PhD Research Proposal Form
China Scholarship Council (CSC) - ENS Group

FIELD: ----------
(eg: Mathematics, Physics, Sociology, ….)

Thesis subject title: Characterizing micronuclei diversity

Name of the French doctoral school : BMIC

Name of the Research team : Ontogenesis and Molecular Interactions
Website : http://igfl.ens-lyon.fr/equipes/s.-merabet-ontogenesis-and-molecular-interactions

Name of the Supervisor : Samir MERABET/Frédéric MARMIGERE
Email : frederic.marmigere@ens-lyon.fr

Lab Language : English/French

Research Proposal Abstract :

Although identified more than a century ago, micronuclei have extensively and mostly been used as end-points to assess genotoxicity and as biomarkers of DNA damage[1–4]. Micronuclei are small nuclear structures poorly characterized aside of the nucleus and arise from lagging chromosomes or chromosome fragments caused by mitotic errors or DNA damage[2]. However, recent discoveries associating micronuclei formation and rupture in different physiological and pathological conditions renewed the interest in micronuclei biology. Now, micronuclei can be pictured as transient cellular organelles involved in normal and pathological cellular functions. Micronuclei frequency progressively increases with age in various tissues including the nervous system[5,6], and there are accumulating evidences that they contribute to carcinogenesis and metastasis[7] but also to normal embryonic development[8–10]. In the nervous system, the role of micronuclei is just starting to be unravelled. Their number are increased in several neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases(Migliore et al. 2011; Sharma et al. 2020). Interestingly, they were also detected in the cerebral cortex and hippocampus of healthy young adult mice[14], suggesting that they also participate to the normal development and function of the nervous system.

Micronuclei are small intracellular structures that contain DNA surrounded by a nuclear membrane. Their diameter ranges from very small micronuclei (0.5–1 µm) to large micronuclei (10-15 µm) depending on whether they carry portions of chromosomes or full chromosome. Beside this variety in size and DNA content, sparse data from the literature indicates that micronuclei also exhibit a great diversity in fate, behaviour and molecular contents, suggesting that they participate to various biological and physiological processes. For instance, the nuclear protein LaminB is differentially expressed among micronuclei, and micronuclei bearing LaminB expression are transcriptionally active[15,16]. Some are expulsed from the cytoplasm and may have paracrine functions, whereas others undergo rupture within the cytoplasm and activate innate immune pathways. Some stand in the cell cytoplasm during the whole cell life or re-integrate the nucleus to induce chromotripsis[4]. Thus,

micronuclei exhibit a large diversity that has to date been poorly investigated, and studies on micronuclei function is hampered by the lack of knowledge on their molecular identity.

Using **Bimolecular Fluorescence Complementation (BiFC)** to identify new Meis2 interactors, we found that Meis2 induces micronuclei formation in HEK cells. Meis2 is a transcription factor with key functions in embryonic development and neurogenesis. Our results indicate that Meis2 differentially regulate micronuclei numbers depending on the Meis2 alternatively spliced-isoforms but also the transcriptional co-factors suggesting that specific transcriptional complexes are carried together with their target DNA within micronuclei. Our BiFC approach allowed thus to visualize micronuclei in an unprecedented manner, opening wide possibilities to investigate in details their architecture, behaviour, molecular contents and functions. The goal of the PhD work is to better characterise micronuclei diversity and during the process of neurogenesis in vitro, and is divided in 3 aims. Aim 1: Set-up a method to **purify micronuclei** from HEK and a neurogenic cell line using **BiFC** combined with sucrose gradient separation and **FACS sorting** as recently reported[17].

