

Graduate School of Advanced Science and Engineering  
Waseda University

# 博士論文概要

## Doctoral Thesis Synopsis

論文題目  
Thesis Theme

Comprehensive Analysis of Homoeolog  
Expression Bias in Allopolyploid Diatom  
*Fistulifera solaris* JPCC DA0580

異質倍数性珪藻

***Fistulifera solaris* JPCC DA0580 株の  
ホメオロガス遺伝子発現の網羅的解析**

申請者  
(Applicant Name)

Tatsuhiko	NOMAGUCHI
野間口	達洋

Department of Advanced Science and Engineering,  
Research on Life Science and Medical Bioscience

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Allopolyploidy is a genomic configuration wherein more than two genomes derived from divergent parental species coexist within an organism. It is known as prevalent genomic structure in terrestrial plants, in particular in agricultural crops, believed as an important source of genetic variation for plant speciation. Previous studies have revealed its intriguing property that it frequently provides advantageous characteristics, such as high environmental adaptability, to plants that render the organism to be more advantageous for agriculture. Therefore, allopolyploidy could be an advantageous genetic factor for improve the productivity of crops or any other plants for agricultural and industrial usage. However, throughout century long history of allopolyploid study, allopolyploidy has never been reported in microalgae, which is prominent hosts for industrial macromolecular production.

Microalgae, a major group of eukaryotic photosynthetic organisms, are one of the most successfully diversified organisms on the planet, which up to several million species are considered to exist worldwide. The study of microalgae has attracted large attention for the last few decades because they are recognized as prominent hosts for production of functional macromolecules which could be converted into useful products for the general publics, such as biofuels and pharmaceuticals. One of the useful microalgal macromolecule is lipids which are mainly accumulated in the form of triacylglycerol (TAG) in their cells. Various microalgal species, include species of Bacillariophyceae (or diatom), are known to naturally accumulate TAGs under certain stressed conditions.

Research group of candidate has previously discovered the oleaginous diatom *Fistulifera solaris* JPC DA0580 strain, which showed one of the highest lipid accumulating ability in reported microalgae (~65%, w/w) with decent growth rate in large-scale outdoor cultivation systems. This diatom has been received attention as a potential host for biofuel production, but in addition, its genomic study revealed that this is the world first allopolyploid discovered in microalgae, possessed 9,007 diversified gene pairs, called homoeologous gene, which has been inherited from two distinct ancestors. Since this diatom shows both allopolyploidy and high potency as a host for lipid production, there is a possibility that allopolyploid contribute to high productivity in microalgae as well as terrestrial plants. However, the study of allopolyploidy in microalgae are completely missing, and also the study was hindered by the difficulty of subgenome classification due to limited genomic resource of diatoms.

In this study, the candidate investigated the allopolyploid genomic structure of *F. solaris* and its expression, especially focusing on the lipid metabolic process. The molecular insights proposed in this study provide the novel insights to the mechanisms of lipid metabolism in highly oleaginous, allopolyploid microalga, and also provide the potential target for genetic engineering to develop the metabolically engineered microalga for lipid production.

This thesis is composed of following chapters:

- Chapter 1: General Introduction
- Chapter 2: Classification of Genome into Subgenomes
- Chapter 3: Homoeolog Expression Bias in Global Transcriptomes
- Chapter 4: Homoeolog Expression Bias in Lipid Metabolic Pathways
- Chapter 5: Summary and Future Prospects

The contents of each chapter are in the following:

### Chapter 1: General Introduction

In this chapter, general information of allopolyploidy and microalgae, especially focusing on diatom, will be described. The information includes 1) previous allopolyploid studies, mainly with terrestrial plants, 2) the microalgal usage in macromolecular production, 3) previous studies of diatoms, 4) strategy for improving lipid productivity in microalgae.

