Investigating the role of the ubiquitin E3 ligase SP1 in chloroplast development in plants

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Background:

Chloroplasts are the organelles in plant cells responsible for photosynthesis – the process that harnesses sunlight energy to power cellular activities. As photosynthesis is the source of essentially all energy in biological systems on earth, these organelles are hugely important, not just to plants but to animals and mankind alike, and for agriculture productivity.

Actually, chloroplasts are members of a broad family of related, but structurally and functionally diverse organelles called plastids[1]. Other plastid types are: amyloplasts, which accumulate starch in seeds, roots and tubers; chromoplasts, which accumulate red, orange or yellow carotenoid pigments in fruits and flowers; etioplasts in dark-grown plants; and gerontoplasts in senescent leaves. Significantly, these different plastid types interconvert during specific phases of development, and these transitions are critical for plant growth.

More than 90% of the ~3000 different proteins that are present in chloroplasts are encoded in the cell nucleus. Thus, the development and operation of chloroplasts and other plastids depends on the import of thousands of nucleus-encoded proteins from the cytosol. Protein import is initiated by multiprotein TOC complexes in the plastid outer envelope membrane, and these exist in different client-specific forms[2]. Action of the TOC machinery controls the proteomic composition, developmental fate, and functions of plastids.

The SP1 protein is a widely-conserved RING-type ubiquitin E3 ligase located in the plastid outer membrane[3]. It regulates the TOC machinery by mediating ubiquitination of TOC components, promoting their degradation by the cytosolic 26S proteasome[4]. By increasing or decreasing SP1 levels in transgenic *Arabidopsis* plants, we showed that plastid processes that involve reorganization of the organellar proteome (and corresponding organism-level developmental changes) can be accelerated or retarded[3]. This indicated a requirement for SP1-mediated reorganization of the TOC machinery to accommodate the different sets of proteins that must be imported. Thus, SP1 orchestrates proteasomal activity to control the plastid's proteomic make-up, development and functions. Recognizing its potential commercial importance in agriculture, we patented SP1 as a tool for the manipulation of various crop plant developmental processes in which plastids play a critical role.

Proposed work:

This project has a dual focus. In the first part (1. below), the student will elucidate the molecular mechanisms underlying the removal of TOC proteins following ubiquitination by SP1. In the second part (2. below), the role of SP1 in fruit ripening in tomato (a process characterized by the conversion of chloroplasts to bright red chromoplasts) will be studied. In both parts, the student will work alongside an experienced postdoctoral scientist, Dr Qihua Ling[3,4]. The work may involve a full range of molecular biology techniques (DNA, RNA and protein extraction, electrophoresis, blotting, molecular cloning, PCR, etc.), as well as fluorescence microscopy, plant physiology and plant genetics.

1. The AAA+ chaperone protein CDC48 (p97) has emerged as an important player in ubiquitin-mediated protein degradation[4]. In the ER-associated degradation (ERAD) pathway, it provides driving force for the extraction of membrane proteins to be degraded by the cytosolic proteasome, and was recently found to act in mitochondrial membrane protein degradation. A role for CDC48 in chloroplasts has not been reported, but we speculate that ubiquitinated TOC components (which are integral membrane proteins) are extracted by CDC48. The student will investigate this possibility, using *Arabidopsis thaliana* as the model system.

2. Transgenic tomato plants that either over- or under- express SP1 have been generated. The student will study these plants to determine the extent to which fruit ripening can be controlled by modifying the activity of SP1. The plants will also be studied to further elucidate SP1's role in other aspects of plant development, in the context of an important crop species.

1. Jarvis, P. & Lopez-Juez, E. Biogenesis and homeostasis of chloroplasts and other plastids. *Nat. Rev. Mol. Cell Biol.* **14**, 787-802 (2013).
2. Jarvis, P. Targeting of nucleus-encoded proteins to chloroplasts in plants (Tansley Review). *New Phytol.* **179**, 257-285 (2008).
3. Ling, Q., Huang, W., Baldwin, A. & Jarvis, P. Chloroplast biogenesis is regulated by direct action of the ubiquitin-proteasome system. *Science* **338**, 655-659 (2012).
4. Ling, Q. & Jarvis, P. Dynamic regulation of endosymbiotic organelles by ubiquitination. *Trends Cell Biol.* **23**, 399-408 (2013).