

l de la Recherche Agronomique

Copper zinc SOD in Anemonia viridis, an animal submitted to daily hyperoxic conditions A. Plantivaux°, P. Furla°, D. Zoccola*, G. Garello°, S. Richier°, PL. Merle°, É. Tambutté*, S. Tambutté* & D. Allemand*°.

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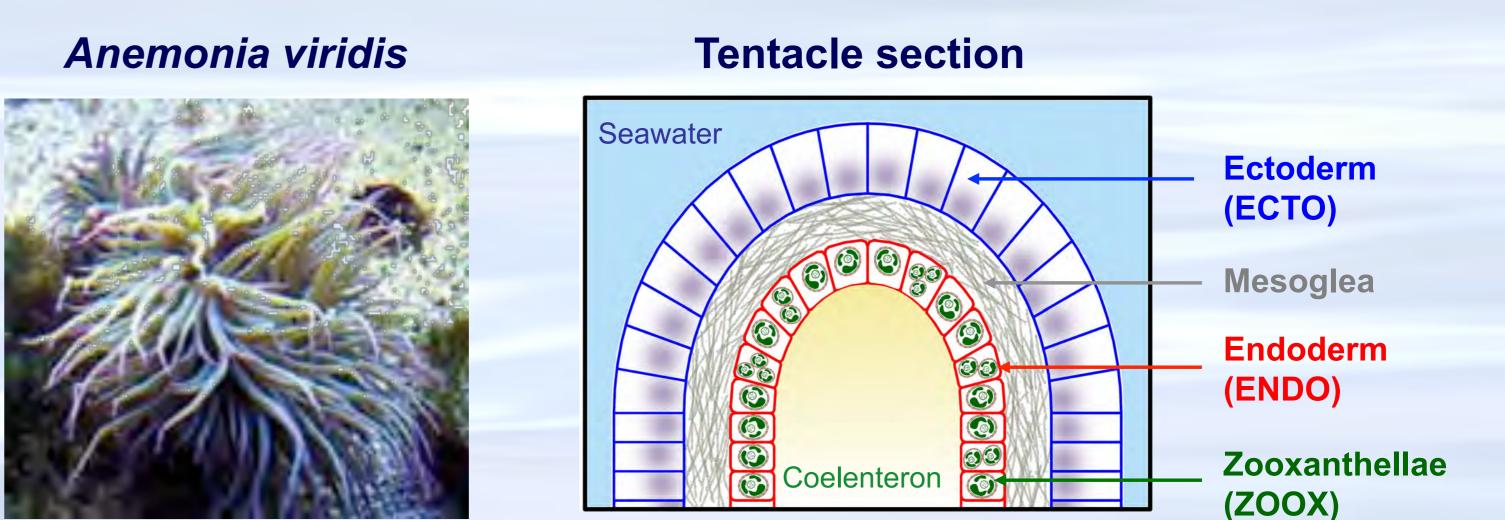




Introduction

Why Anemonia viridis?

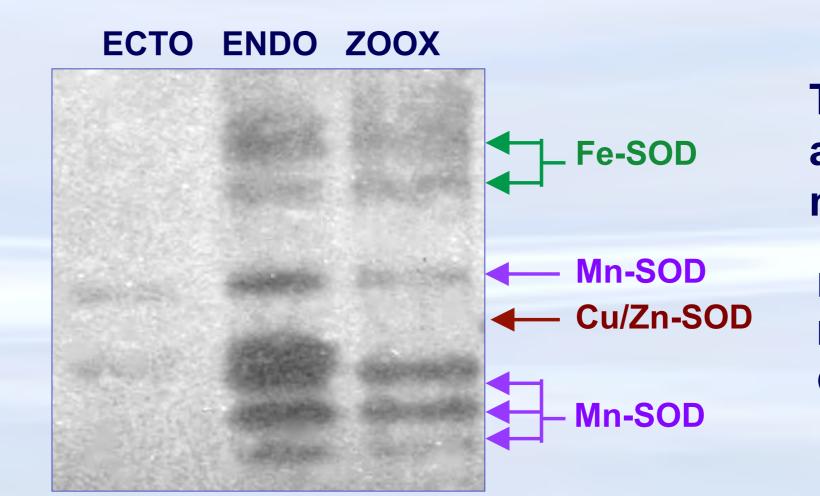
This symbiotic Cnidarian harbors, inside its endodermal cells, protists called zooxanthellae. Those protists living in symbiosis still have a photosynthetic activity and produce oxygen in host cells. Although high oxygen levels induce overproduction of reactive oxygen species (ROS), Anemonia viridis resists well to such an oxidative stress; it is a good animal model for studying ROS detoxifying strategies [Richier et al., 2003].



