RNA Binding of T-cell Intracellular Antigen-1 (TIA-1) 
C-terminal RNA Recognition Motif Is Modified by pH 
Conditions*

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Background: T-cell intracellular antigen-1 (TIA-1) is a key DNA/RNA-binding protein in RNA metabolism.

**Results:** Binding of the TIA-1 RRM3 domain (isolated or fused to RRM2) to RNA is affected by pH changes.

**Conclusion:** The pH dependence of the RRM3/RNA interaction may have a significant effect on the overall TIA-1 function.

**Significance:** Unveiling the auxiliary role of RRM3 in RNA recognition sheds light on the TIA-1 function between cell compartments.

T-cell intracellular antigen-1 (TIA-1) is a DNA/RNA-binding protein that regulates critical events in cell physiology by the regulation of pre-mRNA splicing and mRNA translation. TIA-1 is composed of three RNA recognition motifs (RRMs) and a glutamine-rich domain and binds to uridine-rich RNA sequences through its C-terminal RRM2 and RRM3 domains. Here, we show that RNA binding mediated by either isolated RRM3 or the RRM23 construct is controlled by slight environmental pH changes due to the protonation/deprotonation of TIA-1 RRM3 histidine residues. The auxiliary role of the C-terminal RRM3 domain in TIA-1 RNA recognition is poorly understood, and this work provides insight into its binding mechanisms.

Human T-cell intracellular antigen-1 (TIA-1)3 is a 46-kDa DNA/RNA-binding protein with a dual regulatory role at the level of transcriptional and post-transcriptional processes, shuttling between the nucleus and the cytoplasm (1, 2). In the nucleus, TIA-1 regulates the alternative splicing of pre-mRNA (3–5) and modulates gene transcription by binding to DNA (6). In the cytoplasm, TIA-1 regulates the turnover of several mRNAs (7–9) by repressing their translation. To fulfill this function, TIA-1 binds to 3′-UTRs, which are frequently adenosine/uridine-rich elements. In addition, recent studies have identified 5′-terminal oligopyrimidine tract mRNAs, defined as 4−15-nucleotide CU-rich elements in 5′-UTRs (11, 12), as novel targets for TIA-1/TIAR (TIA-1 related) proteins (10). Under stress conditions, eIF2α is phosphorylated, so the initiation of translation is inhibited, and TIA-1 recruits and escorts non-canonical preinitiation complexes to stress granules (1, 13, 14).

TIA-1 consists of three RNA recognition motifs (RRM1–3) along with a C-terminal glutamine-rich prion-related domain (Fig. 1A). Both the prion-related domain and the N-terminal RRM (RRM1) are involved in U1 small nuclear ribonucleoprotein recruitment to regulated splice sites (4). Isolated RRM1 binds to single-stranded DNA molecules (6) but not to RNA (15). However, recent reports show that the presence of RRM1 enhances TIA-1 affinity for certain RNA sequences (16). RRM2 is necessary for TIA-1 binding to RNAs containing U-rich motifs, whereas RRM3 binds weakly but contributes to improve such binding (15, 16).

All TIA-1 RRMs display the canonical aβ-fold with the consensus ribonucleoprotein (RNP1 and RNP2) residues for RNA binding (17, 18). RRM3 exhibits a non-canonical N-terminal α-helix in a homology model (19) that is conserved in TIAR (Protein Data Bank code 1X4G) and Pub1p, the analogous protein in Saccharomyces cerevisiae (20). Moreover, the C-terminal motif Trp-Gly-(Arg/Lys) is also conserved, and the β-sheet is slightly distorted (Fig. 1, B and C). Due to their common features, TIA-1/TIAR/Pub1p RRM3 domains are considered to be a new type of RRM family called TRRM (TIA-1 C-terminal domain-like RRM) (19, 20). The contribution of RRM3 to RNA recognition is still unclear, although its contribution to binding poly(U) sequences (16) and its role in specifically selecting transcripts (20) have been reported.

