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ORIGINAL ARTICLE

Interactions between RNA-binding proteins and P32 homologues in trypanosomes and human cells

Juan Manuel Polledo[1] · Gabriela Cervini[1] · María Albertina Romaniuk[1] · Alejandro Cassola[1]

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Abstract RNA-binding proteins (RBPs) are involved in many aspects of mRNA metabolism such as splicing, nuclear export, translation, silencing, and decay. To cope with these tasks, these proteins use specialized domains such as the RNA recognition motif (RRM), the most abundant and widely spread RNA-binding domain. Although this domain was first described as a dedicated RNA-binding moiety, current evidence indicates these motifs can also engage in direct protein–protein interactions. Here, we discuss recent evidence describing the interaction between the RRM of the trypanosomatid RBP UBP1 and P22, the homolog of the human multifunctional protein P32/C1QBP. Human P32 was also identified while performing a similar interaction screening using both RRMs of TDP-43, an RBP involved in splicing regulation and Amyotrophic Lateral Sclerosis. Furthermore, we show that this interaction is mediated by RRM1. The relevance of this interaction is discussed in the context of recent TDP-43 interactomic approaches that identified P32, and the numerous evidences supporting interactions between P32 and RBPs. Finally, we discuss the vast universe of interactions involving P32, supporting its role as a molecular chaperone regulating the function of its ligands.

Keywords RNA-binding proteins · RNA Recognition Motif · P32 · C1QBP · HABP1 · Trypanosoma · Trypanosomes · Protein–protein interactions

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✉ Alejandro Cassola
alecassola@gmail.com

[1] Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús, UNSAM-CONICET, Buenos Aires, Argentina

The RNA recognition motif (RRM)

Mature messenger RNA (mRNA) molecules exist as part of ribonucleoprotein (RNP) complexes inside the cell. RNA-binding proteins (RBPs) associate cotranscriptionally with transcripts as these become elongated (Lee and Lykke-Andersen 2013). Some of these RBPs can bind early in the life of the mRNA molecule, and most probably remain associated until the degradation of the transcript, while others recognize the mRNA transiently during specific processes like mRNA splicing, processing, transport, or specific localization (Dreyfuss et al. 2002). For this to happen, RBPs interact with the RNA through dedicated functional domains, such as the RNA Recognition Motif (RRM), CCCH zinc finger domains of RGG boxes, to name a few (Clery and Allain 2012). Particularly, the RRM is the most abundant in vertebrates as well as the most studied biochemically and structurally (Afroz et al. 2015). The information obtained from genome sequencing studies showed that RRM-containing proteins are present in all forms of life (Maris et al. 2005). RRMs are typically comprised by about 90 amino acids in length, consisting of four antiparallel β-strands (eventually they can have one or two short additional strands) that form a β-sheet, packed against two α-helices, adopting the typical β1α1β2β3α2β4 conformation. The β3 and β1 strands of the RRM contain the RNP1 and RNP2 signature sequences, respectively. Arising from these motifs there are three conserved aromatic residues on the β-sheet, generally involved in RNA-binding (Clery et al. 2008). In canonical RRMs, two of the three conserved aromatic side-chains located in the RNP1 and RNP2 sequences accommodate two nucleotides, while the third aromatic ring located in β3 is often found inserted between the two

sugar rings of the dinucleotide (Clery and Allain 2012). Although this disposition allows the interaction with two consecutive single-stranded RNA nucleotides, increased RNA sequence-specificity by RRMs can be achieved by side-chains present on the β-sheet. In some RRMs, increased binding specificity can be achieved using the loops connecting the β-strands or the β+ strands to the α-helices of the RRMs (Clery and Allain 2012). Also, two or more RRMs can be combined in the same molecule to recognize longer stretches of RNA nucleotides, increasing sequence affinity and specificity, as is the case of Poly (A) binding protein (Deo et al. 1999). RBPs synergic interactions aiding in RNA-binding can also occur between RRMs from different molecules, as is the case of the RNA stabilizer HuR, which can form oligomers allowing cooperative association to RNA (Fialcowitz-White et al. 2007). Cooperative binding also occurs in U1A homodimers, where two separate RRMs interact through their C-terminal helices only after binding to RNA (Varani et al. 2000). These examples illustrate the cooperative association of different RRMs with RNA molecules to modulate RNA-binding affinity, but also the capacity of RRMs to engage into direct protein–protein interactions.

