Evaluating the specific contributions of pre-mRNA splicing and polyA selection in TDP-43 autoregulation .

Jeremias S. Herzog, Sara Bembich, Laura De Conti, Emanuele Buratti, Marco Baralle and Francisco E. Baralle
ICGEB, Molecular Pathology Lab, Padriciano 99, 34149 Trieste, Italy.

Introduction

TDP-43 protein contains two RNA Recognition Motifs (RRMs), NLS and NES signals, and a Gly-rich C-terminal region with which it can interact with other hnRNP A/B family members. (Fig.1). Although the best characterized activity of this protein is in alternative splicing control several functions that range from DNA transcription to mRNA translation have been proposed in recent years. Its role in neurodegenerative disease has been recently reviewed by Buratti and Baralle, TiBS, 2013.

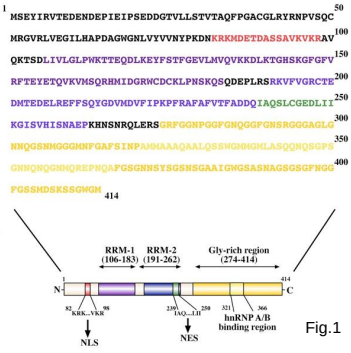

Fig.1

TDP-43 stable cell lines expressing wild-type and mutant TDP-43 proteins display autoregulation at the transcriptional level. Fig.2. Tagged forms of TDP-43 (F-TDP43) were expressed for 24 or 72 hours upon tetracycline (Tet) induction. RNA binding is an absolute requirement for autoregulation to take place.

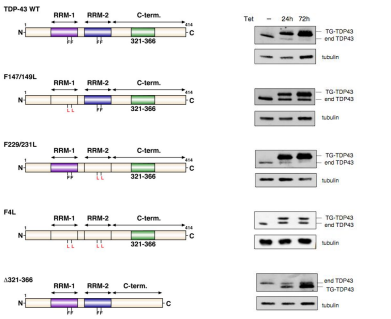

Fig.2

Autoregulation occurs at the mRNA level. Fig.3. Upper panels, show a schematic representation of the TDP-43 gene and polyadenylation signals. The lower panels shows the rapid degradation of TDP-43 mRNAs following the upregulation of the TDP-4343 transgene. Degradation depends on the binding of TDP-43 in a specific region of its 3'UTR called TDPBR (Fig.4). (Ayala et al., EMBO, 2011)

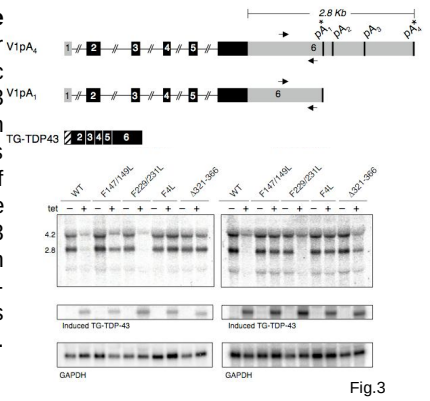

Fig.3

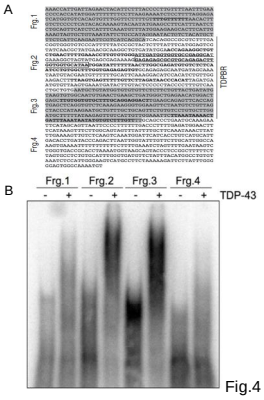

Fig.4

Binding of TDP-43 to the TDPBR leads to Pol II stalling. Fig.5, shows the consequences of low vs. high TDP-43 expression on 3'UTR intron 7 processing and differential PAS usage.

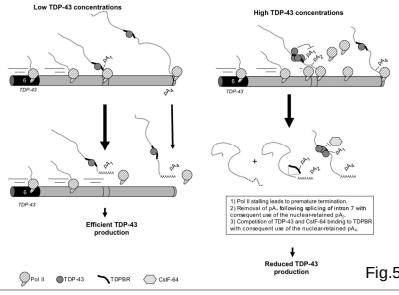

Fig.5

Cis acting elements and importance of PAS sequences in TDP-43 autoregulation. Fig.6, shows the ability to autoregulate of various TDP-43 3'UTR constructs fused to the GFP protein and transfected in HEK-293 cells stably expressing a TDP-43 transgene following Tetracycline induction (+Tet lanes).

