Proc. Arthropod. Embryol. Soc. Jpn. 49, 19–20 (2016)

ISSN 1341–1527

Gene Knockout by Transcription Activator-like Effector Nucleases (TALENs) in the Sawfly, *Athalia rosae ruficornis* Jakovlev (Hymenoptera)*

Masatsugu HATAKEYAMA[1] and Megumi SUMITANI[2]

[1] *Division of Insect Sciences, National Institute of Agrobiological Sciences, Owashi Tsukuba, Ibaraki 305–8634, Japan*
[2] *Genetically Modified Organism Research Center, National Institute of Agrobiological Sciences, Owashi, Tsukuba, Ibaraki 305–8634, Japan*
E-mail: sawfly@affrc.go.jp (MH)

Recent progress in genomics and transcriptomics together with the establishment of new molecular tools enables questions in evolution, development and physiology to be addressed at the molecular level. Experimental methods inducing gene misexpression, such as transgenesis, gene knockdown and knockout, contribute to accelerate gene functional analysis in insects (Daimon *et al*., 2014). In the sawfly, *Athalia rosae ruficornis*, transposon (*piggyBac*)-mediated germline transformation and gene silencing (knockdown) by RNA interference have been established (Sumitani *et al*., 2003, 2005; Yoshiyama *et al*., 2013). Here, we show that gene disruption (knockout) is feasible in this species by using transcription activator-like effector nucleases (TALENs), one of the genome editing tools used in a variety of organisms (Joung and Sander, 2013). A pair of TALENs consist of a DNA-binding protein derived from plant pathogenic bacteria fused to the DNA cleavage domain of a restriction endonuclease *Fok*I, and *Fok*I dimer creates targeted double-strand breaks, resulting in an insertion/deletion mutation upon repair of the break (Christian *et al*., 2010; Miller *et al*., 2011).

In the present study, TALENs were designed using a high efficient construct developed by Cermak *et al*. (2011) and Takasu *et al.* (2013), to bind specifically to the sequences corresponding to the chromophore region of the enhanced green fluorescent protein (EGFP) gene. These TALEN-coding DNA fragments were transcribed to mRNAs *in vitro* and injected into the anterior end of mature unfertilized eggs taken from the transgenic strain carrying an EGFP transgene driven by the 3xP3 eye-specific promoter (Hatakeyama *et al*., 2009). More than a half of the G_0 males (26/41) showed a chimeric phenotype in terms of eye EGFP expression, suggesting that cells bearing the disrupted EGFP gene contributed to development. After progeny testing, four out of 26 chimeric G_0 males produced EGFP-negative G_1 females. As these G_1 females retained the EGFP transgene with deletion by some base pairs at the targeted region, we concluded that these females were originated from EGFP-disrupted sperm of the chimeric G_0 males and, hence, the EGFP-knockout individuals.

By contrast, about 15% of the G_0 males (8/53) showed a chimeric phenotype when the TALEN-mRNAs were injected at the posterior end of the eggs. Six out of eight chimeric G_0 males produced presumable EGFP-knockout G_1 females. It remains to confirm insertion/deletion in the EGFP transgene of these G_1 females. We nevertheless, conclude that gene knockout by TALENs is feasible in *A. rosae ruficornis*.

References

Cermak, T., E. L. Doyle, M. Christian, L. Wang, Y. Zhang, C. Schmidt, J. A. Baller, N. J. Somia, A. J. Bogdanove and D. F. Voytas (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Research*, **39**, e82.

Christian, M.,T. Cermak, E. L. Doyle, C. Schmidt, F. Zhang, A. Hummel, A. J. Bogdanove and D. F. Voytas (2010) Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics*, **86**, 757–761.

Daimon, T., T. Kiuchi and Y. Takasu (2014) Recent progress in genome engineering techniques in the silkworm, *Bombyx mori*. *Development Growth & Differentiation*, **56**, 14–25.

Joung, J. K. and J. D. Sander (2013) TALENs: a widely applicable technology for targeted genome editing. *Nature Reviews Molecular Cell Biology*, **14**, 49–55.

Hatakeyama, M., M. Sumitani, D. S. Yamamoto, J. M. Lee and K. Oka (2009) The sawfly, *Athalia rosae ruficornis* (Hymenoptera) as a model insect for developmental and reproductive biology: What has been done and what should be done? *Proceedings of the Arthropodan Embryological Society of Japan*, **44,** 1–12.

Miller, J. C., S. Tan, G. Qiao, K. A. Barlow, J. Wang, D. F. Xia, X. Meng, D. E. Paschon, E. Leung, S. J. Hinkley, G. P. Dulay, K. L. Hua, I. Ankoudinova, G. J. Cost, F. D. Urnov, H. S. Zhang, M. C. Holmes, L. Zhang, P. D. Gregory and E. J. Raber (2011) A TALE nuclease architecture for efficient genome editing. *Nature Biotechnology*,

* Abstract of paper read at the 50th Annual Meeting of the Arthropodan Embryological Society of Japan, July 3–4, 2014 (Fuchu, Tokyo)

29, 143–148.

Sumitani, M., D. S. Yamamoto, J. M. Lee and M. Hatakeyama (2005) Isolation of *white* gene orthologue of the sawfly, *Athalia rosae* (Hymeoptera) and its functional analysis using RNA interference. *Insect Biochemistry and Molecular Biology*, **35**, 231–240.

Sumitani, M., D. S. Yamamoto, K. Oishi, J. M. Lee and M. Hatakeyama (2003) Germline transformation of the sawfly; *Athalia rosae* (Hymenoptera: Symphyta), mediated by a *piggyBac*-mediated vector. *Insect Biochemistry and Molecular Biology*, **33**, 449–458.

Takasu, Y., S. Sajwan, T. Daimon, M. Osanai-Futahashi, K. Uchino, H. Sezutsu, T. Tamura and M. Zurovec (2013) Efficient TALEN construction for *Bombyx* gene targeting. *Plos One*, **18**, e73458.

Yoshiyama, N., K. Tojo and M. Hatakayema (2013) A survey of the effectiveness of non-cell autonomous RNAi throughout development in the sawfly, *Athalia rosae* (Hymenoptera). *Journal of Insect Physiology*, **59**, 400–407.