

hypothesis of Montgomery and Fire, would be predicted to produce dsRNA. Although this transgene locus did silence the endogenous gene, it could not silence an unlinked single-copy *Chs* transgene present in the same plant, contrary to the expectation of the dsRNA hypothesis. Stam *et al.*⁸ proposed instead that silencing was triggered by a DNA–DNA interaction between the transgene locus and the endogenous locus, as in homology-based gene silencing in filamentous fungi.

An interesting alternative to unintended antisense transcription is post-transcriptional production of copy RNA (cRNA) molecules. This hypothesis proposes that RNA-directed RNA polymerases use cytoplasmic sense transcripts as templates for the synthesis of antisense cRNAs (Refs 10, 11). Thus, if dsRNAs mediate cosuppression in

plants, as is the case in nematodes, their origin could lie in cRNA synthesis¹². Importantly, the cRNA mechanism also helps to explain the phenomenon of systemic acquired silencing in which the cosuppressed state is transported throughout the plant in a gene-specific manner via the phloem and into the cells of surrounding tissues, presumably via the plasmodesmal macromolecular trafficking system of plants^{12–14}. This gene-specific systemic signal is most likely to be a nucleic acid. If so, it might be a dsRNA composed of a sense transcript and a cRNA, or it might simply be a single-stranded cRNA. In either case, production of a signal RNA from any transgene (or endogenous gene) would be straightforward in the copy RNA hypothesis, whereas it would be limited to certain aberrant transgene loci only in the unintended antisense RNA hypothesis.

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Intended inverted-repeat transcripts can trigger cosuppression

A recent study has now directly addressed the possibility that dsRNA might be an efficient trigger of cosuppression by engineering a transgene to produce a sense transcript that contains an inverted duplication and by intercrossing plants containing sense and antisense transgenes. Such circumstances were much more effective at causing gene silencing than was either a sense or an antisense transgene alone, supporting the hypothesis that dsRNA is an efficient trigger of cosuppression. This is clearly an important result even though alternative interpretations still remain to be excluded (e.g. increased transcript stability or altered transcript localization of a sense RNA containing an inverted duplication, either of which might increase the ability of a transcript to trigger the RNA degradation mechanism independent of dsRNA). These results do not alter our conclusion that unintended antisense transcripts rarely, if ever, contribute to cosuppression by standard sense transgenes, but they clearly suggest that further analysis of inverted-repeat-containing transcripts will be very informative about the trigger(s) of cosuppression in plants.

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Jorgensen and colleagues raise a number of interesting points that should generate lively discussion in the field and in the lab. Cosuppression effects are likely to reflect an aggregate of several distinct but perhaps related mechanisms. We certainly had no intention of proposing that all such phenomena were due to unintended antisense. Despite the likely existence of many alternative mechanisms for cosuppression, we still put forward the hypothesis that certain transgene-triggered cosuppression phenomena could involve unintended antisense. The validity of this hypothesis will become clear as mechanistic analysis becomes possible for several examples of cosuppression.

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