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論文内容の要旨

Female mammals have two X chromosomes, whereas males have only one. In general, an increase in dosage can cause higher levels of gene products, leading to cell death or serious disorders, and sex chromosome is not an exception. To compensate for dosage difference in X-linked genes between the sexes, one of the two X chromosomes in females is transcriptionally silenced during early development. Although there are some controversies, prevailing view supports the idea that this chromosomal silencing event known as X-inactivation initiates at the preimplantation stages with the paternal X being preferentially inactivated. This imprinted X-inactivation is maintained in the extraembryonic tissues such as the placenta and a part of the extraembryonic membranes. In contrast, the inactivated paternal X chromosome becomes transiently reactivated in a subset of cells in the inner cell mass (ICM), which are committed to the epiblast lineage giving rise to all tissues of the fetus including germ cells, and subsequently one of the two X chromosomes undergoes inactivation in a random fashion with regard to the parental origin as cells differentiate. Although the X chromosome thus inactivated in the epiblast lineage is stably maintained over successive cell divisions, it is known that the inactivated X chromosome becomes reactivated in those cells that have contributed to primordial germ cells (PGCs).

X-inactivation is mediated by noncoding Xist RNA encoded on an X chromosome. It has been shown that the *Xist* gene is essential for the initiation of X-inactivation and its RNA products expressed from the future inactive X coats the chromosome in *cis* to induce chromosomal silencing upon cellular differentiation. Available evidence suggests that the requirement of Xist RNA for the maintenance

of the inactive state is developmentally regulated. During the early phase of differentiation, Xist RNA is required for the maintenance as well as the initiation of X-inactivation, but once the inactive state is established in differentiated cells, X-inactivation shift to the one that is independent of the RNA. It has been shown that in the process of X-reactivation, Xist RNA accumulated on the X chromosome disappears in developing PGCs prior to meiosis. Although the activity of the X chromosome in PGCs appears to be closely correlated with the presence of Xist RNA on the X chromosome, it is unclear whether a loss of Xist RNA is a cause or a consequence of X-reactivation. Given a critical role of *Xist* in the process of X-inactivation, however, it seems reasonable to assume that X-reactivation could be compromised if the expression of *Xist* is sustained in PGCs. To address the impact of Xist RNA on the activity of the X chromosome and molecular mechanisms of X-reactivation in female germ cells, I attempted to sustain *Xist* expression in female PGCs and examine the effect on X-reactivation. I took advantage of a new *Xist* allele, $Xist^{CAG}$, where the endogenous *Xist* promoter had been replaced with a CAG promoter known to drive gene expression in many types of cells including female germ cells. I expected here that this allele constitutively expresses Xist RNA in PGCs, and perhaps, compromises X-reactivation. In female embryos heterozygous for $Xist^{CAG}$, X-inactivation was confined to the mutated X chromosome (X^{CAG}) in somatic cells, and most probably, in the progenitors of PGCs at the time when they initially arose during development.

I examined in detail the expression of Xist RNA from the $Xist^{CAG}$ allele in PGCs of XX^{CAG} fetuses. RNA-FISH revealed that contrary to my initial expectation, Xist RNA was not detected in PGCs at embryonic day (E) 16.5. It was, however, still

retained on X^{CAG} in a significant proportion of PGCs at E10.5 and E13.5. This contrasted well with the fact that Xist RNA had been disappeared at E10.5 in wild-type female PGCs. The *Xist*^{CAG} allele, therefore, allowed me to study the effect of such prolonged expression of *Xist* on X-reactivation in PGCs. I took advantage of two X-linked transgenes, lacZ and EGFP, introduced on X^{CAG}, both of which had been previously used to monitor the activities of the X chromosome during develoment. The result demonstrated that reactivation of EGFP but not *lacZ* was significantly retarded by the prolonged expression of *Xist*. Subsequently, I examined the activities of the endogenous genes on X^{CAG} in PGCs by allele-specific RT-PCR and found that reactivation of some endogenous X-linked genes was also affected. These results suggested that the prolonged expression and/or retention of Xist RNA retarded the timing that a subset of genes regained the transcriptional activity on X^{CAG} in PGCs. It is, therefore, likely that downregulation of *Xist* is a key event at the onset of X-reactivation in female PGCs. However, synapsis formation between the wild-type X and X^{CAG} at the pachytene stage was not affected and XX^{CAG} females produced mature functional eggs, regardless of their possession of either wild-type X or X^{CAG}, indicating that the observed delay in the reactivation of X^{CAG} compromise neither meiosis nor oogenesis. Unexpected finding of this study was that the CAG promoter, although known to effectively drive gene expression in many types of cells including germ cells, was eventually repressed in female germ cells. Given the fact that the CAG promoter integrated at other loci is functional in PGCs, my failure to drive constitutive expression of *Xist* in PGCs using the *Xist*^{CAG} allele could be ascribed to the effect specific for the *Xist* locus, implying the presence of a locus-specific regulation of *Xist* in female PGCs. Quantitative RT-PCR showed that

