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Abstract of Doctoral Dissertation

Degree requested of Doctor　Life Science　Applicant's name　Matiz-Ceron Luisa

Title of Doctoral Dissertation

Informatics studies on the sex chromosome evolution in genus *Tokudaia*, focusing on the X chromosome inactivation mechanism
(X 染色体不活性化に焦点をあてたトゲネズミ属における性染色体進化の研究)

X chromosome inactivation (XCI) is an essential mechanism for dosage compensation between females and males in mammals. Wherein one of the X chromosomes in females is transcriptionally silenced. This process ensures equitable X-linked gene expression levels in somatic cells. Also, XCI involves silencing one of the two X chromosomes in each cell of a female's body. XCI process is not only necessary to realize the balance between females and males, but the modes of X chromosome regulation, especially mosaicism and skewing of XCI, influence the severity of diseases caused by X-linked mutations. In humans, age-related X reactivation could further influence the diversity of phenotypes observed in diseases. For example, cancer and autoimmune diseases as neurological disorders have been known. In females, XCI is controlled by a complex, conserved locus termed the X inactivation center (Xic), in which the lncRNA *Xist* is the key regulator.

In mice and humans, the Xic encompasses an approximately 660 kb region. Several genes reside in the Xic, including the lncRNAs *Tsx, Xist, Tsix, Jpx*, and *Ftx*, and protein-coding genes *Cdx4, Chic1, Slc16a2*, and *Rlim*. There is a complex relation and interaction between these genes. The cornerstone of Xic regulation is the lncRNA X-inactive specific transcript, *Xist*. *Xist* depends on a series of repeats to perform the early and late steps of XCI. *Xist* is the central transcript in a regulatory network orchestrated by two topologically associating domains (TADs) on the X chromosome, TAD-E, and TAD-D, each playing a vital role in governing the XCI process and playing a positive and negative effect respectively. The mechanism underlying XCI, resulting from the XX/XY constitution, is conserved and widespread among Eutheria.

Nevertheless, a few exceptional mammalian species possess unique sex chromosomes that could mean differences in the conserved process, like the Y-bearing females, Y-loss between, among others. The genus *Tokudaia* (Muridae, Rodentia) stands out as a prime example of Y-loss. This genus includes three species, each endemic to a single island in southernmost Japan, and is faced with significant conservation challenges. The Tokunoshima spiny rat (*Tokudaia tokunoshimensis*, TTO, 2*n*=45, XO/XO) and Amami spiny rat (*Tokudaia osimensis*, TOS, 2*n*=25, XO/XO) lack one X chromosome and the Y chromosome. Both males and females share a single X chromosome, and their genome sequences are nearly identical, with only a male-specific 34 kb duplication upstream of *Sox9* on chromosome 3 in TOS, resulting in equal gene dosage of the X chromosome in the XO/XO species. By contrast, the Okinawa spiny rat (*Tokudaia muenninki*, TMU, 2*n*=44) exhibits the typical XX/XY sex chromosome system found in most mammals. However, the sex chromosomes have fused with a pair of autosomes, forming recently acquired regions called as the neo-X and neo-Y on the X and Y chromosomes, respectively. These neo-X and neo-Y regions display partial genetic differentiation, indicating recombination suppression between them.

Based on the above information, I hypothesized structural and expression changes in the sex chromosomes of species with unusual determination systems, like the loss of one X chromosome and the Y chromosome, even in regions usually conserved in Eutherian, like the Xic. To verify this hypothesis, I used the three species of genus *Tokudaia*, the Okinawa spiny rat (TMU, XX/XY), the Tokunoshima spiny rat (TTO, XO/XO), and the Amami spiny rat (TOS, XO/XO). Using a bioinformatics approach, their reciprocal genome and RNA-Seq were used to analyze gene structure, order, expression, and repeats location of Xic in the X chromosome. In Chapter I, I compared the gene location and order in Xic of the genus Tokudaia to test whether Xic was different between XO/XO species and XX/XY species. I annotated the Xic region and the genes inside by using homology and RNA-Seq-based prediction. Intra and inter-genic regions were evaluated. In Chapter II, I analysed the detailed structure and expression of defined nine genes (*Cdx4, Chic1, Tsx, Xist, Tsix, Jpx, Ftx,*

Rlim, and *Slc16a2*) in Xic of *Tokudaia* compared with mice. Exons, introns, and coding sequence (CDS) were determined. For the key factor, *Xist*, repeats that indicate function were identified. To explore whether the Xic genes operate in the XO/XO species an expression analysis was performed. In Chapter III, to understand whether TEs are the potential elements of mutation in Xic, I performed a repeat analysis on the X chromosome. I identified types of TEs in the X chromosome and regions with high accumulation of repeat sequences. The TEs located in Xic were identified. Rat and mouse chromosomes were compared to the genus *Tokudaia*.

Based on the results obtained through these experiments, I discussed the potential contribution of the loss of one X chromosome and the Y chromosome, the consequences of the degradation of the XCI system, and possible sources of these changes.

My findings indicate that even if the *Xist* and *Tsix* lncRNAs are expressed, they are incapable of producing a successful and lasting XCI in the XO/XO species. I hypothesized that the significant structure change in intergenic region of *Jpx-Ftx* resulted in the inability to perform the XCI, and, as a result, a lack of *Xist* expression. I analyzed the transposable elements in the X chromosome of the genus *Tokudaia*, comparing them with those in mice and rats. The findings of unique transposable elements specific to *Tokudaia* could help us understand the mutational forces that have modified the conserved Xic in mammals. Furthermore, the decreased density of crucial LINE elements in the XO/XO species indicates a loss of the inactivation capacity of the X chromosome, shedding light on the evolutionary and functional dynamics of sex chromosome regulation. My results collectively suggest that structural changes in the Xic occurred in the ancestral lineage of the XO/XO species, likely due to the loss of one X chromosome and the Y chromosome and a consequence of the degradation of XCI system.