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Structure of the central RNA recognition motif of human TIA-1 at 1.95 Å resolution

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Abstract

T-cell-restricted intracellular antigen-1 (TIA-1) regulates alternative pre-mRNA splicing in the nucleus, and mRNA translation in the cytoplasm, by recognizing uridine-rich sequences of RNAs. As a step towards understanding of RNA recognition by this regulatory factor, the X-ray structure of the central RNA recognition motif (RRM2) of human TIA-1 is presented at 1.95 Å resolution. Comparison with structurally homologous RRM-RNA complexes identifies residues at the RNA interfaces that are conserved in TIA-1–RRM2. The versatile capability of RNP motifs to interact with either proteins or RNA is reinforced by symmetry-related protein-protein interactions mediated by the RNP motifs of TIA-1–RRM2. Importantly, the TIA-1–RRM2 structure reveals the locations of mutations responsible for inhibiting nuclear import. In contrast with previous assumptions, the mutated residues are buried within the hydrophobic interior of the domain, where they would be likely to destabilize the RRM fold rather than directly inhibit RNA binding.

Keywords

RRM; RNA binding domain; Pre-mRNA splicing; mRNA translation; TIA-1; TIAR

Post-transcriptional mechanisms for regulating gene expression depend on numerous coordinated interactions among RNA sequences and trans-acting protein factors. The RNA binding protein TIA-1 (T-cell-restricted intracellular antigen-1) functions as a post-transcriptional regulator of gene expression by recognizing uridine-rich RNA sites. In the nucleus, TIA-1 regulates alternative splicing of pre-mRNAs, including those encoding fibroblast growth factor receptor-2 (*fgfr-2*), type II procollagen (*col2a1*), the cystic fibrosis transmembrane conductance regulator (*cftr*), and the pro-apoptotic Fas receptor (*fas*), among others [1;2;3;4;5;6]. In the cytoplasm, TIA-1 regulates mRNA translation [7;8;9;10], and suppresses mRNA translation during environmental stress (reviewed in [11]). The functional importance of TIA-1 is shown by its requirement for viability of DT40 cells [12], and the high rates of embryonic lethality among mice lacking TIA-1 [8;13].

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The TIA-1 polypeptide contains three RNA recognition motifs (RRMs, a prevalent class of RNA binding domain) and a C-terminal, Q-rich domain (Fig. 1) [14;15]. In humans, a second gene encodes the nearly identical TIAR protein [16], and at least two alternative spliceoforms exist for both TIA-1 and TIAR [14;15;17]. The Q-rich effector domain directs TIA-1-associated mRNAs to cytoplasmic stress granules during translational silencing [18], and recruits the U1 spliceosomal component (snRNP) to weak 5' splice sites during nuclear pre-mRNA splicing [19]. Like the Q-rich domain, the N-terminal RRM (RRM1) participates in U1 snRNP recruitment [19], and additionally associates with single-stranded DNA molecules [20]. The C-terminal RRM (RRM3) co-precipitates with cellular RNAs, however interactions of TIA-1–RRM3 with uridine-rich RNAs are not detected *in vitro* [21]. The central RRM (RRM2) of TIA-1 is the established, uridine-specific region, as demonstrated by the ability of isolated TIA-1–RRM2 to bind the *msl-2* 5' splice site region [19], viral RNAs [22], and uridine-rich SELEX RNAs [21]. Furthermore, the RRM2 region is necessary and sufficient for nuclear import of TIA-1 [23]. To investigate the major RNA binding surface of TIA-1, we have determined the structure of RRM2 in the absence of RNA at 2.0Å resolution.

Materials and Methods

Protein purification

The human TIA-1–RRM2 fragment (residues 94-175) was expressed using the pGEX-6p vector (GE Healthcare). Glutathione-S-transferase (GST) fusion proteins were purified by glutathione affinity, and then the TIA-1 fragment was further purified by cation exchange chromatography following removal of the GST tag. The TIA RRM2 and crystallized variant (described below) were concentrated to >15mg/mL in 100mM NaCl, 25mM HEPES pH7.4, 0.2mM TCEP.

