Tracking, Testing, Tuning, and Topping Up the Import Activity of Chloroplast-Transit Peptides

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Plastids, including chloroplasts, are specialized organelles crucial for the regulation of photosynthesis and the provision of essential nutrients to plant cells. These organelles contain plastidial proteins primarily encoded by nuclear genomes, which are later transported into the stroma through an integrated import complex located within the membrane layers of plastids. The efficiency of this import process varies among different plastid-targeting proteins and is rigorously controlled by chloroplast-transit peptides (cTPs) predominantly positioned at the N-terminus of each protein. However, a systematic classification of the import activities of cTPs has not been previously established. In this research, we undertook a comprehensive approach that integrated computational prediction, *in planta* expression, fluorescence tracking, and *in vitro* import assays to systematically categorize 89 sequences of combinatorial cTPs collected from a chloroplast protein library based on their distinct import activities. Notably, we discovered a remarkable cTP with high import efficiency, surpassing other candidates in facilitating the translocation of green fluorescent protein into chloroplasts. Intriguingly, our experimental optimization unveiled the significant roles of specific amino acid sequences and the cleavage site of this cTP in enhancing the import efficiencies of key metabolic enzymes and RNA processing proteins associated with photosynthesis within the chloroplast. Our findings present valuable insights into the potential application of biotechnological strategies in chloroplast engineering by manipulating cTPs in specialized plastidial proteins. Moreover, the identified cTP holds promise for enabling the nanostructure-based biomolecules targeting within the plastids of cultivated plants, marking a significant advancement in plant molecular biology.

Short biography:

Chonprakun Thagun obtained his PhD from the Nara Institute of Science and Technology (NAIST), Japan, in 2016. Following this, he worked as a postdoctoral researcher at RIKEN from 2017 to 2021 and later as a program-specific assistant professor at Kyoto University from 2021 to 2022. Currently, he holds the position of a specially-appointed assistant professor at C-Bio, Utsunomiya University. Primarily a plant biotechnologist, he focuses on the biotechnological engineering of commercially-significant traits in crops, with a particular emphasis on tomatoes, potatoes, and strawberries. His current research centers on enhancing the economic characteristics of Tochigi's commercial strawberry cultivars and Japan's wild strawberries, utilizing genetic engineering and gene-editing technologies.