Jena School for Microbial Communication

JENA CENTRE FOR BIOINFORMATICS

JSMC/JCB Workshop

'Microbes, Models & Methods'

March 9 – 10, 2010

University Main Building, Fürstengraben 1, Jena
Main Lecture Hall "Aula"

with the Graduate Research School in Genomic Ecology (GENECO), University of Lund, Sweden

furthered by the Beutenberg Campus e.V.

Friedrich-Schiller-Universität Jena
seit 1558

JSMC
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Tuesday, March 9, 2010

9:00 **Brief Introduction GENECO, JCB, and JSMC**
Karin Rengefors, Stefan Schuster, Axel Brakhage

9:45 **Douwe Molenaar**, Vrije Universiteit Amsterdam, The Netherlands
"The physical limits that shape life"

vrije Universiteit amsterdam

10:15 **Jörg Linde**, Research Group Systems Biology/Bioinformatics, Hans Knöll Institute
"Regulatory network modelling of iron acquisition by a fungal pathogen during oral Infection"

10:35 Coffee Break

11:05 **Ekaterina Shelest**, Research Group Systems Biology/Bioinformatics, Hans Knöll Institute
"Genome mining for transcription factors and secondary metabolite producers in fungi"

11:30 **Michael Weber**, Reinhard Guthke, Hans Knöll Institute, **Raimund Kinne,** Experimental Rheumatology Unit, FSU Jena
"Network inference from transcriptome monitoring of the response of synovial fibroblasts from rheumatoid arthritis and osteoarthritis patients to TNF-alpha and TGF-beta "

12:00 Lunch Break

13:00 ***Poster Session*** *(University Main Building, Foyer 1st+2nd floor)*

14:00 **Jürgen Sühnel**, Leibniz Institute for Age Research, FLI Jena
"*GenColors: annotation and comparative genomics of prokaryotes made easy*"

14:25 **Sarah Werner**, Department of Bioinformatics, FSU Jena
"A complex model of the communication processes between Mucorales*"*

14:45 **Sabine Brantl**, Institute of Microbiology, FSU Jena
"*Small regulatory RNA SR1: The first dual-function sRNA from* Bacillus subtilis"

15:10 **Thomas Schulze**, Institute of General Botany and Plant Physiology, FSU Jena
"*Functional characterization of the eyespot protein SOUL3 in* Chlamydomonas reinhardtii"

15:30 Coffee Break

16:00 **Karin Rengefors**, Department of Ecology/Limnology, Lund University, Sweden
"Processes generating biogeographic patterns in microorganisms: local adaptation and genetic divergence in Antarctic aquatic protists (Dinophyceae)"

16:30 **Catherine Legrand,** Marine Ecology Group, Linnaeus University Kalmar, Sweden
"Impact of algal allelochemicals in algal-bacteria interactions"

17:00 **Georg Pohnert,** Institute of Inorganic and Analytical Chemistry, FSU Jena
"Chemical communication in plankton revisited: New high throughput analytical methods reveal complex release patterns of metabolites from phytoplankton"

17:25 ***Poster Session with refreshments***

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Wednesday, March 10, 2010

9:00 **Anders Tunlid**, Department of Microbial Ecology, Lund University, Sweden
"*How to kill a nematode – interaction between nematode-trapping fungi and nematodes*"

9:30 **Jure Piškur,** Department of Cell and Organism Biology, Lund University, Sweden
"*Formation of novel chromosomes as a virulence mechanism in yeast* Candida glabrata"

10:00 **Dirk Hoffmeister**, FSU Jena, Associated Department for Pharmaceutical Biology, Hans Knöll Institute
"*Pigment formation in boletoid homobasidiomycetes"*

10:25 ***Coffee Break***

10:50 **Kerstin Voigt,** Fungal-Reference-Center Jena, FSU Jena
"*Ups and Downs in the finalization of a comprehensive fungal phylogeny"*

11:15 **Erika Kothe,** Institute of Microbiology, FSU Jena
"Microbial communities at heavy metal contaminated sites"

11:40 **Dennis Görlich, Peter Dittrich**, Institute of Computer Science, Bio Systems Analysis Group, FSU Jena
"*Accessing the semantic level of cells"*

12:10 **Udo Hahn**, Jena University Language & Information Engineering Lab (JULIE-Lab)
"Biomedical text mining: Extraction of entities and relations"

12:35 ***End (+ another (informal) opportunity to look at the posters)***

Guests are cordially welcome!

Contact

Jena School for Microbial Communication
Phone: 03641-9-30421
Email: JSMC@uni-jena.de
Web: www.jsmc.uni-jena.de

Jena Centre for Bioinformatics
Phone: 03641-9-49584
Email: margit.leitner@uni-jena.de
http://www.jcb-jena.de/

