# Regulatory Network Modelling for Understanding Boron-Induced Transcriptomics Changes within HepG2 Cell Line

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## INTRODUCTION

Boron has a crucial role in plant growth and survival; also, it is suggested as an essential trace element for human physiology. Accumulating evidence show beneficial effects of boron for human health. Along with its benefits to bone and brain health, many findings support the anti-carcinogenic role of dietary boron. Although biochemical significance of boron is evident, relatively few studies focus on boron-induced biological processes and mechanisms at the molecular level.

In this work, we aim to reveal the boron-induced molecular mechanisms in detail, and our preliminary findings of network modelling study is presented. HepG2 cell line is treated with boric acid (BA) at half-maximal inhibitory concentration ($IC_{50}$) for 24 hours. Differential gene expression profile relative to non-treated HepG2 cells is investigated with microarray technology. A regulatory network is build using boric acid induced gene expression data with motif knowledge and known physical interactions among transcription factors.

At half-maximal inhibitory concentration, boric acid treatment lead to a massive **down-regulation of genes which take part in in cell-cycle progression and various metabolic processes**. Regulatory network revealed transcription factor-gene interactions, which will help us to exploit the effected regulatory mechanisms at transcriptomics level in the presence of highly concentrated boron.

**Our results** indicates, a group of genes involved in lipid metabolism might be particularly meaningful since latest research also suggest potential therapeutic activity of boron in lipid dysregulation disorders like fatty liver disease and obesity.

**Next** we plan to validate the key proteins in the regulatory network in cell culture. Moreover, we aim to recapitulate the microarray experiments and carry out subsequent network modelling at lower concentrations of boric acid to study the boric acid related network patterns in a concentration dependent manner.

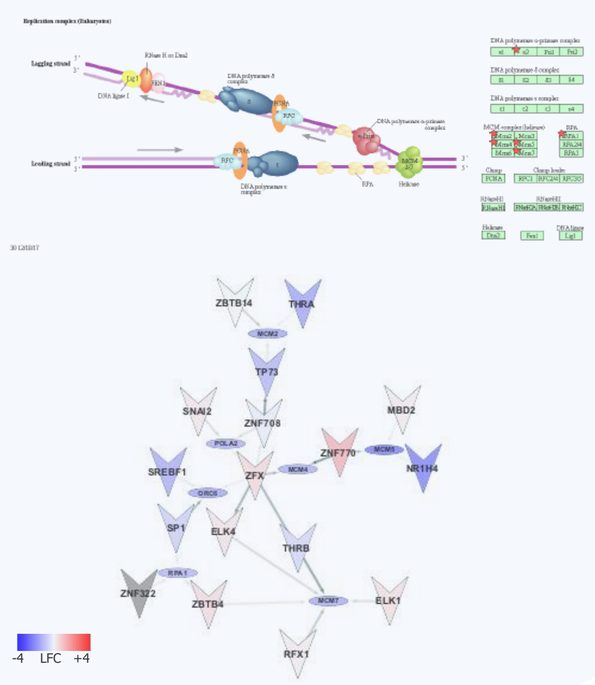
## MATERIALS & METHODS

- **Microarray data**
  - 6 Affymetrix Human Gene 1.0 S.T arrays
  - 3 hybridizations : BA treatment
  - 3 hybridizations: Untreated control
- **Preprocessing of microarray data**
  - Limma package[1]
  - BrainArray-v22 ENST annotation package[2]
  - RMA summarization[3]
- **Finding differentially expressed genes**
  - e-bayes moderated t-test
  - Limma package[1]
  - BH adjusted p<0.05
  - LFC>1 or <-1
- **Motif Data**
  - HOCOMOCOv11[4] Human PWMs-full
  - [-1000,+500] around TSS
  - FIMO[5] search tool
  - Q-val<0.05 selected
- **PPI Data**
  - Obtained from Ravasi et al.[6]
- **Obtaining Network with PANDA[7]**
  - pandaR package[8]
  - Two regulatory networks
    -BA treatment network
    -Control network
  - Differential Network
  - Filtering
    -Scaled edge weight <-2.58, >2.58
    -Average edge weight >2
- **Network Visualization**
  - Cytoscape[9]
  - Colored according to LFC
- **JactiveModules Search[10]**
  - 5 modules
  - Overlap threshold:0.5
  - Depth:3
  - Max depth from start: 3
  - Module with the highest activepath score is selected.

## RESULTS

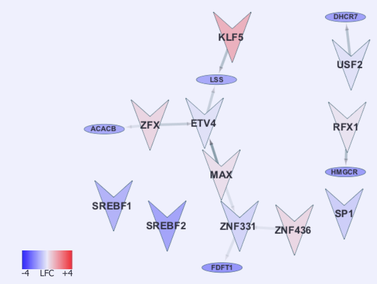
**Boron induced cell cycle arrest might be through downregulation of DNA replication complex**

- **ZFX;** a transcription factor important in cell proliferation, targets many of the genes in DNA replication complex.




