WHAT IS HIGH IMPACT?

High Impact is a new feature in *Plant Physiology* that will spotlight a highly cited paper from a past issue and follow how it has been cited and the impact it has had on research. This section will examine articles in which the highlighted paper has been cited and will include opinions from those authors on how this particular paper affected their research. The goal of this new feature is to show the evolution of a research paper as it is utilized by other researchers to further advance science.

THIS MONTH'S SELECTION

The paper selected this month is ''Evidence for nuclear processing of plant micro RNA and short interfering RNA precursors'' by Papp et al., which appeared in our July 2003 issue. It is evident that conclusions in this article reach beyond the plant community, as it has been cited by researchers using both plant and animal systems. Three articles that cite Papp et al. (2003) will be used as examples to explore different aspects of its research.

BACKGROUND

RNA silencing is a gene-specific inactivation system whose specificity is achieved by double-stranded RNA (dsRNA) cleaved by an RNase III, which has been dubbed Dicer. The resulting small RNAs associate with different silencing complexes that can lead to mRNA degradation, translational repression, or genome modification. Two classes of short RNAs are known to be involved in silencing: short interfering RNA (siRNA) and micro RNA (miRNA).

In plants, RNA silencing can occur either transcriptionally or posttranscriptionally. At the transcriptional level, a class of siRNA 24 to 26 nucleotides in length is believed to be involved in DNA methylation and systemic silencing. A smaller class of siRNAs and miRNAs 21 to 22 nucleotides in length is involved in posttranscriptional gene silencing by targeting mRNAs for degradation via an endonuclease complex.

In contrast with other organisms examined, there are multiple Dicer-like (DCL) proteins in plants. Four members of the Dicer gene family, DCL1 to DCL4, have been found in Arabidopsis (Schauer et al., 2002). Two of these, DCL1 and DCL4, contain nuclear localization signals. Although neither enzyme has been definitively shown to be located in the nucleus, DCL1 has been shown to be required for producing miRNA (Park et al., 2002; Reinhart et al., 2002).

WHAT WAS SHOWN

To address where in the plant cell the dsRNA is processed, Papp et al. used Arabidopsis plants containing a nopaline synthase promoter (NOSpro)-driven locus further transformed to express the tomato bushy stunt virus suppressor of posttranscriptional gene silencing P19, targeted either to the nucleus or to the cytoplasm. P19 recognizes and interacts with RNA cleaved by DCL and has been shown to inhibit targeting of the viral RNAs potentially by preventing incorporation of the short RNA into RNA-induced silencing complex (Baulcombe and Molnar, 2004). P19 has also been shown to prevent siRNA accumulation from transiently expressed dsRNA (Hamilton et al., 2002). It was reasoned that the expression of P19 in either the nucleus or the cytoplasm would enable the determination of the location of nuclear-encoded dsRNA processing due to a decrease in the abundance of the short RNAs in that compartment.

No decrease in quantity of the short RNAs was observed in the plants containing the cytosolic P19. Under this condition, two size classes of both siRNA and miRNA appeared: normal sized and those truncated by two nucleotides. This suggests that although P19 has the effect of shortening the RNAs, the dsRNA is not processed in the cytoplasm because there is no change in the accumulation of the short RNAs. In contrast, those plants containing the nuclear-located P19 displayed a decrease in the accumulation of both sense and antisense NOSpro short RNAs, implying that the DCL cleavage products are in the nucleus and that the processing of the tiny RNAs is in the nuclear compartment.

This observation raised the following question: If there is nuclear processing of the dsRNAs, which DCL is involved? Two of the four Arabidopsis DCLs have nuclear localization signals: DCL1 and DCL4. DCL1, previously implicated in miRNA processing (Park et al., 2002; Reinhart et al., 2002), would be a likely candidate. Transient expression of a DCL1 nuclear localization signal fused with green fluorescent protein in onion was localized in the nucleus. This suggests the nuclear localization signals of DCL1 are functional and DCL1 is located in the nucleus, thus further implicating DCL1 involvement in the processing of the dsRNAs in the nucleus. Indeed, in *dcl1* plants, accumulation of the miRNA miR159 was decreased relative to wild type, again supporting that miRNAs are processed by DCL1. Despite the observed decrease in NOSpro siRNAs in plants expressing nuclear-located P19, there was no change in the accumulation of the

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NOSpro siRNAs in the *dcl1* plants. Therefore, the authors concluded that the siRNAs are processed by a different DCL protein than the miRNAs, possibly DCL4, which also has a nuclear localization signal.

THE IMPACT

As of August 2005, Papp et al. (2003) was cited 55 times according to a cited reference search in Thomson ISI Web of Science (http://www.isinet.com/). Of these, three papers have been selected that use the highlighted article to support findings from their studies.

