LETTER

Source of sugar nucleotides for starch and cellulose synthesis

We wish to correct a potentially misleading impression about the provision of substrate for starch and cellulose synthesis presented in a recent paper by Baroja-Fernández et al. (1).

The plant carbohydrates starch and cellulose are synthesized from ADPglucose and UDPglucose respectively. There is overwhelming evidence that ADPglucose for starch synthesis is made via the plastidial enzyme ADPglucose pyrophosphorylase. Some ADPglucose may also be made via the cytosolic enzyme sucrose synthase (SUS), which catalyses the interconversion of sucrose and UDP or ADP with fructose and UDPglucose or ADPglucose (2). Indirect evidence indicates that UDPglucose for cellulose synthase is made via SUS (e.g., 3).

To investigate the importance of SUS for starch and cellulose synthesis in *Arabidopsis*, we studied the locations of the six SUS isoforms and isolated mutants lacking multiple isoforms (4, 5). We established that combinations of isoforms SUS1, SUS2, SUS3, and SUS4 account for SUS in all cell types except the phloem. Isoforms SUS5 and SUS6 appear to be located exclusively in the phloem, the sucrose transport system of the plant, where they putatively provide UDPglucose for synthesis of the carbohydrate callose. Thus, the *sus1/sus2/sus3/sus4* quadruple mutant has no SUS in any cell type except the phloem. Nonetheless, we found that it has normal starch and cellulose contents. We concluded that SUS is not required for starch or cellulose synthesis. It might participate in these processes in WT plants, but other enzymes can also provide all the sugar nucleotide required.

Baroja-Fernández et al. (1) challenged this conclusion. Surprisingly, they asserted that it was based mainly on measurements of SUS activity in WT and mutant plants, and ignored our findings about the location of SUS isoforms. This is a misinterpretation of our work. We did indeed report that SUS activity in WT leaves was lower than the rate of starch synthesis. However, as discussed above, our conclusion was based principally on discoveries about the location of SUS isoforms rather than measurement of catalytic capacity. It remains valid whether leaf activity is lower or higher than the rate of starch or cellulose synthesis. Baroja-Fernández et al. (1) claimed that our assay for SUS activity was flawed, and have described a new assay. Their measurements (at 37 °C) are higher than ours (at 25 °C) for WT leaves and for the *sus1/sus2/sus3/sus4* mutant but are generally comparable with ours for WT and *sus5/sus6* stems. From this, they concluded that our values were "a gross underestimation."

We agree with Baroja-Fernández et al. (1) that methods for assaying SUS activity may generally require further development. However, this technical issue should not distract attention from the pressing biological question raised by our work. Our results support the conventional view that ADPglucose pyrophosphorylase provides ADPglucose for starch synthesis, but they reveal a major gap in knowledge about the source of UDPglucose for cellulose synthesis. Cellulose is the most abundant carbohydrate on earth, and of crucial importance as a carbon sink and a source of fuel and renewable materials. The focus should now be on elucidation of the pathway of its synthesis.

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