



GÖTEBORGS UNIVERSITET

Gene expression and regeneration in ophiuroids: A study of transcriptional activity and regeneration in the temperate *Amphiura filiformis* and Antarctic *Ophionotus victoriae*.

Mr Gavin Burns

Institutionen för biologi och miljövetenskap
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i Marine Zoology, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras Torsdag den 22 November 2012 kl. 10.30 i Sven Lovén centrum för marina vetenskaper, Institutionen för biologi och miljövetenskap, Kristineberg 566, 451 78 Fiskebäckskil.

ISBN: 978-91-628-8568-7



GÖTEBORGS UNIVERSITET

Abstract

Regeneration, in which lost or damaged tissues are re-grown, is a common phenomenon amongst animals and especially so within the ophiuroids (brittle stars) of the phylum echinodermata. The process of regeneration begins with the injury event followed by a period of cell and tissue reorganization which results in wound healing and tissue re-growth. The genetic programme of regeneration is complex and poorly understood, however the role of gene expression is becoming increasingly well characterised.

The goal of this thesis was to identify genes that are transcribed during the process of regeneration in ophiuroids to determine the pathways and gene families active during the various stages of regeneration. By developing and adapting high throughput genomic techniques for use with ophiuroids the investigation of ophiuroid regeneration was taken from the single gene to whole transcriptome studies. This effort was carried out in two diverse ophiuroid species; the Antarctic *Ophionotus victoriae* and temperate *Amphiura filiformis*. Initially the levels of natural arm damage and rate of regeneration was investigated in *O. victoriae*. Subsequently, the genomic techniques required to explore the transcriptomes of these organisms were developed or adapted. Using these techniques the scale of gene expression and gene networks active during regeneration in both *O. victoriae* and *A. filiformis* were surveyed. Determining the extent of gene expression and identifying gene involvement in regeneration in two diverse ophiuroid species facilitates a deeper understanding of the conservation of this important survival and potentially clinically important mechanism.

Investigation of natural arm damage and the rate of regeneration in *O. victoriae* demonstrated that this stenothermic Antarctic ophiuroid has a slow rate of regeneration. This is preceded by an unexpected and unprecedented delay (approximately 5 months) followed by a long period of regeneration (>1 year) at a reduced rate that is expected for an organism living at low temperatures. Additionally this study discovered that *O. victoriae* has a very high level of natural arm damage with 97% of sampled animals showing some arm damage or signs of previous arm regeneration.

A DNA microarray was constructed to determine the extent of gene expression and the genes and gene networks involved in regeneration in *A. filiformis*. The DNA microarray analysis allowed the measurement of the activity of several thousand genes in each sample to construct a dataset describing transcriptional activity during the early, mid and late stages of regeneration. It was demonstrated that the early stage of regeneration, in which the stem cell rich blastema is formed, is the most active in terms of gene expression followed by the intermediate stage, in which approximately half of the cells are differentiated. The later stage, in which most cells are terminally differentiated, had the fewest differentially expressed clones of the three stages measured. From this dataset genes involved in energy production, structural proteins, cell polarity, segmentation, stem cell maintenance and inhibitors of cell proliferation were putatively identified.

Further measurements of gene expression in amputated arms (explants) of *A. filiformis* that had been double amputated to form a wound site at the proximal and distal end further confirmed the specific regenerative activity of several genes, including SoxB1 and DSP-1 homologues. The results demonstrated a polarity of gene expression in double amputated arm explants and that the mechanisms for forming and maintaining a pool of undifferentiated cells are present in the distal part of the explant.

The genes present during regeneration in *O. victoriae* were identified using next generation sequencing to produce approximately 18,000 contiguous sequences, of which 19% were identified using similarity searching. Few sequences were synonymous between *O. victoriae* and *A. filiformis* but the 111 that were included the SoxB1 and DSP-1 orthologues previously described. The increased depth of sequence data gained compared to that achieved for *A. filiformis* allowed the identification of more gene families and pathways including several Hox gene family members, four SOX genes and members of the Notch, TGF-beta and Wnt signalling families. All of these genes and gene families have been previously shown to be involved in the regenerative process of non echinoderms, providing important comparators that give an insight into the processes occurring during ophiuroid regeneration.

Presented in this thesis is the first large scale study of gene activity in ophiuroids. The data described provides an insight into the genetic control of regeneration in ophiuroids and will facilitate the further characterisation of regeneration in these organisms. On a wider scale, this investigation of the extraordinary regenerative ability of ophiuroids could contribute to the overall understanding of this clinically important process.

Keywords: echinoderm, regeneration, ophiuroid, gene expression, Antarctica.