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Studies on the Molecular Mechanism of Adaptation to Salt-Deficient Condition in *Daphnia magna* - Cloning of Genes Responsive to Salt-Deficient Condition -

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Summary

Salinity concentration is one of the important environmental factors for an aquatic life. Although the adaptation or tolerance to hypersalinity has been intensively investigated in various animals, plants or microorganisms, the adaptation to salt-deficient condition has hardly been studied except in some brackish species or diadromous fish. Consequently, it is speculated that freshwater species has developed some unique functions to maintain and to utilize various salt factors in low salinity condition, which are still unexplained. In this study, we tried to understand the molecular mechanism of adaptation to salt-deficient condition by the comprehensive cloning of genes responsive to salt-deficiency in the water flea, *Daphnia magna*. The salt-deficient conditions were prepared by the serial dilution of COMBO medium (0, 3.125, 6.25, 12.5, 25, 50% of concentration), and the salt-deficient effects on daphnids were observed. According to the observation, then genes responsive to hyposaline condition, that was 10% of salt concentration of COMBO medium, were cloned comprehensively by suppressive subtractive hybridization method. The cloned genes were sequenced and their function were predicted by homology search.

All daphnids in 0% of medium salt (H_2O) dead in 6 hours after the exposure, and 20% dead in 3.125% medium in 36 hours. On the other hand, under the conditions with above 6.25% of medium concentration all daphnids could survive for 36 hours. According to these results, salt-deficient condition was set as 10% concentration of the COMBO medium for cloning suppressive subtractive hybridization experiment. By the functional prediction of sequenced 127 genes, major functional categories were ‘ion binding and transport’, ‘transport’, ‘stress response’, ‘signal transduction’, ‘transcription’, ‘protein production’ and ‘energy production’. Above all, ‘ion binding and transport’ and ‘transport’ categories directly related to the salt-deficient stress. Genes involved in these categories may play important roles in the adaptation to the salt-deficient condition. Especially, some genes related to calcium ion accumulation and transport were cloned, indicating the importance of the response to the decrease in calcium in the adaptation to salt-deficiency in the daphnid.