Development of an RNA interference method in the cladoceran crustacean *Daphnia magna*

Yasuhiko Kato[1], Yasuhiro Shiga[2], Shin-ichi Tokishita[2], Hajime Watanabe[1]
[1]Department of Biotechnology, Osaka University, Japan, [2]School of Life Science, Tokyo University of Pharmacy and Life Sciences, Japan

Daphnids are small crustaceans ubiquitous in fresh water; they have been a subject of study in ecology, evolution, and environmental sciences for decades. To understand data accumulated in daphnid biology at the molecular level, expressed sequence tags and a genome sequence have been determined. However, these discoveries lead to the problem of how to understand the functions of newly discovered genes. Double-stranded RNA (dsRNA)-mediated RNA interference (RNAi) is a useful tool to achieve specific gene silencing in nontransformable species.

We established a technique to inject exogenous materials into ovulated eggs and developed a dsRNA-based RNAi method for *Daphnia magna*. Eggs were collected just after ovulation and injected with dsRNA specific to the *Distal-less* (*Dll*) gene, which functions in appendage development. We found that the dsRNA successfully triggered the degradation of *Dll* mRNAs, which induced the truncation of the second antenna in a dose-dependent manner. This effect was sequence specific in that: (1) an unrelated dsRNA did not induce any morphological abnormalities and (2) two non-overlapping *Dll* dsRNAs generated the same phenotype. This is the first report of an RNAi technique in *D. magna* and will be useful for advancing knowledge of the molecular biology of daphnids.