日本学術振興会に紙媒体で提出する必要はありません。



様　式　Ｆ－７－１

# 科学研究費助成事業（学術研究助成基金助成金）実施状況報告書（研究実施状況報告書）（令和元年度）

| 機関番号 | １４６０３ |
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| 所属研究機関名称 | | 奈良先端科学技術大学院大学 |
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１．研究種目名　若手研究

２．課題番号　19K16168

３．研究課題名　Changes in cellular dynamics of cellulose synthases during secondary cell wall production

４．補助事業期間　令和元年度～令和３年度

５．研究実績の概要

We are currently in middle of writing a draft manuscript on one of our related research projects. This project has looked at the role of a protein closely associated with cellulose synthesis, but who's function is unknown, especially in plant secondary cell walls. Our paper shows data supporting the hypothesis that this protein is important for controlling the speeds at which enzymes produce cellulose. This may cause the defects in the crystallinity of the cellulose being produced when the protein is absent, resulting in the decrease in cellulose strength but not quantity.
In terms of experiments we have carried out a number of key experiments in our project goals. This include carrying out several preliminary trials of different markers for lipid microdomains in our inducible secondary wall system. We have used a variety of commercially available dyes and probes and constructs from collaborating labs in our system to see if these lipid microdomains form or concentrate in areas around secondary walls.
Additionally, we have created photo-convertible fluorophore tagged cellulose synthase enzymes. With this it is now possible for us to track the movement of different populations of the same enzyme within a cell. With this tool we can begin to ask how the cell targets these proteins at the molecular level during different stages of secondary cell wall formation. Combined, these achievements form the foundation of our ongoing and future research.

６．キーワード

Cellulose　Plant Cell Wall　Plant Cell Biology　Molecular Biology　Microscopy　Secondary Cell Walls

７．現在までの進捗状況

区分　(3) やや遅れている。

理由

One of our original goals was to determine whether lipid microdomains surround cellulose synthase enzyme (CESA) complexes. However, a number of our experiments have resulted in negative or inconclusive results which did not support our hypothesis. There could be several causes for this including, that our hypothesis is wrong or that current probes do not work well in plants (many of these were developed for mammalian or yeast systems). These data though will still be used to supplement our other research project outlined in future work.
In terms of our other projects, we have been successful in developing photo-convertible fluorophore tagged CESA enzymes and are in process of carrying out specific experiments designed to answer questions about the intracellular trafficking of CESAs during the onset of secondary cell wall synthesis. These experiments are progressing smoothly and we plan to submit a manuscript by the end of this fiscal year.

８．今後の研究の推進方策

Our future work includes shifting one of our research goals from lipid microdomains to lipid contact sites. We have created lines of plants that contain fluorescently labelled markers for endoplasmic reticulum (ER) - plasma membrane (PM) contact sites. These contact sites are regions where the ER and PM are within 50 nm of each other and are believed to be important for non-vesicular transport and lipid recycling. Our preliminary data shows that there is a dynamic change in how these sites are organized from before and after secondary cell wall formation occurs. Currently we are attempting to elucidate the biological importance of this change in organization.
Our CESA trafficking project continues in elucidating the molecular mechanism for CESA movement and localization during secondary cell wall formation. This work includes carrying out site-directed mutagenesis for potential site for post-translational modifications such as ubiquitination and phosphorylation which may be integral for controlling CESA localization at different stages of its life. With such constructs it should be then easy to see if any movement of CESAs is perturbed when one of these sites are lost.

９．次年度使用が生じた理由と使用計画

Due to the large cost of upgrading our microscope to have both motorized sample stage and automatic focus control, we would like to carry over this year's unused amount to the next year in order for us to have enough funds to purchase such upgrades.
Additionally, new computers are needed to carry out image processing. The raw images produced by our AIRY scan microscope produces large files due to large amount of meta data associated with each file that is needed to do the post-acquisition image processing for airy scan super resolution. Thus, a new computer would need to be purchased in order to handle these large data files and process them efficiently.

１０．研究発表（令和元年度の研究成果）

〔雑誌論文〕　計0件

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　1件）

１．発表者名
Yoichiro Watanabe

２．発表標題
Elucidating the molecular mechanism of COBRA-LIKE4 in secondary cell wall cellulose production

３．学会等名
XV Cell Wall Meeting（国際学会）

４．発表年
2019年

１．発表者名
Ishio Hirono, Yoichiro Watanabe

２．発表標題
Identifying the cellular mechanism for CESA turnover during xylem differentiation

３．学会等名
新学術領域研究「植物構造オプト」第1回若手の会

４．発表年
2019年

〔図書〕　計0件

１１．研究成果による産業財産権の出願・取得状況

計0件（うち出願0件／うち取得0件）

１２．科研費を使用して開催した国際研究集会

計0件

１３．本研究に関連して実施した国際共同研究の実施状況

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１４．備考

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