Why CuZnSOD?

Superoxyde dismutases (SOD) are the first step in the enzymatic detoxifying process. They dismutate the superoxide radical (O_2°) to hydrogen peroxide (H_2O_2) . Up to now, 3 types of SOD, differing by the metallic ion present at their active site, have been identified in eukaryotic cells: CuZnSOD, MnSOD and FeSOD. Previous studies had identified those 3 classes in A. viridis; only the Cu/Zn SOD activity is specific to animal cells [Richier et al., 2003].

Are the CuZnSOD involved in the resistance of *A. viridis* tissues to hyperoxia?



Tissue-specific pattern of SOD activities from A. viridis on a native PAGE

Fe SOD: iron-containing SOD Mn SOD: manganese-containing SOD Cu/Zn SOD: copper and zinc-containing SOD

Cloning of two CuZnSOD genes from A. viridis



Localization of their transcription

Because no data were available for diploblastic animals, we designed degenerated primers, comparing the CuZnSOD sequences from phylogenetically unrelated species. Using RT-PCR, RACE-PCR and the Genome Walker kit (Invitrogen), we investigated cDNA and genomic DNA.

Amino acid sequences aligned with the ClustalW method.

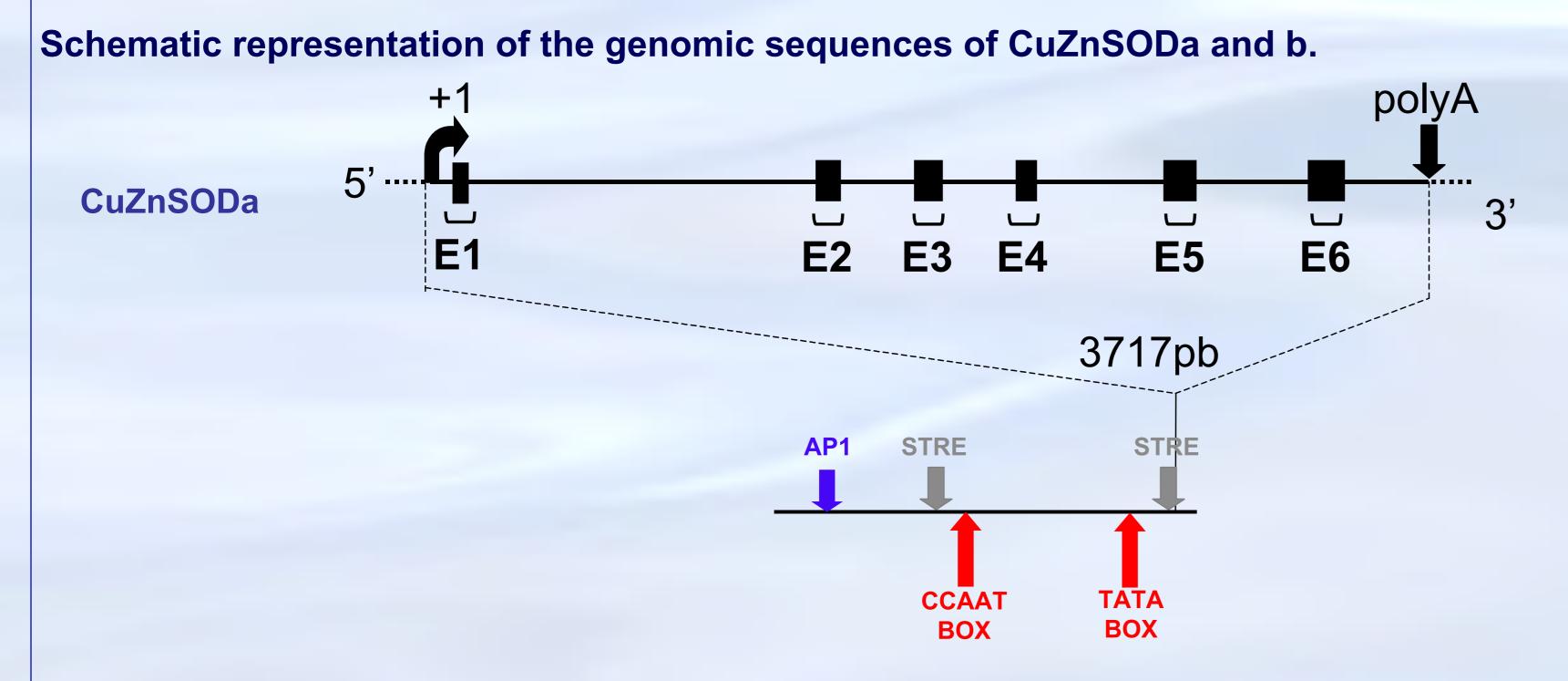
In order to locate where CuZnSOD transcripts are expressed, in situ hybridizations were performed using specific RNA probes on entire tentacles and endodermal cells from A. viridis.

