Transcriptome specific expression of the calcifying tissues in *Corallium rubrum* highlights alternative processes for calcification.

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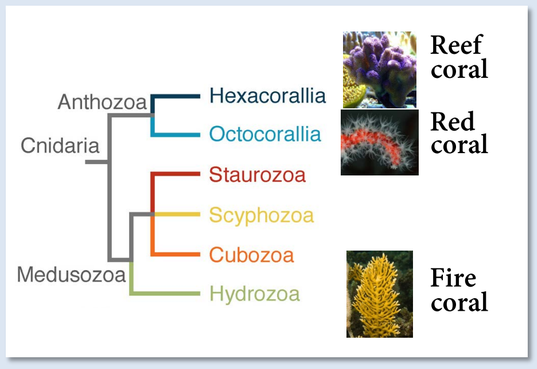
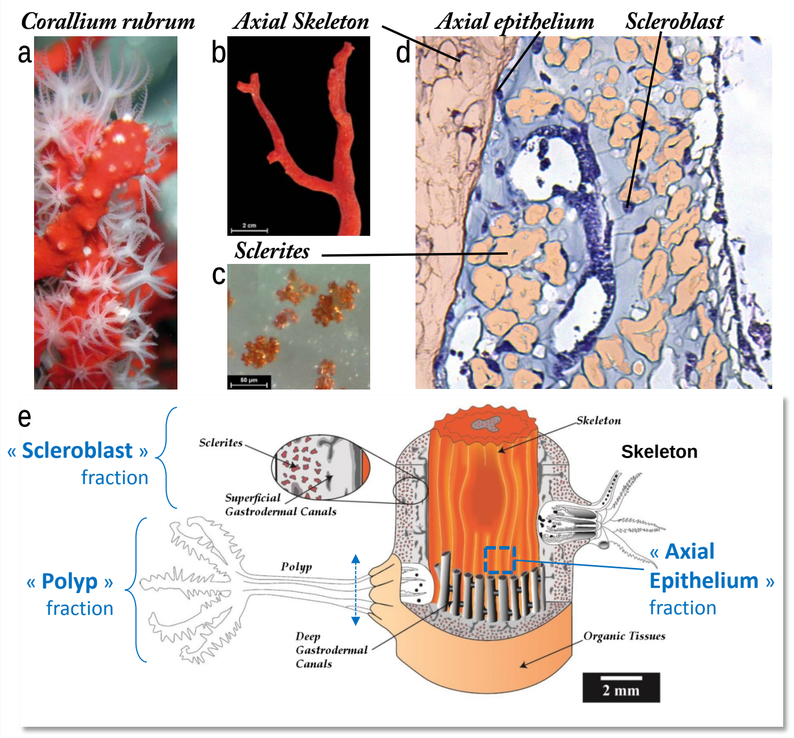
Corals are calcifying organisms represented in diverse taxa of Cnidaria, including Hydrozoa, Hexacorallia, and Octocorallia. Studies on the biologically controlled process of calcification in scleractininan corals have so far highlighted the role of specific proteins including various transporters, carbonic anhydrases, and organic matrix proteins. For a comparative view, we are investigating this process in the octocorallian red coral *Corallium Rubrum*. Using microdissection technics, we were able to separate the polyp and the two calcifying cell types from the rest of the colony. RNAseq analyses from the different "tissue fractions" allowed us to map tissue specific gene expression for the polyps, scleroblasts and axial epithelium versus Total (whole colony) expression.



