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Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease

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1. ABSTRACT

TDP-43 is a RNA/DNA binding protein that structurally resembles a typical hnRNP protein family member and displays a significant specificity for binding the common microsatellite region $(GU/GT)_n$. Initially described as a regulator of HIV-1 gene expression, it has been reported in the past to affect both normal and pathological RNA splicing events. In particular, it has been shown to play a fundamental role in the occurrence of several monosymptomatic/full forms of Cystic Fibrosis caused by pathological skipping of CFTR exon 9 from the mature mRNA. Recently, and in a way probably unrelated to splicing, a hyperphosphorylated form of TDP-43 has also been found to accumulate in the cytoplasm of neuronal cells of patients affected by fronto temporal lobar degenerations. In addition to its role in transcription and splicing regulation, a growing body of evidence indirectly suggests that TDP-43 may be involved in other cellular processes such as microRNA biogenesis, apoptosis, and cell division. The aim of this work is to provide the basic facts about TDP-43 an assessment of the multiple functions ascribed to this protein.

2. INTRODUCTION

In eukaryotes, gene expression requires the coupling of many processes: transcription, pre-mRNA processing, mRNA transport, and finally translation (1, 2). These processes were initially considered to be functionally separate units, however, recent research has uncovered an unexpected high degree of overlap between them (3, 4). The connection between these gene expression steps is not always provided by novel factors, which may function as a "bridge" between different processes. In fact, in many instances several key factors initially thought to be exclusive of a particular processing step have been shown to play different, equally important roles, in other gene expression steps. One of the most striking examples of this recently discovered multiple functionality is found in the SR protein factors (5-7). For many years, these proteins have been predominantly thought to play a fundamental (but rather confined) role in alternative and constitutive splicing control (8-13). Recently, however, they have been shown to participate in a very wide range of processes that include the maintenance of genomic stability, mRNA export, mRNA surveillance, protein translation, and one of

its key members has even been shown to act as a proto-oncogene in tumor transformation and maintenance (14-24). Therefore, even in the case of well known factors it is now very difficult to ascribe to some of them a single major cellular function. In this ever expanding world of hard-to-define proteins a new rising star is certainly represented by the nuclear factor TDP-43.

2.1. The TARDBP gene and its expression

Human TDP-43 (SwissProt no. Q13148, OMIM no. 605078) was first isolated in 1995 during a search for novel transcriptional inactivators binding to the TAR DNA element of the HIV-1 virus, hence the name: TAR DNA-binding Protein with an approximate molecular weight of 43 kDa (25). The gene coding for this protein (*TARDBP*) is present on Chromosome 1 and is made up by six exons some of which may be alternatively spliced to yield a variety of isoforms (26). An interesting feature of this gene is its localization in the *MASP2* locus, a highly conserved gene-rich region (1p36.2-3) in the human genome and its syngenic counterparts in the mouse and rat (27). The TARDBP gene is a highly preserved gene throughout evolution and corresponding homologues of human TDP-43 have been found in all higher eukaryotic species, including distant organisms such as *Drosophila melanogaster*, *Xenopus laevis*, and *Caenorhabditis elegans* (26, 28). This high degree of sequence conservation in unrelated organisms hints towards a fundamental role played by this protein during evolution.

Using Northern blot analysis human TDP-43 mRNA has been shown to be expressed in all tissues analyzed although with varying levels: pancreas, placenta, spleen, testis, ovary, lung, and kidney are the organs in which is mostly present (29). In parallel, using real time PCR to analyze rat TDP-43 mRNA expression showed that a messenger molecule could also be detected in all rat tissues tested (except for the heart) but with a predominant expression in lung, brain, spleen, and thymus (27). Taken together, these results suggest that TDP-43 is a ubiquitously expressed protein in rodents and humans, although with a possible species-specific difference in its expression levels.

2.2. The TDP-43 protein

The full open reading frame of *TARDBP* codes for a nuclear protein of 414 aminoacids with a theoretical MW of 44740 Daltons. The primary aminoacid structure strongly resembles in domain composition the members of the heterogeneous ribonucleoprotein (hnRNP) family (30, 31). In fact, like many members of this family its major structural features are represented by two highly conserved RNA Recognition Motifs (RRM1 and RRM2), flanked by an N-terminal and a C-terminal tail. The C-terminal tail contains a typical Glycine-rich region that is often found to mediate protein-protein interactions. Indeed, it is through this region that TDP-43 has been recently described to contact other members of the hnRNP protein family such as hnRNP A1, A2/B1, and A3 (32). These three proteins are at present the better characterized interactors of TDP-43. The SMN protein has also been reported as a partner of TDP-43, although not through contact with the C-terminal region (33).

The RNA and DNA binding specificities of this protein have been shown to play a decisive role in almost all the functions of this protein described up to now. In particular, previous research has demonstrated that through the RRM1 domain TDP-43 can bind with high affinity single stranded UG or TG repeated motifs (28, 34). In order to achieve efficient binding TDP-43 needs at least five repeated units (UG_5) and its affinity increases with repeat length (Kd for UG_5 = 30±3 nM and for UG_6 = 8.0±0.7 nM) (28). As expected, the RRM-1 domain is highly conserved throughout evolution especially in the RNP-2 and RNP-1 regions that contain the aminoacid residues which make a direct contact with the RNA (Figure 1A, highlighted in yellow). Mutating the key phenylalanine (F147/F149) residues to leucine residues in RNP-1 and deleting the RNP-2 has been shown to result in total loss in RNA binding ability (34). The high sequence conservation in the neighbourhood of these regions has also allowed to identify additional aminoacids that make direct contact with the (UG)m repeated sequence: W113 and R151, highlighted in violet in Figure 1A. Selective mutagenesis of these residues has allowed to conclusively identify them as part of the RRM-1 sequence that makes direct contact with the RNA (28). Taken together, these results have allowed the construction of a three-dimensional *in silico* model of the complex between TDP-43 RRM1 and the (UG)m RNA (Figure 1B) (28).

