Epigenetics of Cnidarian Stem Cells (EpiCS)

The project addressed a new and exciting topic in biology, namely the role of epigenetic factors in shaping stem cell fate. The work utilized an emerging model organism for stem cell biology, the cnidarian *Hydractinia*. This animal possess a remarkable regenerative ability and growth plasticity that are both dependent on a population of adult stem cells called i-cells.

Epigenetics refers to heritable changes in gene expression that are not resulting from changes in DNA sequence. They are related to chemical modification of chromatin, which includes DNA and the proteins (histones) around which it is wrapped. Changes to chromatin include DNA methylation and histone modifications such as methylation, acetylation, and SUMOylation. Such changes can affect gene function, either activating or silencing them, over multiple cell cycles. Most epigenetic marks are erased during sexual reproduction, but some persist across generations and can affect siblings in various ways.

The project's objectives were [1] to identify epigenetic mediator and pluripotency genes in the *Hydractinia echinata* genome using bioinformatics and predict genome–wide CpG islands; [2] to map the methylome and important histone modification sites in stem cells and differentiated cells of *Hydractinia* using Illumina MeDIP-Seq and ChIP-Seq; [3] to verify and functionally assess epigenetic mechanism in *Hydractinia* by mis-expression of selected genes.

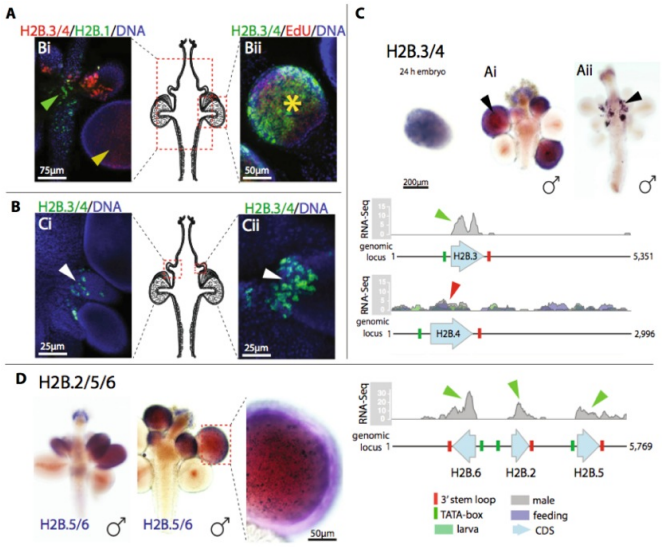
A comprehensive bioinformatic analysis was carried out of the *Hydractinia* genome and transcriptome sequences. They revealed a list of over 130 genes that have clear homologues in other animals and are known to have a role in chromatin modification or interpretation. Phylogenetic analysis of selected gene/protein families suggested conservation of individual members and lineage specific duplications.

Two distinct DNA methylation marks were studied: 5-methylcytosine (5mC) and 6-methyladenosine (6mA). For this, biochemical and computational methods were used to identify the distribution of these epigenetic marks across the *Hydractinia* genome. The pattern of their occurrence is consistent with a role in transposon silencing but also with stabilizing protein-coding gene expression by inhibiting spurious transcription initiation.

Functional studies on *Hydractinia* histones were carried out, in particular on novel sperm-specific histone variants. Genetic gain- and loss of function experiments were complemented by biochemical assays to address the role of these histones in sperm development. It was found that these histone variants cannot substitute for the absence of canonical histones during embryonic development. Biochemical analysis has shown that these histone variants provide stability to sperm chromatin and reduces its accessibility.

The results of this project will benefit those researchers interested in chromatin modifications and histone biochemistry. It will also impact the field of reproductive biology.

The results of the work have been published in international, peer-reviewed journals. The data has also been presented in a number of scientific conferences in the form of oral presentations and posters.



The colony forming cnidarian *Hydractinia echinata* possesses three main polyp types (sexual, feeding and defensive). Here we show the male sexual polyp specific expression of previously unknown histone H2B variants as well as their genomic loci and expression status across life stages (larva, male, feeding). A) Fluorescent in-situ hybridisation (FISH) showing the expression of canonical H2B and male-specific H2B.3/4 (Bi). Proliferating sprematocytes also express H2B.3/4 (Bii); B) FISH showing early spermatocytes (Ci) and late spermatocytes (Cii) expressing H2B.3/4; C) ISH shwoing expression of H2B.3/4 in 24 hour embryos and male sexual polyps (Ai and Aii); D) ISH showing H2B variant H2B.5/6 expression in male sexual polyps; CDS=coding sequence.