Neurons don't appreciate FUSsing in the cytoplasm

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The EMBO Journal (2010) 29, 2769–2771. doi:10.1038/emboj.2010.163

In the amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) neurodegeneration field, two nuclear factors (first TDP-43, then FUS) have recently made researchers realize that alterations at the level of hnRNP proteins and RNA metabolism may have a decisive function in disease origin and progression. However, the exact mechanisms through which this occurs are still largely unknown. In this issue of *The EMBO Journal*, Dormann *et al* have for the first time succeeded in linking ALS-associated mutations in the FUS protein with a specific functional property, that is alterations in nuclear import and stress granule formation. This finding has profound implications for future research efforts in better understanding the pathogenesis mediated by these proteins and eventually developing disease-modifying therapies.

The unexpected appearance of hnRNP proteins, namely TDP-43 and FUS, in the neurodegeneration field has sparked considerable enthusiasm because it has increased our insight on the pathogenic events leading to ALS and FTLD (Buratti and Baralle, 2009; Chen-Plotkin *et al*, 2010; Lagier-Tourenne *et al*, 2010). Indeed, the discovery of TDP-43 brain inclusions in 2006 (Neumann *et al*, 2006) and of FUS inclusions just 1 year ago (Kwiatkowski *et al*, 2009; Vance *et al*, 2009) has already lead to a complete change in disease nomenclature in the FTLD field (Mackenzie *et al*, 2010). The observation that TDP-43 is also the main disease-associated feature in ALS and that FUS inclusions are also present in familial ALS cases has further strengthened the molecular link between these two diseases.

At the moment, disease mechanisms mediated by these two proteins are still largely unknown although many functional studies have highlighted the considerable importance of these two factors in the general RNA metabolism, suggesting that alterations in their sequence, post-translational modifications, and especially nuclear–cytoplasmic localization may have very severe consequences (Ticozzi *et al*, 2010). In parallel, one additional feature that links these proteins with disease is the presence of amino-acid substitutions in some of the patients. In TDP-43, these mutations are predominantly localized in the C-terminal tail (Pesiridis *et al*, 2009), whereas in FUS most mutations are clustered in the first glycine-rich region and in the last 17 amino acids (Lagier-Tourenne *et al*, 2010). To this day, the functional effects of these mutations that mediate disease onset and progression were very much unclear.

In this issue, Dormann *et al* (2010) have successfully unravelled some of the mysteries by clarifying the functional

consequences of several naturally occurring mutations in the extreme C-terminal region of FUS. On the basis of previous observations that had predicted a non-classical nuclear localization signal (NLS) in its C-terminal tail (Lee *et al*, 2006), the authors have confirmed that the 514–526 region of FUS mediates nuclear import through the nuclear import receptor Transportin. They next analysed the effects on this property on a set of disease-associated mutations, and in particular the very severe P525L substitution using a variety of reporter constructs injected in HeLa cells, primary rat neuronal cultures, and zebrafish embryos. The results showed that with respect to the wild-type protein, the mutant proteins were indeed predominantly localized in the cytoplasm to a variable degree. Interestingly, the authors also noted that the mutant proteins did not just loiter aimlessly in the cytoplasm, but following heat shock were gathered in stress granules, which normally represent a defensive mechanism by the cell (Anderson and Kedersha, 2008). The results obtained in this study have allowed them to develop a two-hit model of FUS pathology (Figure 1). In this model, the first hit is provided by a mutation, which hampers the interaction with Transportin and promotes an abnormally increased localization of FUS in the cytosol. In the short term and following cellular stress, this increased cytoplasmic localization causes the formation of FUS-containing stress granules. In the long term, however, it will trigger pathological formation of inclusions similar to those observed in patient's samples. Probably one of the most exciting findings of this work is provided by the observation that in patients carrying NLS-impairing mutations, the age of onset of ALS disease is directly proportional to the degree of abnormal FUS localization in the cytoplasm (i.e. the higher, the earlier).

Of course, like all true groundbreaking studies, the experiments by Dormann *et al* (2010) raise a good deal of additional questions. As acknowledged by the authors, the first question that comes to mind is what causes FUS accumulation in the cytoplasm in the absence of FUS mutations (which represent a small minority of pathological cases). This issue may also be particularly important with regards to TDP-43 accumulation in the cytoplasm, especially as also this protein has been recently reported to be present in stress granules following experimental stress conditions (Colombrita *et al*, 2009). In parallel, there is also the major open question that looms on the horizon and regards the pathological mechanism triggered following the formation of TDP-43 and FUS

Figure 1 Schematic representation of the two-hit model proposed in this issue by Dormann *et al* to explain the pathological mechanism mediated by missense mutations in the non-classical PY NLS at residues 514–526 of FUS.

cytoplasmic inclusions. In both cases, gain-of-function (i.e. toxicity of the inclusions themselves) or loss-of-function effects (i.e. impairment of essential TDP-43/FUS-dependent nuclear processes) are possible and still remain mostly uncharacterized. The correct identification of the processes affected will thus represent a key issue in the development of future therapeutic strategies.

Acknowledgements

The research on these proteins in our laboratory is supported by AriSLA.

Conflict of interest

The authors declare that they have no conflict of interest.

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