

Structure-function relationship of the intrinsically disordered Starmaker protein in the process of calcium carbonate biomineralization.

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Summary

Biom mineralization is a complicated and still poorly understood process of creating minerals by living organisms. Acidic proteins that are intrinsically disordered and have high level of post-translational modifications, fulfill a special role in regulation of biomineralization. Starmaker (Stm) is one of such proteins, which controls biomineralization of zebrafish (*Danio rerio*) otoliths. Otoliths are calcium carbonate biominerals that are involved in sensing changes in linear acceleration and balance in fish. *In vivo*, Stm regulates size and shape of otoliths, and inhibition of the protein expression contributes to changes in otolith polymorph from aragonite to calcite. The recombinant protein undergoes phosphorylation and controls biomineralization of calcium carbonate *in vitro*.

The phosphorylation of Stm *in vivo* was confirmed using proteomics approach. It was also shown that the protein is present in both, aragonite and vaterite otoliths of zebrafish. There are two characteristic fragments in N- and C-end of the protein sequence, which roles have not yet been determined. A method of purification and separation of recombined fragments of Stm was developed. It was proven that regardless the disordered structure of both fragments, they differ in functions. An analysis of phosphorylation and biomineralization tests *in vitro* has shown that the C-end of the protein is responsible for biomineralization activity of Stm. For the first time, a procedure of isolation of Stm homolog from otoliths was developed. Starmaker-like protein from common carp (kStm-l) was purified and preliminary characterized. Analysis have shown that kStm-l has intrinsically disordered structure and appears in phosphorylated form *in vivo*. Stm homolog from carp controls size, amount and polymorph of calcium carbonate crystals *in vitro*. Additionally, the proteomics research contributed to identification of many new proteins present in otoliths.