The Structure of the Zinc Finger Domain from Human Splicing Factor ZNF265 Fold*

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Craig A. Plambeck‡§, Ann H. Y. Kwan§, David J. Adams‡‡, Belinda J. Westman§, Louise van der Weyden§, Robert L. Medcalf***, Brian J. Morris‡, and Joel P. Mackay$$††

From the ‡Basic and Clinical Genomics Laboratory, School of Medical Science and Institute for Biomedical Research, University of Sydney, New South Wales 2006, Australia, the §§School of Molecular and Microbial Biosciences, University of Sydney, New South Wales 2006, Australia, and ***Monash University, Department of Medicine, Box Hill Hospital, Box Hill, Victoria 3128, Australia

Identification of the protein domains that are responsible for RNA recognition has lagged behind the characterization of protein-DNA interactions. However, it is now becoming clear that a range of structural motifs bind to RNA and their structures and molecular mechanisms of action are beginning to be elucidated. In this report, we have expressed and purified one of the two putative RNA-binding domains from ZNF265, a protein that has been shown to bind to the spliceosomal components U1-70K and U2AF35 and to direct alternative splicing. We show that this domain, which contains four highly conserved cysteine residues, forms a stable, monomeric structure upon the addition of 1 molar eq of Zn(II). Determination of the solution structure of this domain reveals a conformation comprising two stacked β-hairpins oriented at −80° to each other and sandwiching the zinc ion; the fold resembles the zinc ribbon class of zinc-binding domains, although with one less β-strand than most members of the class. Analysis of the structure reveals a striking resemblance to known RNA-binding motifs in terms of the distribution of key surface residues responsible for making RNA contacts, despite a complete lack of structural homology. Furthermore, we have used an RNA gel shift assay to demonstrate that a single crossed finger domain from ZNF265 is capable of binding to an RNA message. Taken together, these results define a new RNA-binding motif and should provide insight into the functions of the >100 uncharacterized proteins in the sequence data bases that contain this domain.

The zinc finger architecture has provided a diverse range of structures and functions (1), including recognition of protein, DNA, and RNA targets. Whereas the structural basis for interactions between DNA and classical zinc fingers has been well characterized (2–4), RNA-zinc finger interactions are less well understood, with only limited structural information on the conformation of RNA-binding domains available. Available structures have indicated that in many cases, RNA-binding domains are not completely folded in the absence of their target RNA sequence (5). Conformational changes have been observed to occur in both protein and the RNA target upon binding (5–9), and even completely unstructured domains have been found to fold upon interacting with RNA (10). It has therefore been suggested that rigid proteins may be unfavorable for RNA-binding and flexible structures may increase specificity (5).

In recent years, the functions of many RNA-binding proteins that regulate the process of RNA splicing have been delineated, enhancing our understanding of splice site recognition, spliceosomal coordination, and alternative splicing. Typically, the accessory proteins involved in splicing (SR proteins) contain an N-terminal RNA-binding domain and a C-terminal protein-binding or RS domain that is rich in Arg and Ser residues.

The human SR protein, ZNF265 (or ZIS) (11), can bind to the splicing factors U1-70K and U2AF35 (components of the E-complex that forms early in the splicing process) and is able to stimulate alternative splicing (12). It has also been shown to immunoprecipitate with splicing factors in association with mRNA and to co-localize in the nucleus with components of the transcriptosome (12), indicating that it plays a role in mRNA processing. ZNF265 is a multidomain protein, and is highly conserved in many organisms, including mouse (Zip265) (13), rat (Zip265; formerly Zis) (14), and Xenopus (C4SR) (15). Like other SR proteins, ZNF265 contains a C-terminal RS domain, as well as a nuclear localization sequence and a glutamic acid-rich domain. However, in contrast to other SR proteins, the N terminus of ZNF265 does not contain any of the known RNA recognition motifs, but rather contains 8 Cys residues spaced appropriately to give rise to two putative zinc finger domains, each with a CX_2/CX_2/CX_2/CX_2 topology.

Domains with homology to the putative zinc fingers of ZNF265 have been observed in a wide range of proteins, including ubiquitin ligases such as Mdm2 (16, 17) and RanBP2 (18, 19), as well as RNA-binding proteins such as EWS (20). However, little structural information is available on any of these proteins. In addition, the putative zinc finger domains appear to be capable of carrying out different functions depending on context. In RanBP2, the eight tandem zinc finger domains are essential for exportin-1 binding (21), whereas the double zinc finger domain in C4SR, the Xenopus homolog of ZNF265, binds to RNA (15).

