A (CoA)-thioester intermediates. All three operons are located on the linear 113 kbp plasmid pAL1 [1].
The DNA region comprising the catabolic operons also contains two genes, *qdr1* (quinaldine degradation repressor) (pAL1.016) and *qdr2* (pAL1.024), which code for proteins similar to PaaX, a GntR family transcriptional regulator. This family contains more than 250 members which recognize highly diverse palindromic operator regions [2]. PaaX is the main regulator of the phenylacetate catabolon 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 (EMSA) with recombinant Qdr1 and Qdr2 showed that both regulators bind specifically to the promoter regions of the catabolic operons, and revealed that the dissociation of Qdr-DNA complexes is mediated by anthraniloyl-CoA, i.e., a very late intermediate of 2-methylquinoline degradation. Interestingly, Qdr2 also retards 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 a 40 nt region. However, consensus sequences for PaaX-like or other GntR regulators as reported by Rigali *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* Rü61a 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 exonuclease III footprinting.

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## RSP046

### The redox sensor Rex controls product formation in *Clostridium acetobutylicum*

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The anaerobic 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 Cac2713, which is annotated as "redox sensing transcriptional repressor Rex". The deduced amino acid sequence of Rex shows a high similarity to well-known NADH/$NAD^+$ redox regulators. To analyze the function of Rex in *C. acetobutylicum*, a Rex 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 acid and acetone was significantly reduced. In agreement with the physiological data the genes of several dehydrogenases, including the bifunctional aldehyde/alcohol dehydrogenase AdhE2 (Cap0035) were upregulated as shown by Northern blot analysis. Furthermore, the purified Rex protein was able to bind to putative Rex boxes in front of these genes.
We concluded that Rex 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 rRNA transcription in *Staphylococcus aureus*

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The stringent response is a conserved regulatory system present in almost all bacterial species. Nutrient limitation provokes the synthesis of (p)ppGpp. 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 rRNA synthesis, seems to be regulated by fundamentally different mechanisms dependent on the organisms analysed. For *Bacillus subtilis* it was proposed that a lowering of the intracellular GTP pool leads to transcriptional inactivation of the rRNA operons, which are initiated by iGTP. In *Staphylococcus aureus* three (p)ppGpp synthetases (RSH, 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 (p)ppGpp synthesis provoked by amino acid deprivation is accompanied by a drop of the GTP pool. To analyse rRNA regulation in *S. aureus* we first determined the transcriptional start sites of the *rrn1* operon by RACE (rapid amplification of cDNA ends). The main promoter initiates with an iGTP (P1), the other with an iTTP (P2). For measurement of promoter activity we cloned the single promoters (P1, P2) of the *rrn1* operon in front of a truncated *gfp* gene and integrated these constructs into the chromosome. *Rrn1* transcription was assessed in the WT and in the (p)ppGpp synthetase mutants under different conditions. Analysis of the single 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) This 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 saeRS and initiating with iATP. Thus, both *rrn1* promoters are specifically down-regulated in a RSH-dependent manner. In conclusion, since only one of them initiates with an iGTP, the lowering of the GTP pool can only partially explain the RSH-dependent down-regulation of rRNA synthesis in the human pathogen *S. aureus*.

## RSP048

### A deep sequencing approach to identify sRNAs in *Streptomyces coelicolor*

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Latest 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*. Streptomycetesare filamentous $Gram^+$ bacteria with a high G+C content which produce a large variety of secondary metabolites, especially antibiotics.
We took an RNomics approach to identify sRNAs in *S. coelicolor*. We isolated total RNA and performed deep sequencing using the 454 technology. RNA was prepared from bacteriagrown in rich media to stationary phase. We obtained 58,000 reads from the sequencing and compared them to the *S. coelicolor* genome. After bioinformatic analysis, we obtained 63 candidates with a length from 82-494 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.