At present, much less is known about the function of the N-terminal region that is of varying length in the different species (ranging from an average of 105 residues in most species to 174 residues in *C.elegans*) and seems to be much more loosely conserved than the two RRM regions. In spite of this, however, it has to be reported that selected residues/regions within the N-terminus seem to be very highly conserved. The most striking example of conserved sequence motifs in the N-terminal region is represented by a fifteeen nucleotide long region highly enriched in acidic aminoacids (Figure 2A) localized near the N-terminus of the protein (in the human protein it resides from residues 9 to 23, Figure 2B shows a graphic depiction of this region using Weblogo). The existence of such regions strongly suggests that at least portions of the N-terminus sequence may mediate some highly conserved functions/interactions. Another region whose function still remains elusive is the RRM2 sequence although some indications suggest that it may also participate in RNA binding and that it has been predicted to contain a strong nuclear export signal (aa. IAQSLCGEDLII) (34, 35).

2.3. TDP-43 isoforms and their cellular distribution

A database EST search combined with RT-PCR analysis of TARDBP transcripts from the human and mouse genomes has highlighted the possibility that both genes may express up to eleven different isoforms of the protein (26). However, even though no search has been made to effectively identify all these possible isoforms in humans it has to be noted that Western blot and immunoprecipitation analyses performed on a variety of human cell lines or normal tissues (with both monoclonal and polyclonal antibodies) generally detect only the

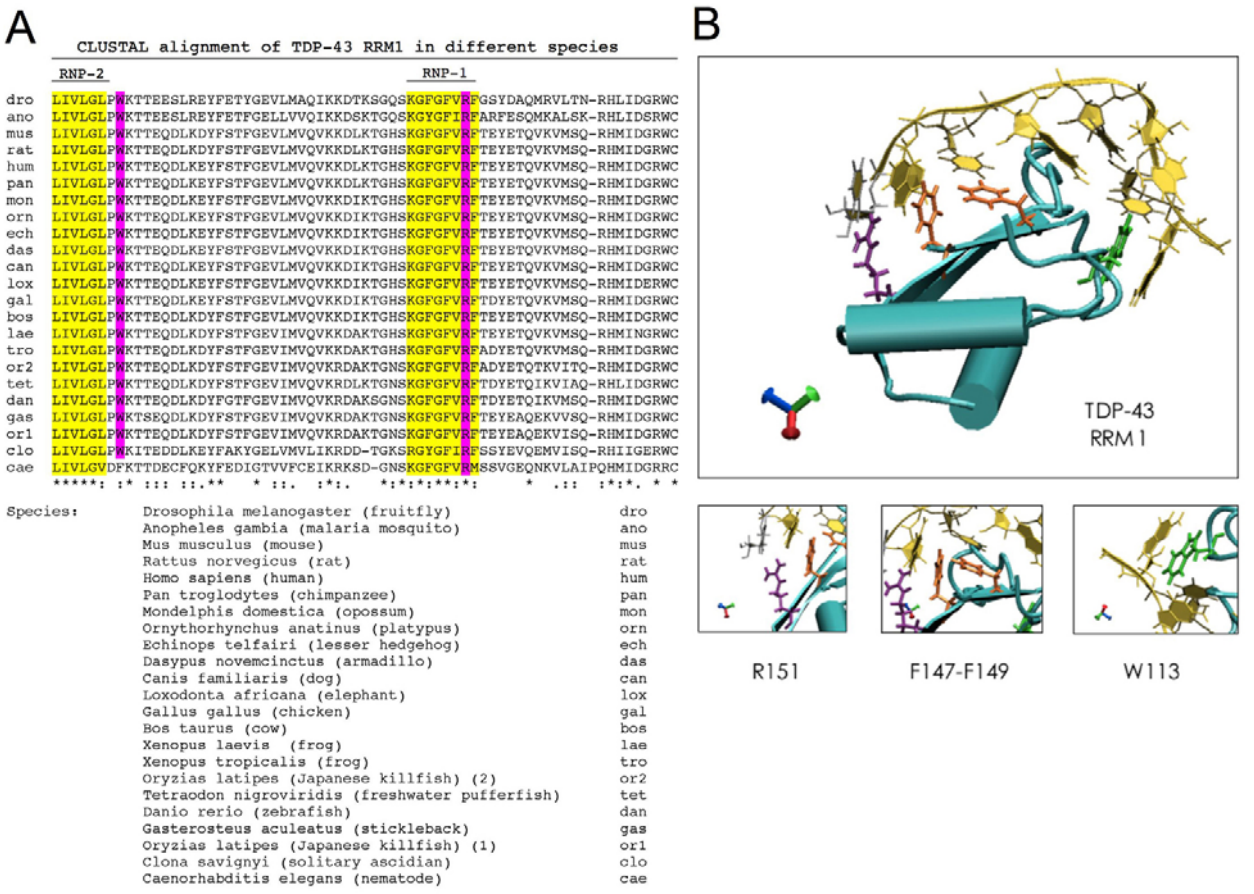

Figure 1. The RRM-1 region is responsible for the (UG)m binding activity of TDP-43. This region is highly conserved throughout evolution as shown by a ClustalW alignment (http://www.ch.embnet.org/software/ClustalW.html) of 23 different RRM-1 sequences obtained from 23 different species (Figure 1A) (sequences are available at http://www.ensembl.org). Highlighted in yellow are the highly conserved RNP-2 and RNP-1 regions whilst highlighted in violet are the W113 and R151 residues that were found to be directly involved in RNA binding. Figure 1B shows a schematic diagram of an *in silico* ribbon-like model of TDP-43 RRM-1 binding to a (UG)m repeated RNA sequence. The lower panels represent a close-up view of the direct protein-RNA interactions of key aminoacid residues (W113, F147-F149, and R151).

