MOLECULAR ASPECTS OF THE FLUORESCENT PROTEIN HOMOLOGUES IN *Acropora millepora*

Thesis submitted by

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ABSTRACT

GFP-like proteins are responsible for some of the most spectacular colours displayed in coral reefs all over the planet. In here, the GFP repertoire from the scleractinian *Acropora millepora* was molecularly and phenotypically characterised in the context of embryonic development.

Phylogenetic analyses demonstrated the existence of two major clades; corresponding to the fluorescent and non-fluorescent chromoprotein genes respectively. The cDNA sequences were identified from a large EST library constructed using the pre settlement stage of *A. millepora* larvae. Both the fluorescent and non-fluorescent chromoprotein genes have highly similar intron-exon structure, thus, indicating a relatively recent common origin. Gene comparisons against the gene bank indicated that the larval GFPs are genetically distinct to their adult homologs.

Representative clones encoding green -amilGFP517 a, b-, red fluorescent -amilRFP602- and two blue chromoproteins -amilCP597 and amilCP601- were expressed in a bacterial system and biochemically characterized. Importantly the recombinant chromoproteins were shown to transform from blue non fluorescence to red fluorescent, after dehydration and further excitation with green/red light.

In situ hybridisation studies indicated that the chromoprotein genes were expressed early in development and predominantly in the endoderm. In contrast, the expression of the genes encoding fluorescent proteins was initiated later and the mRNA was detected mainly in the ectoderm. Although, the high mRNA similarity between GFP and RFP hampered a clear detection of gene expression; the corresponding expression of the proteins was observed to be axially restricted, as in planulae intense green or red fluorescence were associated with the oral and aboral regions and fluorescence patterns were restricted to specific cells. After metamorphosis, the red fluorescence associated with the aboral ectoderm disappeared, but the green signal remained in oral tissue.

Light/dark exposure experiments indicated that embryos reared in darkness showed some reduction of fluorescent-like gene expression whereas chromoprotein expression was unaffected

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