Programme Doctoral Interfaces pour le Vivant

Implicating the LINC complex in collective muscle cell behavior

Context: Heart and skeletal muscles are characterized by specific patterns of collective cell organization, decisive for their function. Force generation relies on the contractile features of the myofibers constituting the muscle tissue. The myofibers are long multinucleated cells resulting from the fusion of precursor myoblasts (**Fig 1A**). They contain contractile myofibrils organized in periodic segments called sarcomeres, which are composed of actin and myosin filaments parallel to the myofiber axis. Emergence of certain order of organization at the collective level is thus paramount in muscle tissue formation and regeneration, and essential to proper force generation. In various biological systems the study of the organizational properties led to an analogy with a well-known phase of physical systems called ``liquid crystals", in particular nematics[1]. Importantly, the orientational order in these active systems is established and maintained across a wide range of scales, from multicellular organisation at the tissue level[2,3] all the way down to subcellular cytoskeleton of the cells[4,5]. Such properties are particularly relevant in the context of muscle assembly. The differentiation process is characterized not only by major changes in collective cell properties, in particular with the formation of myoblasts domains that precede formation of the corresponding myotubes (**Fig 1A**), but also in cytoskeletal organization (**Fig 1B**), with modifications partially driven by the nucleus. The nuclear envelope (NE) is indeed able to interact with both the actin and the microtubule networks through the linker of nucleoskeleton and cytoskeleton (LINC) complex[6]. We have shown that disruption of the LINC complex leads to cytoskeletal disorganization and impaired nuclear movements, resulting ultimately in hindered contraction, characterizing multiple muscle disorders[7–9]. However, the relationship between collective cell behavior and cytoskeletal organization regulation remains unaddressed in the muscle field.

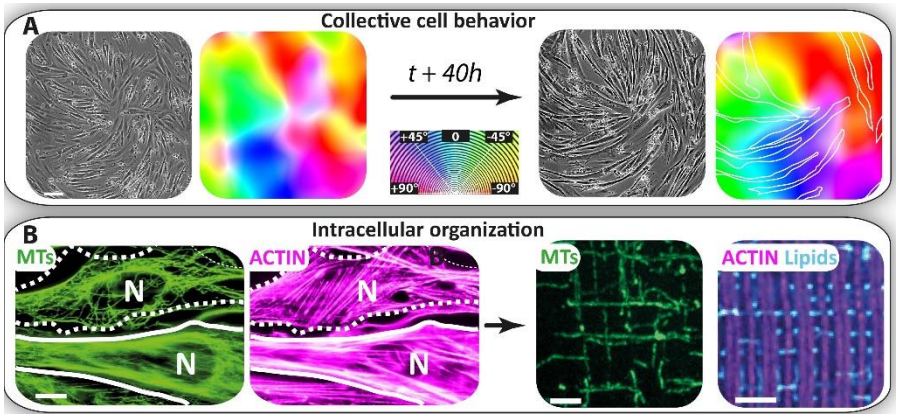

Figure 1: *(**A**) Cultured myotubes are formed in domains where myoblasts had the same orientation (color-coded based on angle).* Bar= 50µm. *(**B**) Microtubules (MTs) and actin reorganize during differentiation from a myoblast (dashed line) to a myotube (continuous line). N*=Nucleus, bar= 10µm. *Strong parallel arrays are formed in mature fibers (right).* Bar= 2µm.

Objectives and working hypothesis:

The PhD project aims at understanding the interplay between the regulation of intracellular architecture and supracellular organization to decipher how skeletal and cardiac muscles are formed and function properly. We hypothesize that intracellular organization driven by the LINC complex is determinant for muscle collective cell arrangement and plays a role in its function. The modeling of cellular assemblies and cytoskeleton by liquid crystals physical equations opens up opportunities to understand cellular arrangement and tissue function.

The project combines complementary expertise ranging from muscle cell biology (**Supervisor1:** Dr Cadot), physics of cell populations (**Supervisor2:** Dr Ladoux) and tissue mechanics (**Co-Supervisor2:** Dr Trichet), and has the following objectives i/ understand the implication of the LINC complex on the cytoskeleton organization, ii/ determine the role of muscle cells population organization on the differentiation process, and iii/ correlate organization at the mesoscale with cell population organization.