In the present study, we show that the conserved Cys-containing motifs in ZNF265 are genuine zinc-binding domains...

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§§ Recipients of National Health and Medical Research Council (NHMRC) C. J. Martin fellowships. Present address: The Wellcome Trust Sanger Institute, Hinxton CB10 1SA, United Kingdom.

†† To whom correspondence should be addressed: School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia. Tel.: 61-2-9351-3906; Fax: 61-2-9351-4726; E-mail: j.mackay@mbm.usyd.edu.au.

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and we have determined the solution conformation of the first zinc finger of ZNF265, using NMR methods. The structure appears to constitute a variant of the zinc ribbon class of zinc-binding domains (1). In addition, we demonstrate that the ZNF265 zinc finger domain can bind cyclin B1 mRNA, indicating that this fold has apparently been adapted for both RNA-binding and protein-binding. Such adaptability appears to be common among zinc-binding motifs.

**EXPERIMENTAL PROCEDURES**

**Sequence Analysis**—All sequence analysis and alignments were performed using the software pileup and prettybox (Australian Genome Information Service).

**Expression and Purification**—The first putative zinc finger region of ZNF265 is located at the N-terminal end of the protein. A construct encoding residues 1–40 of human ZNF265 (accession code NP 005446) was therefore cloned into the Escherichia coli expression vector pGEX 4T-3 (Amersham Biosciences), creating a C-terminal fusion with glutathione S-transferase (GST). Note that DNA sequencing revealed that all 10 isolated clones contained aspartic acid at position 32, rather than asparagine (the residue listed in the GenBank™ sequence). It is likely that the sequence variation indicates either a single nucleotide polymorphism or perhaps a sequencing error in the original paper (11); only one cDNA was sequenced in this initial report. The fusion protein was expressed in the host strain DH5α grown in Luria-Bertani broth. Cells were grown at 37 °C, and expression of ZNF265-F1 was induced at an A600 of 0.90 by the addition of isopropyl-D-thiogalactopyranoside (1 mM).

**Analytical Ultracentrifugation**—Sedimentation equilibrium experiments were performed using a Beckman model XL-A analytical ultracentrifuge equipped with an An-60 Ti rotor. ZNF265-F1 was prepared for NMR by dissolving lyophilized peptide in H2O containing 1 mM tris(2-carboxyethyl)phosphine and 1.7 mM ZnSO4 and the pH was adjusted to 5.8 by dissolving lyophilized peptide in H2O containing 1 mM tris(2-carboxyethyl)phosphine and 1.7 mM ZnSO4 and the pH was adjusted to 5.8 using 0.1 M NaOH. Protein concentrations were determined in the XL-A spectrometer, equipped with a 5-mm triple resonance probe and three-bond 13C-1H heteronuclear spin-echo spectra were acquired over a period of 1 h at three centrifugal speeds, 10,000, 20,000, and 30,000 rpm. Scans were collected at 3-h intervals and compared to determine when the samples reached equilibrium. Analysis of the data was carried out using the program ARIA1.2 (37, 38). Manually assigned NOEs in combination with the remaining ambiguous NOEs were included in the ARIA structure calculations and the latter NOEs were iteratively assigned by the program.

**RNA Electrophoretic Mobility Shift Assay (REMSA)**—Expression plasmids harboring the full-length cyclin B1 cDNA were used to transfect the cyclin RNA polymerase II-deficient CHO cell line with BamHI, 1 μg of template was incubated for 2 h at 37 °C in the presence of 50 μCi of [α-32P]UTP (DuPont), 10 μM UTP, 0.5 mM ATP, 0.5 mM GTP, 0.5 mM CTP, 20 units of RNase inhibitor (Promega), and 50 units of T7 RNA polymerase. RNA probes were purified on a 4% polyacryl-
amid-urea denaturing gel, eluted overnight at room temperature in a solution containing 500 mM NH₄CH₃COO and 1 mM EDTA, ethanol precipitated at −80 °C, and resuspended in water (100 cps/µl) as previously described (45).

For the binding assays, 0.1–5.0 µg of affinity purified GST-ZNF265-F1 protein was preincubated with 150 µg of heparin (Sigma) for 10 min at room temperature in CEB buffer before addition of the RNA probe (200 cps). After 30 min incubation at room temperature, samples were subjected to RNase T1 digestion (1 molar eq of Zn(II)). The substantial increase in chemical shift dispersion in the presence of Zn(II) indicates the formation of stable secondary structure. Spectra were recorded at pH 5.8 and 298 K, on a 600 MHz spectrometer (sample concentration was 0.5 mM).

