GAL4 transcription factor is not a “zinc finger” but forms a $\text{Zn(II)}_2\text{Cys}_6$ binuclear cluster

(^1H NMR/metalloproteins)

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ABSTRACT The DNA-binding domain of the transcription factor GAL4, consisting of the 62 N-terminal residues and denoted GAL4(62*), contains a Cys-Xaa₂-Cys-Xaa₆-Cys-Xaa₆-Cys-Xaa₂-Cys-Xaa₆-Cys motif, which has been shown previously to bind two Zn(II) or Cd(II) ions. Binding of Zn(II) or Cd(II) is essential for the recognition by GAL4 of the specific palindromic DNA sequence to which it binds upstream of genes for galactose-metabolizing enzymes, the UAS_G sequence. On the basis of the ^{113}Cd NMR chemical shifts of the two bound $^{113}\text{Cd(II)}$ ions, we propose a binuclear cluster model for this Zn(II)-binding subdomain. ^1H - ^{113}Cd heteronuclear multiple-quantum NMR spectroscopy and phase-sensitive double-quantum filtered ^1H correlation spectroscopy of the $^{112}\text{Cd(II)}$ - and $^{113}\text{Cd(II)}$ -substituted GAL4(62*) derivatives provide direct evidence that the two bound $^{113}\text{Cd(II)}$ ions are coordinated only by the six cysteine residues, two of which form bridging ligands between the two $^{113}\text{Cd(II)}$ ions. The latter can be identified from the pattern of ^1H - ^{113}Cd J coupling. Thus a binuclear metal ion cluster rather than a “zinc finger” is formed by the six cysteine residues of the GAL4 DNA-binding domain. This model can be directly applied to eight other fungal transcription factors which have been shown to contain similarly spaced Cys₆ clusters. ^1H NMR spectra of apo-GAL4(62*) suggest conformational fluctuation of the metal-binding subdomain upon removal of Zn(II) or Cd(II). Both Cd(II)₂- and Zn(II)₂-containing species of GAL4 can be formed, and the similar ^1H NMR spectra suggest similar conformations.

The GAL4 transcription factor from *Saccharomyces cerevisiae* is a protein of 881 amino acids required for the transcriptional activation, in the presence of galactose, of the genes coding for galactose-metabolizing enzymes (1). The DNA-binding domain, which recognizes a specific nucleotide sequence (UAS_G) upstream of the promoters for these genes, has been localized to the N-terminal 62 amino acids (2–5). This domain of GAL4 contains a cysteine-rich amino acid sequence, Cys¹¹-Xaa₂-Cys¹⁴-Xaa₆-Cys²¹-Xaa₆-Cys²⁸-Xaa₂-Cys³¹-Xaa₆-Cys³⁸, conserved among a group of nine transcription factors isolated from fungi (6). The cysteine cluster in GAL4 and related transcription factors has been proposed to form a single “zinc finger” containing a tetrahedral Zn(II) coordination complex (7), similar to that described for transcription factor TFIIIA from *Xenopus* oocytes (8). The GAL4 “finger” would have 4 cysteine residues as ligands rather than the 2 cysteine and 2 histidine residues found in TFIIIA and related proteins (7, 8).

We have shown previously that cloned N-terminal fragments of GAL4 consisting of 149 and 62 amino acids, denoted GAL4(149*) and GAL4(62*), contain Zn(II) (4, 5). The Zn(II) is essential for the binding of these fragments to the UAS_G DNA sequence, and Zn(II) can be replaced by Cd(II) without

loss of specific DNA binding (4, 5). A single zinc-finger model of GAL4 predicts that the protein contains 1 Zn(II) ion per molecule, but both GAL4(149*) and GAL4(62*) can bind 2 Zn(II) or Cd(II) ions per molecule (4, 5). $^{113}\text{Cd(II)}$ substitution of Zn(II)-binding sites in metalloproteins has served as a powerful probe of the structure of these Zn(II) sites, primarily because of the great sensitivity of ^{113}Cd NMR signals to the nature of the ligands to the metal ion. ^{113}Cd NMR in combination with two-dimensional (2D) ^1H NMR has led to the determination of the complete solution structure of the small Zn(II)- and Cd(II)-binding protein metallothionein (9, 10). This protein is the only example of a Zn(II) or Cd(II) cluster complex found in nature in which several of the cysteine residues act as bridging ligands between two metal ions (10). Metallothionein contains 7 metal ions located in two clusters (11).

