Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Fiche à nommer selon le format Nom_Prenom (sans accents ni cédilles), à enregistrer en format PDF et à renvoyer à l'adresse : edcdv@sorbonne-universite.fr

Nom et prénom du directeur de thèse (et si besoin du co-directeur) : Egée Stéphane
Le directeur de thèse et le co-directeur doivent impérativement avoir l'HDR ou équivalent
Coordonnées Tel : 02 98 29 23 31 e-mail : egee@sb-roscoff.fr

Nom et prénom du co-encadrant (*non HdR*) (s'il y a lieu) : Bouyer Guillaume
Coordonnées Tel :02 98 29 23 82
e-mail : bouyer@sb-roscoff.fr

Y-a-t-il un candidat déjà identifié pour le projet: OUI ~~NON~~

Nom et prénom du responsable de l'équipe : Egée Stéphane

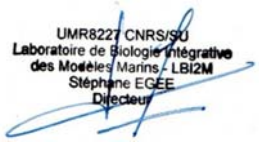
Intitulé de l'équipe : Physiologie et Destin Cellulaire
Nombre de chercheurs et enseignants-chercheurs statutaires de l'équipe titulaires d'une HDR (ou équivalent) : 2

Nom et prénom du responsable d'UMR ou de département: Egée Stéphane

Intitulé et N° d'UMR ou de département : UMR8227 Laboratoire de Biologie Intégrative des Modèles Marins

Titre du projet de thèse *: Diversité et rôle des canaux ioniques dans l'érythropoïèse terminale humaine*

Signature du directeur d'UMR ou de département (vaut avis favorable pour le dépôt du projet) :



Spécialité : *Physiologie, Biologie Cellulaire*

Résumé du projet de thèse *(1 page maximum, en anglais)*
Pour les thèses avec 2 co-directeurs, ou en partenariat entre 2 laboratoires ou structures, indiquer la participation de chaque co-directeur et structure dans la gestion du projet.

Erythrocytes are highly specialized and atypical cells, as evolution has led to the complete loss of intracellular organelles in mature mammal red cells to optimize respiratory functions. Nonetheless, human erythrocytes are far from being a simple bag of hemoglobin, and have to maintain their deformability, volume and intern homeostasis to accomplish with the best efficiency their function of gas transporters.

Membrane ion permeability is thus a key parameter, and mammalian red cells have long been a paradigm to study membrane transport, for two main reasons: they are easily available in massive amounts, and the lack of intracellular organelles facilitates membrane transport studies. Thus several transporters such as ionic pumps ($3Na^+/2K^+$, Ca^{2+}) or aquaporins have first been described using mammalian erythrocytes. The human red cell also possesses a repertoire of facilitated transporters, cotransporters or antiporters that use the gradients built by the pumps. However, among these pathways, ionic channels are far from being fully described and more importantly their physiological role during the 120 days lifespan within circulation is still poorly understood.

Erythrocyte membranes are characterized by an important anionic permeability (thanks to Band 3, a Cl^-/HCO_3^- antiporter), allowing the equilibrium for chloride ions and facilitating the transport of CO_2 via the Jacobs-Stewart cycle.. Surprisingly erythrocytes possess not only anionic channels, but also a repertoire of cationic channels whose full description is still lacking. The most described cation channel in RBCs is the Gárdos channel (KCNN4, hSK4), selective for K^+ ions. More recently, the mechanosensitive PIEZO1 channel, the NMDA Receptor and the TRPV2 channel, all non-selective cationic channels, have been described. Several other channels have also been evoked, such as Ca^{2+} or Na^+ channels. If the implication of the two first ones in several pathologies

of the erythrocyte is starting to get clearer, the various physiological functions of all these channels in mature RBCs are still unclear.

A key to clarify this question is to have a look at late stages of differentiation (when cells are still nucleated) and to seek for channel expression and role during terminal erythropoiesis.

In humans, erythropoiesis occurs within the bone marrow, where cells differentiate from the pluripotent HSC (hematopoietic Stem Cell), into first BFU-E and CFU-E progenitors. From CFU-E, cells differentiate in the erythroblastic island, consisting of a central macrophage surrounded by up to 30 erythroid cells at various degrees of maturation. This terminal differentiation is characterized by progressive accumulation of hemoglobin, decrease in cell size and nuclear condensation ultimately resulting in enucleation. At this step, cells are called reticulocytes, which will be eventually released in the circulation and mature within two days into erythrocytes.