Vockenhuber MP., Scharma CM., Statt MG., Schmidt D., Xu Z., Dietrich S., Liesegang H., Mathews DH., Suess B. (2011) Deep sequencing-based identification of small non-coding RNAs inStreptomyces coelicolor.RNA Biol.,1; 8(3).

## 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) was earlier shown to bind TnrA and disable its activity. During nitrogen-limited growth TnrA is fully membrane bound via an AmtB-GlnK complex [Heinrich *et al*., 2006]. The complete removal of nitrate 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 AmtB- or GlnK-deficient strains, TnrA is present in a soluble state in cytoplasm and does not degrade in response to nitrate depletion. We have found that TnrA forms either a stable soluble complex with GlnK in the absence of AmtB or constitutively binds to GS in the absence of GlnK, and is protected thereby from proteolysis. It was shown previously that the TnrA C-terminus is responsible for interactions with (GS) [Wray *et al*., 2007]. To check whether the C-terminus of TnrA is also required for interaction with GlnK, various truncations of N-terminally $His_6$-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 abrogate 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 TnrA. These data confirm that the interaction of GS or GlnK with TnrA protects it from degradation. Alternatively, if ammonium was added to nitrogen starved cells, TnrA dissociates from GlnK and binds to GS. Interaction of TnrA with GS inactivates the transcription factor. Conversely, TnrA inhibits the GS activity; TnrA 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 promotors in the 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 quantitatively their interaction strength and kinetics using FRET. Additionally, we identify mixed complexes between different sensors and non-cognate 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 stimulation, 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 non-cognate promotor regulation, 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 (sRNA) emerges as an regulatory feature common to most prokaryotes. Recently, biocomputational prediction [1], comparative transcriptional analysis [2] and high throughput pyrosequencing of Synechocystis sp. PCC6803 [3] revealed the existence of many new sRNAs in this cyanobacterial model organism. One of these candidates is the strongly accumulating sRNA SyR1 (Synechocystis ncRNA 1), which is a 130nt long transcript from the intergenic region between the fabX and hoH 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 also 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 abolishes SyR1 accumulation in the overexpression 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 Ci-signaling pathways.

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## 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 non-human pathogenic and therefore classified as S1-organism, it could be used as model organism for related pathogenic bacteria like *Pseudomonas aeruginosa*.

Due to its relevancy 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 over-production strain[1,2]. 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 T7-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 T7-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 T7-Polymerase gene. The transcription of genes downstream this lipase promoter can be induced for example by olive oil[3]. 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.

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## RSP053

### An expression system for the W-containing class II benzoyl-coenzyme A reductases in *Geobacter metallireducens*

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In anaerobic bacteria most aromatic growth substrates are converted into the central intermediate benzoyl-coenzyme A (benzoyl-CoA). Benzoyl-CoA reductases (BCRs) dearomatize benzoyl-CoA to cyclohexa-1,5-diene-1-carboxyl-CoA (dienoyl-CoA). Obligately anaerobic bacteria such as *Geobacter metallireducens* employ class II benzoyl-CoA reductases. The active site components of this W-enzyme, BamBC, have recently been isolated and characterized[1]. A genetic system comprising a suitable expression plasmid was established in *Geobacter metallireducens* that enabled the active production of Strep-tagged BamB, which supposedly contains tungsten. Surprisingly, the electron transferring wild type BamC subunit, containing 3 [4Fe-4S] clusters, was co-purified with Strep-tagged BamB indicating a strong but reversible interaction of the two subunits. The established system enables the efficient production and purification of class II benzoyl-CoA reductase subunits and may enable expression of other W-/metallo enzymes from obligately anaerobic Deltaproteobacteria.

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## RSP054

### Insight into the (de)phosphorylation of the phosphotransferase proteins HPr and Crh in *Bacillus subtilis*

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In *Bacillus subtilis* uptake and utilization of different carbon sources are tightly regulated by carbon catabolite repression (CCR) (1). The global players involved in CCR are HPr and the HPr kinase/phosphorylase. Upon phosphorylation of HPr at Ser~46, CCR is mediated by the CcpA-HPr-