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Synthetic control devices for gene regulation in *Penicillium chrysogenum*

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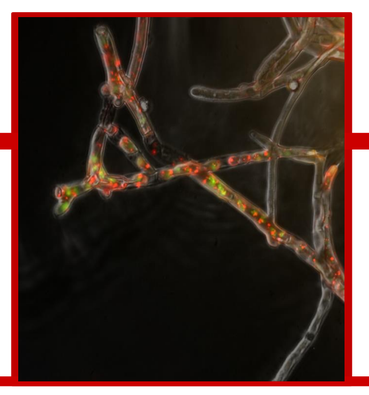

Introduction

Synthetic biology aims at controlled gene regulation that can lead to increased production of chemicals and pharmaceuticals. In this work synthetic control devices were developed for *Penicillium chrysogenum*, a model filamentous fungus and industrially relevant cell factory.

In the synthetic transcription factor (STF) the QF DNA-binding domain of the transcription factor of the quinic acid gene cluster of *Neurospora crassa*[2] is fused to the VP16 activation domain. This synthetic transcription factor controls the expression of genes under a synthetic promoter containing quinic acid upstream activating sequence (QUAS) elements, where it binds prior to a core promoter (CP) (**Fig.1.**).

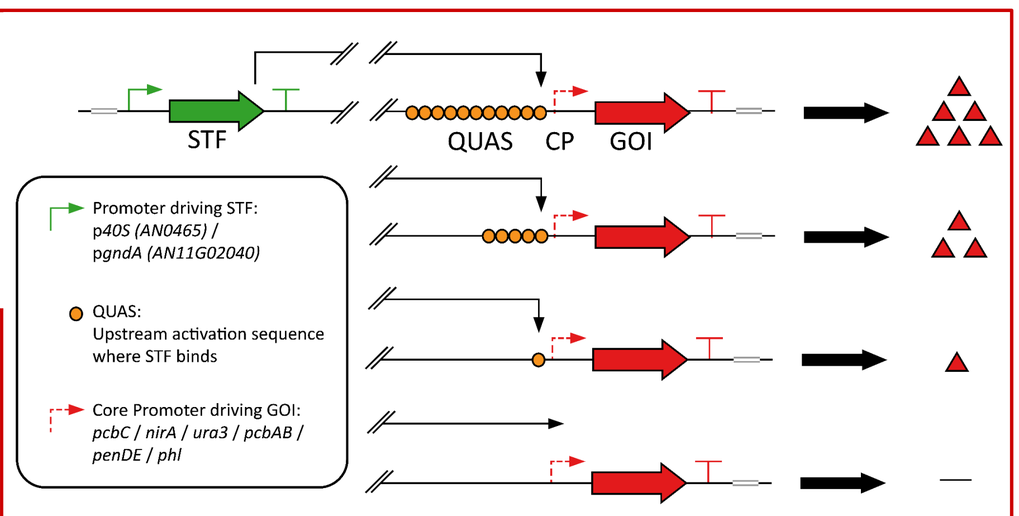

Fig.1. Schematic representation of the control devices.

Results

The strength of the control device can be altered by altering the expression of the transcription factor, the core promoter upstream the QUAS or the number of QUAS elements (**Fig.2.-4.**).

The versatility of the control device was demonstrated by fluorescent reporters (eGFP-NLS, DsRed-SKL) and its application was confirmed by synthetically controlling the production of Penicillin V (**Fig.5.**).

