A Novel Trojan Horse for Molecule Delivery into Plants

The agronomic application of nanotechnology harbors huge potential for future agriculture (Landry and Mitter, 2019). Within the last years, nanocarriers have emerged as vehicles for the delivery of cargo (RNA, DNA, protein, and plant protection substances) into the plant cell (Wang et al., 2016). RNA-induced gene silencing (also known as RNA interference) is a reliable method to study and alter the genetic form and function of plants. Nanocarriers offer the possibility to directly deliver small interfering RNAs (siRNAs; double-stranded RNAs of 20–25 bp) into the plant cell without involving a biological carrier (e.g. viruses) or genetic transformation (Cunningham et al., 2018).

Carbon-based nanostructures such as carbon dots and single-walled carbon nanotubes (Demirer et al., 2019, 2020) are valuable alternatives to common transformation methods, since they do not require genetic transformation (Wang et al., 2016) and avoid heavy metal nanoparticles, usually used for biolistic transformation (Klein et al., 1987). Although single-walled carbon nanotubes and carbon dots share most beneficial properties, they differ in size. Single-walled carbon nanotubes are ~1 nm in diameter and up to 1,000 nm in length; in contrast, carbon dots are on average ~3 nm in size (Demirer et al., 2020; Schwartz et al., 2020). The benefits of carbon-based nanostructures are a high aspect ratio (i.e. the ratio of length to width), good biocompatibility (especially compared with metal nanoparticles), and the ability to protect bound biomolecules from cellular metabolism and degradation (Demirer et al., 2019; Kwak et al., 2019). Furthermore, tissue-specific tracking based on their fluorescent properties and intracellular on-demand cargo release holds great promise for broad application (Wang et al., 2016).

Figure 1. Carbon dot-delivered siRNA-mediated gene silencing in plants. A, Schematic representation of carbon dot leaf entry and intracellular siRNA release. B, Spray application of carbon dot-delivered siRNAs. GFP fluorescence (GFP fluores.) in leaves of a tomato reporter line was imaged after treatment with a non-GFP-specific siRNA (Control) or a GFP-specific siRNA (Treatment). Images were taken 5 d after application. Adapted from Schwartz et al. (2020).
Carbon dot-based siRNA delivery into plant cells expands the spectrum of carbon-based nanoparticles for molecule delivery into plant cells and is an exciting tool for fundamental and applied plant science. The simple spray application makes it well suited for large-scale agricultural use. Furthermore, prepared carbon dots are also persistent and retain overall efficacy for at least 1 week of storage before application. Nevertheless, a comparable high efficacy with less established plant species needs to be shown, and fine-tuning cellular entry and intracellular siRNA release can further optimize broad-spectrum application.

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Cunningham et al., 2018). The cellular uptake of nanocomplexes is facilitated by endocytosis (Fig. 1), and intracellular release of the nanocomplex is caused by an osmotically driven endosome burst, called the proton-sponge effect (Behr, 1997). The utilization of nanoparticles that respond either to intracellular stimuli (pH, redox state, and enzymes) or external stimuli (light or ultrasound) facilitates controlled cargo release within the cell (Wang et al., 2016; Cunningham et al., 2018).

In this issue of *Plant Physiology*, Schwartz et al. (2020) establish the use of carbon dots for the delivery of siRNAs as a novel tool for gene silencing in plants. Simple spray application of carbon dots results in highly efficient reporter and endogenous gene silencing in model and crop species and holds great potential for field application. In their study, Schwartz et al. (2020) optimized the chemical synthesis and purification of carbon dots before application. The low-cost bottom-up synthesis of carbon dots is based on a one-pot reaction using citrate or Glc with branched polyethyleneimine for carbon dot surface functionalization. Size-exclusion chromatography of prepared carbon dots revealed that carbon dots with an average size of 3.8 nm in diameter, combined with 22mer siRNAs, are most efficient for in planta gene silencing.

Whereas previous nanoparticle approaches in plants rely on particle bombardment (Klein et al., 1987) or leaf infiltration (Demirer et al., 2019, 2020), carbon dots with siRNA cargo are efficient for gene silencing upon low-pressure spraying application (Schwartz et al., 2020). GFP transcript and protein abundance were more than 80% reduced in reporter lines of wild tobacco (*Nicotiana benthamiana*) and tomato (*Solanum lycopersicum*) 5 d after application (Fig. 1). Remarkably, systemic spreading of silencing was observed in emerging leaves 12 d after application due to intercellular and long-distance movement of siRNAs (Melnyk et al., 2011).

Reporter gene independent validation was achieved by targeting the endogenous H and I subunits of magnesium chelatase. Magnesium chelatase catalyzes the insertion of magnesium into protoporphyrin IX, an essential step in chlorophyll biosynthesis, and knockdown results in leaf bleaching. Comparable to GFP silencing, endogenous gene silencing also reached an 80% reduction in mRNA level.