Characterization of carbonic anhydrases in the biomineralization of *Corallium rubrum*

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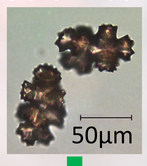
Introduction

The Mediterranean red coral *Corallium rubrum* is a Cnidaria which produce two types of biomineral structures :

Axial skeleton

Exoskeleton

Sclerites

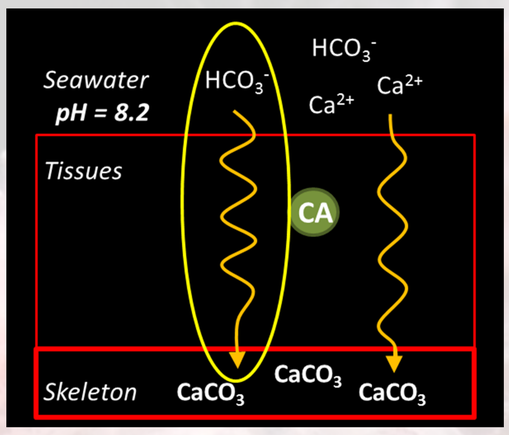


Fragmented endoskeleton

Mineral fraction → $CaCO_3$ **: Calcite**

Organic fraction → Proteins, Lipids, Sugars

Biomineralization in corals



Carbonic anhydrases (CAs) are responsible for the reversible convertion of carbone dioxyde into bicarbonate (1) and are involved in the biomineralization process (2) in corals. *cf. Refs*

(1) $H_2O + \mathbf{CO_2} \overset{CA}{\rightleftharpoons} \mathbf{HCO_3^-} + H^+$

(2) $HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+$

Which isoforms of carbonic anhydrases are involved in the process of biomineralization in *Corallium rubrum* ?

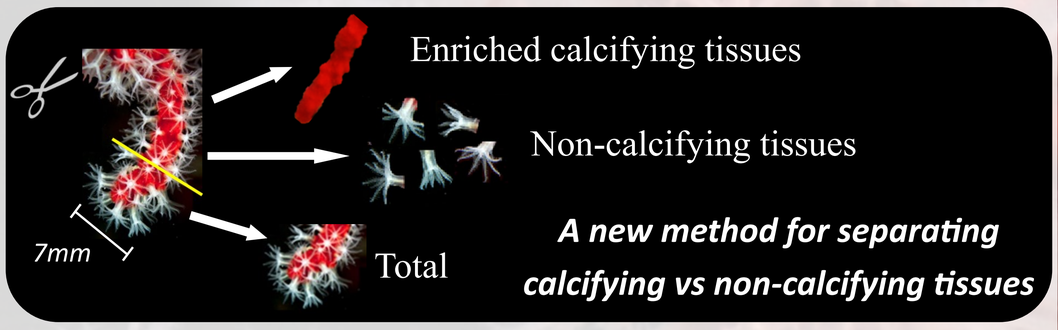
Methods

1. Bioinformatic tools

- Identification of genes coding for carbonic anhydrases in the transcriptome and the genome of *Corallium rubrum*
- Protein structure prediction

2. Gene expression

- Anesthesia : MS222 / action potential blocking
- Dissection



- RNA extraction and real-time PCR

Results

Five out of six carbonic anhydrases of *Corallium rubrum* (CruCAs) show typical structure of either secreted or cytoplasmic CAs with an active site necessary for zinc binding and enzyme activity (Figure 3A). CruCA2 (CARP) lacks two important histidines involved in zinc binding (Figure 3B).

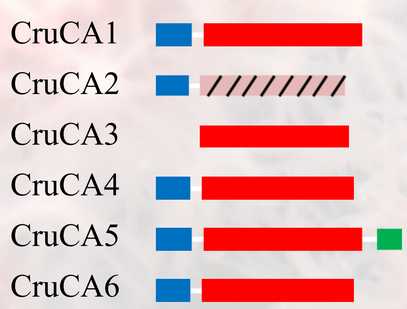


Figure 1: *Corallium rubrum* carbonic anhydrases (CruCAs) structure I prediction.

