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The Congregation of Nano Filament in *Cyanobacteria* for Protein Co-Allotment

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INTRODUCTION

Due to their photoautotrophic capabilities, *cyanobacteria* have great potential as alternative hosts for biotechnological research. However, several crucial aspects, such as product titers, still lag behind established heterotrophic hosts. Nano-biotechnology is a new field with a lot of potential to improve existing hosts, but it hasn't been much looked at in photosynthetic microbial systems so far. Large proteinaceous nano-filaments were found to form in the unicellular model cyanobacterium *Synechocystis sp*., as shown here. PCC 6803 and the *cyanobacteria*l strain *Synechococcus elongatus* UTEX 2973, which has a rapid growth rate. Transmission electron microscopy and electron tomography revealed that *S. elongatus* UTEX 2973's bundles of longitudinally aligned nano-filaments and *Synechocystis sp*. shorter filamentous structures were produced when PduA*, a modified bacterial micro-compartment shell protein, was overexpressed. PCC 6803. Comparative proteomics revealed that nano-filament assembly had little effect on cellular metabolism and that PduA* was at least 50 times more abundant than the second most abundant protein in the cell.

DESCRIPTION

Nano-biotechnology is an emerging field at the intersection of nano and biotechnology that deals with the synthesis, manipulation, and bio-functionalization of nanometer-scale structures. Conceptually, the goal of programming units locally to achieve desired properties globally is intertwined with synthetic biology, a rapidly expanding field. Synthetic biology focuses on using naturally occurring biological units to construct novel structures and functions when applied bottom-up. The desire to control and make use of nanoscale spatial organization is one area where the two fields meet. The scaffolding of biosynthetic pathways is one particularly promising application of the ability to control the spatial organization of biological units. Sequential enzymes of a biosynthetic pathway frequently use cell structural elements, such as lipid membranes and proteinaceous compartments, to form dynamic supramolecular complexes known as metabolons in their native environment. The assembly of enzymes into a metabolon can boost the pathway's catalytic efficiency, reduce crosstalk with native pathways, and prevent the accumulation of toxic intermediates. However, low product titers are frequently the result of the loss of the native structural and regulatory elements required to form the metabolon when biosynthetic pathways are expressed in a heterologous chassis. In an effort to address this, artificial nanostructures, such as scaffolds, have been engineered within heterologous chassis so that enzymes can co-locate to form metabolic hotspots. DNA or protein subunits have been used in the vast majority of enzyme scaffolds. The dockerin and cohesin domains of cellulolytic bacteria, the SH3, PDZ, and GBD domains of metazoan cells, affibodies, and DNA origami tiles are just a few examples. Although scaffolding technology has been utilized successfully in heterotrophic chassis, such as yeast and *E. coli*, photoautotrophic microbes have yet to be developed. Due to their rapid growth and capacity to fix carbon dioxide in the atmosphere, the diverse group of photosynthetic bacteria known as *cyanobacteria* holds great promise as a potential chassis for future manufacturing processes.

CONCLUSION

However, due to difficulties in effective enzyme targeting and co-localization within a cell structure with two membrane systems and a densely packed structure, production titers frequently lag behind heterotrophic production hosts. By introducing a synthetic scaffold into *cyanobacteria* for the first time, we address this issue in this study.

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