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Identification and preliminary characterization of a protein motif related to the zinc finger

(DNA binding/spectroscopy/zinc binding)

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ABSTRACT **We have identified a protein motif, related to the zinc finger, which defines a newly discovered family of proteins. The motif was found in the sequence of the human *RING1* gene, which is proximal to the major histocompatibility complex region on chromosome six. We propose naming this motif the "RING finger" and it is found in 27 proteins, all of which have putative DNA binding functions. We have synthesized a peptide corresponding to the RING1 motif and examined a number of properties, including metal and DNA binding. We provide evidence to support the suggestion that the RING finger motif is the DNA binding domain of this newly defined family of proteins.**

Protein–DNA interactions are involved in many of the fundamental processes that occur inside cells, including transcription, replication, recombination, and restriction. To meet such a large number of functional requirements, a number of protein sequence and/or structural motifs have evolved that allow both specific and nonspecific DNA interaction (1, 2). Among these motifs are zinc fingers, which are autonomously folding domains that require zinc for folding and DNA binding activity (3, 4).

The classic zinc finger motif is characterized by two conserved cysteines and histidines, which bind tetrahedrally to a zinc atom thereby stabilizing the secondary structure comprising an antiparallel two-stranded β-sheet and an α-helix (5, 6). The N-terminal end of the α-helix is responsible for making DNA sequence-specific interactions, with each finger recognizing 3 bp (7) as suggested previously (8). A second class of zinc finger has been described in the steroid/nuclear receptor family of proteins, which differs from the classic zinc finger in that the motif binds two zinc atoms to form a single folded domain with four cysteine ligands for each zinc (for review, see ref. 9). The mode of DNA interaction of this motif differs from that of the classic zinc finger in that the receptor binds as a dimer to a palindromic DNA sequence (10). However, sequence-specific DNA interactions are achieved by placing an α-helix in each of the major grooves of the DNA site (10). Recently, a third class of zinc finger fold has been described in the GAL4 DNA binding domain (11–13). GAL4 binds two zinc atoms through six cysteines, with the metals sharing two of the ligands (14). GAL4 also uses an α-helix in the major groove for sequence-specific DNA binding (11), which appears to be a common feature of the three zinc-mediated folds characterized to date.

Previously, we have reported the identity of a cysteine-rich motif found in the sequence of the *RING1* gene that is related to the zinc finger (15). Here, we report the full sequence of the human *RING1* gene and define further this motif, which we propose to call the "RING finger." We extend the family of proteins containing the motif and characterize a synthetic peptide corresponding to the motif from RING1 in terms of zinc and DNA binding.††

MATERIALS AND METHODS

DNA Sequencing. Sequencing reactions were performed by the primed synthesis chain-termination method using the Sequenase 2.0 enzyme (United States Biochemical) directly from plasmid DNA. The sequence was obtained from the double-stranded cDNA and genomic subclone (first 90 bp), with primers specific to the vectors and inserts.

Cells. All cell lines were obtained from the cell production unit at the Imperial Cancer Research Fund. The following cell lines were used: T cells, HSB.2, Molt-4, and J6; B cells, Namalva, Mann, ROF-NL, and IM9; macrophage, U937; cervical carcinoma, HeLa; fibroblast, HFF; embryonic lung, ICRF-23; erythroid, K562.

RNA Isolation and Northern Blot Analysis. Poly(A)$^+$ RNA was isolated from cell lines with FastTrack (Invitrogen, San Diego). After blotting the RNA onto Hybond N (Amersham), the membrane was hybridized at 42°C to ^{32}P-labeled *RING1* (Multiprime; Amersham; 850-bp *Xba* I fragment) in 50% formamide, 5× standard saline phosphate EDTA/5× Denhardt's solution/0.1% SDS/100 μg of salmon sperm DNA per ml, and washed at 65°C to a stringency of 0.1× standard saline citrate. The filter was exposed to autoradiographic film at −70°C with intensifying screens.

Multiple Sequence Alignment of the RING Finger Family. The RING1 amino acid sequence was used to search the OWL14.0 protein data base with the program PROSRCH (16) on an AMT-DAP computer. The RING finger motif was further refined by using the pattern searching program PROMOT (17). The sequence alignment was obtained automatically by using the program package AMPS (18) with a point accepted mutation matrix of 250 and a gap constant of 12.