Protein–protein interactions involving the RRM

In recent years, it has become clear that RRMs not only interact with nucleic acids, but also participate of protein–protein interactions (Clery and Allain 2012). Many examples of RRM-protein and RRM–RRM interactions have shown to involve different parts of the RRM. In the case of the Polypyrimidine Binding Protein (PTB), the α helixes of RRM3 and RRM4 provide dimerization of the protein (Vitali et al. 2006), while in Y14 it is the β-sheet that interacts with Mago (Fribourg et al. 2003). Members of the U2AF Homology Motifs (UHM) family depict extreme examples of RRM-protein interactions. UHMs share structural and sequence similarities with RRMs, but do not comply with canonical aromatic residues in RNP2. This results in a surface interface available for interaction with UHM-ligand motif found in some splicing-associated RBPs, which is unable to interact with RNA (Kielkopf et al. 2004). Other RRMs make use of protein interactions while free of RNA, as is the case of mammalian polyA binding protein PABPC1 RRM1 + RRM2 or RRM3 + RRM4 with importin alpha (Kumar et al. 2011). In the yeast Retention and Splicing complex, 18 residues in Bud13p interact with the helical surface of the Snu17p RRM domain, suggesting that this interaction can take place at the same time the β-sheet interacts with an RNA molecule (Tripsianes et al. 2014). These evidences show that, besides being dedicated RNA-binding structures, RRMs can also engage in diverse protein–protein interactions, which might have a direct impact in RNA-binding and its regulation.

Interactions between RRMs and P32 homologues: from trypanosomes to man

In our laboratory, we have been studying mechanisms regulating gene expression in trypanosomes. These are pathogenic protozoan microorganisms such as *Trypanosoma cruzi*, the etiological agent of Chagas Disease in the Americas (Rassi et al. 2010), and *T. brucei*, which causes Sleeping Sickness in Sub-Saharan Africa (Brun et al. 2010). Recombination events in *T. brucei* have a central role in the regulation of Variant Surface Glycoproteins expression (Horn and McCulloch 2010), while other recombination events are likely neutral for the evolution of trypanosomes (Beauparlant and Drouin 2014). Protein-coding genes in these parasites have the peculiarity of being organized into large directional gene clusters lacking canonical RNA polymerase II promoters (Daniels et al. 2010; El-Sayed et al. 2005). Coordinated trans-splicing and polyadenylation events process immature polycistronic mRNAs into mature monocistronic mRNAs (Michaeli 2011). Given this independence from transcription initiation events to regulate the final concentration of proteins in trypanosomes, current models describe gene expression regulation based almost exclusively on posttranscriptional events (Clayton 2013). Functionally related mRNAs seem to be controlled in a coordinated fashion as posttranscriptional regulons due to the presence of structural motifs enriched in 3′ untranslated regions (UTR) (Ouellette and Papadopoulou 2009). Different RBPs associate to these motifs forming a ribonucleoprotein (RNP) complex. RNA-bound RBPs can recruit other factors to the RNP complex, dictating the fate of the mRNA inside the cell (Kolev et al. 2014).

One of the most characterized RBPs from *T. cruzi* is TcUBP1, a protein with preference for U and G-rich structural RNA motifs (D'Orso and Frasch 2001; Noe et al. 2008). TcUBP1 has a single RRM with the characteristic βαββαβ fold, and benefits from an additional hairpin (β4–β5) in the β-sheet to display an enlarged RNA-binding surface (Volpon et al. 2005). This RRM is flanked by low complexity Q-rich sequences at the N and C-terminus, which might be involved in protein–protein interactions (D'Orso and Frasch 2001). TcUBP1 uses its RRM to interact with structural motifs found in the 3′ UTR of functionally related transcripts in the cytoplasm (Noe et al. 2008). However, under conditions of nutrient deprivation TcUBP1 is recruited through the RRM to starvation-induced mRNA granules together with many other RBPs (Cassola 2011; Cassola et al. 2007). These granules contain intact mRNA, which is protected from degradation in an untranslated

