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Article Addendum

Dissection of Silencing Signal Movement in Arabidopsis

Lisa M. Smith†

David C. Baulcombe*

The Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, United Kingdom

†Present Address: Max Planck Institute for Developmental Biology; Spemannstraße 37-39; Tübingen 72076 Germany

*Correspondence to: David C. Baulcombe; The Sainsbury Laboratory; John Innes Centre; Colney Lane; Norwich NR4 7UH United Kingdom; Fax: +44.1603.450.011; Email: david.baulcombe@tsl.ac.uk

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Addendum to:

An SNF2 Protein Associated With Nuclear RNA Silencing and the Spread of a Silencing Signal Between Cells in Arabidopsis

Smith LM, Pontes O, Searle I, Yelina N, Yousafzai FK, Herr AJ, Pikaard CS, Baulcombe DC

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ABSTRACT

In our recent paper in *Plant Cell*, we examined the phenomenon of non-cell autonomous RNA silencing through a genetic screen of the requirements for cell-to-cell signal movement.[1] We found a requirement for components of the nuclear and trans-acting RNA silencing pathways in blocking or enhancing the spread of silencing and identified a new SNF2 domain-containing protein, CLSY1, in the nuclear RNA silencing pathway. Here we discuss our data from a broader perspective of other recently published papers.

Concurrent with our study, a similar screen has been carried out by Olivier Voinnet's group.[2-4] Both studies use the SUC2 companion cell-specific promoter to drive transcription of an inverted repeat that triggers silencing of an endogenous target to give a visual phenotype upon movement of the silencing signal.[1,2] Our study uses a *PHYTOENE DESATURASE* (*PDS*) inverted repeat, while the Voinnet lab uses a *SULFUR* (*SUL*) inverted repeat. Both studies have reported that the silencing phenotype is sensitive to mutation of a Dicer-like protein (*DCL4)* but not an RNA-dependent RNA polymerase [*RDR6*[1-3]]. In their latest paper,[4] the Voinnet group has also extended their analysis into the nuclear RNA silencing pathway. Although the results from these concurrent studies are largely in agreement, there are some significant differences in the findings that will be discussed here along with the factors that may have led to these discrepancies.

A loss of some trans-acting siRNAs was reported in a subset of the mutants recovered from each screen.[1,3] However the identities of the trans-acting siRNA mutants recovered from the two studies differs substantially with eight *dcl4* alleles recovered in the P_{Suc2}:*SUL* screen[3,4] and three alleles of a novel factor recovered from the P_{Suc2}:*PDS* screen.[1] Identification of this second factor has not been reported from the P_{Suc2}:*SUL* screen, while no *DCL4* alleles were recovered amongst the fifteen characterized lines from our screen. In our screen, *DCL4* is expected to be recovered if the screen were saturated as introgression of the *dcl4-1* allele leads to a reduction in silencing. We speculate that saturation would lead to identification of the same factors in the two screens and also identify further cofactors in the pathway.

The two studies report formation of 21- and 24-nt siRNAs from the inverted repeat constructs and their targets. However, these two siRNA species are found in approximately equal levels in the P_{Suc2}:*SUL screen*[4] while the 24-nt siRNAs are predominant in silencing triggered by the *PDS* inverted repeat.[1] The study utilizing the *SUL* inverted repeat reports the involvement of a number of proteins of the miRNA pathway in the spread of silencing[4] with reduced silencing due to introgressed *dcl1* and *HUA ENHANCER* (*hen1*) mutations also identified in our studies (LS, unpublished data).

New alleles of *RDR2* and the largest subunit of PolIVa (*NRPD1a*), factors of the nuclear RNA silencing pathway, were recovered from both screens and led to a reduction in silencing. In the P_{Suc2}:*SUL* study these alleles were not associated with a loss of siRNAs from the target and inverted repeat loci.[4] In contrast, in our P_{Suc2}:*PDS* system, a large reduction in both sizes of siRNAs was noted in each of the *nrpd1a* and *rdr2* alleles studied.[1] In addition, our study found a clear role for an ARGONAUTE protein (AGO4) and DCL3 in limiting the spread of RNA silencing, while there was no effect of mutation of these genes in the P_{Suc2}:*SUL* system.[4] We report the identification of CLSY1, a novel factor of the nuclear RNA silencing pathway. *CLSY1* mutations were the most frequent alleles identified in our screen, with six of our fifteen lines carrying mutations within *CLSY1*. This gene has not been reported among the mutants from the Voinnet lab.

Silencing triggered by the P_{Suc2}:*PDS* transgene appears more sensitive to perturbation in the nuclear silencing pathway than the silencing initiated by the P_{Suc2}:*SUL* transgene. Two factors that could affect the pathway through which RNA silencing proceeds are the

endogenous target and the inverted repeat T-DNA. The target of silencing could affect RNA silencing through properties such as GC content, the 5' or 3' position of the targeted RNA, gene expression level or chromatin structure prior to introduction of the transgene. In turn, the structure of the T-DNA insertion may be important in terms of number of independent T-DNA insertion sites, number of copies of the T-DNA at the insertion site(s) and any rearrangements within the T-DNA. In our study, the insertion event in one of the two lines used in the screen is complex with two truncated or rearranged copies of the T-DNA in an inverted repeat structure. The complexity of this insertion may make the T-DNA locus structurally similar to an endogenous nuclear RNA silencing target and so channel RNA silencing predominantly through this pathway. The structure of the P_{Suc2}:*SUL* insertion used in the concurrent study has not been discussed, but we speculate that differences in insertion site architecture may contribute to the different outcomes of these two studies.

In conclusion, these two studies were designed to dissect the cell-to-cell movement of silencing however it is not possible thus far to attribute the affects of the individual factors to perturbation of production, translocation or reception of the signal. To further dissect the effects of these genes it will be necessary to express them specifically in cells initiating, translocating or receiving the silencing signal, which will require redesign of the silencing system used.

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