Studying the effect of natural genetic variation on protein abundance in *C. elegans*

Kapil Dev Singh[1], Bernd Roschitzki[2], Mark Elvin[3], Gino Poulin[3], Basten Snoek[4], Jan E. Kammenga[4], Sabine Schrimpf[1], Michael Hengartner[1]

[1] Institute of Molecular Life Sciences, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
[2] Functional Genomics Center Zurich, University of Zurich and ETH Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
[3] Faculty of Life Sciences, The University of Manchester, Oxford Road, Manchester, M13 9PL, UK
[4] Laboratory of Nematology, Wageningen University, 6708 BP Wageningen, The Netherlands

University of Zurich[UZH]

uzh | eth | zürich
Zurich Ph.D. Program in Molecular Life Sciences

Abstract

Complex diseases are caused by a combination of genetic, environmental as well as lifestyle factors, and are mostly polygenic in nature. To study the influence of natural genetic variation on the development of complex diseases, we are using *Caenorhabditis elegans*, a model organism that has orthologs of many human disease genes. We focus on cancer signaling pathways (Apoptosis, Notch, MAPK and Wnt). From the two highly divergent wild-type strains - Bristol N2 and Hawaii CB4856, recombinant inbred lines (RILs) were generated. Transcriptome analysis of these RILs showed significant heritable variation in gene expression, but very little is known about variation at the protein level. We used Selected Reaction Monitoring (SRM) to determine the effect of natural variation on protein abundance. Our results indicate that for signaling pathways transcriptome variation tends to be higher than proteome variation.

