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The FUS about arginine methylation in ALS and FTLD

Hannah M Kaneb[1,2], Patrick A Dion[1,3] and Guy A Rouleau[1,2,4,*]

[1]Centre Hospitalier de l'Université de Montréal Research Center, Université de Montréal, Montréal, Quebec, Canada, [2]Department of Medicine, Université de Montréal, Montréal, Quebec, Canada, [3]Department of Pathology and Cellular Biology, Université de Montréal, Montréal, Quebec, Canada and [4]Centre Hospitalier Universitaire Sainte-Justine Research Center, Montréal, Quebec, Canada.
*Correspondence to: guy.rouleau@umontreal.ca

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In a time where links between amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) neurodegeneration are becoming increasingly clear, it is important to establish the convergent and divergent mechanisms responsible for this. Accordingly, Dormann *et al* (2012) have identified that methylation of the Fused in sarcoma (FUS) RGG3 domain is involved in the cytoplasmic mislocalisation of ALS-FUS mutants, through a transportin-dependent mechanism. By contrast, hypomethylation in this domain may play a role in the aberrant accumulation of FUS in FTLD-FUS. This work showcases arginine methylation as a phenomenon to watch out for in neurodegenerative pathology.

The nuclear RNA/DNA-binding protein, FUS, first burst onto the ALS scene in 2009 when it was discovered that mutations in this protein are causative for familial ALS (Kwiatkowski *et al*, 2009; Vance *et al*, 2009). It is now thought to be responsible for 4% of familial (and rare sporadic) ALS cases. In ALS patients with *FUS* mutations (ALS-FUS), the FUS protein is deposited in abnormal protein inclusions in neurons and glia and nuclei often show a reduced FUS staining (Lagier-Tourenne *et al*, 2010). Fascinatingly, this abnormal FUS deposition is also observed in several subtypes of FTLD, subsequently termed as FTLD-FUS. However, unlike in ALS-FUS, there are no known FUS mutations in this disease (Da Cruz and Cleveland, 2011).