presence of the major, full length TDP-43 protein, although variably modified post-translationally through phosphorylation, ubiquitination etc. (29, 36-38). Until further studies prove otherwise it is thus reasonable to conclude that in humans all these different possible isoforms either represent an absolute minority of the expressed TDP-43 protein in the cell or that their expression may be selectively confined in a subset of cells/tissues or in particular developmental stages. In this respect, it is therefore interesting to note that three different isoforms of TDP-43 have been recently detected in human brain and spinal cord tissue (35). Beside the full-length protein, in fact, one isofom has been observed to contain a very small 6nt. deletion near the end of the C-terminal region whilst the remaining isoform contains multiple deletions within the protein that destroy both RRM domains and remove part of the C-terminal tail (35). No information is currently available regarding the functional significance of these isoforms.

With regards to its intracellular distribution it has to be noted that the full length human protein has always been considered to display an exclusively nuclear localization (with the exception of the nucleolus). However, careful analysis of its cellular distribution has revealed that a low degree of cytoplasmic TDP-43 may also be observed, (Baralle F.E., Buratti E., Zago P., unpublished results). Furthermore, the observation that pathological forms of this protein may be exclusively present in the cytoplasm of affected neurons in patients affected by Frontemporal Lobar Degenerations (see below) suggests that a more careful evaluation of its distribution within the cellular compartments is needed.

Compared to humans, mouse EST database analysis has detected the presence of two major isoforms, mTDP-L and mTDP-S, that differ in the presence or not of the C-terminal tail containing the Gly-rich region (33). Within the nucleus, mTDP-S has been reported to localize in distinct nuclear substructures, called TDP-bodies (TB), that may act as a bridge between other different kinds of nuclear bodies such as GEM, POD/ND10, Cajal bodies, and SC35 speckles (33). These data suggest that mTDP-43 could be involved in the functional coupling between the

A

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dro   EEEGDEPIELPAEED
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hum   EDENDEPIEIPSEDD
mus   EDENDEPIEIPSEDD
pan   EDENDEPIEIPSEDD
rat   EDENDEPIEIPSEDD
orn   EDENDEPIEIPSEDD
mon   EDENDEPIEIPSEDD
gal   EDENDEPIEIPSEDD
das   EDENDEPIEIPSEDD
can   EDENDEPIEIPSEDD
bos   EDENDEPIEIPSEDD
lox   EDENDELIEIPSEDD
lae   EDENEEPMEIPSEDD
tro   EDENEEPMEIPSEDD
or2   EDENEEPMEIPSEDD
tet   EDENEEPMEIPSEDD
dan   EDENEEPMEIPSEED
gas   EEENEEPMEIPSEDD
orl   EEENEEPMEIPSEDD
cae   DPNGDEPIELPTVDG
clo   DENEEEPIEIPTEEN
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B

Figure 2. Figure 2A shows a linear comparison of a highly conserved fifteen nucleotide region obtained using the ClustalW program. In the 22 species from which N-terminal sequences were available this region has been found to be highly enriched in acidic aminoacids. This can also be better appreciated using the Weblogo schematic diagram reported in Figure 2B (the program is freely accessible at http://weblogo.berkeley.edu/) (89). In this depiction, the overall height of the stack indicates the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino or nucleic acid at that position.

different processes associated with these kinds of nuclear bodies, such as transcription and splicing.

3. FUNCTIONAL ROLES OF TDP-43 IN TRANSCRIPTIONAL AND SPLICING REGULATION

Even though the exact cellular function of TDP-43 is officially unknown (provided that a major one exists at all) there is a number of biological systems in which this protein has already been shown experimentally to play a very important role. At present, these systems either regard the control of gene transcription, of selected splicing processes, and in the maintenance of mRNA stability.

3.1. Transcriptional Regulation

3.1.1. The HIV-1 TAR DNA regulatory region

Proviral gene expression of human immunodeficiency virus type 1 (HIV-1) is regulated many host cellular factors that bind several cis-acting sequences present within the long terminal repeat region (LTR) of its genome (39). In this complex regulatory system, TDP-43 was demonstrated to inhibit the assembly of basic transcriptional factors in correspondence to the TATA element of the HIV-1 LTR, both in the absence or in the presence of the Tat protein (25) (Figure 3A). Using band shift analysis the exact binding site of TDP-43 was identified as the -18/+28 polypyrimidine-rich region of the TAR DNA element (25) (Figure 3A). It is important to note that to this date this poly-C/T sequence represents one of the only two examples of a non-UGm/TGm target binding site for the TDP-43 protein (see Section 3.3 for the second example). The ability to bind the single stranded TAR DNA sequence has been confirmed independently by later studies (34) although competition studies have indicated that the binding ability of TDP-43 for pyrimidine-rich single stranded DNA regions is substantially lower than the one displayed for single-stranded TG-repeats (34). In addition, no binding could be detected for pyrimidine-rich RNA sequences and for the equivalent TAR RNA region. This contrasts the equally efficient binding of TDP-43 for sigle-stranded UG or TG repeats (34).