Peptide Synthesis and Purification. The 55-amino acid peptide corresponding to the RING1 motif (residues 12–66 in the RING1 sequence) was synthesized on a model 430A Applied Biosystems solid-phase synthesizer. The peptide was purified by reverse-phase preparative HPLC on a Brownlee Aquapore ODS C18 column. A few milligrams of the pure synthetic peptide was analyzed by electrospray mass spectrometry (19). To further characterize the synthetic product,

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Abbreviation: DTT, dithiothreitol.

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††The sequence reported in this paper has been deposited in the GenBank data base (accession no. Z14000).

≈5 mg of freeze-dried peptide was reduced and alkylated by the addition of 20 mM iodoacetic acid. Approximately 100 pmol of this material was covalently attached to an arylamine membrane (20) and sequenced for 37 cycles by solid-phase Edman degradation. The observed partial sequence was in exact agreement with that expected from the synthesis, with all expected cysteines positively identified as the corresponding *S*-carboxymethyl derivatives. Reduced RING1 peptide was prepared by the method described by Frankel *et al.* (21), which involved treating the peptide with 250 mM dithiothreitol (DTT) at 90°C for 30 min. The reduced RING1 peptide was stored at −20°C after removal of the DTT and lyophilization and was used for all subsequent experiments.

Binding of Cobalt and Zinc to the RING1 Peptide. A solution of peptide (1 ml) containing 160 μM reduced RING1 peptide (10 mM Tris·HCl, pH 7.0) was titrated with solutions of $CoCl_2$ in the same buffer. The peptide was also titrated against solutions of $ZnCl_2$, $MnCl_2$, $CdCl_2$, $CuSO_4$, $MgSO_4$, and $FeSO_4$ in the presence of 100 μM $CoCl_2$. The binding reaction was monitored on an HP 8452 diode array spectrophotometer using a 1-cm path length at room temperature, and each spectrum was the average of three recorded spectra.

CD and NMR of the RING1 Peptide. The CD spectra comprise the unsmoothed averages of four scans recorded in a 0.1-mm quartz cuvette on a Jasco J-600 spectropolarimeter using an instrumental time constant of 1 sec. The reduced RING1 peptide (0.75 mg/ml) was dissolved in 50 mM $NaClO_4$ (pH 7.0) and the spectra were recorded in the absence and presence of 2 mM Zn^{2+}. The spectrum obtained at 1 mM Zn^{2+} (data not shown) was effectively identical to that recorded in the presence of 2 mM Zn^{2+}. Secondary structure predictions from the CD spectra were performed by the method of Hennessey and Johnson (22). For the NMR experiments, the reduced RING1 peptide (≈200 μM) was dissolved in a ^2H_2O solution containing 10 mM sodium phosphate, 50 mM sodium perchlorate, 100 μM DTT, and 200 μM EDTA (p^2H 7.1) (uncorrected meter reading). Argon was bubbled through the solution to maintain a reduced environment. Data were recorded on a Varian Unity 600 MHz spectrometer at 25°C.

DNA Binding of the RING1 Peptide. The reduced RING1 peptide was resuspended in phosphate-buffered saline and diluted to 0.5 mg/ml in binding buffer [50 mM Hepes, pH 7.5/50 mM KCl/10% (vol/vol) glycerol/0.5 mM $ZnSO_4$] with 1 mM DTT. The DTT was removed by dialysis against the binding buffer. The binding reactions were carried out in 13 μl of the binding buffer with 2 or 4 μg of peptide/5 mM spermidine/1 mM EDTA/0.5% Nonidet P-40/6.25 μg of bovine serum albumin/various concentrations of poly(dI·dC) and ^{32}P-labeled DNA (76-bp oligonucleotide R76; ref. 23). The peptide was preincubated with the nonspecific competitor poly(dI·dC) for 10 min at room temperature before the addition of labeled DNA and a further 50-min incubation. As a control, 200 pg of the labeled DNA was incubated as described above but without the peptide. The binding reactions were then run on 0.5× TBE (1× TBE = 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3)/10% polyacrylamide gels at 8 mA. The gels were dried and autoradiographed for differing times on Kodak XAR-5 film at room temperature.

