

Brief Note on Bacterial Synthetic Biology

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Description

The predictability and robustness of engineered bacteria depend on the many interactions between synthetic constructs and their host cells. Expression from synthetic constructs is an unnatural load for the host that typically reduces growth, triggers stresses and leads to decrease in performance or failure of engineered cells. Work in systems and synthetic biology has now begun to address this through new tools, methods and strategies that characterise and exploit host-construct interactions in bacteria. Focusing on work in *E. coli*, we review here a selection of the recent developments in this area, highlighting the emerging issues and describing the new solutions that are now making the synthetic biology community consider the cell just as much as they consider the construct.

Microbial polyhydroxyalkanoates have been produced as bioplastics for various purposes. Under the support of China National Basic Research 973 Project, we developed synthetic biology methods to diversify the PHA structures into homo-, random, block polymers with improved properties to better meet various application requirements. At the same time, various pathways were assembled to produce various PHA from glucose as a simple carbon source. At the end, *Halomonas* bacteria were reconstructed to produce PHA in changing morphology for low cost production under unsterile and continuous conditions. The synthetic biology will advance the PHA into a bio- and material industry.

Incorporate Noncanonical

Synthetic biology is opening up new opportunities for the sustainable and efficient production of valuable chemicals in engineered microbial factories. Here we review the application of synthetic biology approaches to the engineering of monoterpene/monoterpenoid production, highlighting the discovery of novel catalytic building blocks, their accelerated assembly into functional pathways, general strategies for product diversification, and new methods for the optimization of productivity to economically viable levels. Together, these emerging tools allow the rapid creation of microbial production systems for a wide range of monoterpenes and their derivatives for a diversity of industrial applications.

Stimulated largely by the availability of new technology, biomedical research at the molecular-level and chemical-based control approaches arguably dominate the field of infectious diseases. Along with this, the proximate view of disease etiology predominates to the exclusion of the ultimate, evolutionary biology-based, causation perspective. Yet, historically and up to today, research in evolutionary biology has provided much of the foundation for understanding the mechanisms underlying disease transmission dynamics, virulence, and the design of effective integrated control strategies.

Here we review the state of knowledge regarding the biology of Asian liver Fluke-host relationship, parasitology, phylodynamics, drug-based interventions and liver Fluke-related cancer etiology from an evolutionary biology perspective. We consider how evolutionary principles, mechanisms and research methods could help refine our understanding of clinical disease associated with infection by Liver Flukes as well as their transmission dynamics..

Ms2 Bacteriophage Coat Protein

We recently published a solution to the first of these problems: Membrane less Orthogonally Translating (OT) organelles that are formed by phase separation and targeting to microtubule plus-ends to afford a micron-sized organelle. We define the term organelle as a spatially distinct site in the cell, regardless of its structure or appearance, which nevertheless executes a specific function and has a composition distinct from its surroundings. Phase separation occurs at above the critical concentrations of certain proteins that were fused to PylRS and the ms2 bacteriophage coat protein (MCP), an RNA-binding protein. Although the suppressor tRNA itself is a relatively small molecule, the PylRS-loaded organelle efficiently recruits it, leading to a very high concentration inside the condensate and a very low concentration throughout the rest of the cell. The POI mRNA is labeled in the 3' untranslated region with specific RNA motifs (ms2 loops) that are bound by MCP, thus leading to recruitment of the mRNA into the organelle. Because only the ribosomes processing the recruited mRNA are exposed to a very high concentration of suppressor tRNA^{Pyl}, it is preferentially translated according to an expanded genetic code. By contrast, ribosomes translating mRNA elsewhere in the cell terminate translation once the chosen stop codon is encountered, because no tRNA^{Pyl} is available. Note that

the translational machinery requires that a few hundred factors work smoothly together and no component other than the PylRS and MCP were fused to the organelle scaffold. Thus, despite sharing all other components of translation with the cytoplasm, from which these components are essentially freely accessible, we detected up to 8-fold selectivity for amber suppression of targeted (ms2 tagged) versus untargeted mRNAs. We also showed that the same logic could be applied to reprogramming the opal or ochre codon. Hereafter, we refer to this particular OT organelle-based GCE technology as being mRNA selective.

Schematic representation of the dual-color reporter used to test OT organelles and theoretical FFC plots. mRNAs encoding EGFP and mCherry with amber codons at permissive sites are expressed from one plasmid. The mCherry mRNA is tagged with specific RNA motifs (RMs). In the case of cytoplasmic GCE, both full-length EGFP and mCherry should be produced and give an approximate diagonal in FFC analysis (shown in orange).

If the OT organelle works selectively, only mCherry will be produced, which would result in an mCherry-positive population in FFC analysis (shown in red). Untransfected cells are represented as gray circles.

To achieve this, besides the amber suppressor aaRS/tRNA pair being orthogonal to the host machinery, the orthogonality of multiple, mutually orthogonal OT organelles inside the same cell should be 3-fold. First, the organelles should form independently and not intermix; we term this the “independent assembly” criterion. This criterion considerably extends the previously described general requirement that the mRNA targeted to the organelle is not efficiently translated elsewhere in the cytoplasm by the host’s canonical translation machinery. Second, each OT organelle must recruit a specific subset of mRNAs; this is the “selective RNA recruitment” criterion. Third, each aaRS variant in a respective organelle should selectively utilize a distinct ncAA; this defines the “distinct ncAA specificity” criterion.