Loss-of-function mutation in SCY1 triggers chloroplast-to-nucleus retrograde signaling in Arabidopsis thaliana

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Abstract

The secretory (Sec) pathway is one of the most important systems for transporting proteins across the thylakoid membrane into the lumen. Two Arabidopsis genes encoding SecY translocon proteins, designated SCY1 and SCY2, were characterized in this study. Semi-quantitative RT-PCR and histochemical staining β-glucuronidase (GUS) activity reveal that both SCY1 and SCY2 promoters were active in germinating seeds, etiolated cotyledons, and flowers, but not in roots. In particular, the expression of GUS gene driven by the SCY1 promoter was almost undetectable in green leaves, whereas GUS staining controlled by the SCY2 promoter was clearly detected. Moreover, homozygous scy1-1 plants could grow heterotrophically but appeared sensitive to radiation. Further studies show that chloroplasts of scy1-1 were arrested in early developmental stages with fewer thylakoid membranes. Real-time quantitative RT-PCR reveals that a number of nuclear-encoded genes involved in chlorophyll biosynthesis and photosynthesis were substantially down-regulated in the scy1-1 mutant. All these results indicate that the SCY1/2 genes were regulated developmentally and spatially, and a loss-of-function mutation in SCY1 triggered chloroplast-to-nucleus retrograde signaling in Arabidopsis thaliana.

Introduction

Chloroplasts are responsible for essential plant functions, such as fixation of CO₂, syntheses of many amino acids, fatty acids, secondary metabolites, hormones, etc. (Mueller and Martin 1997, Staehelin and Newcomb 2000, Taylor et al. 2005). Radiation is one of the most important environmental signals controlling chloroplast development and photosynthesis (López-Juez and Pyke 2005, Dias et al. 2013). Although the chloroplast possesses its own genome, it encodes only a small proportion of chloroplast proteins. Most chloroplast proteins are encoded by nuclear genes, translated in the cytosol as precursor proteins, and posttranslationally imported into the chloroplast stroma (Leister 2003). From the stroma, intermediate precursors are translocated into or across the thylakoid membrane by at least four distinct pathways: secretory (Sec), twin arginine translocation (Tat), signal recognition particle (SRP), and spontaneously (Di Cola et al. 2005).

The Sec system is an evolutionarily conserved protein translocation pathway found at the plasma membrane of bacteria and at the thylakoid membrane of plant and algal chloroplasts (Veenaadaal et al. 2004, Vrontou and Economou 2004, Denks et al. 2014). The bacterial Sec system is composed of heterotrimeric SecYEG translocation channel and SecA ATPase subunit (Veenaadaal et al. 2004). In higher plants, cpSecA gene in pea (Nakai et al. 1994), spinach (Berghofer et al. 1995), maize (Roy and Barkan 1998), and Arabidopsis (Liu et al. 2010), cpSecY gene in Arabidopsis (Laidler et al. 1995, Skalitzky et al. 2011), spinach (Berghofer and Klosgen 1996), maize (Roy and Barkan 1998), and pea (Mori et al. 1999), and cpSecE gene in Arabidopsis (Schuenemann et al. 1999, Skalitzky et al. 2011) have been identified. Since the functions of the chloroplast are largely dependent on the function of properly localized proteins of photosynthetic apparatus, disruption of cpSec and other chloroplast

Although the nucleus predominantly determines chloroplast gene expression and controls chloroplast development, the developmentally arrested or damaged chloroplast has been shown to control a set of nuclear genes that encode chloroplast-localized proteins through chloroplast-to-nucleus retrograde signaling (Surpin et al. 2002, Woodson and Chory 2008, Liu et al. 2014). For instance, norflurazon (NF), an inhibitor of carotenoid synthesis, is well-known to cause a photooxidative damage of chloroplasts and leads to a decreased transcription of a set of nuclear photosynthesis-related genes (Gray et al. 2003, Aluru et al. 2009). Mutations in a large number of genes, such as ispD, ispE, EMB1303, PDS3, SPC1, and VAR2, block chloroplast development and result in a significantly decreased expression of nuclear-encoded genes involved in chloroplast function (Dong et al. 2007, Qin et al. 2007, Hsieh et al. 2008, Huang et al. 2009, Putarjunan et al. 2013).