Our initial ^{113}Cd NMR study of Cd₂GAL4 confirmed the presence of two Cd(II)-binding sites in both GAL4(149*) and GAL4(62*) which induce ^{113}Cd chemical shifts of 707 and 669 ppm, consistent with ligation of each $^{113}\text{Cd(II)}$ to at least 3 and probably 4 sulfur atoms (4, 5). We have now used 2D ^1H NMR techniques to demonstrate that the only ligands to the 2 $^{113}\text{Cd(II)}$ ions in $^{113}\text{Cd}_2\text{GAL4(62*)}$ are the 6 cysteine residues. The ^1H - ^{113}Cd J coupling patterns of the cysteine β protons are best explained by a binuclear cluster in which two of the cysteine residues are bridging ligands between the two metal ions. The NMR data supporting this model of the DNA-binding domain of GAL4 are presented in this paper.

MATERIALS AND METHODS

Cloning, Overproduction, and Purification of GAL4(62*). The GAL4(62*) clone was obtained by insertion of a *Spe* I stop-codon linker, 5'-CCCGGCTAGACTAGTCTAGC-CGGG, into the *Hinf*I site of codon 59 in pTPT7G1 (4). The *Spe* I linker replaces the natural sequence Leu-Glu at residues 61–62 by Leu-Asp. We have termed our construct GAL4(62*). Overproduction and purification of GAL4(62*) was as described for GAL4(149*) (4) except that GAL4(62*) was eluted at standard column buffer plus 250 mM NaCl from a Bluegel agarose column (Bio-Rad).

Preparation of Apo- and Cd(II)GAL4(62*). Purified GAL4(62*) contains 1–2 atoms of Zn(II) per molecule. Apo-GAL4(62*) can be obtained as described for apo-GAL4(149*) (4). Cd(II)GAL4(62*) can be prepared by addition of Cd(II) to the apoprotein at pH 8.0 in the presence of excess 2-mercaptoethanol. Alternatively, Zn(II) can be exchanged with Cd(II) as follows: A mixture of 3-fold molar excess of Cd(II) over Zn(II)GAL4(62*) in the presence of excess 2-mercaptoethanol is incubated at room temperature (22°C) for 12 hr. Dialysis against metal-free buffer then removes more than 90% of the Zn(II), which has become free. The exchange

process can be repeated to yield GAL4(62*) with two tightly bound Cd(II) ions.

^1H NMR. The NMR spectroscopy was performed on a Bruker (Billerica, MA) AM-500 spectrometer at 35°C. All samples were stored under nitrogen to prevent oxidation of SH groups. $^{112}\text{Cd}_2$ - and $^{113}\text{Cd}_2\text{GAL4}(62^*)$ were obtained as described above, lyophilized, and redissolved in $^2\text{H}_2\text{O}$. The concentrations for both species were about 3 mM. Apo- and Zn(II)GAL4(62*) were exchanged into buffer in $^2\text{H}_2\text{O}$ (50 mM sodium phosphate/100 mM NaCl, pH 7.8) by a Sephadex G-25 size-exclusion spun column. The acquisition parameters are listed in the figure captions. Chemical shifts are plotted relative to sodium trimethylsilyltetraduteriopropanoate. The Fourier transform NMR program by Dennis Hare (Hare Research, Woodinville, WA) was used for processing the 2D NMR spectra.

RESULTS

^1H - ^{113}Cd Heteronuclear Multiple-Quantum Correlated Spectroscopy (HMQC) of $^{113}\text{Cd}(\text{II})\text{GAL4}(62^*)$. To identify the protons of GAL4(62*) coupled to the bound $^{113}\text{Cd}(\text{II})$ (i.e., protons within three bonds of the ^{113}Cd nucleus), ^1H - ^{113}Cd HMQC was carried out on $^{113}\text{Cd}_2\text{GAL4}(62^*)$ (9). The chemical shifts of all protons observed to be coupled to $^{113}\text{Cd}(\text{II})$ are shown in Fig. 1. Their chemical shifts, 3.8–2.6 ppm, are consistent with the assignment of all the resonances to the β protons of the 6 cysteine residues in the protein. No protons from other residues such as those of the CH_3 group of the N-terminal methionine are coupled to the $^{113}\text{Cd}(\text{II})$ ions.

