Structural Characterization of the Cysteine-rich Domain of TFIIH p44 Subunit*

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In an effort to understand the structure function relationship of TFIIH, a transcription/repair factor, we focused our attention on the p44 subunit, which plays a central role in both mechanisms. The amino-terminal portion of p44 has been shown to be involved in the regulation of the XPD helicase activity; here we show that its carboxyl-terminal domain is essential for TFIIH transcription activity and that it binds three zinc atoms through two independent modules. The first contains a C4 zinc finger motif, whereas the second is characterized by a C2,C2–CF2ADCD motif, corresponding to interleaved zinc binding sites. The solution structure of this second module reveals an unexpected homology with the regulatory domain of protein kinase C and provides a framework to study its role at the molecular level.

Transcription of protein coding genes in eukaryotes requires the formation at core promoters of a multiprotein complex composed of RNA polymerase II and the general transcription factors TFIIA, -IIB, -IID, -IIE, -IIF, and -IHH (reviewed in Refs. 1–3). TFIIH, which possesses nine subunits, plays a critical role in transcription. CAF (the Cdk-activating kinase complex, composed of cdk7, cyclinH, and MAT1) phosphorylates the carboxyl-terminal domain of the RNA polymerase II largest subunit (4); the two DNA helicases, XPB and XPD, are involved in the opening of the DNA template around the start site to allow transcription by RNA polymerase II (5, 6). The TFIIH role in nucleotide excision repair, a DNA repair pathway essential for maintaining the integrity of the genome, is also firmly established (7). Mutations in either XBP or XPD result in DNA repair defects, one of the phenotypes found in three genetic disorders: xeroderma pigmentosum (XP), Cockayne syndrome, or trichothiodystrophy (8, 9). The roles of the four other TFIIH subunits, p62, p52, p44, and p34, are not well understood.

Of these four subunits of TFIIH, p44 (10) is the most characterized. Its yeast homologue, Ssl1, is thought to nucleate the formation of the core TFIIH with TFB1, the yeast counterpart of p62 (11, 12). The human p44 protein interacts with human p34, XPD, XBP (13–15), as well as CSA, a WD repeat-containing protein (16). The p44 amino terminus shares sequence homology with S5a, a proteasome subunit, leading to the proposal that this homologous region could be a ubiquitous regulating motif for both transcription and translation (17). Additionally, the p44 subunit possesses a carboxyl-terminal cysteine-rich region (residues 252 to 395) (10). Its sequence analysis reveals relationships to three different zinc binding motifs: a C4 zinc binding motif followed by either a TFIIIA-like zinc finger or a RING-related sequence (see Ref. 18 and Fig. 2).

Here we demonstrate through biochemical, biophysical, and structural studies that the carboxyl-terminal portion of p44 is essential for the integrity of TFIIH transcriptional activity. This role is supported by two distinct zinc binding modules belonging to the C4 zinc finger family and to the interleaved zinc-binding protein family. The solution structure of this latter module reveals an unexpected homology with the regulatory domain of protein kinase C and provides a framework to study its role at the molecular level.

EXPERIMENTAL PROCEDURES

Recombinant TFIIH Production, Purification, and Transcription Assay—Baculovirus allowing the expression of wild type recombinant TFIIH were generated as described by Tirole et al. (19). The cDNAs encoding truncated forms of the p44 subunit with an amino-terminal His tag were inserted into pVL1392, and corresponding baculoviruses were generated using standard protocols. Recombinant TFIIH complexes were produced in Sf9 cells (typically 107) infected with a combination of baculoviruses expressing XPB (10), XPD (10), p62(1), p52(4), either His-p44 wt, His-p44A (252–395), or His-p44A (321–395) (1), p34(10), cdk7(2), cyclinH (2), and MAT1(1) at a multiplicity of infection indicated under brackets (plaque-forming units/cell). Recombinant TFIIH complexes were immunopurified, and their transcriptional activity was assayed.