Crystallization

Initial crystals of TIA-1–RRM2 grew in clusters of fine needles (<25 × 200 µm) (Supp. Fig. 1A). To improve crystal quality, the surface entropy reduction (SER) server (<http://nihserver.mbi.ucla.edu/SER>) was used to identify suitable modifications of TIA-1–RRM2 to promote crystallization. The site furthest from the putative RNA binding surface was selected for mutagenesis. Improved crystals of the resulting SER variant (E109A-D110A-K112A) grew in 2–3 days by the hanging drop vapor diffusion method at 22°C, from a 1:1 mixture of protein with 15% polyethylene glycol 3350, 0.5M KI, 0.1M Tris pH8.0 (Supp. Fig. 1B).

Data collection, structure determination, and refinement

X-ray data were collected using a MICROSTAR X-ray source with X8 Proteum detector (Bruker-AXS) (Table 1). A homology model of TIA-1–RRM2 [24] based on the N-terminal RRM of poly-A binding protein (PDB ID=1CVJ) [25] was used for molecular replacement in the program Phaser [26;27]. Refinement and model building were respectively completed using CNS [28] and Coot [29;30]. The coordinates and structure factors are available (PDB-code=3BS9).

Fluorescence measurements

The affinities of the wild-type RRM2 and SER-RRM2 domains of TIA-1 were measured for a 3' fluorescein-labeled 20-uridine RNA (U₂₀) (Dharmacon Research). Proteins were titrated into a thermostatted, 0.5cm quartz cuvette containing U₂₀ in 100mM NaCl, 25mM HEPES pH7.4 at 25°C, and the fluorescence anisotropy values were measured using a Fluoromax-3 fluorimeter (Jobin-Yvon). Excitation and emission wavelengths were typically 490/520nm, respectively. Data were fit by non-linear regression assuming single site binding (Fig. 2) to

obtain the apparent K_D with the following equation, where x is the total protein

$$r = r_F + \frac{r_B - r_F}{2[RNA]} \left((K_d + x + [RNA]) - \sqrt{(K_d + x + [RNA])^2 - 4[RNA]x} \right)$$

concentration, $[RNA]$ is the total RNA concentration, r is the observed anisotropy at the i th titration, r_B is the anisotropy at zero protein concentration, and r_F is the anisotropy at saturating protein concentration (floated in fit).

Results and Discussion

Overall structure and comparison with other RRM2s

The structure of TIA-1-RRM2, with a SER modification to improve crystal quality (E109A-D110A-K112A), was determined at 1.95 Å resolution (Table 1). Two, nearly identical TIA-1-RRM2 molecules (residues 94-172, 0.4 Å RMSD between 79 Cα atoms) in the crystallographic asymmetric unit adopt canonical RRM folds (β₁β₂β₃ topology) (Fig. 2A). Accordingly, the TIA-1-RRM2 structure most closely matches the N-terminal RRM2s of the RNA binding proteins poly-A binding protein (PAB) ([25]; PDB-code=1CVJ; Z-score=15.5; RMSD=1.1 Å and 37% sequence identity for residues 11-88) and sex-lethal (SXL) ([31]; PDB-code=1B7F; Z-score=14.6; RMSD=1.3 Å and 28% identity for residues 125-202) (Fig. 2B). Other close matches identified using the DALI protein structure server include hnRNP A1 ([32]; PDB-code=1HA1; Z-score=13.7; RMSD=1.3 Å and 27% identity for residues 13-89), nuclear cap binding protein ([33]; PDB-code=1H6K; Z-score=12.7; RMSD=1.3 and 34% identity for residues 40-117), and PRP24 ([34]; PDB-code=2GHP; Z-score=12.7; RMSD=2.0 and 21% identity for residues 41-115). The β₂-β₃ loop conformations of TIA-1-RRM2, PRP24, and the nuclear cap binding protein are the most variable regions of the structures, whereas β₂-β₃ loop conformations are very similar among the structures of TIA-1-RRM2 and the other single-stranded RNA binding proteins PAB, SXL, and hnRNP A1. The β₂-β₃ loop differences are likely to result from interdomain interactions mediated by the PRP24 β₂-β₃ loop, and removal of the nuclear cap binding protein β₂-β₃ loop by limited proteolysis. All of the aligned structures share a common 3₁₀-helical extension of the first α-helix, and a β-hairpin (β₄-β₅) insertion prior to the terminal β-strand (Fig. 2B).