2D ^1H Correlated Spectroscopy (COSY) of GAL4(62*). The resonances of individual β protons coupled to the protein-bound $^{113}\text{Cd}(\text{II})$ ions can be separated and identified by comparing the phase-sensitive double-quantum filtered (DQF)- ^1H -COSY of $^{112}\text{Cd}_2$ ($I = 0$) and $^{113}\text{Cd}_2$ ($I = 1/2$)-GAL4(62*). Resolution of the additional ^1H - ^{113}Cd scalar coupling of the α - β and β - β cross-peaks of the cysteine side chains allows the precise determination of the number of cysteine residues coordinated to Cd(II), and the magnitude and pattern of the J coupling observed give information on the structure of the $^{113}\text{Cd}(\text{II})$ -binding sites.

^1H - ^{113}Cd Coupling Patterns for the α - β Cross-Peaks of Cysteine Ligands in $^{113}\text{Cd}_2\text{GAL4}(62^*)$. Regions of the phase-sensitive DQF- ^1H -COSY containing the α - β cross-peaks for 5 of the 6 cysteine residues (designated Cys 2–6) are shown in Fig. 2 A and B for $^{112}\text{Cd}(\text{II})_2\text{GAL4}(62^*)$ and $^{113}\text{Cd}(\text{II})_2\text{GAL4}(62^*)$, respectively. In the case of Cys 2 the α - β^a and α - β^b cross-peaks are degenerate due to the similar chemical shifts of the two β protons (Table 1). The change from ^{112}Cd to ^{113}Cd is expected to induce additional scalar coupling only to Cys α - β and β^a - β^b cross-peaks. The α - β cross-peaks of Cys 1 lie outside of the spectral region shown in Fig. 2 and are shown in Fig. 3A. Supportive confirmation for the assignment of Cys 4 in Fig. 2 is shown in Fig. 3B, taken from the other half of the COSY spectrum. The chemical shifts of the cysteine β^a and β^b protons determined from the phase-sensitive ^1H COSY are consistent with those obtained from the ^1H - ^{113}Cd HMQC (Fig. 1, Table 1). The ^1H - ^{113}Cd coupling

FIG. 1. ^1H - ^{113}Cd HMQC [$90^\circ(^1\text{H})$ - τ - $90^\circ(^{113}\text{Cd})$ - $t_{1/2}$ - $180^\circ(^1\text{H})$ - $t_{1/2}$ - $90^\circ(^{113}\text{Cd})$ - τ -FID] of $^{113}\text{Cd}(\text{II})\text{GAL4}(62^*)$ in $^2\text{H}_2\text{O}$; $\tau = 8$ ms. The number of transients was 1024 and the relaxation delay was 1.5 s.

FIG. 2. Comparison of the phase-sensitive DQF- ^1H -COSY of $^{112}\text{Cd}_2\text{GAL4}(62^*)$ (A) and $^{113}\text{Cd}_2\text{GAL4}(62^*)$ (B). For both experiments sweep width was 2790 Hz and 2048 data points in the t_2 and 512 experiments in the t_1 domain were collected. The spectra represent 64 scans each. The relaxation delay was 1.7 s. α - β^a , β^b cross-peaks are shown. Numbers by boxed peaks refer to cysteine residues arbitrarily designated as 1–6. Note that in Figs. 2 and 4 the contours are not phase coded.

observed for the β^a protons of all 6 cysteine residues of GAL4(62*) confirms that all 6 residues are coordinating at least one of the two Cd(II) ions in the protein.

^1H - ^{113}Cd Coupling Patterns for the β^a - β^b Cross-Peaks for Cysteine Ligands in GAL4(62*). The β^a - β^b cross-peaks of Cys 3, Cys 4, Cys 5, and Cys 6 are located in the section of the $^{112}\text{Cd}_2\text{GAL4}(62^*)$ and $^{113}\text{Cd}_2\text{GAL4}(62^*)$ ^1H COSY shown in Fig. 4 A and B, respectively. The detailed ^1H - ^{113}Cd coupling pattern of the β^a - β^b cross-peaks of Cys 5 and Cys 6 are shown in Fig. 5. Metallothionein provides the best guide to the ^1H - ^{113}Cd coupling expected for the $\alpha\beta$ and $\beta^a\beta^b$ ^1H -COSY cross-peaks of cysteine residues coordinated to $^{113}\text{Cd}(\text{II})$ (12). In metallothionein there are 20 cysteine residues coordinated to Cd(II), 8 of which bridge two Cd(II) ions (12). From the ^1H

