氏　　名	渡部　聡朗
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第1251号
学位授与の日付	平成２１年３月２４日
学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第６条第１項該当
学位論文題目	Roles and biogenesis pathways of endogenous siRNAs and piRNAs in mouse germline cells
論文審査委員	主　査　教授　相賀　裕美子 教授　深川　竜郎 准教授　酒井　則良 准教授　池尾　一穂 室長　北條　浩彦 （国立精神・神経センター）

論文内容の要旨

Small RNAs ranging in size between 20 and 35 nucleotides (nt) are found in many organisms including yeasts, plants, and animals. Small RNAs are associated with Argonaute proteins, which are effectors of silencing, and involved in regulation of gene expression through translational repression, mRNA degradation, and chromatin modification in a sequence dependent manner. In mammals, three classes of small RNAs have been found; microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi interacting RNAs (piRNAs). miRNAs are ubiquitously expressed in many tissues. In contrast, piRNAs and siRNAs are expressed mainly in germline cells.

In the first chapter, I describe the results obtained from the analysis of mouse fetal piRNAs. piRNAs are 24-30 nt small RNAs that associate with Piwi proteins, which are a subfamily of Argonaute proteins. piRNAs and Piwi proteins are mainly expressed in the germline and implicated in germline development and silencing of retrotransposons. A recent study in mouse showed that DNA methylation of the 5'-untranslated region of LINE-1 is decreased in Piwi knockout (KO) male germ cells, raising the possibility that DNA methylation is mediated by piRNAs. Although piRNAs have been identified in neonatal and adult testes, they remained uncharacterized in fetal testes, where *de novo* methylation takes place. To examine the link between piRNAs and DNA methylation, I have analyzed piRNAs in fetal testes.

I cloned more than 100,000 small RNAs from fetal testes. A distinct set of piRNAs were expressed in fetal testes and most of them were derived from retrotransposons. L1Md and IAP1 retrotransposons, of which DNA methylation levels are reduced in Piwi KO, were the major classes in fetal piRNAs. This suggests that piRNAs define genomic retrotransposon sequences to be subject to DNA methylation. To further investigate the link between methylation and piRNAs, I generated and analyzed *Zucchini* KO mice. The *Zucchini* gene has been thought to be involved in the piRNA pathway in *Drosophila*. In *Zucchini* mutant mice, the expression level of piRNAs and methylation levels of L1Md and IAP1 were decreased in fetal testes. Furthermore, decreased methylation was also observed at a specific region of the *Rasgrf1* locus. In this region, piRNAs were specifically and densely mapped, and RNAs transcribed from the *Rasgrf1* locus were targeted by these piRNAs. The results of my study are in concordance with the piRNA-mediated DNA methylation in fetal mouse testes and thus help to understand the mechanism of piRNA-mediated DNA methylation.

In the second chapter, I describe the results obtained from the analysis of mouse oocyte endogenous siRNAs. siRNAs are generated from long double-stranded RNAs (dsRNAs) by a ribonuclease called *Dicer* and are mainly involved in defense against molecular parasites including viruses, transposons, and transgenes through RNA interference (RNAi) in plants and worms. RNA dependent RNA polymerase (RdRP) is involved in the generation of precursor dsRNAs from single stranded RNAs. Gene regulation by endogenous siRNAs has been observed only in organisms possessing RdRP.

Despite no report of RdRP activity in mammalian cells, endogenous siRNA molecules were previously observed in mouse fully grown oocytes. However, only a small number of endogenous siRNAs have been identified and their biogenesis and function largely remained unclear. In order to obtain a comprehensive picture of endogenous siRNAs, I have analyzed small RNAs expressed in mouse growing oocytes through deep sequencing.

I identified a large number of both ~25-27 nt piRNAs and ~21 nt siRNAs corresponding to mRNAs or retrotransposons in growing oocytes. piRNAs in oocytes play a role in the regulation of retrotransposons. siRNAs were exclusively mapped to retrotransposons or other genomic regions that produce transcripts capable of forming dsRNA structures. Inverted repeat structures, bidirectional transcription and antisense transcripts from various loci are sources of the dsRNAs. Some precursor transcripts of siRNAs were derived from expressed pseudogenes, suggesting that one role of pseudogenes is to adjust the level of the founding source mRNA through RNAi. Loss of *Dicer* or *Ago2* resulted in decreased levels of siRNAs and increased levels of retrotransposons and protein-coding transcripts complementary to the siRNAs. Thus, the RNAi pathway regulates both protein-coding transcripts and retrotransposons in mouse oocytes.

The results obtained here establish the existence of endogenous siRNAs in mammals, and provide the information on small RNAs expressed in mouse germline cells and new insights into the pathway and function of small RNAs.

論文の審査結果の要旨

渡部君はマウスの生殖細胞系列における小分子 RNA に焦点を絞り、その網羅的同定と機能解析を行った。小分子 RNA は、多くの生物に存在し遺伝子発現制御に関与する。哺乳類では、microRNA(miRNA)、small interfering RNA(siRNA)、Piwi-interacting RNA (piRNA)とよばれる 3 種類の小分子 RNA が存在し、siRNA や piRNA は生殖細胞で特異的に発現し、これら小分子 RNA の機能解析は大変興味深い。

渡部君の博士論文は 2 章からなっている。第一章で、まずマウス胎児期に発現する 10 万以上の小分子 RNA を同定し特に piRNA を解析した。渡辺君はこの時期の piRNA の多くは L1_Md と IAP レトロトランスポゾンに由来しており、これらの piRNA が DNA メチル化の獲得に関与することを示唆する結果を得た。さらに彼は、ショウジョウバエで piRNA 経路に関与する Zucchini 遺伝子のマウスホモログを単離しその欠損マウスを作製し機能解析を行った。その結果、Zucchini 変異マウスで piRNA が減少しレトロトランスポゾンの発現が上昇していること、piRNA 産生に関与する Mili の局在異常が見いだされる事、またインプリント遺伝子 Rasgrf1 領域の DNA のメチル化が減少することを発見し、Zucchini がマウスでも piRNA 経路に関わることを明らかにした。これらの結果は胎生期の精巣において、piRNA が DNA メチル化を受ける配列の決定に働くことを示唆し、そのメカニズムの解明にも道を開いた。

第二章では、マウス卵母細胞で発現する内在性 siRNA の存在を明らかにし、またその機構を示唆する結果を報告した。siRNA は、長い二本鎖 RNA から Dicer とよばれる酵素による切断により生成され、ウイルス、トランスポゾンの制御に働く。これまで、RdRP をもたない哺乳類においては、siRNA による遺伝子発現制御が報告されておらず、内在性の siRNA の存在に関しては懐疑的だった。渡部君は、成熟期のマウス卵子から小分子 RNA を網羅的に解析し piRNA と siRNA を同定した。さらに、siRNA が mRNA とレトロトランスポゾンの配列をもち、その前駆体のいくつかは偽遺伝子領域に由来しており、これらの siRNA が由来遺伝子の発現制御に働いていることを明らかにした。このことから、siRNA 経路は卵母細胞において遺伝子発現調節およびレトロトランスポゾン制御に働いていることがわかった。

このように渡部君の博士論文は、マウス胎生期の精巣で特異的に起こる DNA メチル化に piRNA 経路が深く関わること、哺乳類において偽遺伝子に由来する内在性 siRNA が存在し遺伝子発現を制御していることを明らかにした非常にオリジナリティの高い論文となっており博士論文として十分の内容をもっていると判断した。