

# Symbiosis-dependent expression of a P-type H<sup>+</sup>-ATPase gene in a coral-dinoflagellate association



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## What we know

Free-living marine organisms live in an environment with a relatively large and constant dissolved inorganic carbon (DIC) pool (~2 mM). Due to the pH of seawater (8.2), the majority of this DIC is in the form of HCO<sub>3</sub><sup>-</sup>. Consequently, marine phototrophs have developed carbon concentrating mechanisms (CCM) to increase CO<sub>2</sub> concentration around Rubisco. These CCMs allow them to use, in addition to the small pool of available CO<sub>2</sub> (~10 μM), the much larger pool of HCO<sub>3</sub><sup>-</sup> (~2 mM).

Dinoflagellates from the genus *Symbiodinium* (commonly named **zooxanthellae**) establish an **endosymbiosis** with **most scleractinian corals** underpinning the formation and maintenance of coral reef ecosystems. Since they are isolated in a host-derived vacuole (symbiosome), inside the endodermal cells of their host, **zooxanthellae are not in direct contact with seawater**.

By a pharmacological approach, Al-Moghrabi *et al.* (1996) demonstrated a considerable plasticity in the CCM pathways. They suggested that in symbiotic state (holobiont), CCM depends on a H<sup>+</sup>-ATPase and a Carbonic Anhydrase (CA), whereas these enzymes are absent in the free-living zooxanthellae (Fig. 1).

## What we did

1. We cloned and characterized a gene encoding for a P-type H<sup>+</sup>-ATPase in *Symbiodinium sp.*
2. We have investigated for gene expression according to the symbiotic state of the zooxanthellae (i.e. inside the coral *Stylophora pistillata* or free-living)

## What we found...

### ...about the sequence

This gene encodes for a protein of 975 amino acids with a calculated molecular mass of about 105 KDa. The sequence shares several features with both fungal and plant groups of plasma membrane proton-pumping ATPases (type IIIa PMA).



Fig.2 : ClustalW alignment of putative H<sup>+</sup>-ATPase amino acids sequences from *Symbiodinium sp.*, *S. cerevisiae* and *A. thaliana*.  
Red shade : conserved AAs. Blue shade: AAs conserved in 2 of 3 sequences.  
Dashed : absent AAs. Dashed lines : conserved catalytic autophosphorylation and ATP binding-sites used for primers design. I to X : transmembrane domains. Black boxes : type IIIa P-type ATPases specific motifs.

### ...about the phylogenetics

The cloned H<sup>+</sup>-ATPase is related to the dinoflagellates sister group, Euglenozoa. This is another striking evidence that this protein belongs to the symbiont.

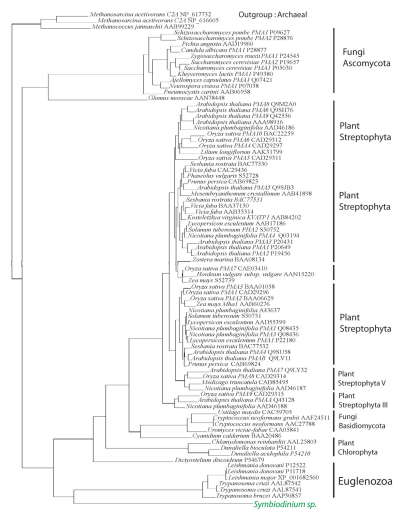


Fig.3 : Phylogenetic relationships with other previously cloned proton-pumping ATPases.

### ...about the gene expression

The H<sup>+</sup>-ATPase cloned in this study is present in the symbionts' genome but specifically expressed when zooxanthellae are engaged in a symbiotic relationship with the coral partner.

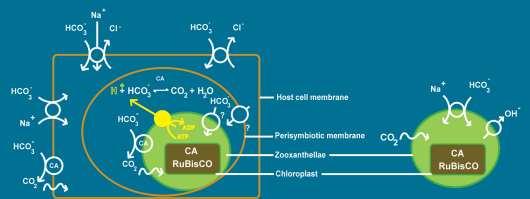
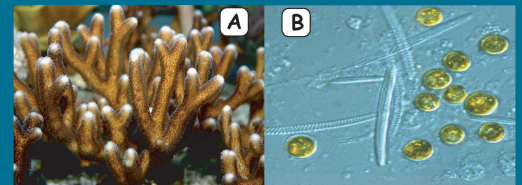


Fig.1 : Model for inorganic carbon uptake in coral / dinoflagellate association (A) and in free-living zooxanthellae (B).  
(A) Colony of the branching coral *Stylophora pistillata*. (B) Cultured zooxanthellae.  
CA = Carbonic Anhydrase

## Holobiont CZ

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## What we learn

This work provides the first example of symbiosis-dependent gene expression in *Symbiodinium sp.*

Our results confirm previous studies of Ci transport and CCM in coral-associated and cultured zooxanthellae. The cloned H<sup>+</sup>-ATPase shares several characteristics with other plasma membrane ATPases and could be involved in the acidification of the perisymbiotic space leading to bicarbonate dehydration by a carbonic anhydrase activity in order to supply inorganic carbon for photosynthesis.

The specific expression of this gene during symbiosis suggests physiological changes between the free-living and the symbiotic forms in *Symbiodinium*.

Future studies are needed to confirm the localization of this protein and characterize some other actors of CCM and inorganic carbon transport in coral / dinoflagellate symbiosis.

These studies will moreover provide answer to the questions :  
"What is the signal responsible for transcriptomic shifting between free and symbiotic zooxanthellae ?"  
"When does this signal occur during symbiosis settlement and bleaching ?"

Ref: Al-Moghrabi S., Goiran C., Allemand D., Speziale N., Jaubert J. (1996) J. Exp. Mar. Biol. Ecol. 199, 227-248.