Table 1. Chemical shifts and ^1H - ^1H and ^1H - ^{113}Cd coupling constants for six cysteine residues of GAL4(62*)

No. of spin system	$^{113}\text{Cd}_2\text{GAL4(62*)}$ at 35°C, pH 8.0										$\text{Zn}_2\text{GAL4(62*)}$ at 35°C, pH 5.4		
	Chemical shift, ppm			^1H - ^1H coupling constants, Hz			^1H - ^{113}Cd coupling constants, Hz				Chemical shift, ppm		
	H_α	H_{β^a}	H_{β^b}	$J_{\alpha\beta^a}$	$J_{\alpha\beta^b}$	$J_{\beta^a\beta^b}$	$J_{\beta^a\text{Cd}^1}$	$J_{\beta^b\text{Cd}^1}$	$J_{\beta^a\text{Cd}^2}$	$J_{\beta^b\text{Cd}^2}$	H_α	H_{β^a}	H_{β^b}
1	4.94	3.73	3.60	11	10	15	25	10			4.67	3.60	3.50
2	4.64	2.95	2.95	—	—	—	≈52	—			4.65	2.84	2.84
3	4.33 [†]	3.04	2.67	11	<2	22	55	26			4.29	3.04	2.55
4	4.22	2.99	3.14	7	<2	16	20	45			4.12	2.88	—
5	3.98	2.82	3.35	10	<2	14	28	14	5 [‡]	3	3.98	2.75	3.25
6	3.75	2.77	3.26	10	<2	14	29	16	2 [‡]	3	3.73	2.73	3.17

Note that by definition β^a is the proton with the larger homonuclear spin-spin coupling (12). A — indicates not obtainable due to lack of resonances or degeneracy of the chemical shifts.

[†]Chemical shift differs from that of $^{113}\text{Cd}_2\text{GAL4(62*)}$ ($\text{H}_\alpha = 4.29$). The reason for such differences is not clear.

[‡]From α - β^a cross-peaks.

COSY of $^{113}\text{Cd}_7$ metallothionein, it is apparent that in the presence of a bridging cysteine, coupling of the β protons to one of the coordinated ^{113}Cd nuclei is generally of much greater magnitude than to the other. In fact, only two of the shared cysteine residues in metallothionein can be readily identified in the ^1H COSY of the $^{113}\text{Cd}_7$ derivative (12). The β^a - β^b cross-peaks of Cys 5 and Cys 6 of GAL4(62*) show splitting patterns similar to the pattern shown by the one "classical" shared cysteine (C14 = Cys⁴⁴ in the sequence) in metallothionein. However, the discernible $^{113}\text{Cd}^2$ - β^b coupling in $^{113}\text{Cd}(\text{II})\text{GAL4(62*)}$, expressed as increased vertical separation of the multiplet on shifting from ^{112}Cd to ^{113}Cd , is quite small, 3–4 Hz for both Cys 5 and Cys 6. Although a $J_{\text{H}\beta^a\text{Cd}^2}$ is not indicated for either Cys 5 or Cys 6 in Fig. 5 ($J_{\text{H}\beta^a\text{Cd}^1} = 28$ and 29 Hz, respectively), the splitting of the α - β^a cross-peaks of these same cysteine residues is ≈33 and 31 Hz (Fig. 2B). This difference in coupling of $^{113}\text{Cd}^1$ to β^a as measured from the β^a - β^b vs. the α - β^a cross-peak suggests that significant coupling (≈2–5 Hz) of β^a to $^{113}\text{Cd}^2$ is present. In addition, partial cancellation of the multiplets in the α - β cross-peaks of Cys 5 and Cys 6 is also suggestive of finite coupling constants to a second ^{113}Cd ion. Such coupling is more directly manifest in the α - β^a than in the β^a - β^b cross-peak. The ^1H - ^{113}Cd coupling constants for GAL4(62*) derived from the phase-sensitive ^1H COSY vary from 2 to 55 Hz (Table 1). The range of values is comparable to that observed for the $^{113}\text{Cd}_7$ derivative of metallothionein (12). The β^a - β^b cross-peak of Cys 1 is located downfield of the section of the COSY spectrum shown in Fig. 4 and has been used to measure the ^{113}Cd - $^1\text{H}_\beta$ coupling constants given in Table 1. There is no β - β cross-peak for Cys 2 due to the degeneracy of the chemical shifts of the β protons (Table 1).