The Cysteine-rich Region of ZNF265 Forms a Monomeric Zinc-dependent Structure—Sequence analysis performed at the time of the initial discovery of rat ZFP265 (‘Zis’ (14)) identified the putative zinc finger domains of this protein, although no experimental verification was made. We therefore subcloned DNA encoding the first such domain from ZNF265 (ZNF265-F1) into the pGEX-4T-3 vector. The protein was expressed as a GST fusion and purified using affinity chromatography and reversed-phase high performance liquid chromatography. One-dimensional ¹H NMR spectra, recorded at pH 5.8 in both the absence and presence of 1 molar eq of Zn(II)) (Fig. 2A), showed a substantial increase in chemical shift dispersion in the presence of Zn(II). This increase is consistent with the formation of persistent secondary structure and demonstrates that these domains are indeed genuine zinc finger domains.

To determine the aggregation state of ZNF265-F1, we used sedimentation equilibrium methods (Fig. 2B). Data were recorded on two different loading concentrations at three speeds, and these data were well fitted by a model incorporating a single, ideal species with a molecular mass of 4.9 kDa (with 95% confidence intervals of 4.5 and 5.2 kDa; M theor = 5,061 Da). Thus ZNF265-F1 is monomeric in solution under these conditions.

Determination of the Structure of ZNF265-F1—The high quality of the ¹H NMR spectra of ZNF265-F1 in the presence of Zn(II) (Fig. 2A) allowed us to determine the three-dimensional solution structure of this domain. ¹H resonance assignments
were achieved by using standard two-dimensional homonuclear NMR experiments. Using DYANA (30), NOEs were iteratively assigned and a preliminary fold determined; no zinc ion was included, and the zinc coordination site was not restrained during these calculations. On the basis of these structures, it was clear that the four cysteine thiol groups (Cys\textsuperscript{15}, Cys\textsuperscript{20}, Cys\textsuperscript{31}, and Cys\textsuperscript{34}) comprised the zinc coordination sphere. Subsequent calculations, carried out using the ARIA protocol (37, 38) implemented in CNS (36), therefore included a zinc ion, together with constraints defining tetrahedral coordination. The remaining ambiguous NOEs were introduced iteratively in ARIA in an automated manner. The final ensemble of the 20 lowest energy structures is based on a total of 709 unambiguous intra- and inter-residue distance restraints derived from two-dimensional NOESY spectra and 22 backbone dihedral restraints. The total number of experimental restraints was 731. Of these, 347 were meaningful inter-residues restraints, representing an average of 12.8 constraints per structured residue (i.e., residues 12–38). From the final ARIA calculations, the 20 lowest energy structures were chosen to represent the solution structure of ZNF265-F1 (Fig. 3A). The structures display good covalent geometry and good non-bonded contacts. Analysis of the structures with PROCHECK (43) shows that, for residues that exhibit \( \phi \) angle order parameters >0.6, over 99.6% of non-glycine and non-proline residues falls into the most favored or additionally allowed regions of the Ramachandran plot. Structural statistics for the ensemble are given in Table I.

**ZNF265-F1 Forms a Zinc Ribbon**—Residues 12–38 of ZNF265-F1 adopt a compact, globular fold that incorporates a single zinc ion. The fold consists of two distorted \( \beta \)-hairpins that each provide two of the zinc ligating cysteines (Fig. 3B); the Zn(II) ion is effectively sandwiched between the two hairpins, which cross each other at an angle of \( \sim 80^\circ \) (Fig. 3C). The first \( \beta \)-hairpin is formed by residues Asp\textsuperscript{12}-Asn\textsuperscript{34} (strands 1 and 2 comprise residues Trp\textsuperscript{12}-Cys\textsuperscript{15}, and Asn\textsuperscript{22}-Asn\textsuperscript{24}, respectively) and displays backbone hydrogen bonds between the H\textsuperscript{N} proton of Cys\textsuperscript{15} and the carbonyl oxygen of Asn\textsuperscript{22}, as well as between the H\textsuperscript{N} proton of Asn\textsuperscript{24} and the carbonyl oxygen of Trp\textsuperscript{13}. In addition there are two hydrogen bonds to the S\textsuperscript{t} atom of Cys\textsuperscript{34}, from the backbone amide protons of Asn\textsuperscript{22} and Gly\textsuperscript{21} (2.1 and 3.1 Å in the lowest energy structure, respectively); and an additional hydrogen bond to the S\textsuperscript{t} atom of Cys\textsuperscript{34}, from the backbone amide protons of Asp\textsuperscript{17} (3.6 Å). These hydrogen bonds are characteristic of the rubredoxin knuckle, which is very common in zinc-binding domains (49). Residues Cys\textsuperscript{15}-Lys\textsuperscript{18} form a type VIII \( \beta \)-turn, judging from backbone \( \phi \) and \( \psi \) angles (although no (i,i+3) hydrogen bond is observed), whereas residues Asp\textsuperscript{17}-Cys\textsuperscript{20} form an interlinked type I \( \beta \)-turn (50). The side chain carbonyl oxygen of Asp\textsuperscript{17} forms hydrogen bonds with the H\textsuperscript{N} protons of Lys\textsuperscript{18} and Lys\textsuperscript{19}, as is commonly observed in type I turns (50).