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The atomic coordinates and structure factors (code 1e53) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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Expression and Purification of Recombinant Proteins—Domains of the human p44 TFIIH subunit with either the wild type or a mutated amino acid sequence were expressed in the *Escherichia coli* Bl21 (DE3) strain as His-tagged or GST fusion proteins from plasmids pE7-15b-p44 (p45/252–385) or pGEX-p44 (p45/252–320) and pGEX-p44 (p45/321–395). In contrast, in vivo transcription system experiments were performed using the *N. crassa* S-288C strain as His-tagged or GST fusion proteins from plasmids pET-15b-p44 (p45/252–385) or pGEX-p44 (p45/252–320) and pGEX-NB, a modified version of pGEX-4T2 (Amersham Pharmacia Biotech), using the NdeI and BamHI restriction sites. Cells were grown at 37 °C to an A600 of 0.6, induced with 0.4 mM isopropyl-β-D-thiogalactopyranoside, and harvested after 4 h of incubation at 25 °C. Cells, resuspended in 20 mM Tris-Cl, pH 8, 100 mM NaCl, 5 mM 2-mercaptoethanol, were disrupted by sonication and centrifuged for 1 h at 4 °C at 100,000 × g. The p44 (p45/252–395) protein was purified with the TALON (CLONTECH™) cobalt affinity resin using a 10 to 250 mM imidazole gradient. The GST-p44 (p45/321–395) proteins were purified using GSH-Sepharose (Amersham Pharmacia Biotech), and the GST fusion protein was cleaved with bovine thrombin (0.25 units/mg of fusion protein). All recombinant proteins were finally subjected to gel filtration chromatography using a Superdex 75 26/60 column (Amersham Pharmacia Biotech) in 20 mM Tris-Cl, pH 8, 50 mM NaCl, 5 mM 2-mercaptoethanol.

**Atomic Absorption Spectroscopy**—Protein samples were dialyzed against 20 mM Tris-Cl, pH 8, 250 mM NaCl, 5 mM 2-mercaptoethanol, 0.1 mM EDTA at 4 °C for 16 h. Measurement of metal content was performed on a Varian AA75 spectrophotometer at the appropriate wavelength and deduced from a standard calibration. Measurement of metal content was introduced into the ion source via a 10-μL acetonitrile mixture (v/v) containing 1% of formic acid. Samples were injected to drive the iterative assignment of the full set of NOE distances between cysteine residues Cys-360 and Cys-363 and residues His-376 and His-380, as described in Shang et al. (20). Protein and 4-2-pyridyldiazoresorcinol λ 280 as described in Shang et al. (20). Protein and 4-2-pyridyldiazoresorcinol concentrations were 3 μM and 0.1 mM.

**Cysteine Modification**—Protein in 20 mM Tris-Cl, pH 8, 50 mM NaCl, 5% glycerol was titrated at room temperature with increasing amounts of p-(hydroxymercuri)benzene-sulfonate reagent (PMPS; Sigma-Aldrich). Mercaptide bond formation was monitored at 250 nm. A first analysis of the NOESY spectra, 366 NOE cross-peaks; 166 NOEs cross-peaks retained their ambiguities assignment at the end of the procedure. Inter-proton distance restraints were obtained by classifying peak volumes as strong, medium, weak, and very weak, corresponding to distances of 2.5, 3.2, 4.5, and 5 Å respectively. The standard pseudo-atom corrections were applied.

**Structural Calculations**—Two runs of structure calculation were performed using the restrained simulated annealing protocol implemented in the program X-PLOR 3.851 (24, 25). A first set of 50 structures was generated using only NOE distances as experimental restraints. This set of structures allowed the identification of secondary structure elements, hydrogen bond acceptors compatible with slow exchanging amide protons and the four cysteines involved in the first zinc coordination site (ZNI). For the second zinc binding site (ZNI), six possible coordinating ligands (Cys-360, Cys-363, His-376, His-380, Cys-382, and Cys-385) were located in the vicinity of the zinc ion. This set of structures pointed out a few inconsistencies in the NOE-derived distances between ZNI-II possible ligands. Two experimentally observed NOE distances between cysteine residues Cys-360 and Cys-363 and Cys-382 and Cys-385 had to be discarded to build a Cys-2-His-2 coil coordination scheme that was based on eight long range NOEs between residues Cys-360 and Cys-363 and residues His-376 and His-380.

A second set of 50 structures was then generated using 1082 NOE distance restraints, 12 hydrogen bond constraints, and 6 S=Zn distance restraints. The structures were further refined using 25 dihedral angle restraints and geometric constraints around zinc according to tetrahe-
Structural Characterization of p44 Carboxyl Terminus

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The amino-terminal portion of the p44 subunit of TFIIH is involved in the regulation of XPD helicase activity (15). To investigate the function of the carboxyl-terminal portion of p44, we generated, based on limited proteolysis, two recombinant TFIIH complexes in which the p44 subunit was deleted at its carboxyl-terminal end.