3.1.2. The mouse SP-10 gene promoter

Regulation of gene expression represents a critical factor during mammalian spermatogenesis and involves both an activation of transcription at the correct developmental stage and its repression in inappropriate cell types. The mouse SP-10 gene codes for an acrosomal protein and its promoter represents just such an example of transcriptional repression. In fact, transcriptional repression is relieved exclusively in round spermatids between selected stages of development (40). In particular, two ACACAC regions within the SP-10 promoter were demonstrated to be fundamental for the negative regulation of SP-10 gene expression (41) (Figure 1B). An expression cloning procedure to screen for interactors of this region identified TDP-43 as a specific binder together with the Pur-α and Musashi2 proteins (42). Band shift analyses confirmed specific binding of TDP-43 to the antisense strand of the SP-10 promoter that contained two complementary TGTGTG motifs, and binding could not occur when these motifs were mutated (Figure 3B). At present, no molecular mechanism has been proposed to explain this inhibitory action as the authors provided binding data only with single-stranded DNA probes. Since TDP 43 does not bind to double stranded DNA evidence is needed for the unwinding of the GT repeat sequence possibly as a consequence of the formation of the Pol II initiation complex that may display a single stranded target for TDP 43 interaction. In any case, the tissue distribution of mouse TDP-43 protein in testis cross-section revealed that there was a very good correlation between levels of mTDP-43 protein expression in the various types of testicular cells and expression of the SP-10 gene (42).

3.2. Splicing Regulation

3.2.1. *CFTR* exon 9

CFTR exon 9 is an alternatively spliced exon that needs to be included in the final *CFTR* mRNA transcript in order to obtain a functional protein (43, 44). As shown in Figure 4, *CFTR* exon 9 is embedded in numerous splicing regulatory elements (SREs) localized both inside and around this particular exon. The major SREs identified so far are a (UG)m(U)n regulatory region near the 3'ss, two Composite Regulatory Exonic Regions (CERES) within the

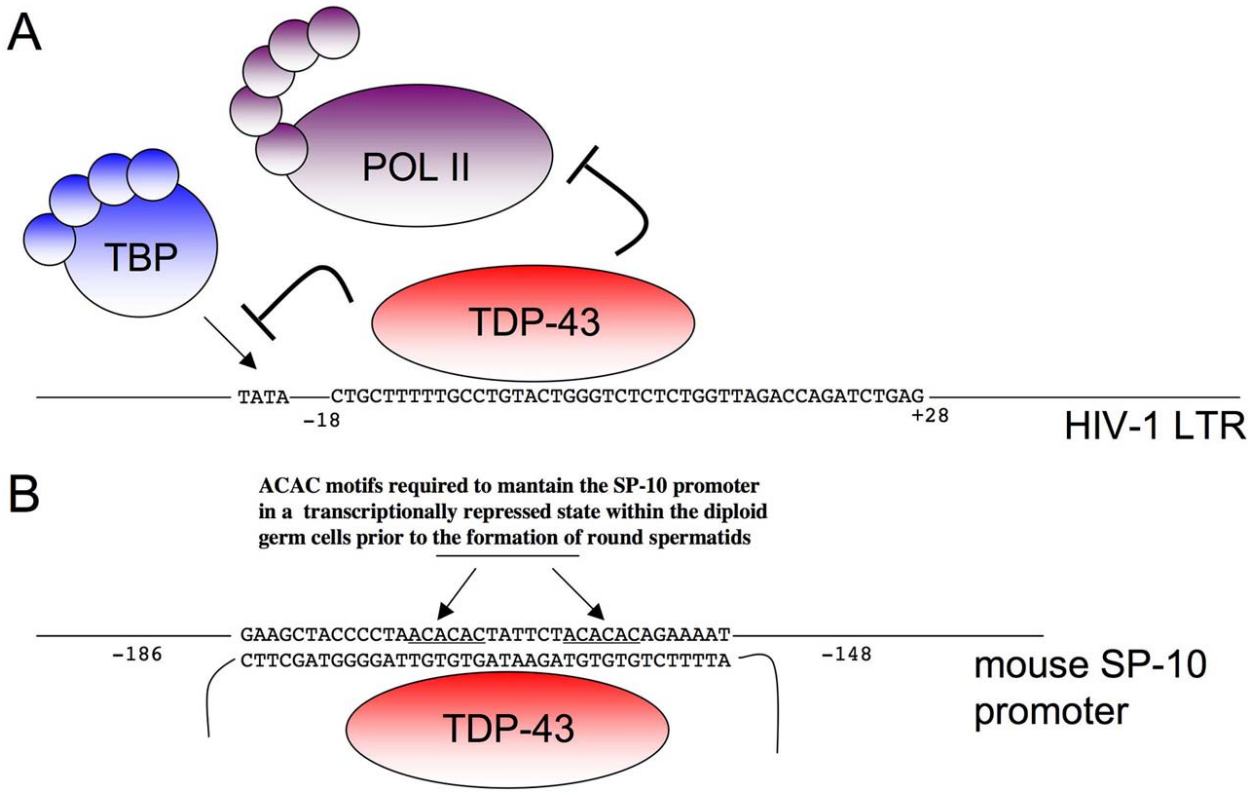

Figure 3. TDP-43 and transcriptional regulation. Figure 1A shows a schematic diagram of TDP-43 inhibition of the HIV-1 LTR promoter following its binding to the TATA element localized in the TAR DNA region. The diagram shows that binding of TDP-43 to this element can inhibit the recruitment of basic transcriptional factors to this element. Figure 1B shows the ability of TDP-43 to bind the negative strand of the mouse SP-10 gene promoter. This strand contains two TGTGTG repeats that are complementary to the ACACAC motifs in the positive strand and are required to repress SP-10 transcription in all mouse tissues or developmental stages with the exception of specific spermatogenic steps.

exon itself , a Py-rich enhancer region (PCE) near the 5'ss, and an Intronic Silencer Element (ISS) in IVS9 (45-49).

