Characterisation of anterior patterning genes in the staghorn coral, *Acropora millepora* (Cnidaria; Anthozoa; Scleractinia)

Thesis submitted by

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III. ABSTRACT

To a surprising extent, molecular mechanisms underlying many aspects of development are conserved across the higher (bilateral and triploblastic) Metazoa, but the degree to which similar mechanisms apply to non-bilateral and diploblastic animals such as cnidarians is unclear. The aim of this thesis was to characterise genes homologous to those that play key roles in anterior patterning in bilaterians, in the basal cnidarian *Acropora millepora*.

In *Acropora*, a gene clearly related to the *ems/Emx* family was shown to be expressed in a subset of presumed neurons, restricted along the oral-aboral (O/A) axis to the aboral end of the planula larva, in a pattern overlapping that of *cnox2-Am*, the *Acropora ind/Gsx* homolog. Hence, key components of both anterior-posterior (A/P) and dorsal-ventral (D/V) patterning systems of higher animals are differentially expressed along the single (O/A) overt body axis of the Acropora planula. During metamorphosis, *emx-Am* expression is significantly reduced, corresponding to the degeneration of the nervous system observed prior to settlement (Ball et al., unpublished). After settlement, expression of *emx-Am* is again detected in a subset of neurons, although an axis-related pattern of distribution is not apparent. Yeast-one hybrid and electrophoretic mobility shift assays (EMSA) were used to identify candidate target sites for the Emx-Am homeodomain and to examine its DNA-binding behaviour *in vitro*. Yeast-one hybrid experiments identified three regions within the *Acropora* genome containing putative Emx-Am binding sites, and further characterisation of these regions attempted to identify potential downstream targets of the Emx-Am homeodomain. Data regarding downstream targets of Ems/Emx proteins is limited, but EMSA provided novel evidence that Ems/Emx homeodomains may be able dimerise on TAAT half sites, a characteristic feature of many ANTP-related proteins.

In *Drosophila*, the Tailless nuclear receptor is a direct regulator of *ems*; for this reason the expression of *tlx-Am*, an *Acropora* gene encoding a protein clearly related to Tailless, was studied. In addition to its patterns of expression, the *tlx-Am* locus was also characterised, but this analysis revealed that a similar regulatory relationship with *ems* is unlikely to exist in the coral.

Attempts to clone the *Acropora* homolog of a *Hydra prdl-a* gene led to the fortuitous identification of four *paired*-like genes. Although none of these appears to be strictly orthologous with *prdl-a*, the sequences and expression patterns of these genes provided some novel insights into the evolution of the Paired superfamily of homeodomain proteins. One of these genes (*hbn-Am*) is orthologous with *Drosophila homeobrain*, and appears to be a very early ectodermal marker during coral development. *hbn-Am* is expressed in a region corresponding to the presumptive ectoderm, in a pattern that is complimentary and mutually exclusive to that of the coral *snail* homolog (*snail-Am*), suggesting that these genes interact.

Two of the *paired*-like genes identified are clearly related and were shown to be organised in tandem in the *Acropora* genome. Two other cases of tightly linked pairs of related genes were also identified, suggesting that a high proportion of coral genes may be organised in this way.

IV. STATEMENT OF CONTRIBUTIONS BY OTHERS

I, the undersigned author of this thesis, wish to acknowledge the collaborative research efforts by others that has contributed to the data presented here in this thesis. The individual research contributions of these people are outlined below, and are additionally acknowledged throughout this thesis.

Chapter 3

Heather Dodd	Generation of *emx* homeobox PCR product
Patricia Pontynen	Isolation and sequencing of *emx-Am* cDNA clone
Dr. Julian Catmull	Isolation of *emx-Am* genomic clone
Dr. Eldon Ball	Collaborative *emx-Am in-situ* hybridization experiments

Chapter 4

Lauretta Grasso	Isolation and sequencing of the *tlx-Am* cDNA clone
Dr. David Hayward	Northern blot analysis of *tlx-Am* temporal expression
Christine Dudgeon	Isolation of a partial *tlx-Am* genomic clone
Dalma Soebok	Sequencing of a partial *tlx-Am* genomic clone

Chapter 5

Dalma Soebok	Isolation and sequencing of the *hbn-Am* and *arx-Am* cDNA clones

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Nikki R. Hislop Date

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IX. STATEMENT OF SOURCES

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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