Cubilin is transcriptionally modulated in the spinal cords of salamanders following traumatic resection.

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In nature, there are extraordinary creatures that have the ability to regenerate spinal cord tissue following traumatic injuries^{1,2,3,4}. We mined published microarray data^{5,6} to understand in an unbiased fashion how this process transpires, by studying transcription at the systems level in the spinal cord tissue of axolotl salamanders following traumatic cord resection. We identified CUBN, encoding cubilin, as among the genes whose expression changes most significantly in the spinal cords of salamanders during the regenerative process. CUBN is likely one of many molecules whose modulation at the level of gene expression, in a complex, multi-step process, is critical for rebuilding the spinal cord.

Keywords: paralysis, spine, spinal cord regeneration, central nervous system regeneration, CUBN, cubilin.

1 In humans, the peripheral but not central nervous system has regenerative capacity⁷. Thus, humans
2 cannot regenerate spinal cord tissue following a severing injury. This is the basis by which humans
3 become paralyzed following acute trauma in civilian or military life. To understand the molecular basis of
4 spinal cord regeneration in nature, we studied the transcriptional response to spinal cord trauma in a
5 creature capable of spinal cord regeneration using published microarray data^{5,6} from a severing injury
6 model in the axolotl.

7 **Methods**

8 We used microarray datasets GSE79299⁵ and GSE71934⁶ for this global differential expression analysis of
9 the response to spinal cord injury during spinal cord regeneration in the axolotl salamander. GSE79299
10 was generated using Affymetrix Ambystoma mexicanum AMBY_002 20k array technology with $n=3$
11 control samples, $n=3$ at day 2 postinjury, $n=3$ day 14 post-injury, and $n=3$ at day 21 post-injury; analysis
12 was performed using platform GPL15153. GSE71934 was generated using Affymetrix Ambystoma
13 mexicanum AMBY_002 20k array technology with $n=3$ control samples, $n=3$ at day 1 post-injury, $n=3$ at
14 day 3 post-injury, and $n=3$ at day 7 post-injury; analysis was performed using platform GPL15153. The
15 Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but
16 raw p -values were used to assess statistical significance of global differential expression.
17 Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation
18 was used.

19 **Results**

20 We identified cubilin, encoded by CUBN, as among the genes most differentially expressed in
21 spinal cord tissue of the axolotl salamander following a severing injury, studying tissues before injury, and
22 at day 2, day 14, and day 21 post-injury using published microarray data⁵. When sorting each of the genes
23 expressed in the spinal cord at day 2, day 14, and day 21 post-injury based on significance of difference as
24 compared to baseline, CUBN ranked 2 out of 20080 total transcripts (Table 1), equating to 99.99%
25 differential expression. Differential expression of CUBN in the spinal cord of the axolotl salamander
26 following severing injury was statistically significant (Table 1; $p=5.85e-11$).

27 We studied a second microarray dataset⁶ to validate differential expression of CUBN in the axolotl
28 salamander in response to a severing injury and again observed significant, transcriptome-wide differential
expression of CUBN during the spinal cord regenerative response. When sorting each of the genes
expressed in the spinal cord at day 1, day 3, and day 7 post-injury based on significance of difference as
compared to baseline, CUBN ranked 1910 out of 20080 total transcripts (Table 2), equating to 91.8%
differential expression. Differential expression of CUBN in the spinal cord of the axolotl salamander
following severing injury was again statistically significant (Table 2; $p=1.17e-09$).

Thus, by mining published microarray data^{5,6} in an unbiased fashion and at the systems-level, we
identified cubilin as among the genes whose expression changes most significantly in central nervous
system tissues of the salamander during the process of spinal cord regeneration.

Discussion

Spinal cord injury resulting in paralysis has traditionally been viewed as a final injury with no chance of recovery. Amphibians including the salamander are capable of regenerating the spinal cord following a traumatic injury, and we used published microarray data to understand how this occurs, with hopes that studying how this process occurs in nature can guide humans in their efforts to treat those with spinal cord injuries. We demonstrated here that cubilin (CUBN) is transcriptionally modulated following severing spinal cord injury in a creature capable of spinal cord regeneration, the axolotl salamander, during a period in which the central nervous system regenerates itself. Changes in CUBN expression during regeneration of the salamander spinal cord are likely an important factor in the transcriptional biology of central nervous system regeneration in nature. Future systematic dissection of the most significant transcriptional changes that transpire during spinal cord regeneration in amphibians may perhaps provide insights that inform therapeutic approaches to treating spinal cord injury in humans.

References

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Rank: 2

Probe ID: axo4567-r_at

F: 383.4

p-value: 5.85 e-11

Gene: CUBN

Gene name: cubilin

Table 1: CUBN is differentially expressed in the spinal cord tissue of the axolotl salamander following traumatic injury and during the course of central nervous system regeneration.

The rank of global differential expression out of 20800 total transcripts, the probe ID of the differentially expressed gene, the F-statistic, the *p*-value of global differential expression, the gene and gene name are listed here.

Rank: 1910

Probe ID: axo4567-r_at

F: 138.02946

p-value: 1.17e-09

Gene: CUBN

Gene name: cubilin

Table 2: CUBN is differentially expressed in the spinal cord tissue of the axolotl salamander following traumatic injury and during the course of central nervous system regeneration.

The rank of global differential expression out of 20800 total transcripts, the probe ID of the differentially expressed gene, the F-statistic, the *p*-value of global differential expression, the gene and gene name are listed here.

CUBN: Cubilin

Figure 1: CUBN is transcriptionally induced in the spinal cord of the salamander during spinal cord regeneration between days 2 and 14 post-injury.

The mean expression value of CUBN messenger RNA transcript is depicted prior to injury, at 2 days post-injury, at 14 days post-injury and 21 days post-injury.