Of particular prominence amongst these regions is the (UG)m(U)n polymorphic sequence because of its diagnostic importance for predicting the occurrence of several mono symptomatic and full forms of cystic fibrosis (50). TDP-43 was identified as the trans-acting factor binding specifically to the (UG)m region and inhibiting the recognition of this exon (29, 34). Interestingly, TDP-43 is particularly abundant in the tissues most affected by these monosymptomatic forms of CF (29) and in cell lines derived from the most affected type of tissues such as testis (36). Recently, the importance of the Gly-rich C-terminal region in mediating this inhibitory ability has been demonstrated by several studies and has been observed to correlate with the ability to interact with other members of the hnRNP A/B protein families with well known splicing inhibitory properties (26, 28, 32). The inhibitory effect can be tentatively explained by the working model shown in Figure 4 in which the TDP-43 protein binding in correspondence to the (UG)m repeated motif can recruit, through its C-terminal tail, an hnRNP-rich inhibitory complex that interferes with the earliest stages of spliceosome assembly.

From a functional point of view, the use of antisense oligos or siRNA reagents to inhibit expression of TDP-43 *in vivo* and its depletion in a *in vitro* splicing system has resulted in an increased inclusion of this exon, conclusively demonstrating the inhibitory role played by this protein in CFTR exon 9 recognition (29, 51, 52). Interestingly, removal of TDP-43 from transiently transfected cell lines using siRNA has also beneficial effect in correcting splicing alterations due to mutations in the other exon 9 regulatory elements (51). This dominant inhibitory role of TDP 43 role over other exon 9 splicing regulatory elements may hopefully be exploited for specific therapeutic targeting once the biological role of this protein in cell metabolism is fully understood.

3.2.2. Apo AII Exon 3

Exon 3 of the human Apolipoprotein A-II (*Apo AII*) gene is normally constitutively spliced in the final *Apo AII* mRNA and, at present, no human diseases have been associated with skipping of this exon. However, this exon represents an interesting splicing model because it is very rich in SRE elements that promote its inclusion during the splicing process (Figure 5). In particular, experimental studies have identified the presence of a very powerful ESE enhancer element within this exon that can recruit

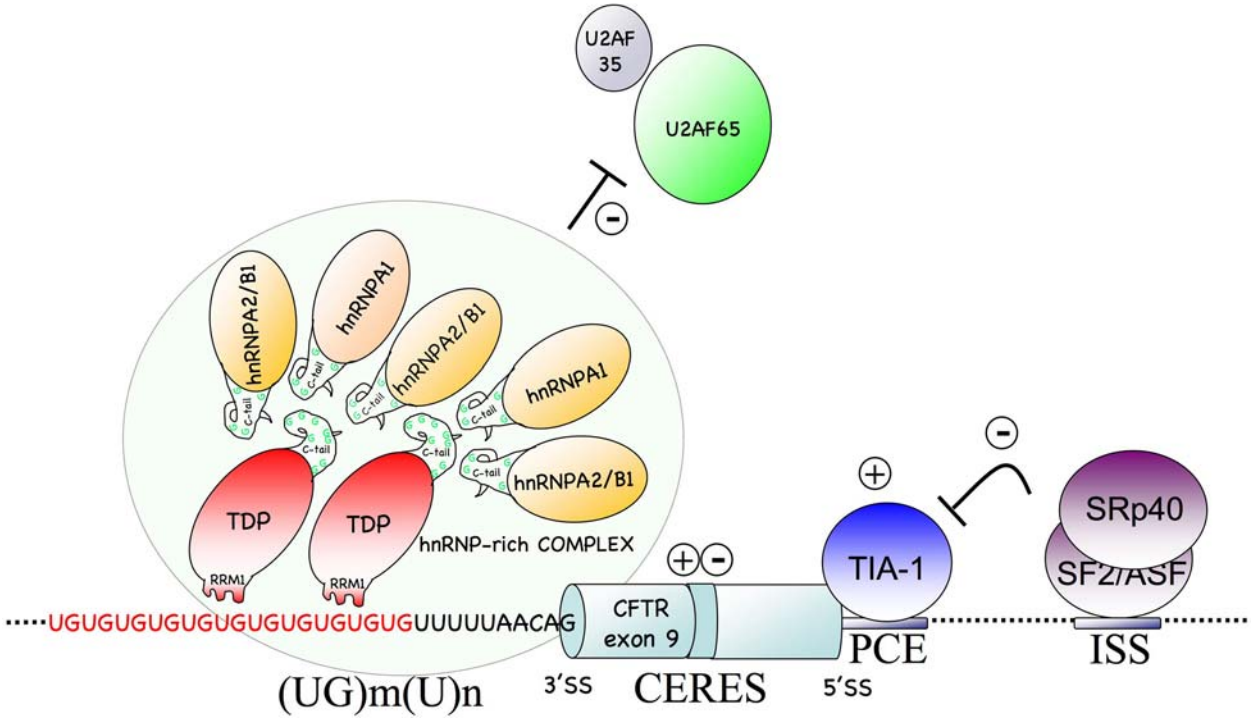

Figure 4. This figure represents a schematic diagram of the major trans acting factors (and their binding sites) that have been found to bind near the 3'ss and 5'ss elements of CFTR exon 9. As shown in this picture, TDP-43 binding to the UG-repeats near the 3'ss can successfully inhibit recognition of the basic splicing factors to this site. This inhibition is dependent on the presence of the C-terminal tail of this protein that can specifically recruit several other hnRNP A/B proteins in the vicinity of the 3'ss. Other trans acting factors important for exon 9 splicing regulation are the TIA-1 protein binding to the PCE region near the 5'ss and the SR proteins binding to the ISS region in IVS9.