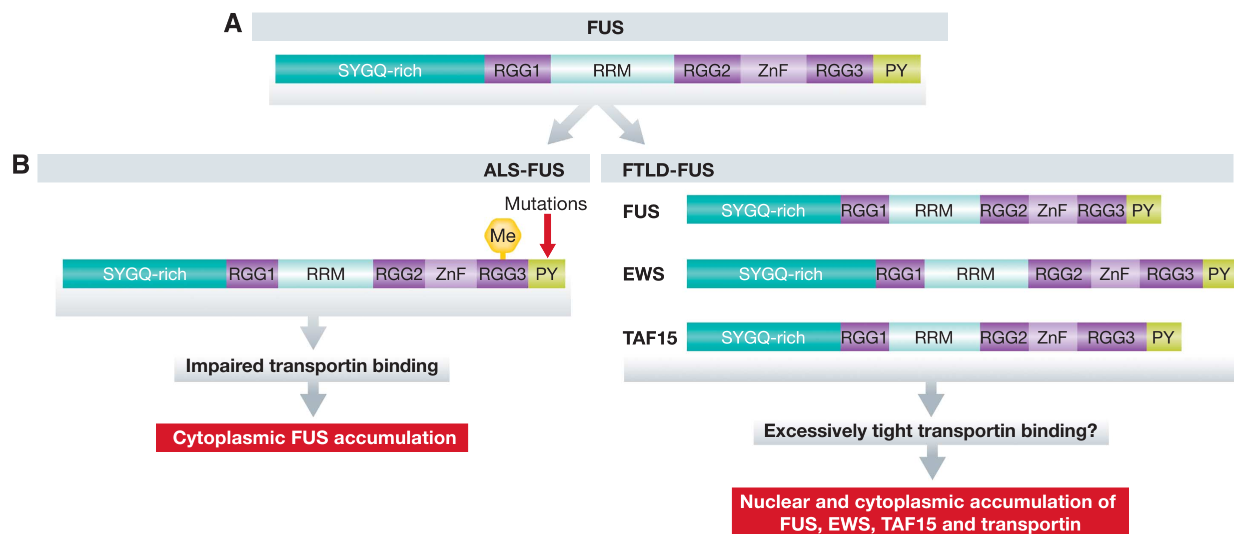

Figure 1 (**A**) Schematic diagram showing the domain structure of FUS. SYGQ-rich, serine, tyrosine, glycine, glutamine-rich domain; RRM, RNA recognition motif; ZnF, zinc finger; PY, proline-tyrosine nuclear localization signal (PY-NLS). (**B**) Schematic diagram summarizing the divergent mechanisms by which arginine methylation (or absence thereof) may bring about FUS mislocalization and accumulation in ALS-FUS and FTLD-FUS. In ALS-FUS caused by FUS mutations, neuronal cytoplasmic inclusions contain methylated FUS, but are negative for EWS, TAF15 and TRN. Dormann *et al* (2012) propose that these FUS-specific inclusions result from the combination of a genetic defect (point mutations in the PY-NLS that impair transportin binding) and post-translational modification (arginine methylation in RGG3 domain that also impairs transportin binding). By contrast in FTLD-FUS, neuronal cytoplasmic inclusions contain all three FET proteins and transportin, but are not immunoreactive with meFUS-specific antibodies. It is therefore possible that hypomethylation of the FET proteins and thus increased transportin binding may be involved in the co-deposition of these proteins in FTLD-FUS.

Dormann *et al* (2012) now broaden our knowledge of how ALS-causing mutations in FUS lead to its abnormal cytoplasmic deposition in neurons and glia, and for the first time suggest a mechanism through which this could also occur in FTLD-FUS, in the absence of FUS mutations.

The majority of pathogenic mutations identified in ALS-FUS are located at the C-terminus of the protein within a region identified to be a proline-tyrosine nuclear localization signal (PY-NLS) (Figure 1). The PY-NLS binds to the nuclear import receptor transportin (TRN), which facilitates FUS transport into the nucleus. Pathogenic *FUS* mutations affect key residues of the PY-NLS or completely delete the signal sequence and thus impair nuclear import of FUS (Dormann *et al*, 2010). This nuclear transport defect is likely directly involved in pathogenesis, as mutations that cause a very severe nuclear import deficit (e.g., FUS-P525L), are associated with earlier disease onset and a rapid disease course (Dormann *et al*, 2010).

FUS and other related PY-NLS-containing FET proteins such as Ewing sarcoma (EWS) protein and TATA-binding protein-associated factor 15 (TAF15) have been described to undergo extensive asymmetric dimethylation in their arginine-glycine-glycine (RGG) domains (Araya *et al*, 2005; Hung *et al*, 2009; Jobert *et al*, 2009). In recent times, it has been established that this arginine methylation can affect their nuclear localization (Araya *et al*, 2005; Jobert *et al*, 2009; Tradewell *et al*, 2012). In this issue, Dormann *et al* (2012) sought to delve further into the mechanism by which this occurs. They began their studies in a similar fashion to Tradewell and colleagues, by confirming that inhibiting global arginine methylation using the general methylation inhibitor, adenosine-2,3-dialdehyde (AdOx) could restore the lost nuclear localization of both HA-tagged cytoplasmic ALS-causing mutants and cytoplasmic EWS and TAF15 point mutants in HeLa cells. As AdOx is capable of inhibiting protein, DNA and lipid methylation, they also investigated the effect of specifically preventing protein methylation on the localization of the severe FUS-P525L ALS mutant. In these experiments, siRNA-mediated silencing of PRMT1, the protein arginine methyltransferase responsible for the majority of cellular protein arginine dimethylation, successfully restored the nuclear localization of FUS-P525L, thus confirming the importance of arginine methylation in the cytoplasmic localization of ALS-FUS mutants. After performing this groundwork, Dormann and colleagues then began an elegant set of experiments to unravel the mechanism behind their results. They first wanted to identify whether TRN was involved. Co-expression of GFP-tagged TRN inhibitor peptide with HA-tagged FUS-P525L in HeLa cells lead to a complete prevention of the normal nuclear accumulation of FUS-P525L upon AdOx treatment. Thus, identifying a critical role for TRN in the observed nuclear import of ALS-FUS mutants upon demethylation.

