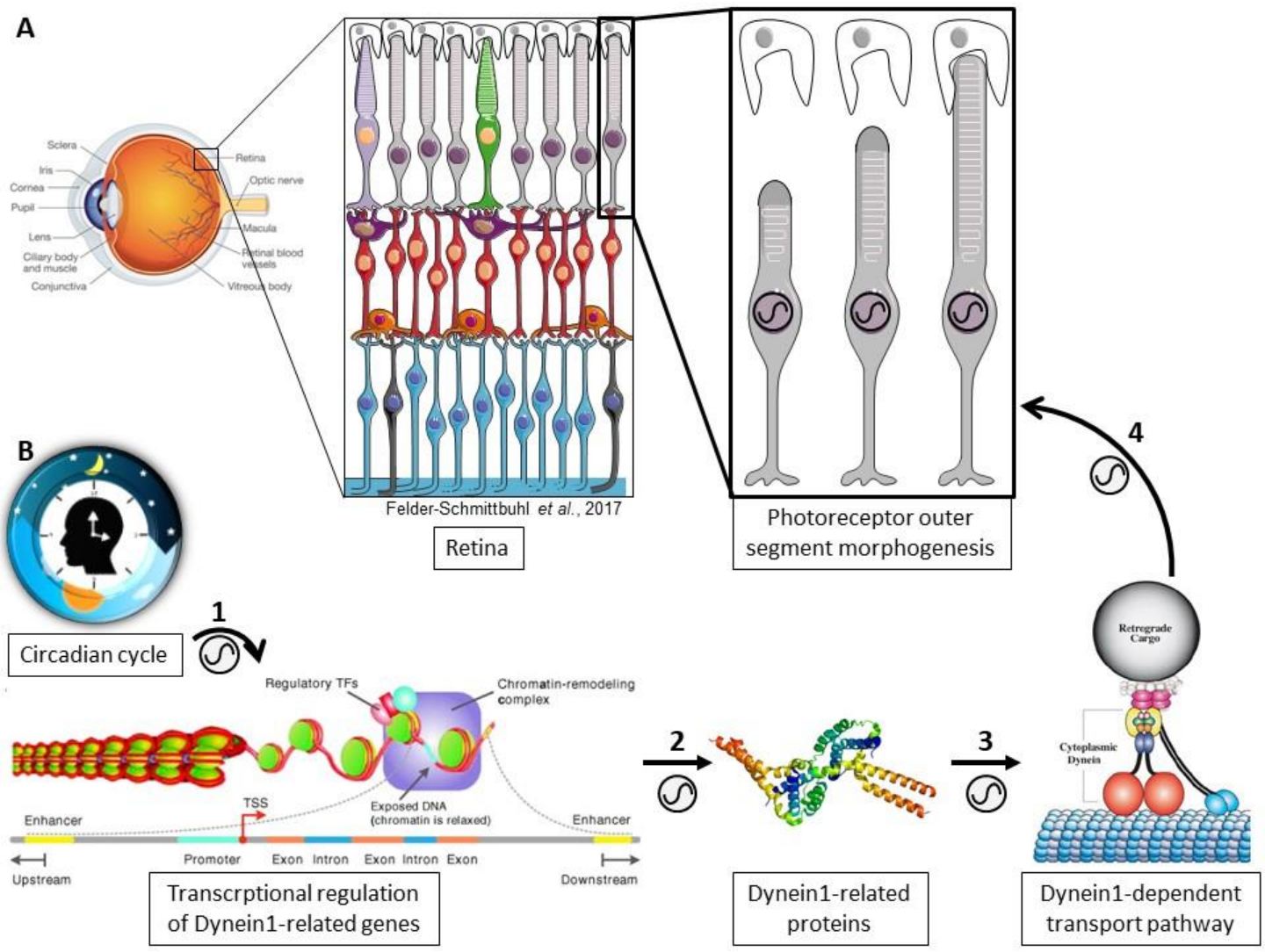
CRePIT

Cis-regulation of photoreceptor neogenesis by the retinal clock in association with the intracellular transport signalling



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Title : *CRePIT : Cis-regulation of photoreceptor neogenesis by the retinal clock in association with the intracellular transport signalling*

Key words : *Photoreceptor renewal, Circadian clock, Dynein1, Intracellular transport, Transcription*

Projet :

Daily rhythms in behavior and physiology are programmed by biological clocks widely distributed in mammalian tissues and together constituting the circadian system. Circadian clocks are cell-autonomous mechanisms involving clock genes which take part in interlocked transcriptional/post-translational feedback loops. Clock factors in turn drive cyclic expression of "clock-controlled genes", thereby enabling rhythmic adaptations in physiology. They regulate gene expression through complex enhancer-promoter interactions with co-recruited cell-specific transcription factors (TF) and epigenetic remodelers. Dysregulation of the circadian system (*i.e.*: artificial light, shift work, …) can lead to long term health deficits. In the eye, clock gene malfunction blunts the adaptation of vision to the Light/Dark cycle (L/D) and promotes the development of myopia and diabetic retinopathy.

