氏　　名	西村 俊哉
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論 文 審 査 委 員	主　査　教授 吉田 松生 准教授 田中 実 教授 上野 直人 客員教授 近藤 寿人 京都産業大学 総合生命科学部

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論文内容の要旨
Summary of thesis contents

Sperm and eggs are the only cells with the capacity to produce the next generation and are derived from cells of common origin called germ cells. Sex determination is an essential step in determining the fate of germ cells, at which point they commit to becoming either sperm or eggs. In vertebrates, the sex determination genes identified thus far are expressed in somatic cells surrounding the germ cells. Influenced by these somatic cells, germ cells become either sperm or eggs. However, the mechanism by which the sexual fate of germ cells is determined in vertebrates has not yet been revealed. The objective of this study was to unveil the mechanism by which germ cells acquire sexual differences and identity at the molecular level, using medaka as a model.

To identify the essential factors affecting the sex of germ cells, germ cells of both sexes at different stages of development during sex differentiation were isolated using a fluorescence-activated cell sorter (FACS). Then, global gene expression of these cells was analyzed using microarray and RNA-seq. I identified two intrinsic factors affecting germ cell sex in medaka.

In medaka, a sex determination gene on the Y chromosome, *DMY*/*dmrt1bY*, is expressed in gonadal somatic cells; it regulates the sexual identity of germ cells after gonad formation. Sexual differentiation of germ cells is initiated after the somatic sex has been determined; however, analysis of a novel gene, *Sdgc* (Sex chromosome–dependent Differential expression in Germ Cells) revealed that medaka germ cells could exhibit sexually different characters cell-autonomously prior to gonad formation. This study revealed a novel mechanism underlying sex differences between germ cells, but the sexual differences seemed not to have a critical effect on the sexual identity of germ cells after gonad formation. Next, in the course of screening sexually different genes in germ cells after gonad formation, I identified *SDiG* (Sex Determination in Germ cells) as an essential gene for the sperm–egg fate decision and demonstrated the mechanism by which germ cells acquire their sexual identity.

Prior to gonad formation, the first factor, *Sdgc*, was highly enriched in early XY germ cells compared to XX germ cells. Chimeric analysis revealed that sexually different expression of *Sdgc* was controlled in a germ cell–autonomous manner by the number of Y chromosomes. Unexpectedly, *DMY*/*dmrt1bY* was expressed in germ cells prior to gonad formation, but knockdown and overexpression of *DMY*/*dmrt1bY* did not affect *Sdgc* expression. I also found that XX and XY germ cells isolated before the onset of *DMY*/*dmrt1bY* expression in gonadal somatic cells behaved differently *in vitro* and were affected by *Sdgc*. This finding suggests that cells can autonomously exhibit sexually different characters by a mechanism independent of a sex determination gene, even in animals in which sex is determined by that gene.

After gonad formation, the second factor, *SDiG* was detected in premeiotic germ cells of both male and female at the early stage of gonadal sex differentiation but completely disappeared during male development. The XX mutants with disruption of *SDiG* in germ cells initiated spermatogenesis precociously in the female gonadal environment, not awaiting the normal timing of male puberty. Sperm were continuously produced and filled the expanded germinal epithelium of the ovary, while a few oocytes were present in the stromal compartment. Artificial insemination demonstrated that the sperm were fully fertile. Therefore, this study demonstrated that suppressing the initiation of spermatogenesis is essential for acquisition of sexual identity during oogenesis.

In somatic cells, antagonism between male and female pathways is a key element in the establishment and maintenance of sexual identity during embryonic development and in adults, respectively. The antagonistic regulation of sex determination may also extend to germ cells. Furthermore, this study also suggest that even if this antagonism in germ cells results in the establishment of the sex opposite to that in somatic cells, germ cells could initiate the gametogenesis independent of the somatic sex. Therefore, it is ultimately germ cells that instruct the adaptation of sexual fates in gametogenesis, and somatic signals are “cues” that create the imbalance between female and male signals in germ cells.