siRNA: A New Reverse Genetics Tool

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The heterogeneous process of reversible, photoprotective energy dissipation [commonly referred to as nonphotochemical quenching of chlorophyll fluorescence (NPQ)] is a major route of light utilization on the planet. In higher plants, NPQ occurs in Photosystem II, requires the xanthophyll zeaxanthin, and the dominant (qE) phase appears and relaxes on a time scale of seconds. Despite considerable effort in several laboratories, the mechanism of NPQ remains obscure. A significant development in this field was the discovery that the 22-kiloDalton nuclear-encoded psbS protein is essential for qE. Structure/function analysis of psbS using transformants of a null psbS line of Arabidopsis is hampered by the laborious nature of this approach. To address this challenge, we have developed a new high throughput system for analysis of psbS structural variants in Nicotiana benthamiana. First, virus-induced gene silencing (VIGS) employs small interfering RNAs (siRNA) to efficiently suppress expression of endogenous N. benthamiana psbS and thereby create a de facto deletion phenocopy. Secondly, Agrobacterium-mediated transient expression of native (eg. N. benthamiana) or orthologous Arabidopsis psbS resulted in accumulation of psbS and partial complementation of NPQ-deficiency in pre-silenced N. benthamiana leaves. An important implication of this effort is that siRNA introduced by VIGS can facilitate creation of a knockout phenocopy for any nuclear gene and thereby enable systematic molecular dissection of multi-component systems for functional analyses.