MOLECULAR AND CELLULAR BIOLOGY, Dec. 1987, p. 4582–4584
0270-7306/87/124582-03$02.00/0

Vol. 7, No. 12

RNA Polymerase II Transcription Factors H4TF-1 and H4TF-2 Require Metal To Bind Specific DNA Sequences

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Specific DNA-binding and in vitro transcription activities of H4TF-1 and H4TF-2 are inactivated by chelating agents. Binding activity is restored by addition of Zn^{2+}, and H4TF-2 is also reactivated by Fe^{2+}. In contrast, preformed factor-DNA complexes are resistant to chelators. Therefore, metal ions are a required component of the H4TF-1 and H4TF-2 DNA-binding domains.

H4TF-1 and H4TF-2 bind specifically to a human histone H4 promoter 95 and 50 base pairs, respectively, upstream of the transcription start site (2). The region bound by H4TF-1 is necessary for maximal expression of the H4 gene in vitro, whereas H4TF-2 binds to highly conserved sequences in the H4 promoter (the H4 subtype specific element) immediately upstream of the TATA box (2, 8).

Fractionation of HeLa nuclear extract on a phosphocellulose (P11) column (3) separates H4TF-2 and H4TF-1 into the flowthrough (FT) and 0.5 M KCl fractions, respectively (2). However, after chromatography and subsequent dialysis, we observed a greater than 70% loss of H4TF-1 DNA-binding activity by the gel shift assay. Addition of EDTA (5 mM) completely abolishes H4TF-1-binding activity within 3 h, whereas 5 mM $MgCl_2$ stabilizes it (data not shown).

On the basis of these results, we investigated whether H4TF-1 requires a metal ion(s) for its DNA-binding activity by the gel shift assay (2, 4, 5). The ^{32}P-labeled DNA probe used spans H4 promoter sequences from −196 to +46 and contains the binding sites of both H4TF-1 and H4TF-2 (2). Probe DNA was mixed with samples of P11 0.5 M KCl fraction which had been incubated for 10 min on ice with $ZnCl_2$, as indicated in Fig. 1. The reaction mixtures were incubated for 10 min at room temperature, and 10 μl from each reaction was fractionated on a polyacrylamide gel (2).

Incubation of the P11 0.5 fraction with $ZnCl_2$ causes a dramatic increase in H4TF-1-binding activity (Fig. 1A). If this effect is due to restoration of Zn^{2+} to H4TF-1 molecules which were depleted of the ion by EDTA during chromatography, then H4TF-1 activity should also be susceptible to the Zn^{2+} chelator 1,10-phenanthroline. Samples of P11 0.5 M KCl fraction were incubated on ice for 15 min with 1,10-phenanthroline (Sigma Chemical Co.) at the concentrations indicated in Fig. 1B and 2A. Metal ions (as indicated in Fig. 1B and 2) were then added, and incubation was continued on ice for 15 min. A 2-μl portion from each of the treated samples was assayed in the binding reactions.

As shown in Fig. 1B, 1,10-phenanthroline abolished H4TF-1-binding activity, but activity could be restored by subsequent addition of $ZnCl_2$. Furthermore, no other cations tested (including Co^{2+}, data not shown) restored activity to the 1,10-phenanthroline- (Fig. 2A) or EDTA- (data not shown) treated H4TF-1 preparations. H4TF-1, therefore, requires a metal ion, probably zinc, for its specific interaction with DNA.

Poor recovery of H4TF-2 activity after dialysis suggested that this factor also requires metal ions. Treatment of the P11 FT fraction with 1,10-phenanthroline severely reduces H4TF-2 DNA binding (Fig. 2B). However, H4TF-2 activity is restored by Fe^{2+} as well as Zn^{2+}. We cannot conclude from this result which ion is associated with H4TF-2 in vivo or if one ion is able to functionally substitute for the other when provided after chelation.

In analogous studies on the RNA polymerase III transcription factor TFIIIA, zinc ions could be chelated from the free factor but not from TFIIIA complexed to RNA within a 7S ribonucleoprotein particle (7). This finding led us to test the stability of bound H4TF-1 and H4TF-2 to 1,10-phenanthroline. Binding reactions were assembled with 2 μl of P11 0.5 M KCl or P11 FT fraction. After 10 min, 1,10-phenanthroline was added and incubation was continued for 5 min on ice.