**SREBF pathway genes in lipid biosynthesis are significantly enriched in the Boron-induced subnetwork.**

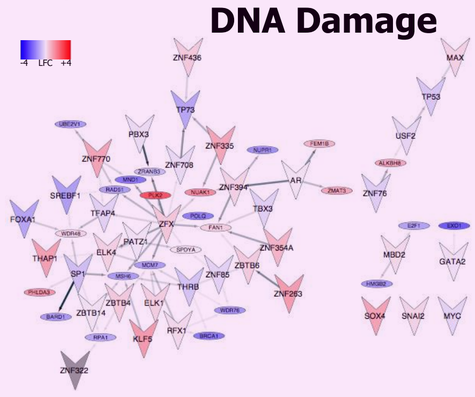
- The genes are known regulatory targets of SREBF1 and SREBF2, however, interactions of the genes with these TFs are not observed in current network.
- TF-gene relations might be overfiltered.



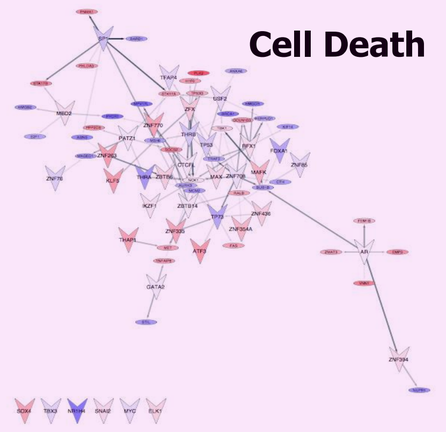

**Genes involved in DNA damage response and cell death share many TFs as their regulators.**

- DNA damage response genes are mostly downregulated.
- Cell death genes are mostly upregulated.

**DNA Damage**

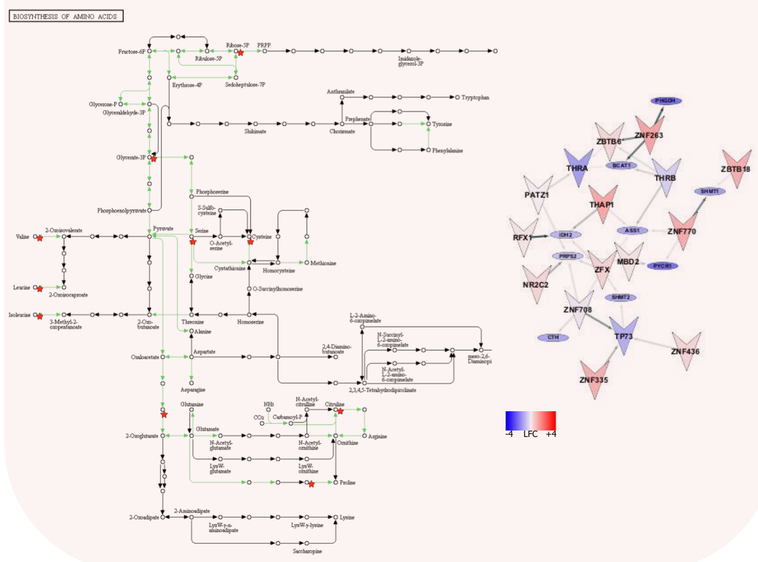



**Cell Death**



**Amino acid biosynthesis pathway is downregulated at a wide scale**

- Genes involved in amino acid biosynthesis are down-regulated at different points within the metabolic pathway.
- Many of the regulators in amino acid metabolism also target DNA replication complex.




## CONCLUSIONS / FUTURE STUDIES

- Boric acid induced cellular mechanisms were investigated via regulatory network modelling.
- According to preliminary results, boric acid at $IC_{50}$ level triggers a plethora of TF-gene interactions which might be responsible in boric acid related expression profile.
- In particular boric acid treatment at $IC_{50}$ concentration leads to a halt in cell cycle progression accompanied by hampered amino acid and lipid metabolism as well as activation of apoptotic genes. According to the network model different cellular events observed in boric-acid related gene profile might be affected by a common group of transcription factors.
- X-linked Zinc finger protein (ZFX) seems to be a central regulator in many boron treatment related outcomes. In cancer cells, ZFX is reported as a regulator involved in adjusting the balance between cell proliferation and apoptosis[11]. Herein ZFX is potentially involved in down-regulation of proteins in DNA replication complex assembly leading to cell cycle arrest and inhibition of cell growth. Some down-regulated genes in amino acid and lipid metabolism are also targetted by ZFX.
- Additional microarray studies are needed to increase the statistical power in network analysis and to understand boric acid specific mechanisms at lower doses. Hypotehetical TF-gene relations need to be validated with in vitro assays.

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