Given the pleiotropic role of human TIA-1 in the control of critical cellular events, understanding how RNA recognition occurs and the associated biophysical features has great relevance. This work suggests that the auxiliary role, in RNA recognition, of isolated RRM3 or RRM3 in the RRM23 context is modulated by slight environmental changes in terms of pH, as small changes in protonation/deprotonation of conserved RRM3 histidine residues induce drastic effects on TIA-1 binding to RNA.
EXPERIMENTAL PROCEDURES

Protein and RNA Oligonucleotide Preparation—The RRM2 domain (residues 80–173), RRM3 domain (residues 190–288), and RRM23 construct (residues 80–288) were obtained by PCR from a plasmid containing full-length TIA-1 (kindly provided by Dr. P. Anderson, Harvard Medical School) and cloned into the pETM-11 vector (Invitrogen) as described previously (19). Isotopically 15N- and 13C-labeled proteins were expressed in Escherichia coli BL21(DE3) cells in minimal medium (M9) supplemented either with 15NH4Cl or with 15NH4Cl and [13C]glucose following the protocol described by Aroca et al. (19) for LB medium. His-tagged TIA-1 constructs were purified by nickel affinity chromatography (Ni Sepharose 6 Fast Flow, GE Healthcare). The His tag and tobacco etch virus (TEV) protease cleavage site were left in the constructs because no differences in secondary structure with or without the His tag were observed (19). Samples were concentrated up to 1 mM in 20 mM potassium phosphate buffer and 50 mM KCl (pH 7.0). Protein concentration was determined by spectrophotometry with predicted extinction coefficients. RNA oligonucleotides (5'/H11032-AUUUA-3'/H11032 and 5'/H11032-AUUUUAUUUAUUUAUUUAUUU-3'/H11032) were chemically synthesized (Integrated DNA Technologies).

NMR Spectroscopy—NMR samples of TIA-1 RRM3 were prepared in 90% H2O and 10% D2O solutions of 20 mM potassium phosphate buffer and 50 mM KCl (pH 7.0). Protein concentration was determined by spectrophotometry with predicted extinction coefficients. RNA oligonucleotides (5'-AUUUA-3' and 5'-AUU-AUUUAUUUAUUUAUUU-3') were chemically synthesized (Integrated DNA Technologies). Standard three-dimensional NMR experiments (HNCACB, HNCA, and HNCO) were used to assign backbone resonances of the RRM3 domain. The coverage of the backbone assignment was 98%. The assigned chemical shifts were deposited in the Biological Magnetic Resonance Data Bank with accession number 18829 and were used to generate the three-dimensional protein structure at the CS23D2.0 web server (24). The file generated with NMR chemical shift assignments for the three-dimensional structure was used for data discussion and to build Figs. 1, 4, 6, and 7.

The pH effects on the TIA-1 RRM2 and RRM3 domains were followed by recording 15N heteronuclear single quantum correlation (HSQC) spectra on 700-MHz Bruker AVANCE III and 800-MHz Varian INOVA spectrometers during titrations of a 0.01 mM sample of the 15N-labeled RRM2 domain and a 0.05 mM sample of the 15N-labeled RRM3 domain, respectively, in 20 mM potassium phosphate buffer and 50 mM KCl (pH 3.8 for RRM2 and pH 5.0 for RRM3) with small aliquots of 0.1M NaOH to gradually increase the pH. RNA binding of TIA-1 RRM3 was monitored by acquiring 15N HSQC spectra during titrations of the 5'-AUUUA-3' RNA oligonucleotide into a 25 mM sample of the 15N-labeled RRM3 domain in 20 mM potassium phosphate buffer and 50 mM KCl at both pH 5.5 and 7.5. The pH value of the sample was verified after each titration step. Weighted average values of 1H and 15N chemical shift perturbations (Δδavg) of each resonance were calculated as Δδavg = ((ΔδH)² + (ΔδN/5)²)/2, where ΔδH and ΔδN are chemical shift increments of 1H and 15N, respectively. The threshold value, used to consider
significant chemical shift perturbations from the titration data, was defined as the average of $\Delta \delta_{\text{avg}}$ values plus 2 S.D. ($2\sigma_{\text{avg}}$).

The pH effect on RNA structure was controlled by registering one-dimensional NMR spectra on the 700-MHz Bruker AVANCE III spectrometer at pH 5.5 and 7.5. Samples contained 50 mM 24-mer AU-rich oligonucleotide (5'-AUUUAUUUAUUUAUUU-3') and were resuspended in the buffer described above using either 90% H$_2$O and 10% D$_2$O or 100% D$_2$O as solvent. Comparison of the spectra at different pH values (data not shown) revealed no significant changes in line width or chemical shift perturbations.

**pH Titration Curves**—Titration curves were obtained by plotting the change in chemical shift as a function of pH for all of the detectable backbone amide protons ($^1H$) and nitrogens ($^15N$) of TIA-1 RRM3. Data from 12 of 18 affected residues were fitted to a sigmoidal curve model describing a single protonation event.

