氏　　名	鈴木　敦
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論文審査委員	主　査　教授　佐々木　裕之 教授　広海　健 助教授　酒井　則良 助教授　平田　たつみ チームリーダー　斎藤　通紀（理化学研究所）

論文内容の要旨

Germ cells are highly specialized for transmitting genetic information to the next generation. In the mouse, primordial germ cells (PGCs) are segregated from the somatic cell lineage at an early gastrulation stage. After the formation of PGCs, they continue to proliferate and migrate through somatic tissues to reach the gonad. Although germ cells are sexually bi-potential at the migrating stage, after their colonization in the gonad, the interactions between the germ cells and somatic cells regulate the sex-specific development and differentiation of the germ cells; the female germ cells enter into meiosis and proceed to the diplotene stage of meiotic prophase I, whereas the male germ cells become arrested at G1/G0 and undergo genome-wide DNA methylation and paternal imprinting. In 2006, it was reported that somatically produced retinoic acid (RA) induces an RA-responsive gene Stra8 in female germ cells and that this in turn initiates meiosis in the embryonic ovary. By contrast, in the embryonic testis, RA is degraded by a metabolizing enzyme Cyp26b1 before it reaches to the germ cells. Thus, although some factors regulating the sex-specific phenotype of male and female germ cells are beginning to be revealed, the molecular basis of the sex determination of germ cells is largely unknown.

Nanos is one of the evolutionarily conserved proteins known to be involved in germ cell development. The mouse has three Nanos gene homologues (Nanos1-3), in which Nanos2 and Nanos3 are essential for germ cell development. Nanos2 mRNA is first detected in male germ cells that have colonized in the embryonic gonad at around E13.5, when germ cells begin to interact with gonadal somatic cells. Although Nanos2 mRNA is transiently down-regulated at later embryonic stages, it is again detected in spermatogonia at 1 week after birth. When Nanos2 was disrupted by the gene knockout technology, the Nanos2-null male mutants showed a complete loss of germ cells due to apoptosis that occurred as early as E15.5. No phenotype was observed in the Nanos2-null female mutants.

To reveal the male-specific functions, other than the suppression of apoptosis, of Nanos2, I first investigated the effects of the Nanos2 knockout on the sexual differentiation of male germ cells. I found that the Nanos2-null male germ cells do not undergo cell-cycle arrest and continue to proliferate. Interestingly, a meiosis-specific marker Scp3 was highly up-regulated in these male germ cells. Since it was difficult to analyze the developmental fate of Nanos2-null male germ cells due to the apoptosis caused by the mutation, I tried to suppress it by introducing a mutation in the pro-apoptotic factor Bax. Once the apoptosis was successfully suppressed, the Nanos2-null male germ cells formed axial-cores along the chromosomes, a meiosis-specific nuclear morphology, and proceeded at least to the zygotene stage of meiotic prophase I. These cells expressed not only Scp3 but also another meiosis-specific marker Dmc1, further supporting the notion that Nanos2-null male germ cells entered into meiosis. Next, I attempted to express

Nanos2 ectopically in the female embryonic germ cells. In the Nanos2-expressing female germ cells, I observed disruption of the axial-core formation and induction of other events specific to the male germ cells, including the up-regulation of TDRD1 and Dnmt3L, enlarged nuclear morphology, and suppressed di-methylation of histone H3 Lys9. These data suggest that Nanos2 promotes male germ cell differentiation by preventing meiosis. Moreover, I found that Stra8, a key factor involved in the initiation of meiosis, is normally suppressed by Nanos2 in male germ cells. In addition, a forced expression of Nanos2 suppressed Stra8 even in female germ cells. These data indicate that Nanos2 prevents male germ cells from entering meiosis via suppression of RA signaling.

Next, I tried to reveal the molecular action of Nanos2. Although Nanos is known to co-localize with Tudor and Vasa and function to maintain germ cells in Drosophila, I found that Nanos2 does not co-localize with their mouse homologues TDRD1 and MVH. Instead, Nanos2 localized to P-bodies, the central place for RNA degradation. I also found that the number and size of P bodies were not changed in Nanos2-null male germ cells, suggesting that Nanos2 is not involved in the assembly of P-bodies. To reveal the function of Nanos2 in P-bodies, I identified some Nanos2-interacting mRNAs: one of them was found to be Stra8 mRNA. These data suggest that Nanos2 transports Stra8 mRNA to P bodies, resulting in its degradation and then suppression of meiosis. Taken together, my present results suggest that Nanos2 plays a critical role in establishing the sexual identity of male germ cells via RNA degradation.

論文の審査結果の要旨

哺乳類の発生過程において、雌の生殖巣に到達した始原生殖細胞は減数分裂へと移行するのに対して、雄の生殖巣に到達したものは一旦細胞周期を停止する。このような性差は、体細胞側から供給される性特異的なシグナルによってもたらされることが知られているが、その後の生殖細胞で起こる性分化の分子的基盤は不明である。本学位論文において、鈴木敦君は、マウスのRNA結合蛋白質の1つであるNanos2が生殖細胞の性分化を制御する重要な因子であることを明らかにした。

マウスのNanos2は胎生期の雄性生殖細胞に発現し、そのノックアウトマウスの雄は、生殖細胞がアポトーシスによって死滅するため不妊となる。鈴木君は、まずノックアウトマウスの解析により、(1) Nanos2が胎生期の雄性生殖細胞においてアポトーシスだけでなく減数分裂も抑制していることを示した。このことは、雌性生殖細胞に強制発現させたNanos2が正常な減数分裂を抑制することからも支持された。ところが、驚くべきことに、(2) Nanos2を雌性生殖細胞に強制発現すると、雄性生殖細胞に特異的なマーカー遺伝子やクロマチン修飾を誘導することが分かった。この発見は、Nanos2が生殖細胞の雄性分化に関わる重要な因子であることを示唆している。さらに、ノックアウトマウスや強制発現マウスの解析から、(3) 減数分裂を促進するレチノイン酸シグナルはNanos2の発現を抑制すること、逆に、Nanos2はレチノイン酸シグナルの下流にあって減数分裂を促進するStra8を抑制することが分かった。この結果をもとに、鈴木君は、生殖細胞の性分化の分子基盤に関する新たなモデルを提唱した。最後に、(4) Nanos2はRNAの分解に関わる細胞質内構造物であるP-bodyに局在し、Stra8などの標的遺伝子のmRNAと相互作用することで、それらの発現をpost-transcriptionalに抑制するであろうこと、を示した。これらの結果から、Nanos2は、RNAの代謝を介して減数分裂を抑制すると同時に、生殖細胞の雄性分化を誘導すると考えられた。

審査員全員でこの論文を審査し、マウスの生殖細胞の分化におけるNanos2の役割と作用機序に関する知見を大きく前進させ、生殖細胞の性分化について今後の研究の指針となる新たなモデルを提唱するに至ったことを高く評価し、本大学院の学位の水準を十分に満たす論文であると判断した。