The SecY, the translocon protein of Sec system, is encoded in Arabidopsis by two homologous genes, SCY1 and SCY2 (Skalitzky et al. 2011). The aim of this study was to analyze the tissue specific expression pattern of SCY1/2 as well as to characterize the promoter activity of SCY1/2 genes and a previously reported mutant with T-DNA insertions in SCY1 gene of Arabidopsis (Skalitzky et al. 2011) in more details.

Materials and methods

Plants and growth conditions: Arabidopsis thaliana L. ecotypes, Columbia-0 (Col-0) and Wassilewskija (Ws), were used for the experiments. The T-DNA insertion mutant scy1-1 (CS16483) was obtained from the Arabidopsis Biological Resource Center (Alonso et al. 2003). Seeds were surface sterilized in a solution of 10 % (v/v) sodium hypochlorite plus 0.01 % (m/v) Triton X-100 for 10 min, and 15 times washed in a sterilized water. Following a 3-d stratification in the dark at 4 °C, these seeds were germinated, and seedlings were grown on plates containing a half-strength Murashige and Skoog (1/2 MS) medium with 0.75 % (m/v) agar and 1 % (m/v) sucrose (pH 5.7) at a temperature of 22 ± 1 °C, a 16-h photoperiod, and an irradiance of 90 µmol m−2 s−1.

For a low irradiance treatment, 5-d-old scy1-1 and wild-type seedlings grown on a 1/2 MS medium under a normal irradiance of 90 µmol m−2 s−1 were shifted to a low irradiance of 5 µmol m−2 s−1 for 12 or 24 d. Phenotypes were then recorded with a scanner (Epson perfection V30). For a heterotrophic growth analysis, scy1-1 and wild-type seeds were germinated and grown in the dark for 5 d, then transferred to an irradiance of 90 µmol m−2 s−1 and grown for 7 d. The lengths of the primary root and the hypocotyl of each seedling were measured.

 Constructs and plant transformation: To construct a SCY1::GUS fusion gene, a 2 085-bp promoter fragment of SCY1 gene (At2g18710) was amplified from an Arabidopsis genomic DNA by PCR. The pair of primers used in PCR was Pscy1-F and Pscy1-R (Xba I and Nco I sites were introduced). A specific PCR fragment was then inserted into a binary vector pCAMBIA1301 between Xba I and Nco I sites, replacing the CaMV 35S promoter, to create a recombinant transcription unit SCY2::GUS. The recombinant plasmids were then introduced into Agrobacterium tumefaciens strain GV3101 and transformed into wild-type Arabidopsis plants using the floral dip method (Clough and Bent 1998). Primers presented in this paper are listed in Table 1 Suppl.

 RNA extraction, cDNA synthesis, and gene expression analysis: A total RNA was extracted from plant tissues with TRI reagents, followed by a treatment with RNase-free DNase I (TaKaRa, Dalian, China) to degrade a genomic DNA at 37 °C for 1 h. A first strand cDNA was synthesized from 2.0 µg of the total RNA using ImProm-II reverse transcriptase (Promega, Madison, WI, USA) in 0.01 cm3 of a reaction mixture following the manufacturer’s instructions with minor modifications. For a semi-quantitative RT-PCR analysis, a PCR reaction contained 0.001 cm3 of a template, 1.5 U ExTag (TaKaRa), dNTPs (0.2 mM each), and primers in a final volume of 0.03 cm3. A TIP41-like gene, one of the recommended new quantitative RT-PCR standards (Czechowski et al. 2005, Udvardi et al. 2008), was used as an internal control. A real-time RT-PCR analysis was performed as described previously (Liu et al. 2010). The TIP41-like gene and ACTIN2 gene were used as internal controls, respectively. Primers presented in this paper are listed in Table 1 Suppl.

Transmission electron microscopy (TEM): Sections of leaf tissue were prepared for electron microscope analysis as previously described (Choy et al. 2008) with minor modifications. Briefly, Arabidopsis leaf tissue was fixed with glutaraldehyde followed by osmium tetroxide and dehydrated in an ethanol series before being infiltrated with Spurr’s resin. Polymerization was conducted at 70 ºC for 8 h. Specimens were sliced to yield ultra-thin sections and stained with uranyl acetate and alkaline lead citrate before being examined with a transmission electron