ウェブサイト公開用

（様式 10）
（海外特別研究員事業）
平成３１年　４月１１日

海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人 日本学術振興会 理事長　殿

採 用 年 度　平成２９年度
受 付 番 号　２１０
氏　　名　棟方涼介

（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：　ナンシー　（国名：　フランス　）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
フラノクマリン生合成をつなぐ柑橘のプラスチド包膜輸送メカニズムの解明

3. 派遣期間：平成　２９年　４月　１日　～　平成　３１年　３月　３１日

4. 受入機関名及び部局名
University of Lorraine

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

Background and Purpose

The production of metabolites within plant cells goes through biosynthetic pathways that are complex depending on the families of molecules. This synthesis is done in several steps generally catalyzed by different enzymes and which can be carried out in different subcellular compartments. Thus, a molecule can transit among different compartments during its synthesis. Although many families of biosynthetic enzymes have already been characterized, very little has been described about intracellular transport mechanisms of plant specialized metabolites.

The main purpose of this project is to investigate the intracellular transport machinery to integrate the transport events of the metabolite intermediates into the canonical plant specialized metabolic maps, which mainly consist of only individual biosynthetic reaction steps.

To achieve this goal, I selected the biosynthesis of bergamottin in *Citrus* species as a metabolic model (A. Dugrand *et al.*, 2013, *J. Agric. Food. Chem.*). The synthesis of this major furanocoumarin (FC) derivative in *Citrus* starts from a coumarin derivative, umbelliferone, which is imported from the cytosol into the plastid where it is converted to demethylsuberosin (DMS) by a U6DT, the first prenyltransferase (PTs) (F. Karamat *et al.*, 2014, *Plant J.*, R. Munakata *et al.*, 2016, *New Phytol.*). In a second step, DMS moves back to the cytosol where it is transformed in bergaptol by several cytochrome P450s (P450s) (R. Larbat *et al.*, 2007, *J. Biol. Chem.*). Finally this molecule is imported to plastid again for the last reaction catalyzed by an *O*-prenyltransferase (*O*-PT) to yield bergamottin (R. Munakata et al., 2012, *Biosci. Biotechnol. Biochem.*) (Figure 1). P450s are bound to ER membranes whereas PTs are embedded in the inner envelope of plastids. The synthesis of this molecule could involve three different transporters which I defined here as Plastid envelope-localized FC intermediate transporters (PFIT) 1, 2, and 3 for a plastidial umbelliferone importer, a plastidial DMS exporter, and a plastidial bergaptol importer, respectively (Figure 1).

To carry out this project several elements are necessary. The first one relies on screening of candidate genes encoding transporters. The second aspect consists of developing the tools necessary to realize the functional characterization of these membrane-bound proteins.

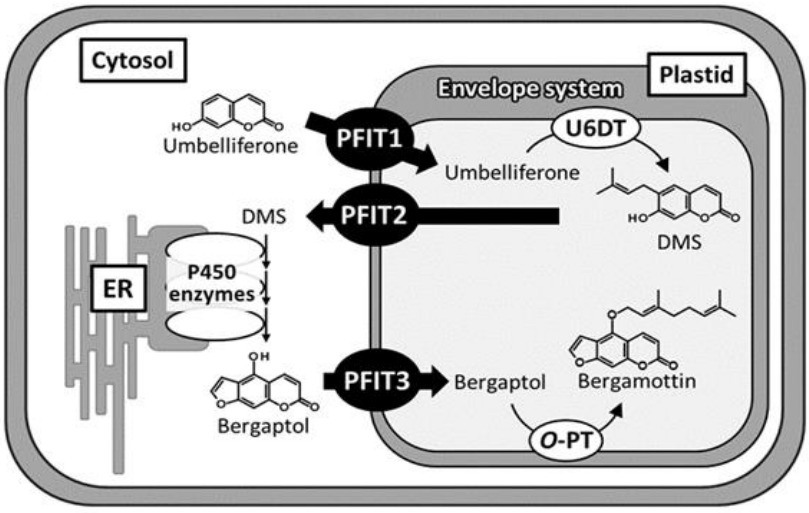

Figure 1. Roles of PFITs in bergamottin biosynthesis in *Citrus*.
U6DT: prenyltransferase specific to umbelliferone
O-PT: prenyltransferase specific to bergaptol