### Chapter 2: Classification of Genome into Subgenomes

In this chapter, allopolyploid genome of *F. solaris* was investigated. In previous study, the genome of *F. solaris* was reported as an allopolyploidy, due to abundance of duplicated genes with highly diversified sequence similarity, called homoeologous genes, which was considered to be generated from interspecies hybridization. However, its genomic information was intermingled with different ancestral genome, which could not be separated into distinct subgenomes due to lack of genomic resource of diatoms. This caused a difficulty of analyzing allopolyploid genome of *F. solaris*.

To overcome this challenge, we tentatively classified the genome of *F. solaris* into two pseudo-parental subgenome by analyzing GC content ratio and codon usage bias. Comparison of these species specific genomic characteristic enabled to divide the genome into two distinct fractions, which were tentatively called pseudo-parental subgenomes Fso\_h and Fso\_l. This classification enabled to study a biased gene expression in allopolyploid, known as homoeolog expression bias, which are explained in next chapter.

This Chapter is based on the article “Homoeolog Expression Bias in Allopolyploid Oleaginous Marine Diatom *Fistulifera solaris*”.

### Chapter 3: Homoeolog Expression Bias in Global Transcriptomes

In this chapter, homoeologous gene expression in *F. solaris* during lipid accumulation was comprehensively analyzed, based on classification in previous chapter. Previous allopolyploid studies in higher plants revealed that homoeologous genes from each parent frequently show unequal expression level. This biased expression, called homoeolog expression bias in allopolyploid, is considered to be important factor for allopolyploid to have diversified phenotype from its ancestors. However, since *F. solaris* is only genome-read allopolyploid microalga, study of homoeolog expression bias in microalgae is completely missing.

The transcriptome study revealed that more than half of the homoeologous gene pairs in *F. solaris* show homoeolog expression bias. It was also discovered that many genes showed homoeolog expression bias consistently towards specific subgenome, suggesting that some homoeologous genes are preferably expressed from one of the genome. Further expression analysis showed that homoeolog expression bias are occurring in specific metabolic pathways, which in case of *F. solaris*, lipid metabolism shows the homoeolog expression bias. Further analysis on homoeolog expression bias in lipid metabolic pathways will be described in chapter 4. This Chapter is based on the article “Homoeolog Expression Bias in Allopolyploid Oleaginous Marine Diatom *Fistulifera solaris*”

#### Chapter 4: Homoeolog Expression Bias in Lipid Metabolic Pathways

In this chapter, homoeolog expression bias of genes involved in lipid metabolic pathways were investigated. To study the expression bias in lipid metabolism observed in chapter 3, we looked the gene expression during lipid accumulation and degradation, and revealed that genes involved in lipid synthetic pathway in chloroplast and endoplasmic reticulum (ER) showed trend of biased expression towards subgenome Fso\_l, while genes involved in lipid degradation in peroxisome and mitochondria showed biased towards the other subgenome Fso\_h.

For analysis of lipid degradation pathway, sequence analysis was performed for discover putative TAG lipases. In initial step of lipid degradation, TAG is degraded to diacylglycerol (DAG) and free fatty acids by catabolic enzyme TAG lipases [EC3.1.1.3]. However, studies of TAG lipase in microalgae is limited, that only two TAG lipases were functionally characterized in model diatom species in recent studies.

Based on sequence analysis, 42 putative TAG lipases were discovered in *F. solaris*. In addition, these putative TAG lipases showed homoeolog expression bias towards Fso\_h during lipid degradation, which follow the trend discovered in chapter 3. This supports the results that lipid biosynthesis and degradation in *F. solaris* were strongly contributed by different subgenomes, suggesting that oleaginous phenotype of *F. solaris* could be generated by admixture of these subgenome.

This Chapter include informations from the article “Homoeolog Expression Bias in Allopolyploid Oleaginous Marine Diatom *Fistulifera solaris*” and “Comprehensive analysis of triacylglycerol lipase in the oleaginous diatom *Fistulifera solaris* JPC DA0580 with transcriptomics under lipid degradation-induced culture”.