RESULTS AND DISCUSSION

The RING1 Motif. The *RING1* gene was identified in association with a CpG island at the centromeric end of the human major histocompatibility complex on chromosome 6p21.3 and maps 95 kb proximal to the HLA-DPB2 gene (24). The predicted amino acid sequence of RING1 (377 amino acids; Fig. 1*A*) shows a glycine-rich region (27%) in the C-terminal two-thirds and a cysteine-rich domain (residues 15–64) found near the N terminus. A putative nuclear localization signal (KRPR) is also found associated with the N-terminal cysteine-rich domain (25). Northern blot analysis of the RING1 mRNA (Fig. 1*B*) shows that the gene is expressed at similar levels in a variety of cell lines as a 1.6-kb transcript, which correlates well with the predicted cDNA sequence without a poly(A)$^+$ tail. Initial protein sequence

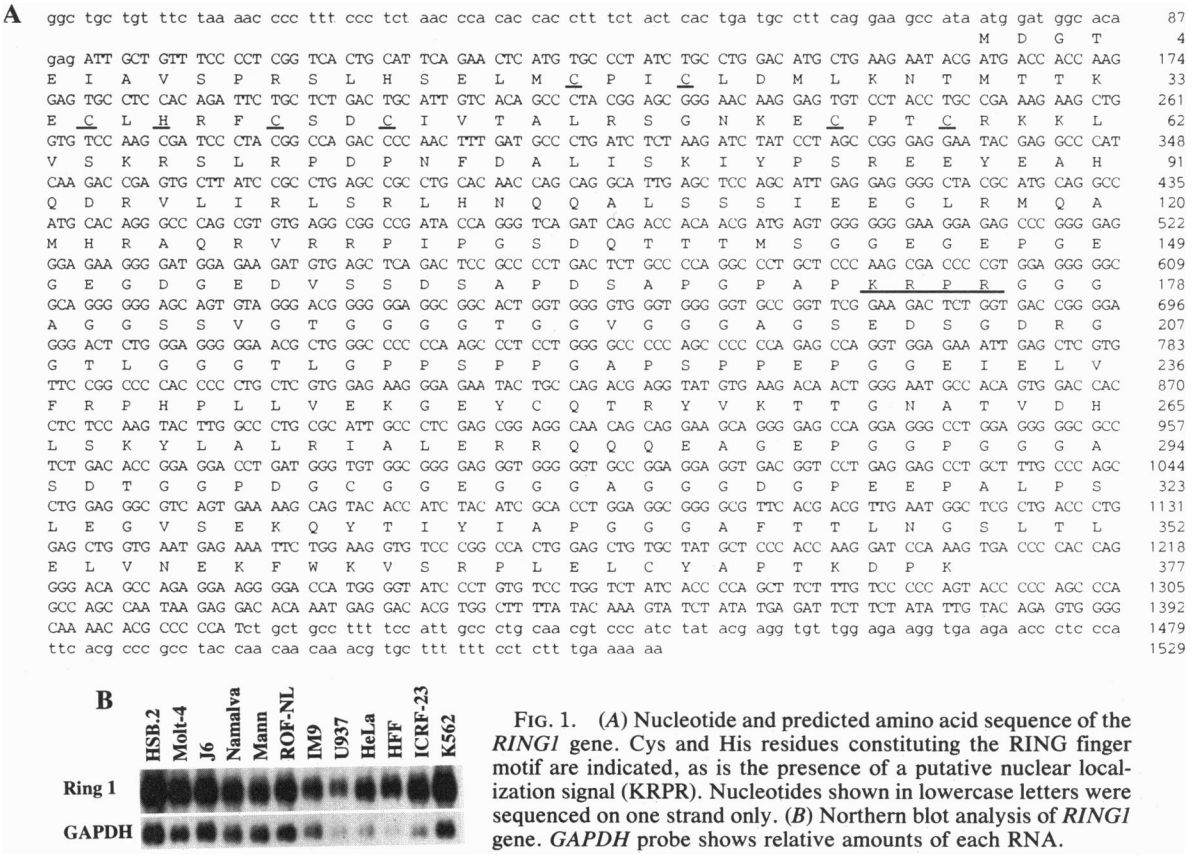

FIG. 1. (*A*) Nucleotide and predicted amino acid sequence of the *RING1* gene. Cys and His residues constituting the RING finger motif are indicated, as is the presence of a putative nuclear localization signal (KRPR). Nucleotides shown in lowercase letters were sequenced on one strand only. (*B*) Northern blot analysis of *RING1* gene. *GAPDH* probe shows relative amounts of each RNA.