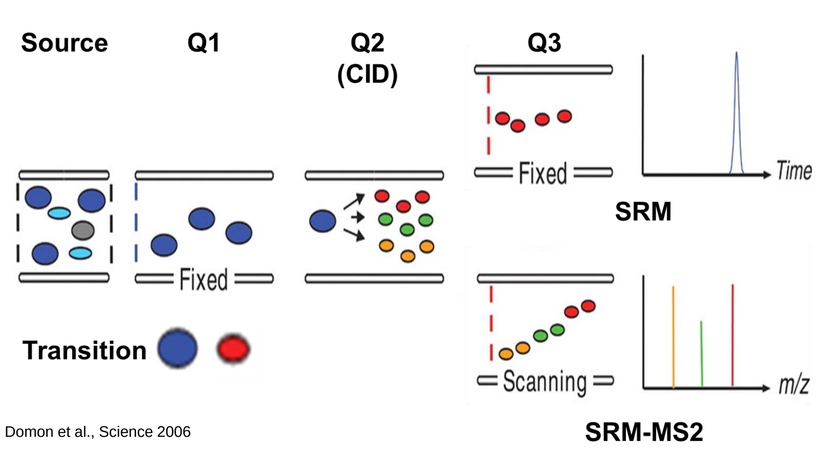

Figure 1: Schematic representation of SRM and SRM-MS2 experiments

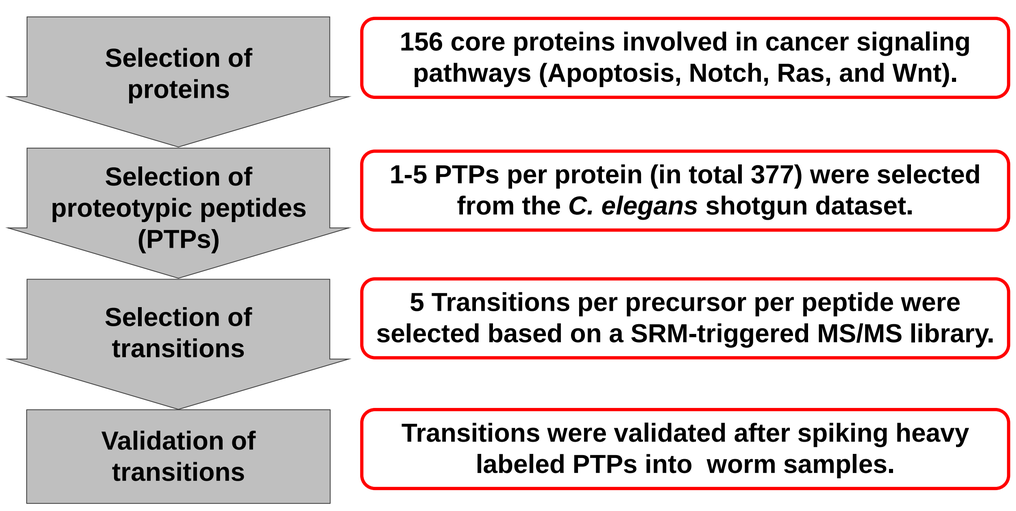

Figure 2: Workflow for SRM method development

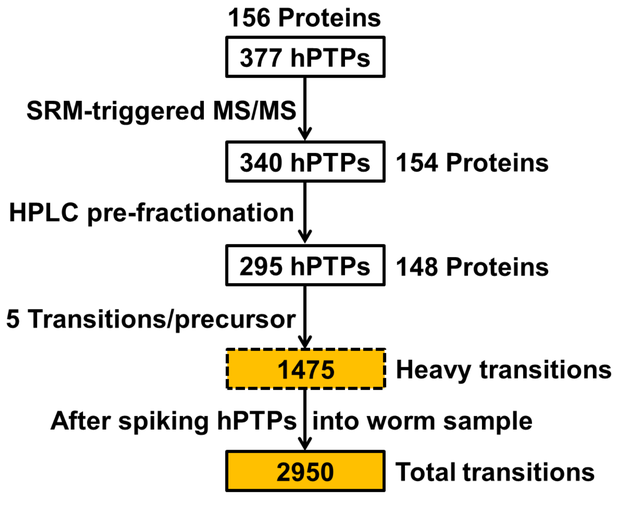

Figure 3: Number of transitions

Heavy labeled (13C and 15N at C-terminal Arginine and Lysine) proteotypic peptides [hPTPs, from JPT technologies, Berlin] were used as internal standards for SRM. SRM-triggered MS/MS measurements resulted in identification of 356 (94.4 %) peptides with 3.3 % FDR at peptide level, and a consensus MS/MS spectra library for 340 (90.2 %) peptides.

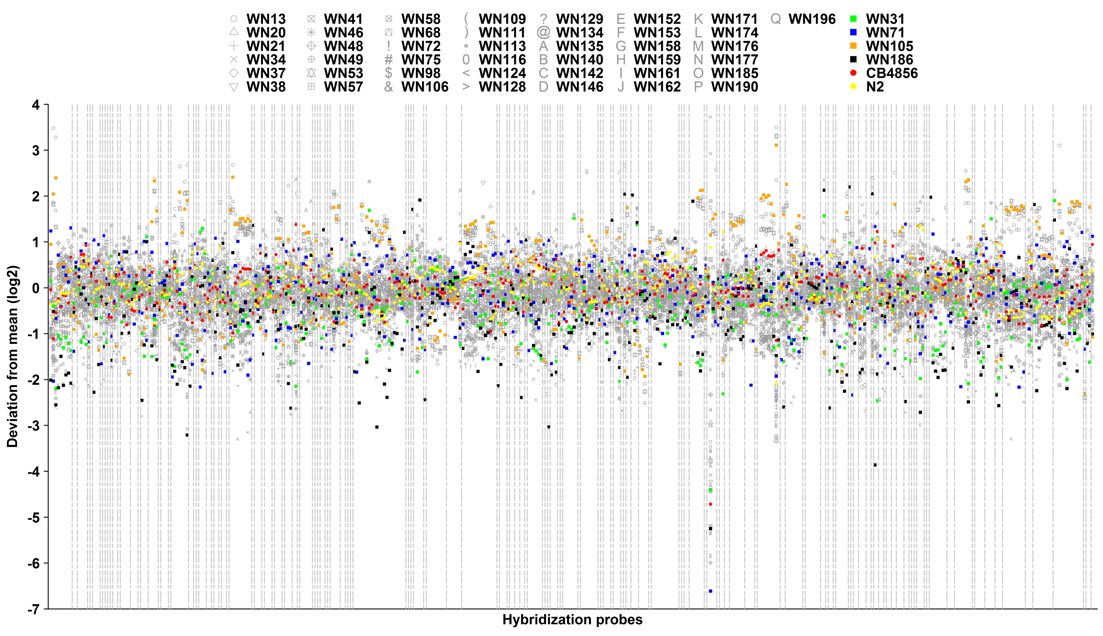

Figure 4: Selected RILs have more transcriptome level variation than parents. **Gene expression was quantified by two color microarray using 413 different hybridization probes (representing 148 genes of interest). Log2 scaled deviation of the probe expression value to the mean of probe expression values across all samples indicate the variation in expression. Vertical dashed-lines separate probes of one gene from another. Four RILs (WN31, WN71, WN105 and WN186), were empirically selected for initial experiments.**

