Actin-based alterations of nuclear dynamics

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Introduction

This PhD project addresses how **actin-based forces applied to the exterior of the nucleus affect internal dynamics, including nuclear condensate remodeling, chromatin reorganization and changes in gene expression in the context of cell differentiation.** Indeed there is mounting evidence that perinuclear actin impacts DNA dynamics and phase separation in the nucleus, which in turn can affect gene expression (*1-4*). Forces produced by perinuclear and cytoplasmic actin cytoskeleton are, in many cases, transmitted to the nucleus via the LINC (LInker of Nucleoskeleton and Cytoskeleton) proteins, nesprin and SUN, which attach the lamin nucleoskeleton to the actin and microtubule cytoskeletons of the cell (*4-7*).

We will work with primary radial glial cells (RGCs) from mouse pups. Preliminary results of Nathalie Delgehyr/Alice Meunier suggest that RGC differentiation into multiciliated ependymal cells (ECs) depends on nuclear deformation through actin reorganization (Figure 1). This is dependent on the actin polymerization nucleator, the Arp2/3 complex, and the LINC complex. **In this PhD project, we will confirm and characterize further this result in mouse brain and in cultured primary RGCs (Task 1).** However a caveat of observations such as these in living animals and cells is that impairing actin dynamics affects many other cell processes (e.g. cell adhesion, endocytosis, motility). To side-step this problem, we will **isolate nuclei from cultured RGCs and subject them to controlled actin-based forces in a microfluidic device (Task 2).**

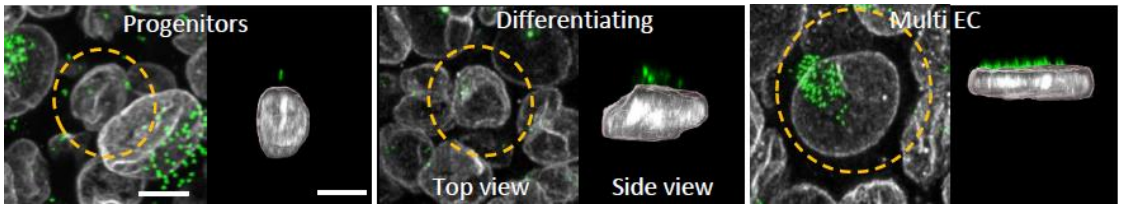

Figure 1: Nuclear lamin (grey) and centrioles (green) at different stages of RGC differentiation to multiciliated ECs. Top views are maximum projections of z-stacks, and side views are Imaris 3D reconstitutions of the differentiation RGC nuclei (orange dashed lines). Scale bars 5 μm.

What we seek in this PhD project, using a dual *in vivo/in vitro* approach, is clear evidence that actin dynamics on or around the nucleus surface can produce forces that change what is going on inside the nucleus, and contribute to cell differentiation.

Methodology

Task 1: Actin-based alterations of the nucleus in differentiating RGCs *in vivo* and in primary cultures.

We will address how the nucleus deforms and contributes to EC differentiation *in vivo* directly in the brain of animals by perturbing the actin cytoskeleton and the LINC complex at differentiation onset and assessing the consequences on nuclear morphology (lamin staining), and differentiation (centriole amplification or transcription factor/cell cycle markers). Given our preliminary result that knocking down the Arp2/3 complex impairs nuclear deformation and differentiation, we will analyze mutant mice lacking the Arp2/3 complex inhibitor Arpin (already obtained from the Lennon-Duménil lab) to see whether increasing actin enhances nuclear deformation and accelerates differentiation. Other preliminary results using a dominant negative construct of the LINC complex (KASH-domain) to sever the nucleus-actin connection blocks nuclear deformation and differentiation without affecting actin organization in RGCs, suggesting that nuclear

deformations, via the LINC complex, trigger EC differentiation. We will further characterize this result via shRNA against specific LINC components.

It is known that changes in DNA methylation are associated with differentiation processes (*8*). Some of these changes, such as the H3K9me2-3 transition to H3K27me3, have been shown to occur upon nuclear deformation mediated by actin, softening chromatin to avoid DNA breakage (*9*). We will characterize chromatin modifications in differentiating RGCs, and assess whether methylation changes are dependent on actin-nuclear interactions. We will also prevent histone methylations by performing shRNA against methylases, and observe the effect on nuclear dynamics and RGC differentiation. Finally, these methylations are in part dependent on nuclear condensates such as HP1 (H3K9me2-3) (*10*) or PRC2 (H3K27me3) (*11*), and nuclear condensates are known to be influenced by nuclear deformation (*3*). To examine this in our system, we will transfect cultured RGCs with markers for nuclear condensates such as nucleoli, nuclear speckles, HP1 or PRC2 to observe changes in these compartments over the course of differentiation and if this is affected by altering actin and/or actin-nuclear attachment.

This part of the project will evaluate nuclear deformation induced by the actin cytoskeleton *in vivo*, how this affects DNA and nuclear condensate reorganization, and how reorganization correlates with differentiation.