state (Cassola et al. 2007), and which can reenter translation if growing conditions are resumed (De Gaudenzi et al. 2011). Additionally, TcUBP1 RRM also functions as a structural nuclear localization signal (NLS), allowing this RBP to completely accumulate into the nucleus of arsenite-treated parasites (Cassola and Frasch 2009). This accumulation is reversible, showing that this RBP shuttles between the nucleus and the cytoplasm, thus suggesting that TcUBP1-RNP complexes biogenesis probably occurs in the nucleus (Cassola et al. 2010). Given the importance of the RRM for the function of this protein, we aimed to identify other protein factors that could be interacting with it, and which could be affecting its association with mRNA. For this, we used a recombinant TcUBP1-RRM fused to GST to perform affinity chromatography purifications using RNA-treated extracts, in order to avoid RNA-mediated interactions with proteins (Cassola et al. 2015). As a result, we obtained a very defined protein band that corresponded to TcP22 protein (Fig. 1a), which was completely absent from the GST control chromatography (Cassola et al. 2015). Additional characterizations of this interaction showed that both endogenous proteins could be immunoprecipitated together, and were found colocalizing in some foci in the cell (Cassola et al. 2015). In vivo and in vitro experiments showed that TcP22 could be displacing TcUBP1 from mRNA through a direct interaction with the RRM β-sheet, the same surface used by this RBP to interact with RNA (Cassola et al. 2015). Furthermore, we could also detect direct protein–protein interactions by GST-pull down assays between recombinant TcP22 (rTcP22) and other *T. cruzi* RRM-containing RBPs such as TcUBP2, TcRBP4, TcRBP5, and TcRBP6 (Fig. 1b), all belonging to the same protein family as TcUBP1. However, we could only detect a very weak interaction with TcRBP3, and no interaction at all with TcCBP20, the nuclear cap-binding protein (Fig. 1b), suggesting that rTcP22 can interact with different RRMs, but not with all of them. In *T. brucei*, the P22 ortholog was shown to interact with RBP16 through a cold-shock domain (Hayman et al. 2001), and with the RRM-containing protein TbRGG2 (Sprehe et al. 2010).

The human homolog of trypanosomatid P22 proteins is P32 (Hayman et al. 2001). Evidence for this is supported by sequence identity analysis, showing that all members of this family possess a Mitochondrial acidic matrix protein (MAM33) domain. Quaternary protein structure showed that every P32 homolog studied to date form homotrimers, resembling a doughnut shape (Jiang et al. 1999; Pu et al. 2011; Sprehe et al. 2010), with an unusual asymmetric charge exposed on the surface. This protein has been proposed as a multifunctional and multicompartmental protein since it has been also identified as Complement component 1 Q subcomponent-binding protein (C1QBP) (Ghebrehiwet et al. 1994), and Hyaluronan- binding protein 1 (HABP1) (Deb and Datta 1996). It is believed to affect different cellular functions through direct protein–protein interactions (Ghebrehiwet et al. 2001) (see below). In recent years, there have been numerous high-throughput approaches to determine the interaction map of human P32 in order to better understand the function of this multifunctional protein. Besides interactions involving P32 in ribosome biogenesis (Yoshikawa et al. 2011) and chemotaxis and metastasis (Zhang et al. 2013), these screenings identified numerous RBPs and mRNA metabolism-related proteins (Table 1). These potential interactions, added to the numerous confirmed and putative associations between P32 homologs and RBPs, many of which contain one or more RRMs, clearly show that this protein is intimately involved with the function of RBPs. It is of course possible that P32 could be interacting with just a few of these RBPs, and that the rest could be just dragged as a complex, or through RNA-mediated associations. It has also been proposed that P32 can bind RNA (Yagi et al. 2012), so it is possible that many of these interactions could be mediated by RNA molecules instead of being direct protein–protein interactions. However, P32 was originally isolated as a protein that binds and inhibits the essential cellular splicing factor ASF/SF2 through a direct protein–protein interaction (Krainer et al. 1990; Petersen-Mahrt et al. 1999). This interaction probably occurs through one or both of ASF/SF2 RRMs (Petersen-Mahrt et al. 1999), although the specific protein moieties involved in this interaction have not been described in detail. Intriguingly, P32 showed up in two independent high-throughput screenings for interactors of TAR DNA-binding protein 43, better known as TDP-43 (Dammer et al. 2012; Freibaum et al. 2010). TDP-43 is a splicing-associated factor containing two RRMs. TDP-43 RRM1 recognizes UG-rich RNA regions with high affinity, but also can recognize single chain TG-rich DNA sequences (Ayala et al. 2005). RRM2 is also able to interact with both nucleic acids, and could have functions involved in chromatin organization (Ayala et al. 2008). These domains are contiguous, followed by a Gly-rich domain to the carboxyl terminus (Da Cruz and Cleveland 2011). This is a nuclear RBP, and like TcUBP1, shuttles between the nucleus and the cytoplasm (Ayala et al. 2008). In the cytoplasm, it is able to form protein aggregates with RNA, which have recently been associated with the development of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) (Da Cruz and Cleveland 2011). Currently, it is not clear whether the occurrence of the disease is caused by the presence of aggregates per se, or by the lack of function of the protein when sequestered in the cytoplasmic aggregates (Xu 2012).