RESULTS AND DISCUSSION

The amino-terminal portion of the p44 subunit of TFIIH is involved in the regulation of XPD helicase activity (15). To investigate the function of the carboxyl-terminal portion of p44, we generated, based on limited proteolysis, two recombinant TFIIH complexes in which the p44 subunit was deleted.

parallel, the corresponding carboxyl-terminal domains were cloned, expressed, and purified for biophysical and structural studies.

The p44 Carboxyl-terminal Domain Is Essential for Transcription Activity—Two recombinant TFIIH complexes in which the p44 subunit was deleted at its carboxyl-terminal end from amino acid 252–395 (p44(252–395)) and from 321–395 (p44(321–395)) were generated. After being overexpressed in the baculovirus expression system, rIIH9-wt, rIIH9-p44(252–395) and rIIH9-p44A(321–395) were purified by immunoprecipitation using a monoclonal antibody directed toward the XPD helicase activity. Together, these data demonstrate a crucial in-

2. DINO, Visualizing Structural Biology, available on the Internet.

3. POVRay: the Persistence Of Vision Ray Tracer, Version 3, available on the Internet.
volvement of the carboxyl-terminal domain of p44 in the TFIIH transcriptional activity.

The p44 Cysteine-rich Domain Binds Three Zinc Atoms—A sequence similarity search using human p44(252–395) as the query sequence retrieved eight homologous proteins that were aligned. As shown in Fig. 2, 13 of the 14 cysteines ( residues) and 4 of the 9 histidines ( residues) present in the human p44 are conserved among all species from Homo sapiens to Saccha-

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**Fig. 3.** Mass spectrometry measurements of p44(252–395), p44(252–320), and p44(321–395) (electrospray ionization-mass spectrometry). A, Coomassie Blue-stained SDS-polyacrylamide gel electrophoresis of p44(252–395), p44(252–320), and p44(321–395) (lanes 1, 2, and 3, respectively). B–H, mass spectra of the p44 fragments under native (ammonium acetate) and denaturing conditions (HCOOH, formic acid). B and C, mass spectra of p44(252–395) recorded under denaturing (B) and native conditions (C). D and E, same as B and C for p44(252–320). F, G, and H, mass spectra acquired in native conditions of the same p44(321–395) sample before treatment (F), after EDTA treatment (G), or after EDTA treatment followed by back addition of an excess of zinc acetate (H). Peaks marked * and † correspond to sodium and 2-mercaptoethanol adducts, respectively.
Two blocks of conservation can be defined: Block I (Gly-288 to Leu-328) and Block II (Cys-345 to Cys-385) are separated by an insertion of 12 to 23 residues. Block I contains five cysteines and one histidine and possesses a typical sequence C\textsubscript{X\textsubscript{2}}C\textsubscript{X\textsubscript{2}}C\textsubscript{X\textsubscript{2}}C amino acid sequence that corresponds to a C4 zinc binding motif (36). Block II contains eight cysteines and two histidines and possesses a typical sequence C\textsubscript{X\textsubscript{2}}C\textsubscript{X\textsubscript{2}}A FCACD (A is an aliphatic residue) that is found only in human p44 and its orthologues. Block II also exhibits similarities with a consensus TFIIIA zinc finger (37) and with a derived C3HC4 RING finger motif (38) (Fig. 2).