In the paper "Nuclear processing and export of microRNAs in Arabidopsis," Park et al. (2005) describe the distribution of miRNAs and tRNAs within cells of Arabidopsis along with the effect of *hasty* (*hst*) and *paused* (*psd*) mutations on their accumulation. The two genes *HST* and *PSD* were identified among genes shown to be necessary for the transition from juvenile to adult in Arabidopsis. Both are members of the importin β family of nucleocytoplasmic transport receptors and are orthologs of yeast or mammalian receptors. The function of HST in plants was uncertain, as HST orthologs in mammals and yeast have different functions. Among the functions in mammals, the ortholog Exportin-5 was shown to be involved in the export of pre-miRNAs from the nucleus to the cytosol. Export of pre-miRNA from the nucleus would imply cytoplasmic processing and thus processing by a DCL other than DCL1.

Park et al. sought to determine the site of miRNA processing by isolating nuclear and cytoplasmic fractions from Arabidopsis overexpressing the putative precursor of miR156 and then probing them with miR156 oligos. In both fractions, a 20-nucleotide transcript corresponding to the mature size of miR156 was found. The presence of the mature-sized miRNA in the nucleus supports the proposal of nuclear processing of miRNAs. The possibility that the mature-sized miRNA in the nuclear fraction was the result of cytoplasmic contamination was raised, but because different sizes of the miRNA were found in the fractions, this was not believed to be the case. Other miRNAs were examined in *hst* and *psd* mutant backgrounds, and with all of the miRNAs examined the mature size was again found in both the nuclear and cytoplasmic fractions. In agreement with the results of Papp et al. (2003), the authors also propose the processing of miRNAs in the nucleus.

In their study, Papp et al. used the virus suppressor protein P19 to examine the subcellular location of dsRNA. These observations were important to Vargason et al. in their 2003 paper published in *Cell* titled "Size selective recognition of siRNA by an RNA silencing suppressor." These authors determined the crystal structure of tomato bushy stunt virus P19 viral suppressor bound to a 21-nucleotide siRNA. In parallel, they conducted biochemical and in vivo assays of P19 function. They concluded that P19 acts as a "molecular caliper" and selects siRNA based on length with P19 having a higher affinity to smaller classes of siRNA, specifically those of 21 nucleotides.

Affinity to the smaller classes of siRNA was also observed by Papp et al., who found that P19 did not bind to larger classes of siRNA, as they were unaffected by either the nuclear- or cytosolic-located P19. It was the smaller classes of siRNAs that were disrupted, either with a significant reduction of small RNAs in the nuclear P19 plants or by truncation with cytosolic P19.

The smaller class of siRNAs is proposed to generate RNA silencing via mRNA degradation, whereas the larger siRNAs are responsible for systemic silencing, retrotransposon silencing, and methylation of homologous DNA. Vargason et al. inferred that the preference of P19 to bind the shorter class of siRNAs would serve to protect the viral genome, as these would be the siRNAs targeting the viral genome. This inference is further supported by Papp et al.'s observation that there was no change in the methylation status of promoters in plants expressing P19, i.e. the larger size class was unaffected by P19.

A side-by-side comparison of P19 and four other unrelated viral silencing suppressors and their effects on siRNA and miRNA pathways was examined by Dunoyer et al. (2004) in their paper titled "Probing the microRNA and small interfering RNA pathways with virus-encoded suppressors of RNA silencing," which was published in *The Plant Cell*. For their study, they transformed Arabidopsis with RNA interference of an endogenous transcript that was activated with one of five viral silencing suppressors. Suppressors were selected from five unrelated viruses: p1-HcPro (*Turnip mosaic virus*), p38 (*Turnip crinkle virus*), p25 (*Potato virus X*), p15 (*Peanut clump virus*), and P19 (*Tomato bushy stunt virus*).

Similar to Papp et al., they also observed the appearance of truncated miRNAs with a mobility shift of one to two nucleotides in plants expressing P19. In addition, a truncating of siRNAs, similar to that of miRNA, was also observed. Both groups postulate that this truncated form could be the result of targeting of P19-bound miRNA or siRNA by an exonuclease, which could cleave exposed nucleotides, consistent with the observed shift in mobility. The binding of the RNA by P19 could prevent its incorporation into RNA-induced silencing complex, thus inhibiting cleavage of target RNA. Dunoyer et al. went further to hypothesize that the 21-nucleotide classes of siRNAs and miRNA share biosynthetic steps due to both being affected similarly by P19.

The results reported by Papp et al. on the nuclear localization of DCL1, the processing of miRNAs in the nucleus, and the effects of P19 have all been used by other researchers in support of their work. In addition to being cited in research articles, this work has been highly cited in review articles, particularly the work on DCL1 localization to the nucleus. Papp et al. (2003) represents an additional piece in the puzzle of RNA

silencing and is furthering our understanding of this phenomenon.

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