searches using the N-terminal 139 residues identified sequences that contained a conserved cysteine-rich domain (15). Subsequent searches combined with a review of recent sequences identified 27 proteins that contained the motif (Fig. 2). The extended motif, which we propose to name the RING finger, can now be summarized as Cys^1-Xaa-hydrophobic aa-Cys^2-Xaa_{9-27}-Cys^3-Xaa_{1-3}-His-Xaa-hydrophobic aa-Cys^4-Xaa_2-Cys^5-hydrophobic aa-Xaa_{5-47}-Cys^6-Xaa_2-Cys^7.

The Cys and His residues are absolutely conserved in all of the RING finger family (Fig. 2). Further conservation of sequence is observed one residue before Cys^2, Cys^3, Cys^4, and Cys^7 and one residue after Cys^5, Cys^6, and Cys^7. These amino acids are generally hydrophobic, with notable preferences for Phe before Cys^4 and for Pro after Cys^6. All of the conserved residues may be important in secondary and tertiary structure interactions and in positioning and orienting the metal binding ligands. Four members of the family show insertions of generally small amino acids between Cys^3 and the central His residue (Fig. 2). The average length of the first "loop" (11±1aa) excluding the two *Baculovirus* sequences (CG30 and PE38) is similar to that of the second loop (10±3aa) excluding the T18 sequence. Thus, the motif could be reviewed as partially symmetric, which is a common structural arrangement for many protein–DNA binding motifs. However, greater variation in length and sequence conservation is observed within the second loop. Interestingly, the RING finger motif generally occurs near the N termini of member proteins.

Similarity of the RING Finger to Other Zinc Finger-like Motifs. A comparison of the RING finger with the consensus zinc finger motif shows that the first part of both motifs—namely, Xaa_2-Cys^1-Xaa_2-Cys^2-$Xaa_{11\pm1}$-Cys^3—are similar. A number of hydrophobic residues are conserved between both motifs (Fig. 2), and the lengths of the loop regions are also similar (12aa for a consensus zinc finger). However, the RING finger cannot be viewed simply as a motif of two tandemly linked zinc fingers, as the central linking region between both fingers would be too small and is probably involved in zinc binding. The RING finger motif is also similar to the two-metal binding motif of glucocorticoid and estrogen receptor proteins (9). Although the spacing of the metal ligands is different, there are common features to both motifs, including the different sizes of the two loops and the requirement for two zinc atoms to form a single folded domain.

The most similar motif to the RING finger consensus sequence is the LIM domain (26). The domain is found in a number of homeodomain-containing proteins from *Caenorhabditis elegans* as well as in rat insulin I gene enhancer binding protein (27). Although the LIM and RING motifs appear similar in terms of cysteine spacings, there is no sequence homology between both consensus sequences. Interestingly, the LIM motif has recently been shown to bind both iron and zinc (28), although the domain has not been shown to bind to DNA. It remains to be seen whether the LIM and RING finger domains have any functional similarity.