To test whether TDP-43 RRMs could interact directly with proteins, we performed a similar biochemical

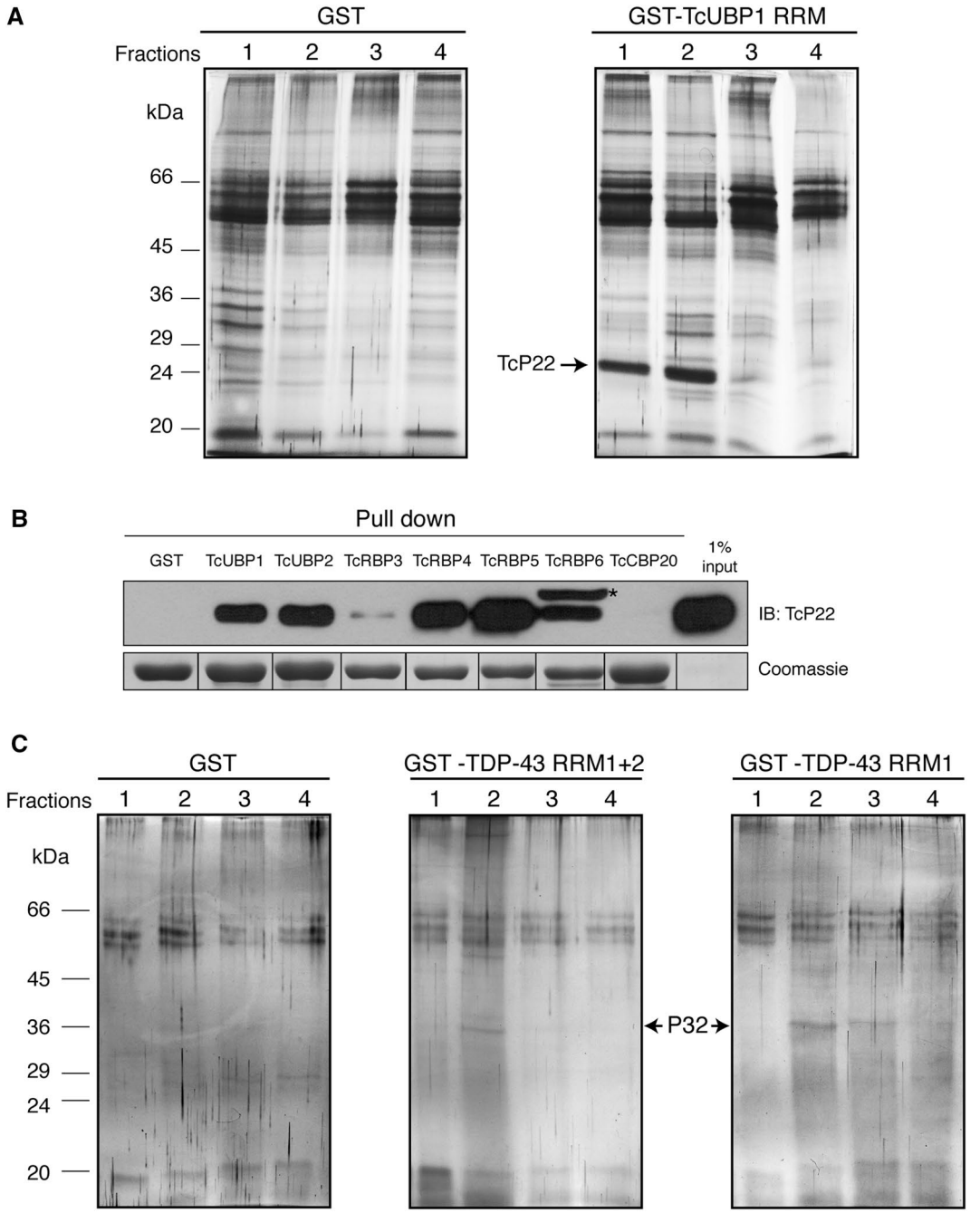

Fig. 1 RBPs interactions with trypanosomatid P22 and human P32. **a** Silver-stained SDS-PAGE showing all four eluted fractions from control (GST) and TcUBP1 RRM from affinity chromatography columns. All the procedure was as described by Cassola and coworkers (Cassola et al. 2015). The position of TcP22 is shown on the gel slice submitted to mass spectrometry. **b** GST-pull down using different trypanosomatid RBPs GST fusion proteins and recombinant TcP22. TcP22 was detected by immunoblot (ib) using a specific antibody. Coomassie staining of the same samples determined GST fusions input loading. **c** Silver-stained SDS-PAGE showing all four eluted fractions from control (GST) and TDP-43 RRM1+2 (amino acids 101–265) and RRM1 (amino acids 101–191) from affinity chromatography columns. Both proteins were expressed from the pGEX-2T vector in which coding sequences were cloned into the BamHI site. All the procedure was as described by Cassola and coworkers (Cassola et al. 2015). The position of P32 is shown on the gel slice submitted to mass spectrometry

approach as the one we did for TcUBP1. We screened for proteins interacting with recombinant TDP-43 RRMs fused to GST by affinity chromatography using a HEK293 RNA-depleted protein extract. As a result, we found a single band that was present in the eluate of TDP-43 RRM1 + 2 samples, and absent in the control GST eluate

Table 1 Interacting partners of P32 homologues from different species

Protein description	Domain type	Species	Reference
TAR DNA-binding protein 43 (TAR-DBP)	RRM (2)	*Homo sapiens*	(Dammer et al. 2012; Freibaum et al. 2010)
Serine/arginine-rich splicing factor 1 [ASF/SF2] (SRSF1)	RRM (2)	*Homo sapiens*	(Krainer et al. 1991; Petersen-Mahrt et al. 1999; Yoshikawa et al. 2011)
Serine/arginine-rich splicing factor 9 (SRSF9)	RRM (2)	*Homo sapiens*	(Petersen-Mahrt et al. 1999)
Isoform 2 of Splicing factor, arginine/serine-rich 15 (SFR15)	RRM	*Homo sapiens*	(Yoshikawa et al. 2011; Zhang et al. 2013)
Splicing factor U2AF 35 kDa subunit (U2AF1)	RRM and Zinc Finger C3H1 (2)	*Homo sapiens*	(Zhang et al. 2013)
