

## Molecular characterization of *Hydra* acetylcholinesterase and its catalytic activity

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The enzyme acetylcholinesterase (AChE) catalyses the hydrolysis of the neurotransmitter acetylcholine (ACh) to choline and acetic acid. This enzyme exists primarily in nerve cells involved in cholinergic synaptic transmission, but is also found in a variety of non-neuronal cells. Although its function is not yet clear, the non-neuronal cholinergic system appears to be involved in the regulation of several biological functions, including proliferation, differentiation, organization of the cytoskeleton, cell-cell contact and immune functions. In the present study, we investigated the presence of AChE in *Hydra*, a member of the phylum Cnidaria. We found a *Hydra* AChE (HyAChE) that has sequence homology to AChEs in other animals. Furthermore, we obtained direct evidence for catalytic activity of this enzyme in HyAChE-expressing *Xenopus* oocytes. The gene was expressed in both ectodermal and endodermal epithelial cells except for the tentacles and basal disk. AChE gene expression was not detected in the regenerating tips in either the head or the foot, indicating that regeneration is controlled by the non-neuronal cholinergic system in *Hydra*.

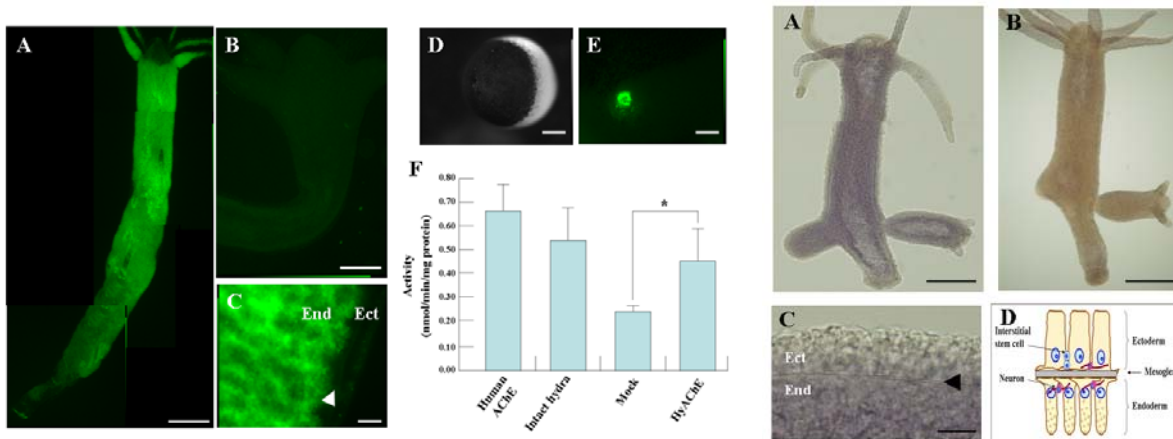


Fig. 1

Fig. 2

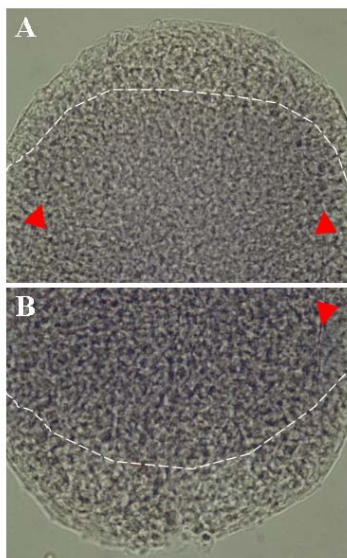


Fig. 3

**Fig. 1.** Localization of HyAChE with Ph-F1 in both *Hydra* and HyAChE-expressing oocytes and its catalytic activity. (A) Localization of enzymatically active HyAChE with a Ph-F1 probe in *Hydra*. (B) The upper part of *Hydra* without any Ph-F1 probe. (C) Enlargement of epithelial cell layers of the body column. Endoderm (End) is left, and Ectoderm (Ect) is right. Arrowhead indicates the basement membrane mesoglea. (D) Mock-injected oocyte. (E) Localization of HyAChE activity with a Ph-F1 probe in an HyAChE-expressing oocyte. (F) AChE activity was assayed in homogenates of intact *Hydra* and in oocytes microinjected with (HyAChE) or without (Mock) HyAChE cRNA. **Fig. 2.** Expression of HyAChE transcripts in *Hydra*. (A) Whole-mount in situ hybridization (WISH) (B) Whole *Hydra* hybridized with a sense probe. (C) Enlargement of a transverse section of the body column. Ectoderm (Ect) is above, and endoderm (End) is below the basement membrane mesoglea (arrowhead). (D) Schematic representation of a cross-section of the body column indicating the location of neurons and interstitial stem cells between the epithelial cells. **Fig. 3.** Expression of HyAChE during head and foot regeneration. HyAChE expression during head regeneration (A), and foot regeneration (B), was analyzed by in situ hybridization 3 hours after decapitation. Arrowheads indicate mesoglea. Dotted lines indicate the border between expression and non-expression.