The RING Finger Family. A common feature of the RING finger family of proteins is that their functions, where known, can be mediated through DNA binding. These functions include regulation of gene expression, DNA recombination, and DNA repair. Four proteins of the family are associated with the regulation of development in *Xenopus* (XNF-7; ref. 29) or *Drosophila* [sina, ref. 30; Psc and Su(z)2, refs. 31 and 32]. *DG17* (*Dictyostelium*; ref. 33) expression is also developmentally regulated. *MEL18* (34) and *BMI-1* (35, 36) are highly homologous human oncogenes (37). *BMI-1* acts as a cooperating oncogene with *MYC* in the acceleration of pre-B-cell lymphomagenesis and the expressed protein is predominantly localized to the nucleus (35, 36). Mel18 protein is also localized to the nucleus and has been shown to bind to DNA-cellulose (34). *Rpt-1* (mouse; ref. 38), *PAS4* (yeast; ref. 63), and *TLR* (*Trypanosoma brucei*; refs. 39 and 40) have been suggested to regulate gene expression with *Rpt-1* implicated in down-regulation of the interleukin 2 receptor (38). *IE110* (41), *VZ61* (42), *CG30* (43), *PE-38* (44), and *EP0* (45) are viral early genes with known or suggested transactivation properties. *LCMV* (46) and *P11* (47) are related viral genes. *RAG-1* (human; ref. 48) is involved in activation of DNA recombination and *RAD18* (yeast; ref. 49) is involved in DNA repair. *SS-A/Ro* (human; ref. 50) gene product forms part of a ribonucleoprotein complex, although there is no evidence for direct nucleic acid interaction by SS-A/Ro. *RET* (51), *T18* (52), and *CBL* (53) are known human oncogenes. *RAD16* (yeast) has homology with proteins known to be involved in chromatin structure and possesses a helicase domain motif (64). The functions of the *RING1* (human) and *PML* (human; refs. 54–57) genes are as yet unknown. Interestingly, *PML* is disrupted and fused to the retinoic acid receptor α gene in patients with acute promyelocytic leukemia (e.g., see ref. 56). One unusual member of the family is peroxisome assembly factor (PAF-1), which has been shown to promote the biogenesis of peroxisomes (58). The presence of the RING finger domain in PAF-1 could suggest that PAF-1 functions via DNA interaction, although it does not exclude the possibility that the PAF-1 RING finger has alternative properties.

To investigate the functional properties of the RING finger motif, we have synthesized and purified a 55-amino acid peptide (residues 12–66 in the RING1 sequence) corresponding to the RING1 finger. The purity and sequence of the