Isoform 2 of Splicing factor U2AF 65 kDa subunit (U2AF2)	RRM (3)	*Homo sapiens*	(Zhang et al. 2013)
Splicing factor U2AF 26 kDa subunit (U2AF1L4)	RRM and Zinc Finger C3H1 (2)	*Homo sapiens*	(Heyd et al. 2008)
U1 small nuclear ribonucleoprotein 70 kDa homolog (SNP1)	RRM	*Saccharomyces cerevisiae*	(Ho et al. 2002)
U2 small nuclear ribonucleoprotein auxiliary factor 35 kDa subunit-related protein 2 (ZRSR2)	RRM	*Homo sapiens*	(Huttlin et al. 2015)
Splicing regulatory glutamine/lysine-rich protein 1 (SREK1)	RRM	*Homo sapiens*	(Li et al. 2003)
Heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1)	RRM (2) and RRG-box	*Homo sapiens*	(Yoshikawa et al. 2011)
Heterogeneous nuclear ribonucleoprotein D (HNRPD)	RRM (2)	*Homo sapiens*	(Lehner and Sanderson 2004)
Isoform 3 of Heterogeneous nuclear ribonucleoproteins C1/C2 (HNRPC)	RRM	*Homo sapiens*	(Zhang et al. 2013)
RALY (hnRNP C-related protein)	RRM	*Homo sapiens*	(Tenzer et al. 2013)
THO complex subunit 4 (THOC4)	RRM	*Homo sapiens*	(Zhang et al. 2013)
RNA annealing protein (YRA1)	RRM	*Saccharomyces cerevisiae*	(Krogan et al. 2006)
Isoform 2 of Polyadenylate-binding protein 2 (PABP2)	RRM	*Homo sapiens*	(Zhang et al. 2013)
Poly(U)-binding-splicing factor (PUF60)	RRM (3)	*Homo sapiens*	(Yoshikawa et al. 2011)
RNA-binding protein 23 (RBM23)	RRM (2)	*Homo sapiens*	(Yoshikawa et al. 2011; Zhang et al. 2013)
RNA-binding motif protein 26 (RBM26)	RRM	*Homo sapiens*	(Yoshikawa et al. 2011; Zhang et al. 2013)
RNA-binding protein 27 (RBM27)	RRM	*Homo sapiens*	(Yoshikawa et al. 2011; Zhang et al. 2013)
RNA-binding protein 39 (RBM39)	RRM (3)	*Homo sapiens*	(Yoshikawa et al. 2011)
RNA-binding region containing 2, isoform CRA_b (RNPC2)	RRM (3)	*Homo sapiens*	(Zhang et al. 2013)
U-rich RNA-binding protein 1 (UBP1)	RRM	*Trypanosoma cruzi*	(Cassola et al. 2015)
U-rich RNA-binding protein 2 (UBP2)	RRM	*Trypanosoma cruzi*	This work
RNA-binding protein 4 (RBP4)	RRM	*Trypanosoma cruzi*	This work
RNA-binding protein 5 (RBP5)	RRM	*Trypanosoma cruzi*	This work
RNA-binding protein 6 (RBP6)	RRM	*Trypanosoma cruzi*	This work
Trypanosoma brucei RGG2 (TbRGG2)	RRM	*Trypanosoma brucei*	(Sprehe et al. 2010)
G-rich sequence factor 1 (GRSF1)	RRM (3)	*Homo sapiens*	(Jourdain et al. 2013)