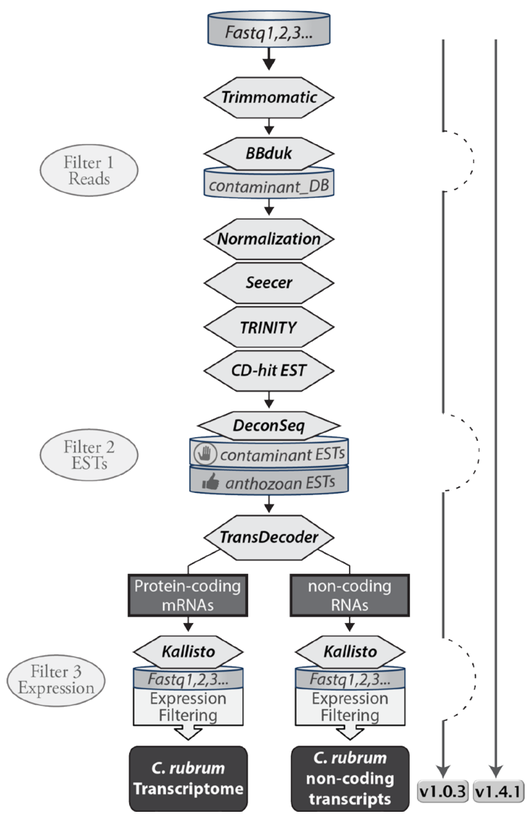
Transcriptomic



Coralium rubrum colonies (a) elaborate two biominerals, the axial skeleton (b) and the sclerites (c), produced by the axial epithelium and the scleroblasts (tissue section, d), respectively. Anatomy of the red coral as well as the different tissue fractions produced by micro-dissection are depicted in (e). A colony fragment removed from most of its polyps is called the skeletogenic fraction.

Transcriptome assembly pipeline

Samples used in this analysis: whole colony (Tot)= 3 ; polyps (Pol)= 2 ; skeletogenic (Skel)= 2 ; axial epithelium (Axe)= 3 ; scleroblast (Scl)= 3



Taxonomic distribution of the Ribosomal Proteins

Eukaryotic 40S + 60S

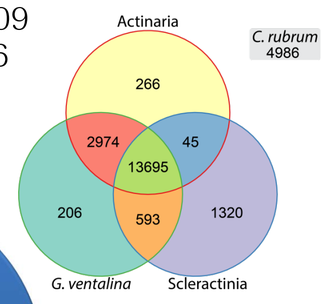
	v1.0.3	v1.4.1
Cnidarians	46	46
Other invertebrates	146	22
Vertebrates	12	7
Unicellular	22	0
Plants and Fungi	46	0
NA's	1	0

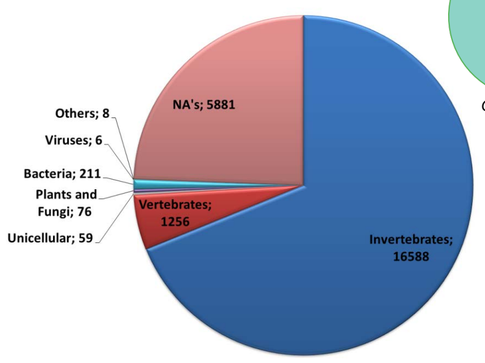
Prokaryotic 30S + 50S

	v1.0.3	v1.4.1
Cnidarians	7	8
Bacteria	17	1
NA's	2	0

C.rubrum protein-coding Transcriptome

Genes number : 24085
Transcripts numbers : 28309
Longest transcripts : 31196
N50 genes : 2329
%GC : 40.64