### Table I

| Structural statistics for ZNF265-F1 |
|-----------------------------------|
| **Experimental input**           |
| Total NOE restraints              | 723 |
| Total unambiguous restraints      | 709 |
| Intraresidue                     | 384 |
| Sequential                       | 158 |
| Medium range                     | 53 |
| Long range                       | 114 |
| Total ambiguous restraints        | 14 |
| Torsion angle constraints         |     |
| Dihedrals \( \phi \)              | 22  |
| Dihedrals \( \chi \)              | 0   |
| **Quality control**               |
| PROCHECK statistics; residues in most favored regions | 63.5% |
| Residues in allowed regions       | 32.7% |
| Residues in generously allowed regions | 3.3% |
| Residues in disallowed regions    | 0.4% |
| R.m.s.d. of backbone atoms        | 0.67 ± 0.16 |
| R.m.s.d. of all heavy atoms       | 1.54 ± 0.26 |
| Mean deviations from ideal geometry | 0.0051 ± 0.0008 Å |
| Bond lengths                      | 0.54 ± 0.04 Å |

**Fig. 3. The solution structure of ZNF265-F1.** A, ensemble of best 20 structures of ZNF265-F1 (backbone atoms only). Structures are superimposed over the backbone atoms (C\textsuperscript{\alpha}, C\textsuperscript{\beta}, and N) of residues 12–38 (residues 2–11 and 39–43, which are unstructured, are omitted for clarity). The zinc chelating side chains and the zinc atom are shown. B, ribbon diagram of one of the lowest energy structures of ZNF265-F1 showing elements of secondary structure as recognized in the program MOLMOL. Both diagrams are shown in wall-eyed stereo format. C, diagram showing the crossing of the two \( \beta \)-hairpins that form the zinc-binding site. The hairpins are angled at \( \sim 80^\circ \) relative to each other. D, diagram showing the distribution of conserved residues in the first CF domain of ZNF265. Residues that are highly conserved are shown in space filling representation and are labeled. The most highly conserved residues appear to be involved in maintaining the fold of the domain, whereas others lie on the protein surface, indicating a functional role.
The second β-hairpin consists of residues Thr29-Lys38 but is not recognized by the Kabsch and Sander (51) secondary structure prediction algorithm (as implemented in MOLMOL; 42) in any of the final conformers. A closer manual inspection of the structures, however, reveals that many of the hydrogen bonding patterns observed across the first β-sheet are also maintained here. For example, hydrogen bonds can be observed between the H proton of Ser29 and the side chain carbonyl oxygen of Glu37, the H proton of Lys38 and the carbonyl oxygen of Thr28, and between the H proton of Cys31 and the carbonyl oxygen of Arg38. This hairpin is recognized in the family of conformers that are obtained prior to water refinement, but the slight loosening of the ensemble that takes place in the water refinement puts the hydrogen bond parameters just outside the tolerances set by the Kabsch and Sander (51) algorithm. Consequently, we have not shown this hairpin in Fig. 3. The two strands of this “hairpin” are connected by residues Cys31-Cys34, which form a half-knuckle with a hydrogen bond linking the S atom of Cys31 to the backbone amide proton of Arg33. A small hydrophobic core is formed by Trp13, Cys16, Pro16, Asn22, Asn24, Cys31, and one face of the aliphatic side chain of Lys40.

