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Applications of Plant Physiology and Biology

Srdjan Wagner*

Department of Biology, University of Patras, Patras, Greece

***Corresponding author:** Email: wagner_s@gmail.com

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Description

Biotechnology applications require engineering complex multi-genic traits. The lack of knowledge on the genetic basis of complex phenotypes restricts our ability to rationally engineer them. However, complex phenotypes can be engineered at the systems level, utilizing directed evolution strategies that drive whole biological systems toward desired phenotypes without requiring prior knowledge of the genetic basis of the targeted trait. Recent developments in the synthetic biology field accelerates the directed evolution cycle, facilitating engineering of increasingly complex traits in biological systems. In this review, we summarize some of the most recent advances in directed evolution and synthetic biology that allows engineering of complex traits in microbial systems. Then, we discuss applications that can be achieved through engineering at the systems level.

Ribosome Engineering

Microbes have been successfully employed in a broad range of applications, including in the food, pharmaceutical, petrochemical and bioremediation industries. Increasingly complex applications are demanding more sophisticated designs, that consider optimization of multiple phenotypes such as tolerance, pathway flux, and growth rate, each of which is governed by multiple genes and their interaction with environmental factors. The lack of knowledge on the genetic basis of these complex traits restricts our ability to rationally engineer them. An alternative approach to manipulate these complex phenotypes is to engineer at the systems level, driving entire systems toward desired phenotypes without deep a priori knowledge of the underlying mechanisms.

Recent technological developments facilitate engineering of microbes at the systems level. These advances come primarily through the fields of directed evolution, metabolic engineering, and synthetic biology, with successful applications already at the gene, pathway, genome and even multi-organism (consortia) level. This approach sets the stage to explore diverse and complex functions for novel biotechnology applications. In this review, we discuss strategies that can be used to engineer complex traits in microbial systems. Then, we provide examples and applications of biological systems engineering on increasingly complex scales.

Peptidyl Transferase Center

The ribosome has evolved over billions of years to accelerate the rate of amide bond formation between α-amino acids by more than 107-fold. In order to enable the incorporation of non-α-ncAAs and facilitate the synthesis of polymers comprised solely of such monomers, the ribosomal active site, or the peptidyl transferase center (PTC) will likely have to be redesigned. The PTC is a dynamic pocket that adjusts conformations and interaction of the 3′ terminal peptidyl group of the bound peptidyl-tRNA with the 5′ terminal group of the α-amino group and serves as proofreading for aminoacylated-tRNAs.

In vivo, orthogonal aaRSs that are capable of specifically charging their cognate tRNAs with an ncAA of choice are essential in order for the ncAA to be site-specifically incorporated into a peptide. The orthogonal aaRSs must not cross-react with any canonical amino acids or native tRNAs, requiring that the aaRSs are products of extensive protein engineering and optimization and/or derived from a sufficiently phylogenetically distant organism (often archaea) such that cross-reactivity is innately low. To achieve the necessary properties for orthogonal translation systems, engineering strategies for tRNA charging systems require the use of both positive and negative selections for orthogonal pairs; a positive selection to ensure that the pair successfully can incorporate an ncAA at the amber codon and a negative selection to confirm that the pair is specific to the given ncAA and does not incorporate canonical amino acids.

This can be used to create a sub-population of orthogonal ribosomes in cells that is available for engineering and is independent of wild-type ribosomes that support cell life. For example, Orelle et al. first demonstrated the utility of this system for ribosome evolution by introducing mutations into the tethered ribosome's PTC that improved translation of a problematic protein and would otherwise have been dominantly lethal.

In vivo-based ribosome-engineering strategies offer much potential but present their own challenges associated with cell viability and the requirement that ncAAs must permeate the cell membrane. In vitro, or cell-free, strategies have thus also emerged for ribosome synthesis and evolution.

More recently, the ribosomal subunit linker sequences were optimized to improve activity such that the tethered ribosome could support cellular growth at rates comparable to the wild-type ribosome. Importantly, both tethered and stapled ribosomes have now been shown to be functionally isolated and do not cross assemble to form hybrids. Orthogonal ribosomes have also since been used to design a “flipped” orthogonal system in which the tethered ribosomes translate the proteome and leave the untethered ribosome available for engineering and translation of o-mRNAs.

Notably, this system can be used to introduce mutations that would otherwise be dominantly lethal into the untethered ribosome's rRNA, allowing for evolution of the ribosome to incorporate previously inaccessible ncAAs and produce novel proteins.