Our team "Light, vision and brain" has made significant contributions to understand the mechanisms regulating retinal physiopathology downstream of the clock, including the identification of layer-specific oscillators and characterization of the rhythm in photoreceptor outer segment (POS) renewal. This turnover prevents harmful accumulation of products resulting from phototransduction. While cyclic degradation of the POS has been well characterized, the involvement of circadian clocks in anabolic aspects of POS morphogenesis (*i.e*. constituents synthesis/trafficking towards the POS) remains to be demonstrated. Several studies evidenced the crucial role of dynein1-dependent intracellular transport in POS renewal. However, the question of its circadian regulation, including at the genomic and epigenomic levels, remains largely unanswered.

The aim of the project is **to decipher the mechanism of transcriptional regulation of genes involved in the intracellular transport in photoreceptors and to provide new insights into the regulation of POS neogenesis, thus into therapies of retinal degeneration.**

This objective will be reached through 3 steps corresponding to a 3 year PhD training and involving the development of molecular approaches and *in vitro* model.

Step 1: Selection of candidate genes with rhythmic regulation

The first year aims to identify potential regulatory regions linked to daily rhythms of transcription by using Cut & Run assay and an histone mark that is specific of enhancer/promotor (collaboration: D Duteil, IGBMC). To do so young postnatal rodent rod photoreceptors (PRs) will be collected at distinct time points over 24h. DNA associated with H3K27ac will be isolated and sequenced in order to found clock's target genes and regulatory regions. The results will allow the characterization of the transcriptional control of POS renewal (Step 3).

Step 2: Spatio-temporal characterization of *dynein1-dependent transport*

In parallel to step1, the spatio-temporal distribution of organelles and dynein1 motor protein will be assessed in *in vitro* model of mouse retinal explants and/or cryosections of mouse retina by using immunolabeling at different time points (Mockel et al 2012; Rodriguez-Muela et al 2015). Thus, the kinetic over 24h of dynein1-dependent transport pathways (lysosomes, …) will be established and their disruption by altering target genes identified in step1 will be further assessed in step 3.

Step 3: Cis-regulatory network

A motif-enrichment search will be performed on regulatory regions characterized in step 1 in order to identify TF binding sites. The potential enhancer activity of those features will be assessed in the *in vitro* model by using transfection of an enhancer-reporter vector in which GFP expression is under the control of a minimal promoter and of the sequence of interest. In addition, mutagenesis assays will allow identifying regulatory TF binding sites, thus pathways regulated by daily rhythm. The step 3 will take place in the third year.

This study should provide the first *cis*-regulatory mechanism of PR intracellular transport pathways regulated by the circadian clock.

Mandatory requirement: M2 defense before June 20th, 2022

Special care of candidate's readiness will be provided before Doctoral School ED414 presentation

Wished skills :

Requirements include Master degree in neuroscience or molecular biology, notions in mechanisms of transcriptional regulation, chronobiology and some technical skills: collecting animal tissues, molecular and cellular approaches, immunohistochemistry and microscope. He or she had experience in *in vitro* culture and quantitative data analysis of gene expression, useful to compare results of Cut&Run and those previously obtained from qRT-PCR of genes associated to dynein1-dependent transport. Familiarity with programming language will be highly appreciated. Good communication skills English (both written and spoken).

Expertises which will be acquired during the training :

After the 3-year project, the candidate will be expert in mechanisms of photoreceptor turnover because of retinal in vitro culture and approaches for identifying transcriptional regulation and *in silico* analyses of transcription factor binding sites. He or she will develop skills in transfection of tissues/cells, immunohistochemistry of organelles and confocal imaging (Image J), as well as in bioinformatic analyses of sequencing data by collaborating with a bioinformatics scientist. Finally, the candidate will learn how to manage a project and acquire the ability to work as part of a team.