Table 1 continued

Protein description	Domain type	Species	Reference
La-related protein 4 isoform d (LARP4)	RRM	*Homo sapiens*	(Zhang et al. 2013)
La-related protein 4B (LAR4B)	RRM	*Homo sapiens*	(Zhang et al. 2013)
Isoform 1 of La-related protein 7 (LARP7)	RRM	*Homo sapiens*	(Zhang et al. 2013)
Single-stranded nucleic acid-binding protein (SBP1)	RRM (2) and RGG-box	*Saccharomyces cerevisiae*	(Hsieh et al. 2007)
Isoform 2 of Protein LSM14 homolog B (LSM14B)	DFDF box	*Homo sapiens*	(Yoshikawa et al. 2011; Zhang et al. 2013)
Putative RNA-binding protein Luc7-like 1 (LUC7L)	Coiled coil (2)	*Homo sapiens*	(Yoshikawa et al. 2011)
Isoform 2 of Putative RNA-binding protein Luc7-like 1 (LUC7L2)	Coiled coil	*Homo sapiens*	(Zhang et al. 2013)
60 kDa SS-A/Ro ribonucleoprotein isoform 4 (TROVE4)	TROVE	*Homo sapiens*	(Zhang et al. 2013)
Isoform 2 of Fragile X mental retardation syndrome-related protein (FXR1)	KH and RGG-box	*Homo sapiens*	(Zhang et al. 2013)
Fragile X mental retardation syndrome-related protein 2 (FXR2)	KH and RGG-box	*Homo sapiens*	(Yoshikawa et al. 2011; Zhang et al. 2013)
Isoform 5 of Fragile X mental retardation 1 protein (FMR1)	KH and RGG-box	*Homo sapiens*	(Yoshikawa et al. 2011; Zhang et al. 2013)
Nuclear polyadenylated RNA-binding protein (NAB2)	RGG-box	*Saccharomyces cerevisiae*	(Batisse et al. 2009)
Pre-rRNA-processing protein TSR1 homolog (TSR1)	Bms1-type G	*Homo sapiens*	(Zhang et al. 2013)
Isoform 3 of Plasminogen activator inhibitor 1 RNA-binding protein (PAIRB)	Hyaluronan/mRNA-binding family	*Homo sapiens*	(Zhang et al. 2013)
U4/U6.U5 small nuclear ribonucleoprotein 27 kDa protein (SNR27)	Arg-rich	*Homo sapiens*	(Zhang et al. 2013)
U4/U6.U5 tri-snRNP-associated protein 1 (SART1)	Coiled coil (2)	*Homo sapiens*	(Zhang et al. 2013)
U3 small nucleolar ribonucleoprotein protein (IMP3)	S4 RNA-binding domain	*Homo sapiens*	(Zhang et al. 2013)
RNA-binding protein 16 (RBP16)	Cold-shock domain and RGG-box	*Trypanosoma brucei*	(Hayman et al. 2001; Miller and Read 2003)