It is well known in other cellular models that ion channels are essential for differentiation and morphological changes (1, 2), but this has never been addressed in the differentiating erythroblast, where ion membrane permeability is known to switch from mainly cationic to anionic at the end of differentiation (3), according to the late strong expression of Band 3. The channels responsible for this initial cation permeability, however, are not clearly identified. Several ion channels have been described in erythroblasts, but only the activity of a TRPC3 channel (Transient Receptor Potential Cation channel) in BFU-E cells, stimulated by Erythropoietin (EPO) and leading to Ca^{2+} influx at specific stages (4-6) has been more deeply investigated, notably using electrophysiology techniques. A putative role for Gárdos or PIEZO1 channels during terminal erythropoiesis is strongly supported by the recent studies on erythropoiesis of PIEZO1 mutant, which showed a prolonged process in patient differentiating cells (7, 8), or by the volume variation of the cells along differentiation.

Therefore the proposed thesis project aims at determining the roles of ion channels in the process of terminal erythroid differentiation by following three lines of study:

1) Determine the expression repertoire of ion channels and the expression levels of mRNAs during terminal erythropoiesis.

2) Quantify the relative protein expression levels during this differentiation and determine when proteins are addressed to the plasma membrane.

3) Establish the activity and the roles of these channels during key steps of terminal differentiation. This will include electrophysiology studies to monitor the activity of channels, but also functional studies along differentiation with either pharmacological or genetic tools.

1. Girault A, Brochiero E. *Am J Physiol Cell Physiol*. 2014;306(4):C307-C19.
2. He L, Ahmad M, Perrimon N. *Experimental cell research*. 2019;374(2):259-65.
3. Kirk RG, Lee P. *J Membr Biol*. 1988;101(1):173-8.
4. Cheung JY, Zhang XQ, Bokvist K, Tillotson DL, Miller BA. *Blood*. 1997;89(1):92-100.
5. Tong Q, Hirschler-Laszkiewicz I, Zhang W, Conrad K, Neagley DW, Barber DL, et al. *J Biol Chem*. 2008;283(16):10385-95.
6. Miller B, Cheung J, Tillotson D, Hope S, Scaduto RJ. *Blood*. 1989;73(5):1188-94.
7. Moura PL, Hawley BR, Dobbe JGG, Streekstra GJ, Rab MAE, Bianchi P, et al. *Haematologica*. 2019:haematol.2019.231159.
8. Caulier A, Jankovsky N, Demont Y, Ouled-Haddou H, Demagny J, Guitton C, et al. *Haematologica*. 2019:haematol.2019.218503.

Faisabilité du projet de thèse (*1/2 page maximum, en anglais)*

Expliciter la faisabilité du projet en terme d'expertise de l'équipe d'accueil, des collaborations potentielles qui pourront être mises en place pour certains aspects du projet, de la disponibilité des appareils nécessaires au bon déroulement du projet...

The protocol for the differentiation of human erythroid progenitors *ex vivo* has been implemented in the lab since 5 years, in the frame of a collaboration with F. Verdier (Institut Cochin) and is now routinely run in the lab. $CD34^+$ progenitor cells are obtained from human donors, collected from G-CSF mobilized peripheral blood after cytapheresis and purified by an immunomagnetic procedure.

In order to answer the first item of the thesis topic, we have prepared mRNA batches to have a representative sample collection by temporal follow-up. The conditions for rt-qPCR study on these batches are currently been set out in the team as part of an M2 internship. All the studies carried out to date based on omics approaches (transcriptomics or proteomics) are fragmentary regarding the quantification of these membrane proteins (1, 2). Indeed, ion channels are present only at a very low copy number, making their detection in a holistic approach somewhat random.