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List of Posters 1/3

1. **Dittrich, Peter,** Institute of Computer Science, FSU Jena
   "*Bio Systems Analysis Group - Current Research*"
2. **Görlich, Dennis**, Institute of Computer Science, FSU Jena
   "Accessing the semantic level of cells"
3. **Grünert, Gerd**, Institute of Computer Science, FSU Jena,
   "Rule-based modelling in space"
4. **Münchberg, Ute**, Institute for Physical Chemistry, FSU Jena
   "Identification of fungal spores with micro-Raman spectroscopy"
5. **Vogler, Nadine**, Institute of Photonic Technology e.V., FSU Jena
   "Multimodal imaging for investigating morphochemistry of tissue samples"
6. **Walter, Angela**, Institute of Physical Chemistry, FSU Jena
   "Investigation of soil microbes by means of Raman spectroscopy in combination with statistical analysis"
7. **Kemmler, Michael**, Department of Mathematics and Computer Science, FSU Jena
   "Classification of microorganisms via Raman spectroscopy using Gaussian processes"
8. **Schumacher, Wilm**, Institute of Physical Chemistry, FSU Jena
   "Chemometric techniques for identification of bacteria"
9. **Mai, Juliane**, Helmholtz Centre for Environmental Research - UFZ, Leipzig
   "Analysis of spatio-temporal dynamics by FRAP data"
10. **Faisal, Saadia**, Helmholtz Centre for Environmental Research - UFZ, Leipzig,
    "Reverse engineering of gene networks by multilinear polynomials"
11. **Bodenstein, Christian**, Department of Bioinformatics, FSU Jena
    "Calcium Oscillations and Jensen's Inequality"
12. **Böcker, Sebastian and Hufsky, Franziska,** Department of Bioinformatics, FSU Jena
    "From center gene cluster computation to center string problem"
13. **Bohl, Katrin**, Department of Bioinformatics, FSU Jena
    "Mutualistic interactions among amino acid exchanging bacteria studied by evolutionary game theory"
14. **de Figueiredo, Luis F.**, Department of Bioinformatics, FSU Jena
    "*Computing metabolic pathways with K-shortest EFM*"
15. **Franke, Mathias**, Leibniz Institute of Plant Genetics and Crop Plant Research, AG Systembiologie, Gatersleben
    "Steady-state 13C metabolic flux analysis of developing crop seeds"
16. **Reinicke, Martin**, Institute of Microbiology, FSU Jena
    "Adaptation of microbial cells and communities to heavy metal contamination"

List of Posters 2/3

17. **Schindler, Frank**, Institute of Microbiology, FSU Jena
"Bioremediation and bioimmobilization"
18. **Burkhardt, Eva-Maria**, Institute of Microbiology, FSU Jena
"Interaction of Fe(III)-reducing bacteria with heavy metals in a contaminated soil"
19. **Madhavan, Soumya**, Institute of Microbiology, FSU Jena
"Genetic manipulation of Schizophyllum commune *for investigating fungal influence on weathering"*
20. **Seifert, Anne-Gret**, Institute of Microbiology, FSU Jena
"Contribution of different microbial groups to black shale degradation"
21. **Boisselet, Tsilla**, Institute of Geosciences, FSU Jena
"*Biological factors influencing heavy metal mobility through analysis of REE fractionation patterns"*
22. **Wadke, Namita**, MPI for Chemical Ecology, Jena
"Functional genomics of the bark beetle associated fungus Ceratocystis polonica*"*
23. **Wackler, Barbara**, Department Pharmazeutische Biologie at the Hans-Knöll-Institute, FSU Jena
"Secondary metabolites of Ralstonia solanacearum*, a bacterial phytopathogen"*
24. **Schäuble, Sascha,** Department of Bioinformatics, FSU Jena
"Modelling changes in amino acid metabolism during day-night cycles"
25. **Kötzing, Martin; Kaleta, Christoph; Bartl, Martin; Schuster, Stefan**, Department of Bioinformatics, FSU Jena, in cooperation with Ilmenau University of Technology
"Just in time activation of a metabolic pathway under consideration of enzyme synthesis"
26. **Bettenbrock, Katja; Kremling, Andreas; Jahreis, Knut; Rinas, Ursula; Schuster, Stefan; Pfaff, Michael; Guthke, Reinhard,** Hans-Knöll Institute, Jena, Research Group Systems Biology / Bioinformatics, FORSYS-Partner Project
"Dynamics and regulation of the metabolic balance in Escherichia coli*"*
27. **Schmidt-Heck, Wolfgang; Zellmer, Sebastian; Bauer, Alexander; Hengstler, Jan; Gebhardt, Rolf; Guthke**, **Reinhard,** Hans-Knöll Institute, Research Group Systems Biology/Bioinformatics Jena
"Network inference applications for cultivated hepatocytes"
28. **Jaradat, Sameh**, Department of Dermatology, University Hospital Jena
"Investigation of B-defensin genes copy number association with skin fungal infections and periodontal diseases"
29. **Lüttich, Anja**, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Jena
"Microevolution of Candida albicans *and* C. glabrata *during co-incubation with immune cells"*