**FIGURE 2.** NMR pH titration of the TIA-1 RRM3 domain at pH 5–8. Upper, overlaid HSQC spectra acquired during titration showing the most affected residues. HSQC spectra are colored according to pH as follows: red, pH 5.0; orange, pH 5.5; dark green, pH 6.0; light blue, pH 6.5; purple, pH 7.0; light green, pH 7.5; and dark blue, pH 8.0. Lower, selected regions from overlaid HSQC spectra showing substantial chemical shift perturbations of some residues.

**FIGURE 3.** Representative $pK_a$ curves indicating the pH dependence of the chemical shifts of backbone amides of TIA-1 RRM3. Data were fitted to a sigmoidal curve model and describe a single protonation event.
tonation event with an associated pKₐ value: δ = (K_a δ_H + [H⁺] δ_L)/(K_a + [H⁺]) where δ_H and δ_L are the chemical shift values at high and low pH, respectively. Fits were obtained using Origin 8.0 (OriginLab, Northampton, MA) by nonlinear least squares fitting on the proposed model. The pKₐ value was the average of two independent pH titrations with estimated errors of ±10%. The threshold value, used to identify significant perturbations when data from the titrations were analyzed together, was defined as the averaged chemical shift perturbations (∆δ_avg) for the system plus 2 S.D. (2Sᵢ₋₋). The reported pKₐ values of TIA-1 RRM2 histidines were calculated as described for RRM3. 15N HSQC spectra were assigned according to the work of Kuwasako et al. (17) (Protein Data Bank code 2RNE and Biological Magnetic Resonance Data Bank accession number 11376).

**CD Spectroscopy**—All CD spectra were recorded on a Jasco J-815 spectropolarimeter equipped with a Peltier temperature control system. RRM3, RRM23, and the RNA oligonucleotide samples were prepared in 20 mM potassium phosphate buffer and 50 mM KCl at both pH 5.5 and 7.5. Each spectrum was an average of 20 scans.

The secondary structure analysis of RRM3 was performed by recording far-UV CD spectra (190–250 nm) of 3 μM samples at 25 °C. RNA binding was monitoring in the UV range of 240–330 nm by the addition of increasing amounts of the TIA-1 RRM23 construct to a 3 μM sample of the 24-mer oligonucleotide (5′-AUUUAUUUAUUUAUUUAUUU-3′). A temperature of 10 °C was chosen to optimize the signal change upon protein binding. The integral of the CD signal between 255 and 265 nm was plotted against the ratio [RRM23]/[24-mer AU-rich RNA] and fitted to a 1:1 binding site model as reported previously (25).

**Fluorescence Measurements**—Emission spectra were recorded at 290–500 nm with an excitation wavelength of 282 nm on a PerkinElmer LS-5 fluorometer equipped with a water thermostat cell holder at 25 °C. 10 μM RRM3 samples were prepared in 20 mM potassium phosphate buffer and 50 mM KCl at pH 5.5 and 7.5. The slit wavelength was 5 nm for both excitation and emission, and the path length of the cell was 1 cm. The background signal from the buffer was subtracted.

**TABLE 1**

| Residue | pKₐ | ¹⁵N | ¹H |
|---------|-----|-----|-----|
| Glu-193 | 6.20 ± 0.60 | 6.20 ± 0.60 |
| Val-210 | 6.40 ± 0.60 | 6.40 ± 0.60 |
| Thr-211 | 6.40 ± 0.60 | 6.40 ± 0.60 |
| Ser-250 | 6.70 ± 0.70 | 6.70 ± 0.70 |
| His-253 | 6.90 ± 0.70 | 6.90 ± 0.70 |
| Val-256 | 6.40 ± 0.60 | 6.50 ± 0.70 |
| Thr-262 | 6.40 ± 0.60 | 6.30 ± 0.60 |
| Glu-264 | 6.40 ± 0.60 | 6.80 ± 0.70 |
| Gly-265 | 6.20 ± 0.60 | 6.20 ± 0.60 |
| His-266 | 6.40 ± 0.60 | 6.40 ± 0.60 |
| Val-267 | 6.30 ± 0.60 | 6.30 ± 0.60 |
| Lys-269 | 6.50 ± 0.70 | 6.50 ± 0.70 |

**FIGURE 4.** Distribution of averaged chemical shift perturbations for pH-affected backbone amides of TIA-1 RRM3. A, the ∆δ_avg values (ppm) of signals were calculated as described under “Experimental Procedures” at high (7.5) and low (5.0) pH values. The red asterisk indicates the His-248 resonance, which is broadened beyond the detection limit. B, mapping of the ∆δ_avg on the TIA-1 RRM3 ribbon. Each view is rotated 180° around the vertical axis. Residues with ∆δ_avg > 0.05 ppm are shown in orange. Residues with ∆δ_avg below the threshold value are shown in blue, whereas prolines or non-assigned residues are shown in gray. The three exposed histidines are marked with their side chain traces in yellow. Note that the ∆δ_avg of His-248 was not calculated due to the broadening of this amide signal at pH 7.5 beyond the detection limit, as pointed out by the arrow. C, details of the environment of the three histidines showing the residues located within a distance of 3.5 Å.
RESULTS AND DISCUSSION