A search of known protein structures, using ZNF265-F1 as a template and the program DALI (52), did not reveal any structural homologs, indicating that ZNF265-F1 constitutes a new protein fold. Because the fold consists of two hairpins crossing each other, we have termed this new fold a crossed finger or CF domain. However, DALI searches can yield false negatives when dealing with small domains (1). Inspection of the structure reveals that it falls into the zinc ribbon class of zinc-binding domains, as defined by Krishna et al. (1). Members of the zinc ribbon class all possess two β-hairpins, but otherwise display significant sequence and structural variability. Typically, a third β-strand adjoins one of the hairpins. Many classical zinc ribbon proteins are involved in the transcriptional/ translational machinery and are capable of interacting with either DNA or RNA (1). Overlays of ZNF-F1 with typical zinc ribbons (e.g. the ribosomal protein L37E; PDB number 1JJ2) give RMSDs of 2–3 Å.

Within the family of crossed finger domains, a number of amino acids, other than the zinc ligands, are highly conserved (Fig. 1). Fig. 3D illustrates the distribution of these conserved amino acids on the structure of ZNF265-F1. Two of these, Trp13 and Asn24, are ~90% buried, and are likely to be important for maintaining the folded structure of the CF domain. Indeed, the buried side chain amide protons of Asn24 form hydrogen bonds with the backbone carbonyl oxygens of Trp13 and Ile14. Lys38 also packs against Trp13 and probably contributes to fold stability, whereas the remaining most highly conserved residues, Asp12, Lys15, Arg27, Arg32, and Phe25, are largely exposed. These latter residues may therefore serve a functional role.

**Could the CF be a Novel RNA-binding Domain?**—Analysis of the surface of the ZNF265-F1 structure reveals a number of conserved amino acids that are presumably not involved in specifying the folded conformation of the domain. Two lysines (Lys18 and Lys19) and three arginine (Arg27, Arg28, and Arg33) residues that lie within the well defined portion of the domain and are well conserved are highlighted in Fig. 4A together with the exposed phenylalanine residue Phe25. Strikingly, the spatial arrangement of a number of these residues is remarkably similar to the RNA-binding surface identified in the double stranded RNA-binding module of PKR (Fig. 4B). A large body of structural (53) and mutagenic (54–56) data has shown that a solvent-exposed phenylalanine, together with several lysine and arginine residues are used to contact RNA.

**The ZNF265 Crossed Finger Domain Can Bind mRNA**—The structural homology of the CF domain to a known RNA-binding protein suggested to us that this domain might function in RNA recognition. Interestingly, crossed finger domains are found in a number of other proteins that have either been shown directly to be involved in RNA metabolism or contain domains with RNA-related functions. The double-CF domain of C4SR, the Xenopus homolog of ZNF265, has been shown to stably interact with a cyclin B1 mRNA transcript (15). To determine whether ZNF265 could bind to the same cyclin transcript, a REMSA analysis was performed using an in vitro transcribed 32P-labeled full-length cyclin B1 transcript and purified ZNF265 protein. As shown in Fig. 5, addition of increasing concentrations of ZNF265 to the cyclin transcript pro-
duced a protein-RNA complex (indicated by arrow). This was seen clearly when using 5 μg of purified material (lane 4), but was also evident at a lower concentration (i.e., 100 ng) after prolonged exposure of the film. These results indicate that ZNF265 is able to bind to the cyclin transcript.

**DISCUSSION**

**The Structure of ZNF265-F1**—The crossed finger domain seems to be a subclass of the zinc ribbon fold, and it adds to the already large family of zinc-binding domains in which the zinc probably serves only a structural (rather than a catalytic) role (1). There are at least eight classes of such domains for which structural information is available (1), and the structures that they form are diverse. A recurring theme among these structures is the fact that the zinc ligands are divided into two pairs in the amino acid sequence. The spacing between the two ligands within a pair is generally 2 to 5 amino acids, whereas the sequence connecting the pairs may be up to 50 amino acids in length. It is clear that these H/C-X\(_{2,5}\)/H/C motifs take up a limited number of conformations, and presumably form a stable macrocyclic structure when bound to a zinc ion. Motifs containing histidine residues often take up a helical conformation (e.g., in classical zinc fingers), whereas C-X\(_{2,5}\)-C sequences typically form part of a turn or take up an irregular conformation. Given the diversity of folds that contain these motifs, it is interesting to speculate about why they have apparently arisen so frequently during evolution. It is possible that when two H/C-X\(_{2,5}\)/H/C units arise by random sequence variation and are spaced less than ~50 amino acids apart, there is a relatively high probability that this sequence will be able to ligate a zinc ion, thereby forming some kind of persistent structure (high at least compared with the chance of persistent structure forming in the absence of directing influences like metal-binding motifs).