List of Posters 3/3

30. **Heiland, Ines, Hinze, Thomas, Voytsekh, Olga, Mittag, Maria, Schuster, Stefan**, Department of Bioinformatics, and Institute of General Botany and Plant Physiology, FSU Jena
"Temperature regulation of circadian rhythms"
31. **Schau, Benedict; Hinze, Thomas; Lenser, Thorsten; Heiland, Ines; Schuster, Stefan,** Department of Bioinformatics, FSU Jena
"Control system-based reverse engineering of circadian oscillators"
32. **Vidoudez, Charles; Pohnert, Georg**, Institute of Inorganic and Analytical Chemistry, FSU Jena
"A growth phase specific release of polyunsaturated aldehydes by the diatom Skeletonema marinoi*"*
33. **Barofsky, Alexandra; Paul, Carsten; Pohnert, Georg,** Institute of Inorganic and Analytical Chemistry, FSU Jena
"High variability of the exo-metabolome of intact diatoms suggests possible release of infochemicals"
34. **Schmid, Daniela**, Department of Bioorganic Chemistry, MPI for Chemical Ecology
"Sex-related proteins of the brown alga Scytosiphon lomentaria *– bioinformatic tools to compare two proteomes"*
35. **Härter, Andrea; Melzer, Rainer; Theissen, Günter,** Department of Genetics, FSU Jena
"Floral homeotic protein interactions in basal angiosperms: from fading borders to fuzzy interactions"
36. **Wang, Yong-Qiang; Melzer, Rainer; Theißen, Günter**, Department of Genetics, FSU Jena
"Evolutionary origin of 'Floral Quartets' as revealed by molecular interactions"
37. **Eckart, Martin; Hoffmann, Kerstin**, Fungal Reference Center, FSU Jena
"Single locus phylogenies on anaerobic fungi (Neocallimastigomycota)"
38. **Hoffmann, Kerstin; Wagner, Lysett**, Fungal Reference Center, FSU Jena
"Zygomycetes revisited: A phylogenetic revision of Mortierella*"*
39. **Böcker, Sebastian , Griebel,Thasso , Hüffner, Falk , Truss, Anke , Wahlström, Magnus,** Department of Bioinformatics, FSU Jena
"Comparing phylogenetic trees via tanglegrams"
40. **Griebel, Thasso; Brinkmeyer, Malte; Böcker, Sebastian**, Department of Bioinformatics, FSU Jena
"EPoS - A modular software framework for phylogenetic nalysis"
41. **Huehne, R.; Suehnel, J.,** Biocomputing Group, Leibniz Institute for Age Research - Fritz Lipmann Institute, Jena
"Jena3D - A Versatile Jmol-based 3D Structure Viewer"

Abstracts – Talks

(in order according to the program)

The physical limits that shape life

Douwe Molenaar, Pinar Öztürk, Evert Bosdriesz

Systems Bioinformatics, Vrije Univeriteit Amsterdam, The Netherlands

Evolution is a force that, to an unknown extent, seems to optimize organisms for certain tasks. This effect can, for certain phenomena, be used to explain characteristics of organisms in terms of fitness maximization. The success of the application of optimization principles in biology depends on the knowledge of the fitness, or objective functions, but also on the knowledge of the trade-offs that play a role in the organism. For bacteria, we assume that the growth rate is an important fitness component. Using this assumption, and taking into account a minimal number of constraints concerning the physical and biochemical limits of the material that life is made of, it is possible to predict certain characteristics of microorganisms. We used such an approach to predict shifts in central metabolic pathways (1), but also to predict actual the actual growth rate and size of a bacterium with the metabolic make-up similar to an *Escherichia coli*. For that, we built modular models of self-replicating entities that, we hope, grasp just enough of the complexity of the problem to understand the observed behavior.

(1) Molenaar, D. et al. (2009) Mol. Syst. Biol. 5:323

Regulatory network modelling of iron acquisition by a fungal pathogen during oral infection

Jörg Linde[1], Duncan Wilson[2], Bernhard Hube[2], Reinhard Guthke[1]

(1) Research Group: Systems Biology / Bioinformatics, Leibniz-Institute for Natural Product Research and Infection Biology-Hans-Knoell-Institute, Jena
(2) Department Microbial Pathogenicity Mechanisms, Leibniz-Institute for Natural Product Research and Infection Biology-Hans-Knoell-Institute, Jena

Candida albicans is one of the most important human fungal pathogens. Iron is an essential mineral required as cofactor for several proteins, as well as for a number of biochemical processes. However, within the human host, iron is bound to storage proteins, making the acquisition of this mineral an important virulence attribute of most pathogens. This is reflected by the *C. albicans* genome containing more iron acquisition genes than that of the non-pathogenic relative, *Saccharomyces cerevisiae,* and colonization, as well as proliferation, are only possible if sufficient amounts of this mineral are accessible[1].

I*n vitro* studies have identified a number of genes and regulators involved in the response of *C. albicans* to limited iron[2,3]. Network inference reverse engineers regulatory networks with help of high-throughput data and has been successfully applied in a number of studies[4,5].

In this study we propose the first *ex vivo* model of regulatory interactions during iron limitation using high-throughput gene expression time series data during human oral infection [6]. Interestingly, “iron transport” is the most significantly enriched Gene Ontology process for the differentially expressed genes during infection.

Our modelling approach is based on nonlinear differential equations and utilizes selection criteria as sparseness and robustness[4]. The integration of different data sources has been shown to improve the reverse engineering approach[7]. Hence, our model softly integrates three kinds of prior knowledge: Transcription factor binding sites[9] and *in vitro* expression data under limited iron[2], as well as from transcription factor knockout mutants[10,11]. The final model consists of a number of gene regulatory relationships. Some of them are validated by literature, while others reveal yet unknown biological relevant interactions. For example, a number of target genes for the transcription factors Rim101p, Ssn6p and Tup1p during iron limitation are predicted.