**pH Effect on the TIA-1 RRM3 Domain**—pH NMR titration of TIA-1 RRM3 over a pH range of 5–8 showed remarkable chemical shift differences in superimposed $^{15}$N HSQC spectra at high and low pH values (Fig. 2). A set of signals corresponding to 18 residues changed upon increasing pH. Other residues underwent negligible chemical shift changes because they were insensitive to pH effects. A single resonance was observed for each amide over the tested pH range, suggesting that the protonation and deprotonation of side chains are fast on the NMR time scale. Different sigmoidal curves were obtained for perturbed residues with titration data (Fig. 3), but most of them could be fitted (see “Experimental Procedures”) to single $pK_a$ values for both $^1$H and $^{15}$N dimensions. As shown in Table 1, $pK_a$ values were in the range of 6.3–6.9.

The $\Delta \delta_{\text{avg}}$ values at high (pH 7.5) and low (pH 5) pH (see “Experimental Procedures”) give evidence of sensitivity of a particular nucleus to a protonation event. The largest values for backbone amides were observed for His-266 (numbering according to the full-length TIA-1 protein) (Fig. 4A). 18 perturbed amides showed significant perturbations ($\Delta \delta_{\text{avg}} > 0.05$ ppm) distributed mainly along four regions of RRM3. Two sequential stretches at the N terminus involved Asp-192 and Glu-193 on one side and the Val-210–Thr-215 stretch except for Leu-214 on the other side. The C-terminal residue stretch included Ser-250, His-253, Val-256, and Thr-262–Lys-269. Residues that overcame the threshold of 0.05 ppm in $\Delta \delta_{\text{avg}}$ were mapped onto the TIA-1 RRM3 backbone (Fig. 4, B and C). As was expected, the strongest effects were found for the three solvent-exposed histidines (His-248, His-253, and His-266). In fact, the His-248 chemical shift perturbation could not be determined due to signal broadening beyond the detection limit at pH 7.5, which is clear evidence of a pH-dependent change. Other residues surrounding the three histidines were also perturbed, as in the case of Thr-211, which is in close proximity to His-266.

To check that the structure of RRM3 remained unaltered upon pH changes, far-UV CD spectra and fluorescence emission measurements of the single tryptophan of this domain (Trp-272) were obtained (Fig. 5). No substantial changes appear in the CD spectra, indicating that the secondary structure of RRM3 is not affected by pH. In addition, the fluorescence maximum appears at $\sim 330$ nm at both pH values, which corresponds to a partially buried Trp-272 residue. The absence of red-shifted fluorescence ($\sim 350$ nm) shows no substantial conformational changes or protein unfolding.

**pH Effect on RNA Binding of the TIA-1 RRM3 Domain**—To assess the effect of pH on RNA recognition by the TIA-1 RRM3 domain, its interaction with a short 5-mer AU-rich RNA oligonucleotide (5’-AUUUA-3’) was studied. Because the $pK_a$ values for histidine protonation events are $\sim 7$ (Table 1), NMR experiments were carried out at low (5.5) and high (7.5) pH values. Averaged chemical shift perturbations inferred from the titration of $^{15}$N-labeled RRM3 with 5’-AUUUA-3’ revealed not only that RRM3 bound RNA using the canonical platform, which comprises aromatic residues localized mainly at three of four strands from the $\beta$-sheet, but also that it occurred at both pH values. Due to the small calculated $\Delta \delta_{\text{avg}}$ suggesting a moderate affinity of RRM3 for the tested oligonucleotide, the dissociation constant could not be obtained. However, striking differences are obvious from data comparison at the tested pH values (Fig. 6). Fig. 6 (A and B, upper) shows details of the superimposed $^{15}$N HSQC spectra during titrations for the same residue revealing chemical shift perturbations that suggest stronger binding at pH 5.5 than at pH 7.5. These differences were observed for all perturbed residues. Fig. 6 (middle and lower) also shows the mapping of significantly shifted RRM3 residues ($\Delta \delta_{\text{avg}} \geq 0.025$ ppm) upon RNA binding over the backbone and surface representations, respectively.