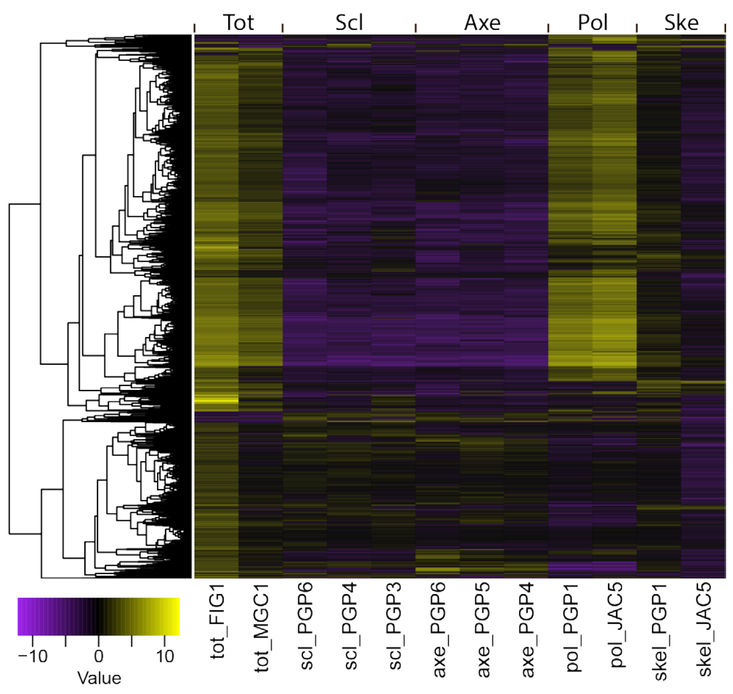




Illumina paired-end reads obtained from mRNA sequencing of the different tissue fractions were submitted to a pipeline designed to eliminate most of the « non-*C. rubrum* » transcriptome (holobiont/food). From the initial non-filtered v1.0.3 version to the final v1.4.1 version, many contaminants were cleared out as exemplified by the analysis of the ribosomal proteins content. The final protein-coding transcriptome was further annotated (see pie chart for taxonomy distribution) and compared to other cnidarian transcriptomes (venn diagram, blast (<1e-25) analysis against representative transcriptomes of actinarians, scleractinians and the octocoral *Gorgonia ventilana*). Out of the 24085 genes, 4986 were unique to *C. rubrum*.

RNAseq

HeatMap (P<0.01; fold_change>2; log2.centered)



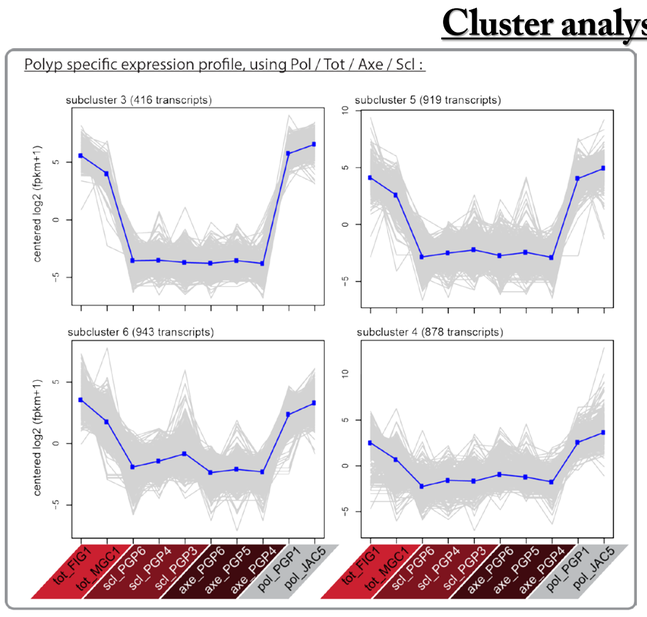
Values for published genes (Debreuil, 2012; LeGoff, 2016)

Name	TMM					axe_vs_pol	
	Tot	Scl	Axe	Pol	Ske	**logFC**	*FDR*
Scleritin	829.3	531.3	11311.6	78.4	1086.5	**11.6**	*9.29E-22*
CruCA4	22.6	23.9	614.6	0.4	48.6	**10.7**	*3.71E-20*
CruCA5	267.4	15.6	15.6	375.9	101.4	**-4.3**	*1.22E-12*

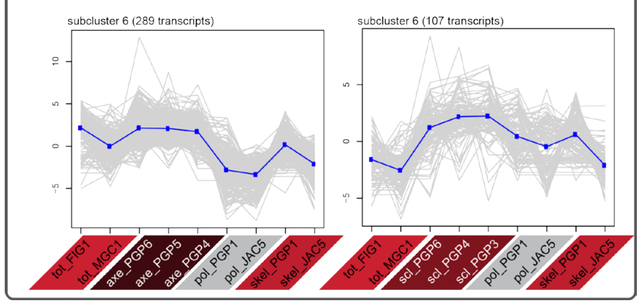
Reads were mapped back to the reference transcriptome and tissue (fraction) specific expression levels were monitored using RSEM/EdgeR packages (Heatmap on the left). Expression levels of the organic matrix Scleritin gene, as well as 2 Carbonic Anhydrases (CruCA) know to be differentially expressed validated the method. Genes specifically involved in calcification are supposedly highly expressed in the calcifying tissues, lesser in the skeletogenic, then in the whole colony and finally little expressed in the polyps, and *vice versa*. We therefore choose to analyze clusters of expression profiles across fractions of interest. This approach identified 3155, 289, and 106 genes preferentially expressed in the polyp, axial epithelium, and scleroblast fractions, respectively. Unexpectedly, none of these 3 fractions showed enrichissement in genes shared with the calcifying scleractinians as opposed to the non-calcifying actinarians (see conclusion). Further, we did a GO term enrichment analysis (Fisher's Exact test; P<0,05) between the GO identifiers associated with the Axe fraction *versus* the Pol fraction. Several GO IDs were enriched (see word clouds), associated to a number of genes and functions.