To determine whether the p44 carboxyl-terminal portion binds a metal through the detected motifs, three expression vectors that enable the production of p44(252–395) (Blocks I and II) as well as p44(252–320) (Block I) and p44(321–395) (Block II) were constructed (Fig. 3A, lanes 2 and 3; see also “Experimental Procedures”). The p44(252–320) and p44(321–395) fragments were chosen on the basis of limited proteolysis of p44(252–395). The metal content of the purified recombinant polypeptides was analyzed. Atomic absorption measurements revealed the presence of zinc atoms within the protein fragments, whereas no trace of metal such as Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, Zn\textsuperscript{2+}, or Mn\textsuperscript{2+} could be detected. The accurate zinc stoichiometry was determined from mass spectrometry measurements by comparing the mass measured for the denatured protein (Fig. 3, B and D) with that of the native one (Fig. 3, C and E) for p44(252–395), p44(252–320), p44(321–395) (see also Table I). Apart from the primary ion peaks, minor peaks belonging to sodium (Fig. 3, *) or 2-mercaptoethanol (Fig. 3, ◆) adducts were detected. The mass spectrometry data obtained under denaturing conditions fit the theoretical values. Mass spectrometry measurements of the native protein fragments show additional molecular masses of 191.1, 63.8, and 127.9 Da for p44(252–395), p44(252–320), and p44(321–395), respectively (Fig. 3, C, E, and F). These correspond to 3, 1, and 2 zinc atoms associated with the three fragments, respectively (Table I).

Upon treatment with EDTA, we observed that the 9015.9-Da protein peak (Fig. 3F) gives rise to two different species of 8888.0 Da and 8950.9 Da corresponding to the loss of two and one zinc atoms, respectively (Fig. 3, F and G). The addition of a large excess of zinc acetate restores the native state of the protein, leading to a species of 9015.7 Da, which corresponds to the back addition of the two zinc ions (Fig. 3H). The zinc binding to the protein is specific since no additional species is observed. Together, these data demonstrate that p44 carboxy-terminal domain contains three zinc atoms coordinated by two independent domains that correspond to p44(252–320) and p44(321–395).

**Identification of Zinc Binding Amino Acids**—To identify the zinc coordinating amino acids, p44(252–395), p44(252–320), and p44(321–395) were treated with p-(hydroxymercuri)benzene sulfonate (PMPS) or diethylpyrocarbonate (DEPC), which react with cysteine and histidine residues, respectively, and the amount of zinc released upon treatment was quantified. These experiments revealed that only cysteines are involved in zinc chelation for p44(252–320), whereas in p44(321–395), both cysteine and histidine residues bind zinc (Table II).

In a second set of experiments, we overexpressed and purified p44 variants where conserved cysteines and histidines were mutated to alanine. Their metal content was then analyzed using mass spectrometry and by chemical modification experiments (Fig. 2 and Table II). Mutation of His-275 and Cys-299 does not modify the zinc content of p44(252–320), whereas mutations at positions Cys-291, Cys-294, Cys-305, and Cys-299, Cys-308 do not modify the zinc content of p44(252–320), whereas in p44(321–395), both cysteine and histidine residues bind zinc (Table II). Cys-308 yield an overexpressed insoluble protein. These data suggest that these four cysteine residues are putative zinc ligands. Mutations in p44(321–395) at positions Cys-294, Cys-305, and His-371 alter the zinc content of the protein, leading to the loss of one zinc atom. Mutations of Cys-360 and Cys-363 make the protein insoluble, thus providing evidence for the structural role of these cysteine residues. Mutations of His-376, His-380, Cys-382, and Cys-385 residues do not alter the zinc content nor the solubility of the protein. However, a double mutation at position His-376/His-380 results in an insoluble polypeptide. It was also found that the non-conserved Cys-331 did not substitute for zinc binding (data not shown), nor did the conserved His-334. Together, these data suggest that Cys-345, Cys-348, Cys-360, Cys-363, Cys-368, and Cys-371, and at least one histidine (His-376 or His-380) are involved in the coordination sphere of the two zinc atoms in p44(321–395).

Three-dimensional Solution Structure of p44(321–395)—The three-dimensional structure of p44(321–395) was determined by NMR spectroscopy using both unlabeled and uniformly \textsuperscript{15}N-labeled protein ("Experimental Procedures"). Backbone and side chain protons are assigned for 72 residues out of the 75. One unique set of resonance is observed for all residues; how-

| TABLE I |
| --- |
| **Mass spectrometry measurements and deduced zinc ratios for p44(252–395), p44(252–320), and p44(321–395)** |
| **Theoretical mass (Da)** | **Denaturing condition (Da)** | **Native condition (Da)** |
| 16,450.9 | 7,705.9 | 8,888.0 |
| 16,451.4 | 7,705.9 | 8,888.0 |
| 16,452.5 | 7,709.7 | 9,013.9 |
| **\( \Delta \) mass** | | |
| 191.1 | 63.8 | 127.9 |
| **Zinc atoms per molecule** | | |
| 2.92 | 0.98 | 1.95 |