For detection at the protein scale we intend to use a targeted approach by western-blot and immunolocalization using confocal microscopy. This first approach will allow us to quantify, if not in an absolute way at least relatively, the number of copies present at each stage of differentiation. Furthermore, immunolocalization will provide an essential element to our study by allowing us to determine whether the expressed proteins are really addressed to the plasma membrane. The specificity of commercial antibodies and the optimization of the working conditions are also currently being carried out.
Additionally, electrophysiological approaches (patch-clamp) to determine the activity and role of ion channels in the process of terminal differentiation will be used. The team has a long standing experience on the use of these techniques on mature red blood cells, in physiological or pathological situations (ion channel mutant, *Plasmodium falciparum* infection) (3,4). We have already adapted and applied these techniques to erythroid progenitors and we obtained preliminary results along the 15 days of differentiation, in the frame of a M2 internship. The approaches envisaged are to determine the dominant conductances throughout the differentiation. In addition, pharmacological tests associated to electrophysiology will be considered in order to correlate protein expression to the roles of these channels, either by using known inhibitors of these channels or, more precisely in the context of this thesis, by using potential activators corresponding to the essential factors used to ensure differentiation (EPO, interleukins, Stem Cell Factor, etc.).
Finally, once the temporal cartography of channel expression at the membrane will be described, the roles of these channels along differentiation will be tested. We are considering two strategies to silence targeted channel activity at specific moments of differentiation: (i) by adding specific inhibitors to the culture at various steps and monitor their effects on proliferation / differentiation and (ii) by a silencing RNA (shRNA) approach for channels of particular interest, followed by monitoring and phenotyping transformed cell lines; our collaborator Frédérique Verdier at Cochin Institute has a recognized expertise on these tools applied to erythroid progenitors (5).

1. Gautier EF, Ducamp S, Leduc M, Salnot V, Guillonneau F, Dussiot M, et al. *Cell Reports*. 2016;16(5)**,** 1470-1484. doi: https://doi.org/10.1016/j.celrep.2016.06.085.
2. Li J, Hale J, Bhagia P, Xue F, Chen L, Jaffray J, et al. *Blood.* 2014;124(24)**,** 3636-3645. doi: 10.1182/blood-2014-07-588806.
3. Bouyer G, Barbieri D, Dupuy F, Marteau A, Sissoko A, N'Dri ME, et al. *Commun Biol*. 2020;3(1):726.
4. Rotordam GM, Fermo E, Becker N, Barcellini W, Brüggemann A, Fertig N, et al. *Haematologica*. 2018;doi: 10.3324/haematol.2018.201160.
5. Richard C, Viret S, Cantero Aguilar L, Lefevre C, Leduc M, Faouzi EH, et al. *Am J Hematol.* 2021;doi:10.1002/ajh.26104

Thèses actuellement en cours dans l'équipe

Tous les encadrements doivent être indiqués (y compris les co-directions avec un autre HDR pour des doctorants d'une autre ED, et les encadrements dans le cadre de programmes doctoraux tels qu'IPV, FDV...)

Nom et Prénom du doctorant	Directeur(s) de thèse	Année de 1ère inscription	ED	Financement
HATEM Aline	EGEE Stéphane	2020	515	ITN Evidence (Europe)
DELEHOUZE Claire	BACH Stéphane	2020	515	CIFRE (SeabeLife)

Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Trois publications récentes du directeur de thèse (du co-directeur ou du co-encadrant s'il y a lieu). Mettre en gras le nom du directeur de thèse.

Mansour-Hendili, L., **Egée, S.**, Monedero-Alonso, D., **Bouyer, G.**, Godeau, B., Badaoui, B., Lunati, A., Noizat, C., Aissat, A., Kiger, L., Mekki, C., Picard, V., Moutereau, S., Fanen, P., Bartolucci, P., Garçon, L., Galactéros, F., Funalot, B., 2021. Multiple thrombosis in a patient with Gardos channelopathy and a new KCNN4 mutation. Am. J. Hematol. 96, E318–E321. **https://doi.org/10.1002/ajh.26245**

Monedero Alonso, D., Pérès, L., Hatem, A., **Bouyer, G.**, **Egée, S.**, 2021. The Chloride Conductance Inhibitor NS3623 Enhances the Activity of a Non-selective Cation Channel in Hyperpolarizing Conditions. Front. Physiol. 12, 1705. **https://doi.org/10.3389/fphys.2021.743094**

Pérès, L., Alonso, D.M., Nudel, M., Figeac, M., Bruge, J., Sebda, S., Picard, V., Nemer, W.E., Preudhomme, C., Rose, C., **Egée, S.**, **Bouyer, G.**, 2021. Characterisation of Asp669Tyr Piezo1 cation channel activity in red blood cells: an unexpected phenotype. Br. J. Haematol. 194, e51–e55. **https://doi.org/10.1111/bjh.17467**

Docteurs encadrés par le directeur de thèse ayant soutenu entre la date de dépôt de ce dossier et il y a 5 ans et publications relatives à leur sujet de thèse. Mettre en gras le nom du directeur de thèse et celui du docteur.