Domain type assignation for each protein was performed according to the information obtained from Uniprot (http://www.uniprot.org)

(Fig. 1c). Mass spectrometric analysis of this band determined that it corresponded to P32. We further determined that P32 was also present in the eluate fractions of TDP-43 RRM1 (Fig. 1c), but not in the ones of RRM2 (not shown). Our experimental setup prevents RNA-mediated interactions due to the addition of RNase A to the HEK293 protein extract; the omission of this treatment showed a completely different result, with many ribosomal proteins in the eluted fractions (not shown). Although we have only scratched the surface of the relevance of this interaction, it serves as a proof of concept to determine that the TDP-43 interaction with P32 is mediated by RRM1. At difference with our approach, Freibaum and coworkers performed an in vivo approach isolating TDP-43 by immunoprecipitation (Freibaum et al. 2010); Dammer and coworkers analyzed the protein content of the detergent-insoluble fractions formed by the aggregation prone TDP-S6 variant by stable-isotope labeled (SILAC) and liquid chromatography coupled to mass spectrometry (Dammer et al. 2012). While these approaches identified putative in vivo interactions, our approach might be confirming a direct protein–protein interaction between TDP-43 RRM1 and P32. Moreover, we can conclude that this interaction happens in the absence of RNA.

P32 multifunctional nature and localization

As mentioned before, P32 has been identified in association with numerous different proteins by different interaction screenings. Although this could be interpreted as interactions happening between a sticky protein (P32 homologues) and any cellular protein, it is intriguing to find so many RNA-binding and mRNA metabolism-related proteins as interactors. At least for *T. cruzi* recombinant P22, which can be obtained in high amounts in a soluble state, we have not found any bacterial protein copurifying with it, suggesting it does not have a tendency to interact with unspecific proteins (Cassola et al. 2015). Moreover, we demonstrated that the interaction between TcUBP1 RRM and TcP22 is abolished when three RNP1 amino acids were mutated to alanine (Cassola et al. 2015). These results suggested two conclusions: first, that the interaction is highly specific, involving specific amino acids; second, that the interaction occurs through the same surface used to bind RNA. Thus, it is tempting to speculate that P32 participates in numerous, yet specific, interactions by preventing the correct function of its ligand. In the case of the TcUBP1-TcP22 interaction, we found that it could prevent the association of this RBP to endogenous RNP complexes (Cassola et al. 2015). In analogy, in human cells, P32 binding to ASF/SF2 prevents its phosphorylation on the RS domain by either SRPK1 or Clk/Sty (Petersen-Mahrt et al. 1999). The unphosphorylated ASF/SF2 has reduced RNA-binding affinity, thus suggesting a role of P32 in the splicing function of ASF/SF2. Thus, both TcP22 and P32 have a similar impact onto TcUBP1 and ASF/SF2, respectively, although through different mechanisms. Whether the interaction between P32 and TDP-43 RRM1 has an impact on its association with RNA will require deeper digging into the detailed physiological interplay of these two proteins.