We have shown previously by circular dichroism that removal of Zn(II) causes substantial conformational change in GAL4(63) (5). Considerable chemical shift changes and reduction of linewidth of several signals are observed when Zn(II) or Cd(II) is bound to apo-GAL4(62*) (Fig. 6). The protons of Zn(II)- and Cd(II)GAL4(62*) have very similar chemical shifts, suggestive of nearly identical conformations for both species.

DISCUSSION

The N-terminal fragments of the transcription factor GAL4, either 147 or 63 amino acids in length, form domains which fold independently and bind to the specific DNA sequence, UAS_G, recognized by GAL4 (4, 5). Both fragments bind 1 to 2 Zn(II) ions, and they maintain the binding of the second Zn(II) when the free Zn(II) concentration is 5 μM (5). When the Zn(II) is replaced with $^{113}\text{Cd}(\text{II})$, the ^{113}Cd NMR of both fragments shows two ^{113}Cd NMR signals, each of which integrates to one $^{113}\text{Cd}(\text{II})$ ion (4, 5). Thus there are two metal-ion-binding sites on the DNA-binding domain of GAL4.

GAL4(62*) has a high-resolution proton NMR spectrum uncomplicated by the oligomerization shown by GAL4(149*) when it is present at the concentrations of protein required for ^1H NMR (5). The GAL4(62*) clone produces a protein structurally and functionally identical to GAL4(63) obtained from GAL4(149*) by partial proteolysis (5). GAL4(62*) is soluble to concentrations of several millimolar and thus ideal for 2D ^1H -NMR studies. The fact that cloned GAL4(62*) is highly soluble, while GAL4-(1–74) is not (5), also suggests that GAL4(62*) forms an independent domain. The ^1H - ^{113}Cd HMQC establishes that only the six cysteine residues appear to be ligands to metal ions (Fig. 1). The DQF- ^1H -COSY

FIG. 3. Phase-sensitive DQF- ^1H -COSY regions showing α - β^a , β^a - β^b cross-peaks of Cys 1 (A) and α - β^a cross-peak of Cys 4 (B) (see text). For Cys 1 and ^{113}Cd , additional splitting makes resonance 1b of low intensity.

FIG. 4. Phase-sensitive DQF- ^1H -COSY as in Fig. 2. β^a - β^b cross-peaks of Cys 3, Cys 4, Cys 5, and Cys 6 are shown. (A) $^{112}\text{Cd}_2\text{GAL4}(62^*)$. (B) $^{113}\text{Cd}_2\text{GAL4}(62^*)$. The cross-peak for Cys 4 in both derivatives and half the cross-peak of Cys 3 in the ^{113}Cd derivative are badly overlapped with other resonances, but they can be separated in expanded and phase-coded plots. The expanded plots were used to measure the coupling constants given in Table 1.

shows that two of these cysteine residues (designated 5 and 6) appear to be bridging ligands between the two $^{113}\text{Cd}(\text{II})$ ions (Figs. 4 and 5). Among the six $^{113}\text{Cd}(\text{II})$ -coordinated cysteine residues of GAL4(62*), Cys 5 and 6 must occupy almost identical and unique coordination environments, since they both have nearly identical and relatively large coupling of both β protons to one of the $^{113}\text{Cd}(\text{II})$ ions (Fig. 5), a pattern which is also observed for the two well-resolved β^a - β^b cross-peaks of bridging cysteine in metallothionein (12). One can conclude that the torsional angles describing the configuration of the three-bond fragment between the β protons and the coordinated $^{113}\text{Cd}(\text{II})$ ions must be the same for Cys 5 and Cys 6. A possible model of the binuclear complex (cloverleaf) formed by GAL4(62*) is shown in Fig. 7. This model assumes Cys²¹ and Cys³⁸ are the bridging ligands, which places them in equivalent configurations, as

FIG. 5. Expanded region from Fig. 4 showing the coupling patterns derived from the β^a - β^b cross-peaks of Cys 5 and Cys 6.

their J coupling suggests. The model building suggests that the five peptide loops necessitated by the cluster structure pack most easily with this choice of bridging ligands. A preliminary sequential assignment of Zn(II)GAL4(62*) supports the assignment of Cys 5 and 6 to residues 38 and 21, respectively, but this result must be considered tentative.