Upon pH 5.5 titration (Fig. 6A), residues undergoing the largest shifts were located at Tyr-206–Gly-208 and Lys-238–Tyr-240, along with Val-243, as part of the central $\beta$-strands ($\beta_1$ and $\beta_2$). In addition, the Lys-269–Lys-274 stretch from $\beta_3$ was also involved in RNA recognition. Note that the resonance of Phe-242 overlapped with that corresponding to Gln-221, so the $\Delta \delta_{\text{avg}}$ values could not be accurately calculated, although significant line broadening was observed. Moreover, the easily assigned N$^\alpha$ of the indole ring from Trp-272 experienced large

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**FIGURE 5.** Negligible effects of pH on the TIA-1 RRM3 structure. A, far-UV (190–250 nm) CD spectra of the RRM3 domain. The solid line indicates the spectrum registered at pH 5.5, whereas the dotted line indicates the spectrum at pH 7.5. deg, degrees. B, fluorescence emission of the RRM3 single tryptophan (Trp-272). A.U., arbitrary units.
chemical shift perturbations, even though its amide signal could not be identified. The titration at pH 7.5 (Fig. 6B) showed almost the same perturbed residues of the RRM3 domain but with significant differences: Tyr-206 and Phe-242 amide resonances were neither shifted nor broadened. Indeed, the perturbation level of highly affected residues at pH 5.5 (avg δavg > 0.050) was lower at pH 7.5 (0.025 ≤ δavg ≤ 0.050) (Fig. 6B), with the exception of Tyr-271, as a result of weaker binding affinity between TIA-1 and AU-rich RNA at the latter pH value.

An in-depth analysis of the TIA-1 RRM3/RNA binding event (Fig. 6) revealed that aromatic residues from the β1- and β3-strands (Tyr-206, Tyr-240, and Phe-242) constituted the usual RNA-docking platform in most RRMs. In addition, Tyr-271 and Trp-272 (at least its indole amide) from the end of β4, along with the motif formed by Gly-273 and Lys-274 at the C-terminal region, were also involved in RNA interaction. Indeed, the C-terminal stretch Gly-273–Lys-274, which is well conserved among TIA-1 analogs, has been previously described to be involved in RNA recognition by the Pub1p C-terminal domain (Fig. 1C) (20). Moreover, it also takes part in forming hydrogen bonds with RNA in the RRM-like polypyrimidine tract-binding protein family, even though the C-terminal stretch differs from that mentioned above (26, 27).

Trp-272 is part of a pocket in which His-248 from α3 and Gln-197 from α1 are in close proximity (Fig. 4C). Hence, the His-248/Trp-272 interaction may pack the extra N-terminal α1-helix on the RRM3 domain (19, 20). Within this interaction network, protonation/deprotonation events of His-248 could

FIGURE 6. Binding of TIA-1 RRM3 to the 5'-AUUUA-3' RNA at pH 5.5 (A) and pH 7.5 (B). Upper, details of the superimposition of 15N HSQC spectra recorded during titration. The spectrum for free protein is shown in cyan, and the 1:4:1 and 3:1 RNA/protein ratios are represented in orange and red, respectively. Middle, ribbon representations of TIA-1 RRM3 colored according to the chemical shift changes undergone by their amide resonances upon RNA binding. Residues with 0.025 ≤ δavg ≤ 0.050 ppm are shown in orange, whereas those with δavg > 0.050 are shown in red. Residues with δavg < 0.025 ppm are marked in blue; prolines or non-assigned residues are shown in gray. Lower, TIA-1 RRM3 surface with the same orientation and color code as described for the ribbon representation. The asterisk indicates the resonance of Trp-272 corresponds to the N° nucleus in the indole ring.
hinder the anchorage of Trp-272 by disrupting cation–π contacts, thus affecting the RNA recognition platform of TIA-1 RRM3, as suggested by the intrinsic tryptophan residue fluorescence (Fig. 5). In fact, the chemical shift perturbation experienced by the Trp-272 N\(^\text{\text{145}}\) nucleus of the indole ring is substantially larger at acidic pH (Fig. 6). As a consequence of protonation/deprotonation of TIA-1 RRM3 histidine residues, electrostatic potential surfaces change at both pH values (Fig. 7), especially those protein regions surrounding histidines. Electrostatic potential changes around the His-248 environment can explain differences in the RNA binding affinity of TIA-1 RRM3 due to pH variations. Thus, the disruption of the RNA recognition platform can explain the lower binding of TIA-1 RRM3 at pH 7.5.