Amongst the plethora of ascribed functions of P32, it has been proposed to have a role in viral infections. Evidences for this include the inhibition of splicing of HIV transcripts by interacting with the acetylated form of the viral transactivator Tat (Berro et al. 2006). It has also been proposed to interact directly with the basic domain of the HIV regulatory protein Rev, and to specifically relieve the inhibition of splicing exerted by the basic domain of this regulator in vitro (Tange et al. 1996). Additionally, it was proposed that P32 promoted herpes simplex virus type 1 nuclear egress by interacting with the virulence factor ICP34.5 (Wang et al. 2014). P32 was also proposed to be involved in Adenovirus major late transcription and to cause hyperphosphorylation of RNA polymerase II (Ohrmalm and Akusjarvi 2006). In other pathogenic conditions such as cancer, P32 has been proposed to play an important role in immune evasion, angiogenesis, and metastasis (Peerschke and Ghebrehiwet 2014). Furthermore, it has been also proposed as a marker of tumor cells and as a possible target for cancer therapy (Fogal et al. 2008). It has also been proposed that P32 could be regulating tumor metabolism by regulating the shift from oxidative phosphorylation to glycolysis (Fogal et al. 2010). Regarding non-pathogenic conditions, P32 has been proposed to interact with several members of the PKC kinase family, and to regulate PKCμ kinase activity by steric hindrance (Storz et al. 2000). In another report by Bialucha and coworkers, it was proposed that P32 forms a bridging interaction with lethal giant larvae (Lgl) and PKCζ, thus promoting Lgl phosphorylation (Bialucha et al. 2007). Storz and coworkers proposed P32 as a molecular chaperone in order to cope with all these proposed interactions and functions assigned to this protein (Storz et al. 2000). Here, the use of the term Chaperone is not referred to a facilitator of protein folding, but rather to a promoter or preventer of additional protein–protein interactions. Given the variety of P32 interactions, it is tempting to speculate that the effect of P32 on its interacting protein ligands might not always have the same outcome. It is possible that certain activities might become enhanced when P32 plays a role as an escort, while other activities or capacities might become abolished, resulting in the regulation of the function of the P32-interacting protein.

Localization of P32 and its orthologs has been a matter of debate. Numerous reports have detected human P32 exclusively in the mitochondria (Dedio et al. 1998; Muta et al. 1997; Yagi et al. 2012), while others have detected it in numerous other locations (Berro et al. 2006; Ghebrehiwet et al. 2014; Heyd et al. 2008; Majumdar et al. 2002). When analyzing the above effects of P32 on viral infections, it is clear that this protein could not be involved in so many facets without leaving the mitochondria. Van Leeuwen and O'Hare have proposed that the surface localization of P32 could be the result of an alteration of the correct recognition of the N-terminal mitochondrial signal peptide (van Leeuwen and O'Hare 2001). These authors could relocalize P32 inside the cell by adding a short tag to the N-terminus of the protein, affecting the recognition the N-terminal mitochondrial signal peptide. It was because of this that we have used specific antibodies in *T. cruzi* to detect endogenous TcP22, showing it is distributed throughout the cell including mitochondria (Cassola et al. 2015). It seems likely that P32 and its orthologs can adopt different cellular locations depending on the cell type and external stimuli. Murine P32 was shown to accumulate in the nucleus of a monocyte/macrophage cell line upon stimulation with a mitogen (Majumdar et al. 2002), while adenovirus infection of human cell lines also promotes the translocation of P32 from the mitochondria to the nucleus (Matthews and Russell 1998). P32 has been found to be necessary for nuclear translocation of U2AF26, an alternative subunit of U2AF splicing factor, through the interaction with

a novel nuclear localization sequence (Heyd et al. 2008). Moreover, the human P32 interactome analysis revealed its association to many cytoplasmic and nuclear components (Zhang et al. 2013). These evidences argue in favor for extra-mitochondrial localizations and functions for P32 and its orthologs. However, P32 orthologs analyzed up to date have shown to possess an N-terminal mitochondrial targeting peptide (Soltys et al. 2000), and no mechanism for mitochondrial protein export has been described up to now in mammalian cells (Schmidt et al. 2010) or trypanosomes (Schneider et al. 2008). As for TcP22, we could only detect a single band with a molecular mass compatible with a cleaved mature form (Cassola et al. 2015), suggesting efficient targeting to the mitochondria. Notwithstanding this, some mitochondrial proteins can suffer retrograde transport while being pulled into this organelle, as is the case for fumarase in yeast cells (Knox et al. 1998). This inversion in the transport direction is a consequence of rapid protein folding, which prevents the transport across the mitochondrial membrane, and culminates with a cleaved protein in the cytoplasm (Yogev and Pines 2011). Whether or not retrograde transport of proteins from mitochondria could explain cytoplasmic or nuclear localization of P32 homologues remains to be determined.

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Compliance with ethical standard

Conflict of interest The authors have no conflict of interest to declare.

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