| TABLE II |
| --- |
| **Zinc content of wild type and mutants p44(252–320) and p44(321–395) deduced from mass spectrometry measurements and amount of zinc released upon p-(hydroxymercuri)benzene sulfonate (PMPS) or diethylpyrocarbonate (DEPC) treatment** |
| **NS, insoluble protein.** |
| **Mass** | **PMPS** | **DEPC** |
| p44(252–320)-wt | 1 | 1 | 0 |
| H275A | 1 | 1 | 0 |
| C291A | NS | NS | NS |
| C294A | NS | NS | NS |
| C299A | 1 | 1 | 0 |
| C305A | NS | NS | NS |
| C308A | NS | NS | NS |
| C382A | 2 | 2 | 1 |
| C345A | 1 | 1.1 | 0.6 |
| C348A | 1 | 1.2 | 0.8 |
| C360A | NS | NS | NS |
| C363A | NS | NS | NS |
| C368A | 1 | 1.2 | 1 |
| C371A | 1 | 1.1 | 0.8 |
| H376A | 2 | 1.9 | 1.1 |
| H380A | 2 | 1.9 | 0.8 |
| C381A | 2 | 2 | 1 |
| C382A | 2 | 2.1 | 1.2 |
| C385A | 2 | 2.1 | 1.3 |
| H376A/H380A | NS | NS | NS |
| C382A/C385A | 2 | 1.8 | 1.1 |
| H376A/C382A | 2 | 1.8 | 0.9 |
| H376A/C385A | 2 | 1.9 | 1.1 |
| H380A/C382A | 2 | 2.1 | 1.2 |
| H380A/C385A | 2 | 2 | 1 |
ever, line broadening is found to occur for some residues located in the carboxyl and amino termini of the sequence. This phenomenon is due to an intermediate time scale conformational exchange and leads to a complete coalescence of resonances for amino-terminal residues including histidines 323 and 324. A significant line broadening is also found to affect carboxyl-terminal residues located in the vicinity of zinc binding site. Large line-widths are found for amide protons of serine 378 and glycine 383, which are bracketed by residues potentially involved in the binding of the zinc atom (His-376 to His-380 and

**Fig. 4. Solution structure of p44(321–395).** A, the number of short-range (i, i+1, cyan bars), medium range (i, i+(2–4), yellow bars), and long range (orange bars) inter-residue NOE-deducted distance restraints is represented as a function of residue number. The average root mean square deviation (Rmsd) value from the mean structure for 23 structures of p44(321–395) is indicated as a solid line. B, overlay of the Ca backbones from the 23 simulated annealing structures in stereo for residues 328 to 386. The mean positions of the Zn$^{2+}$ atoms are represented by orange (ZNI) and blue (ZNII) sphere. C, ribbon diagram of the average solution structure. In the left panel, residues 328 to 375, which form the well defined core as well as side chains from residues involved in zinc chelation (yellow) and buried hydrophobic residues (green), are represented. Zinc atoms from ZNI and ZNII are drawn in orange and blue, respectively. In the right panel, the distribution of charges at surface of the molecule is illustrated for the face covered by the α-helix (conserved charged residues are labeled). No charge was assigned to zinc. The color scheme shows negatively charged atoms as red, positively charged atoms areas as blue, and neutral areas as white. Left and right panels represent 180°-rotated views of the molecule.
Cys-382 to Cys-385, respectively). This dynamic behavior affects the structure precision of the tail residues of p44(321–395) but not those located in the core of the structure (Fig. 4A). Structure calculations were performed using dynamic simulated annealing using 456 intra-residual, 382 medium, and 244 long range restraints (Fig. 4A) to generate a set of 50 structures, 23 being retained and shown in the stereo view (Fig. 4B) (See “Experimental Procedures” and Table III).

