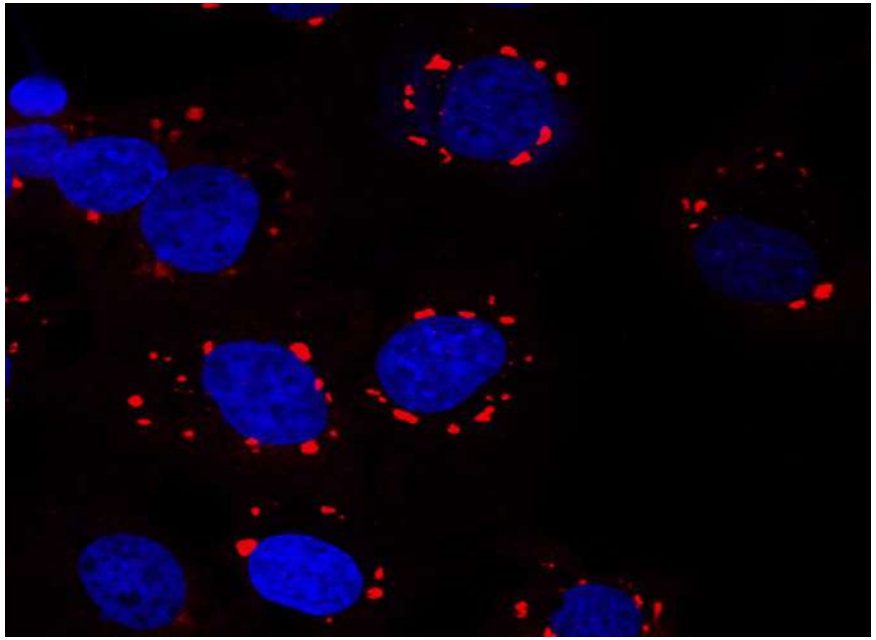
DEFINING THE COMPONENTS STRESS GRANULES *IN VITRO* and *IN VIVO* CONDITIONS

Gilles M *(1), Roger K (2), Renault S (1), Pasho E (1), Jung V (3), Guerrera C (3), Ciura S (1) and Kabashi E (1)*

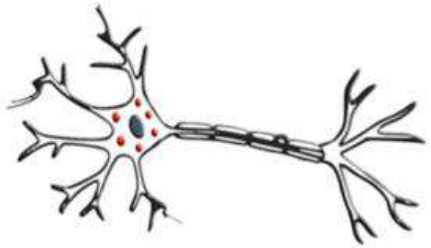
(1) INSERM U1163, Institut Imagine, University Paris Descartes, Hospital Necker-Enfants Malades, Paris (France)
(2) Faculté de Médecine, Université de Paris, Paris (France)



1. INTRODUCTION

1. What are stress granules ? :

Stress granules (SG) are cytoplasmic protein-RNA aggregates assembled in response to a cellular stress, and associated to the development of several neurodegenerative diseases including Amyotrophic Laretal Sclerosis (ALS) but their role in the pathogenesis is still uncompletely understood.



Persistent granules → Neurodegeneration

2. AIM : Defining new stress granules components

→ **To develop a new method of SG purification working *in vitro* and *in vivo*.**

→ **To validate new candidates / compare with chronic-like stress condition.**

Tools : G3BP1 was used as stress granules marker.
Stress granules activators : Sodium arsenite, Heat shock or bortezomib (proteoasome inhibitor).

