Challenges and opportunities for modeling signal transduction pathways using immunohistochemistry data

at a multi-species approach to study the impact of mild stress on healthy ageing (http://www.jenage.de).
Towards this goal we set out to systematically characterize the changes of transcriptomes for five species ranging fromCaenorhabditis elegansover two fish models and mice to man at different ages. Data have been acquired by RNA-seq using the Solexa/Illumina platform. First results characterizing the process of normal ageing within and between species by fuzzy c-means clustering, gene set enrichment analysis, machine learning of non-linear models by induction of decision trees as well as correlation network inference will be presented.
As a proof of principle, we identified genes that were found to be uniformly up- or down-regulated with age inC. elegans, zebrafish and mouse. Next, worms were cultivated on bacteria containing candidate-specific RNAi constructs and lifespan was determined. Remarkably, the knock-down of the majority of the candidate genes led to alterations of lifespan inC. elegans, thus underscoring the suitability of our approach.

P2.19: In silico evidence for gluconeogenesis from fatty acids in humans

C. Kaleta[1], L. F. de Figueiredo[2], S. Schuster[2]
[1]*University of Jena, Research Group Theoretical Systems Biology, Jena, Germany*
[2]*University of Jena, Department of Bioinformatics, Jena, Germany*

Question: The question whether even-chain fatty acids can be converted into glucose in humans has been a topic of intense debate until the mid of the last century. In particular, a potential link to diabetes has spurred this discussion. Since the discovery of the glyoxylate shunt that allows this conversion in bacteria, plants, fungi and nematodes but is absent in higher animals, the expected answer to the question whether gluconeogenesis from even-chain fatty acids is possible in humans is "No".
Methods/Results: Using recent advances in Systems Biology in the form of large-scale metabolic reconstructions of humans that encompass the entire set of metabolic reactions known to take place in humans, we reassessed this question. By utilizing elementary flux pattern analysis, a tool for pathway discovery in genome-scale metabolic network, we found numerous pathways on which gluconeogenesis from fatty acids is feasible in humans and other mammals. On these pathways, four moles of acetyl-CoA are converted into one mole of glucose and two moles of CO2. Analyzing the detected pathways in detail we found that their energetic requirements potentially limit their capacity.
Conclusion: Results from our work have manifold applications for the understanding of the nutritional state of mammals during times of limited carbohydrate supply as it occurs during starvation, prolonged exercise, upon carbohydrate free diets as this of the inuit as well as during hibernation. In particular, an improved understanding of how humans can survive with a practically carbohydrate-free diet as it is traditionally consumed by natives of the arctic regions is of high relevance. Moreover, the energetic loss associated to the usage of gluconeogenesis from fatty acids can help explain the efficiency of carbohydrate reduced and ketogenic diets such as the Atkins diet.

References

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P2.20: Application of identifiability-based model refinement to analyze the function of SHP-2 in early IL-6-induced Jak/STAT signaling

A. Dittrich[1], T. Quaiser[2], M. Mönnigmann[2], F. Schaper[1]
[1]*Otto-von-Guericke-University Magdeburg, Department of Systems Biology, Magdeburg, Germany*
[2]*Ruhr University Bochum, Automatic Control and Systems Theory, Bochum, Germany*

Question: Deregulated interleukin-6 (IL-6)-induced Jak/STAT signaling is found in many inflammatory and proliferative diseases. Under non-pathological conditions the activation and duration of Jak/STAT signaling is tightly controlled by a complex network of regulators. One of these proteins is the tyrosine phosphatase SH2-containing phosphatase (SHP2). Although SHP2 is known to be a negative regulator of IL-6-induced Jak/STAT signaling, its exact molecular function is still undefined. To elucidate the role of SHP2 in IL-6 signaling and to gain further insights into the dynamics of early IL-6-induced signaling we followed a systems biology approach, in which modeling and experimental analyses are closely linked.
Methods: Starting with a detailed, yet unidentifiable model of the IL-6-induced Jak/STAT signaling we refined the model by an iterative process of
1) generating quantitative biochemical data,
2) estimating parameters of the actual model using a multi-start approach,
3) ranking the parameters based on their identifiability,
4a) using 2) and 3) to guide model refinement or
4b) defining more informative biochemical experiments to be executed in 1) using optimal experimental design.
The quantitative biochemical experiments covered the analysis of the stoichiometry and the dynamics of early IL-6-induced Jak/STAT signaling in wild type and SHP2 knockdown cells with Western blots, FACS analysis and ELISA.
Results: We derived an identifiable model of early Jak/STAT signaling that adequately describes the data and proved to be predictive. The identifiability-based model refinement implied that 1) the stepwise assembly of the IL-6-receptor complex, 2) the dimerization of STAT3 at the receptor, and 3) a nonlinear phosphorylation kinetic of SHP2 are essential for the dynamics of early IL-6-induced signal transduction. Moreover, we found that SHP2 does not act as early feedback inhibitor of IL-6-induced Jak/STAT signaling. However, experimental data revealed that SHP2 may act as repressor of cytokine-independent basal receptor activity.
Conclusion: Generating quantitative biochemical data together with the application of identifiability-based model refinement indicates that the phosphatase SHP2 is not a feedback inhibitor of early IL-6-induced Jak/STAT signaling. However, SHP2 seems to serve as a basal repressor of spontaneous receptor activation in the absence of IL-6. Hence, SHP2 might have an essential function in the repression of constitutive cytokine-independent Jak/STAT pathway activation, which is associated with chronic inflammatory diseases and tumor development.

