Adeno-associated virus (AAV) serotype 8 is of particular interest as a vector used in gene therapy for neuromuscular disorders, e.g. Duchenne muscular dystrophy (DMC) and X-Linked Myotubular Myopathy (MTM1). However, toxicities have recently emerged with high-dose AAV gene therapies in clinical trials for these myopathies (High-dose AAV gene therapy deaths, Nature Biotechnology 2020). Therefore, optimization studies are needed to reach maximal therapeutic benefit with the lowest dose of vector. In this context, basic knowledge is missing to understand the mechanism of AAV-mediated transduction in diseased muscle, especially regarding the intracellular trafficking of AAV which is a rate-limiting step of AAV transduction for many cell types.

AAV vectors reach the nucleus through multiple intracellular trafficking events including binding to their receptors at the plasma membrane, dynamin-dependent receptor-mediated endocytosis, trafficking through the endosomal system and trans-Golgi network by exploiting the microtubule cytoskeleton, and endomembrane escape before nucleus entry (Jalish M. Riyad et al, Gene Ther 2021). In addition, AAVs containing single-stranded genomes, need to be uncoated in endosomes and/or nucleus in order to allow synthesis of the second strand of DNA and subsequent transgene expression in the nucleus. Interestingly, our studies performed on animal models of DMD showed that AAV-mediated transgene expression is drastically lower in this pathological muscle compared to healthy controls (Peccate et al, Hum Mol Genet 2016). Our recent work summarized in a manuscript in preparation (phD project of Julie Chassagne 2016-2019) pointed to the importance of the endo-lysosomal pathway for AAV8-trafficking in muscle cells and identified defects of this pathway in cell and animal models of DMD. An impairment of AAV-mediated transduction is not restricted to DMD as we also showed such deficiency in a knock-in mouse model expressing the most frequent autosomal dominant Centronuclear (CNM) myopathy mutation (KI-Dnm2(R465W)) (Trochet et al, Embo Mol Med, 2018).

CNM is a rare congenital myopathy resulting from mutations in the DNM2 gene which encodes dynamin 2 (DNM2) (Bitoun M. et al. Nat Genet 2005). DNM2 is involved in clathrin-dependent and clathrin-independent endocytosis at the plasma membrane, in the formation of vesicles from endosomes and trans-Golgi network and regulates microtubule dynamics. Remarkably, whereas AAV treatment rescued muscle structure and function in young KI-Dnm2 mice, it was less efficient when started at 6 months of age, when mice exhibit a more pronounced
muscle phenotype. This limitation may be overcome by increasing dose of vector in agreement with a decreased entry and/or a defective intracellular trafficking of the AAV.

Using muscle cells derived from CNM patients and from an animal model of this myopathy, the current project aims at defining the main processes occurring from the plasma membrane to the nucleus which underlie the AAV-mediated transgene expression in CNM by tackling the following questions

- **Is the AAV entry into the cells efficient?**

  We will determine expression levels of AAV8 receptor and the co-receptor as well as their proper localization at the plasma membrane. The entry of AAV will be measured using fluorescent viral particles.

- **What is the intracellular route taken by AAV towards the nucleus?**

  The time required for fluorescent AAV particles to reach the nucleus will be measured and the route taken by the AAV particles from the plasma membrane to the nucleus will be determined through co-immunostaining of the AAV capsid with markers of intracellular compartments (early endosomes, late endosomes, lysosomes, and trans-Golgi network). Involvement of microtubules will be also investigated.

- **Is the AAV properly processed for mediating transgene transcription in the nucleus?**

  We will investigate the uncoating of the viral genome and its proper nuclear localization. We will use an AAV-reporter gene allowing to correlate the amount of uncoated viral genome with the transduction efficiency through activity and expression of the reporter gene. In addition, efficiency of synthesis of the second strand of the viral genome will be studied.

The project is the first step towards treatments ameliorating future AAV clinical use by associating the best transduction efficiency with the lowest AAV dose.

**References:**

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

Explicitier 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...

This project is based on methods and tools already developed in the laboratory and in its scientific environment (Sorbonne Université and Institute of Myology):

**Funding:** This project is funded by Sorbonne University “emergence”

**Expertise:** The PhD student will benefit from the combining expertise of the two supervisors; Marc Bitoun (expertise: CNM, intracellular trafficking) and Sofia Benkhelifa-Ziyyat (expertise: DMD, biology of AAV vectors, intracellular trafficking).

**AAV vector production and quantifications:** The Myology Research Center gives access to the AAV production facility headed by S. Benkhelifa-Ziyyat (co-director of the thesis) in the host laboratory. The host laboratory has a solid expertise in the vector biology, viral genome quantification in cells and animal tissues.

**Human cells:** In the Myology Research Center, the host laboratory collaborates with Vincent Mouly (head of the human cell culture platform) who gives access to a variety of human cells (immortalized and primary) derived from muscle biopsies of CNM patients and control with the technical expertise of their use.

**Animals and biopsies:** CNM and controls are bred within the animal facilities of the “Faculté de médecine Pitié-Salpêtrière” (CEF) in the basement of the building where the host laboratory is located. At the beginning of the thesis, the student will follow a training for animal experimentation (level 1) to be able to perform injections in animals and dissections for the project.

**Microscopy:** The Myology Research Center provides access to the High resolution and electron microscopy equipments in an Imaging platform headed by Bruno Cadot (expertise: live cell imaging, muscle cell culture system), another researcher of the Bitoun’s team.

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...)

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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.


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 : Chassagne Julie
Date de soutenance : 11/10/2019
Durée de thèse (en mois) : 36 mois
Ecole Doctorale : Complexité du vivant

Publications :