[1] Sutak R, et al(2008] :Crusade for iron: iron uptake in unicellular eukaryotes and its significance for virulence.Trends Microbiol,16,261--268
[2] Lan CY, et al (2004) :Regulatory networks affected by iron availability in Candida albicans.Mol Microbiol,53,1451--1469
[3] BaekYU, Davis DA (2008): Candida albicans ferric reductases are differentially regulated in response to distinct forms of iron limitation by the Rim101 and CBF transcription factors.
Eukaryot Cell,7,1168--1179
[4] Guthke R, et al. (2005): Dynamic network reconstruction from gene expression data applied to immune response during bacterial infection. Bioinformatics, 21, 1626-1634
[5] Guthke R, et al. (2007) Discovery of gene regulatory networks in *Aspergillus fumigatus*. Lect Notes Bioinf 4366, 22-41

[6] Zakikhany K, et al (2007): In vivo transcript profiling of *Candida albicans* identifies a gene essential for interepithelial dissemination. Cell Microbiol,9,2938—2954
[7] Hecker M, et al (2009): Integrative modeling of transcriptional regulation in response to antirheumatic therapy. BMC Bioinformatics, 10:262
[8] Hecker M, et al (2009) :Regulatory Network Inference - Data Integration in Dynamic Models - A Review.BioSystems, 96:86-103
[9]Ramón AM, Fonzi WA(2003): Diverged binding specificity of Rim101p, the Candida albicans ortholog of PacC.Eukaryot Cell,2,718—728
[10] Bensen ES, et al (2004): Transcriptional profiling in Candida albicans reveals new adaptive responses to extracellular pH and functions for Rim101p.Mol Microbiol,54,1335-1351
[11] García-Sánchez S, et al (2005): Global roles of Ssn6 in Tup1- and Nrg1-dependent gene regulationin the fungal pathogen, Candida albicans.Mol Biol Cell,16,2913--2925

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Genome mining for transcription factors and secondary metabolite producers in fungi

Ekaterina Shelest

Systems Biology/Bioinformatics research group, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knoell Institute Jena

Genome mining is a powerful approach to predict a potential role and functionality of genes and gene clusters. Analyses of fungal genomes have revealed numerous examples of 'cryptic' biosynthetic gene clusters, which can potentially be responsible for the production of the novel, structurally complex natural products. On the other hand, for many secondary metabolites the corresponding biosynthetic pathways are unknown. In this talk I will speak about some problems and prespectives of the genome mining for secondary metabolite genes clusters. Besides polyketide synthase (PKS) or non-ribosomal peptide synthetase (NRPS) genes, these clusters contain several other more or less “characteristic” genes, most typically transcription factors. Fungal transcription factors are in a special focus of the research in our group. In many respects, the repertoire of transcription factors (TFs) determines the life and functionality of the cell. For a better understanding of regulatory mechanisms, it is essential to know the entire repertoire of TFs of a species. The increasing number of sequenced genomes together with the development of computational methods allow us not only to predict whole sets of TFs but also to analyse and compare them. First of all, it is important to know which TFs can in general be found in fungi, and which of them are strictly fungal-specific. Other interesting questions regard the evolutionary relationships of fungal TFs with other kingdoms and the functions of fungal-specific TFs. The analysis of predicted occurrences of DNA-binding domains in 62 fungal genomes reveals a set of 37 potential ‘fungal’ TF families. Six families are fungal-specific, i.e. they do not appear in other kingdoms. Interestingly, the fungal-specific TFs are not restricted to strictly fungal-specific functions.

After having described the general picture of distribution of TFs in fungi, we concentrated on the investigation of the TFs in *Candida* species. Two species, *C.albicans* and *C.dubliniensis*, are of particular interest because, in spite of having very similar genome structures, they demonstrate very different life style. It is believed that this dissimilarity is due to the differences on the regulatory rather than on the structural level. Thus, we decided to compare the TF repertoirs of these two species taking as a reference *C.glabrata* and *S.cerevisiae*, which are known to be taxonomically far from those two and close to each other. Applying the methods of comparative genomics, we could identify TFs unique for each of the four considered species. The functionality of some unique genes of *C.dubliniensis* is confirmed in a microarray experiment.

Network inference from transcriptome monitoring of the response of synovial fibroblasts from rheumatoid arthritis and osteoarthritis patients to TNF-alpha and TGF-beta

M. Weber[1], R. Guthke[1], R.W. Kinne[2]

[1] Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute (HKI), Systems Biology/Bioinformatics Group
[2]Experimental Rheumatology Unit, Friedrich Schiller University Jena

Introduction

Rheumatoid arthritis (RA) and osteoarthritis (OA), respectively, are the most common inflammatory/degenerative joint diseases in the adult population worldwide. RA is characterised by chronic inflammation, whereas OA shows degenerative changes accompanied by occasional phases of inflammation. Although comprehensive research has led to detailed descriptions, the precise molecular nature of both disorders is still not fully understood.

Synovial fibroblasts are particularly involved in the pathogenesis of RA and OA. In the synovial environment, fibroblasts are affected by pro-inflammatoric cytokines like TNF-alpha, as well as suppressive cytokines like TGF-beta. Network inference shall reconstitute molecular interactions of disease-related proteins (cytokines) and, in addition, generate hypotheses to promote further investigations.

Methods

Fibroblasts from RA and OA patients suffering from mild or severe disease states (n=3 each state and disease) were stimulated by TNF-alpha or TGF-beta (10 ng/ml)
Affymetrix Gene Chips h133PLUS2 were employed to obtain gene expression profiles before as well as 1h, 2h, 4h and 12h after stimulation.
Therefore, data obtained from 120 microarrays were analysed to identify differently expressed genes. Filtering of candidate genes was achieved by setting up a fold change cut-off and by further eliminating non-significantly altered intensities applying the SAM statistic tool.
Using KEGG, immune system-related pathways were analysed with respect to significant overrepresentation of gene subsets. Finally, potential links between the expression profiles of selected genes were analysed by construction and simulation of ordinary differential equation (ODE) systems using the network inference tool NetGenerator.

