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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Form 3

Abstract

Title: Studies on reproductive-related seminal plasma proteins of cichlid fishes
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Studies on reproductive-related seminal plasma proteins of cichlid fishes via mass spectrometry are a good way to identify and investigate those 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. It is reported that several reproductive proteins evolved rapidly in terms of non-synonymous/synonymous substitutions (ω). Among identified seminal plasma proteins, I selected "rapid evolving gene(s)" that encoding seminal plasma proteins in terms of the rates of molecular evolution (ω). Five proteins (SPP120, ZP3-like, ccdc59, GTPase IMAP, and LAMP2-like) showed evidence of positive selection. Among these proteins, it is reported that SPP120 immobilizes sperm motility, but functions of the other 4 proteins has not yet been reported. To investigated the other 4 proteins function on sperm motility, I examined an effect of the other 4 proteins on sperm motility. Among assayed 4 proteins, ZP3-like protein agglutinated sperm in a dose- and Ca^{2+}-dependent manner. ZP proteins are usually localized in the egg surface but mRNA of *ZP3-like protein* was expressed in the testes. Therefore, it is likely that ZP3-like proteins functions to mediate sperm motility in seminal plasma. Function of SPP120 in terms of glycosylation was also investigated. SPP120 was major protein in seminal plasma and heavily glycosylated in the oral fertilization cichlid *O. mossambicus*. Moreover, glycosylated SPP120 was associated with semen viscosity, which could contribute fertilization success only in the oral fertilization manner. To evolutionary correlation in between oral fertilization and SPP120 glycosylation, I examined the glycosylation states of 40 species in Lake Tanganyika. Among 40 species showing distinctive fertilization manner from oral fertilization, most of the oral fertilization species had glycosylated SPP120 in their seminal plasma. 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.

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