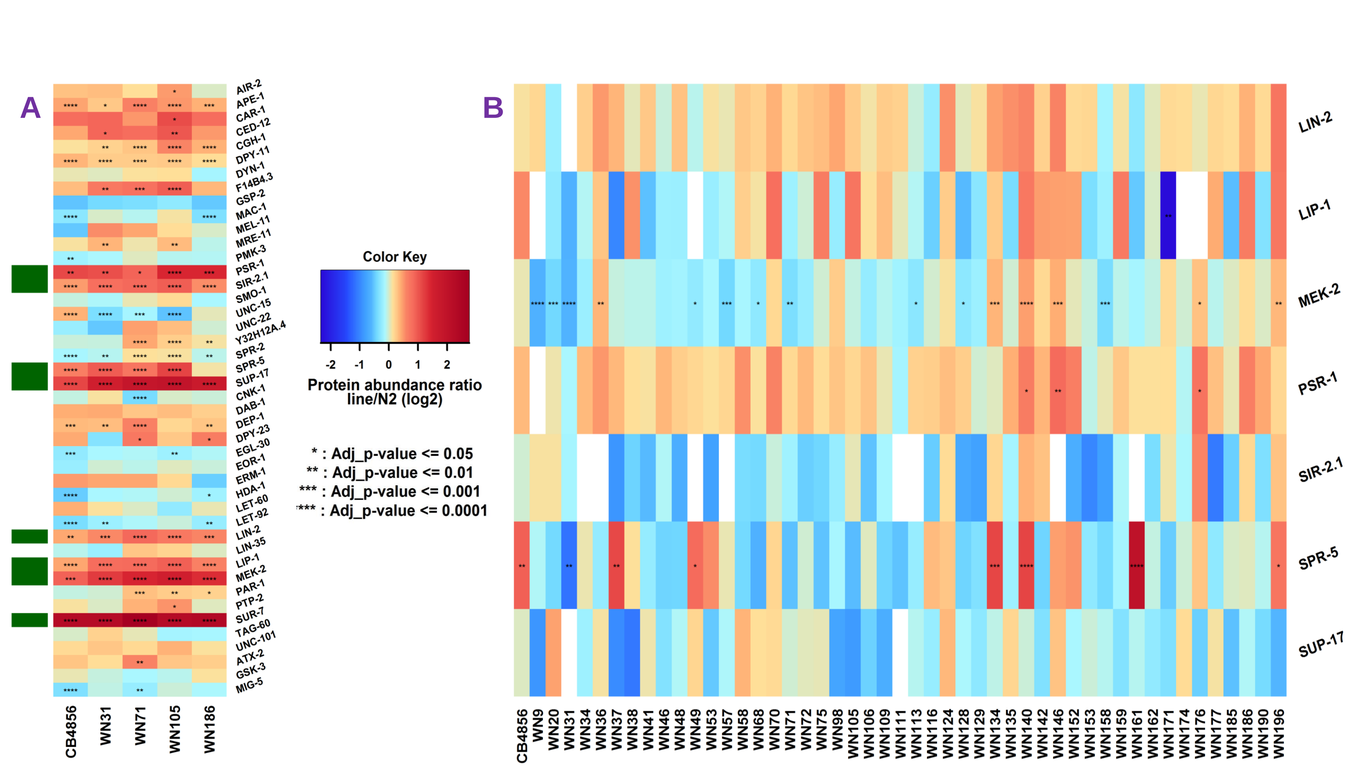

Figure 5: Targeted protein tends to be up-regulated in RILs compared to N2. **Protein abundance was quantified by SRM. Data analysis was performed using mProphet software, followed by protein significance analysis using R-package MSstats. (A) Differential abundance of 44 selected proteins in CB4856 and selected RILs relative to N2. Green bands on left side indicate proteins selected for QTL mapping. (B) Differential abundance of 7 selected proteins in CB4856 and 48 RILs relative to N2.**