Research Plan:

I.Link between collective cell behavior and cytoskeleton organization

i. Following **Supervisor2** recent work, we hypothesize that muscle cells can self-organize in emergent patterns to control their interactions and the fusion process. Comparing 2D muscle organization with active nematics, the PhD student will focus on the emergence of domains of cellular alignment (**Fig A**). He/she will thus correlate collective muscle cell behaviors with differentiation processes by following the evolution of domain sizes, formation of domain walls, local order breakdown, and assemblies of topological defects. He/she will characterize and model the sequential steps required for proper formation of myotubes. He/she will use live cell imaging, develop analytical tools including segmentation methods based on machine learning (**Supervisor2**), determine cell shapes, cell packing and tissue flows. He/she will combine these studies with force and stress measurements applied by cells on the substrate using techniques mastered by **Supervisor2**[3,10]. In parallel, with the **Supervisor1**, he/she will identify the changes in cytoskeletal organization, known to follow active nematics, using super resolution microscopy and live cell imaging. Then, cytoskeletal perturbations will be applied through drugs (*Nocodazole, Taxol, Latrunculin or Blebbistatin*) or modulation of known regulators (*Myosin, Arp2/3, Formins, microtubules motors)*, to correlate differentiation with collective cell and cytoskeletal organization.

ii. We postulate that muscle cell organization and domains of cellular alignment can be tuned by substrate geometry. He/she will thus investigate the impact of confinement-generated forces on cytoskeleton organization, by combining different adhesion pattern widths (**Co-Supervisor2**) with perturbation of the cytoskeleton. He/she will monitor cytoskeletal changes *(time-lapse super resolution microscopy)* during the differentiation process resulting in myotubes formation (**Supervisor1**). He/she will further combine micropatterning with force and stress measurements to determine the contribution of patterning in force load and myotube formation. *This part will define how cytoskeletal organization is tightly linked to the collective cell behavior required for myotube formation.*

II. LINC complex-related mechanisms involvement in muscle cell organization

i. **Supervisor1** has shown that disruption of the nucleo-cytoskeleton connection negatively impacts normal myotube organization[11], but that it rescues the dilated cardiomyopathy and damaged nuclear phenotypes of the *Lmna*^{KO} mice (submitted research paper). Interestingly, an increase in homogenous cell alignment in diseased hearts appears to be detrimental for their function[12]. Using the tools developed in the first part, he/she will follow time-dependent myoblasts collective alignment and myotubes formation in situations where the LINC complex is impaired (*Knock-down, overexpression of Lamins, Nesprins, SUNs or dominant-negative KASH domain*). He/she will investigate the effects on cytoskeleton organization (*EB3-GFP, Utrophin-RFP*), but also the consequences on nuclear deformation and force production and active nematic characteristics of these multicellular systems.

ii. Based on our preliminary results indicating that the removal of forces applied on the NE can rescue fragilized NE, he/she will characterize several available pathological *in vitro* models, such as the *Lmna*$^{p.H222P}$, *Lmna*$^{p.dK32}$, *Nesprin-1*$^{23560G>T}$, Emerin^{KO} [11,13] for their collective cell and cytoskeleton organization. He/she will then remove LINC complex proteins one by one in these backgrounds to further decipher the relationship between the nucleo-cytoskeleton connection and collective cell organization. This will allow us to identify the best targets for a structural and functional rescue.

iii. To further understand the coupling between molecular components and tissue formation, he/she will determine if such organization is conserved in a context closer to a physiological situation. For this, he/she will use a microfabricated platform recently developed by **Supervisor2** and **Co-Supervisor2** to produce 3D muscle organoids between two flexible pillars[14]. This technology enables the control of the cells physical and chemical microenvironment and the monitoring of the forces produced by the obtained microtissues during spontaneous contractions, with the possibility to recapitulate pathological phenotypes and potential therapeutical strategies (**Fig 2**). The influence of mechanical parameters on the cellular alignment and possible formation of well-organized sheets of different orientations, like in the healthy heart as opposed to pathological backgrounds (*LMNA* and LINC complex mutations), will be

studied. This will allow us to determine how the modification of the nucleo-cytoskeleton connection has an impact on cell population organization and proper organ function.

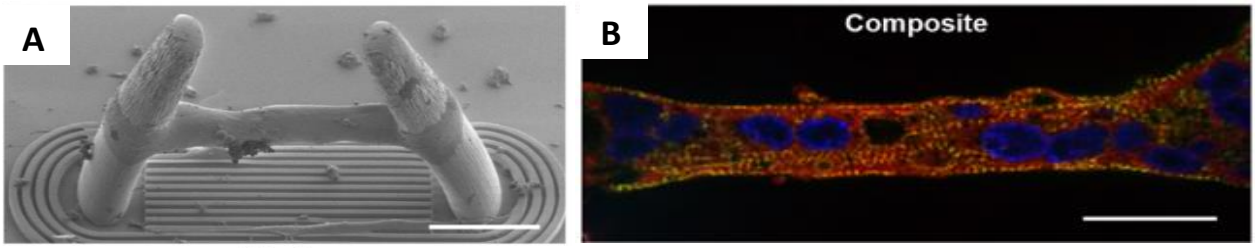

*Figure 2: **(A)** SEM image (tilted view 45°) of 3D myotubes obtained from human immortalized myoblasts after 7 days of differentiation. Scale bar = 50 µm. **(B)** A 3D myotube stained for Titin(red), a-Actinin (green) and Nuclei (Blue). Scale bar = 25 µm.*

The novelty of our approach lies in the observation of cellular processes at multiple scales, from the cytoskeletal organization to the arrangement of cells in the tissue, a parameter known to be decisive for the function. By combining biological and biophysical expertise, we aim at bringing novel and solid knowledge about the collective behavior of cells in heart and muscle. In fine, we expect to find new therapeutical strategies to fight severe diseases, in particular dilated cardiomyopathies, based on the modulation of mechanical forces applied to the nuclear enveloppe.