**pH Effect on TIA-1 Binding to RNA**—The assays performed by NMR (Fig. 6) with the RRM3 domain to evaluate its interaction with RNA showed chemical shift perturbations that were too small to be used for quantitative assessment of binding affinities. To overcome this issue, an additional binding experiment was performed to quantify the pH-dependent effect and to evaluate whether the overall TIA-1 binding to RNA is significantly affected. A construct that comprises the RRM2 and RRM3 domains (RRM23) was used, which is actually the responsible unit in TIA-1/RNA recognition (15). As the RRM2 domain contains two histidines (His-94 and His-96) (17), the \(pK_a\) values of their –NH groups were first checked by NMR pH titrations (His-94 \(pK_a \approx 5.3\) and His-96 \(pK_a \approx 5.4\)) (Fig. 8). This is in agreement with the \(pK_a\) prediction of the imidazole group for the RNA-binding His-96 residue in the context of RRM23 (His-96 \(pK_a \approx 5.1\)) using the PROPKA3 server (28). Similar \(pK_a\) values \((<5.3)\) were measured for –NH resonances of those RRM2 residues surrounding both His-94 and His-96. These results indicate that only the three histidines from RRM3 in RRM23 experience the protonation/deprotonation events at the two pH values (5.5 and 7.5) used in the RNA binding studies. We next assessed the affinity of TIA-1 RRM23 for the 24-mer AU-rich RNA by CD (Fig. 9). The protein bound to RNA more strongly at pH 5.5 than at pH 7.5 (\(K_D \approx 4.4\) and 58.5 nM, respectively). This demonstrates that RRM23 binding to RNA is pH-dependent and that this effect is caused mainly by the RRM3 domain because the deprotonation of RRM2 in the context of RRM23 was negligible at both pH values used in the RNA binding studies.

As previous studies have described, the three domains of the TIA-1 protein contribute to RNA binding, with the RRM2 and RRM3 modules as the main recognition platforms (16), as in other RNA-binding proteins, such as the central domain units of KSRP (KH-type splicing regulatory protein) (29) and the N terminus of HuR (human antigen R) RRM modules (25). Whereas TIA-1 RRM2 dominates the interaction, RRM3 enhances it, playing an auxiliary role. The pH influence on exposed histidine residues of RRM3 (at positions 248, 253, and 266) in protonation/deprotonation events around pH 7 is clear.
Notably, the histidine residues are highly conserved among TIA-1 homologs, and in the particular case of His-248, it has remained unchanged even in species such as *Arabidopsis thaliana* and *S. cerevisiae* during evolution (Fig. 1C). These observations are well correlated with RNA binding, which decreases at pH 7.5, leading to shortening of the RNA platform by His-248 deprotonation. It is tempting to speculate that the canonical folding of RRM3 modulates the RNA binding capability of TIA-1 according to small pH changes (around the physiological value). Even though the extra RRM3 α-helix (α4) fails in binding directly to RNA oligonucleotides, its relative orientation regarding α3 and β4 could facilitate stable RNA binding (30). A similar effect has been recently reported for the transcription factor EGR1 (early growth response protein 1) in its binding to DNA (31). His-382 is well conserved and modulates protein/DNA binding, acting as a pH-dependent molecular switch. Another well studied example of molecular switches with regard to environmental pH is the hemagglutinin fusion peptide of the influenza virus (32, 33), which is responsible for viral penetration into host cells by membrane fusion. Altogether, this pH-dependent mechanism may interfere with the overall RNA binding of TIA-1 given the auxiliary role of the RRM3 domain and the different binding affinities of RRM23 for RNA depending on the pH.

Given that the pH between the cytoplasm and nucleus ranges from ~7.2 to 7.7 (34), the evaluation of the pH effect on multidomain DNA/RNA-binding proteins that act in both compartments is highly relevant. This is the case for the KH domain-containing protein vigilin, whose biological functions are poorly understood (35–37), and for the TIA-1 protein studied here. Overall, our findings suggest that these little changes around the physiological pH in such cell compartments could control the RRM3 domain packing and, eventually, the RNA recognition by TIA-1.