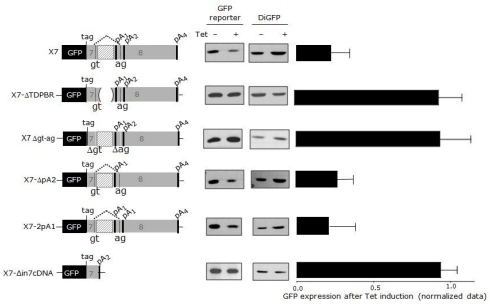

Fig.6

3'RACE analyses. Fig.7A shows the primers used for 3' RACE analysis. Fig.7B to 7F show the results of the 3'RACE analyses in –Tet and +Tet conditions from the various hybrid constructs shown in Figure 6. These initial results suggest that the key feature that allows TDP-43 autoregulation is represented by the intron 7 splicing event or at least to spliceosome assembly in intron 7 splice sites, and is not strictly linked to the quality of the PAS sites that can be found in the vicinity.

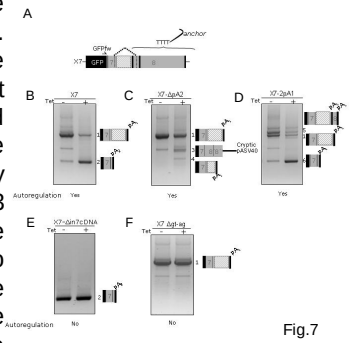

Fig.7

Improving intron 7 donor and acceptor splice sites. Fig.8A shows the intron 7 splice sites in the improved version (X7-sup5'-3'). Fig.8B 3'RACE analysis of X7-sup5'-3' in normal conditions (left) and CHX treatment to rule out eventual effects by NMD (right). Fig.8C shows a Northen blot analysis of the X7-sup5'-3' mutant cotransfected with X7. Results show that compared to the X7 this mutant displays a 50% reduction in mRNA expression (Fig.8D) and of 75% in protein production (Fig.9).

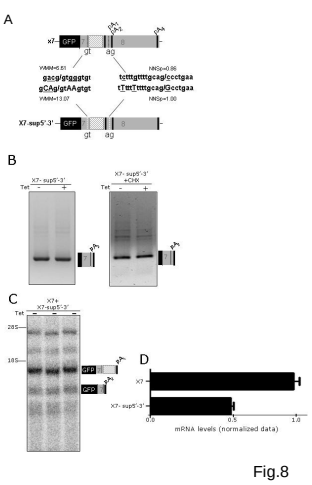

Fig.8

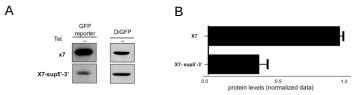

Fig.9

Cellular distribution of RNA from these different constructs. The additional drop in protein production (Fig.9) could be explained by RNA FISH analysis, In fact, Fig.10 showed that whilst X7 and X7-Δin7cDNA RNA are mostly cytoplasmic the X7-sup5'-3' RNA is equally nuclear and cytoplasmic.

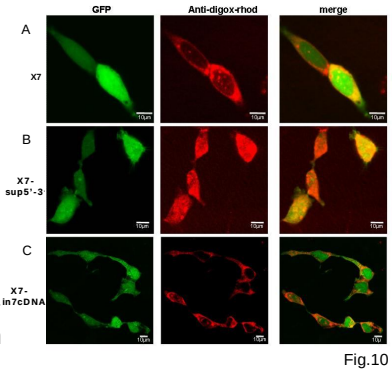

Fig.10

Conclusions. Our results show that the intron removal and/or the TDP-43 mediated spliceosomal assembly represent the major events mediating autoregulation (Fig.11A), and not the intrinsic quality of the various PAS site (Fig.11B).

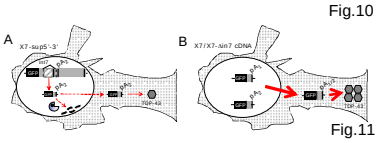

Fig.11