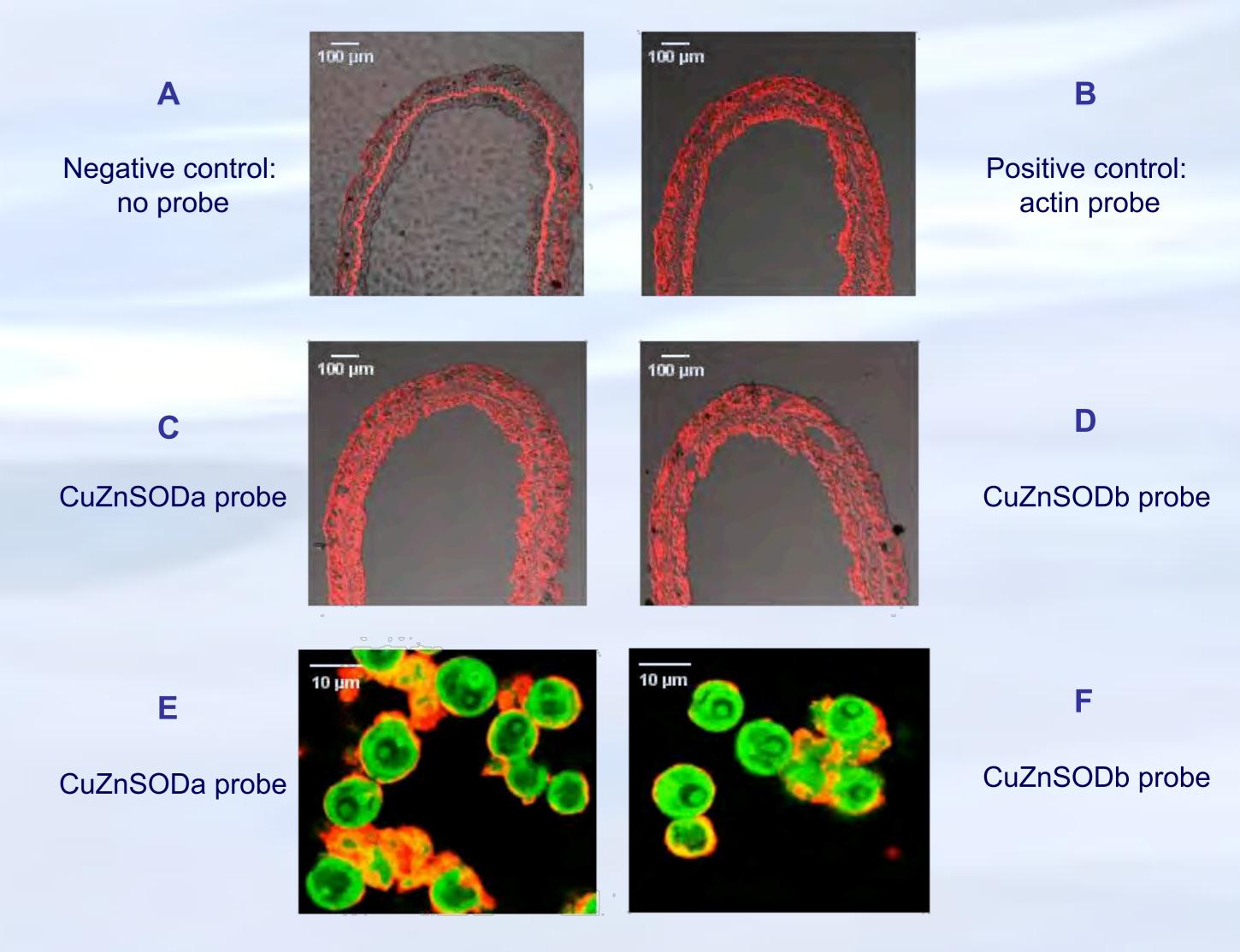
In situ hybridization.