the expression levels of both splice and nascent transcript was much lower in germ cells heterozygous for *Xist*^{CAG} than those in surrounding somatic cells, suggesting that the *Xist*^{CAG} was somehow trasncriptionally repressed in germ cells. In addition, bisulfite sequencing revealed that this was not apparently due to methylation of CpG sites in the CAG promoter. Intriguingly, the endogenous *Xist* promoter was found to be essentially unmethylated in wild-type PGCs despite the fact *Xist* expression was totally repressed. These findings suggest that the unexpected repression of the CAG promoter in PGCs is ascribed to a locus specific, perhaps DNA methylation-independent, mechanism effective only at the *Xist* locus. My study would provide some insight into our understanding of the molecular mechanism of X chromosome reactivation in female PGCs and facilitate further studies in the future.

博士論文の審査結果の要旨

哺乳類の雌はX染色体を２本持ち、その一方が初期発生の過程で不活性化され、この不活性化状態が細胞分裂後も維持される。X染色体不活性化は、この染色体の遺伝子発現量における雌雄間の差を少なくするのに貢献している。X染色体の不活性化には、蛋白質をコードしない転写産物であるXist RNAが必要なことがわかっている。また、不活性化されたXは、雌の生殖細胞で再活性化され、両方のXが活性化された状態で減数分裂がおこる。この再活性化に先立ち，Xist RNAが減少することが知られているが、このXistの消失とX染色体再活性化との因果関係や、減数分裂に対する影響については、これまで探索されていなかった。

尼川裕子さんは、XistによるX染色体活性制御の発生過程における役割に興味を持ち研究を行なった。Xistの発現を操作するため、野生型Xistのプロモーターを、構成的に働くプロモーターであるCAGプロモーターで置き換えたX染色体（以降、X-CAGと表す）を持つマウスを用いた。これによって、Xist発現様式を変化させ、その効果を調べた。

体細胞においては、常にX-CAGアレルからXistが発現し，それにより、X-CAG染色体の複製時期が遅れること、複数のＸ連鎖遺伝子の発現が抑制されることを見出した。したがって，体細胞ではX-CAGからのXistの発現によってX染色体の不活性化を引き起こせたと考えられる。

さらに、雌の生殖細胞におけるX-CAGの挙動を調べたところ、野生型アレルでXistの発現が抑制されている時期になっても、X-CAG上にはXistが持続して存在していた。このようなXでは、X染色体の再活性化が遅延した。このことは、Xistの消失がX染色体再活性化を引き起こす可能性を示唆している。一方、X染色体の再活性化の遅延にもかかわらず、X-CAGを持つ細胞においても、減数分裂は正常におこり，受精能を持つ卵子も形成された。

構成的と考えられるプロモーターであるCAGを用いているにもかかわらず、何日か遅れて、生殖細胞においてXist RNAが検出されなくなった。この興味深い現象が転写の抑制によるのか、転写後のRNAの挙動の変化によるのかを知るため、スプライシング前のXist RNAを定量したところ、やはり生殖細胞でのRNAレベルが顕著に低下しており，転写段階で生殖細胞特異的な発現抑制がおこったことが示唆される。この観察は、生殖細胞におけるXist発現制御機構に関して重要な知見を提供すると考えられる。

この研究は、生殖細胞におけるX染色体の再活性化にXist発現が影響しうることを示すなど、X染色体再活性化の制御機構という重要な問題において、これまでの知見を大きく前進させるものである。審査員全員で審査した結果、本大学院における学位授与の水準を十分に満たす論文であると判断した。