

Internship offer
M2 Musculo-Skeletal system, Locomotion, Exercise (MuSkLE)

Title of the Internship: Role of the reticulum-mitochondria Ca^{2+} coupling in the excitation-energetics coupling in adult rat cardiomyocytes

Laboratory: CarMeN - U1060, <https://carmen.univ-lyon1.fr/en/>



Research team: MERISM / future INTERACT team - Mitochondria-reticulum coupling, liver-heart crosstalk, and cardio-metabolic diseases (starts on 01/01/27); <https://carmen.univ-lyon1.fr/en/teams/team-merism/> (not updated)

Supervisor to contact: Dr Mélanie PAILLARD, melanie.paillard@inserm.fr

Project description:

Mitochondrial calcium (mtCa^{2+}) concentration plays a key role in the control of mitochondrial bioenergetics, by regulating the mitochondrial respiratory chain activity, ATP production and the activity of key mitochondrial dehydrogenases^{1, 2}. In the cardiomyocyte, mtCa^{2+} uptake has been shown to assure the **excitation-energetics coupling** by regulating mitochondrial ATP production to match the energy demand upon cell contraction^{3, 4}. Sarcoplasmic reticulum-mitochondria (SR-Mito) Ca^{2+} coupling is also involved in the control of the excitation-energetics coupling^{5, 6}, since mitochondrial metabolism is dynamically controlled by Ca^{2+} signals, which are propagated to the mitochondria locally at the SR-Mito contacts^{7, 8}. Indeed, the distribution of mitochondria in the cardiomyocytes is highly ordered with a strategic positioning close to the reticulum. **The mitochondrial Ca^{2+} uniporter (mtCU) represents here the key structure, which controls Ca^{2+} entry inside the mitochondrial matrix.** Importantly, a strategic **positioning of the mtCU at the SR-Mito interface** has been demonstrated to enhance the excitation-energetics coupling⁹.

Using an **obesogenic diet-induced mouse model of diabetic cardiomyopathy, reproducing the early clinical features of type 2 diabetic patients with heart failure with preserved ejection fraction**, our team has shown a defective reticulum-mitochondria Ca^{2+} coupling as an early, causal, and reversible trigger of cardiac alterations¹⁰, further confirmed in patient cardiac biopsies¹¹. How this reticulum-mitochondria Ca^{2+} miscommunication rearranges the excitation-energetics coupling remains underexplored.

The goal of this project is to **assess the role of each Ca^{2+} signaling players (IP3R, RyR, mtCU) in the excitation-energetics coupling** in adult rat cardiomyocytes under physiological and pathological conditions.

The project will consist in 3 main aims:

- 1) Effect of the **modulation of Ca^{2+} signaling player** activity on excitation-energetics coupling and reticulum-mitochondria Ca^{2+} coupling, using a pharmacological approach to target
- 2) Effect of the **modulation of reticulum-mitochondria Ca^{2+} coupling** on excitation-energetics coupling and expression/localization of Ca^{2+} signaling players, using genetic spacers and linkers
- 3) Analysis of reticulum-mitochondria Ca^{2+} coupling, excitation-energetics coupling and expression/localization of Ca^{2+} signaling players **under “HFpEF-like” conditions** (palmitate, phenylephrine)

The Master 2 student will learn the different techniques:

- isolation and culture of adult rat cardiomyocytes
- adenoviral infection
- Ca^{2+} imaging: IonOptix system to measure simultaneously cytosolic or mitochondrial Ca^{2+} transients and sarcomere shortening
- reticulum-mitochondria Ca^{2+} coupling analysis: proximity ligation assay, electron microscopy
- Western Blot, immunofluorescence

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References: in bold from the team

1. McCormack JG, et al. Role of calcium ions in regulation of mammalian intramitochondrial metabolism, *Physiological reviews*. 1990
2. Moreno-Sanchez R, et al. Dependence of cardiac mitochondrial pyruvate dehydrogenase activity on intramitochondrial free Ca²⁺ concentration, *Biochem J*. 1988
3. Wust RC, et al. Rapid frequency-dependent changes in free mitochondrial calcium concentration in rat cardiac myocytes, *J Physiol*. 2017
4. Yaniv Y, et al. Matching ATP supply and demand in mammalian heart: in vivo, in vitro, and in silico perspectives, *Ann N Y Acad Sci*. 2010
5. Rossini M, et al. Sarcoplasmic Reticulum-Mitochondria Kissing in Cardiomyocytes: Ca²⁺, ATP, and Undisclosed Secrets, *Front Cell Dev Biol*. 2020
6. Seidlmayer LK, et al. Inositol 1,4,5-trisphosphate-mediated sarcoplasmic reticulum-mitochondrial crosstalk influences adenosine triphosphate production via mitochondrial Ca²⁺ uptake through the mitochondrial ryanodine receptor in cardiac myocytes, *Cardiovascular research*. 2016
7. Csordas G, et al. Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface, *Mol Cell*. 2010
8. Garcia-Perez C, et al. Physical coupling supports the local Ca²⁺ transfer between sarcoplasmic reticulum subdomains and the mitochondria in heart muscle, *J Biol Chem*. 2008
9. De La Fuente S, et al. Strategic Positioning and Biased Activity of the Mitochondrial Calcium Uniporter in Cardiac Muscle, *J Biol Chem*. 2016
- 10. Dia M, et al. Reduced reticulum-mitochondria Ca²⁺ transfer is an early and reversible trigger of mitochondrial dysfunctions in diabetic cardiomyopathy, *Basic Res Cardiol*. 2020**
- 11. Cherpaz M, et al. Myocardial transcriptomic analysis of diabetic patients with aortic stenosis: key role for mitochondrial calcium signaling, *Cardiovascular diabetology*. 2024**

Skills required:

We are seeking a motivated student with a background in physiology or cell biology. A previous internship in a lab would be a plus.