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P11      (35-82)    GRYNCKCCWFADKN      LITC SDHYLCLRCHQ     IMLRNSEL    CNICWKPLPT
LCMV     (28-74)    GPLSCKSCWQKFDS      LVRC HDHYLCRHCLN     LLLSVSDR    CPLCKYPLPT
DG17     (23-72)    NKYTCPICFEFIYKK   QIYQCKSGHHACKECWE     KSLETKKE    CMTCKSVVNS
SINA     (69-113)   SLFECPVCFDYVLPP     ILQCSSGHLVCVSCRS      KLTC      CPTCRGPLAN
EPO      (42-90)    TVMDCPICLDVAATEA    QTLPC  MHKFCLDCIQ     RWTLTSTA    CPLCNARVTS
VZ61     (15-63)    SDNTCTICMSTVSDLG    KTMPC  LHDFCFVCIR     AWTSTSVQ    CPLCRCPVQS
IE110    (112-162)  EGDVCAVCTDEIAPHLRCDTFPC  MHRFCIPCMK     TWMQLRNT    CPLCNAKLVY
PAS4     (286-334)  ASRKCILCLMNMSDP    SCAPC  GHLFCWSCLM     SWCKERPE    CPLCRQHCQP
CBL      (377-425)  TFQLCKICAENDKD V    KIEPC  GHLMCTSCLT     SWQESEGQG   CPFCRCEIKG
BMI-1    (14-62)    PHLMCVLCGGYFIDAT    TIIEC  LHSFCKTCIV     RYLETSKY    CPICDVQVHK
MEL18    (14-62)    PHLMCALCGGYFIDAT    TIVEC  LHSFCKTCIV     RYLETNKY    CPMCDVQVHK
PSC      (259-307)  PHIICHLCQGYLINAT    TIVEC  LHSFCHSCLI     NHLRKERF    CPRCEMVINN
SU(z)2   (31-79)    DLITCRLCRGYNIDPT    TVDYC  YHTYCRSCIL     KHLLRAVY    CPECKASGGK
SS-A/Ro  (12-60)    EEVTCPICLDPFVEPV     SIEC  GHSFCQECIS     QVGKGGGSV   CAVCRQRFLL
RPT-1    (11-64)    EEVTCPICLELLKEPV     SADC  NHSFCRACITLNYESNRNTDGKGN   CPVCRVPYPF
XNF7     (141-190)  EELTCPLCVELFKDPV     MVAC  GHNFCRSCID     KAWEGNSSFA  CPECRRESIT
RAD16    (533-586)  GVVICQLCNDEAEEPI     ESKC  HHKFCRLCIKEYVESFMENNNKLT  CPVCHIGLSI
RET      (12-62)    QETTCPVCLQYFAEPM     MLDC  GHNICCACLA     RCWGTAETNVS  CPQCRETFPQ
RING1    (15-64)    SELMCPICLDMLKNTM     TTKEC  LHRFCSDCIV     TALRSGNKE   CPTCRKKLVS
RAG1     (289-337)  KSISCQICEHILADPV      ETNC  KHVFCRVCIL     RCLKVMGSY   CPSCRYPCFP
RAD18    (24-71)    TLLRCHICKDFLKVPV      LTPC  GHTFCSLCIR     THLNNQPN    CPLCLFEFRE
PE38     (82-144)   FKFECSVCLETYSQQ(8)IPTTC  DHGFCFKCVINLQSNAMNIPHSTVCCPLCNTQVKM
PAF-1    (240-289)  SGKECALCGEWPTMP     HTIGC  EHVFCYYCVK     SSFLFDMYFT  CPKCGTEVHS
T18      (47-135)   LLDTCAVCHQNIQSRVP    KLLPC  LHSFCQRCLP(36)PFATQVGVIR   CPVCSQECAE
TLR      (5-51)     TYGMCAVCREPWAEGAV    ELLPC  RHVFCTACVV     QRWR        CPSCQRRIGG
CG30     (4-68)     VKLQCNICFSVAEI(17)ELDTC  KHQLCSMCIR      KIRKRKKVP   CPLCRVESLH
PML      (53-97)    QFLRCQQCQAEAKCP     KLLPC  LHTLCSGCLE     ASGMQ       CPICQAPWPL
                     $  * $*$ $$         $$ *   * $*  *$$      $           *$$*   $
                        1  2               3      4  5                      6  7
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FIG. 2. Multiple sequence alignment of the RING finger family. Identical residues are shown in boldface. Metal binding ligands, which are absolutely conserved between family members, are indicated (★), as are the positions of conserved hydrophobic residues ($). Numbering below each conserved Cys refers to metal binding ligands Cys^1–Cys^7. See text for reference to each sequence. Amino acid insertions are indicated in parentheses. Gaps have been inserted automatically by the AMPS program to maximize sequence homology.

synthetic peptide were confirmed by mass spectrometry and solid-phase peptide sequencing. We then examined a range of structural and functional properties of the peptide.

Metal Binding to the RING1 Finger. Divalent metal binding properties of peptides and proteins can be determined by studying the optical absorption spectra of specific cobalt–peptide/protein complexes (21, 59–61). The RING1 peptide–cobalt complex exhibits absorption maxima at λ = 307 and 340–350 nm (Fig. 3*A*), which correspond to the S–Co(II) charge transfer bands and indicate that the cysteines are involved in metal binding. Several maxima are also observed at λ = 605, 655, and 690 nm, which correspond to the Co(II) *d–d* transitions and are consistent with tetrahedral coordination of the cobalt by cysteine ligands. No spectral change was observed during the cobalt titration. From direct titrations with low peptide concentrations, the molar ratio of cobalt to peptide at saturated binding is estimated as 1.8:1, which is in general agreement with the predicted ratio of 2:1, as determined from the number of potential metal ligands. Cobalt binding to the RING1 peptide is tighter than previously observed for classical zinc finger peptides ($K_d \approx 1$ μM; ref. 60), with a measured dissociation constant of 10 (±6) nM.

The preferential binding to the peptide of zinc over cobalt is also shown in Fig. 3*A*. Increasing concentrations of zinc diminish the intensities of the characteristic cobalt transitions, even in 10-fold molar excess of cobalt over zinc. The same experiment was carried out using iron, copper, manganese, magnesium, and cadmium as the competitor metal to cobalt (data not shown). No binding was observed for iron, manganese, and magnesium. Some binding was observed for copper but the binding was less tight than that for zinc. However, cadmium bound as tightly as zinc, which has also been observed for GAL4 (62). The RING1 finger therefore binds zinc preferentially to other physiological divalent metal ions and at least 10 times more tightly than cobalt, with a dissociation constant in the nanomolar range.