At least three of the classes of zinc-binding domains have been demonstrated to bind specifically to RNA. The retroviral nucleocapsid domain that is essential for RNA packaging comprises a C-X\(_{2,5}\)-C-X\(_{2,5}\)-H-X\(_{2,5}\)-C zinc-binding domain (57). The zinc ribbon fold is found in many ribosomal proteins and recognizes RNA, and a number of proteins containing classical zinc fingers, such as TFIII A, also recognize RNA (58). Of these, structural alignments indicate that the zinc ribbon resembles most closely the ZNF265 domain.

**ZNF265 as an RNA-binding Protein**—The REMSA data presented here indicate that the ZNF265 CF domains are capable of interacting with an mRNA transcript from the cyclin B1 gene. These data are in accord with the previous demonstration by Ladomery et al. (15) that the zinc finger region from X. laevis C4SR, a ZNF265 sequence homolog (~98% similarity in the double CF domain region), can stably bind the same RNA message.

Several themes have emerged from studies of RNA-binding proteins to date and at least two of these are reflected in ZNF265. First, the importance of both basic and exposed aromatic amino acids for RNA recognition has become clear (5). The CF domains of ZNF265 contain many basic residues (seven in ZNF265-F1), as well as an exposed phenylalanine (Phe\(_{25}\)) and, remarkably, a number of these residues combine to form a surface that is rather similar to that observed in unrelated RNA-binding proteins such as PKR. Indeed, the use of a surface phenylalanine for the recognition of a ribonucleotide base is rather common among RNA-binding proteins; the trans-criptional termination factor Rho is another example of this phenomenon (59).

Second, RNA recognition motifs (and double stranded RNA-binding modules) are often found in pairs separated by a flexible linker; and large conformational changes, such as the freezing of a flexible linker between two RNA-binding domains, often occur upon RNA binding (47, 60). This is in contrast to DNA-binding zinc fingers that typically have much shorter linkers, usually of seven amino acids between adjacent fingers (61). ZNF265 contains two CF domains separated by a 24-residue linker, and interestingly PKR has two double stranded RNA-binding modules that are connected by a 22-residue flexible linker, consistent with the observed RNA-binding function of ZNF265. It has previously been suggested that widely spaced zinc fingers may allow greater flexibility in binding site selection (62, 63).

We have shown that the zinc fingers of ZNF265 form an RNA-binding motif, and the protein therefore conforms to the standard architecture of SR protein splicing factors (64, 65). It has been shown that ZNF265 has a role in directing alternative splicing and is able to bind to both U1-70K and U2AF\(_{35}\) (12). However, the binding to these proteins alone is insufficient for directing alternative splicing, because U1-70K and U2AF\(_{35}\) bind at their respective sites in all introns. Thus, to enable splice site selection the zinc finger domain of ZNF265 may selectively bind RNA targets; we are currently in the process of defining the sequence specificity for the ZNF265-RNA interaction.

**The CF Domain in Other Proteins**—Sequence analysis of the proteins that contain CF domains indicates a likely role for many CF-containing proteins in RNA metabolism. Whereas a number of these proteins have no known function, at least 30 contain other motifs such as the well characterized RNA recognition motif, or RRM (66), further linking this domain to RNA metabolism. The presence of multiple CF domains in the nuclear transport factor RanBP2 is particularly interesting. RanBP2 is a 358-kDa nucleoporin that is involved in nuclear export (18, 67). The presence of CF domains in this protein may indicate that it is involved not only in the export of protein cargo but also in shuttling RNA in and/or out of the nucleus. It should be noted, however, that few of the positively charged residues found in ZNF265-F1 are conserved in either the RanBP2 or the other ubiquitin ligase CF domains. This may argue for the adaptation of the CF fold for more than one function, in the same way that many other classes of zinc-binding domains appear now to be capable of a variety of roles (68).

In summary, a combination of structural, REMSA, and sequence analysis data have shown that the zinc-binding domains of the splicing factor ZNF265 are zinc ribbons that bind to RNA, most likely via a mechanism that bears some resemblance to the binding modes of other RNA-binding proteins. These data point to an interesting convergence of function among a wide variety of proteins involved in RNA metabolism.

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The Structure of the Zinc Finger Domain from Human Splicing Factor ZNF265 Fold
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