P2.21: Challenges and opportunities for modeling signal transduction pathways using immunohistochemistry data

C. Colmekci Oncu[1], J. Vanlier[1], P. A. J. Hilbers[1]
N. A. W. van Riel[1]
[1]*Eindhoven University of Technology, Biomedical Engineering, Eindhoven, Netherlands*

Question: Modeling signal transduction pathways is challenging and accurate quantification of experimental data is an essential prerequisite for parameterization of such models. Western blotting is widely used to measure proteins and their phosphorylation levels. However, Western blotting is laborious and results in data with a low signal-to-noise ratio. On the other hand immunohistochemistry techniques with automated fluorescent imaging provide possibilities for a higher signal-to-noise ratio, a higher time resolution to capture the dynamics and also determine the localization of the (phosphorylated) proteins. Image analysis is crucial and challenging for quantification of the fluorescence. Moreover,

it is important to know how the biological variability and measurement noise associated with immunohistochemistry fluorescence imaging can be statistically described. The error model will determine which mathematical function should be used as likelihood function in model fitting to provide accurate parameter estimates (maximum likelihood estimation).
Methods: We consider mathematical models of signal transduction pathways consisting of a set of ordinary differential equations and parameters that need to be parameterized with quantified experimental data. In this study, immunohistochemistry data is being used to parameterize a mathematical model of the insulin signaling pathway. The data set consists of the frequently sampled time courses of phosphorylation of multiple signaling cascade intermediates. Fluorescence images are quantified based on signal intensity distributions. The error model will be used to account for systematic sources of error in experimental data and develop an appropriate estimator.
Results: Intensity based quantification provides a distribution of measurements/protein phosphorylation which allows us to construct an error model to represent the experimental error and biological variability. The error model provides the means to be able to parameterize a mathematical model based on immunohistochemistry data which can be obtained at high temporal resolution. For additive and normally distributed errors, the sum of square errors weighted by the data variance can be used to parameterize the model. Else a suitable estimator will be developed.
Conclusion: Preliminary results showed that intensity based quantification of fluorescent images obtained by immunehistochemistry techniques, has a high potential for high-throughput collection of signal transduction data that can be used in systems biology modeling. Developing adequate error models is critical to adequately propagate uncertainty in the experimental data to uncertainties in model parameter values and predictions.

P2.22: Xenobiotic -Induced Signaling Influences Physiolo-gically Relevant Pathways

S. Trump[1], S. Rudzok[1], J. Michaelson[2], A. Beyer[3], I. Lehmann[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Environmental Immunology, Leipzig, Germany*
[2]*University of California, San Diego, Dept. of Psychiatry, San Diego, United States*
[3]*Biotec, TU Dresden, Cellular Networks & Systems Biology, Dresden, Germany*