Nom Prénom : MONEDERO ALONSO David

Date de soutenance : 25 novembre 2019

Durée de thèse (en mois) : 39

Ecole Doctorale : ED 515 CDV

Publications :

1- Buks, R., Dagher, T., Rotordam, M.G., **Monedero Alonso, D.**, Cochet, S., Gautier, E.-F., Chafey, P., Cassinat, B., Kiladjian, J.-J., Becker, N., Plo, I., **Egée, S.**, El Nemer, W., 2022. Altered Ca2+ Homeostasis in Red Blood Cells of Polycythemia Vera Patients Following Disturbed Organelle Sorting during Terminal Erythropoiesis. Cells 11, 49. https://doi.org/10.3390/cells11010049

2- Fermo, E., **Monedero-Alonso, D.**, Petkova-Kirova, P., Makhro, A., Pérès, L., Bouyer, G., Marcello, A.P., Longo, F., Graziadei, G., Barcellini, W., Bogdanova, A., **Egee, S.**, Kaestner, L., Bianchi, P., 2020. Gardos channelopathy: functional analysis of a novel KCNN4 variant. Blood Adv. 4, 6336–6341. https://doi.org/10.1182/bloodadvances.2020003285

3- Filser, M., Giansily-Blaizot, M., Grenier, M., **Monedero Alonso, D.**, Bouyer, G., Pérès, L., **Egée, S.**, Aral, B., Airaud, F., Da Costa, L., Picard, V., Cougoul, P., Palach, M., Béziau, S., Garrec, C., Aguilar-Martinez, P., Gardie, B., Girodon, F., 2021. Increased incidence of germline PIEZO1 mutations in individuals with idiopathic erythrocytosis. Blood 137, 1828–1832. https://doi.org/10.1182/blood.2020008424

4- **Monedero Alonso, D.**, Pérès, L., Hatem, A., Bouyer, G., **Egée, S.**, 2021. The Chloride Conductance Inhibitor NS3623 Enhances the Activity of a Non-selective Cation Channel in Hyperpolarizing Conditions. Front. Physiol. 12, 1705. https://doi.org/10.3389/fphys.2021.743094

5- Nader, E., **Monedero Alonso, D.**, Robert, M., Skinner, S., Stauffer, E., Cibiel, A., Germain, M., Hugonnet, J., Scheer, A., Joly, P., Renoux, C., Connes, P., **Égée, S.**, 2020. Impact of a 10 km running trial on eryptosis, red blood cell rheology, and electrophysiology in endurance trained athletes: a pilot study. Eur. J. Appl. Physiol. 120, 255–266. https://doi.org/10.1007/s00421-019-04271-x

6- Pérès, L., **Monedero Alonso, D.**, Nudel, M., Figeac, M., Bruge, J., Sebda, S., Picard, V., Nemer, W.E., Preudhomme, C., Rose, C., **Egée, S.**, Bouyer, G., 2021. Characterisation of Asp669Tyr Piezo1 cation channel activity in red blood cells: an unexpected phenotype. Br. J. Haematol. 194, e51–e55. https://doi.org/10.1111/bjh.17467

1 autre article en cours de preparation :

- **Monedero Alonso D**, Pérès L, Rotordam M, Bouyer G, Fermo E, Becker N, Rapedius M, Kaestner L, Bianchi P, **Egée S.** Instantaneous changes in membrane potential as fast, accurate and reliable method for red cells functional characterization of channelopathies. En preparation pour soumission à *Frontiers in Physiology*

Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Nom Prénom :	Date de soutenance : Durée de thèse (en mois) : Ecole Doctorale :
Publications :	