3.2 Influence of the SER mutations on RNA binding and crystallization

As predicted based on homology with other RRM2s, the SER-modified residues are located on the exterior of the first α-helix, distant from the putative RNA binding surface presented by the β sheet (Fig. 2C). To ascertain that the SER-RRM2 binds RNA comparably to unmodified RRM2 of TIA-1, fluorescence anisotropy assays were used to compare the affinities of the wild-type and SER-RRM2 for uridine-rich RNA. The SER mutations had no detectable effect on the polyuridine-binding characteristics of TIA-1-RRM2 (Fig. 2D), demonstrating the utility of these mutations for structural investigations of TIA-1-RNA binding.

Rather than mediating crystallographic symmetry-related interactions, the SER residues are located at the interface of two RRM2 molecules in the asymmetric unit (Fig. 2C). Juxtaposition of the SER alanine-replacements for Glu109, Asp110, and Lys112, coupled with surrounding hydrophobic residues, created a nonpolar iodide-binding site. An iodide with a significant anomalous difference Fourier signal (22σ) makes eight contacts ranging from 3.4 Å (H₂O---I⁻) to 4.6 Å (Glu109Ala methyl group---I⁻) in length, consistent with the experimentally determined 3.6–3.7 Å distances of iodide hydration [35] and coordination distances of iodides bound nonspecifically to proteins [36]. Anti-parallel association of the N-terminal α-helices about the noncrystallographic two-fold axis places the β-sheet surfaces of the two molecules on the equivalent face of the RRM2 dimer. Although the SER mutations promote a noncrystallographic interaction between two TIA-1 RRM2s, this interaction in turn positions the molecules appropriately to build the crystal lattice.

3.3 Symmetry-related protein-protein interactions mediated by RNP motifs

Although the level of primary sequence identity among most RRM motifs is low (<25%), two ribonucleoprotein consensus motifs (RNP1 and RNP2) in the central β -strands commonly display aromatic stacking interactions with bound nucleic acids. Nevertheless, the RNP motifs of certain RRM motifs engage in protein-protein rather than protein-RNA recognition (reviewed in [37;38]), including the Y14-mago [39;40;41] and Upf3-Upf2 [42] complexes. Remarkably, the TIA-1 RNP1 and RNP2 motifs interact extensively with the α -helices of a symmetry-related molecule (Fig. 3A). The buried solvent-accessible surface area is large for a crystal packing interface (945 Å² compared with 570 Å² average crystal packing interface), but slightly less than expected for an oligomeric protein complex (1200–2000 Å²) [43]. Despite low primary sequence homology (27%), the symmetry-related TIA-1 contacts are qualitatively similar to the Y14 interactions with the α -helices of the mago partner [39;40;41] (Fig. 3B). In contrast, the Upf3-Upf2 orientation [42] differs from that of the symmetry-related TIA-1 RRM motifs. Although the TIA-1–RRM2 domain is likely to bind RNA in a canonical manner given its established role in RNA binding [19;21;22], the extensive symmetry-related interface mediated by the TIA-1 RNP motifs illustrates the versatile potential of RNP motifs to engage in protein-protein as well as protein-RNA interactions.