Secondary Structure of the RING1 Finger. Preliminary NMR studies of the reduced RING1 peptide showed the peptide to be structured in the absence of zinc. Unexchanged amide protons are observed in spectra of reduced RING1 peptide that has been freshly dissolved in ^2H_2O, EDTA, and DTT, indicating stable hydrogen bonding even in the absence of zinc. However, upon addition of zinc the peptide undergoes a conformational change as indicated by increased aggregation and subsequent precipitation with resonances shifting from their values in the reduced state (data not shown). The extent of the conformational change will have to await a detailed NMR analysis, although we have no evidence to suggest that the observed aggregation/precipitation is caused by oligomerization.

CD studies of the reduced RING1 peptide in the absence of zinc also show the peptide to be structured with ≈20% α-helix and ≈20% β-sheet (Fig. 3*B*). The addition of zinc causes a small but reproducible increase in ellipticity (Fig. 3*B*), consistent with a slight increase (≤5%) in α-helix content. This suggests a possible structural rearrangement upon zinc binding and could indicate a zinc-induced stabilization of secondary structure. Our preliminary structural observations of the RING1 peptide therefore suggest that the RING1 finger has significant secondary structure in the absence of zinc, as shown directly by NMR measurements, and that zinc binding promotes a small conformational change.

DNA Binding of the RING1 Finger. The DNA binding properties of the reduced RING1 peptide were analyzed by gel mobility-shift assays using a 76-bp oligonucleotide containing a central 26-bp randomized sequence (23) (Fig. 4). The RING1 peptide retards the migration of the oligonucleotide and forms one major discrete band in the presence of excess zinc (Fig. 4*A*, lane 2). A number of fainter lower molecular weight bands are also observed, which could represent complexes with different ratios of peptide to DNA. Some labeled DNA remains in the wells, which we attribute to nonspecific aggregation. The retardation also appears to be zinc dependent, as adding high concentrations of EDTA or DTT diminishes the formation of the specific band (lanes 4–8). However, boiling and cooling the RING1 peptide–DNA complex does not affect binding (lane 3), suggesting that the "competent" peptide can refold into an active species. Titration of the nonspecific competitor poly(dI·dC) against the specific RING1 peptide–DNA complex shows inhibition of DNA retardation at a 1000-fold weight excess of competitor to labeled DNA (Fig. 4*B*). From the titration, we measure the association constant of the RING1 peptide for poly(dI·dC) to be in the micromolar range, which suggests that binding of the peptide to poly(dI·dC) is nonspecific. However, it is clear that the RING1 peptide binds to a subset of randomized DNA sequences at least 10-fold more tightly than to poly(dI·dC), as binding is unaffected in excess competitor (lanes 2 and 3). Although a 100-fold molar excess of peptide to DNA was used in the DNA binding reactions, we observed significant peptide precipitation in the peptide–DNA reaction mixture prior to loading the gel. We were therefore unable to determine the actual concentration of active peptide available for DNA binding. However, it is clear that RING1–DNA com-