2. METHODS

1. SH-SY5Y cells stably overexpressing G3BP1-GFP

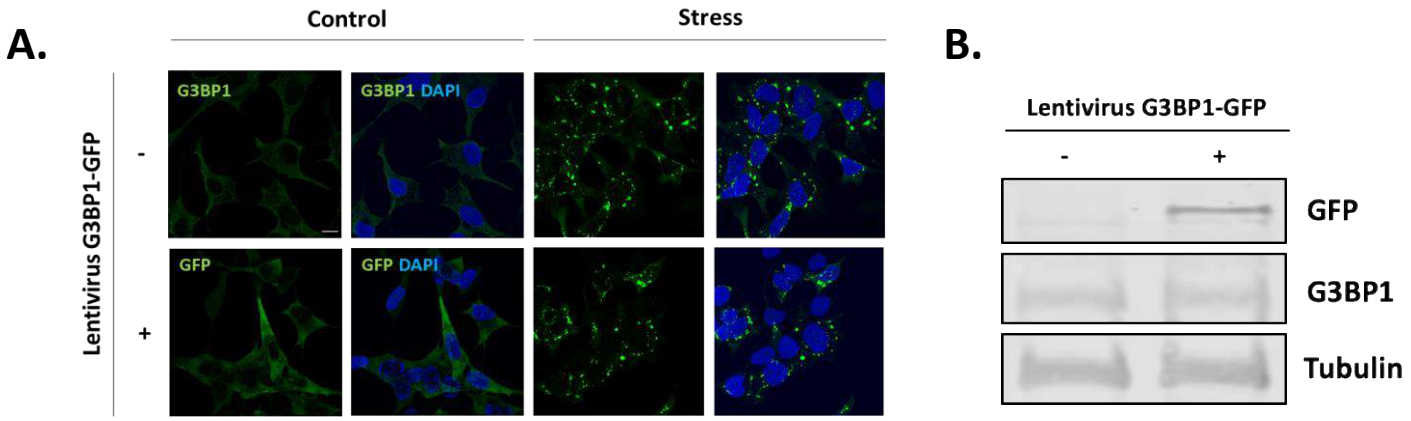
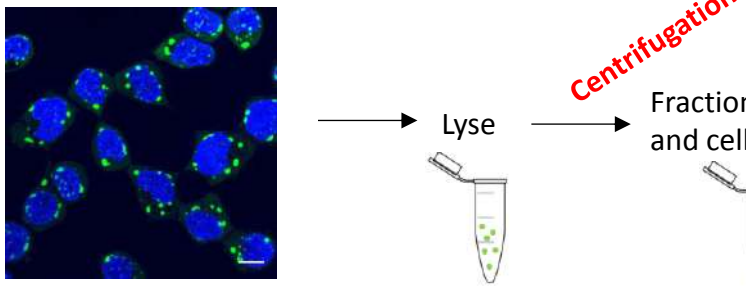


Figure 1 : (A) SH-SY5Y cells were infected (+) or not (-) with lentivirus containing G3BP1 fused to GFP. G3BP1-GFP stress granules are observed only in stress condition (green spots) while a diffuse staining is shown in control condition. Spinning-disk image, scale bar 10µm **(B)** Western blot validating G3BP1-GFP expression only in lentivirus G3BP1-GFP (+) condition.

2. FAPS method – experimental setup



Cells with fluorescent SG

3. RESULTS : Proteomic analysis of FAPS stress granules purification

Figure 2 : Validation of stress granules purification by FAPS method. Volcano-plot showing the high level of proteins purified in stressed condition (arsenite or heat shock) compared to the control. Already known stress granules proteins are highlight in red and are mainly present in stressed condition. Same results have been found in U2OS G3BP1-GFP cell line.

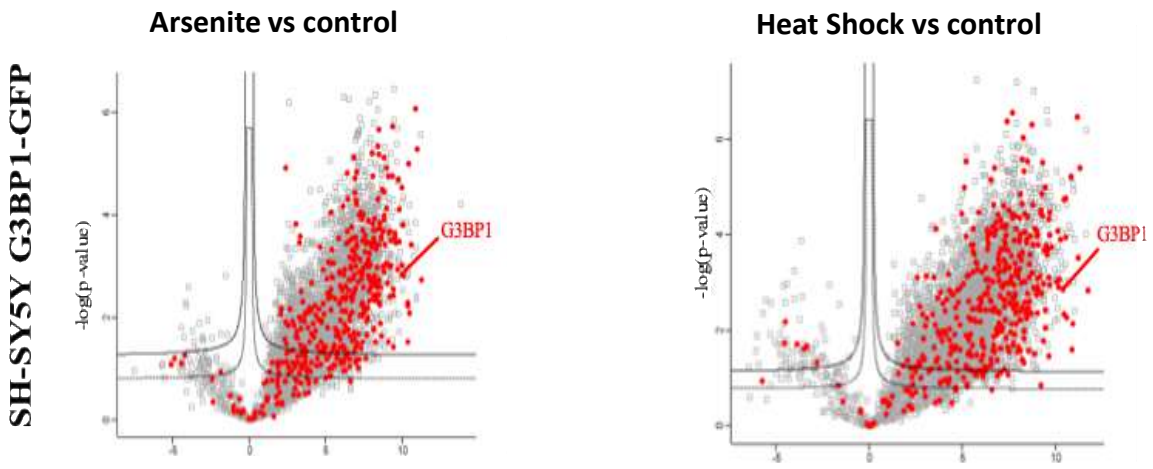


Figure 4. ALS genes in FAPS stress granules. Around 25% of ALS genes have already been found to localize in stress granules. Using the FAPS method we show that the majority of ALS genes (60%) represent stress granule components that we are validating in ALS models.