Validation by fluorescent reporters

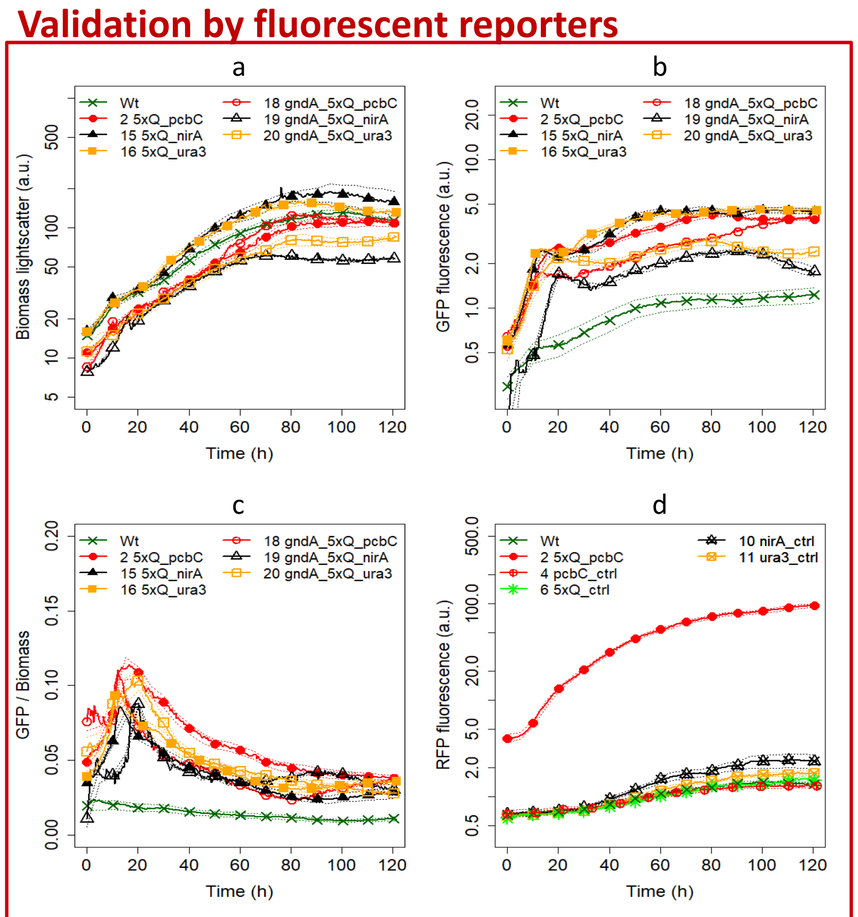

Fig.2. Development of biomass **(a)**, GFP fluorescence **(b)**, GFP fluorescence/biomass **(c)** and RFP (DsRed) fluorescence **(d)** over time of selected *P. chrysogenum* strains in BioLector micro-bioreactor system[3]. Promoter driving STF p*40S* (when not indicated differently). Parental strain DS68530 (Wt) shown in dark green.

Validation of synthetic promoters

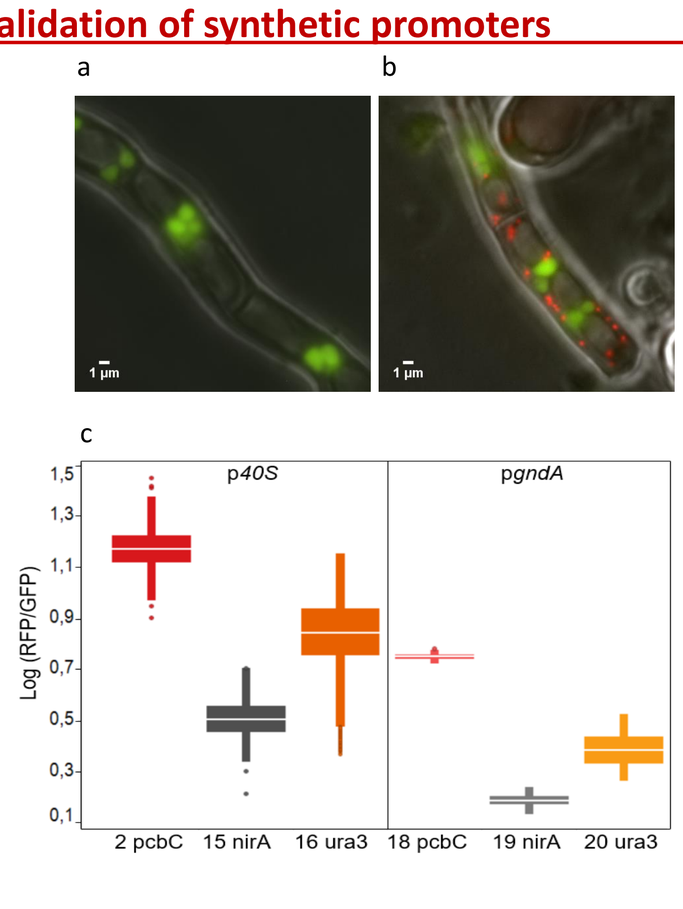

Fig.3. Fluorescence microscopy images of STF(-eGFP-NLS) expressing strains with **(a)** and without **(b)** 5xQUAS binding sites prior core promoter driving *DsRed*. **c)** Ranking of the expression of 6 control devices. All devices contain 5xQUAS; promoters driving the STF (on top) and CPs (on bottom).