3.4 Comparison of putative RNA binding surfaces

The close structural matches of TIA-1–RRM2 with the N-terminal RRM motifs of PAB and SXL provide an opportunity to compare the residues involved in RNA interactions. Six prominent residues responsible for RNA interactions in both the PAB and SXL complexes are also conserved in the TIA-1–RRM2 (Fig. 3C–D), despite slight differences among the RNA sequences preferred by these factors. Three hydrophobic residues of the RNP motifs (RNP2-Phe98; RNP1-Tyr138; RNP1-Phe140) mediate conserved base- and sugar-stacking interactions of PAB and SXL, as well as the majority of other canonical RRM motifs [38]. Despite amino acid conservation, the remaining three residues are involved in distinct types of RNA interactions in the PAB compared with SXL complexes, due to differences in the local conformations of the RNA ligands. Most prominently, an adenosine in the PAB complex is rotated 180° relative to the SXL-bound uridine counterpart. Altogether, the PAB residues corresponding to Arg125, Asp129, and Arg167 of TIA-1 respectively engage in base stacking interactions, a specific hydrogen bond with an adenosine exocyclic amine, and a phosphate salt bridge [25], whereas the corresponding residues of SXL respectively hydrogen bond with a ribose 2' hydroxyl, lack direct RNA interactions, or form a specific hydrogen bond with a uracil base [31]. These differences underscore the difficulties confronting accurate prediction of RRM-RNA interactions, despite an expanding number of RRM-nucleic acid structures [44].

3.5 Locations of RNP residues implicated in nuclear import

Previously, the RRM2 RNP motifs of the TIA-1 homologue, TIAR, were mutated to investigate the importance of RNA binding for nuclear import [23]. Given that the RRM2 regions of TIA-1 and TIAR share 93% sequence identity, the availability of the TIA-1–RRM2 structure provides the opportunity to examine the locations of the mutated residues (Fig. 4). The nuclear localization of two mutant proteins was investigated, each with a cluster of mutations in either RNP1 (Gly137Asp/Tyr138Asp/Phe140Asp/Val141Glu, TIA-1 numbering), or RNP2 (Phe98Asp/Gly100Pro/Leu102Asp). Although several of the residues are solvent-exposed and may interact with RNA, both Leu102 (RNP2) and Val141 (RNP1) are buried in the hydrophobic protein interior (Fig. 4), where charged aspartate and glutamate side chains introduced by the site-directed mutagenesis would be likely to interfere with domain folding. Thus, the conclusion that TIAR RNA binding ability is important for nuclear accumulation is premature. Instead, the TIA-1–RRM2 structure presented here supports the conclusion that the

folded RRM2 domain is necessary for nuclear accumulation, as shown by deletion analysis of both TIA-1 and TIAR [23].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Domain structure of human TIA-1 above a sequence alignment of TIA-1-RRM2 from representative homologs. Sequences are colored by percent identity: 100%=green, <90%=sea green, <80%=lime, <70%=bright green, <60%=yellow, and <50%=white. Secondary structure elements are indicated above the alignment [45]. The RNP1 and RNP2 are boxed and labeled below the alignment. Surface residues of human Tia-1 mutated to facilitate crystallization are highlighted by blue filled circle. Residues that interact with RNA in both PAB and SXL complexes are marked with red filled circle. Accession numbers for aligned sequences include: *Homo sapiens* (NP_071320), *Mus musculus* (NP_035715), *Gallus gallus* (XP_001233215), *Caenorhabditis elegans* (NP_495121), *Neurospora crassa* (XP_962723), *Arabidopsis thaliana* (NP_188026), *Bombyx mori* (NP_001036895), *Entamoeba histolytica* (XP_649094), *Saccharomyces cerevisiae* (CAA46011).

Fig. 2.

Overall structure of TIA-1-RRM2. (A) Ribbon diagram of the two TIA-1-RRM2 molecules in the asymmetric unit of the crystal. Molecules 'A' and 'B' are distinguished by subscripts of the residues labels. Secondary structure elements of molecule 'A' are labeled. Composite omit electron density (light blue) at 1σ contour level surrounds key aromatic residues of the RNP motifs. Anomalous difference Fourier maps (magenta) at 3σ contour level indicate the iodide positions. (B) Superposition of the top five related structures identified using DALI [46]: TIA-1-RRM2 (orange), PAB (PDB-code=1CVJ, cyan), SXL (PDB-code=1B7F, magenta), hnRNP A1 (PDB-code=1HA1, green), PRP24 (PDB-code=2GHP, marine), and nuclear cap binding protein (PDB-code=1H6K, red). (C) Noncrystallographic contacts mediated by the SER mutations, viewed 180° about the y-axis relative to (A). Ball-and-stick diagrams are shown for SER residues (green) and other residues of the iodide binding sites (orange). The iodides bound at the interface are shown as magenta spheres, and the water molecules directly coordinated to these iodides are shown as blue spheres. (D) Comparison of TIA-1 RRM and SER TIA-1-RRM2 binding RNA using fluorescence anisotropy. Overlay of nonlinear least square fits of TIA-1-RRM2 (orange) and SER TIA-1-RRM2 (green) binding 5' fluorescein-labeled 20mer polyuridine. The average apparent equilibrium dissociation constants (K_D) and standard deviations of 2–3 experiments are inset.

