Redirecting photosynthetic reducing power

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Photosynthesis drives the production of ATP and NADPH mainly used to fix CO_2. Surplus of redox power can be exploited for biotechnology such as for production of high-value compounds. Important natural products are often synthesized in low quantities by their host organism and can be difficult and expensive to produce by chemical synthesis because of their complex structures. The cytochromes P450 (P450s) situated in the endoplasmic reticulum play key roles in natural product biosynthesis, and are powered by electron transfers from NADPH. We have shown that plant P450s can be expressed in chloroplasts and cyanobacteria can be directed to the thylakoid membrane and that photosynthetic electron transport will support P450 catalytic activity independent of NADPH and dedicated reductases. In order to route reducing power more efficiently to P450s, we have fused them with ferredoxin (Fd) or flavodoxin-like FMN domains. These fusions allow the P450s to obtain electrons for catalysis directly from the photosynthetic electron transport chain by interacting with photosystem I and make them competitive with all the natural occurring ferredoxin requiring enzymes in the chloroplast. Further dedicated redirection of reducing power can be obtained by scaffolding all the enzymes of a pathway on the thylakoid membrane. In a novel strategy, we have fused enzymes with transmembrane domains of TatB and TatC from the chloroplast twin arginine translocation system. This reduced the accumulation of unwanted intermediates and side products and increased the accumulation of the end product fivefold. This work shows that photosynthetic organisms have many attractive features for metabolic engineering, and suggests much unexplored potential for engineering of photosynthetic electron transfer chains to accommodate heterologous enzymes.