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A close-up photograph of a coral colony, *Acropora millepora*, showing its characteristic rounded, dome-like structures. The coral is illuminated with a green light, causing it to fluoresce with a bright green glow. The background is dark, making the glowing coral stand out prominently.

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

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Feb 2010

For the degree of **Doctor of Philosophy**
In the School of Pharmacy and Molecular Sciences
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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 -amiGFP517 a, b-, red fluorescent -amiRFP602- and two blue chromoproteins -amiCP597 and amiCP601- 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

TABLE OF CONTENTS

STATEMENT OF ACCESS	ii
STATEMENT ON SOURCES DECLARATION	iii
ACKNOWLEDGEMENTS	iv
ABSTRACT	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	ix
LIST OF TABLES	x
Chapter 1	1
General Introduction	1
1.1 The colour in cnidarians	1
1.2 GFP-like protein structure and classification	3
1.3 Evolution of the GFP-like proteins	3
1.4 Ecological and biological aspects of GFP-like proteins in corals	5
1.5 Importance of evolutionary developmental studies (Evo-Devo)	6
Chapter 2	8
Material and Methods	8
2.1 Nuclear gene cloning, sequencing and assembling	8
2.1.1 [α - ³² P] radioactive probe generation by PCR	8
2.1.2 Agarose gel electrophoresis and storage of radioactive probes	9
2.1.3 Competent cell preparation for viral infection	9
2.2 Titering the genomic libraries	10
2.2.1 Phage library titration	10
2.2.2 Cosmid library titration	10
2.2.3 Acropora genomic Cosmid library amplification and storage	11
2.3 Genomic λ lambda library screening	11
2.3.1 Blotting and marking of membranes	11
2.3.2 Blotting and marking of membranes (Cosmid library)	11
2.3.3 Preparation of the membranes for phage DNA hybridisation	12
2.3.4 Preparation of the membranes for cosmid DNA hybridisation	12
2.4 Hybridisation and DNA recovery of the positive plaques	12
2.4.1 DNA Hybridisation	12
2.4.2 Washing the unbound products and detection	13
2.4.3 Secondary screening	14
2.4.4 DNA purification from the nuclear genomic clones	14
2.4.5 DNA precipitation and storage	15
2.4.6 Restriction enzyme digestions genomic fragments to detect differences ..	15
2.4.7 Southern blot and subcloning	15
2.4.8 Sequencing and gene assembling	16
2.5 Recombinant protein characterization	17
2.5.1 PCR amplification of the amilGFP517a from the presettlement cDNA library	17
2.5.2 Gel extraction and ligation into PCR-based propagation vector	18
2.5.3 Cloning of the GFP-like proteins from the EST collection	18
2.5.4 PCR-based introduction of restriction sites and subcloning into expression vector	19
2.5.5 Dephosphorylation of the expression vectors	20
2.5.6 Cell strain preparation and transformation	20
2.5.6.1 Competent cells preparation and its storage	20
2.5.6.2 Bacterial transformation with plasmid vectors	21
2.5.7 Screening and protein purification	21

2.5.7.1 Bacterial visual screening for the presence of target insert.....	21
2.5.7.2 Bacterial re-protein purification	22
2.5.7.3 Histidine affinity chromatography	23
2.5.8 Recombinant protein characterization	23
2.5.8.1 Electrophoretic characterization in SDS-PAGE assays	23
2.5.8.2 Spectroscopic characterization of the recombinant proteins.....	24
2.6 <i>In vivo</i> and <i>in situ</i> detection of the GFP-like genes	25
2.6.1 Coral colony sampling and general embryo manipulation	25
2.6.2 Live fluorescence microscopy and embryo fixation during the coral development	25
2.6.3 Riboprobe creation (DIG labelling protocol).....	26
2.6.4 Riboprobe fractionation	27
2.6.5 Whole mount <i>in situ</i> hybridisation.....	28
2.6.5.1 Coral embryo preparation	28
2.6.6 Whole mount <i>in situ</i> hybridisation.....	28
2.6.7 Washing the unbound material form the preparations	29
2.6.8 Detection and documentation of the spatial mRNA signal.	29
2.7 Northern blotting procedures.....	30
Chapter 3	31
Characterisation of two GFP loci from the coral <i>Acropora millepora</i>	31
3.1 Introduction	31
3.1.1 Statement of Goals	32
3.2 Results.....	33
3.2.1 Gene structure comparison across the phylum Cnidaria	33
3.2.3 Evolutionary relationships between GFP-like genes	39
3.3 Discussion.....	43
3.3.1 <i>Acropora</i> GFP-like loci: An ancient gene structure	43
3.3.2 Possible transcriptional GFP regulation	45
3.3.3 Phylogenetic relationships of <i>Acropora</i> GFPs	46
3.4 Conclusion	49
Chapter 4	50
Biochemical and spectral analyses of different recombinant GFP-like proteins from the <i>Acropora</i> presettlement cDNA library	50
4.1 Introduction	50
4.1.1 Statement of Goals	52
4.2 Results.....	53
4.2.1 GFP-like protein diversity observed in larvae ESTs	53
4.2.2 3D representation of the observed diversity in each contig	57
4.2.3 Molecular masses of the proteins.....	62
4.2.4 Spectral characterization of GFP-like recombinant proteins from <i>Acropora</i> <i>millepora</i>	64
4.2.4.1 Spectral signature of <i>A. millepora</i> green FP (AmilGFP517a, b).....	64
4.2.4.2 Spectral signature of <i>A. millepora</i> red FP (AmilRFP602).....	66
4.2.4.3 Spectral signature of <i>A. millepora</i> CP (AmilCP597).....	68
4.2.4.4 Spectral signature of <i>A. millepora</i> CP (AmilCP601)	68
4.2.4.5 Spectral characterization of the recombinant proteins under extreme pH conditions.....	70
4.3 Discussion.....	72
4.3.1 Amino acid substitutions and the functions and phenotypes of the protein	72
4.3.2 <i>Acropora</i> GFP-like protein electrophoretic signature	80
4.3.3 The AmilRFP602 spectral signature: similarities and hypotheses	81
4.3.4 Larval AmilGFP517a, b: The most common emission for a coral GFP-like protein	82
4.3.5 The amilCP products: similarities with other cnidarian proteins and potential FPs.....	83