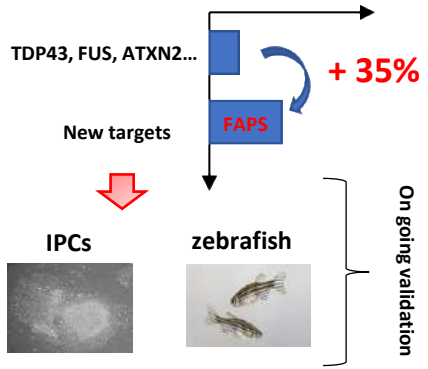
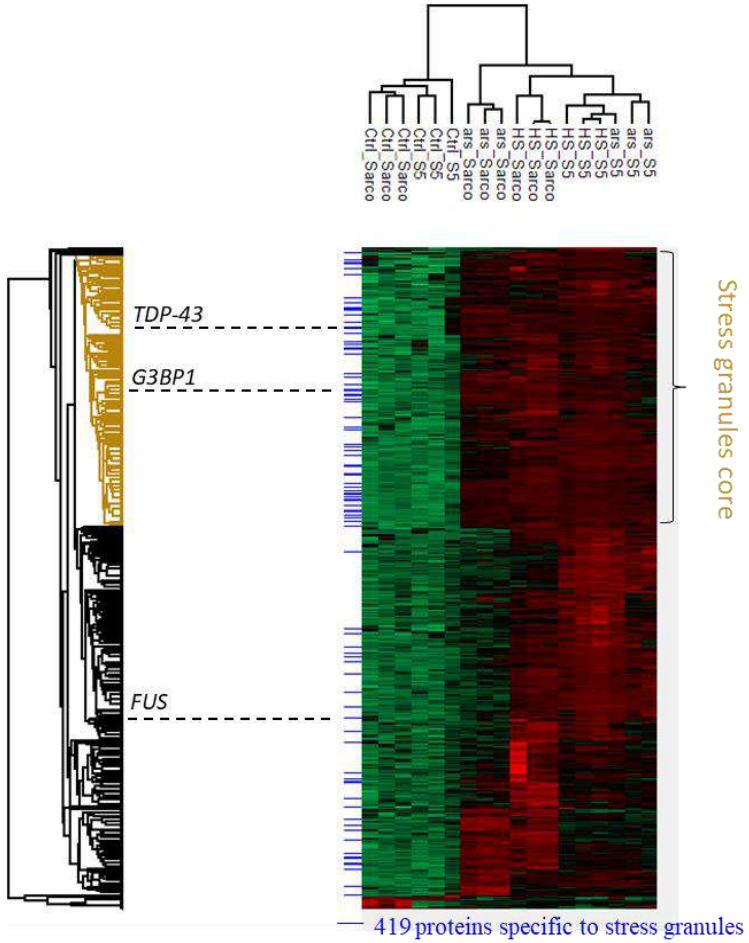
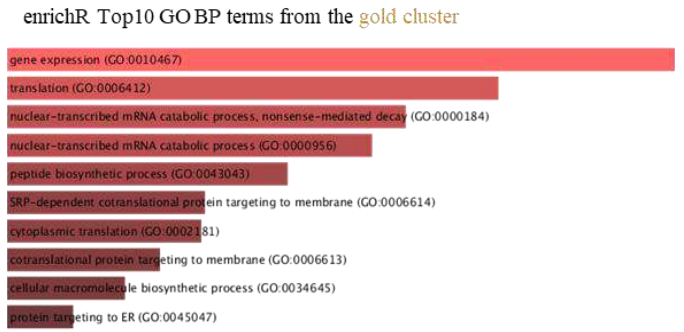


Figure 3 : A commun pool of proteins identified in both cells/stress reveals a stress granule core. Heatmap revealing a cluster of proteins upregulated in the both stressed cell lines (gold cluster) corresponding to the core of the stress granules. Already known proteins are mainly found in the gold cluster (blue proteins) as G3BP1 and TDP43. Biological Process enrichment has been done for the gold cluster showing a main involvement in gene expression and translation. Fus is present in the black cluster (cells or stress specific proteins). (Ctrl : control condition, ars : arsenite, HS : heat shock, sarco : U20S cells and S5 : SH-SY5Y).





3. RESULTS : Impact of stress granules formation on zebrafish model

Figure 5 : Establishment of G3BP1-GFP knockin zebrafish model. Schematic representation of the gene editing strategy used to insert GFP into zebrafish G3BP1 locus.

