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# Cnidarian Sox genes and the evolution of function in the Sox gene family

**Thesis Submitted by**

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**in October 2007**

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**Chuya SHINZATO**

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# Abstract

Members of the Sox transcription factor family have a wide variety of roles in the development of higher animals, including neural development and early embryogenesis. To better understand both the evolution of the Sox family and the roles of these genes in cnidarians, we are studying the Sox gene complement of the coral, *Acropora millepora* (Class Anthozoa). Based on overall domain structures and HMG box sequences, the *Acropora* Sox genes clearly fall into four of the five major Sox classes. Each of these genes has a clear *Nematostella* ortholog, but in most cases the expression pattern observed in *Acropora* differs significantly from that reported in *Nematostella*. AmSoxC is expressed in the ectoderm in a cell specific manner during development, with expression beginning much earlier than in *Nematostella*. During gastrulation, AmSoxB1 and AmSoxB3 transcripts are detected only in the presumptive ectoderm whereas AmSoxE1 transcription is restricted to the presumptive endoderm, suggesting that these Sox genes might play roles in germ layer specification. Again, the expression patterns reported for the corresponding *Nematostella* genes differ in many respects from those observed in *Acropora*. These differences may reflect diversity both in fundamental developmental processes and the underlying molecular mechanisms within the anthozoan Sub-Class Hexacorallia (Zoantharia).

Wnt/β-catenin-signalling has important and multiple roles during early metazoan embryogenesis, including axial patterning or early embryogenesis. Upon receipt of the Wnt signal, β-catenin protein, which acts as a transcriptional regulator, is translocated into nuclei. Interactions between Sox proteins and β-catenin protein during germ layer formation have been reported, in a number of higher animals. To better understand the molecular mechanisms of early embryogenesis in *Acropora* and the ancestral roles of Wnt/β-catenin signalling during early embryogenesis, the distribution of nuclear β-catenin protein was investigated during early development. In *Acropora*, whole-mount immunohistochemistry revealed that, unlike most other animals, including the sea anemone *Nematostella*, β-catenin protein accumulates in nuclei in the presumptive ectoderm of the blastula stage. At the 256–512-cell stage, β-catenin starts to accumulate in nuclei, and nuclear localization is observed in the presumptive ectoderm of the blastula stage. To investigate the function of β-catenin and its potential role as a

regulator of Sox gene expression during embryogenesis, *Acropora* embryos were treated with alsterpaullone, a specific inhibitor of the Wnt/β-catenin signalling inhibitor GSK3β. Alsterpaullone treatment significantly inhibited gastrulation in *Acropora* embryos and suppressed the expression of AmSoxB1 and AmSoxE1, suggesting that AmSoxB1 and E1 are downstream targets of Wnt/β-catenin signalling. These results indicate that Wnt/β-catenin signalling and several of the Sox genes play important roles in gastrulation and/or in germ layer formation in *Acropora*. As in the case of the Sox genes, the ectodermal accumulation of nuclear β-catenin in *Acropora* embryos again illustrates the surprisingly diversity of molecular mechanisms involved in early development of cnidarians.