MOLECULAR CHARACTERIZATION OF CARBONIC ANHYDRASES IN THE RED CORAL *CORALLIUM RUBRUM*: DIFFERENT ISOFORMS WITH SPECIFIC TISSUE-EXPRESSION

LE GOFF CARINE , GANOT PHILIPPE, ZOCCOLA DIDIER, ALLEMAND DENIS, TAMBUTTE SYLVIE

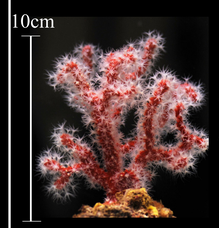
CENTRE SCIENTIFIQUE DE MONACO

Centre Scientifique de Monaco
8 quai Antoine 1er
MC 98000 Monaco

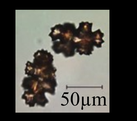
+377 97 77 44 74
carine@centrescientifique.mc

OBJECTIVES

Corallium rubrum is a marine invertebrate (Cnidaria phylum) which produces two types of biomineral structures :



Axial skeleton



Sclerites

Organic fraction
Proteins, lipids, sugars...

+

Mineral fraction
$CaCO_3$:
Mg-Calcite

Carbonic anhydrases (CAs) :

- Component of the « biomineralization toolkit »
- Convert metabolic CO_2 into HCO_3^- (1) which is a source of inorganic carbon for $CaCO_3$ deposition (2) in the calcification process

CA

(1) $H_2O + CO_2 \rightleftharpoons HCO_3^- + H^+$

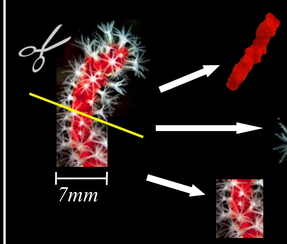
$HCO_3^- \longrightarrow CO_3^{2-} + H+$

(2) $CO_3^{2-} + Ca^{2+} \longrightarrow CaCO_3$

Which isoform of alpha-CAs is involved in the process of calcification in *Corallium rubrum* ?

METHODS

1. Characterization of *Corallium rubrum* carbonic anhydrases (CruCAs) using genomic and transcriptomic databases
2. *In silico* analyses
3. Phylogenetic tree
4. Gene expression: Anesthesia + Dissection + qPCR



Tissues enriched in calcifying cells

Non-calcifying tissues = Polyps

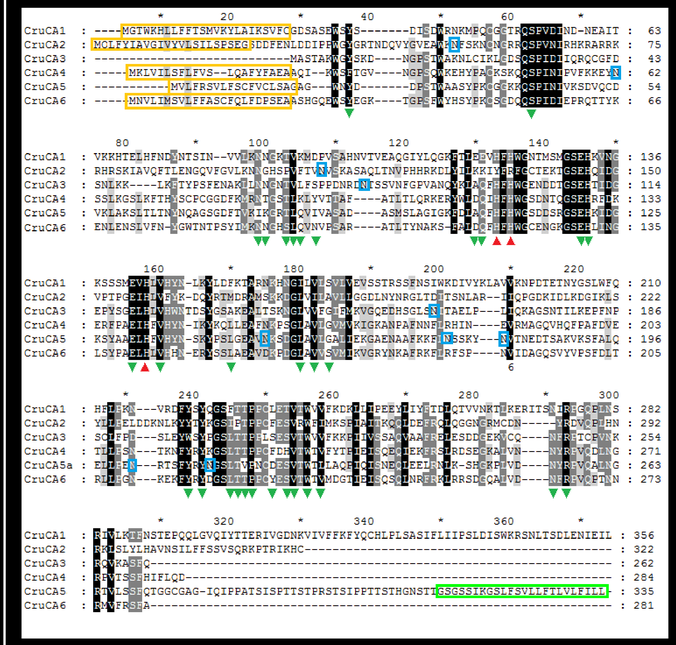
Total

New method for separating calcifying *vs* non-calcifying tissues

RESULTS

1 6 CruCAs identified

2 5 CruCAs show typical structure of either secreted or intracellular CAs with an active site for zinc binding and enzyme activity. CruCA2 (CARP) lacks two important zinc-coordinating histidine residues.



