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Role of SMN and FUS in Nucleolar Homeostasis

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Sujet de stage :

Spinal Muscular Atrophy (SMA) is a debilitating autosomal recessive neuromuscular disorder characterized by the degeneration of motoneurons, leading to severe motor impairments. The disease arises from bi-allelic mutations in the SMN1 gene, which encodes the Survival of Motor Neuron (SMN) protein. Children affected by SMA confront challenges in essential motor functions such as crawling, walking, breathing, and swallowing.

The SMN protein, traditionally known for its pivotal role in RNA processing and splicing, has recently emerged as a multifaceted participant in various cellular processes. Within the cell nucleus, SMN localizes in Cajal bodies (CBs), known to associate with nucleoli—the central hubs for ribosomal biogenesis. Nucleoli play a critical role in ribosomal DNA (rDNA) transcription, orchestrated by RNA Polymerase 1 (RNAP1), and early ribosomal RNA (rRNA) maturation. Perturbations in nucleolar organization can significantly impact ribosome biogenesis, subsequently affecting proper protein production.

Dynamic alterations in nucleolar structure have been observed in response to DNA damage, a phenomenon where nucleolar DNA and proteins relocate to the periphery of the nucleolus.

The restoration of the nucleolar organization occurs only upon the completion of DNA repair processes. Our recent findings have shed light on the crucial role of SMN in orchestrating the restoration of nucleolar structure post DNA repair completion, with SMN shuttling from CBs to the nucleolus during nucleolar DNA repair-driven reorganization [1].

While these discoveries have provided a foundational understanding of nucleolar organization after DNA damage, numerous molecular aspects of this process remain undisclosed. This research project aims to unravel the precise molecular mechanisms underlying nucleolar homeostasis orchestrated by SMN and its partners.

The protein Fused in Sarcoma (FUS) has become a focal point of our investigation, prompted by its known physical interaction with SMN as previously reported. FUS, recognized as a ubiquitously expressed DNA/RNA binding protein, plays a multifaceted role in RNA processing and remarkably in DNA repair of DNA Double Strand Breaks and oxidative damage. The link between FUS and neurodegenerative disorders is underscored by its association with Amyotrophic Lateral Sclerosis (ALS), where mutations in FUS have been identified. Significantly, ALS shares similar phenotypes and patho-mechanisms with SMA, amplifying the importance of unraveling the intricate relationship between SMN and FUS in the context of nucleolar homeostasis.

In the initial stages of our investigation, immunofluorescence (IF) assays provided crucial insights into the dynamic localization of FUS in response to DNA damage. Without damage, FUS predominantly localizes in the nucleoplasm. However, following DNA damage (3-6 hours post-UV-irradiation: PUVI), we observed a distinct accumulation of FUS at the periphery of the nucleolus. Strikingly, after completion of DNA repair, FUS returned to its original localization within the nucleoplasm.

An interesting observation was made during these experiments, as FUS's accumulation at the nucleolar periphery was found to be independent of SMN. This hinted at a potential upstream role of FUS in influencing SMN dynamics during the DNA repair process.

To validate this intriguing result, we employed siRNA-mediated knockdown of FUS. Remarkably, when FUS was depleted, we observed a significant impact on SMN shuttling, indicating an interdependence between FUS and SMN in this cellular process. Additionally, the recovery of RNAP1 to its proper position within the nucleolus after DNA repair was compromised in the absence of FUS. These findings strongly support the notion that FUS plays a pivotal role in nucleolar homeostasis after DNA repair, establishing it as a major protein of interest in our ongoing research.

Given the significant insights gleaned from our preliminary findings on FUS (as detailed earlier), we will accord special priority to the investigation of FUS in nucleolar homeostasis and its relationship with SMN in this newly identified SMN function. To facilitate this study, we will generate an inducible shFUS cell line. Additionally, we plan to create and express FUS-GFP constructs, allowing us to track the dynamic behavior of FUS in living cells both after DNA damage induction and during the DNA repair process. This focused approach aims to unravel the intricate interplay between FUS and SMN in the context of nucleolar homeostasis.

Modèle et techniques utilisées :

We aim herein to translate our results in a relevant cellular model for SMA and ALS, namely differentiated post-mitotic cells: motoneurons. In these cells, we will verify whether the absence of SMN and FUS impairs nucleolar reorganization and we will verify in WT cells whether SMN and FUS shuttle within the nucleolus after DNA damage. For this scope, we have recently produced two iPSC cell lines that express inducible shRNA against SMN and selected different clones with distinct steady state levels of SMN. These new inducible shSMN iPSC lines will allow to mimic SMN levels found in patients. In fact, residual functional SMN levels are known to be inversely associated with the severity of SMA disease. We will produce shFUS iPS cells in the same manner.

Techniques frequently used: Cell culture, Immunofluorescence, Western Blot, Confocal Microscopy, FRAP, iPS differentiation into motoneurons.

Publications d'intérêt :

1. S. Musawi *et al.* (2023) *Nat Commun* doi:10.1038/s41467-023-42390-4