The p44(321–395) core domain consists of a three-stranded anti-parallel β-sheet packed against a small α-helix, the carboxyl terminus being spatially close to the amino terminus (Fig. 4C, left). Residues Gln-332 to Ile-334, Val-357 to Val-359, and Val-366 to Cys-368 form strands β1, β2, and β3, respectively. One face of the β-sheet is partially covered by the α-helix, the other by a loop that begins with one turn of a 3_10 helix. The domain is stabilized by two small clusters of buried and partially buried hydrophobic residues located on both sides of the β-sheet. The first cluster includes the conserved aromatic residues Phe-331, Tyr-346, Tyr-358, and Phe-367, stabilizing the face covered by the α-helix. The second cluster located on the other face consists of Tyr-339, Val-357, Val-359, and Val-368. The well defined compact core of p44(321–395) domain partially exposes conserved hydrophobic residues including Phe-331, Tyr-346, Tyr-358, and Phe-367, stabilizing the face covered by the α-helix. The determined solution structure of the second module reveals homologies with inter-leaved zinc binding domains, whereas the second is characterized by a C2 CX3–4FCADCD motif. In addition, the determined solution structure of the second module reveals homologies with inter-leaved zinc binding domains.

As inferred from spatial localization of conserved cysteines and histidines, p44(321–395) possesses two independent zinc binding sites that are separated by 14 Å and located on the face of the β sheet covered by the α-helix. The first zinc binding site (ZNII), organized into a C2 type, is composed of the first pair and the third pair of conserved cysteines (Cys-345/Cys-348 and Cys-368/Cys-371). When mutated, these residues prevent zinc binding. The second zinc binding site (ZNIII) involves the cysteine residues Cys-360 and Cys-363 together with His-376 and His-380, which are the best candidates for the zinc binding ligands on the basis of the observed NOE. The possible presence of a minor conformation involving one of the two cysteines Cys-382 or Cys-385 could not be discarded and may be responsible of the localized line broadening.

How is the cysteine-rich carboxyl-terminal domain of p44 involved in TFIIH transcriptional activity? Zinc binding motifs allow accurate folding of domains (43) whose functions include DNA binding (44), ligand binding (40, 42, 45), and protein-protein interactions (46, 47). The p44 protein is a subunit of TFIIH, a stable macromolecular assembly known to interact with both proteins and DNA. The charge distribution of p44(321–395) rules out a possible role in the recognition of zinc atoms via specific cysteine and histidine residues through two independent modules. The first contains a C4 zinc binding motif, whereas the second is characterized by a C2 CX3–4FCADCD motif. In addition, the determined solution structure of the second module reveals homologies with inter-leaved zinc binding domains.

Conclusion—In the present study, we establish that the carboxyl-terminal cysteine-rich region of the p44 TFIIH subunit is necessary for transcription. This domain binds exactly three zinc ions via specific cysteine and histidine residues through two independent modules. The first contains a C4 zinc binding motif, whereas the second is characterized by a C2 CX3–4FCADCD motif. In addition, the determined solution structure of the second module reveals homologies with inter-leaved zinc binding domains.

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TABLE III

| Restraint type (number of restraints) | VDW, van der Waals; TOR, dihedral torsion angle. |
|-------------------------------------|--------------------------------------------------|
| intra-residue                       | 451                                              |
| inter-residue sequential            | 271                                              |
| inter-residue medium range (i–j < 5)| 111                                              |
| inter-residue long range (i–j ≥ 5)| 249                                              |
| Hydrogen bond constraints           | 6 x 2                                            |
| Dihedral angle restraints           | 13 ϕ + 12 ψ                                     |
| Average root mean square deviation  | NOE restraints (Å) 0.0345 ± 0.0031                 |
|                                    | Bonds (Å) 0.0034 ± 0.0005                        |
|                                    | Angles (°) 0.52 ± 0.04                           |
|                                    | Improper (°) 0.36 ± 0.04                         |
|                                    | Dihedral angle restraints (°) 1.359 ± 0.329      |
| Average Energy                      | E_{NOE} (kcal mol⁻¹) 65.6 ± 12.0                 |
|                                    | E_{Bonds} (kcal mol⁻¹) 0.015 ± 0.007             |
|                                    | E_{VDW} (kcal mol⁻¹) −14.9 ± 28.3               |

Structure statistics of 23 structures of p44(321–395)
Fig. 5. Structural neighbors of p44(321–395). A, ribbon overlay of the β strands in p44(321–395) (gray) and PKC (black). The structural alignments were performed automatically using only Ca. The positions of the zinc atoms, not used for the superimposition, are indicated. B, topology diagrams of RING finger, Vsp27p, p44(321–395), and PKC/Raf-1 (arrows for β-sheets and black rectangles for α-helices). Equivalent β strands are drawn in gray.

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