Results and Discussion

After identification of differentially expressed genes, transcription factors and their genetic targets were selected as potential nodes for network model construction. The initial model was improved by integrating prior signal pathway knowledge from the KEGG pathway database into the network inference algorithm.
Time-dependent, differential expression of certain matrixmetalloproteases (MMP1, MMP3, MMP10) following stimulation with TNF-alpha/TGF-beta was exclusively found in patients suffering from severe RA. This finding led to enhancement of the ODE model and may provide further information on how RA evolves from a mild to a severe degree.

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GenColors: Annotation and analysis of prokaryotic genomes made easy.

Jürgen Sühnel

Biocomputing Group, Leibniz Institute for Age Research – Fritz Lipmann Institute, Jena Centre for Bioinformatics and Jena Centre for Systems Biology of Ageing

GenColors (gencolors.fli-leibniz.de) is a web-based software/database system aimed at an improved and accelerated annotation of prokaryotic genomes considering information on related genomes and making extensive use of genome comparison [1,2]. It offers a seamless integration of data from ongoing sequencing projects and annotated genomic sequences obtained from GenBank. A variety of export/import filters manages an effective data flow from sequence assembly and manipulation programs (e.g., GAP4) to GenColors and back as well as to standard GenBank file(s). The genome comparison tools include best bidirectional hits, gene conservation, syntenies, and gene core sets. Precomputed UniProt matches allow annotation and analysis in an effective manner. In addition to these analysis options, base-specific quality data (coverage and confidence) can also be handled if available. The GenColors system can be used both for annotation purposes in ongoing genome projects and as an analysis tool for finished genomes. GenColors comes in two types, as dedicated genome browsers and as the Jena Prokaryotic Genome Viewer (JPGV). Dedicated genome browsers contain genomic information on a set of related genomes and offer a large number of options for genome comparison. The system has been efficiently used in the genomic sequencing of *Borrelia garinii* and is currently applied to various ongoing genome projects. Examples for freely accessible dedicated browsers are the Spirochetes Genome Browser (sgb.fli-leibniz.de) with Borrelia, Leptospira, and Treponema genomes and engene (engene.fli-leibniz.de) with genomes of enterobacteria. The others will be released after finalization of the corresponding genome projects. JPGV (jpgv.fli-leibniz.de) offers information on almost all finished bacterial genomes, as compared to the dedicated browsers with reduced genome comparison functionality, however. The system provides versatile quick and advanced search options for all currently known prokaryotic genomes and generates circular and linear genome plots. Gene information sheets contain basic gene information, database search options, and links to external databases. GenColors is also available on request for local installation.

1GenColors: annotation and comparative genomics of prokaryotes made easy. Romualdi A, Felder M, Rose D, Gausmann U, Schilhabel M, Glöckner G, Platzer M, Sühnel J., Methods Mol Biol. (2007) 395:75-96.

2GenColors: accelerated comparative analysis and annotation of prokaryotic genomes at various stages of completeness. Romualdi A, Siddiqui R, Glöckner G, Lehmann R, Sühnel J., Bioinformatics. (2005) 21(18): 3669-3671.

A complex model of the communication processes between *Mucorales*

Sarah Werner, Sebastian Vlaic, Anja Schröter, Stefan Schuster

Department of Bioinformatics, Friedrich Schiller University Jena, Germany
Sarah.Werner@uni-jena.de

The communication processes during the sexual mating of *Mucorales* have some fascinating facets. The pheromone trisporic acid has been previously identified as a key player in these processes. Its synthesis pathway includes an exchange of mating type-specific precursors of trisporic acid between the two mating types. By this exchange and the recognition of the end-product trisporic acid *Mucorales* find their mating partners. Trisporic acid also effects the formation of sexually induced hyphae, called zygospores.

Furthermore, some species of *Mucorales* use the trisporic acid signaling system to find potential hosts of the same order for a specific form of parasitic interactions during which the parasite fuses with the host on cellular level, therefore it is called fusion parasitism.

Since these processes are influenced by the environment in which they take place, of course, spatial conditions have to be considered in the diffusion of trisporic acid and its precursors, the growth of mycelium and the contacting of the fungi.

Due to this fact we are about to develop a complex model, which connects all these aspects. Different sub-models will be integrated, for example, a spatial simulation for growth and an ODE model for the production of trisporic acid.

The final aim is a 3D simulation of *Mucorales,* including mating and parasitism, expanding the knowledge about the whole system, which can be potentially helpful for other fields like medicine and pharmacology.

Small regulatory RNA SR1: The first dual-function sRNA from *Bacillus subtilis*

Nadja Heidrich[1], Matthias Gimpel, Ulrike Mäder[2], Hans Krügel[3] and **Sabine Brantl**

AG Bakteriengenetik, Friedrich-Schiller-Universität Jena, Philosophenweg 12, D-07743 Jena, Germany
[1]present address: Institute of Cell and Molecular Biology, Uppsala University, S-75124 Uppsala, Sweden
[2] Interfaculty Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt-Universität Greifswald, Walter-Rathenau-Str. 49A, D-17489, Germany
[3]Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institut, Beutenbergstraße 11, D-07745 Jena, Germany

Small noncoding RNAs (sRNAs) have been found to regulate gene expression in all three kingdoms of life. So far, relatively little is known about sRNAs from Gram-positive bacteria. SR1 is a regulatory sRNA from the *B. subtilis* chromosome (1) that inhibits by basepairing translation initiation of *ahrC* mRNA encoding a transcriptional activator of the arginine catabolic operons by a novel mechanism (2, 3). Interestingly, SR1 is

expressed under gluconeogenic conditions and repressed under glycolytic conditions, and this repression is mediated mainly by CcpN and, to a minor extent, by CcpA (1, 4, 5,). This sugar-dependent regulation prompted us to search for additional targets of SR1 involved in central carbon metabolism.

