Holde, K. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 3520–3529