References (Publications from the supervisors are in red)

1. Doostmohammadi, A. & Ladoux, B. Physics of liquid crystals in cell biology. *Trends Cell Biol.* (2021). doi:10.1016/j.tcb.2021.09.012
2. Kawaguchi, K., Kageyama, R. & Sano, M. Topological defects control collective dynamics in neural progenitor cell cultures. *Nature* **545,** 327–331 (2017).
3. Saw, T. B., Doostmohammadi, A., Nier, V., Kocgozlu, L., Thampi, S., Toyama, Y., Marcq, P., Lim, C. T., Yeomans, J. M. & Ladoux, B. Topological defects in epithelia govern cell death and extrusion. *Nature* **544,** 212–216 (2017).
4. Maroudas-Sacks, Y., Garion, L., Shani-Zerbib, L., Livshits, A., Braun, E. & Keren, K. Topological defects in the nematic order of actin fibres as organization centres of Hydra morphogenesis. *Nat. Phys.* **17,** 251–259 (2021).
5. Trichet, L., Le Digabel, J., Hawkins, R. J., Vedula, S. R. K., Gupta, M., Ribrault, C., Hersen, P., Voituriez, R. & Ladoux, B. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. *Proc. Natl. Acad. Sci. U. S. A.* **109,** 6933–6938 (2012).
6. Crisp, M., Liu, Q., Roux, K., Rattner, J. B., Shanahan, C., Burke, B., Stahl, P. D. & Hodzic, D. Coupling of the nucleus and cytoplasm: role of the LINC complex. *J Cell Biol* **172,** 41–53 (2006).
7. Metzger, T., Gache, V., Xu, M., Cadot, B., Folker, E. S., Richardson, B. E., Gomes, E. R. & Baylies, M. K. MAP and kinesin-dependent nuclear positioning is required for skeletal muscle function. *Nature* **484,** 120–124 (2012).
8. Cadot, B., Gache, V., Vasyutina, E., Falcone, S., Birchmeier, C. & Gomes, E. R. Nuclear movement during myotube formation is microtubule and dynein dependent and is regulated by Cdc42, Par6 and Par3. *EMBO Rep.* **13,** 741–749 (2012).
9. Gache, V., Gomes, E. R. & Cadot, B. Microtubule motors involved in nuclear movement during skeletal muscle differentiation. *Mol. Biol. Cell* mbc.E16-06-0405 (2017). doi:10.1091/mbc.E16-06-0405
10. Nier, V., Jain, S., Lim, C. T., Ishihara, S., Ladoux, B. & Marcq, P. Inference of Internal Stress in a Cell Monolayer. *Biophys. J.* **110,** 1625–1635 (2016).
11. Gimpel, P., Lee, Y. L., Sobota, R. M., Calvi, A., Koullourou, V., Patel, R., Mamchaoui, K., Nédélec, F., Shackleton, S., Schmoranzer, J., Burke, B., Cadot, B. & Gomes, E. R. Nesprin-1α-Dependent Microtubule Nucleation from the Nuclear Envelope via Akap450 Is Necessary for Nuclear Positioning in Muscle Cells. *Curr. Biol.* **27,** 2999-3009.e9 (2017).
12. Masuyama, K., Higo, T., Lee, J.-K., Matsuura, R., Jones, I., Bakal, C., Higo, S., Morimoto, S., Miyagawa, S., Sawa, Y. & Sakata, Y. Homogeneous 2D and 3D alignment of cardiomyocyte in dilated cardiomyopathy revealed by intravital heart imaging. *Sci. Rep.* **11,** 14698 (2021).
13. Macquart, C., Jüttner, R., Morales Rodriguez, B., Le Dour, C., Lefebvre, F., Chatzifrangkeskou, M., Schmitt, A., Gotthardt, M., Bonne, G. & Muchir, A. Microtubule cytoskeleton regulates Connexin 43 localization and cardiac conduction in cardiomyopathy caused by mutation in A-type lamins gene. *Hum. Mol. Genet.* doi:10.1093/hmg/ddy227
14. Rose, N., Sonam, S., Nguyen, T., Grenci, G., Bigot, A., Muchir, A., Ladoux, B., Grand, F. L. & Trichet, L. *Bioengineering a Miniaturized In Vitro 3D Myotube Contraction Monitoring Chip For Modelization of Muscular Dystrophies*. 2021.06.15.448543 (2021). doi:10.1101/2021.06.15.448543