Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Fiche à nommer selon le format Nom_Prénom_ProjetED19, à enregistrer en format PDF et à renvoyer à l'adresse: edcdv@upmc.fr.

Nom et prénom du directeur de thèse : Choquet Yves
Coordonnées           Tel : 01 58 41 50 75            e-mail : choquet@ibpc.fr

Nom et prénom du co-encadrant (non HdR ) (s’il y a lieu) : Coordonnées     Tel :                e-mail :

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

Nom et prénom du responsable de l’équipe : Falciatore Angela

Intitulé de l'équipe : Biologie du Chloroplaste et Perception de la Lumière chez les Microalgues
Nombre de chercheurs et enseignants-chercheurs statutaires de l’équipe titulaires d’une HDR (ou équivalent) : 5

Nom et prénom du responsable d'UMR ou de département: Falciatore Angela

Intitulé et Nº d'UMR ou de département: UMR7141 Chloroplast Biology and Light Sensing in Microalgae
Signature du directeur d'UMR ou de département (vaut avis favorable pour le dépôt du projet) :

Titre du projet de thèse : Assessing the interplay between OTAF and ncRNAs in the regulation of chloroplast gene expression

Spécialité : Biologie moléculaire, génétique

Résumé du projet de thèse (1 page maximum, en anglais)

**Background:** Chloroplasts (Cp) evolved from free-living cyanobacteria engulfed by a primitive eukaryotic cell about 1.5 My ago. Compared to their cyanobacterial ancestors, modern chloroplasts have undergone massive gene transfer to the nucleus of the host and retain only tiny genomes (50-200 genes) that encode a fraction only of the proteins required for their own expression or for photosynthesis. The chloroplast gene expression machinery is still prokaryotic in essence, as most proteins involved are actually the products re-imported into the chloroplast of ancestral cyanobacterial genes that have been transferred to the nucleus. In bacteria, non-coding regulatory RNAs (ncRNAs) have recently emerged as a new class of post-transcriptional regulators that modulate the translation or stability of transcripts. They notably participate in the control of photosynthetic activity in cyanobacteria. Antisense and ncRNAs also accumulate in chloroplasts, as shown by our recent analysis of the chloroplast transcriptome in the unicellular green alga Chlamydomonas reinhardtii (1). A few old studies suggested that they may impact the expression of their target gene (2-6). However, their functional significance in the global regulation of chloroplast gene expression has never been addressed, even if our own results suggests they can modify the processing and the stability of some chloroplast transcripts.

Despite this prokaryotic background, Cp are not just cyanobacteria that have transferred most of their genes to the host: unique regulatory mechanisms have evolved to allow the eukaryotic cell to control the activity of its ancient symbiont. Indeed, Cp gene expression is now under the hierarchical control of nucleus-encoded, Organelle-imported, Trans-Acting Factors (OTAF) that assist, in a gene specific manner, every post-transcriptional step of organelle gene expression, such as transcript Maturation/stability (M factors) or Translation (T factors). Our laboratory has greatly contributed to the study of M and T factors (7-9), in particular via the genetic analysis of Chlamydomonas photosynthesis mutants. Most of these factors pertain to families of helical repeats proteins, almost exclusively involved in organelle gene expression, such as the PPR and OPR (Pentatrico- and Octotrico- Peptide Repeats, respectively) protein families. Each PPR/OPR motif interacts with one nucleotide via specificity-determining residues (SDR) at specific positions, allowing these factors to recognise a specific target sequence. It has been proposed that as- and nc-RNA, by competing with OTAFs for the binding to their target sequence, could modulate the action of OTAFs (10-11).

**The PhD Project** aims at addressing the interplay between OTAF and ncRNAs in the regulation of chloroplast gene expression:

a) In bacteria, ncRNAs are usually expressed conditionally under stress conditions to modulate the expression of their target mRNAs, while our transcriptomic study was performed on unstressed and actively growing cells. To better unravel the physiological importance of chloroplast ncRNAs, transcriptomic studies should be performed in growth conditions leading to an increased RNA turn-over and a reduced protein synthesis, such a nutriment starvation, high light stress, heat or cold shock.
b) The expression of some of the ncRNAs identified in the previous task will be prevented by site-directed mutagenesis (e.g. promoter deletion) to study the consequences of these mutations at the transcript, protein and physiological levels.

c) The effect of ncRNA will also be tested directly by introducing in the chloroplast genome artificial transcription units that will express RNA fragments complementary to the various regions of a chloroplast RNA (5' or 3'UTRs, CDS), and by studying their effect on the expression of the target gene. This study will be more specifically performed on three chloroplast genes encoding ATP synthase subunits: \textit{atpA}, \textit{atpB} and \textit{atpH}, for which we have recently characterised the cognate OTAFs (9,10; 2 manuscripts en prep.). In particular, we will study if and how small RNA fragments complementary or identical to these OTAFs binding site will modulate their binding and activity.

**Methodologies:** Our laboratory uses a multidisciplinary approach of chloroplast biology and the candidate should be interested in combining genetic and molecular approaches (random and site-directed mutagenesis, transformation of the chloroplast and nuclear genomes, analysis of transcript accumulation, transcriptomics), biophysical screens of mutants by time-resolved absorption and fluorescence spectroscopy, biochemical assays (rate of synthesis and accumulation of photosynthetic proteins) and bio-informatics analyses to tackle a question of broad relevance: the interplay of OTAF and noncoding RNAs in the regulation of organelle gene expression.

**References:**

Publications co-authored by the PhD director are written in blue.

**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 host laboratory (UMR7141, IBPC-Paris) is a world leader in studies of nucleo-chloroplastic interactions and of the expression, assembly and function of the major oligomeric proteins of the photosynthesis apparatus in the chloroplast. The PhD director (DR2-CNRS) has several decades of internationally recognized expertise in the study of chloroplast biogenesis, with a strong focus on genetic approaches for the characterization of photosynthetic mutants.

All the techniques required to carry out the project (chloroplast and nuclear transformation; functional and molecular characterisation of photosynthetic mutants) are and routinely used in the laboratory, which is fully equipped with home-made devices for transformation, spectroscopic molecular and biochemical analyses of photosynthetic mutants... Transcriptomic analyses will be performed in collaboration with the bioinformatics platform, recently established at IBPC.

Would our hypothesis of a regulation of chloroplast gene expression fall short of experimental support or only lead to ambiguous results, the doctorant will switch to the analysis of the mode of action of the recently characterised OTAFs controlling the expression of the \textit{atpA}, \textit{atpB} and \textit{atpH} genes.

**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.

   
   Cet article a fait l'objet d'un commentaire par le bureau éditorial de Plant Cell: Mach J. (2015) In brief: Twice as NCC: Two Octotricopeptide Repeat Proteins and the Regulation of Chloroplast Gene Expression. Plant Cell, 27, 947


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.

Pas de soutenance les cinq dernières années.