As shown in Fig. 3, protein-DNA complexes of H4TF-1 or H4TF-2 challenged with increasing amounts of 1,10-phenanthroline remain resistant to the chelator, indicating that the metal ions are relatively inaccessible to chelation when the factors are complexed to DNA. This result would presumably occur only if the metal ions were actually a component of the DNA-binding domains of H4TF-1 and H4TF-2.

These results predict that the in vitro transcription activity of H4TF-1 and H4TF-2 should also be sensitive to chelation. Therefore, the wild-type (wt) H4 promoter, an H4 promoter with base changes in the H4TF-1-binding site (mutant 2-4), and 2606, an H4 promoter lacking both H4TF-1- and H4TF-2-binding sites (2, 8) were assayed for transcription activity in vitro (10, 15) in the absence and presence of 1,10-phenanthroline. In the absence of chelator, the wt H4 promoter and the H4TF-1-defective promoter (mutant 2-4) are transcribed ninefold and twofold more efficiently, respectively, than the deletion mutant 2606 which contains only the H4 TATA box (Fig. 4A). Thus, H4TF-1 stimulates transcription approximately four- to fivefold. However, in the presence of 0.6 mM 1,10-phenanthroline, the ratio of transcription of the wt, 2-4, and 2606 promoters falls from 9:2:1 to 2:1:1 (Fig. 4B). The decrease is not due to inhibition of general transcription activity, since transcription of the 2606 promoter decreases by only 35% in the presence of chelator. The large decrease in transcription efficiency of the wt H4 promoter relative to the 2-4 promoter in the presence of 1,10-phenanthroline strongly suggests that metal ion binding is important for transcription activity of H4TF-1. The small change in the 2-4 and 2606 transcription ratio is

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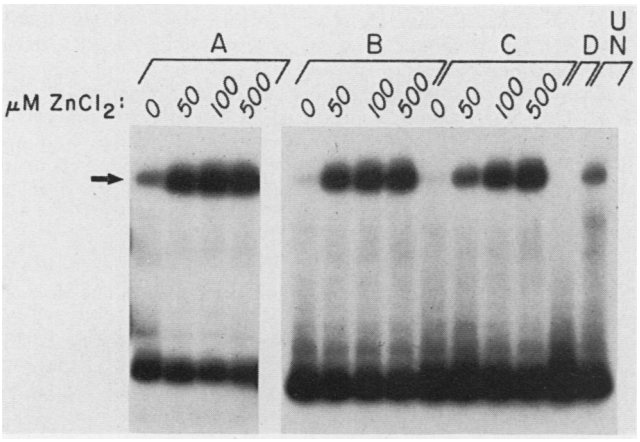

FIG. 1. Restoration of H4TF-1 DNA-binding activity by $ZnCl_2$ after chelation. HeLa nuclear extract was fractionated on a P11 column as previously described (3). $MgCl_2$ (5 mM) was added to the P11 0.5 M KCl fraction to stabilize H4TF-1 against the effects of 0.5 mM EDTA present in the chromatography buffers. (A) Samples of P11 0.5 M KCl fraction were incubated on ice with the indicated amount of $ZnCl_2$, and 2 μl (6 μg of protein) from each sample was assayed in the binding reaction as described previously (2). (B) Samples of the P11 0.5 M KCl fraction were treated with 0.5 mM (final) 1,10-phenanthroline, and subsequently $ZnCl_2$ was added at the concentration shown. In lane 0, the fraction was treated with 1,10-phenanthroline only. The H4TF-1 in 2-μl portions from each sample was then assayed for DNA binding. (C and D) Fractions were treated with 1.0 mM or 2.0 mM phenanthroline, respectively, as described in the legend to Fig. 1B. UN, Untreated 0.5 M KCl P11 fraction. →, Specific H4TF-1-DNA complex.