Here, we identify the glycolytic *gapA* operon mRNA as a new target of SR1. Both microarray and Northern blot analyses show that the amount of *gapA*-operon mRNA is significantly higher in the presence of SR1 when cells were grown in complex medium till stationary phase. Translational *lacZ* fusions and toeprinting analyses indicate that SR1 does not promote translation of *gapA* mRNA. By contrast, the half-life of *gapA*-operon mRNA is strongly reduced in the *sr1* knockout strain. SR1 does not act as a basepairing sRNA on *gapA* operon mRNA. Instead, the 39 aa peptide encoded by SR1, SR1P, is responsible for the effect of SR1 on the *gapA* operon. We demonstrate that SR1P binds GapA, thereby stabilizing the *gapA*-operon mRNA by a hitherto unknown mechanism. SR1 is the first dual-function sRNA found in *B. subtilis* (6).

References:

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2. N. Heidrich, A. Chinali, U. Gerth and S. Brantl. The small untranslated RNA SR1 from the *B. subtilis* genome is involved in the regulation of arginine catabolism.*Mol. Microbiol*. 62, 520-536 (2006).
3. N. Heidrich, Moll I., and S. Brantl. *In vitro* study of the interaction between the small RNA SR1 and its primary target *ahrC* RNA. *Nucl. Acids Res*. 35, 4331-4346 (2007).
4. A. Licht, R. Golbik, and S. Brantl. Identification of ligands affecting the activity of the transcriptional repressor CcpN from *Bacillus subtilis*. *J. Mol. Biol.* 380, 17-30 (2008).
5. A. Licht and S. Brantl. The transcriptional repressor CcpN from *Bacillus subtilis* uses different repression mechanisms at different promoters. *J. Biol. Chem*. 284, 30032-30038 (2009).
6. M. Gimpel, N. Heidrich, U. Mäder, H. Krügel, and S. Brantl. A dual function sRNA from *B. subtilis:* SR1 acts as a peptide encoding mRNA on the *gapA* operon.In revision for *Mol. Microbiol.* (2010).

Functional characterization of the eyespot protein SOUL3 in *Chlamydomonas reinhardtii*

Thomas Schulze[1], Georg Kreimer[2], and Maria Mittag[1]

[1]Friedrich Schiller University Jena, [2]Friedrich Alexander University Erlangen-Nürnberg

Chlamydomonas reinhardtii is a flagellate green algae, which has a primitive visual system, the eyespot. This system allows the cell to phototax and thereby respond to environmental light signals. In addition, the phototactic movement is controlled by the endogenous circadian clock that is synchronized by the daily light-dark cycle. Proteomic approaches of the purified eyespot of *C. reinhardtii* revealed 202 different proteins

including photoreceptors, retinal(I)-related proteins, as well as members of putative signalling pathways for photo- and chemotaxis. Among them, a protein with similarities to the SOUL-heme binding protein (HBP) family, named SOUL3 was identified. Interestingly, *soul* mRNA is specifically expressed in the retina and pineal gland in chicken. Due to the presence of SOUL-heme binding proteins in the visual system from green algae up to animals, these proteins might be involved in light- and/or circadian signalling. Until now, we could show

that His-tagged SOUL3 of *C. reinhardtii*, which was overexpressed and purified from *E. coli,* can bind to hemin-agarose indicating its heme binding ability. Additionally, sucrose gradient experiments revealed that SOUL3 exists in different complexes during subjective day and night. Since the eyespot apparatus is localized at the edge of the chloroplast we investigated the suborganellar localization of SOUL3 *via* immunofluorescence and Western analysis of different chloroplast and eyespot fractions with anti-SOUL3 antibodies. The results show that SOUL3 is purely localized in the eyespot and not present in any other part of the cell. Furthermore, silencing of SOUL3 by RNAi revealed a different phototactic behaviour during the circadian cycle, indicating that SOUL3 is involved in circadian signalling.

Processes generating biogeographic patterns in microorganism: local adaptation and genetic divergence in Antarctic aquatic protists (Dinophyceae)

Karin Rengefors, R. Logares, and J. Laybourn-Parry

Limnology Section, Dept. of Biology, Lund University, Lund, Sweden

A fundamental question in ecology is whether microorganisms exhibit biogeographic patterns in the same way that multicellular organisms do. It has been argued that microorganisms have unrestricted dispersal and no biogeographies, due to their small size and high abundance. Consequently, microbial diversity is low. This view was formulated into the hypothesis “everything is everywhere – but the environment selects” by Baas-Becking in 1934. Recently, with the advent of molecular tools, there has been a growing amount of evidence showing that endemism does occur in microorganism, that microbial diversity is very high, and that genetic divergence increases with geographical distance. A crucial point in this debate is whether or not the processes that lead to speciation, are faster than the rates of dispersal and colonisation. If speciation is faster than dispersal, then there will be no gene flow among populations, and biogeographic patterns will be shaped.

Here, we approached this question by investigating possible genetic divergence due to either local adaptation and/or geographic isolation, i. e. mechanisms which may lead to speciation. As a model system, we used aquatic protists (dinoflagellates) in recently formed Antarctic saline lakes. Clonal strains of two different species were isolated from lakes of different salinities. To study local adaptation, we investigated salinity tolerance in marine and limnic strains. The genetic difference among the strains was determined using the DNA fingerprinting technique, Amplified Fragment Length Polymorphism (AFLP). The two species were the bipolar marine *Polarella glacialis* and an organism closely related to the brackish-water species *Scrippsiella hangoei*. The AFLP analyses indicated that the lake *P. glacialis* strains were more closely related to each other than to the marine strains. The salinity tolerance experiments showed that the limnic strains had a wider salinity tolerance than the marine strains, and that the limnic strains were adapted to the salinity ranges of their lake of origin. We tentatively suggest that the limnic populations have undergone local adaptation and may be genetically isolated from the marine populations.