Fig. 3.

Comparisons with the TIA-1 RNP motifs. (A) Symmetry-related interactions between two TIA-1-RRM2 molecules, respectively colored yellow and green. Key interacting residues are shown with ball-and-stick representation, and consensus residues of the RNP motifs responsible for mediating the intermolecular interaction are colored magenta. Primed italics distinguish residue labels of the symmetry-related molecule. (B) View of the Y14 (red)-mago (blue) complex (PDB-code 1000). Regions of the mago subunit outside the α -helical interactions with the Y14 RRM are omitted for clarity. (C) Superposition of TIA-1-RRM2 with PAB residues 11-88 (PDB-code 1CVJ). (D) Superposition of TIA-1-RRM2 with SXL residues 125-202 (PDB-code 1B7F). TIA-1 is colored yellow-orange, PAB is blue, and SXL is green. The RNA strands are colored magenta. The view is rotated 90° clockwise about the y-axis relative to (A). Residues of TIA-1 (Phe98, Arg125, Asp129, Tyr138, Phe140, Arg167) are labeled. Corresponding residues of PAB (Tyr14, Arg41, Asp45, Tyr54, Tyr56, Arg83) and SXL (Ile128, Arg155, Asp159, Tyr168, Phe170, Lys197) are shown as ball-and-stick representations.

Fig. 4. Site-directed mutations used to investigate TIAR nuclear localization mapped on the TIA-1–RRM2 structure. Side chains of solvent-exposed mutated residues are shown with ball-and-stick representation and colored orange. The mutated glycines are indicated by spheres. Leu102 of the RNP2 motif (blue) and Val141 of the RNP1 motif (red) are buried within the RRM interior. Residues that contact Leu102 are shown with ball-and-stick representation, and colored salmon. Residues that contact Val141 are also shown, and colored light blue. Residues that contact both Leu102 and Val141 are colored violet.

Table 1
Crystallographic data and refinement statistics.

A: Data collection statistics^a	
Space group	P6 ₁
Unit cell	
$a=b, c$ (Å)	56.50, 76.70
Resolution limit (Å)	1.95
R_{sym}^b (%)	7.0 (27.9)
$I/\sigma(I)$	15.5 (3.5)
Completeness (%)	99.8 (99.8)
Redundancy	5.2 (3.2)
B: Refinement statistics	
Resolution range (Å)	20.0–1.95
No. reflections (working/test set)	9648/489
$R_{\text{cryst}}/R_{\text{free}}^c$ (%)	21.6/24.5
No. protein atoms	1221
No. water molecules	118
No. iodide ions	7
RMSD bond lengths (Å)	0.006
RMSD bond angles (°)	1.31
Average B-factors (Å ²)	
all atoms	15.2
protein atoms	14.2
water molecules	23.2

^aValues in parenthesis are for the highest resolution shell (2.04–1.95 Å).

^b $R_{\text{sym}} = \frac{\sum_{hkl} \sum_i |I_i - \langle I \rangle|}{\sum_{hkl} \sum_i I_i}$, where I_i is an intensity I for the i th measurement of a reflection with indices hkl and $\langle I \rangle$ is the weighted mean of all measurements of I .

^c $R_{\text{cryst}} = \frac{\sum_{hkl} \sum_i |F_o(hkl) - k|F_c(hkl)|}{\sum_{hkl} |F_o(hkl)|}$ for the working set of reflections; R_{free} is R_{cryst} for 5% of the reflections excluded from the refinement