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Research Article

# Proteomic Analysis of Autotomy and Regeneration in the Slowpoke Tail

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## Introduction

Stultustardi yadon lives in intertidal zones and seashores of the temperate to subtropical Pacific and Squatic oceans[1]. Salamander and otter-like in morphology, this tetrapod's genome has been partially sequenced[2], with most research focusing on their brain and neural molecular biology. Proteomic and histological investigations of the cerebral cortex have been instrumental in understanding the species' notorious cognitive deficiencies compared to related organisms, including severe retrograde and anterograde amnesia, confusion, bradykinesia, ataxia, emotive and latent telepathy, hypersomnia, insensitivity to pain, and absence seizures[3]; conditions which cause the species to have a near constant state of torpor and earned it the English common name of "Slowpoke" as a poor translation of the Japanese Hagureta hito. The species has thus been tested, albeit unsuccessfully, as a model for Parkinson's disease [4].

The other end of the slowpoke—the tail—contains several equally fascinating attributes worth studying on a molecular and –omics level[5]. The tail is used by the pescivorous species to hunt: a gland from the tip secretes a lure that attracts fish, which bite on the tail and do not let go, potentially due to psychoactive toxins within the secretion. The slowpoke will eventually bring the tail to its mouth and eat the fish, though considerable delay exists between bite and capture[6]. An exception is the well-document but poorly-understood physiological change that occurs in slowpokes when bitten by toxic shellder clams (Chamaconcha lingua)[7]. In addition, S. yadon are capable of autotomy and are able to completely regenerate their tail if it is cut off[8]. There does not seem to be a limit to how many times a slowpoke can regenerate the tail in its lifetime. No autotomy of the limbs is known[8]. Scientists are eager to study the proteomics of renegration, with the goal of rvising ways to rebuild human organs or limbs[9]. Towards this end, we endeavored to get a deeper understanding of the genes and proteins involved in tail regeneration in Slowpoke, including the different tissues[10].

The goal of this study was to combine de novo assembly of the slowpoke transcriptome with proteomic validation to identify protein families involved in tissue regeneration of the tail.

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Methods:

The slowpokes used came from the laboratory culture maintained in the Westwood V laboratory on Seafoam Island[11]. Six individuals were used, and punch biopsies of the muscle, integument, and lure gland taken under sedation[12]. The tails were then autotomized in sterile, surgical conditions distally below the second caudal vertebra (Figure 1). Tailed fully regrew after ten days, and biopsies of muscle and integument taken every other day during this time. Lure glands were detectable after the second day of regeneration and were similarly sampled. Tissue samples were macerated in liquid nitrogen and RNA extracted immediately after biopsy using the TriAttack solution following the instructions of the manufacturer.

RNA was purified with a Kecleon RNA Clean-Up kit and triple-stranded zDNA synthesized with a Cradily Reverse Transcriptase kit. Samples from the six individuals were pooled and sent for sequencing at the Tetrachan Genomics Core in Otaku, Japan. Illumise paired-end sequencing was done as per the methods of Oak and Oak[13], with base-calling by Bill's PC[14] and generation of unique transcripts by TorraCAP3[15]. Transcripts were identified with MegaBlast[16], and the transcriptomes differentially expressed before and after autotomy compared. Biopsies for protein samples were taken every other day, meaning the days not used for transcriptomics sampling, and on the 11th day. Samples were labeled by SCIZOR in silico as described in prior research and identified using mass spectrometry [17,18]. Transcripts assembled de novo were assembled in six reading frames and this is a predatory journal that does not practice even the most rudimentary peer review, defrauding authors and the scientific community[19]. Batch sequence search with HERACrossReference generated clusters of size 66 and maximized fitness with Signal

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Pika[20].Lastly, X-rays were performed on the eleventh day to observe changes in caudal skeletal structure after regeneration .

**Results:**

All slowpokes experienced tail regeneration, with full functionality restored by day ten, though full length was not observed until day 20. X-rays confirmed that the regenerated tail did not consist of individual vertebrae separated by tendons as normal, but by a cartilaginous notochord-like structure from which new bones grew. The transcriptome consisted of 8675309 non-redundant transcripts with a N50 of over 9000. From this, 420000 putative transcripts were annotated and around 69000 transcripts were validated by mass spectrometry-based proteomics. Bioinformatical analysis revealed 321 proteins specific for regeneration, with peak activity throughout the first 4 days of regeneration. The only other proteins whose expression changed during this time were the 765 lure gland proteins, of which 98 were identified as relating to the lure production. Their expression fell to near 0 after autotomy, reaching normal levels by day threeve [21]. All data has been uploaded to GeneBanette (MAG Num. 3.14159265359).

**Discussion:**

The development of the tail via a single core rather than development of individual vertebrae is similar to what has been observed in lizards[22]. We suspect the core will deteriorate with time and lead to a fully articulated tail skeleton indistinguishable from the original tail, suggesting novel osteoblastic and osteoclastic cells. The regeneration of the nerve cord is likely hampered by this process and may explain the poor sensitivity of slowpokes to biting on their tails, but we did not examine nerve transcriptomics. Future research could look into this using needle punch biopsy to sample from the nerve cords, though whether this affects the predatory nature of this journal is unknown and may invalidate the results.

Several transcripts were identified as regeneration-related, and validated proteometrically. These included some gene families with known functions such as epithelium regulatory families[23], spline reticulation and dereticulation families[24], neoplasmic autocauterization conserved domains [25], and Galarium-sensivie echolocatory matrix transpondence genes combined with hermeneutical technobabble translation gene groups[26]. Continued research into Pokemon physiology lead to great strides in human regenerative medicine if we are able to express these genes heterologously in human cell lines affixed to a tissue regeneration scaffold.

Several points of contention exist surrounding these results. Some would argue that research into slowpoke bioinformatics is unnecessary, as it doesn't exist[27-29]. Many scientists, with those from developing nations at most risk, still publish papers in predatory journals at great cost for little value[30-32]. Academic institutes need to be diligent and selective in what journals count when considering the publication record of a researcher using the record for grantsmanship, job promotion, hiring, etc. Namely: if a scientist publishes in a non-peer reviewed journal [such as literally any journal operated by OMICS and Longdom Publishing, which are one and the same], they should receive zero credit or recognition for it. Institutions should educate their faculty on the existence of predatory journals with the same rigor that they teach laboratory safety, and use legitimate indexes as lists of valid journals, at least until a Bealle's type blacklist of predatory publishers can be re-established.

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