

# Transcriptional regulatory networks in neuronal regeneration

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## Introduction

Neurons in the peripheral nervous system (PNS) have a remarkable capacity to regenerate after injury, whereas neurons in the central nervous system (CNS) generally do not. An excellent model system to study these dramatic differences in regenerative capacity between PNS and CNS neurons is provided by the dorsal root ganglion (DRG). Neurons in the DRG have branches extending both into the PNS and CNS and the differences in response to injury of these branches are profound. A lesion of nerve fibers in the sciatic nerve (SN) triggers a response that eventually leads to neurite outgrowth and establishment of new functional contacts, whereas a similar lesion of the dorsal root (DR) does not lead to successful regeneration. Over the past decades some of the conditions required for successful neuronal growth have been identified. However, the underlying molecular mechanisms are still poorly understood. Important insights are expected to be gained by studying early transcriptional events that are triggered by nerve trauma. Differences in (regulation of) transcription factor activity and target genes in the immediate stages following injury are responsible for differences in the intrinsic capacity of CNS and PNS neurons to regenerate. Our main goal is to elucidate the transcriptional network that underlies successful regeneration. We try to achieve this by combining experimental and computational-statistical modeling approaches.

## Microarray analysis

We characterized the temporal changes in gene expression in DRG neurons following either SN crush (successful regeneration) or DR crush (unsuccessful regeneration). We used 44K rat whole-genome microarrays from Agilent, and expression was measured at 12h, 24h, 3 days and 7 days after the crush and compared with unlesioned control DRGs. We ranked genes according to evidence of differential regulation across the two paradigms. This was done using a recent method based on a moderated two-sample Hotelling  $T^2$  statistic developed specifically for short replicated timecourse microarray studies by Tai and Speed [2]. We subsequently clustered the top 2000 ranked genes into six different clusters using k-means to reveal specific gene expression response patterns, see Figure 1. The next step was to identify (specific combinations of) DNA regulatory elements corresponding to DNA binding sites for transcription factors that may underlie these specific gene expression responses.

## Transcription factor binding site prediction

In silico prediction of transcription factor binding sites was performed using a large collection of vertebrate non-redundant TRANSFAC [1] matrices. We used up to 5000 bp of upstream

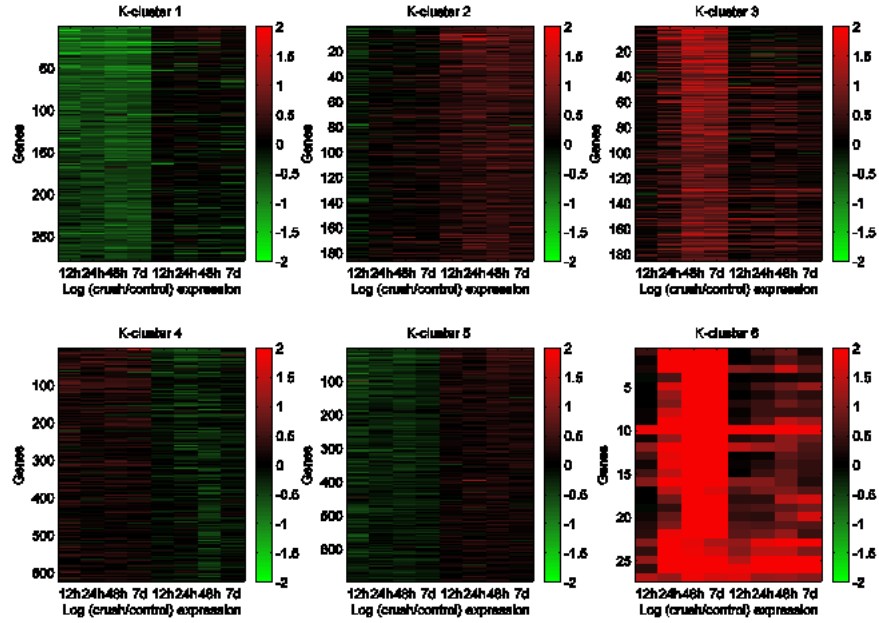


Figure 1: Heatmap of gene expression in SN crush (left) and DR crush (right) experiments

sequence and 2000 bp downstream of the transcription start site of genes present on the array. This resulted in a dataset of 15506 genes for which both expression and regulatory sequence data are present.

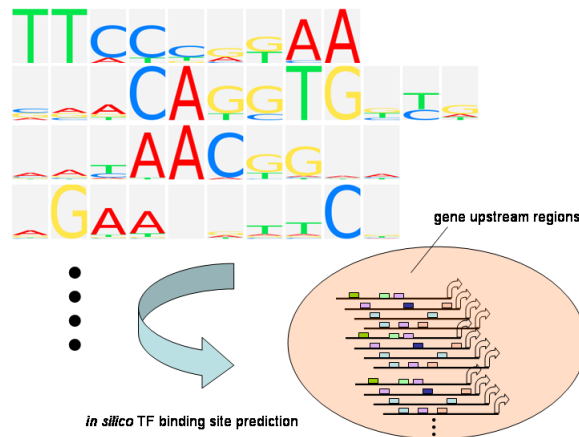


Figure 2: *In silico* binding site predictions

### Statistical analysis

The data we observe, i.e. the binding site predictions, are a mix of false positive predictions and true TF binding sites, only a fraction of which are functionally involved in regeneration. We have carried out a statistical analysis to generate hypotheses of combinatorial regulation based on enrichment of singles, pairs and triples of TF binding sites in clusters. In the

future, we plan to functionally validate the most promising regulation hypotheses with new experiments.

## References

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