Dormann *et al* (2012) next sought to determine how arginine methylation may impact the nuclear import of FUS by TRN. Their first step was to determine which arginine residues in FUS were involved. With this came the ground-breaking finding that it is actually arginine residues in the RGG3 motif N-terminal to the PY-NLS (Figure 1), rather than in the PY-NLS itself that can modulate TRN-dependent nuclear import of mutant FUS. Through NMR spectroscopy, Dormann and colleagues were then able to show that arginine residues in the FUS RGG3 motif can bind directly to TRN. These observations were taken further via studies of the interaction of recombinant FUS-RGG3 domain or synthetic FUS peptides with TRN by isothermal titration calorimetry. Here, they showed that the FUS-P525L mutant bound weakly or not at all to TRN, but in the presence of the unmethylated RGG3 domain the binding affinity of the mutant was rescued to WT-like levels. These observations were validated by the fact that the unmethylated RGG3 domain alone was able to bind TRN in the absence of a C-terminal PY-NLS with an affinity similar to that of the WT PY-NLS. Methylation of the RGG3 peptide completely prevented this binding. Through these experiments Dormann *et al* (2012) were able to show for the first time that residues outside of the PY-NLS can be involved in FUS nuclear import. Furthermore, they were able to establish a working model by which this occurs: they propose that in normal situations, the PY-NLS anchors the FUS C-terminus to TRN and the adjacent RGG repeats stabilize the interaction. Methylation of the RGG repeats interferes with TRN binding, but in WT FUS the affinity of the PY-NLS for TRN is sufficient to allow nuclear import to continue. By contrast, in the methylated P525L mutant, weak binding of both the methylated RGG domain and the c-terminus of the PY-NLS to TRN abrogates FUS nuclear import, thus causing its cytoplasmic accumulation.

On the basis of their model, Dormann *et al* (2010) predicted that cytoplasmically mislocalized ALS FUS mutants would be methylated in their RGG3 domains. To test this hypothesis, they generated two monoclonal antibodies specific to the methylated RGG3 domain (meFUS antibodies). Cytosolic FUS mutants, such as FUS-P525L, were recognized by the meFUS-specific antibodies in HeLa cells, thus strongly suggesting that methylation of FUS mutants is a contributing factor to their cytoplasmic retention.

Having generated the valuable tools of meFUS-specific antibodies, Dormann *et al* (2010) next turned their attention to the analysis of whether the intriguing shared property of cytoplasmic FUS accumulation in ALS-FUS and FTLD-FUS could be related to arginine methylation. In direct support of their cell-culture experiments, they discovered that there was a strong and consistent co-labelling of all FUS-positive cytoplasmic neuronal and glial inclusions with the meFUS antibody in post-mortem tissue from ALS-FUS patients. However, the surprise came when they began analysing post-mortem tissues from various subtypes of FTLD-FUS patients. Here, they could not see any labelling of FUS-positive neuronal and glial cytoplasmic and intranuclear inclusions with the meFUS-specific antibody. This strongly suggests that a different FUS cytoplasmic retention mechanism is in play in FTLD-FUS patients compared to ALS-FUS patients. In support of this, inclusions in ALS-FUS patients have recently been identified to contain only the FUS protein, while inclusions in FTLD-FUS show a clear co-deposition of all FET proteins (FUS, EWS, and TAF15) along with TRN itself (Neumann *et al*, 2012). From this, Dormann *et al* (2012) conclude that mislocalization of FUS in ALS is likely a phenomenon resulting directly from mutations in the nuclear localization signal that is exacerbated by arginine methylation in the RGG3 domain, whereas FUS mislocalization in FTLD-FUS may result from a more general defect in TRN-mediated nuclear import of FET proteins (Figure 1). Indeed, they

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speculate that in FTLD, hypomethylation of the FET proteins by a yet to be determined mechanism may lead to excessively tight binding of these proteins to TRN. This in turn may lead to impaired dissociation of FET-TRN complexes, which could over time lead to the co-deposition of FET proteins and TRN in cytoplasmic and nuclear inclusions in FTLD patients. The results of future experiments performed to test this hypothesis will doubtless be of huge significance for the FTLD field. Furthermore, this study raises many questions about the normal cellular role of FUS arginine methylation. Is it for example involved in the fine tuning of the shuttling of FUS into and out of the nucleus and if this is the case could arginine methylation in the RGG1 and RGG2 domains, one of which (RGG1) is close to the FUS nuclear export signal, also be involved in regulating FUS localization? All this remains to be seen…

Conflict of interest

The authors declare that they have no conflict of interest.

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