Question: How does xenobiotic-induced cellular signaling influence physiologically relevant pathways?
The aryl hydrocarbon receptor (*Ahr)* is an evolutionary highly conserved transcription factor (TF). Originally, Ahrwas discovered because of its role in activating phase I and II metabolization following exposure with xenobiotic compounds. A wide range of different environmental pollutants has been described to bind and thereby activate the *Ahr*. This initiates the translocation of the receptor to the nucleus, where it binds in the promoter region of specific genes subsequently altering their transcription. Since among these genes are those coding metabolizing enzymes, it is a common phenomenon that *Ahr*-ligands are converted to toxic metabolites.
Therefore, monitoring global differential gene expression upon activation of *Ahr* is comprised of a complex mixture of a direct TF-dependent response and possible side effects. The direct *Ahr*-dependent effect is likely to include the activation of other TFs, consecutively initiating a transcriptional cascade. To decipher the complex process ofAhrligand activation and its influence on transcriptional regulation, we carried out genome-wide profiling of *Ahr* DNA binding complemented with chromatin state and gene expression measurements. These data provided the basis for deciphering an *Ahr* initiated transcriptional cascade influencing pathway far beyond detoxification processes.
Methods: Transcriptional changes caused by benzo(a)pyrene (BaP) activatedAhrwere observed using exon arrays (Affymetrix). Significant genes were identified by 2-way ANOVA. Based on Random Forests, primaryAhrtargets were separated from genes regulated due to secondary effects. *Ahr*-ChIP-Seq and -DNase1-Seq were carried out for control and BaP stimulated samples.Ahrbinding and DNase1 accessibility sites were determined on a genome-wide scale and putative TF motifs were identified. Connections between the primary and secondary response were derived from meta- and literature/database- analysis.
Results: Separating primaryAhrtargets from the secondary transcriptional response enabled us to identify a transcriptional network initiated by *Ahr* activation. Several TFs were among the direct target genes of *Ahr* involved in different cellular pathway like oxidative stress, differentiation or hormone signaling. Complementing our transcriptional data, ChIP-Seq motif analysis identified so far unknown consensus sequences related toAhractivation.
Conclusion: In conclusion, the involvement of *Ahr* in cellular signaling goes further than its role in detoxification. Our results point to a far broader role of *Ahr* in physiological and pathophysiological processes than previously known.

P2.23: Whole genome array analysis of cultured mouse hepatocytes identifies a JNK pathway mediated Acute Phase Response (APR) signature

A. Widera[1], W. Schmidt-Heck[2], G. Campos[1], K. Rochlitz[1] R. Guthke[2], N. Blüthgen[3], S. Dooley[4], R. Gebhardt[5] J. G. Hengstler[1], P. Godoy[1]
[1]*Leibniz Research Centre for Working Environment and Human Factors, Toxicology, Dortmund, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute (HKI), Jena, Germany*
[3]*Charite - Universitätsmedizin Berlin, Institut für Pathologie, Berlin, Germany*
[4]*Medical Clinic Faculty of Medicine at Mannheim, Heidelberg, Germany*
[5]*Institute of Biochemistry, Medical Faculty, University of Leipzig, Leipzig, Germany*

Background: The liver is an organ acting as a first line of defense against a number of pathological conditions including sepsis via an acute phase response (APR). Primary hepatocytes are a fundamental tool to fully understand the complex circuitry of signal transduction and gene regulation in APR. However, it is known that primary hepatocytes undergo a dedifferentiation process, i.e., apoptotic resistance and epithelial to mesenchymal transition. These alterations are being partly repressed in a 3D extracellular matrix (e.g. collagen sandwich, CS). In order to fully understand the process of dedifferentiation and its influence in inflammatory responses, we performed a transcriptome analysis of cultivated mouse hepatocytes comparing it to that of freshly isolated hepatocytes and liver tissue.
Methods and Results: Whole genome array analysis of hepatocytes in CM (sub-confluent and confluent) and CS cultures through a 7 day period, using freshly isolated hepatocytes as reference, revealed that a signature of genes typically induced during an APR was observed in all culture systems (e.g. SAA3, Cebp/d, Lcn2). qPCR and Western blot analysis confirmed the identified de-regulated genes. The extent of inflammatory features in vitrowas compared to an *in vivo* model of sterile inflammation (CCl_4 I.P). Spearman analysis showed a strong correlation between all culture systems with the *in vivo* model of inflammation, particularly at 24h after CCl_4 injection with CM cultures having the highest correlation (r=0,623; p-value=0), whereas CS had a lower but highly significant correlation (r=0,436; p-value=0). Additionally, loss of function features such as down regulation of HNF4 and bile salt metabolism gene clusters were observed in CCl_4 inflammed mouse liver *in vivo* and in cultivated hepatocytes. By screening a series of pathway inhibitors, we were able to show that JNK is a main player causing such inflammatory signatures.
Conclusions: Isolation and culture of primary mouse hepatocytes activates an inflammatory response similar to that of sterile inflammation *in vivo*, which is partly mediated by the JNK pathway.