Emerging role of SGT1 as a regulator of NB-LRR-receptor nucleocytoplasmic partitioning

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Plants have evolved multiple defense mechanisms against viruses that interfere with the infection process at several key stages. Potato plants carrying Rx, which encodes CC-NB-LRR type R protein, establish an extreme resistance (ER) to potato virus X (PVX) and severely attenuate virus multiplication. In resistant tobacco plants, tobacco mosaic virus (TMV) multiplies in inoculated cells and moves intercellularly before triggering a hypersensitive response (HR) mediated by N protein. The function and stability of both Rx and N depend on the activity of a chaperone complex containing SGT1 (Table 1). We recently showed that localization of SGT1 exclusively in the nucleus shifted the cytoplasmic N protein pool toward the nucleus whereas forced cytoplasmic localization of SGT1 did not reduce nuclear N levels.5 Previous reports suggested that Rx trafficking might be forced cytoplasmic localization of SGT1 did not reduce nuclear the cytoplasmic N protein pool toward the nucleus whereas showed that localization of SGT1 exclusively in the nucleus shifted equilibrium between these pools is required for full resistance, suggesting tight regulation of nucleocytoplasmic receptor shuttling. We recently showed that SGT1, a protein that controls NB-LRR receptor stability and activity, facilitates nuclear import of N protein, which is a TIR-NB-LRR receptor. In this addendum, we show that the subcellular localization of Rx, a CC-NB-LRR protein, reflects the positions of SGT1 ectopic variants in the cell. This suggests that SGT1 might have a general role in maintaining the nucleocytoplasmic balance of NB-LRR receptors. We discuss these results in light of differences in the N and Rx systems of effector-triggered immunity.

To determine the functional relationship between SGT1 and Rx, we transiently expressed SGT1 variants with forced cytoplasmic or nuclear localization and monitored the effects on cellular Rx distribution. First, endogenous SGT1 was silenced in *Nicotiana benthamiana* plants using virus-induced gene silencing (VIGS). Subsequently, YFP-Rx was transiently expressed in systemic leaves via bombardment in the presence of AtSGT1b, which carried either nuclear localization signal (NLS; PKKKRKV), nuclear export signal (NES; NELALKAGLDINK), or mutated versions thereof (nls and nes, respectively). As previously described, NLS-AtSGT1b showed nuclear or nucleocytoplasmic distribution, NES-AtSGT1b was detected predominantly in the cytoplasm, whereas control constructs with the mutated targeting signals in some cells were found in the cytoplasm, but in others were distributed between the nucleus and cytoplasm. The images showed that Rx distribution exactly mirrored that of ectopic AtSGT1b variants (Figure 1A), and this was supported by measurements of relative fluorescence intensities (Figure 1B, Pearson correlation coefficient (r), calculated for cells co-expressing Rx and AtSGT1b, equals 0.8). This suggests that SGT1 facilitates both Rx import into and export from the nucleus, in contrast to that for N protein, which was relocated only toward the nucleus in our experiments.5 In *SGT1*-silenced plants, N protein has a nucleocytoplasmic distribution pattern similar to that in wild-type plants, which suggests that SGT1 is not essential for nuclear import of N protein but modulates its trafficking. These results may reflect the involvement of Rx and N receptors in distinct resistance responses to viral infection (i.e., ER and HR), in which either the cytosolic or nuclear receptor pool plays a predominant role. Another scenario that cannot be excluded is that, in addition to homodimers composed of full-length N protein, two N forms (e.g., full-length and truncated) encoded by alternatively spliced transcripts, or two truncated forms could associate as other types of hetero- or homo-dimers, respectively. This would add significant system complexity because the different complexes might have different degrees of sensitivity to SGT1 regulation.

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Figure 1. For legend, see next page.
In summary, Rx and N belong to different classes of plant NB-LRR receptors, and confer distinct types of resistance to viral infection, which include ER and HR, respectively. However, recent results and Figure 1 show that nucleocytoplasmic receptor shuttling might be regulated in both systems by SGTL1 in the LRR-dependent manner (Table 1). This reveals a novel role of SGTL1 in effector recognition by NB-LRR receptors, in addition to its role in the control of steady-state levels and activities of the receptors. We proposed that partitioning of the receptors can be finely tuned by phosphorylation of SGTL1, which might establish another surveillance system. However, the exact mode of SGTL1 action in the translocation process remains to be elucidated.

This model does not exclude that the proper equilibrium between nuclear and cytoplasmic receptor pools can be maintained by other means. Multiple levels of regulation might provide specificity for each pathosystem. For example, the observation that the cytoplasmic Rx pool seems to play a dominant role in potato resistance to PVX has been consistent with the fact that cytoplasmic transport of Rx is also controlled by RanGAP2. We speculate that during tobacco defense responses, the N protein might be regulated by dynamic association of the full-length N protein with the truncated N form encoded by alternatively spliced transcripts. Work is underway to test this proposal.

Due to space constraints, we have not focused on SGTL1 role in nucleocytoplasmic shuttling mediates nuclear import and export (Fig. 1).

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