Peptide signal;

Active site;

GPI anchor.

```
__FALDQFHFHWGCENGKGSEHLINGLSYPAELHLVHHN__
__YILKKIYFRFGCTEKTGSEHQIDGVPTPGEIHLVFYK__
__FDLAQFHFHWGSDDSRGSEHKIDGKSYAAELHFVHYN__
__YWLDQIHFHWGSDNTQGSEHRFDKERFPAEIHFVHYN__
__YKLAQFHFHWGENDDTGSEHTIDGEPYSGELHLVHWN__
__FTLEEVHFHWGNTMSMGSEHKVNGKSSSMEVHLVHYN__
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Figure 2: Alignment of the zinc binding site of the six CruCAs.

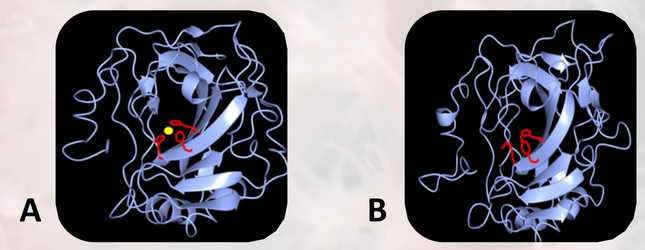

Figure 3: Computed 3D structure of an active isoform (A) and CruCA2 (B). Yellow ball in A represents Zn^{2+}.

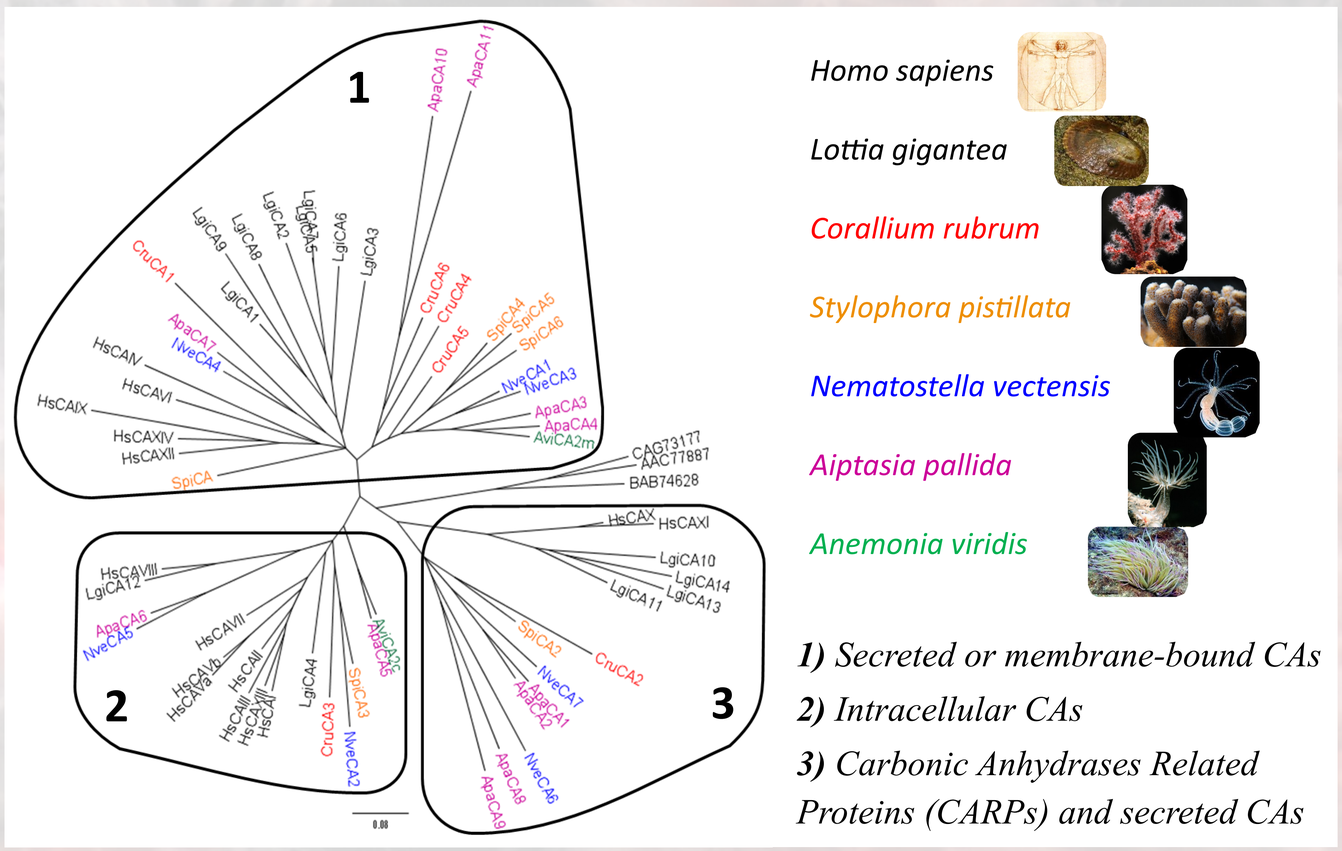

Figure 4: Bayesian phylogenetic tree of carbonic anhydrases protein sequences. Cnidarian carbonic anhydrases cluster in different fonctional families although isoforms are represented both in calcifying and non calcifying organisms.

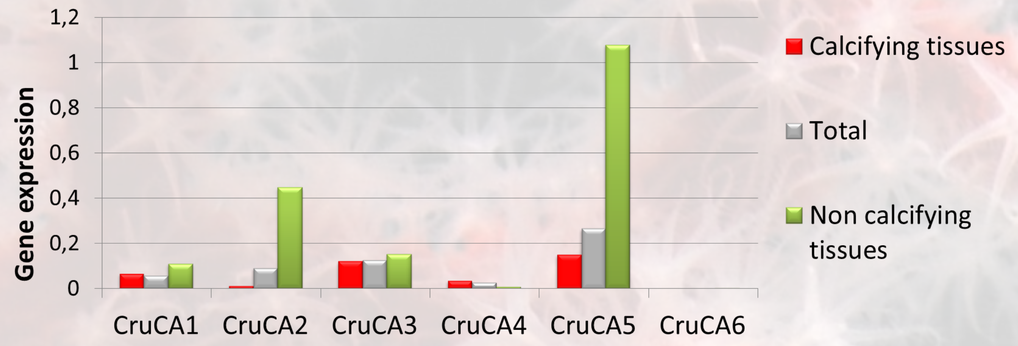

Figure 5a: Gene expression in the different tissue fractions.
The pattern of carbonic anhydrases expression is different among tissues.

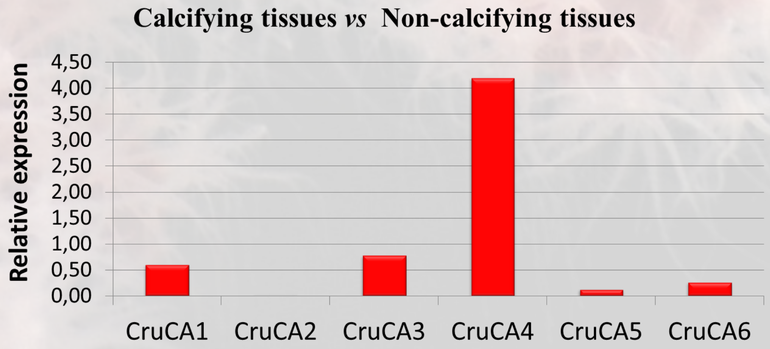

Figure 5b: Relative gene expression in the calcifying tissues *vs* non-calcifying. CruCA4 shows preferential expression in calcifying tissues.

Conclusions

We have identified **six carbonic anhydrases** in the Mediterranean red coral. One of them, **CruCA4**, is secreted and preferentially expressed in the calcifying tissues suggesting a **potential role in the biomineralization process**. Next step will be to determine by immunolocalization the cells responsible for its secretion. Enzyme activity will also allow determining its kinetic and pharmacological properties.

References : **Allemand, D., & Grillo, M.-C. (1992)** Biocalcification mechanism in Gorgonians : 45Ca uptake and deposition by the Mediterranean Red Coral *Corallium rubrum*. *The Journal of Experimental Zoology*, *262*, 237–246. **Allemand, D. (1996)** Dynamics of Calcification in the Mediterranean Red Coral , *Corallium rubrum* (Linnaeus). *The Journal of Experimental Zoology*, *276*, 270–278. **Bertucci, A., et al (2013)** Carbonic anhydrases in anthozoan corals-A review. *Bioorganic & Medicinal Chemistry*, *21*, 1437–1450.