**REFERENCES**

1. Kedersha, N., Cho, M. R., Li, W., Yacono, P. W., Chen, S., Gilks, N., Golan, D. E., and Anderson, P. (2000) Dynamic shuttling of TIA-1 accompanies the recruitment of mRNA to mammalian stress granules. *J. Cell Biol.* **151**, 1257–1268
2. Zhang, T., Delestienne, N., Huez, G., Kruys, V., and Gueydan, C. (2005) Identification of the sequence determinants mediating the nucleo-cytoplasmic shuttling of TIAR and TIA-1 RNA-binding proteins. *J. Cell Sci.* **118**, 5453–5463
3. Förch, P., Puig, O., Kedersha, N., Martínez, C., Granneman, S., Séraphin, B., Anderson, P., and Valcárcel, J. (2000) The apoptosis-promoting factor TIA-1 is a regulator of alternative pre-mRNA splicing. *Cell* **6**, 1089–1098
4. Förch, P., Puig, O., Martínez, C., Séraphin, B., and Valcárcel, J. (2002) The splicing regulator TIA-1 interacts with U1-C to promote U1 snRNP recruitment to 5′ splice sites. *EMBO J.* **21**, 6882–6892.
5. Izquierdo, J. M., Majós, N., Bonnal, S., Martínez, C., Castelo, R., Gúigó, R., Bilbao, D., and Valcárcel, J. (2005) Regulation of Fas alternative splicing by antagonistic effects of TIA-1 and PTB on exon definition. *Cell Mol. Biol.* **19**, 475–484
6. Suswam, E. A., Li, Y. Y., Mahtani, H., and King, P. H. (2005) Novel DNA-binding properties of the RNA-binding protein TIA1. *Nucleic Acids Res.* **33**, 4507–4518
7. Piecyk, M., Wax, S., Beck, A. R. P., Kedersha, N., Gupta, M., Maritim, B., Chen, S., Gueydan, C., Kruys, V., Streuli, M., and Anderson, P. (2000) TIA-1 is a translational silencer that selectively regulates the expression of pro-inflammatory cytokine and their regulation. *Mol. Cell. Biol.* **5453–5463
8. López de Silanes, I., Galbán, S., Martindale, J. L., Yang, X., Mazan-Mamczarz, K., Indig, F. E., Falco, G., Zhan, M., and Gorospe, M. (2005) Identification and functional outcome of mRNAs associated with RNA-binding protein TIA-1. *Mol. Cell. Biol.* **5**, 9520–9531
9. Kawai, T., Lal, A., Yang, X., Galbán, S., Mazan-Mamczarz, K., and Gorospe, M. (2006) Translational control of cytochrome c by RNA-binding proteins TIA-1 and HuR. *Mol. Cell. Biol.* **36**, 3295–3307
10. Damgaard, C. K., and Lykke-Andersen, J. (2011) Translational coregulation of 5′ TOP mRNAs by TIA-1 and TIAR. *Gene Dev.* **25**, 2057–2068
11. Avni, D., Shama, S., Lorenzi, F., and Meyuhas, O. (1994) Vertebrate mRNAs with a 5′-terminal pyrimidine tract are candidates for translational repression in quiescent cells: characterization of the translational cis-regulatory element. *Mol. Cell. Biol.* **14**, 3822–3833
12. Hamilton, T. L., Stoneley, M., Spriggs, K.A., and Bushell, M. (2006) TOPs and their regulation. *Biochem. Soc. Trans.* **34**, 12–16
13. Kedersha, N. L., Gupta, M., Li, W., Miller, I., and Anderson, P. (1999) RNA-binding proteins TIA-1 and TIAR link the phosphorylation of
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eIF-2α to the assembly of mammalian stress granules. J. Cell Biol. 147, 1431–1442
14. Anderson, P., and Kedersha, N. (2002) Stressful initiations. J. Cell Sci. 115, 3227–3234
15. Dember, I. M., Kim, N. D., Liu, K. Q., and Anderson, P. (1996) Individual RNA recognition motifs of TIA-1 and TIAR have different RNA binding specificities. J. Biol. Chem. 271, 2783–2788
16. Bauer, W. J., Heath, J., Jenkins, J. L., and Kielkopf, C. L. (2012) Three RNA recognition motifs participate in RNA recognition and structural organization by the pro-apoptotic factor TIA-1. J. Mol. Biol. 415, 727–740
17. Kuwasako, K., Takahashi, M., Tochio, N., Abe, C., Tsuda, K., Inoue, M., Terada, T., Shirouzu, M., Kobayashi, N., Kigawa, T., Taguchi, S., Tanaka, A., Hayashizaki, Y., Güntert, P., Muto, Y., and Yokoyama, S. (2008) Solution structure of the second RNA recognition motif (RRM) domain of murine T cell intracellular antigen-1 (TIA-1) and its RNA recognition mode. Biochemistry 47, 6457–6460
18. Kumar, A. O., Swenson, M. C., Benning, M. M., and Kielkopf, C. L. (2008) Structure of the central RNA recognition motif of human TIA-1 at 1.9 Å resolution. Biochem. Biophys. Res. Commun. 367, 813–819