Cluster analyses

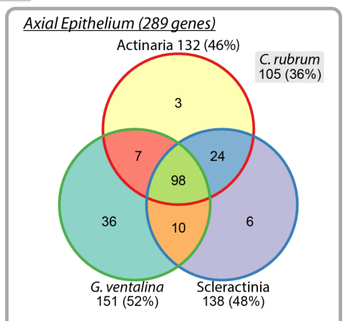
Polyp specific expression profile, using Pol / Tot / Axe / Scl :



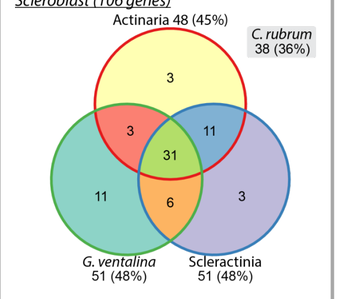
Axial Epithelium (left) and Scleroblastic (right) specific expression profile, using Axe/Tot/Pol/Ske and Scl/Tot/Pol/Ske, respectively



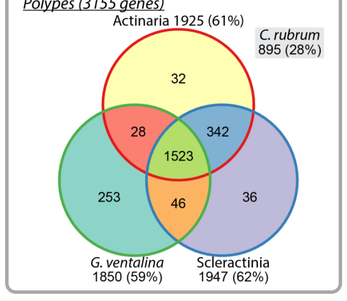
Axial Epithelium (289 genes)



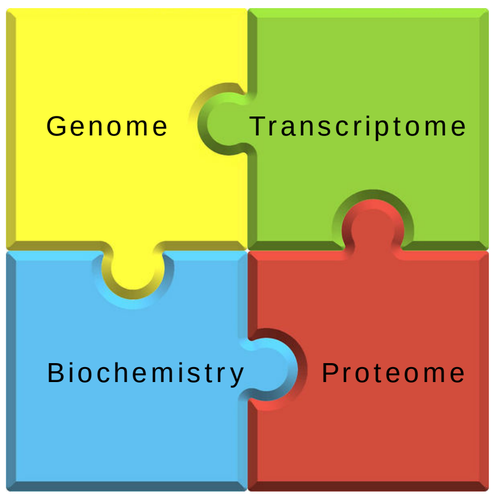
Scleroblast (106 genes)



Polypes (3155 genes)



This approach is combined with genomics, proteomic and biochemical studies of the skeleton organic matrix, to better understand the mechanisms governing calcification process in *C. rubrum*.



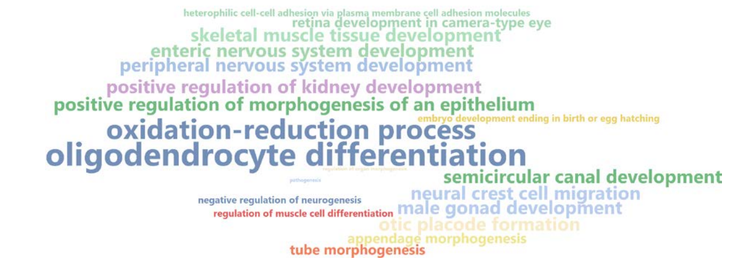
-> We are characterizing various gene families likely controlling the calcification process in *C. rubrum*, ranging from control of gene expression, regulators of the protein post-translational modification and secretory pathway, ions transporters, novel organic matrix proteins…

-> A large majority of the genes of interest found specifically expressed in calcifying tissues are gene duplication(s) of an ancestral copy expressed preferentially in the polyps (neofunctionalization).

-> The outcomes of our present studies are likely transposable to biomineralization of most corals.

GO IDs Enrichment analysis

Biological Process Enriched Word cloud



Molecular Function Enriched Word cloud