

Ascidian *Styela rustica* Proteins' Structural Domains Predicted to Participate in the Tunic Formation

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Abstract : Ascidiacea is the most numerous class of the Tunicata subtype. These chordates' distinctive feature of the anatomical structure is a tunic consisting of cellulose fibrils, protein molecules, and single cells. The mechanisms of the tunic formation are not known in detail; tunic formation could be used as the model system for studying the interaction of cells with the extracellular matrix. Our model species is the ascidian *Styela rustica*, which is prevalent in benthic communities of the White Sea. As previously shown, the tunic formation involves morula blood cells, which contain the major 48 kDa protein p48. P48 participation in the tunic formation was proved using antibodies against the protein. The nature of the protein and its function remains unknown. The current research aims to determine the amino acid sequence of p48, as well as to clarify its role in the tunic formation. The peptides that make up the p48 amino acid sequence were determined by mass spectrometry. A search for peptides in protein sequence databases identified sequences homologous to p48 in *Styela clava*, *Styela plicata*, and *Styela canopus*. Based on sequence alignment, their level of similarity was determined as 81-87%. The correspondent sequence of ascidian *Styela canopus* was used for further analysis. The *Styela rustica* p48 sequence begins with a signal peptide, which could indicate that the protein is secretory. This is consistent with experimentally obtained data: the contents of morula cells secreted in the tunic matrix. The isoelectric point of p48 is 9.77, which is consistent with the experimental results of acid electrophoresis of morula cell proteins. However, the molecular weight of the amino acid sequence of ascidian *Styela canopus* is 103 kDa, so p48 of *Styela rustica* is a shorter homolog. The search for conservative functional domains revealed the presence of two Ca-binding EGF-like domains, thrombospondin (TSP1) and tyrosinase domains. The p48 peptides determined by mass spectrometry fall into the region of the sequence corresponding to the last two domains and have amino acid substitutions as compared to *Styela canopus* homolog. The tyrosinase domain (pfam00264) is known to be part of the phenoloxidase enzyme, which participates in melanization processes and the immune response. The thrombospondin domain (smart00209) interacts with a wide range of proteins, and is involved in several biological processes, including coagulation, cell adhesion, modulation of intercellular and cell-matrix interactions, angiogenesis, wound healing and tissue remodeling. It can be assumed that the tyrosinase domain in p48 plays the role of the phenoloxidase enzyme, and TSP1 provides a link between the extracellular matrix and cell surface receptors, and may also be responsible for the repair of the tunic. The results obtained are consistent with experimental data on p48. The domain organization of protein suggests that p48 is an enzyme involved in the tunic tunning and is an important regulator of the organization of the extracellular matrix.

Keywords : ascidian, p48, thrombospondin, tyrosinase, tunic, tunning

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