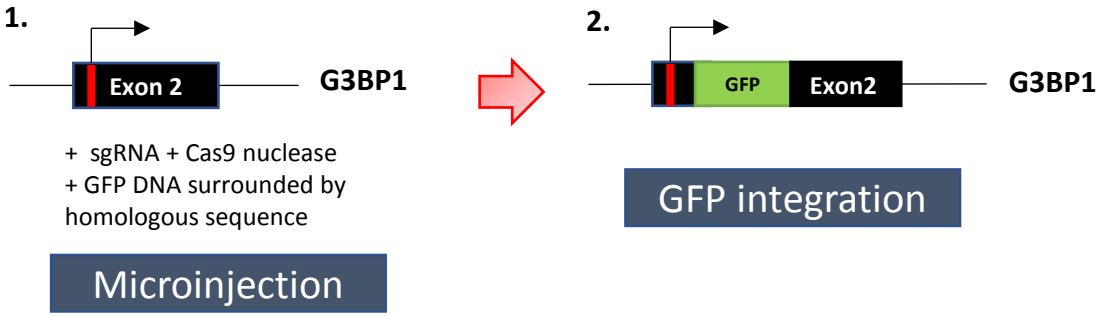


Figure 6 : 24 hours stress induces G3BP1 aggregates in zebrafish brain area. Zebrafish embryos at 24hpf were dechorionated and treated with bortezomib at a final concentration of 50µM for 24 hours. At 48hpf, a TEER test was done (ref **fig 7**), then they were fixed and immunostaining was realized on sagittal sections. G3BP1 aggregates are present in brain area only in stressed condition. Same results have been obtained for spinal cord sections.

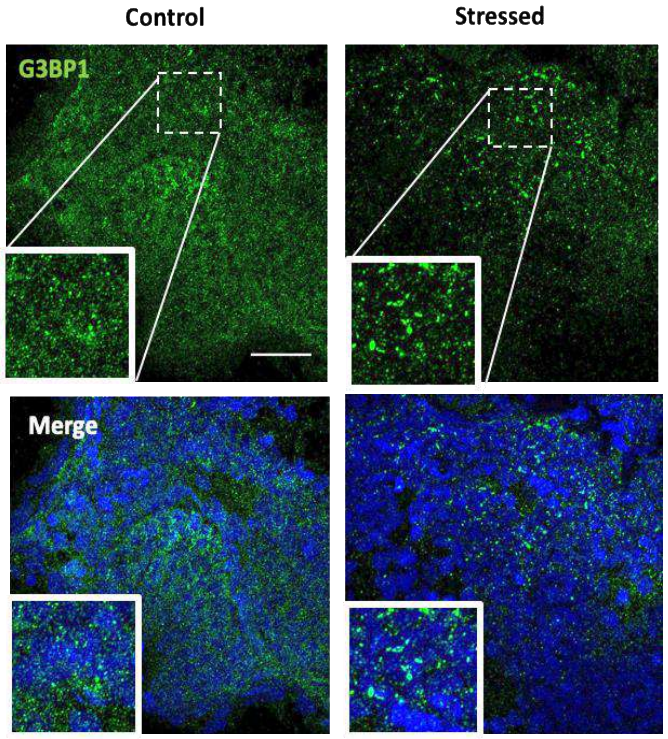
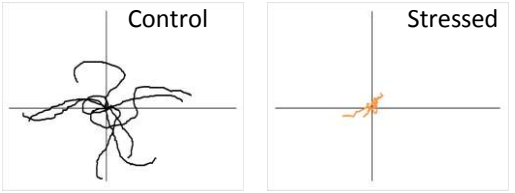
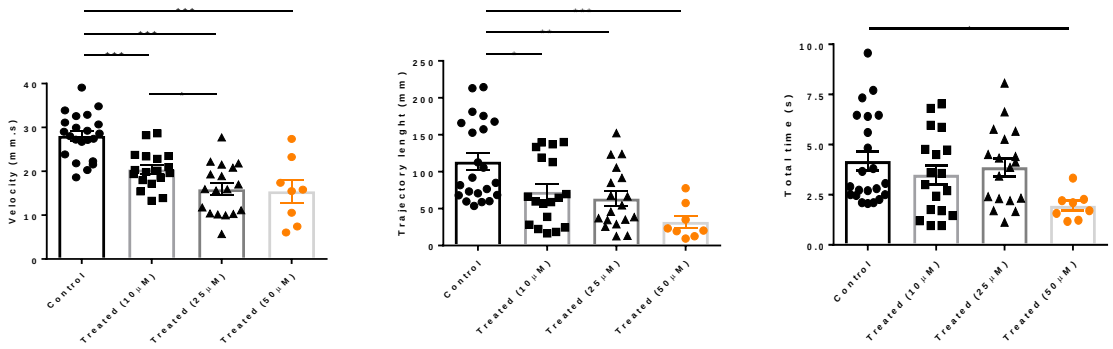


Figure 7 : 24 hour stress induces motor phenotype in zebrafish. (A) Representative swimming trajectories from TEER test showing a strong disability in stressed condition. **(B)** TEER test results for control and stressed conditions showing a significant decrease of the velocity, trajectory lenght and total time of swim for treated fish 50µM (orange color) compared to the control.

A.



B.



4. CONCLUSION

This study permits the development of a new method of stress granules purification which could be adaptable in our zebrafish model. The results should allow us to better understand stress granules role in ALS as well as to emerge new potential therapeutic targets.