様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　５ 年　 ６ 月 １６ 日現在

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研究課題名（和文）Protein import into endosymbionts becoming organelles

研究課題名（英文）Protein import into endosymbionts becoming organelles

研究代表者
ＨＵＳＮＩＫ　Ｆｉｌｉｐ（Husnik, Filip）

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研究成果の概要（和文）：このプロジェクトの枠組みの中で、私はコナカイガラムシで見られるユニークな細菌内細菌真核生物内共生における宿主共生生物の統合に焦点を当ててきました。 オミクスと 3D イメージングの組み合わせにより、システム内でどの遺伝子、タンパク質、代謝物が局在しているか、そして三者間の相互作用がどのように推進されるのかを理解することができました。 これらすべての先進的な手法からのデータを統合することは、単一の共生種では前例のないことであり、細胞小器官のような相互依存性の高い共生における宿主と共生生物の統合（細胞分裂の調整やタンパク質の移入など）について強力な結論を引き出すことが可能になります。

研究成果の学術的意義や社会的意義
Understanding how highly integrated symbioses function is essential(and relates to human health) since one organelle in our cells, the mitochondrion, has originated from a symbiotic bacterium.

研究成果の概要（英文）：In the frame of the project, I have focused on host-symbiont integration in a unique bacterium-within-bacterium-within-eukaryote symbiosis found in mealybugs. Via the combination of omics (genomics, transcriptomics, proteomics, and metabolomics) with 3D imaging (uCT, confocal FISH imaging, SBF-SEM, FIB-SEM), we managed to understand which genes, proteins, and metabolites are localized in the system and how they drive the tripartite interaction. Integrating data from all these advanced methods is unprecedented for a single symbiotic species and allows us to draw strong conclusions about host-symbiont integration (e.g. cell division coordination and protein import) in highly interdependent, organelle-like, symbioses.

研究分野：evolutionary cell biology

キーワード：symbiosis　metabolomics　proteomics　FIB-SEM　uCT　SBF-SEM　genomics　FISH

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Background of the project. Long-term intracellular symbiosis results in massive dependence on the host. Mitochondria and plastids, the oldest known endosymbionts, are well-recognized to rely on the import of thousands of diverse compounds from the host cytoplasm. In fact, the import of many proteins is often used as the main feature distinguishing these cellular organelles from 'host-independent' endosymbionts. However, recent results show that endosymbionts with highly reduced genomes can also rely on the import of some compounds from the host cell, blurring the line between endosymbionts and organelles. Unfortunately, systematically investigating all proteins and metabolites imported into endosymbionts is experimentally challenging and is thus rarely attempted. In addition, the subcellular composition of most intracellular symbioses is not understood at the 3D level, hampering correlations between diverse -omics (genomics, transcriptomics, proteomics, and metabolomics) and ultrastructural imaging data. This project was designed to overcome these difficulties and to understand the functioning of one such symbiotic system.

２．研究の目的

Purpose of the project. Understanding how highly integrated symbioses function is essential since one organelle in our cells, the mitochondrion, has originated from a symbiotic bacterium. The purpose of this project was to establish mass spectrometry methods in a highly integrated symbiotic system and to link the metabolomics and proteomics data to 3D subcellular imaging.

３．研究の方法

Research method. The tripartite bacterium-within-bacterium-within-insect symbiosis of the mealybug *Planoccocus citri* was used as a model system of a tightly interdependent symbiosis. The system was characterized by diverse omics and imaging methods, greatly expanding the experimental toolkit for the system and generating comprehensive data

４．研究成果

Research outcomes. Apart from already published articles, the main research outcomes of the project are multiple manuscripts in preparation, focusing on the genomics of scale insects and their symbioses (Tejeda Mora et al., in preparation and Choi et al., in preparation) and integrating 3D imaging with metabolomics/proteomics (Tejeda Mora et al., in preparation).