4.3.6 Chemical nature of the GFP-like chromophore: hypsochromic pH-dependent shift	84
4.3.7 The 3D structure of GFP and its nearest structural protein neighbour	85
4.4 Conclusions	85
Chapter 5	86
Temporal and spatial mRNA expression and fluorescence analyses of GFP-like genes during <i>Acropora millepora</i> embryogenesis	86
5.1 Introduction	86
5.1.1 Statement of Goals	90
5.2 Results	90
5.2.1 The major GFP gene types are differentially expressed in early coral development	90
5.2.2 Fluorescence microscopy of GFP variants	93
5.4 Discussion	96
5.4.1 GFP-like message temporal regulation	96
5.4.2 Nature of the observed colouration in the early stages	96
5.4.3 Green-red signal distribution in planulae	97
5.4.4 Non-endodermal <i>in situ</i> staining; no fluorescent product was observed ...	98
5.4.5 GFP-like protein-expressing cell types in <i>Acropora</i>	98
5.4.6 Possible interaction of position-governing genes and differential GFP-like protein expression along the anteroposterior axis in <i>Acropora</i> embryos	101
5.4.7 Cell surface array	102
5.5 Conclusions	103
Chapter 6	104
General discussion	104
6.1 Differential exon evolution, functionality, origins and regulators of the GFP-like genes	104
6.2 Protein world	105
6.3 Too much attention to the core	106
6.4 Environmental influences on early embryogenesis: does light controls GFP expression?	106
6.5 Future directions	108
References	109
Appendix	119

LIST OF FIGURES

Figure 1.1 Adult <i>A. millepora</i> corals observed under fluorescence (GFP2 filter).	2
Figure 3.1 Cloning of the nuclear GFP-like genes.	35
Figure 3.2 Comparison of the intron position over the amino acid alignment from several sequenced cnidarian GFP-like genes.	36
Figure 3.3 Upstream region (from the start codon) comparison of GFP-like genes from anthozoans.	38
Figure 3.4 Phylogenetic analyses of the GFP-like genes using the neighbour joining algorithm to construct the tree.	41
Figure 3.5 Schematic representation of the <i>Acropora millepora</i> GFP-like loci and putative gene neighbours.	47
Figure 3.6: Schematic representation of the <i>Nematostella vectensis</i> GFP-like loci.	48
Figure 4.1 Amino acid alignments of all complete clones that belong to the Chromo-like contig.	55
Figure 4.2 Amino acid alignments of all complete clones that belong to the Fluorescent-like contig.	56
Figure 4.3 3D model of the CP recombinant proteins and the substitutions observed in the whole contig.	60
Figure 4.4 3D model representation of fluorescent recombinant proteins, including the observed diversity of amino acid substitutions inside the contig.	61
Figure 4.5 Phenotypic comparison of the expressed recombinant GFP-like proteins.	63
Figure 4.6 Spectral characterization of amilGFP517a and amilGFP517b proteins in solution (PBS, pH 7.2) at room temperature.	65
Figure 4.7 Spectral characterization of recombinant AmilRFP602 in solution (PBS, pH 7.2) at room temperature.	67
Figure 4.8 Spectral characterization of two recombinant CPs measured in solution (PBS, pH 7.2) at room temperature.	69
Figure 4.9 pH-dependent behaviour of the recombinant proteins in solution at room temperature.	71
Figure 4.10 Amino acid alignment from representative GFP homologues across Metazoa.	73
Figure 5.1 Embryological development of <i>Acropora millepora</i>	88
Figure 5.2 <i>In situ</i> and <i>in vitro</i> time-dependent detection of the GFP-like mRNAs in <i>Acropora</i> development.	92
Figure 5.3 <i>In vivo</i> observation of the principal morphological and fluorescence characteristics of <i>Acropora millepora</i> embryogenesis.	95
Figure 6.1 Spectral characteristics of the recombinant proteins from <i>Acropora millepora</i> presettlement library.	119
Figure 6.2 Box shade alignment between larval and adult <i>Acropora</i> fluorescent proteins.	120
Figure 6.3 Prawn chip coloured embryos.	120
Figure 6.4 38h-FITC coral embryo.	120
Figure 6.5 Red granulations in planula cells.	120
Figure 6.6 Planula section stained against amilFP.	120

LIST OF TABLES

Table 1. Primers characteristics; CP correspond to chromoproteins and FP to fluorescence genes.....	19
Table 2. Filters used for live fluorescence	26
Table 3. Developmental stages of <i>Acropora millepora</i>	28
Table 4. Information related to all GFP-like proteins used to construct the general interphylum evolutionary tree.....	42