Sequential assignment of the GAL4(62*) spectrum by 2D NMR techniques is not yet complete. While the ^1H - ^{113}Cd heteronuclear J coupling will help establish landmarks in the spectra of this sequence, which has an abundance of lysine and arginine residues, at present its most important aspect is the establishment of the two-metal-ion cluster structure. From the complete COSY and nuclear Overhauser enhancement (NOE) spectroscopy (NOESY) spectra in H_2O , all the $\text{NH}-\text{H}_\alpha$ connectivities can be identified. Strong $\text{NH}_i-\text{NH}_{i+1}$ NOEs in the NOESY spectrum of Zn(II)GAL4(62*) have established two separate short stretches of α -helix (unpublished results). Thus a large part of the backbone of GAL4(62*) must be unstructured or β -sheet-like, not incompatible with the model shown in Fig. 7. The regions N-terminal (residues 1-10) and C-terminal (residues 49-62) to the cluster subdomain are likely to form the α -helices suggested to be present by the NOESY data on Zn(II)GAL4(62*) and the earlier circular dichroism studies (5).

The model of GAL4 in Fig. 7 places the preponderance of positive charge, six lysine and one arginine residues, on one side of the cluster formed by the two loops Cys¹⁴-Cys²¹ and Cys²¹-Cys²⁸. Comparison of the sequences shows that the positive charge distribution in the Cys¹⁴-Cys²¹ loop is main-

FIG. 6. ¹H NMR spectra of a 0.9 mM solution of apo-GAL4(62*) (A), Zn(II)GAL4(62*) (B), and ¹¹³Cd(II)GAL4(62*) (C). The number of transients is 16 and the relaxation delay was 2.5 s. TSP, sodium trimethylsilyltetradecuteriopropionate.

tained in all nine fungal transcription factors of this class; in fact, the positions of three of the lysine residues are absolutely conserved in this loop. This suggests that these residues within the cluster (Cys¹¹–Cys³⁸) may function in nonspecific DNA binding and that the recognition determinants may be outside this region. The specific contact region could be provided by a turn and α-helix following the most C-terminal cysteine residue. The specific DNA recognition could involve a cloverleaf–turn–helix structure with turns in the region of Pro⁴² and Pro⁴⁸. Genetic studies already suggest that amino acid

FIG. 7. Model of the Zn(II)₂Cys₆ or Cd(II)₂Cys₆ cluster (cloverleaf) of the DNA-binding domain of GAL4. Portions of the amino acid sequences of transcription factors LAC9 and PPR1 are also shown. Loops I, II, and III each contain six amino acid residues.

residues within the Zn(II)₂Cys₆ cluster of GAL4 are not directly involved in specific DNA recognition (13).

Partial amino acid sequences of two other fungal transcription factors in the regions of the DNA-binding domains corresponding to that of GAL4(62*) are compared in the model (Fig. 7). One of them, LAC9, must be homologous to GAL4, since they both recognize the UAS_G sequence (14, 15). The second, PPR1, does not recognize this sequence but maintains the Cys₆ motif (16). All three factors maintain similar charge and amino acid sequences within the metal ion cluster. As shown for PPR1, the most marked deviation of sequence in this group of transcription factors occurs in the region immediately downstream from the cluster. Of the eight other factors, only the more closely related LAC9 has a sequence in this region similar to that of GAL4 (Fig. 7). This region of the DNA-binding domain may participate in the sequence-specific DNA interactions.

A binuclear zinc cluster complex is a structure different from those previously proposed for the zinc-finger transcription factors. The highly conserved arrangement of cysteine residues in GAL4 and the other fungal transcription factors almost certainly means that this structure is also present in the others. Whether such a binuclear complex or a variant of it is present in other so-called Cys₂Cys₂ zinc-finger transcription factors, such as the steroid receptor proteins (containing a total of nine conserved cysteine residues), remains speculative. Cd(II) forms a stable binuclear cluster with GAL4 and the binding is highly cooperative (4, 5). Not surprisingly, the Zn(II) analogue is more labile, and one of the two Zn(II) ions dissociates when free Zn(II) is less than micromolar. This raises the interesting possibility that DNA-binding affinity and therefore transcriptional regulation might depend under some circumstances on the cellular Zn(II) concentration. Whether the structural changes as a function of Zn(II) content necessary for such a postulate are a feature of GAL4 requires further investigation.

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