Spinal Cord Regeneration in *Xenopus*: A Transcriptomics Analysis

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Spinal cord injury (SCI) results in motor and sensory loss- usually irreversible, as mammals lose their limited regenerative faculty shortly after birth. *Xenopus laevis* presents an interesting model for studying SCI, because its regenerative capacity is also limited to an, albeit longer, defined period. Regeneration occurs during larval stages before metamorphosis, but not after its conclusion. Our hypothesis is: **SCI induces a regenerative-permissive transcriptome in the *X. laevis* spinal cord, which is absent after metamorphosis has concluded. The overexpression or repression of key genes determines the regenerative abilities of pre-metamorphic stages.**

We performed high-throughput RNA sequencing (RNA-Seq) of the spinal cord transcriptome induced 2 and 6 days after injury, in *Xenopus* regenerative and non-regenerative stages (Fig 1). Normalization and differential gene expression analyses between samples were performed using the edgeR package from Bioconductor and MySQL queries. Furthermore, selected genes were validated using RT-PCR.

**Fig. 1:** Samples isolated for RNA sequencing (a) and RNA-Seq flowchart (b). Sham-operated (used as control) refers to animals whose skin and muscle were incised without injuring the spinal cord.
Our results show functionally related groups of genes that are regulated differently in regenerative and non-regenerative stages (Fig. 2). For example, mRNA levels of nestin (a neural stem cell and progenitor marker) were modestly up regulated after SCI in pre-metamorphic stages. However, this increase was more markedly observed in post-metamorphic stages. Furthermore, cell cycle and extracellular matrix related genes were up regulated at 2 days after injury, whereas the main increase in non-regenerative stages was observed at 6 days after injury. Therefore, up regulation of these genes occurs earlier in regenerative stages than in non-regenerative stages.

Fig. 2: Differential gene expression analyses of RNA-Seq results. MA plots showing differential gene expression in pre-metamorphic and post-metamorphic stages, 2 and 6 days after spinal cord transection. All transcripts are shown in blue, housekeeping genes (green), neural stem and progenitor cell markers (red), cyclins and cell cycle related genes (yellow) and extracellular matrix related genes (gray). 2dps- 2 days post sham-operation; 2dpt- 2 days post transection; 6dps- 6 days post sham-operation; 6dpt- 6 days post transection.

Differential transcript levels may account for pre-metamorphic regenerative abilities, and the lack thereof after metamorphosis. These will help us to better understand the process of regeneration, for the eventual development of regenerative therapies in mammals.

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