A. Metabolomics and proteomics were carried out for the P. *citri* system. Especially metabolomics was fully optimized, including the data analysis with a computational pipeline tailored for such a host-symbiont system. Hundreds of metabolites were identified in the system and assigned to biosynthetic pathways on the four genomes (*Tremblaya*, *Moranella*, the insect host, and the host plant).
B. Genome data for the system were massively improved by PacBio HiFi long reads and chromatin capture (HiC) scaffolding methods so that protein and metabolite identification can be now fully assisted with a high-quality reference genome. RNA-Seq data with proper biological replicates (single individuals) were also generated for the bacteriome tissues to improve the robustness of differential gene expression analyses.
C. Several complementary 3D imaging methods were newly established to reconstruct the system in 3D at different levels of resolution -- uCT of the whole insect, confocal FISH imaging of the whole bacteriome, SBF-SEM of many bacteriocytes, and FIB-SEM of a single *Tremblaya*.

The impact of the results and unanticipated findings. The results of the project greatly improve our understanding of the transition from an endosymbiont to a cellular organelle that was essential for the origin of the eukaryotic cell. Via the combination of omics with 3D imaging, we managed to understand which genes, proteins, and metabolites are localized in

the system and how they drive the tripartite interaction. Integrating data from all these advanced methods is unprecedented for a single symbiotic species and allows us to draw strong conclusions about host-symbiont integration (e.g. cell division coordination and protein import) in highly interdependent, organelle-like, symbioses. From several unanticipated findings, I would highlight two: (1) There are fewer *Tremblaya* cells per host than previously reported since the cells are blob-like and connected; (2) Even though our proteomics results are still inconclusive about the amount of protein exchange, our metabolomics results suggest much higher metabolite exchange than anticipated. Protein and metabolite exchange are thus currently under further investigation with advanced spatial mass spectroscopy-based methods (MALDI-MSI and LOPIT-DC).

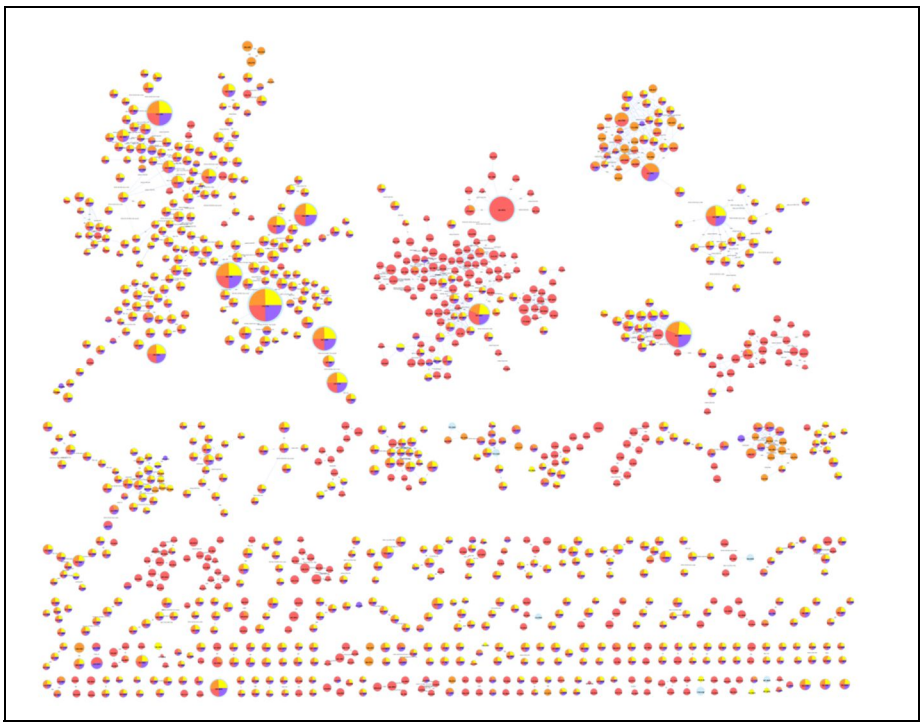

Figure 1. A molecular network of metabolites identified in the mealybug symbiotic system. 1,496 spectra were analyzed, and 951 library hits were detected. Yellow: bacteriome; orange: gut; red: remaining insect tissues; purple: host plant (potato sprouts); blue: blank control.