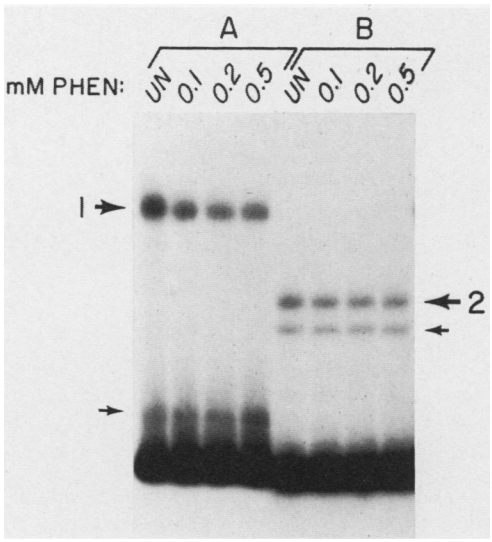

FIG. 3. Resistance of the factor DNA complexes to 1,10-phenanthroline. (A) Binding reactions were assembled with 2 μl of P11 0.5 M KCl fraction and then treated with 1,10-phenanthroline (PHEN) at the concentrations indicated as described in the text. (B) 2 μl of P11 FT protein was used in a binding reaction and treated as described in the legend to Fig. 3A. UN, Untreated fraction; 1, H4TF-1 protein-DNA complex; 2, H4TF-2 protein-DNA complex. Small arrows indicate nonspecific protein-DNA complexes.

consistent with a concomitant decrease in H4TF-2 transcription activity. However, the possibility that other transcription factors (e.g., Sp1 or NF-KB [11, 16]; Fig. 4A) may bind the H4 promoter precludes a definitive statement on the metal ion requirement for H4TF-2 transcription activity.

Taken together, the simplest interpretation of these results is that both H4TF-1 and H4TF-2 are metalloproteins which require bound metal for the integrity of their DNA-binding domains. Zinc is most likely the metal ion involved, since only zinc restored activity to both H4TF-1 and H4TF-2. The $ZnCl_2$ used contains only trace amounts of other metals (for example, <0.001% iron) and none of these alone in much higher quantities could restore binding activity to H4TF-1. Fe^{2+} restored binding to H4TF-2, but the amount of iron contaminant present in the $ZnCl_2$ (<5 nM) is far below that needed to restore H4TF-2 binding in our assay.

Zinc is a required component of the TFIIIA-binding domain (7, 13). As proposed by Miller et al. (13), amino acid

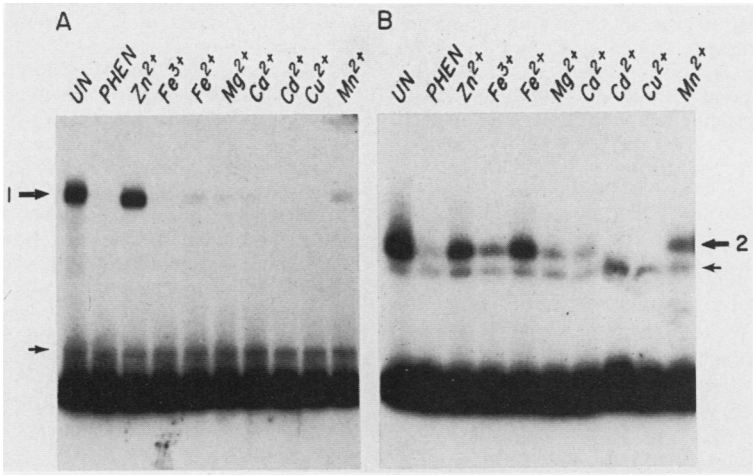

FIG. 2. Ability of different cations to restore H4TF-1- and H4TF-2-binding activity following chelation. Samples of P11 0.5 M KCl fraction (A) or P11 FT (B) fraction were treated with 0.5 mM 1,10-phenanthroline and then incubated in the presence of a 0.5 mM concentration of no addition (PHEN), $ZnCl_2$ (Zn^{2+}), $FeCl_3$ (Fe^{3+}), $Fe(NH_4)_2(SO_4)_2$ (Fe^{2+}), $MgCl_2$ (Mg^{2+}), $CaCl_2$ (Ca^{2+}), $CuSO_4$ (Cu^{2+}), $CdCl_2$ (Cd^{2+}), or $MnCl_2$ (Mn^{2+}) as described in the text. After treatment, H4TF-1 (1 μl of P11 0.5 M KCl fraction) and H4TF-2 (2 μl of P11 FT fraction; 5 μg of protein) activities were assayed in binding reactions. The signal for H4TF-2 binding was aberrantly high in the sample containing Mn^{2+}, since in several repetitions of this experiment, Mn^{2+} had no effect. UN, Untreated fraction; 1, H4TF-1 protein-DNA complex; 2, H4TF-2 protein-DNA complex. Small arrows show nonspecific complexes.