CuZnSODa	MKLLAFLLVC	SSVVQTCAEV	AAMCYMKPNP	VLPDTIDTKV	TGTVMLSQKS	PLHKIKITLN	LK GL PPNTP H	GF H V H QYGDI	DTNG C QSAGP	H FNPFGATRG	100
CuZnSODb		MVV	KAVCCLIG	EV	KGTISFSQEG	DGKPCQITGE	VT GL TEG-K H	GF H I H QYGD-	NTNG C TSAGS	H FNPFGKTHG	72
		#-*	*#*##	*	_**#_#**_#	#**	#-**#*	***#****	_***#* <mark>*</mark> *	***** *#*	

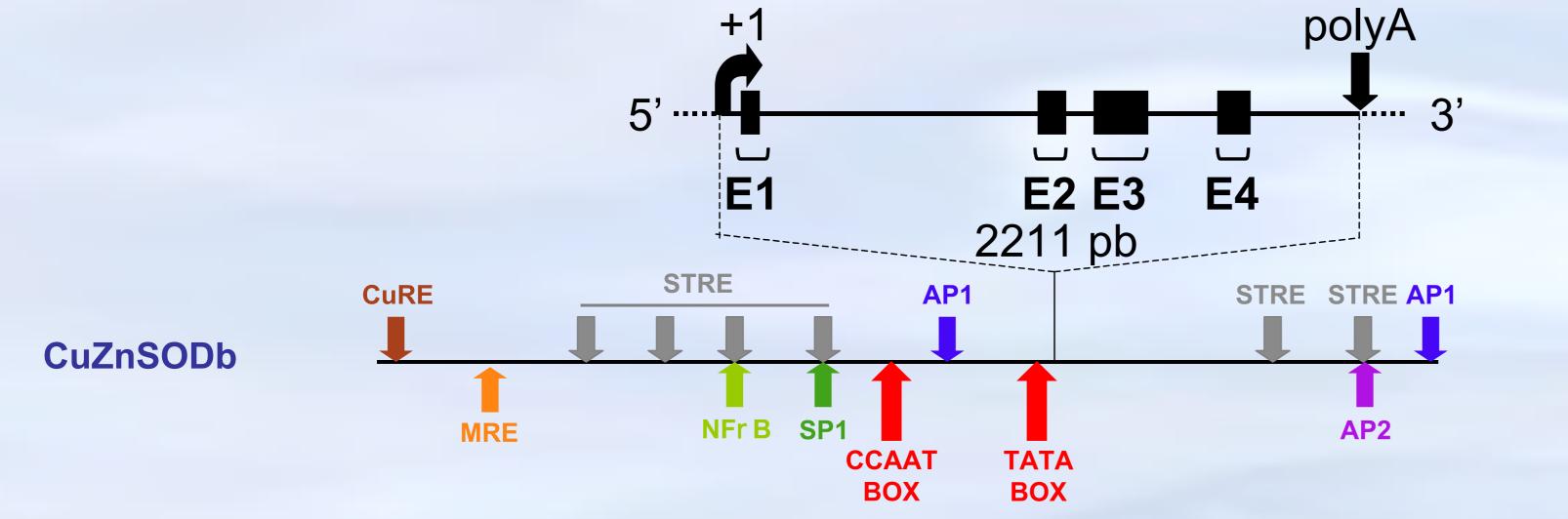
GPQDDEKHRH VGDLGNVMSN AEGRIKIMLS DYLVSLYGPY SVIGRSFVIH AKIDDLGRGT GAARKESLKT GNAGARLACC TIVHAAPAAA LK 192 GP--DDTERH VGDLGNIVAG KDGVAKVDMS DSQVTLLGEH SVVGRSVVVH VGEDDLGKG- --GHDDSLTT GHAGGRLACG -VIGICPV 153 #_#**_* *_**_**# ##_#_*#

- * Residue identical in both sequences
- # Residue similar in both sequences
- **x** Residue involved in cofactor fixation, substrate fixation or oligomerization.
- Potential cleavage site for excision of a peptide-signal.





A to D: sections of entire tentacles. E and F: endodermal cells. Specific RNA probes were visualised by measuring the fluorescence of Alexa Fluor 568 (red) and zooxanthellae were



> We sequenced two CuZnSOD cDNA that we called CuZnSODa and CuZnSODb. The two proteic sequences shared 40 % of homology. The two genes showed different genomic organizations. The putative transcriptional binding sites were different, suggesting two different regulations.

Note : CuZnSODa and CuZnSODb sequences can be found on NCBI server (AY 164663 and AY 164664).

Reference: Richier S., Merle P.L., Furla P., Piozzi D., Sola F., Allemand D., Biochimica et Biophysica Acta, 2003, 1621: 84-91.

- localized by the chlorophyll fluorescence (green).
- expressed both in endoderm and ectoderm, but not in zooxanthellae. Those results are in accord with the native PAGE experiment shown above.



- CuZnSODa and CuZnSODb are two superoxyde dismutases encoded by the genome of A.viridis and expressed in endodermal and ectodermal cells.
- The potential binding sites for transcription factors were different for CuZnSODa and CuZnSODb genes, suggesting two different regulations.
- The molecular study of CuZnSOD here presented could help to understand the resistance of symbiotic Cnidarians to hyperoxic conditions.

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