Task 2: Actin polymerization on and around nuclei to produce nuclear modifications *in vitro*

To analyze the direct role of actin on nuclei, a prerequisite is to isolate minimally perturbed nuclei for *in vitro* manipulation. So far, the Delgehyr/Meunier team has employed two different purification approaches using primary cultures of RGCs: cell lysis via hypotonic buffer with a sucrose cushion to remove cell debris (*12*) and cell lysis by enucleation, involving centrifugation of adherent cells in a home-made slide holder device, giving plasma membrane-wrapped nuclei (nucleoplasts) (*13*). In the first condition, the nuclear membrane is accessible but exposed to mechanical insults, while the second condition protects the nuclei and prevents passive diffusion of molecules (including actin) through nuclear pores, but covers the native nuclear membrane.

To handle a large number of isolated nuclei, the Yamada team has developed a novel fabrication protocol to make microfluidic chips that allow single nuclei to be collected in microwells, permitting exchange of solutions with minimal perturbation and small volumes. This chip enables high throughput observation (4800 wells per channel, 2 parallel channels per chip) at high magnification, minimizing surface adhesion of nuclei and evaporation during hours of observation.

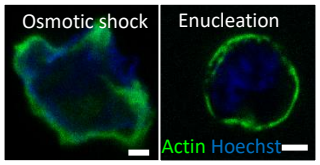

Figure 2: Actin polymerized on nuclei purified by osmotic shock (Left) or by enucleation (Right). Scale bar = 2μm Delgehyr, Plastino and Yamada, 2021.

To test whether actin polymerization on nuclei or in solution can alter internal nuclear dynamics, we will develop two approaches: targeted actin polymerization to nuclei surfaces and bulk actin polymerization. The first method is inspired by previous actin network reconstitution studies on beads/liposomes (an area of expertise for the Plastino team), which show that actin polymerization can deform soft surfaces and that changing the protein mix or the actin polymerization nucleator at the surface can change actin network properties and its effect on the surface *(14, 15)*. As a proof of concept, we have started such experiments on nuclei isolated from RGCs. Using a biotin-streptavidin link, the pVCA domain of human WASP that activates the Arp2/3 complex was attached to the nuclei. Upon loading into the microfluidic chip and then injection of an actin polymerization mix containing fluorescently labeled actin monomers, we observed the growth of an actin layer around the nuclei, attaining a thickness 0.2-1 μm over the course of 1 hour (Figure 2). Using nuclei purified by osmotic shock, we observed nuclei fragmentation. However, this could be a consequence of actin polymerization inside the nuclei, as observed in the star fish oocyte *(16)*. To overcome this issue, we used nuclei isolated by enucleation, and observed more subtle deformations (Figure 2).

During this PhD project, we will continue these experiments, and also employ a second approach where nuclei in microwells are embedded in growing and static actin networks that are not

attached to the nuclei surface. We will then inject myosin, with or without different actin filament bundlers, to assess whether actin or actomyosin networks similar to those in the cell cytoplasm are capable of altering nuclei shape and internal organization.

In all cases of actin polymerization on and around nucleoplasts from RGCs, we will assess changes in DNA packaging, nuclear condensate dynamics and gene expression changes. As concerns the first point, our preliminary results suggest that actin polymerization on the nucleus induces a reorganization of the DNA towards a less dense state, suggesting a decrease in heterochromatin (Figure 3). This will be confirmed by staining with markers of modified histone. Using nuclei isolated from the transfected cultured RGCs mentioned in Task 1, carrying markers for nuclear condensates, we will observe how these compartments are altered by actin polymerization on/around the nuclei.

A final part of the PhD project will address how deforming the nucleus changes gene expression, evaluated via mRNA sequencing. We will coat a large number of nuclei with actin (either at the surface of the nuclei or in the bulk) in the microfluidic chip in order to isolate their mRNA, sequence it and compare it with the mRNA of unperturbed nuclei. A single channel of the chip allows the simultaneous treatment of about 4800 nuclei. Considering that a cell has around 10 to 30 pg of mRNA of which 10% is in the nucleus, the lysis of the nuclei in the chip should allow for the collection of a few ng of total mRNA, an amount sufficient to run mRNA sequencing, in collaboration with the genomic platform of ENS. We will look, in particular, for genes known to protect the genome, to change nuclear properties and for genes important for differentiation into ECs.

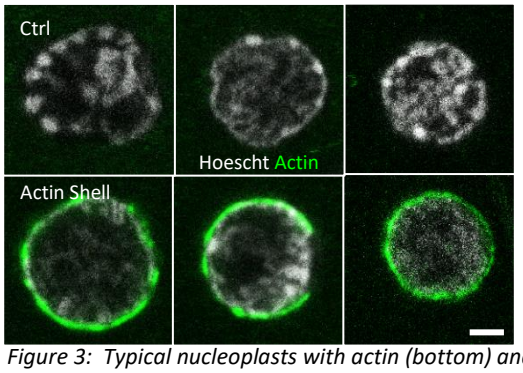

Figure 3: Typical nucleoplasts with actin (bottom) and without actin (top) polymerizing on their surfaces. Actin in green and Hoechst in white. Right shows quantification of the size of the Hoechst dense staining, which overall are significantly smaller in the presence of the actin shell. Scale bar=2 μm. Delgehyr, Plastino and Yamada, 2022.

This part of the project will evaluate *in vitro* how actin cytoskeleton affects DNA and nuclear condensate reorganization in the nucleus, and how these changes are reflected in gene expression alterations.

References

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