specifically the SF2/ASF and SC35 SR proteins (53). In addition, an ISE element in the downstream intron that specifically recruits the SR proteins SRp40 and SRp55 has also been associated with promoting the inclusion of the particular exon (54). The evolutionary conservation of these elements in primates, especially in humans, has found a functional explanation in the need to offset the negative effects on exon 3 inclusion of the atypical acceptor splice site constituted by seven to seventeen UG repeats (54) (Figure 5). As with CFTR exon 9, the negative effects of this peculiar acceptor site are due to the fact that these UG repeats represent a high affinity binding site for TDP-43 (54). In fact, removal of TDP-43 following siRNA treatment has the effect of rescuing exon 3 inclusion even in the presence of mutations that cause the complete inactivation of the ESE and ISE elements (54). These results are very similar to the results obtained in the CFTR exon 9 context in which removal of TDP-43 by RNAi can rescue the effects of mutations in both intronic and exonic enhancer elements of exon 9 (47, 51).

Taken together, these results demonstrate that both *Apo AII* exon 3 and *CFTR* exon 9 definitions can tolerate the disruption of splicing enhancers on condition that the inhibitory action of TDP-43 is removed from their local contexts. This observation possesses considerable importance when assessing the potential reletionships between evolutionary pressure and maintenance of coding ability within these highly regulated coding regions.

3.3. mRNA stability

TDP-43 has also been recently described to be involved in promoting the mRNA stability of the *hNFL* transcript (35). This new property of TDP-43 has been charcaterized both at the cis-acting and at the functional level. In fact, co-immunoprecipitationa and band-shift analyses have demonstrated that TDP-43 can specifically bind the 3'UTR of the *hNFL* transcript in correspondence to the 135-185 region after the stop codon (Figure 6). Interestingly, this region does not contain any UG repeated motifs although the exacts nucleotides involved in this interaction have not yet been mapped. In addition, from the functional point of view, Strong *et al.*, have shown that overexpressing TDP-43 in HEK293T and Neuro2a cell lines has the effect of stabilizing the *hNFL* mRNA by preventing its degradation (35). At present, no data are available regarding also the eventual effects of TDP-43 on the translation efficiency of this mRNA.

From a human disease point of view, *hNFL* is part of a highly conserved family of neurofilament proteins that represent the major structural proteins of neurons (55). Alterations in their aggregation profile may lead to motor neuron degeneration in a variety of model systems. Indeed, one of the characteristics of Amyothophic Lateral Sclerosis (ALS) is the presence of intraneuronal neurofilamentous aggregates that may in part be caused by alterations in *hNFL* mRNA stability (56). The observation that TDP-43

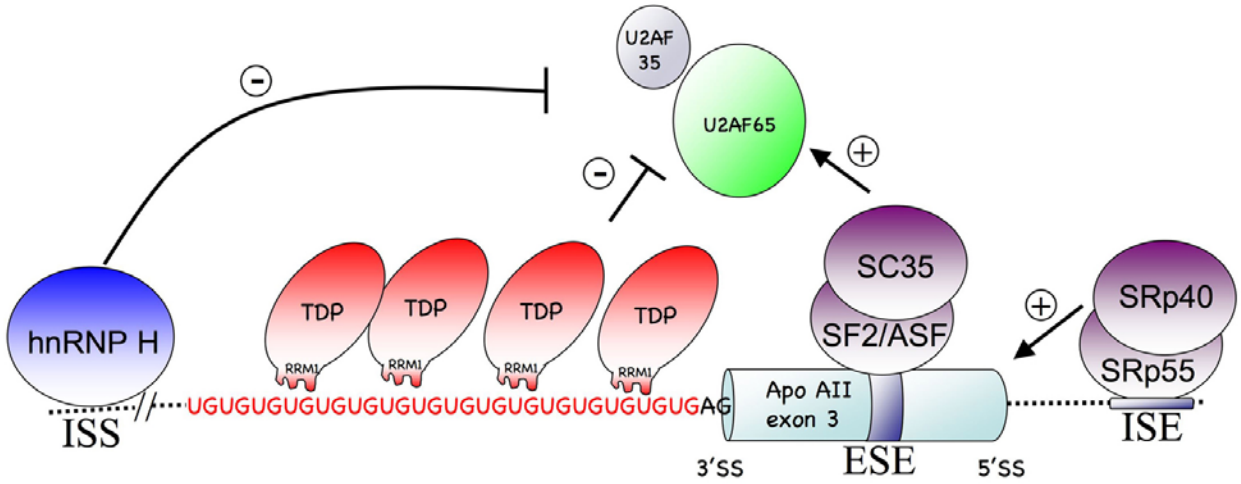

Figure 5. Apo AII exon 3 splicing controlling regions. This figure shows the trans acting factors that play a decisive role in the recognition of this exon by the splicing machinery. As shown in this figure, the negative effects mediated by hnRNP H binding to a ISS element in IVS2 and by TDP-43 binding to the peculiar 3'ss sequence of this exon are offset by an internal exon enhancer element (ESE) and by a intronic splicing enhancer element (ISE) in IVS3. Both these regions bind a specific set of SR protein family members.

binds to th *hNFL* transcripts and participates in its stability may thus provide a potential link between the formation of neurofilament aggregates in ALS (35).

