Silicatein interactors and structure-guided biosilica formation

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The formation of the siliceous sponge spicules involves the formation of an organic cylinder-like structure in the extraspicular space, containing the enzyme silicatein and a calcium-dependent lectin. Applying yeast two-hybrid library screening and a newly developed solid-phase pull-down assay, we discovered, in addition to silicatein, two silicatein-associated proteins that contribute to spicule formation: (i) Silintaphin-1 a molecule that is involved in the shape-determination of the polymeric silica synthesized by silicatein; and (ii) silintaphin-2, a Ca^{2+}-binding protein. Silintaphin-2 is processed from a longer-sized 15-kDa precursor to a truncated, shorter-sized 13 kDa calcium-binding protein via proteolytic cleavage, mediated by the bone morphogenetic protein-1 (BMP-1). In primmorphs from *Suberites domuncula* retinoic acid causes a strong up-regulation of the expression of the gene encoding BMP-1. The expression levels of silicatein-α and silintaphin-2, which are strongly increased in the presence of silicate, are not affected by retinoic acid. More important, immunogold electron microscopy revealed that only in the presence of both silicate and retinoic acid the organic cylinder is formed, that surrounds the spicules and allows the radial apposition of new silica layers and hence the growth of the spicules. We conclude that retinoic acid regulates the formation of the organic cylinder. The two key enzymes of the retinoid pathway, the β-carotene dioxygenase and the retinal dehydrogenase have been cloned from *S. domuncula*. Based on these results, we propose a new scheme for spicule formation.

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