Tunability of the synthetic control device

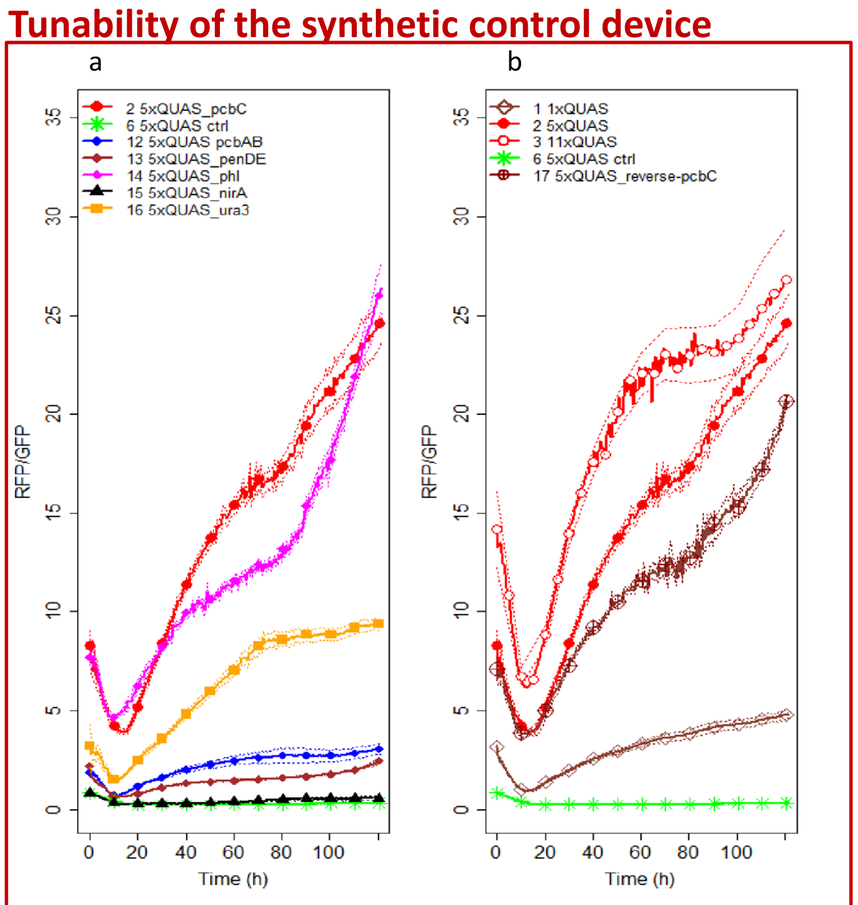

Fig.4. Development of DsRed/GFP fluorescence over time during growth of *P. chrysogenum* strains **a)** Effect of different CPs driving *DsRed* with 5xQUAS. **b)** Strains expressing DsRed under a synthetic promoter containing 0, 1, 5 or 11 QUAS elements upstream the *pcbC* CP.