4. INVOLVEMENT OF TDP-43 IN NEURODEGENERATIVE DISORDERS

In this respect, one of the most interesting finding regarding TDP research consists in the identification of TDP-43 as the major disease protein for two neurodegenerative disorders: frontotemporal lobar degeneration with ubiquitin-positive neuronal inclusions (FTLD-U) and ALS, also called Lou Gehrig's disease (37, 38, 57-61). For general recent reviews of these pathologies and their characteristics see (62-67). All these research teams confirmed that TDP-43 was abnormally accumulated in central nervous system regions (hippocampus, neocortex, and spinal cord) of individuals diagnosed with either disease. Interestingly, TDP-43 accumulation was only present in the cytoplasm of affected neurons, whilst unaffected neurons retained the predominantly nuclear distribution (37) (Figure 7). Compositional analysis of these TDP-43 cytoplasmic aggregates showed that they consisted in a hyperphosphorylated form of the protein, ubiquitinated, and cleaved to generate C-terminal fragments (37, 38). An analysis of the staining pattern in affected cells performed in a later study suggests that these modifications prevent the crossing of the nuclear membrane by this misfolded TDP-43 rather than be the cause of an abnormal translocation from the nucleus to the cytoplasm (59).

Recently, TDP-43 accumulation has also been confirmed to be a characteristic feature in cases of frontotemporal dementia with inclusion body myopathy and Paget disease of bone, in the white matter of FTLD-U brains, in cases of familial amyotrophic lateral sclerosis (FALS), in cases of Guam parkinsonism-dementia complex (G-PDC), hippocampal sclerosis, and in cases of frontotemporal dementia accompanied by motor neuron disease (FTD+MND) (68-74).

In all of these conditions the pathogenic role played by TDP-43 accumulation in the cytoplasm of the affected cells is still unknown and the subject of lively debate (75, 76). However, as it is only to be expected, most of the evidence collected up to now is of a negative nature (ie. tending to rule out possibilities rather than confirming any particular mechanism).

For example, recent reports tend to rule out an association between common variations in the *TDP-43* gene architecture and disease in FTLD patients, although (as acknowledged by the authors) this does not of course rule out the existence of rare pathological mutations in the TDP-43 gene that might correlate with the occurrence of disease (77). In this respect, it has to be noted that a recent gene expression analysis of post-mortem brain tissues obtained from FTLD-U/FTLD-MND patients has highlighted that TDP-43 mRNA expression levels seem to be 1.5 fold elevated with respect to controls (78). In addition, a recent analysis has also suggested that the distribution and levels of TDP-43 binding partners identified up to now (hnRNP proteins and SMN) do not seem to be affected in the brain regions of patients with familial and sporadic FTLD-U (79). Interestingly, no TDP-43 mislocalization has been detected in mutant transgenic mice lines that are used as a model of ALS and carry mutations within the *SOD1* gene, indicating that these mice do not express all the features of the human ALS case (in which prominent TDP-43 inclusions have been observed) (80). This may mean that neuronal degeneration in these ALS mouse models may be not completely identical to the mechanism(s) taking place in human ALS cases.

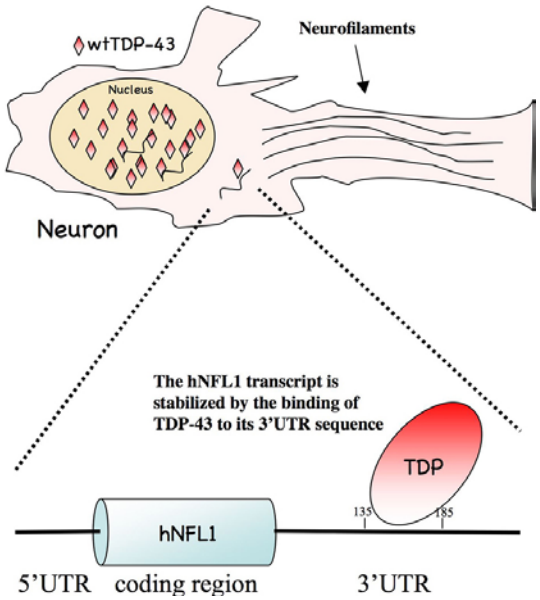

Figure 6. This figure shows the neurofilament (NFs) aggregates that represent the most abundant cytoskeletal elements in large, myelinated axons. NFs run in parallel along the axon and interact with each other through their side domains. By binding to a 3'UTR region of the low molecular weight neurofilament isoform transcript (hNFL) TDP-43 stabilizes this mRNA and prevents its degradation, thus presumably contributing to the maintenance of the correct NF proteins stoichiometry.

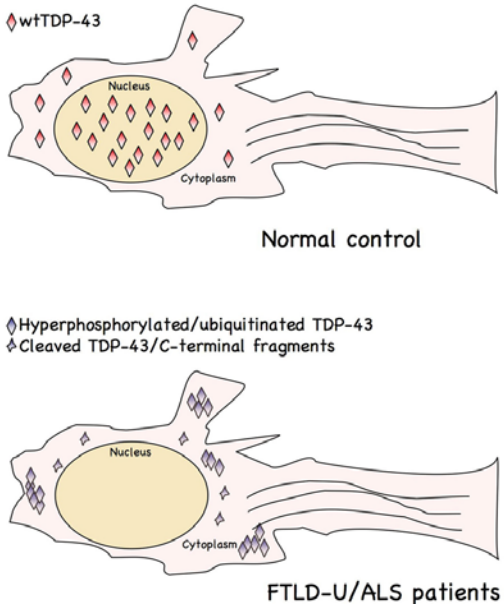

Figure 7. TDP-43 in FTLD-U and ALS. This figure shows the different cellular distribution of TDP-43 in normal cells (top) as opposed to neuronal cells from patients affected by FTLD-U or ALS (bottom). In these patients, no wild type TDP-43 protein can be found within the nucleus and all the pool of intracellular TDP-43 can be found accumulating in the cytoplasm. The modifications to which this cytoplasmic TDP-43 has been subjected include hyperphosphorylation, ubiquitination, and cleavage to generate C-terminal fragments.

