A (CoA)-cholester intermediates. All three enzymes are located on the linear 1.3 kbp plasmid pAL1 [1]. The DNA recombination catalyzing the catobolic operons also contains two genes, qdr1 (quinazoline degradation repressor) (pAL1.016) and qdr2 (pAL1.034), which code for proteins similar to PaaX, a GntR family transcriptional regulator. This family contains more than 250 members which recognize highly divergent palindromic operon regions [2]. PaaX is the main regulator of the phenylacetate catabolism of Escherichia coli [3] and Pseudomonas putida [4] and acts as transcriptional repressor in the absence of its specific effector phenylacetyl-CoA. Electrophoretic mobility shift assays [EMSAs] with recombinant Qdr1 and Qdr2 showed that both regulators bind specifically to the promoter regions of the catobolic operons, and revealed that the dissociation of Qdr-DNA complexes is mediated by arachinol-CoA, i.e., a very late intermediate of 2-methylquinoline degradation. Interestingly, Qdr2 also represses the migration of qdr1 and qdr2 promoter fragments. Analysis of the promoter region of the operon comprising pAL1.007-011 by EMSA with different competitor DNA fragments enabled us to narrow down the recognition site of Qdr2 to 15 bp. Furthermore, the putative sequences for PaaX-like or other GntR regulators as reported by Righi et al. [2] were not evident. The differential roles of Qdr1 and Qdr2 in the regulation of the 2-methylquinoline degradation pathway of A. nitroguajacolicus RUBa-1 are not yet fully understood. Particularly the presumed auto- and/or reciprocal regulation of the qdr genes by their own gene products requires further investigations. For this purpose the interactions between Qdr1 and Qdr2 and all promoter regions are currently being studied by EMSA, antibody supershift analysis, and gel retardation techniques [1].


RSP046 The redox sensor Rlx controls product formation in Clostridium acetobutylicum
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The anoxic bacterium Clostridium acetobutylicum is well known for its biphasic fermentation metabolism. The exponential growth is characterized by acetic and butyric acid formation and during the stationary phase the solvents acetone, butanol and ethanol are the main products. However, very little is known about regulatory and molecular mechanisms controlling the carbon and electron flow during the metabolic shift. The sensing of the redox status of the cell is expected to play an important role within this regulatory network. The genome of Clostridium acetobutylicum encodes the protein Cac27131, which is annotated as "redox sensing transcriptional repressor Rlx." The deduced amino acid sequence of Rlx shows a high similarity to well-known NADH/NAD Redox regulators. To analyze the function of Rlx in C. acetobutylicum, a Rlx negative mutant of C. acetobutylicum was constructed by insertional inactivation of the gene. The mutant exhibited an interesting phenotype. In batch culture this strain produced high amounts of ethanol and butanol production started earlier at higher pH-value compared to the parental strain. The production of butyric and acetic acid was significantly reduced. In agreement with the physiological data the genes of several dehydrogenases, including the bifunctional aldehyde/alcohol dehydrogenase AdhE2 (Cap035) were upregulated as shown by Northern blot analysis. The putative redox protein was able to bind to putative Rlx boxes in front of these genes.

We concluded that Rlx plays an important role in product formation by sensing the redox status of the cell and adjusting the metabolic flux accordingly.

RSP047 The impact of the stringent response on RNA transcription in Staphylococcus aureus
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The stringent response is a conserved regulatory system present in almost all bacterial species. This response provokes the synthesis of pppGpp (pppGpp). The mechanisms by which these molecules result in the profound reprogramming of the cell physiology are still much debated. The most conserved feature of the stringent control, namely down-regulation of RNA synthesis, seems to be regulated by fundamentally different mechanisms dependent on the organisms analyzed. For Bacillus subtilis it was proposed that a lowering of the intracellular GTP pool leads to transcriptional inactivation of the RNA operons, which are initiated by IGGT. In S. aureus three pppGpp synthetases (Rsp, RelP and RelQ) are present. We have constructed in-frame deletion mutants in rsh, relP and relQ as well as a double and a triple mutant. The (pppGpp synthesis provoked by amino acid deprivation is accompanied by a drop of the GTP pool. To analyze RNA regulation in S. aureus we first determined the transcriptional start sites of the rnas operon by RACE (rapid amplification of cDNA ends). The main promoter initiates with an IGGT (P1), the other with an ITTF (P2). For measurement of promoter strength we cloned the simple promoters (P1, P2) of the rnas operon in front of a twofold divergent P1 gene and integrated these constructs into the chromosome. Rnas transcription was assessed in the WT and in the (pppGpp synthetase mutants under different conditions. Analysis of the simple promoters revealed that: I) In the WT both the P1 and P2 promoters are clearly down-regulated within 1 h of amino acid deprivation. II) The down-regulation is RSH-dependent, since in the rsh mutant the P1 and P2 originating transcripts are even up-regulated under stringent conditions. III) Such an effect was not observed using a control promoter driving the two-component system sensor histidine kinase and regulator which initiates with IATP. Thus, both rna promoters are specifically down-regulated in a RSH-dependent manner. In conclusion, since only one of them initiates with an IGGT, the lowering of the GTP pool can only partially explain the RSH-dependent down-regulation of RNA synthesis in the human pathogen S. aureus.