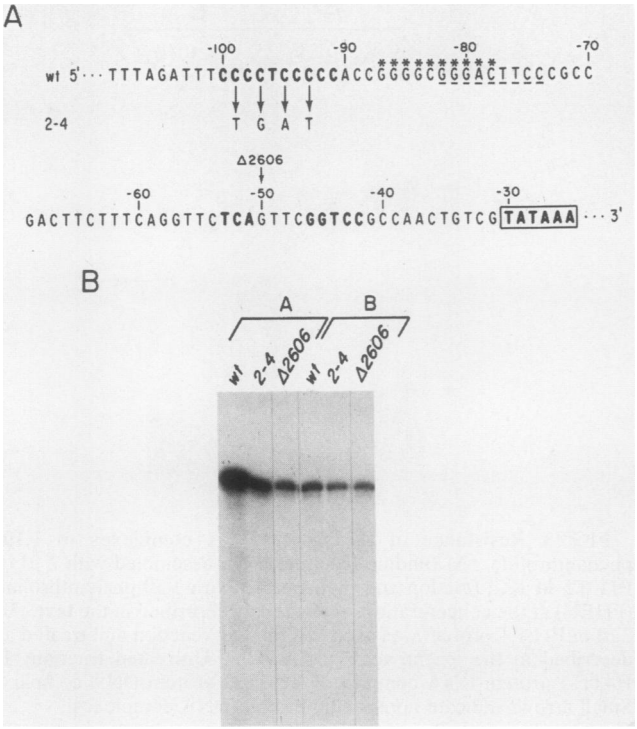

FIG. 4. Effect of 1,10-phenanthroline on transcription from the H4 promoter. (A) The revised H4 promoter sequence of pHu4A (8) as determined by Maxam and Gilbert (12) sequencing is shown. Numbers indicate distance (in base pairs) from the transcription start site. The H4TF-1 recognition sequence around −95 (determined by methylation interference experiments; data not shown) and a conserved sequence between −53 to −42 within the H4TF-2 footprint (2) are indicated in bold print. The 5′ end of the 2606 deletion (8) is indicated at −50. An Sp1 consensus element (11) is starred, and sequences similar to the NF-KB-binding site (16) are underlined. 2-4 is a mutant H4 promoter which contains the indicated base changes. Binding assays (data not shown) had indicated that mutant 2-4 is unable to bind H4TF-1, and previous results (2) have shown that mutant 2606 is unable to bind either factor. (B) Transcription reactions were performed as described (10, 15) using 10 μg of circular template per ml containing the wt or mutant promoter ligated to the C_2AT cassette sequence (15). In Fig. 4B, 0.6 mM (final) 1,10-phenanthroline was also included. wt indicates wt $H4C_2AT$, 2-4 indicates the point mutant 2-4 C_2AT, and 2606 indicates the 2606 C_2AT transcription template DNAs used in each reaction.

repeats within the TFIIIA DNA-binding domain may form "fingers" that are structurally maintained by the coordination of Zn^{2+} ions in the protein. Other proteins contain repeated regions which resemble the finger domain of TFIIIA (1, 6, 9, 14, 17, 18). However, there is little experimental evidence to indicate whether these latter proteins bind metal. It is possible that the H4TF-1 and H4TF-2 metal-binding domains will exhibit the finger structure. Our results thus support the prediction that metal ions are required cofactors for binding of some RNA polymerase II transcription factors.

We thank Robert G. Roeder and the members of his laboratory, especially Michele Sawadogo, Claus Scheidereit, and Mike Van Dyke, for critical discussions related to this work. We also thank Colin Fletcher for synthesis of the oligonucleotides used to construct the H4 promoters.

This work was supported by Public Health Service grant GM 32544 from the National Institutes of Health to N.H., American Cancer Society Postdoctoral Fellowship PF-2739 to L.D., National Institutes of Health Postdoctoral Fellowship GM 11028 to L.D., and American Cancer Society Postdoctoral Fellowship PF-2940 to S.B.R. N.H. was also supported by PeW Scholars Award.

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