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Editorial – preview

Streptomycetes are the main source for a huge repertoire of small molecules, which are in use as antibiotics, antifungals, antiparasitics, or as antitumor and immunosuppressive drugs. As outlined in minireviews and original articles within this special issue, cloning, designed mutations, the comparisons of transcripts, proteomes and metabolites have provided considerable insights into the biosynthesis of metabolites, and the characterization of global and specific regulators, and carbon fluxes. These and future studies will be the basis of systems biology for streptomycetes. The availability of genome sequence information from *Streptomyces* species facilitates the discovery of an additional large gene pool of clusters for cryptic secondary metabolites and for orphan proteins of unknown functions. An increasing number of investigations have provided novel insights into extracellular protein complexes, signalling and interactions. To manipulate streptomycetes for an increasing number of applications, it will be important that researchers elucidate many more complex biological aspects, under laboratory and environmental conditions. In the following, we summarize the main results and implications of the research presented within the articles of this special issue.

The results of numerous studies have provided an emerging picture of control mechanisms for primary and secondary metabolism in streptomycetes. Within their minireview, Martín and colleagues (2011) present an integrated view on current knowledge about interactions between the global nutritional regulators PhoP, GlnR, DasR and AfsR, and other poorly known nutritional regulators in the model species *Streptomyces coelicolor* A3(2). From the diversity of *Streptomyces* species, reflected in differences between the respective genomes, they infer species-specific interactions between these global regulators. They conclude that a deeper understanding of molecular interactions and the integration of signals is an essential basis for ongoing systems biology investigations into the complexity of regulatory networks.

Important signals in this context are the γ-butyrolactones. In *S. coelicolor*, the γ-butyrolactone synthase ScbA is responsible for the biosynthesis of signalling molecules called SCBs (*S. coelicolor* butanolides). D'Alia and colleagues (2011) showed by transcriptome analysis that the *scbA* mutant had a strong perturbation in the expression of gene clusters for three pigmented antibiotics, and that the synthesis of the siderophore desferrioxamine is also under SCB control. The expression of some genes for the primary metabolism occurred earlier in the mutant, indicating mutual control of both primary and secondary metabolism.

Siderophore-mediated iron acquisition has received increased attention. The siderophore-binding receptor DesE is abundant among streptomycetes, while the CdtB counterpart is only found in certain *Streptomyces* species. Based on the analyses of mutants, Tierrafría and colleagues (2011) conclude that DesE and CdtB have overlapping ferrioxamine specificities. Due to additional studies, and the fact that genes for synthesis of desferrioxamines are abundant among streptomycetes, the authors speculate about an additional regulatory or signalling role of siderophores.

Less well studied to date in terms of their contribution to global regulation are the many serine-threonine proteins kinases (STPKs) encoded by streptomycetes. *Streptomyces coelicolor* encodes 42 predicted STPKs and Jones and colleagues (2011) demonstrate how loss of function of two genes encoding Forkhead-Associated domain proteins FhaAB, which function as likely 'brake' proteins, can reveal the role of the genetically linked STPK PknB in regulating key enzymes in central carbon metabolism, increasing carbon flux to secondary metabolism.

The complexity of regulation likely reflects the diverse challenges streptomycetes meet as free-living soil microorganisms. Pérez and colleagues (2011) discovered that the Gram-negative bacterium *Myxococcus xanthus* preys on *S. coelicolor* hyphae. As a result, *M. xanthus* enumerates streams of ordered cells that lyse the substrate hyphae. This process provokes *S. coelicolor* to produce high levels of the polyketide actinorhodin, and to form aerial hyphae, indicating a response that alters expression of many genes. The elucidation of the underlying signal cascade(s) is a challenging future task.

In addition to small biologically active molecules, extracellular proteins determine the environmental and biotechnological role of streptomycetes. Schrempf and colleagues (2011) discovered differently sized 80–400 nm extracellular *Streptomyces* vesicles. These contain different protein arrays that are required for a range of processes: the acquisition of inorganic as well as organic phosphate, iron-ions, and of distinct carbon sources, energy metabolism and redox balance, defence against harmful compounds, modification of secondary

metabolite(s), folding and assembly of proteins, and different signalling cascades. The findings shed novel views on the extracellular biology of streptomycetes.

The diversity of streptomycete secondary metabolites and their biotechnological importance is reflected in several contributions. In the form of a minireview, Olano and colleagues (2011) summarize the knowledge on the chemical structures of antitumor compounds that are produced by streptomycetes and a few other actinobacteria, as well as the corresponding gene clusters. They outline strategies for gene inactivation, gene expression, heterologous expression of the clusters, and genetic techniques to improve production yields. Combinatorial biosynthesis can exploit knowledge of the functions of each characterized pathway. Some resulting bioactive analogues have improved antitumor properties. The authors suggest expanding this strategy to provide increasing numbers of safer and more specific antitumor drugs for chemotherapy.