19. Aroca, A., Díaz-Quintana, A., and Díaz-Moreno, I. (2011) A structural insight into the C-terminal RNA recognition motifs of T-cell intracellular antigen-1 protein. FEBS Lett. 585, 2958–2964
20. Santivari, C. M., Mirasou, Y., Rico-Lastres, P., Martinez-Lumberas, S., and Pérez-Cañadillas, J. M. (2011) Pub1p C-terminal RRM domain interacts with Tif631p through a conserved region neighbouring the Pab1p binding site. PLoS ONE 6, e24481
21. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J. Biomol. NMR 6, 277–293
22. Vranken, W. F., Boucher, W., Stevens, T. J., Fogh, R. H., Pajon, A., Llinas, M., Ulrich, E. L., Markley, J. L., Ionides, J., and Laue, E. D. (2005) The CCPN data model for NMR spectroscopy: development of a software pipeline. Proteins 59, 687–696
23. Goodard, T. D., and Kneller, D. G. (2006) SPARKY 3. University of California, San Francisco
24. Wishart, D. S., Arndt, D., Berjanskii, M., Tang, P., Zhou, J., and Lin, G. (2008) CS23D: a web server for rapid protein structure generation using NMR chemical shifts and sequence data. Nucleic Acids Res. 36, W496–W502
25. Scheiba, R. M., Aroca, A., and Díaz-Moreno, I. (2012) HuR thermal stability is dependent on domain binding upon phosphorylation. Eur. Biophys. J. 41, 597–605
26. Oberstrass, F. C., Auweter, S. D., Erat, M., Hargous, Y., Henning, A., Wenter, P., Reymond, L., Amir-Ahmady, B., Pitsch, S., Black, D. L., and Allain, F. H.-T. (2005) Structure of PTB bound to RNA: specific binding and implications for splicing regulation. Science 309, 2054–2057
27. Clery, A., Blatter, M., and Allain, F. H.-T. (2008) RNA recognition motifs: boring? Not quite. Curr. Opin. Struct. Biol. 18, 290–298
28. Olsson, M. H. M., Sanderberg, C. R., Rostkowski, M., and Jensen, J. H. (2011) PROPKA3: consistent treatment of internal and surface residues in empirical pKa predictions. J. Chem. Theory Comput. 7, 525–537
29. Díaz-Moreno, I., Hollingsworth, D., Kelly, G., Martin, S., García-Mayoral, M., Briata, P., Gherzi, R., and Ramos, A. (2010) Orientation of the central domains of KSRR and its implications for the interaction with the RNA targets. Nucleic Acids Res. 38, 5193–5205
30. Nettter, C., Weber, G., Benecke, H., and Wahl, M. C. (2009) Functional stabilization of an RNA recognition motif by a noncanonical N-terminal expansion. RNA 15, 1305–1313
31. Mikles, D. C., Bhat, V., Schuchardt, B. J., Deegan, B. I., Seldeen, K. L., McDonald, C. B., and Farooq, A. (2013) pH modulates the binding of early growth response protein 1 transcription factor to DNA. FEBS J. 280, 3669–3684
32. Doms, R. W., Helenius, A., and White, J. (1985) Membrane fusion activity of the influenza virus hemagglutinin. The low pH-induced conformational change. J. Biol. Chem. 260, 2973–2981
33. Loréau, J. L., Louis, J. M., Schwieters, C. D., and Bax, A. (2012) pH-triggered, activated-state conformations of the influenza hemagglutinin fusion peptide revealed by NMR. Proc. Natl. Acad. Sci. U.S.A. 109, 19994–19999
34. Seksek, O., and Bolard, J. (1996) Nuclear pH gradient in mammalian cells revealed by laser microspectrofluorimetry. J. Cell Sci. 109, 257–262
35. Kruse, C., Grüneweller, A., Notbohm, H., Kügler, S., Parchke, W. G., and Müller, P. K. (1996) Evidence for a novel cytoplasmic tRNA-protein complex containing the KH-motif protein vigilin. Biochem. J. 320, 247–252
36. Kruse, C., Willkomm, D. K., Grüneweller, A., Vollbrandt, T., Sommer, S., Busch, S., Pfeifer, T., Brinkmann, J., Hartmann, R. K., and Müller, P. K. (2000) Export and transport of tRNA are coupled to a multi-protein complex. Biochem. J. 346, 107–115
37. Vollbrandt, T., Willkomm, D., Stossberg, H., and Kruse, C. (2004) Vigilin is co-localized with 80S ribosomes and binds to the ribosomal complex through its C-terminal domain. Int. J. Biochem. Cell Biol. 36, 1306–1318
38. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612
39. Koradi, R., Billeter, M., and Wüthrich K. (1996) MOLMOL: a program for display and analysis of macromolecular structures. J. Mol. Graph. 14, 51–55