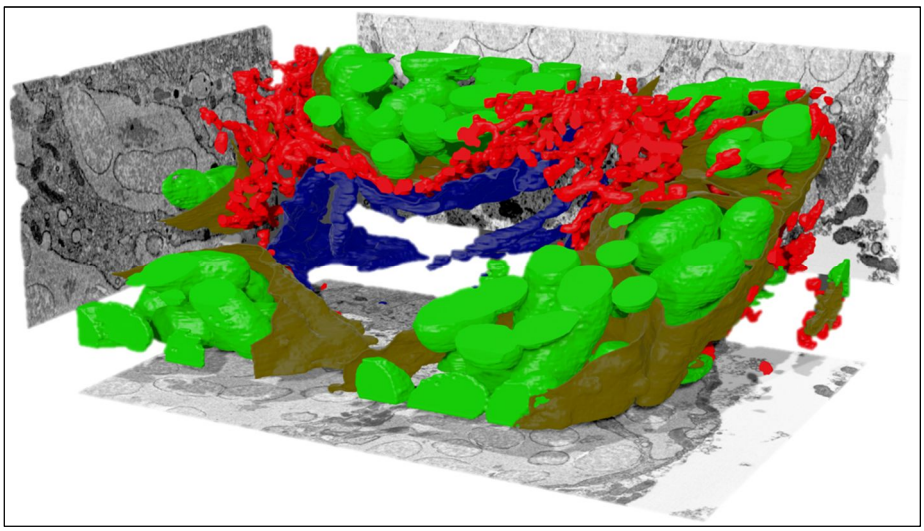

Figure 2. Segmented FIB-SEM data from several *Tremblaya* cells. Light green: *Moranella*; blue: nucleus; red: mitochondria; dark green: *Tremblaya*.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Husnik F, Tashyreva D, Boscaro V, George E, Lukes J, Keeling P	４．巻 13
２．論文標題 Bacterial and archaeal symbioses with protists: functional and evolutionary comparisons with animal models	５．発行年 2021年
３．雑誌名 Current Biology	６．最初と最後の頁 862-877
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.cub.2021.05.049	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Syberg-Olsen et al.	４．巻 39
２．論文標題 Pseudofinder: detection of pseudogenes in prokaryotic genomes	５．発行年 2022年
３．雑誌名 bioRxiv	６．最初と最後の頁 msac153
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2021.10.07.463580	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Husnik F	４．巻 33
２．論文標題 Organellogenesis: Host proteins control symbiont cell divisions	５．発行年 2023年
３．雑誌名 Current Biology	６．最初と最後の頁 R22-R25
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.cub.2022.11.028	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計8件（うち招待講演　7件／うち国際学会　5件）

１．発表者名 Filip Husnik
２．発表標題 Bacterial and archael symbioses with protists
３．学会等名 The DOE JGI Symposium ‘From New Lineages of Life to New Functions’（招待講演）
４．発表年 2021年

１．発表者名
Filip Husnik

２．発表標題
Bacterial and archael symbioses with protists

３．学会等名
The 34th Annual Meeting of the Japanese Society of Microbial Ecology（招待講演）

４．発表年
2021年

１．発表者名
Filip Husnik

２．発表標題
Bacterial and archael symbioses with protists

３．学会等名
The 4th Asian Congress of Protistology（招待講演）（国際学会）

４．発表年
2021年

１．発表者名
Choi, J., Palanichamy, P., Masukagami, Y., Tejeda, J.A., Husnik, F.

２．発表標題
Symbiont replacements are the rule rather than the exception in scale insects

３．学会等名
10th Congress of the International Society of Symbiosis（国際学会）

４．発表年
2022年

１．発表者名
Filip Husnik

２．発表標題
Bacterial and archaeal symbioses with protists

３．学会等名
EMBO Workshop on Comparative genomics of unicellular eukaryotes: Interactions and symbioses（招待講演）（国際学会）

４．発表年
2022年

１．発表者名
Filip Husnik

２．発表標題
Organellogenesis: Provide, Divide, and Rule

３．学会等名
International Society of Evolutionary Protistology (ISEP) biennial meeting（招待講演）（国際学会）

４．発表年
2023年

１．発表者名
Filip Husnik

２．発表標題
Two billion years of protist symbioses

３．学会等名
International Society of Protistology (ISOP) student meeting（招待講演）（国際学会）

４．発表年
2023年

１．発表者名
Filip Husnik

２．発表標題
Organellogenesis: Provide, Divide, and Rule

３．学会等名
ERATO Evolving Symbiosis Project International Seminar Series #21（招待講演）

４．発表年
2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関