In vivo engineering of polyketide synthases

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Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

DTU Bioengineering
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In vivo engineering of polyketide synthases

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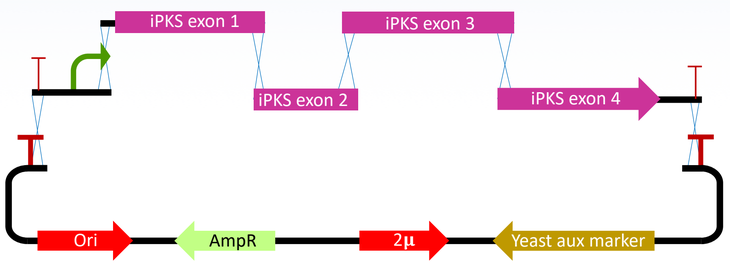
Introduction

Polyketides form the basic building blocks of numerous natural products, which are in use in pharmaceuticals, food additives and other fine chemicals.
Polyketides derived from fungi are formed by type I iterative PKSes (iPKSes). The common domain structure of a non-reducing iPKS (NR-PKS) is SAT-KS-AT-PT-ACP-TE, which enables the NR-PKS to produce very complex polyaromatic compounds. Studies have revealed the general catalytic properties of these domains, and for some even the specificity can be predicted based solely by bioinformatics.[1,2]
Some attempts have been made to investigate and engineer NR-iPKSes, but these have focused on *in vitro* assays. [1,2] To speed up construction and screening the present study focusses on *in vivo* analysis in *S. cerevisiae* of native and engineered iPKSes. To engineer the NR-iPKSes the combination of SAT-KS-AT and PT-ACP-(TE) tridomain units of different origin should create new compounds. The used linker between the tridomain units has been designed by multiple alignment of all the studied NR-iPKSes and by HMM investigation of the region between the AT and PT domains. This revealed a 12 amino acid long conserved region. This region is used as a uniform linker in the synthetic chimeric iPKSes as it will not extend the overall amino acid chain, thus native protein structure should be conserved.

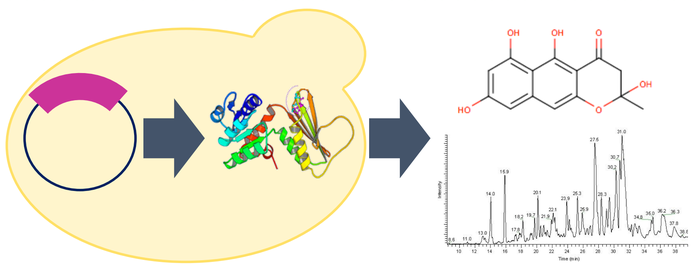
In vivo assembly

Fungal genes are often a mix of introns and exons. To overcome risk of splicing errors of the pre-mRNA in yeast, the iPKSes are assembled by homologous recombination without introns.



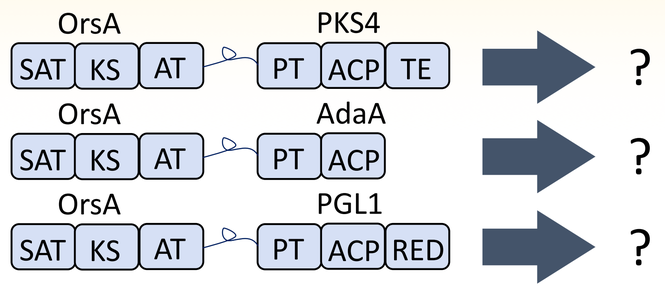
iPKS expression

Expression of the native NR-iPKS will show if the native compound is produced in *S. cerevisiae*. The analysis of choice will be HPLC-HRMS as this will be able to identify breakdown patterns of specific metabolites.



Chimeric iPKSes

By combining the SAT-KS-AT from one iPKS with the PT-ACP-(TE) domain from another novel compounds could be formed.



Overview of investigated iPKSes

The domain structure, product length and first ring formation are shown. Right column requires an unloading enzyme as the iPKSes are lacking a TE/RED domain.

OrsA from *Aspergillus nidulans*
SAT KS AT PT ACP ACP TE C_8 – C2-C7

PKS1 from *Botrytis cinerea*
SAT KS AT PT ACP ACP TE C_{10} – C2-C7

AioG from *A. oryzae*
SAT KS AT PT ACP ACP TE C_{12} – C2-C7

PGL1 from *Fusarium fujikuroi*
SAT KS AT PT ACP ACP RED C_{14} – C4-C9

wA from *A. nidulans*
SAT KS AT PT ACP ACP TE C_{14} – C2-C7

PKS4 from *F. fujikuroi*
SAT KS AT PT ACP TE C_{18} – C2-C7

ACAS from *A. terreus*
SAT KS AT PT ACP C_{16} – C6-C11

MdpG from *A. nidulans*
SAT KS AT PT ACP C_{16} – C6-C11

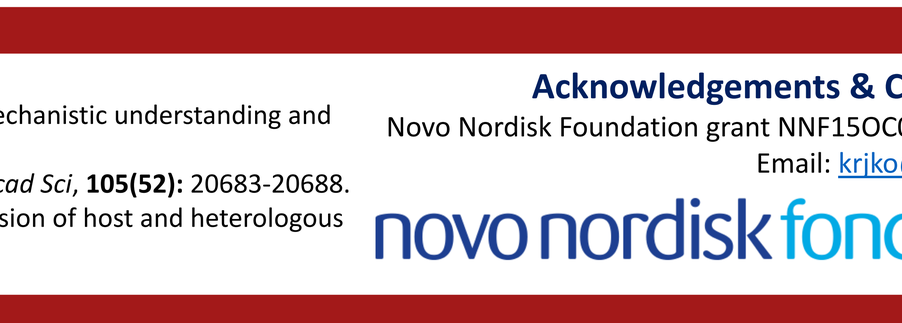
AptA from *A. nidulans*
SAT KS AT PT ACP C_{18} – C6-C11

AdaA from *A. niger*
SAT KS AT PT ACP C_{20} – C6-C11

VrtA from *Penicillium*
SAT KS AT PT ACP C_{19} – C6-C11

Chemical logic of chimeric iPKSes

The SAT-KS-AT tridomain is proposed to control chain length while PT-ACP-(TE) tridomain control the folding pattern. By combining non-native tridomains it may be possible to engineer novel polyketide scaffolds.



Outlook

The combination of tridomain modules form vastly different NR-iPKSes will generate insight into the biochemical logic of the NR-iPKSes. As an example, the combination of tridomains from long chain and short chain iPKSes will show whether short chain ACPs can carry long chain products or if the PT-ACP-TE domain dictates premature release. If the designed linker is found to render the enzymes inactive, another design could focus on investigating a longer more flexible linker. This has been previously shown to enable different enzymes to function better in an assembly line fashion.[3] Another option would be to mimic previous *in vitro* assays and express NR-iPKS domains individually.[1,2]

References
[1] Newman et al. (2014). Systematic domain swaps of iterative, nonreducing polyketide synthases provide a mechanistic understanding and rationale for catalytic reprogramming. *J Am Chem Soc*, **136(20)**: 7348-7362.
[2] Zhang et al. (2008). Engineered biosynthesis of bacterial aromatic polyketides in Escherichia coli. *Proc Nat Acad Sci*, **105(52):** 20683-20688.
[3] Albertsen et al. (2011). Diversion of flux toward sesquiterpene production in *Saccharomyces cerevisiae* by fusion of host and heterologous enzymes. *Appl Environ Microbiol* **77(3)**: 1033-1040.

Acknowledgements & Contact
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