Impact of algal allelochemicals in algal-bacteria interactions

Catherine Legrand[1,2], Astrid Weissbach[1], Karin Holmfeldt[1], Urban Tillmann[2], Allan Cembella[2]

[1]Marine Science Center, Linnæus University, SE-39182-Kalmar, Sweden
[2]Alfred Wegener Institute, Am Handelshafen 12, D-27570 Bremerhaven, Germany

Trophic interactions between bacteria and phytoplankton in aquatic systems are well documented in the literature, as the microbial loop plays a critical role in important processes such as carbon flux and nutrient regeneration. In contrast, chemical interactions between bacteria and harmful/toxic phytoplankton and their potential impact on the production of biotoxins and population dynamics have received increasingly, yet still relatively little attention. While bacteria can play a role in the production of algal biotoxins or bloom termination, phytoplankton can produce biologically active compounds, i.e. allelochemicals that affect bacterial diversity.

In recent projects, we have quantified the antibiotic properties of allelochemicals in model algal-bacterial communities by combining physiological, chemical and molecular approaches. Depending on the trophic composition of microbial food webs, algal allelochemicals can temporary affect bacterial growth and production, either direct inhibition or indirectly by decreasing bacterivory or providing DOM through lysis of protists.

Our results contribute to an integral understanding of complex microbiological and chemical processes, essential to understanding fundamental processes in marine systems, specifically primary and microbial production and decomposition of organic matter. It focuses especially on interactions of algae and bacteria at the cellular and population level, which is crucial for revealing micro-scale processes that have meta scale consequences, e.g. in coastal waters, open ocean, polar regions.

Chemical communication in plankton revisited: New high throughput analytical methods reveal complex release patterns of metabolites from phytoplankton

Georg Pohnert

Institute for Inorganic and Analytical Chemistry, Department for Bioorganic Analytics, Friedrich Schiller University Jena

Chemical communication and allelopathy are recognized as important factors mediating the interaction of plankton species. However, our knowledge about the release of potential infochemicals by intact phytoplankton cells is very limited and only few approaches focus on the determination of specific metabolites in the water. Using high throughput metabolomic methods we are able to survey the water-borne metabolites released by cells in algal cultures, mesocosms and in the phytoplankton. A surprisingly complex pattern of specific metabolites was detected in all investigated cultures. Metabolite release is fluctuating over time and some metabolites show similar kinetics as quorum sensing mediators from bacteria. Release patterns and first experiments towards the function of the exo-metabolome are presented.

How to kill a nematode – interaction between nematode-trapping fungi and nematodes

Anders Tunlid and Dag Ahrén

Department of Biology, Section of Microbial Ecology, Lund University

Nematode-trapping fungi are parasites on nematodes. They are commonly found in soils and enter the parasitic stage by developing specific morphological structures called traps. The traps develop from hyphal

branches and they can be formed either spontaneously or be induced in response to signals from the environment, including peptides and other compounds secreted by the host nematode.There is large variation in the morphology of trapping structures, even between closely related species. Despite this variation, phylogenies inferred from molecular data have shown that a majority of nematode-trapping fungi belong to a monophyletic group placed in the family of Orbiliaceae, Ascomycota.

Following the development of traps, the infection process proceeds through a sequence of events: attachment of the trap cells to the surface of the nematode, penetration of the cuticle, digestion, and assimilation of the nutrients obtained from the killed nematode. Trapped nematodes become paralyzed (immobilized) after adhesion, when the fungus starts to penetrate the nematode cuticle. The molecular background to the paralysis is not well known. Early studies showed that nematode-trapping fungi produce subtilisins with nematotoxic activites. More recently, DNA microarrays were used to identify fungal genes that are specifically expressed by the fungus *Monacrosporium haptotylum* during the killing of the nematode *Caenorhabditis elegans*. Among these genes were subtilisins, several homologues to genes known to be differentially regulated in other pathogenic fungi, but also a large cohort of genes that displayed no significant homologs to genes present in other fungi. These orphans were of two different classes: those translating into presumably functional peptides and those with no apparent protein coding potential (noncoding RNAs). Recently, the genome of M. *haptotylum* has been sequenced in our group. We are now using bioinformatic tools to further characterize the genes being differentially regulated during infection. In parallel, candidates including genes encoding subtiltisins have been selected for heterologous expression in the yeast *Pichia pastoris*.

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Formation of novel chromosomes as a virulence mechanism in yeast *Candida glabrata*

Silvia Poláková, Christian Blume, Julián Álvarez Zárate, Marek Mentel , Dorte Jørck-Ramberg, Jørgen Stenderup and **Jure Piškur**

Cell and Organism Biology, Lund University, SE-22362 Lund, Sweden

In eukaryotes, the number and rough organization of chromosomes is well preserved within isolates of the same species. Novel chromosomes and loss of chromosomes are infrequent and usually associated with pathological events. Here we analyzed forty pathogenic isolates of a haploid and asexual yeast *Candida glabrata*, for their genome structure and stability. This organism has recently become the second most prevalent yeast pathogen in humans. While the gene sequences were well conserved among different strains, their chromosome structures differed drastically. The most frequent events reshaping chromosomes were translocations of chromosomal arms. However, also larger segmental duplications were frequent and occasionally we observed novel chromosomes. Apparently, this yeast can generate a new chromosome by duplication of chromosome segments carrying a centromere and subsequently adding novel telomeric ends. We show that the observed genome plasticity is connected with the anti-fungal drug resistance and it is likely an advantage in the human body, where the environmental conditions fluctuate a lot.