#### Chapter 5: Summary and Future Prospects

In final chapter, the information of previous chapter was summarized and conclusion of this study was described. In addition, future prospect of this study was described.

## 早稲田大学 博士（理学） 学位申請 研究業績書

(List of research achievements for application of doctorate (Dr. of Science), Waseda University)

氏名 野間口 達洋 印( )

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種 類 別 (By Type)	題名、発表・発行掲載誌名、発表・発行年月、連名者（申請者含む） (theme, journal name, date & year of publication, name of authors inc. yourself)
Academic paper○	1. <u>Tatsuhiro Nomaguchi</u> , Yoshiaki Maeda, Tomoko Yoshino, Toru Asahi, Leila Tirichine, Chris Bowler, and Tsuyoshi Tanaka, "Homoeolog Expression Bias in Allopolyploid Oleaginous Marine Diatom <i>Fistulifera solaris</i> ", <i>BMC Genomics</i> , <b>19</b> (1), 330 (2018).
Academic paper○	2. <u>Tatsuhiro Nomaguchi</u> , Yoshiaki Maeda, Yue Liang, Tomoko Yoshino, Toru Asahi, Tsuyoshi Tanaka, "Comprehensive analysis of triacylglycerol lipase in the oleaginous diatom <i>Fistulifera solaris</i> JPC DA0580 with transcriptomics under lipid degradation", <i>J. Biosci. Bioeng.</i> <b>126</b> (2), 258-265 (2018).
Academic paper	3. Tomoko Yoshino, Natsumi Kakunaka, Yue Liang, Yasuhito Ito, Yoshiaki Maeda, <u>Tatsuhiro Nomaguchi</u> , Tadashi Matsunaga, and Tsuyoshi Tanaka, "Production of ω3 fatty acids in marine cyanobacterium <i>Synechococcus</i> sp. strain NKBG15041c via genetic engineering", <i>Appl. Microbiol. Biotechnol.</i> , <b>101</b> (18), 6899-6905 (2017).

## 早稲田大学 博士（理学） 学位申請 研究業績書

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種 類 別 By Type	題名、発表・発行掲載誌名、発表・発行年月、連名者（申請者含む） (theme, journal name, date & year of publication, name of authors inc. yourself)
Lectures (International)	<ol style="list-style-type: none"> <li>1. Yoshiaki Maeda, <u>Tatsuhiko Nomaguchi</u>, Tomoko Yoshino, Toru Asahi, and Tsuyoshi Tanaka, "Biased contribution of the homoeologous subgenomes to lipid metabolisms in the marine allodiploid diatom <i>Fistulifera solaris</i>", Oral, Asia-Pacific Marine Biotechnology Conference (APMBC) 2017, Hawaii, USA, 2017.5.</li> <li>2. <u>Tatsuhiko Nomaguchi</u>, Yoshiaki Maeda, Tomoko Yoshino, Toru Asahi, Leila Tirichine, Chris Bowler, and Tsuyoshi Tanaka, "Structural and Functional Analysis of Allopolyploid Genome in Marine Diatom <i>Fistulifera solaris</i>", Oral, International Conference "Molecular Life of Diatoms", Kobe, Japan, 2017.7.</li> </ol>
Lectures (National)	<ol style="list-style-type: none"> <li>1. <u>野間口達洋</u>, 前田義昌, 吉野知子, 朝日透, 田中剛, "海洋珪藻 <i>Fistulifera solaris</i> の異質倍数性ゲノムの解析", 口頭, 第三回分子珪藻研究会, 兵庫, 2016.12.</li> <li>2. <u>野間口達洋</u>, 前田義昌, 吉野知子, 朝日透, Chris Bowler, 田中剛, "海洋珪藻 <i>Fistulifera solaris</i> の異質倍数性ゲノムの解析", ポスター, NGS 現場の会 第五回研究会, 宮城, 2017.5.</li> </ol>