RSP048 A deep sequencing approach to identify sRNAs in Streptomyces coelicolor
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Recent studies have revealed that bacteria encode a wide range of small noncoding RNAs (sRNAs) and more and more are being discovered. The function of most of these sRNAs is still unclear though they are increasingly recognized as important regulators in bacteria. In the majority of cases they act as antisense riboregulators at the post-transcriptional level. They are usually encoded in the intergenic regions of the genome and their expression pattern is often linked to different points in time during development or to specific stress conditions. We were interested in sRNAs of Streptomyces coelicolor. Streptomycetes filamentous Gram+ bacteria with a high G+C content which produce a large variety of secondary metabolites, especially antibiotics.

We took an RNomeics approach to identify sRNAs in S. coelicolor. We isolated total RNA and performed deep sequencing using the 454 technology. RNA was prepared from bacteria grown in rich media to stationary phase. We obtained 89,000 reads from the sequencing and compared them to the S. coelicolor genome. After bioinformatic analysis, we obtained 63 candidates with a length from 82-694 nt. In addition, we were able to detect 192 transcriptional start sites. We selected 24 interesting candidates, which are located in intergenic regions of the genome and are at least 80 nt in length and highly expressed, for further experiments. The expression of the putative sRNAs was validated by Northern blot.

We will present data of sRNA candidates which show a growth phase dependent expression. We now intend to identify their targets by analyzing knock down and overexpression mutants in collaboration with Vockelhober MP, Schäfer CM, Stutt MG, Schmidt D, Xu Z, Gierisch S, Lauwe HG, Matthews GH, Naes B (2011) Deep sequencing-based identification of small non-coding RNAs (sRNAes) of Streptomyces coelicolor RNA 1, 393.4.

RSP049 The interaction of transcription factor TnrA with glutamine synthetase and PII-like protein GlnK
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TnrA is the major transcription factor in Bacillus subtilis that controls gene expression in response to nitrogen availability [Wray et al., 2001]. When the preferred nitrogen source is in excess, feedback-inhibited glutamine synthetase (GS) is earlier shown to bind TnrA and disable its activity. During nitrogen-limited growth TnrA is fully membrane bound via an AmB/GlnK complex [Heinrich et al., 2006]. The complete removal of nitrater from the medium leads to rapid degradation of TnrA in wild-type cells. We suppose that binding of TnrA to GlnK or GS is required for both regulation of TnrA activity and its protection from proteolysis. In the AmB- or GlnK-deficient strains, TnrA is present in a soluble state in cytoplasm and does not degrade in response to nitrogen depletion. We have found that TnrA forms either a stable soluble complex with GlnK in the absence of AmB or constitutively binds to GS in the absence of GlnK, and is protected thereby from proteolysis. It was shown previously that the TnrA-Cterminus is responsible for correct positioning of TnrA (Kujanov et al., 2007). To check whether the C-terminus of TnrA is also required for interaction with GlnK, various truncations of N-terminally His-tagged TnrA (lacking 6, 20 and 35 amino acids from C-terminus) were
constructed and overexpressed in E. coli cells. By pull-down analysis it was established that deletion of already 6 C-terminal amino acids abrogates GS binding. The region between 20 and 35 amino acids from the C-terminus is required for GlnK interaction as well as for proteolysis of TarA. These data confirm that the interaction of GS or GlnK with TarA protects it from degradation. Alternatively, if an ammonium was added to nitrogen starved cells, TarA is released from GlnK and binds to GS. Interaction of TarA with GS inactivates the transcription factor. Conversely, TarA inhibits the GS activity; TarA represses in vitro the biosynthetic activity of GS, independently of the presence of AMP or glutamine.