Polakova et al., 2009, Proc Natl Acad Sci U S A. 106(8) : 2688-2693.

Pigment formation in boletoid homobasidiomycetes

Dirk Hoffmeister

Associated Department for Pharmaceutical Biology, Hans Knöll Institute, Friedrich Schiller University Jena

The genetic and biochemical basis of natural product formation in homobasidiomycetes is still poorly understood, compared to Aspergilli and other ascomycetes. In search of a representative compound to investigate natural product assembly we chose the terphenylquinone pigments, produced by many species within the boletoid clade which follow mainly a saprophytic or symbiotic lifestyle. Terphenylquinones have been found in other homobasidiomycetes as well.

Atromentin is a simple terphenylquinone and represents a common intermediate of a broad variety of mushroom pigments. Based on results made with Aspergilli, the atromentin biosynthetic genes *atrA* (encoding the quinone synthetase) and *atrD* (encoding an aminotransferase) were identified in *Tapinella panuoides* (Boletales). The corresponding enzymes were heterologously expressed and biochemically characterized, so atromentin biosynthesis was reconstituted *in vitro*.

The boletales are particularly attractive for research on pigments. Some genera (e.g. *Suillus* and *Paxillus*) seem to follow biochemical strategies related yet not identical to atromentin synthesis to assemble their specific natural products. Therefore, this fungal order may represent a model to study evolution and diversification of natural products in homobasidiomycetes.

Ups and Downs in the finalization of a comprehensive fungal phylogeny

Kerstin Voigt

University of Jena, Institute of Microbiology, Fungal Reference Centre, Neugasse 25, 07743 Jena, Germany; E-mail: kerstin.voigt@uni-jena.de

The reconstruction of the phylogeny of fungi was very much hampered by a lack of suitable gene loci providing a sufficient phylogenetic signal over all major groups of the fungi. Among the higher-rank taxa most of the genera are polyphyletic, a fact which annihilates the modern concept of a natural monophyly-based classification scheme. Since the initialization of the Assembling the Fungal Tree of Life (AFToL: http://aftol.org/) project the search for a most likely solution for the phylogenetic relationships among the fungi and their allies became a challenge within recent years. Based on the establishment of a six-gene phylogeny [1] informal trees implementing phylogeny with taxonomic substitution were reconstructed [2], which are discussed in the light of new taxa and novel phylogenetic relations [3]. All analyses conclude in common that a reliable and comprehensive classification of fungal organisms is an indispensable prerequisite for deep-level phylogeny of eukaryotes. The present study addresses the resolution of the evolution of fungi with emphasis on the basal lineages of fungi.

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[2] Hibbett *et al.* (2007) A higher-level phylogenetic classification of the Fungi. *Mycol. Res.* 111:509-547.
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Microbial communities at heavy metal contaminated sites

Kothe, Erika

Institute of Microbiology, Friedrich Schiller University Jena

The influence of heavy metals on microbial populations and their ability to shape the environment is investigated within the frame of the Research Training Group “Alteration and element mobility at microbe-mineral interfaces”. In this talk, ectomycorrhizal communities in different contaminated environments will be shown and the molecular response of different fungi to heavy metal and ROS stress will be discussed as one part. In a second part, the bacterial community and specifically the streptomycetes present at the former uranium mining site in Eastern Thuringia will be shown and processes identified as major players structuring heavy metal mobility and bioavailability will be shown. These, on a molecular scale, include resistance factors of the microbes like siderophores binding not only iron, but also nickel or cadmium thus facilitating release of plant stress. The role of streptomycetes in sequestering heavy metals by biomineralization, intracellular storage and cell wall sorption will also be discussed.

Accessing the semantic level of cells

Dennis Görlich

Bio Systems Analysis Group, Institute of Computer Science, Friedrich Schiller University Jena

Without doubt cellular biological systems, like microorganisms, perform information processing and decision-making. Recent studies apply Shannon's information theory to quantify these aspects of a system. Beside the information aspect it seems even more important to understand the semantics and pragmatics of biological information processing systems. Shannon neglected semantics explicitly for information theory (Shannon, 1948). The concept of organic codes accesses the semantic level of biology and might be a good framework for this kind of analysis. We base our approach on the concept of organic codes, reviewed by Barbieri (2008), and give a formal definition of molecular organic codes in the context of reaction networks. We survey different types of systems, gene regulatory networks (GRN), metabolic networks (MN) and protein-protein-interaction networks (PPI) and describe their semantic capacity, i.e. the potential to form molecular organic codes. We find that the semantic capacity of networks is different and that GRN are likely to have a higher semantic capacity than PPIs and MN. Arising from this we hypothesize that a semantic system can be formed, if (1) the molecular species of a system have a modular structure and (2) the system provides operations that can perform the exchange of these modules. Beside this our definition of molecular organic codes allows the development of algorithms for the code-based analysis of systems. In future we will be able to derive precise mathematical tools (in analogy to Shannon's information theory) that characterizes the semantic aspects of biological systems and use these to study the evolution and dynamics of molecular organic codes.