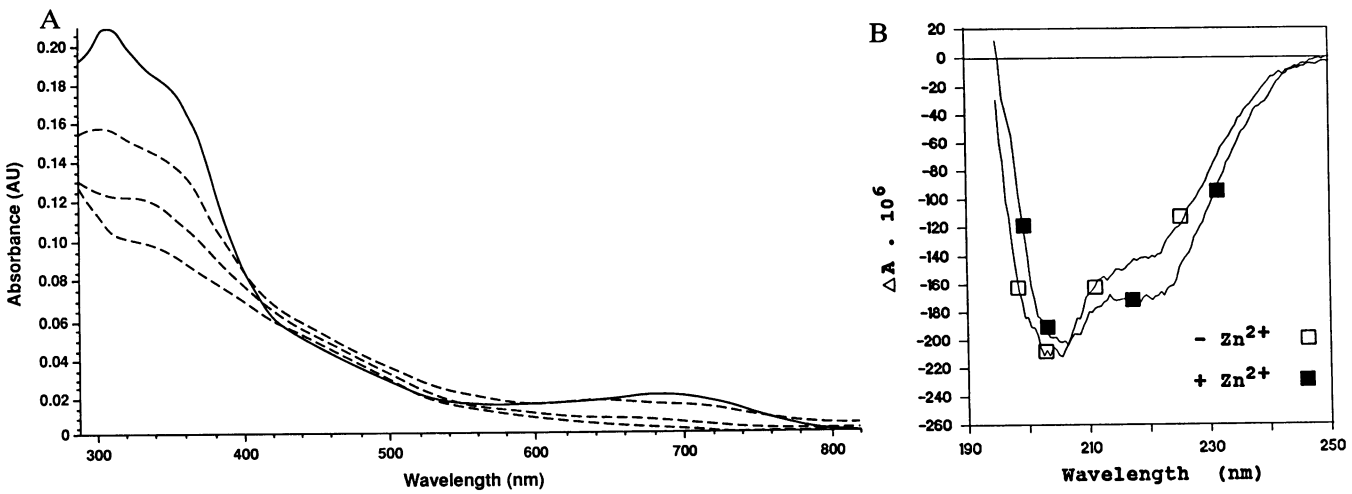

FIG. 3. Physical characterization of the RING1 peptide. (*A*) Binding of cobalt and zinc to the RING1 peptide. Spectrum for the peptide–cobalt complex is shown as a solid line. Dashed lines represent spectra after addition of increasing amounts of zinc. (*B*) Effect of zinc on the far-UV CD spectrum of the RING1 peptide. Spectra shown are unsmoothed averages of four scans.

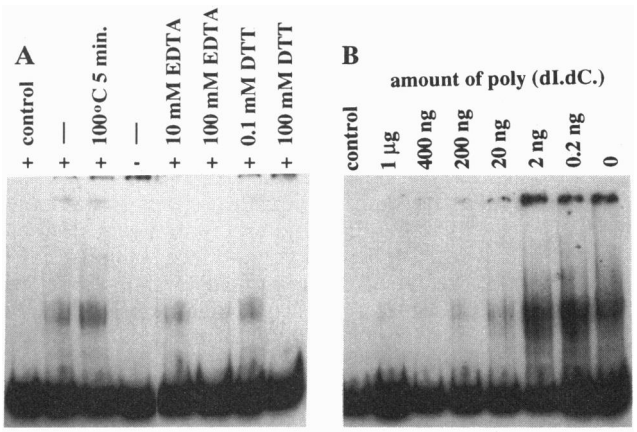

FIG. 4. DNA binding of the RING1 finger. (*A*) Zinc dependence of RING1 peptide–DNA binding. Each lane contained 4 μg of peptide, 200 pg of labeled oligonucleotide R76 DNA, and 260 ng of poly(dI·dC). Label above each lane refers to different conditions for each of the binding reactions, with — indicating standard conditions. Lanes +, addition of 0.5 mM zinc sulfate, which was added to all but one lane; lane −, binding reaction for peptide dissolved in zinc-free binding buffer; lanes 5–8, dialyzed peptide was preincubated with EDTA or DTT for 30 min before starting the binding reaction. (*B*) Effect of nonspecific competitor concentration on RING1 peptide–DNA binding. Each lane contained 4 μg of peptide and 200 pg of labeled DNA. Increasing amounts of poly(dI·dC) were added to the peptide–DNA as indicated.

plex formation is zinc dependent and that the complex is unaffected by boiling. These results, however, do not provide direct evidence for specific RING1 finger–DNA interaction and furthermore do not exclude the possibility that the RING1 finger binds to RNA preferentially.

In conclusion, spectroscopic studies show that the RING1 peptide is structured and binds zinc tightly with tetrahedral coordination and that cysteines are involved in metal liganding. Preliminary gel-retardation studies provide evidence for zinc-dependent DNA binding by the RING1 peptide. These results support the proposal that the RING1 finger motif is a zinc-dependent DNA binding domain found in this newly defined family of proteins.

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