Mithramycin and chromomycin A3 are two structurally related antitumor compounds. García and colleagues (2011) have characterized a chromomycin acetyltransferase of a *Streptomyces griseus* strain that accepts several acyl-CoA substrates. With an engineered *S. griseus* strain, they generated novel derivatives of mithramycin that differ from their parental compounds by the presence of one, two or three acetyl groups. Some of the compounds showed improved activities against glioblastoma or pancreatic tumour cells. The data reveal the value of using this enzyme to enhance the natural structural diversity of related antitumor compounds.

Streptomyces pristinaespiralis Pr11 produces a streptogramin antibiotic consisting of two chemically unrelated compounds, pristinamycin I and pristinamycin II. Mast and colleagues (2011) used previous and newly added sequence information to identify all genes for the pristinamycin biosynthetic pathway. Interestingly, the pristinamycin gene cluster is interspersed by a cryptic cluster that likely codes for a glycosylated aromatic polyketide. Gene inactivation experiments revealed that this cluster has no influence on pristinamycin production. The authors provide new insights into both pristinamycin biosynthesis and the organization of largest (210 kb) largest antibiotic 'supercluster' characterized to date.

Streptomyces cinnamonensis produces furanonaphthoquinone I (FNQ I) and isoprenylated phenazines (endophenazines). Seeger and colleagues (2011) report that the corresponding biosynthetic genes for these isoprenoid metabolites reside atypically not only within a previously described larger gene cluster, but in addition, within a newly identified smaller locus. The latter comprises six genes of the mevalonate pathway, as well as the gene *epzP*. Genetic and biochemical studies indicate that the EpzP protein is a member of a recently discovered class of prenyltranferases. Notably, EpzP prenylates a dihydrophenazine derivative but not a phenolic substrate.

The genome of *Streptomyces avermitilis* encodes the pathway for *epi*-isozizaene or its oxidized derivatives, albaflavenols and albaflavenone; however, Takamatsu and colleagues (2011) did not encounter their production. To activate the silent genes, they introduced the gene for the *epi*-isozizaene synthase under control of a selected promoter (*rpsJp*) together with a P450-encoding gene into *S. avermitilis* SUKA16 that has a large chromosomal deletion. The known oxidized *epi*-isozizaene metabolites (*4R*)- and (*4S*)-albaflavenols and albaflavenone, and one previously unknown doubly oxidized *epi*-isozizaene derivative were successfully produced by the well-designed transformants.

Gomez-Escribano and Bibb (2011) deleted the clusters for actinorhodin, prodiginine, CPK and CDA biosynthesis from *S. coelicolor* M145, and introduced point mutations into the genes *rpoB* and *rpsL*. Re-introduction of the native actinorhodin gene cluster or introduction of gene clusters for the heterologous production of chloramphenicol and congocidine led to dramatic increases in antibiotic production. The usage of these genetically engineered strains is likely to play an important role in the *de novo* discovery of compounds encoded by cryptic gene clusters, and in the high-level production of selected compounds.

Within *Streptomyces clavuligerus* Nárdiz and colleagues (2011) discovered a rhodanese-like enzyme RhlA. They found that it diverges from a standard rhodanese type as it is not involved in thiosulfate utilization. Notably, *rhlA* defective mutants are impaired in holomycin production, and produce lower levels of cephamycin C and clavulanic acid than the parental strain. The authors suggest that the rhodanese-like enzyme might be involved in the oxidoreduction of disulfide bonds. In addition to a general cellular role, RhlA might catalyse the formation of the S–S bridge in holomycin, possibly via a sulfocysteine intermediate. These data will stimulate further studies on the rhodanese-like protein.

Medema and colleagues (2011) compared the genome-wide gene expression of an industrial *S. clavuligerus* strain, an overproducer of clavulanic acid obtained through iterative mutagenesis, with that of the wild-type strain. They showed that most changes were due to the upregulation of various antibiotic biosynthetic gene clusters. A few additional transcriptional changes in primary metabolism at key points seem to divert metabolic fluxes to the biosynthetic precursors for clavulanic acid. The results provide key information for how researchers could improve antibiotic titres also in other species.

J. Davies (2011) reflects that the experimental use of antibiotics and their resistance genes has been of inesti-

mable value in contributing to the major advances made in the life sciences and biotechnology during the past three decades. Unfortunately, the spread of antibiotic resistance has increased dramatically, and led to an enormous limitation to treat infectious diseases worldwide. On the other hand, studies have demonstrated that antibiotics may have direct applications other than infectious disease treatment. These include restoring defective gene function in the mitigation of several human genetic diseases.

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