CRISPR/Cas9-based mutagenesis of neuropeptide receptors potentially involved in nociception in *Drosophila melanogaster*

Dvyne Issei Nosaka（筑波大学 生物学類）　古久保-徳永 克男（筑波大学 生命環境系）

Introduction

Nociception is a vital function for the survival of organisms. Because it is essential, many components of nociception are expected to be evolutionarily conserved. *Drosophila melanogaster* is a suitable model for studying the molecular basis of sensory systems including nociception. In our lab, using Drosophila, a genetic screen has been performed to identify neuropeptide genes important for nociception and several candidate neuropeptides genes have been successfully identified. However, there are currently no neuropeptide receptor mutants for most of candidate neuropeptides that have been isolated from the screen. Therefore, I performed a CRISPR/Cas9-mediated mutagenesis for potentially nociceptive neuropeptide receptors.

Materials and Methods

Mutagenesis with CRISPR/Cas9 genome editing

The genetic crossing that was carried out to obtain candidate mutant strains is shown in Fig. 1 (Kondo and Ueda , 2013). nos-Cas9 strains were crossed to gRNA (guide RNA) strains to obtain offspring expressing both Cas9 and gRNA in germline cells, where Cas9-based genome editing is thought to be performed (Step 1 and 2). To preserve potential mutant genes, the offspring at the next stage are all isolated because there are variations of mutations for the same gene (Step 2 and 3). At the final stages, the potential mutant gene is preserved permanently by crossing offspring with the potential mutant and the balancer gene to another set of balancer flies, thus creating a permanent, isolated stock for the potential mutagenized gene (Step 4). Finally, the target region of gRNA was PCR amplified and sequenced to determine whether mutagenesis was successful.

Results and Discussion

I have generated more than 10 candidate mutant lines for three different neuropeptide receptor genes. Mutations are still being confirmed and the results will be reported in the presentation. Future research plans are to observe behavioral nociception phenotypes of these mutants.

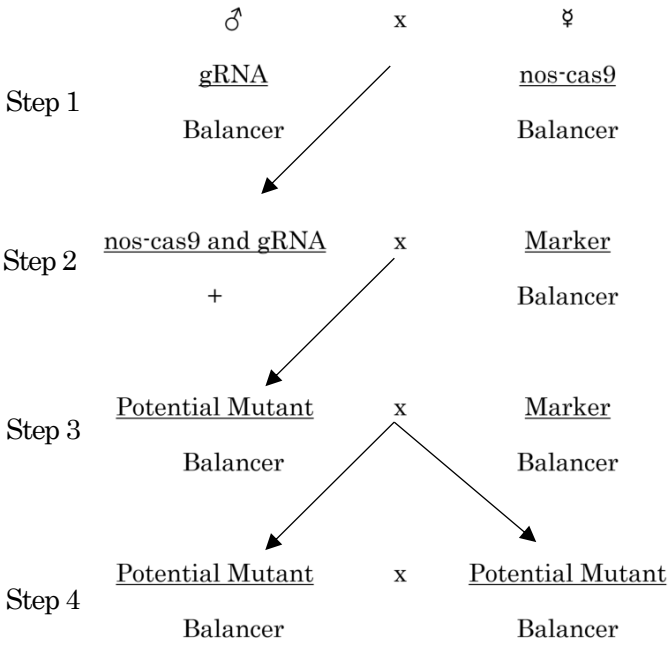

Fig. 1 Crossing scheme to isolate potential mutant lines

References

1) Kondo, Ueda. Highly Improved Gene Targeting by Germline-Specific Cas9 Expression in Drosophila. *Genetics*. 1, November, 2013. Pages 715-721.