Validation by Penicillin V production

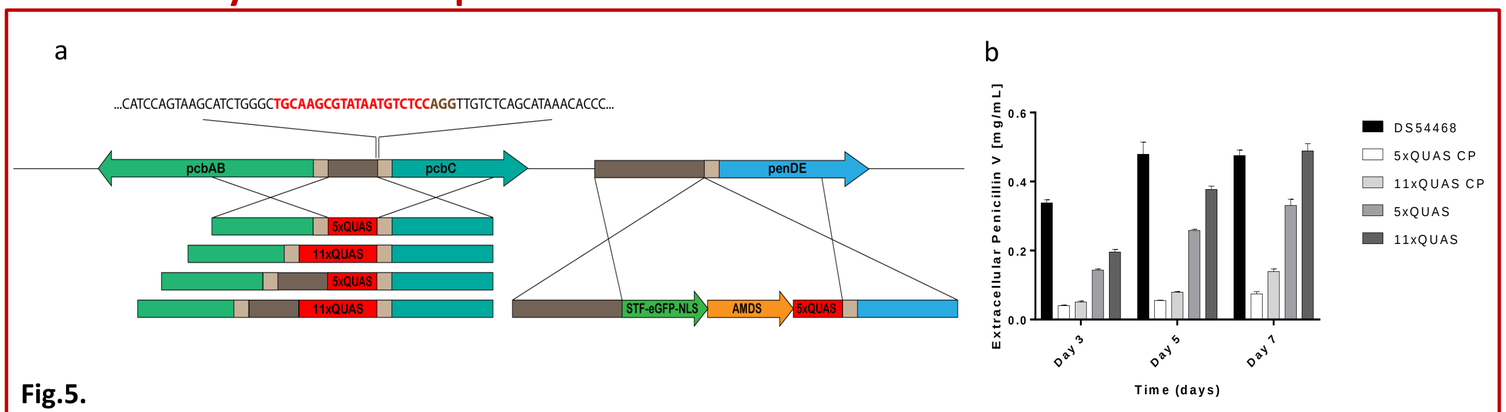

Fig.5.
a) Schematic representation of CRISPR/Cas9 based integration[4] and co-transformation of the synthetic control device and QUAS elements into the penicillin cluster of DS54468. Designed sgRNA targets boundary between the promoter of *pcbAB* and the core promoter of *pcbC*. **b)** Extracellular Penicillin V production of strains where the penicillin biosynthesis cluster is under control of the control device and parental strain DS54468 in shake flask cultivation in penicillin producing medium. Data shows 3 independent cultures, measured in replicates.

Envisioned silent promoter activation with CRISPRa

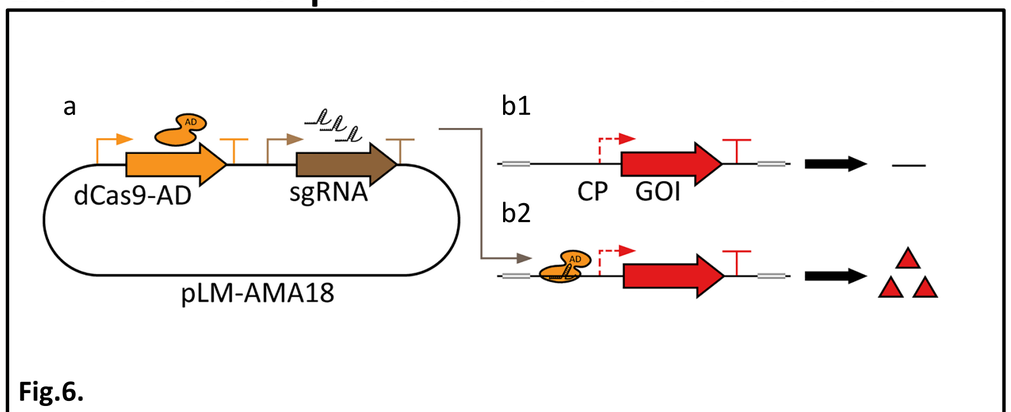

Fig.6.
a) Schematic representation of plasmid delivery of the CRISPRa components (dCas9m4-AD and sgRNA) where AD stands for transcriptional activator domain.
b1) Representation of synthetic silent promoter: no transcription from the gene of interest (GOI) without the integrated QUAS sequences (Figure 1).
b2) sgRNA guided dCas9-AD binding upstream the synthetic silent promoter, promoting transcriptional activation.

Conclusions/Outlook

- Modular, synthetic control devices were developed for *P. chrysogenum* and their function was demonstrated with fluorescent reporters and Penicillin V production.
- The strength of the control devices can be altered by altering the expression of the STF, the core promoter upstream the QUAS or the amount of QUAS elements, leading to expression ranging from barely detectable to similar the highest expressed native genes.
- We anticipate that these well-characterized and robustly performing control devices are highly useful tools in the development of filamentous fungi as production hosts.
- Silent promoter activation was envisioned with the CRISPRa system. This genome editing free transcriptional regulatory tool could be further expand the fungal toolbox of synthetic regulatory systems and could be used to interrogate transcriptionally silent fungal gene clusters.

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