

Proteomic Profiling of the Blastogenic Cycle in *Botryllus schlosseri*, an Emerging Model Organism

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Introduction

Botryllus schlosseri is a marine colonial tunicate, features a unique life cycle which includes both asexual and sexual reproduction. Its close evolutionary relationship to vertebrates, combined with its remarkable regenerative abilities, positions it as an invaluable model for studying regeneration, tissue development, and aging. This study marks the first molecular characterization of *B. schlosseri*'s blastogenic cycle. By using quantitative Data-Independent Acquisition (DIA) proteomics, we investigate the proteome across various stages to reveal the molecular and cellular mechanisms driving the asexual reproduction cycle. Our hypothesis propose distinct proteome patterns at each stage, with a significant upregulation of pro-proliferative proteins in the newly forming tissue.

Methods

To promote consistent colonial growth through asexual reproduction, *B. schlosseri* colonies were reared in stable laboratory conditions with key controlled parameters including temperature, salinity, light, and nutrition. Dissected zooids or buds from identical clones and developmental stages were pooled for proteomic analysis.

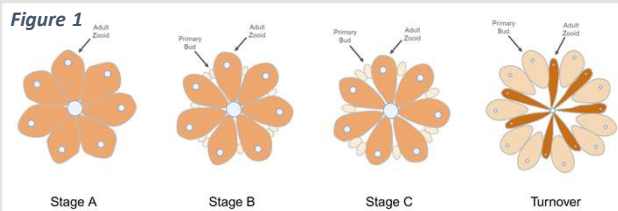


Figure 1: Depiction of the weekly blastogenic cycle in four stages, culminating in the turnover phase where adult zooids regress and are replaced by maturing primary buds.

Quantitative proteomics workflow was established using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) with Data-Independent Acquisition (DIA).

PCA of *B. schlosseri* Stages by Proteome Profiles

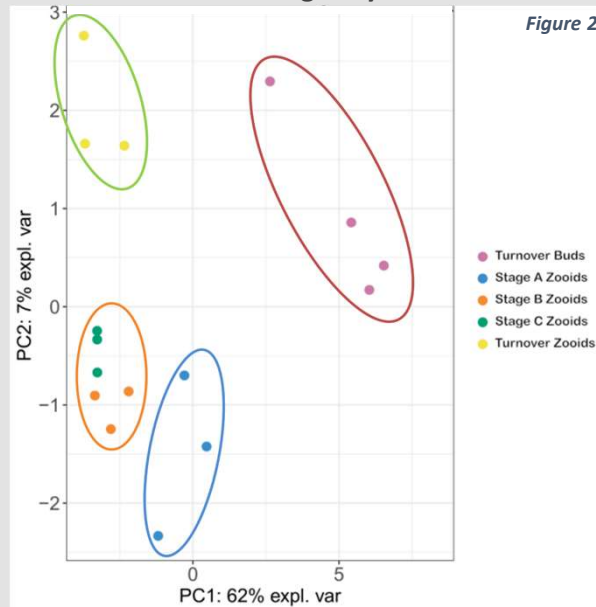


Figure 2: Principal Component Analysis (PCA) performed on proteins that are significantly differentially expressed in at least two conditions across all blastogenic stages.

Differential Protein Expression in Zooids vs. Buds

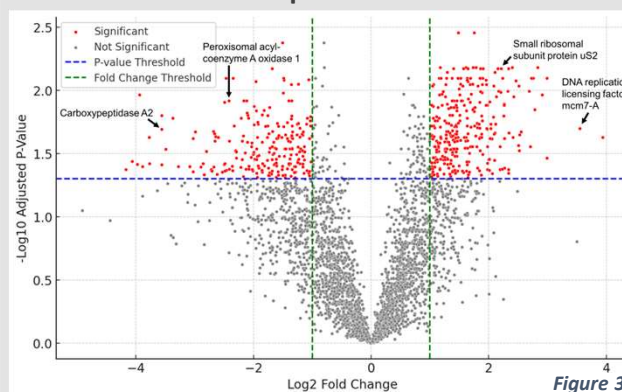


Figure 3: Showcases 317 up-regulated and 224 down-regulated proteins, marking the molecular distinctions between Turnover zooids and buds in *B. schlosseri*.

Pathway Enrichment in Turnover Zooids vs. Buds

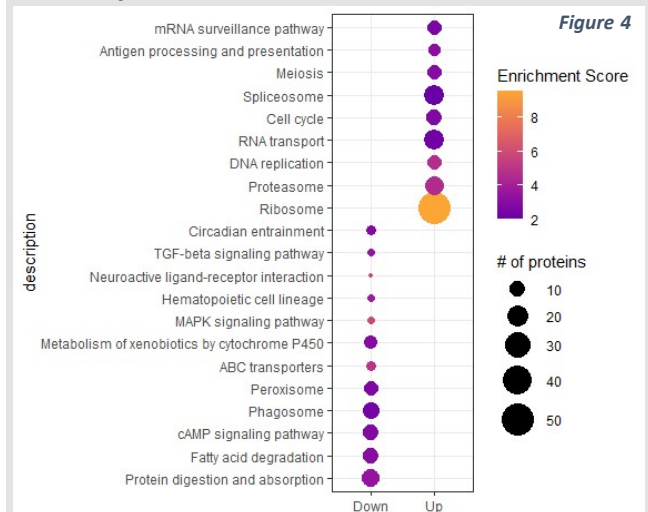
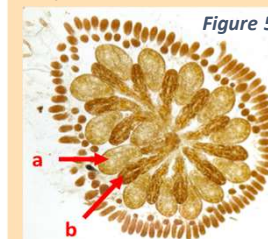


Figure 4: Depicts enriched KEGG pathways (p -value<0.05) based on differentially expressed proteins between turnover zooids and buds in *B. schlosseri*. Bubble size represents the number of proteins involved, while color intensity indicates the enrichment score of each pathway.

Future Directions

The results support our hypothesis that pro-proliferative pathways are active in emerging zooids, while degenerative pathways predominate in zooids undergoing reabsorption. By analyzing differential expression between turnover zooids (Figure 5b) and



buds (Figure 5a), we aim to identify essential regulators of pro- and anti-proliferative pathways. Such insights will direct us toward viable targets for the modulation of regenerative processes.

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