### Titre de la thèse
Early weaning and feeding behavior: study of the serotonergic system.

### 3 mots-clés
- Circadian clock
- Nutritional programming
- miRNA

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### Contexte socioéconomique et scientifique
Breastfeeding is defined as the supply of breast milk to the newborn, regardless of the form of supply, direct or indirect (WHO, 2008). Breastfeeding is recognized as a health benefit for mothers and children, but mothers in Brazil and France are known for their short lactation (only 30% and 10% of babies are breastfed at 12 months in Brazil and in France, respectively (Victora et al., 2016 PMID: 26869575)). Early termination of lactation compromises significant nutritional and maternal contact, promoting morphofunctional changes in newborns (such as anxiety, depression, stress) that can last a lifetime. Similarly, inadequate nutrition of premature infants received in the intensive care unit poses a problem (Boquien 2018 PMID: 30386758) that can lead to the onset of a metabolic syndrome in adulthood. The thesis aims to explore on a rat model, the immediate and long-term effect of 2 perinatal stress. We will attempt to evaluate the effects of microRNA supplementation capable of disrupting the epigenetic profile of intestinal cells. The targeting of specific tissues to manipulate the epigenetic profile of intestinal cells during the perinatal period with miRNAs embedded in nano-biomimetic vehicles may pave the way for the development of preventive strategies for metabolic syndrome induced in intensive care unit leading to diabetes (Beuzelin & Kaeffer, 2018).

### Hypothèses et questions posées
Following a long collaboration between Nantes and Recife, we use the same rat model and we intend to explore 2 perinatal stresses with their consequences on epigenetics and physiology, from the digestive tract to the brain. The first stress is protein restriction in mothers during pregnancy and lactation, which triggers nutritional programming (Orozco-Solis et al., 2011; Nascimento et al., 2013; Ferro et al., 2016). The second is early weaning, which is a nutritional and emotional stress. Gabriel A Tavarez will explore the effects of oral supplementation of rats with non-coding micro-ribonucleic acid (miRNA). Among miRNAs in breast milk, it has been proposed that hsa-miR-320-3p plays an important role in epigenetic regulation by opening up new avenues for therapeutic purposes (Beuzelin & Kaeffer, 2018) or by testing the acquisition of Exogenous non-coding RNA on host physiology with presumed consequences over several generations [Cech & Steitz, 2014 PMID: 24679528; Junien et al., 2016]. Oral miRNA delivery is a major challenge as nucleic acids are not stable in the gastric environment. We have used lipid derivatives of previously developed natural aminoglycosides that have been shown to be effective for intracellular delivery of siRNA, DNA or mRNA [Desigaux et al., 2007; Mével et al., 2012, Collombani et al., 2016, Habrant et al., 2016]. Our goal is to follow the stability of epigenetic profiles in rats after either of the 2 perinatal stresses compared to standard breeding conditions.

### Grandes étapes de la thèse
In the continuation of our Franco-Brazilian collaboration dating from 2009, our rat model is relevant to study the nutritional programming of the circadian clock. Our goal is to test the long-term stability of epigenetic profiles in 2 models of perinatal stress. One stress will use the protein restriction model (Orozco-Solis et al., 2011; Nascimento et al., 2013; Ferro et al., 2016) and another will use early weaning taking advantage of
our recent findings on an oral bolus of miRNA included in lipid derivatives of aminoglycosides. Indeed, we have selected a biomimetic vector that can be loaded with any miRNA, non-cytotoxic when administered per os to the pups. The vector delivers functional exogenous miR-320-3p into the cytoplasm of gastric cells, thereby altering the circadian epigenetic regulation of a promoter (Beuzelin, Pitard, Kaeffer, submitted).

In the French part of the thesis, we intend to study the CLOCK protein and its transcription partner BMAL1 at the level of the transcript and by Chromatin immunoprecipitation by targeting histone complexes H3K9 and H3K14. The CLOCK protein is itself a histone acetyltransferase (Doi et al., 2006 PMID: 16678094). The expression of circadian genes is also regulated by the dynamic methylation of H3K4 by the histone methyltransferase enzymes MLL1 and MLL3 (Katada and Sassone-Corsi, 2010 PMID: 21113167, Valekunja et al., 2013 PMID: 23297224); a regulation that we found impaired during oral supplementation with miR-320 / lipid aminoglycosides in the gastric mucosa (Beuzelin, Pitard, Kaeffer, submitted).

Compétences scientifiques et techniques requises par le candidat (2 lignes):
The doctoral fellow, Gabriel Araujo Tavares, is a trained neurobiologist who will focus on the circadian rhythm of cerebral peptides (hypothalamus, hippocampus) and the serotonergic system.

3 publications de l’équipe d’accueil relatives au domaine (5 dernières années):


Collaborations nationales et internationales:


Collaboration brésilienne : Sandra Lopès de Souza Department of Anatomy, Biological Sciences Center of the Federal University of Pernambuco (UFPE), Recife, PE, Brazil

Collaboration ligérienne : Bruno Pitard (Inserm UMR-1232/In Cell Art), Nantes