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Using promoter architecture to guide engineering the strongest known fatty acid inducible hybrid promoter in *Yarrowia lipolytica*

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Recently, there have been significant improvements in the genetic toolbox for the industrial oleaginous microbe, *Y. lipolytica*. These tools range from CRISPR-Cas9 mediated genome editing to libraries of hybrid promoters made from strong upstream activating sequences for tunable strength; however, strong inducible promoters remain undeveloped. In addition to tunable strength, having control of timing of gene expression can improve cellular efficiency by separating the growth and production phase. Inducible regulation is particularly important when certain chemicals produced could be toxic and inhibit growth, or when requiring the ability to switch on production at stationary phase. Here, we demonstrate the development of the only known and strongest fatty acid inducible promoter system in *Y. lipolytica*. The hybrid promoter has nearly 50-fold induction strength relative to glucose with expression ranging from 2 to 10-fold higher than the commonly used native inducible acyl CoA oxidase promoter. We show that this range of tunable induction strengths can be accomplished by different functional elements comprising the hybrid promoter. While repressed in the absence of fatty acids, the hybrid promoter lacks catabolite repression in the presence of either glucose or glycerol in fatty acid containing media. Furthermore, this inducible genetic switch can be strongly activated at stationary phase with low concentrations of a wide range of fatty acids ranging from oleic acid to eicosapentaenoic acid. The hybrid promoter activity correlates well with changes in intracellular fatty acid pools, suggesting its use as a tool for strain engineering.

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Mechanosensitive signaling pathways detection during TCFA formation

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A surge of recent studies have highlighted the role of blood flow in determining plaque growth and plaque composition, both in animal studies and recent clinical trials. Despite these compelling data, the underlying mechanism for flow-induced plaque formation is currently unknown. Blood flow is sensed by endothelial cells, and these cells react to blood flow by changing their gene signature. Initial studies showed that approximately 2000 genes are regulated by blood flow, distributed over 40 signaling pathways regulating 15 transcription factors and their changes during plaque development are currently unknown. In this presentation, we report the development of a novel platform that enables to study the adaptation of endothelial gene networks in vascular biology. This platform consists of state-of-the-art ultra-high resolution, small animal imaging (μCT, μMRI and US) coupled to finite element methods to determine the endothelial shear stress and strain fields during the development of the vulnerable plaque. These maps subsequently gave a robot-driven laser-capture machine (Zeiss, PALM MicroBeam) which isolated groups of endothelial cells exposed to a certain shear and strain field during plaque development. Next, RNA was isolated, amplified with linear amplification kits, and libraries are prepared for deep RNA sequencing (Illumina HiSeq 2500). After this, in-house developed bioinformatics tools were applied to decipher gene networks and gene modules. Entire gene networks were subsequently tested with a newly in-house developed siRNA microfluidics system for cardiovascular studies, which consists of a programmable robot-dispenser and a bespoke, in-house developed combined flow-cell and microporator. In parallel to this, live cell imaging tools are developed to determine network dynamics from single cell measurements, which was coupled to the siRNA microfluidics system to steer synthetic network design. In a first series of studies, we have developed a synthetic gene network to determine the activity of a GPCR-dependent mechanosensor and we have coupled this novel technology to a microfluidic chamber to test a library of small drugs to decipher a novel treatment of flow-induced TCFA.

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