琉球大学学術リポジトリ

カワスズメ科魚類における生殖行動に関連した精しょうタンパク質に関する研究

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Abstract

Proteomic studies on seminal plasma of cichlid fishes via mass spectrometry are a promising way to identify and investigate reproductive-related seminal plasma proteins. Despite the importance of this approach in the study of seminal plasma protein functions, such as sperm motility regulation, it is difficult to select candidates from many proteins. Analyzing the rate of molecular evolution is a good strategy for selecting candidate(s), and expressing the protein allows the examination of its function. In this study, the seminal plasma proteins of the cichlid *Oreochomis mossambicus* were investigated. Seminal plasma proteins of *O. mossambicus* were separated by 2-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE) and 19 major proteins were identified by mass-spectrometry. Since reproductive proteins are often under positive selection and evolve rapidly, they were distinguished from non-reproductive related proteins through analyses of the rates of molecular evolution of all the identified proteins. The rates of molecular evolution were analyzed in terms of non-synonymous/synonymous substitutions (ω). Among the 19 proteins, six proteins (SPP120, ZP3-like, ccdc59, GTPase IMAP, LAMP2-like and blastomere cadherin-like) showed evidence of positive selection. Functional analyses of two proteins, SPP120 and ZP3-like were investigated. The function of glycosylation of SPP120 in several cichlid species was investigated in terms of its correlation with fertilization manners. The results showed that the glycosylation of SPP120 was species-dependent – SPP120 was glycosylated only in species that show oral fertilization behavior; thus indicating that glycosylation of SPP120 is useful for oral fertilization. Results of functional assay of purified ZP3-like protein on sperm showed that ZP3-like protein regulates sperm aggregation in a dose- and Ca^{2+}-dependent manner in *O. mossambicus*. The mRNA of ZP3-like protein was also expressed in the ovary, suggesting its possible role in fertilization. This study demonstrated the possibility of studying protein functions through an integrative approach, and has given insights into the understanding of diversification of reproductive behavior of cichlids at the molecular level.