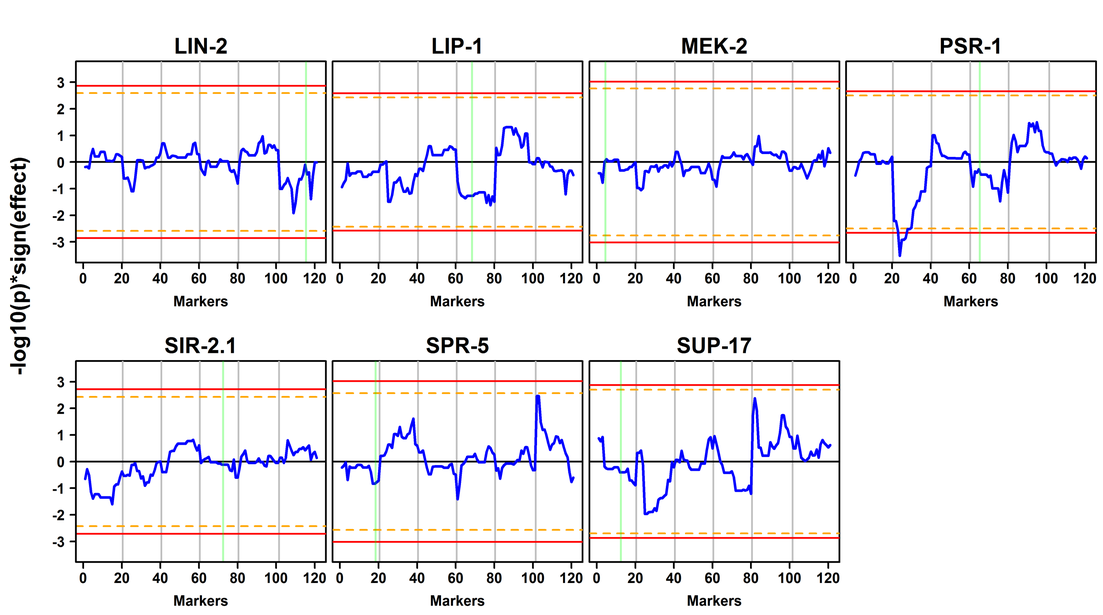

Figure 6: Selected proteins shows no significant QTL. **Positive direction of blue curve indicates the effect of N2 alleles, whereas negative direction indicates effect of CB alleles. Horizontal orange and red lines are 0.1 and 0.05 FDR threshold respectively. Vertical grey lines separate chromosomes each with 20 markers. Light green vertical bands indicate position of the genes.**

Results

From our initial experiment with parents (N2 and CB4856) and four RILs (WN31, WN71, WN105 and WN186) we were able to quantify between 71 and 116 proteins. 44 proteins could be quantified in all samples. These 44 proteins (represented by 114 PTPs) were measured additionally in 3 biological replicates of the above mentioned 6 lines (Figure 5A). Most of the proteins were non-significant and/or below fold-change cutoff, but 8 proteins (LIN-2, LIP-1, MEK-2, PSR-1, SIR-2.1, SPR-5, SUP-17 and SUR-7) showed significant differential expression. These 8 proteins (represented by 15 PTPs) were measured additionally in one sample of 48 RILs (Figure 5B) to perform protein quantitative trait locus (QTL) mapping. Only for PSR-1 we found significant weak trans-QTL on chromosome 2 (Figure 6).

Conclusions

Based on our data we conclude that protein levels of the analyzed signaling pathway are under stronger evolutionary control than transcript levels.

Funding:

EU-FP7

SEVENTH FRAMEWORK PROGRAMME

PANACEA contract nr 222936
http://www.panaceaproject.eu

PANACEA

Acknowledgments

This project is a part of an EU-FP7 funded collaborative project between six laboratories (at the universities of Groningen, Wageningen, Zurich, Manchester and Microsoft Research Cambridge) in three countries (Netherlands, Switzerland, U.K.) entitled 'Quantitative pathway analysis of natural variation in complex disease signaling in *C. elegans* (PANACEA)'.

All mass spectrometric and HPLC experiments were performed at the Functional Genomics Center Zurich (FGCZ).