Chapter 1. Identification and isolation of candidate genes of *PFITs*

a- Characterization of PFIT activities in native plastids from FC-producing plants

Biochemical properties of native PFITs may provide important information that could help to seek for candidate genes. Therefore, in the first attempt, I planned to prepare intact plastid from grapefruit outer peel (flavedo), which accumulates large amounts of bergamottin. Whereas the experiments were successful for spinach, the results obtained with citrus tissues led to the isolation of too low yields to be used for any biochemical analysis (Figure 2). Then, grapefruit leaves, which accumulates bergamottin at the similar levels as flavedo, were also tested, resulting in the similar low yield. Since some citrus tissues are known to be sometime difficult to work with, I extended my experiments to other plant species accumulating FC derivatives such as lemon, *Ruta graveolens*, which both belong to Rutaceae, and parsley, parsnip, anise, celery which are Apiaceae (RDH Murray *et al.,* 1982). Unfortunately, I couldn't manage to isolate enough plastids from any of them to perform any functional characterization.

b- Transcriptomic-based strategy

The second strategy I developed to identify PFIT relied on the differential transcriptomic analysis. In addition to the first material, citrus, I newly focused on fig (*Ficus carica*, Moraceae) because of newly available comparable EST libraries prepared from latexes of different organs of fig, where FC contents largely differ (S. Kitajima *et al.*, 2018, *Planta*). In an attempt to identify candidate genes, I restricted my investigation to ABCG members of the ABC transporter family. These proteins have been reported to transport phenolic compounds including coumarins and additionally include plastid-localized members (M. Geisler, 2014). From both *Citrus* and fig EST-libraries, candidate genes were found by *in silico* analysis.

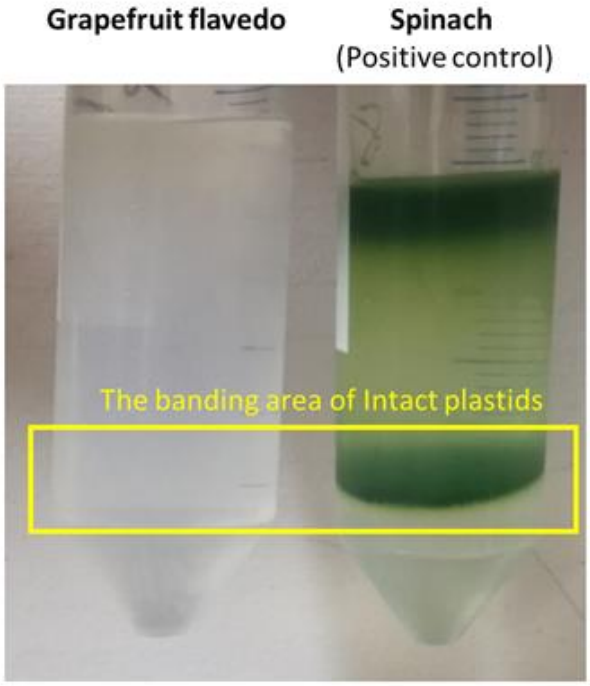

Figure 2. Intact plastid preparation from grapefruit flavedo
Spinach was used as a positive control of the preparation.

Chapter 2. Functional characterization of PFITs candidates: a transport assay

To perform functional characterization of PFITs, it is necessary to have a reliable screening system. I chose to develop a tool based on budding yeast. I engineered a yeast transformant suitable for the screening of PFIT candidates (Figure. 3). Now I am screening *PFITs* using the new strain.

Summary

I developed several methods to identify candidate genes involved in the intracellular transport of phenolics. Several strategies were investigated at the same time. Although the preparation of intact plastids from FC-producing plants was not successful I could find candidate genes by *in silico* analysis of RNA-seq from grapefruit and fig. I also constructed an original system based on yeast which will be highly valuable for screening and functional characterization of PFITs. Using this system PFITs would be discovered.

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