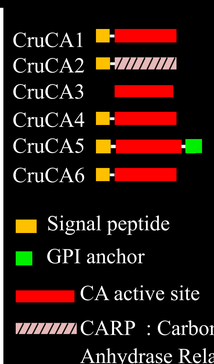


Figure 1: Alignment of the CruCAs. Amino acids conserved at 100%, 80%, and 60% across the alignment are shaded in black, dark gray, and light gray, respectively.
Signal peptides are framed in **orange**
The zinc-binding histidine residues are indicated by **red triangles**
The active site residues are indicated by **inverted green triangles**
Potential N-glycosylation sites are framed in **blue**

3 The metazoan α-CA isoforms involved in calcification are evolutionary distant

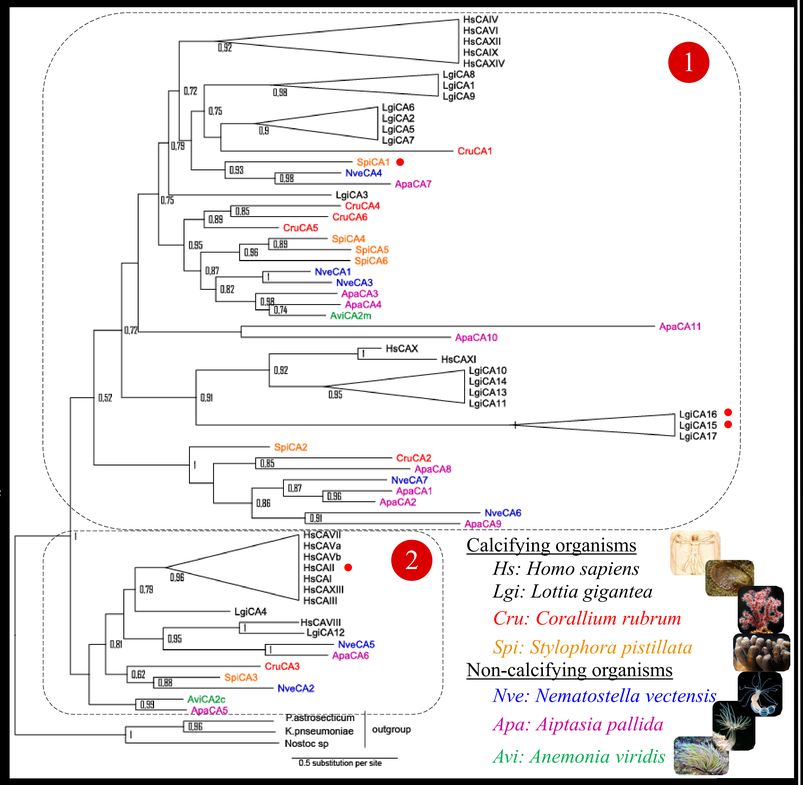


Figure 2: Phylogenetic analysis of the α-CAs of calcifying and non-calcifying organisms, using maximum likelihood method with non-metazoan CAs as an outgroup. Only bootstrap values above 50% are indicated.

- α-CAs involved in calcification

1. Secreted or membrane-bound α-CAs
2. Intracellular α-CAs

4 CruCA4 shows preferential expression in tissues enriched in calcifying cells.

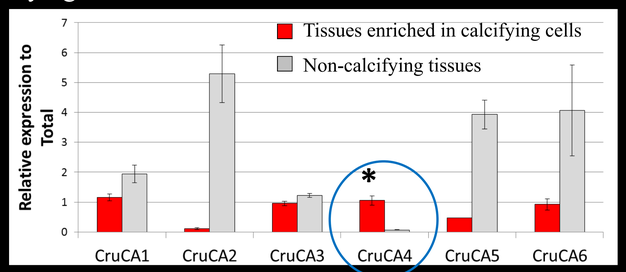


Figure 3: Gene expression of the CruCAs isoforms in each fraction relative to Total.

CONCLUSION

- **6 α-CAs isoforms** in the Mediterranean red coral *Corallium rubrum*
- **CruCA4** : secreted and preferentially expressed in tissues enriched in calcifying cells suggesting a **potential role in the calcification process**
- Independent recruitment of α-CAs for calcification process in Metazoa is also suggested with the phylogenetic analysis

References :

Allemand D. and Grillo MC. 1992. J. Exp. Zool., 262, 237–246. **Allemand D. 1996**. J. Exp. Zool., 276, 270–278. **Jackson D. et al. 2007**. Science, 316, 1893-1895. **Bertucci A. et al. 2013**. Bioorg. Med. Chem., 21, 1437–1450.

Acknowledgments:

The work was funded by the Government of the Principality of Monaco and supported by the Fondation Paul Hamel. The authors would like to thank Nathalie Techer and Natacha Segonds for their technical assistance.