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RSP050
Cross-interactions between two-component signal transduction systems in E. coli

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Microorganisms commonly use 'two-component' signaling systems for sensing environmental conditions. Prototypical two-component systems are comprised of a sensory histidine kinase and a response regulator protein that is phosphorylated by the kinase. The regulator typically acts as a transcription factor regulating gene expression. Apart from a few studies performed in vitro, the signaling properties of a whole prokaryotic two-component network in vivo remains largely unclear. We use a system level approach to characterize the interactions between sensors, regulators and promoters in a minimal model bacterium Escherichia coli on different levels, using in vivo fluorescence resonance energy transfer (FRET) microscopy and flow cytometry. We measure a set of labelled sensor dimers and sensor-regulator combinations at physiological expression levels and describe qualitatively their interaction strength and kinetics using FRET. Additionally, we identify mixed complexes between different sensors and non-co-ordinate sensor-regulator pairs exhibiting in vivo interactions. These findings indicate possible interconnections between different signaling pathways. We demonstrate that in some of the cases interactions are sensitive to specific stimuli, suggesting that changes in protein arrangement play a role in signal processing. Using flow cytometry and transcriptional reporters, we further observe several cases where sensors have an effect on other non-co-ordinate sensor regulator interaction, indicating the physiological relevance of the identified interconnections between different signal transduction pathways. Our results should help to establish an integral picture of cell signalling, which is of general importance for single cellular organisms.

RSP051
SyR1 - a sRNA regulating photosynthesis in cyanobacteria

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Post-transcriptional gene regulation by trans encoded small RNAs (sRNAs) emerges as an regulatory feature common to most prokaryotes. Recently, bioinformatical prediction [1], comparative transcriptional analysis [2] and high throughput pyrosequencing of Synecocystis sp. PCC8003 [3] revealed the existence of many new sRNAs in this cyanobacterial model organism. One of these candidates is the strongly accumulating sRNA SyR1 (Synecocystis sRNA 1), which is a 130nt long transcript from the intergenic region between the fabX and hofH genes. More detailed investigation on SyR1 showed that this sRNA is upregulated under high-light stress and CO2 depletion [2] and that a strain overexpressing SyR1 exhibits a bleaching-phenotype lacking photosynthetic pigments. A homology search revealed SyR1 candidates in other cyanobacteria while a bioinformatical target prediction implies that the predominant interaction site, which is the most conserved sequence element of SyR1, potentially binds to the transcripts of photosynthesis genes. Moreover, gel mobility shift assays provide evidence for a direct interaction between SyR1 and psaL and ongoing mutational analysis of the putative SyR1 binding site aims to verify the post-transcriptional regulation of this target gene. Furthermore, preliminary results indicate that long-term SyR1 overexpression leads to a down-regulation of genes involved in the high-affinity uptake of inorganic carbon (CI) while the aeration of cultures with 5% CO2 quickly abolished SyR1 accumulation in the overexpressing strain and complements the bleaching-phenotype. For these findings we speculate that SyR1-dependent gene regulation affects photosystem biosynthesis and homeostasis and possibly integrates light and C1-signaling pathways.


RSP052
Utilization of metabolic regulation for the production of heterologous proteins in Burkholderia glumae

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Burkholderia glumae is a Gram-negative proteobacteria. Although initially proposed to be part of the Pseudomonas genus, this strain was transferred along with others like Pseudomonas cepacia and Pseudomonas gladioli to the new genus Burkholderia. Since the rice pathogen B. glumae is not a pathogenic bacteria and therefore classified as S1-organism, it could be used as model organism for related pathogenic bacteria like Pseudomonas aeruginosa.

Due to its relevance for agriculture, most of the scientific investigations with regard to B. glumae focused on the mechanisms the rice-pathogenicity is based on. Besides, B. glumae has an interesting industrial application range. The BASF company has developed B. glumae by classical strain improvement as a lipase overproduction strain [1]. Thus, there is the possibility to produce large amounts of functional enzyme and we want to gain access to this production capacity for heterologous protein production by establishing B. glumae as a novel expression strain. Expression systems based on the TT-Polymerase are able to produce large amounts of proteins, for example in E. coli, but lead in some cases to inactive enzymes accumulated in inclusion bodies. Here, posttranslational modification, folding, and secretion of proteins may be crucial steps in successful production of proteins and active enzymes. We want to avoid these problems by inducing the TT-Polymerase expression at a time B. glumae is able to handle large amounts of produced proteins, like its lipase. Therefore, we have created an expression strain which exhibits a lipase promoter controlled TT-Polymerase gene. The transcription of genes downstream this lipase promoter can be induced for example by oleic acid. Since we have shown that the lipase promoter is controllable and inducible by the choice of additional carbon sources in the culture medium, we have also constructed a vector-based expression system for B. glumae containing a lipase promoter. The production capacity and prevention of inclusion bodies for difficult-to-express genes will be determined in further studies.