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Growth of calcareous nanograins within layered glycoprotein gels: A common model for Molluscan prisms and Coral fibres

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Three different results contribute to emphasize similarity between two calcareous skeleton units that are still considered very different with respect to mineralization mechanisms. In Scleractinian corals, purely crystalline structure of fibres is still widely admitted as resulting of a "spherulitic crystallization process", whereas molluscan prisms exemplify at best the "matrix mediated" biominerals.
In contrast, structural, biochemical and AFM data suggest a largely similar mode of formation.

1- Structural evidences: similarity of growth mode by superimposition of micron-thick layers.

Pictures produced by etching process on polished surfaces were the first data allowing a rapprochement to be made between the two crystal-like units. Continuity of growth lines between adjacent fibres or prisms has demonstrated that the basal ectoderm of coral polyps exerts on fibre growth the same type of global control than the external cell layer of the mollusc mantle does on prism growth surfaces.

2- Biochemical evidences: concordance between mineral growth layers and biochemical zonation.

In both coral fibres and molluscan prisms, XANES mapping has shown that organic matrices exhibit a layered repartition that corresponds to the mineral growth units. TGA data have shown that water is structurally associated to organic compounds, the resulting hydrated gel representing about 3 to 5-7 % in weight of the calcareous skeleton.

3- Evidencing the granular structure of the mineral phase.

At AFM scale, the two crystal-like units exhibit a comparable granular organization. Dimensions of nanograins are in the range of the tenth-microns. The thickness of layered sulfated polysaccharides and some phase contrast AFM pictures allow to admit that, in both cases, nanograins were developed within the hydrated gel as the last step of the biomineralization process for each growth unit.

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Biological control of skeleton formation in scleractinian corals

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The use of stable isotopes or other tracers to reconstruct paleoclimates requires sound knowledge of the biological factors controlling incorporation of these parameters into the skeleton. However, although coral skeletons are assumed to be good environmental archives and coral calcification one of the most important biological and geological process, control mechanisms of skeleton formation remain poorly understood.

We have demonstrated, using the branched scleractinian coral, *Stylophora pistillata* as a model organism that calcification is highly regulated, since both ion supply and crystal nucleation by organic matrix are controlled by the calicoblastic cells. The process is very rapid for both mineral and organic fractions: ion incorporation into skeletal structures occurs within 1-2 minutes after addition to the external medium while uptake of amino acid precursors, incorporation into tissular proteins and then into skeleton take less than 20 min. Pools of carbon and calcium for calcification, if they exist, are very small and must have high turn-over rates. Carbon supply for symbiont photosynthesis is regulated by the animal host cells, by carrier-mediated transepithelial pathways. This process leads to the polarization of the oral epithelial layers and, hence may play a role in the phenomenon of light-enhanced calcification. Delivery of calcium ions to the calcification site involves a transepithelial transport through the calicoblastic epithelium. Entry into these cells is controlled by an L-type Ca^{2+} channel proteins. Ca^{2+} export from the calicoblastic cells to the skeleton is performed by a plasma membrane Ca^{2+}-ATPase gene belonging to the P type IIB family. Regulation of biomineralization also implies the fine control of pH within the site of mineralization Using inhibitors we have suggested that a H^{+}-ATPase is specifically involved in the biomineralization process, being necessary for the elimination of H^{+} resulting from the deprotonation of HCO_3^{-} during $CaCO_3$ precipitation. Using specific anti-organic matrix antibodies, we also demonstrated that organic matrix present within the skeleton results from a specific polarized biological secretion and not from an unspecific trapping of coral soft tissues within the skeleton. Inhibition of organic matrix synthesis or protein glycosylation results in immediate inhibition of calcification. While it is generally thought that this organic matrix only represents a minor fraction of skeletal weight, we have revealed by thermogravimetric analysis and incorporation of radioactive amino acids, that these measurements are largely underevaluated. Finally, we have started a molecular analysis of these organic matrix proteins.