Alternatively, it may represent an indication that neuron degeneration in human ALS cases is not directly linked to TDP-43 accumulation/mislocalization and in this case TDP-43 may represent simply a microscopic marker of still uncharacterized pathological mechanisms.

In conclusion, since the discovery in 2006 that TDP-43 is involved in FTD almost 30 peer-reviewed articles have been published on the subject. These articles, however, have for the main part addressed the descriptive part of TDP-43 accumulation in various pathological neudegenerative settings. This should not be underestimated, as an exact characterization of TDP-43 accumulation may well prove to be a very valuable tool to differentiate between the different neuron pathologies described in FTD cases, as described in recent reports (81, 82). Nonetheless, its importance in the pathophysiological sense of the word will be hopefully clarified in future studies aimed at addressing directly the role of TDP-43 in these neudegenerative diseases.

5. MISCELLANEOUS PROCESSES

The examples reported in this final section probably represent one of the most fascinating aspects of current TDP research. Although none of these studies specifically targets TDP-43 for detailed analysis the wider complexes and processes that are described manage to convey interesting glimpses into what may become the future investigative trends regarding this protein.

First and foremost, it is interesting to find TDP-43 as part of a group of 19 proteins that are specifically associated with the Microprocessor complex Drosha/DGCR8 (83). As the name suggests, the Microprocessor complex is one of the fundamental molecular machines that are involved in the generation of miRNAs, a class of small (approx. 22nt.) non coding RNAs that are involved in the control of gene expression in many different organism, including humans (84). The role played by these Drosha-associated proteins in the functioning of this complex remains to be determined and TDP-43 may thus become a likely target to further investigate the functional properties of the Micorprocessor complex.

Another interesting feature of human TDP-43 is represented by the observation that it is a specific caspase substrate in apoptotic Jurkat T lymphocytes (85). These specific proteolytic events represent the major players in the initiation and execution of apoptosis and, as a consequence, the resulting cleavage products are often stable in cells and potentially acquire different functions/localizations. In this particular case, as many other proteins specifically identified in this study belonged to the spliceosomal complex (14 out of 92 total identified targets), TDP-43 may be specifically targeted because of its role in splicing regulation although it may still be related to its other cellular functions.

With respect to viral life-cycle regulation, it has also to be noted that TDP-43 has also been recently shown to be upregulated in cells infected by the Respiratory Syncytial Virus (RSV) (86). In this case, however, a direct

involvement of TDP-43 in regulating the RSV life cycle directly seems unlikely as this process is entirely cytoplasmic. However, its upregulation in RSV infected cells has been associated with a disruption of the POD/ND10 structures and redistribution of PML and Sp100 proteins in the cytoplasm, possibly representing a specific cellular response to viral infection (86).

Finally, the *Xenopus* homologue of human TDP-43, Mitotic Phosphoprotein 39 (MP39), was identified as part of a screening of cyclin-dependent protein phosphorylation events in *Xenopus* interphase and mitotic egg extracts (87, 88). Also in this case, the exact significance of MP39 phosphorylation in *Xenopus* mitosis is currently unknown. However, what makes this case particularly interesting is the potential connections with the hyperphosphorylated state of TDP-43 in the neurodegenerative disorders described in Section 4.

6. CONCLUSIONS AND PERSPECTIVES

Based on the previous sections, we can stress a few pointers regarding future TDP-43 research trends:

1. First, the role of TDP-43 in splicing, transcriptional regulation, and mRNA stability will certainly benefit from a systematic analysis of additional examples in which this protein may play a regulatory role. In this respect, the increasing amount of sequence collections that contain annotated genomic (ie. promoter) or expressed mRNA species (ie. splicing regulated events) now provides us with a great wealth of untapped data-mining possibilities. In fact, by centering our research on conserved UG/TG motifs it would be easy to screen for likely targets. Furthermore, the availability of efficient siRNA reagents to selectively knock down expression of TDP-43 will allow a relatively easy way to check for functionality in the *in silico* identified potential targets.

2. A second issue that will probably greatly benefit several areas of TDP research concerns further experimental efforts to systematically study the cellular distribution of TDP-43, its potential isoforms, and its secondary modifications (phosphorylation probably representing a priority, because of its relationship with the neurodegenerative form of the protein). For much the same reasons, also the mechanisms that underlie its cellular distribution will certainly require further studies, especially since TDP-43 accumulates in the cytoplasm of affected neurons in FTD/ALS patients. A systematic search of this issues is sorely needed. In fact, present expression data has been obtained in a rather unconnected fashion, using different techniques, different cell lines, different tissues, and even different species. Although this has already allowed to obtain a feeling for general trends (as described in Section 2.3 of this review) it is clear that further effort is clearly demanded in this regard.

3. A final issue concerns novel potential functions of TDP-43. As described in Section 5, there are already a number of interesting processes in which this protein may be an active participant. Several of these processes, especially the microRNA generation mechanisms or the control of mitosis, probably deserve a greater degree of attention in the near future.

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Key Words: TDP-43, TARDBP, alternative splicing, CFTR, Apo AII, transcription, HIV-1, SP-10, mRNA stability, NFL, frontotemporal dementia, FTD, amyotrophic lateral sclerosis, ALS, Review

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