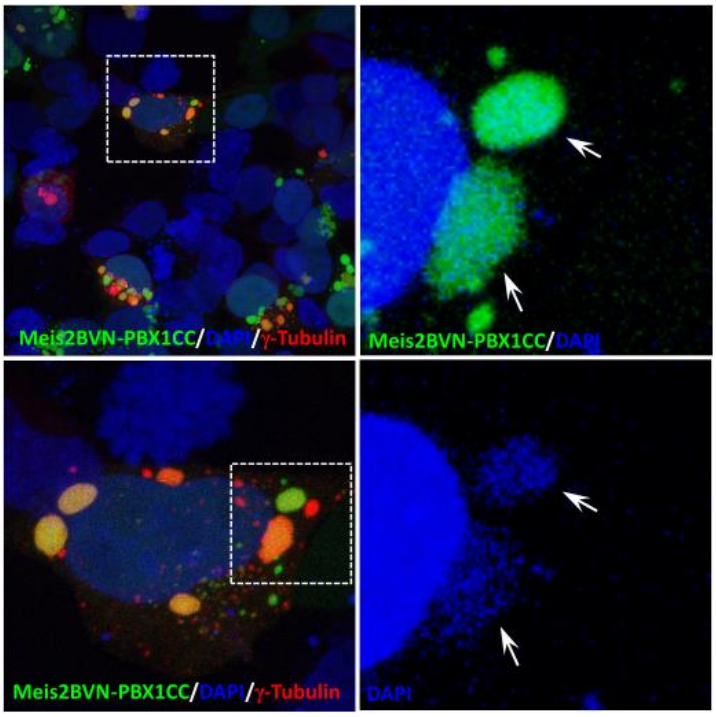

Figure 1: Visualisation des micronuclei par BiFC

Aim2: Characterize the molecular signature of micronuclei and their behaviour in different conditions and different cell lines. Upon successful micronuclei purification, their proteins, DNA and RNA contents will be characterized and compare using **proteomic approaches**, **RNAseq** and **DNAseq**. Their behaviour will be investigated by **live-imaging**. Aim3: to correlate and investigate micronuclei function during in vitro neurogenesis and neuronal differentiation. The results product will help better understanding of carcinogenesis, ageing and development of the nervous system.

References :

1. Krupina, K., Goginashvili, A. & Cleveland, D. W. Causes and consequences of micronuclei. *Current Opinion in Cell Biology* vol. 70 91–99 (2021).
2. Kwon, M., Leibowitz, M. L. & Lee, J. H. Small but mighty: the causes and consequences of micronucleus rupture. *Experimental and Molecular Medicine* vol. 52 1777–1786 (2020).
3. MacIejowski, J. & Hatch, E. M. Nuclear Membrane Rupture and Its Consequences. *Annual Review of Cell and Developmental Biology* vol. 36 85–114 (2020).
4. Hintzsche, H. *et al.* Fate of micronuclei and micronucleated cells. *Mutat. Res. Mutat. Res.* **771**, 85–98 (2017).
5. Miller, K. N. *et al.* Cytoplasmic DNA: sources, sensing, and role in aging and disease. *Cell* vol. 184 5506–5526 (2021).
6. Laffon, B., Bonassi, S., Costa, S. & Valdiglesias, V. Genomic instability as a main driving factor of unsuccessful ageing: Potential for translating the use of micronuclei into clinical practice. *Mutation Research - Reviews in Mutation Research* vol. 787 (2021).
7. Guo, X., Dai, X., Wu, X., Cao, N. & Wang, X. Small but strong: Mutational and functional landscapes of micronuclei in cancer genomes. *International Journal of Cancer* vol. 148 812–824 (2021).
8. Wang, J. *et al.* Comprehensive Chromosome End Remodeling during Programmed DNA Elimination. *Curr. Biol.* **30**, 3397-3413.e4 (2020).
9. Chmielewska, M. *et al.* The programmed DNA elimination and formation of micronuclei in germ line cells of the natural hybridogenetic water frog Pelophylax esculentus. *Sci. Rep.* **8**, (2018).
10. Dedukh, D. *et al.* Micronuclei in germ cells of hybrid frogs from Pelophylax esculentus complex contain gradually eliminated chromosomes. *Sci. Rep.* **10**, (2020).
11. Migliore, L., Coppedè, F., Fenech, M. & Thomas, P. Association of micronucleus frequency with neurodegenerative diseases. *Mutagenesis* vol. 26 85–92 (2011).

12. Droppelmann, C. A., Campos-Melo, D., Moszczynski, A. J., Amzil, H. & Strong, M. J. TDP-43 aggregation inside micronuclei reveals a potential mechanism for protein inclusion formation in ALS. *Sci. Rep.* **9**, (2019).
13. Sharma, M., Rajendrarao, S., Shahani, N., Ramírez-Jarquín, U. N. & Subramaniam, S. Cyclic GMP-AMP synthase promotes the inflammatory and autophagy responses in Huntington disease. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 15989–15999 (2020).
14. Yano, S. *et al.* A MATLAB-based program for three-dimensional quantitative analysis of micronuclei reveals that neuroinflammation induces micronuclei formation in the brain. *Sci. Rep.* **11**, (2021).
15. Okamoto, A., Utani, K. I. & Shimizu, N. DNA replication occurs in all lamina positive micronuclei, but never in lamina negative micronuclei. *Mutagenesis* **27**, 323–327 (2012).
16. Utani, K. I., Kawamoto, J. K. & Shimizu, N. Micronuclei bearing acentric extrachromosomal chromatin are transcriptionally competent and may perturb the cancer cell phenotype. *Mol. Cancer Res.* **5**, 695–704 (2007).
17. Toufektchan, E. & Maciejowski, J. Purification of micronuclei from cultured cells by flow cytometry. *STAR Protoc.* **2**, (2021).

Type of PhD :

1.Full PhD

- Joint PhD/cotutelle (leading to a double diploma) : NO
- Regular PhD (leading to a single French diploma) : YES

2. Visiting PhD (for students enrolled at a Chinese institution who will be invited to a French institution to carry out a mobility period) : NO