

博士論文（要約）

Developmental Cell Biology of the Molecular Motor, KIF3

(分子モーターK I F 3による発生の制御機構の研究)

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論文の内容の要旨

論文題目 : **Developmental Cell Biology of the Molecular Motor, KIF3**
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The kinesin superfamily proteins (KIFs) have been reported to play crucial roles in neuronal function and tissue development. KIF3B, a classical member of the kinesin 2 family, has been reported to form a heterotrimeric KIF3 complex with KIF3A motor and the associated protein, KAP3. As previously reported, *Kif3b* knockout mice (KO mice) show severe developmental phenotypes, including loss of cilia, randomization of left-right determination, abnormal neural tube development, pericardial swelling, developmental retardation, and midgestation lethality. Due to this midgestation lethality, the physiological relevance of KIF3B in the adult has been poorly understood.

In order to circumvent this problem, I established *Kif3b* hypomorph KO mice, on the way to generation of three-*loxP*-type conditional knockout mouse. Because the first P-loop exon was very long, I inserted one of the *loxP* sequences in frame into the coding region of motor domain (P-loop). Floxed positive selection cassette carrying a splicing acceptor sequence was inserted in the first intron. The targeting vector was constructed

by using the *Kif3b* genomic DNA and electroporated to J1 mouse ES cells and selected by G418 antibiotics for neo-resistant clones.

They were then subcloned and expanded to be screened for the homologous recombinant clones by genomic Southern blotting. The homologous recombinant ES clones were then expanded and subjected to blastocyst injection to raise chimeric mice which were backcrossed to laboratory mouse of C57BL/6 strains, and maintained in a specific pathogen free environment at University of Tokyo under the local restriction.

Homozygous mice for this *3loxP* allele were delivered alive, but they did not follow the Mendel's law suggesting a partial embryonic lethality. 12.3% of pups (n = 65) were turned out to be homozygotes according to the PCR genotyping on 10.5 dpc. KIF3B expression was downregulated in KIF3B *3loxP* homozygous mice due to a gene trap by the splicing-acceptor-carrying positive selection cassette of gene targeting. It circumvented the midgestation lethality and the laterality of internal organs was normal. I could not detect any remarkable morphological changes in the nodal monocilia. This was consistent with the results of the loss of left-right randomization phenotype in this hypomorph mice. On the other hand, the hypomorph mouse revealed a polydactyly phenotype in their limbs. Interestingly, the ciliogenesis of hypomorph limb buds appeared to be normal, suggesting that this mouse model will serve as a new

polydactyly model without an apparent defect in ciliogenesis.

Thus, this hypomorph mouse will be a new model for analyzing the relevance of KIF3B motor in developmental patterning, as an essential molecular basis for studying the relevance of this molecular motor in developmental signal transductions.