DICER-LIKE1: blind men and elephants in *Arabidopsis* development

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Genetic studies of embryo, ovule and flower development in *Arabidopsis thaliana* have led to the independent isolation of different mutant alleles of a single gene (*SIN1*/*SUS1*/*CAF*, now renamed *DCL1*) that encodes a complex RNA-processing enzyme. *DCL1* shows similarity to the *Dicer* group of genes, which are required for RNA silencing in *Drosophila* and *Caenorhabditis*. These recent findings identify a novel but conserved mechanism of post-transcriptional gene regulation that is important for development in eukaryotes.

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It was six men of Indostan,
To learning much inclined,
Who went to see the Elephant
(Though all of them were blind),
That each by observation
Might satisfy his mind…

'God bless me! but the Elephant
Is very like a wall!' '…a spear!'
'…a snake!' '…a tree!'
'…a fan!' '…a rope!'

And so these men of Indostan
Disputed loud and long…
Though each was partly in the right,
And all were in the wrong! [1]

Genetic analysis of mutants with interesting and informative defects is crucial to the understanding of plant development. Mutant alleles of different strengths, however, often exhibit different phenotypes and, as a result, are discovered by different investigators using different types of mutant screens [2]. The situation is particularly complex when the mutations are pleiotropic and the gene has a broad regulatory function. One striking example is the multidomain RNA-helicase/nuclease gene known variously as *EMBRYO DEFECTIVE76* (*EMB76*), *SHORT INTEGUMENTS1* (*SIN1*), *SUSPENSOR1* (*SUS1*) and *CARPEL FACTORY* (*CAF*). Here, we summarize the diverse phenotypes associated with mutations in this gene, describe the predicted gene product and attempt to unify these observations in the context of the predicted biochemical function. We have renamed this locus *DICER-LIKE1* (*DCL1*) because the predicted protein sequence is structurally similar to the *Drosophila melanogaster* DICER and *Caenorhabditis elegans* DCR-1 proteins.

Genetic analysis of *DCL1*: a short history

The sus1 *alleles*

Mutations in *DCL1* were first identified by David W. Meinke through the screening of T-DNA insertion lines generated by Kenneth A. Feldmann for embryo-defective (*emb*) mutants [3,4]. Two mutant alleles from this collection and another allele generated by X-irradiation were mapped to the top of chromosome 1 and shown to define a single locus named *EMB76* [5]. The mutant embryos fail to develop beyond the heart stage and cannot be rescued in culture, consistent with an essential role for this gene in growth and development (Fig. 1) [4,6]. The most striking defect observed in mutant seeds is continued proliferation of the suspensor following developmental arrest of the embryo proper [6]. This pattern of growth usually occurs when a mutation interferes with further development of the embryo and indirectly removes an inhibitory signal normally used by the embryo to limit further growth of the suspensor. The *EMB76* locus was renamed *SUS1* to highlight this suspensor defect [6]. Several additional alleles with similar embryo phenotypes have recently been recovered from a collection of T-DNA insertion lines [7]. Detailed information on these mutants is available at (http://www.seedgenes.org/).

The sin1 *alleles*

An ethyl methane sulfonate (EMS) mutagenesis screen conducted by Robert E. Pruitt yielded a series of homozygous recessive mutations causing defects in female reproductive development. One of these mutant lines, TH1073, was characterized further in the laboratories of Charles S. Gasser and of Animesh Ray. The original isolate of TH1073 in the Landsberg (*erecta*) background displayed abnormal ovules that failed to expand their integuments (Fig. 1), so the mutation was termed *short integuments1*

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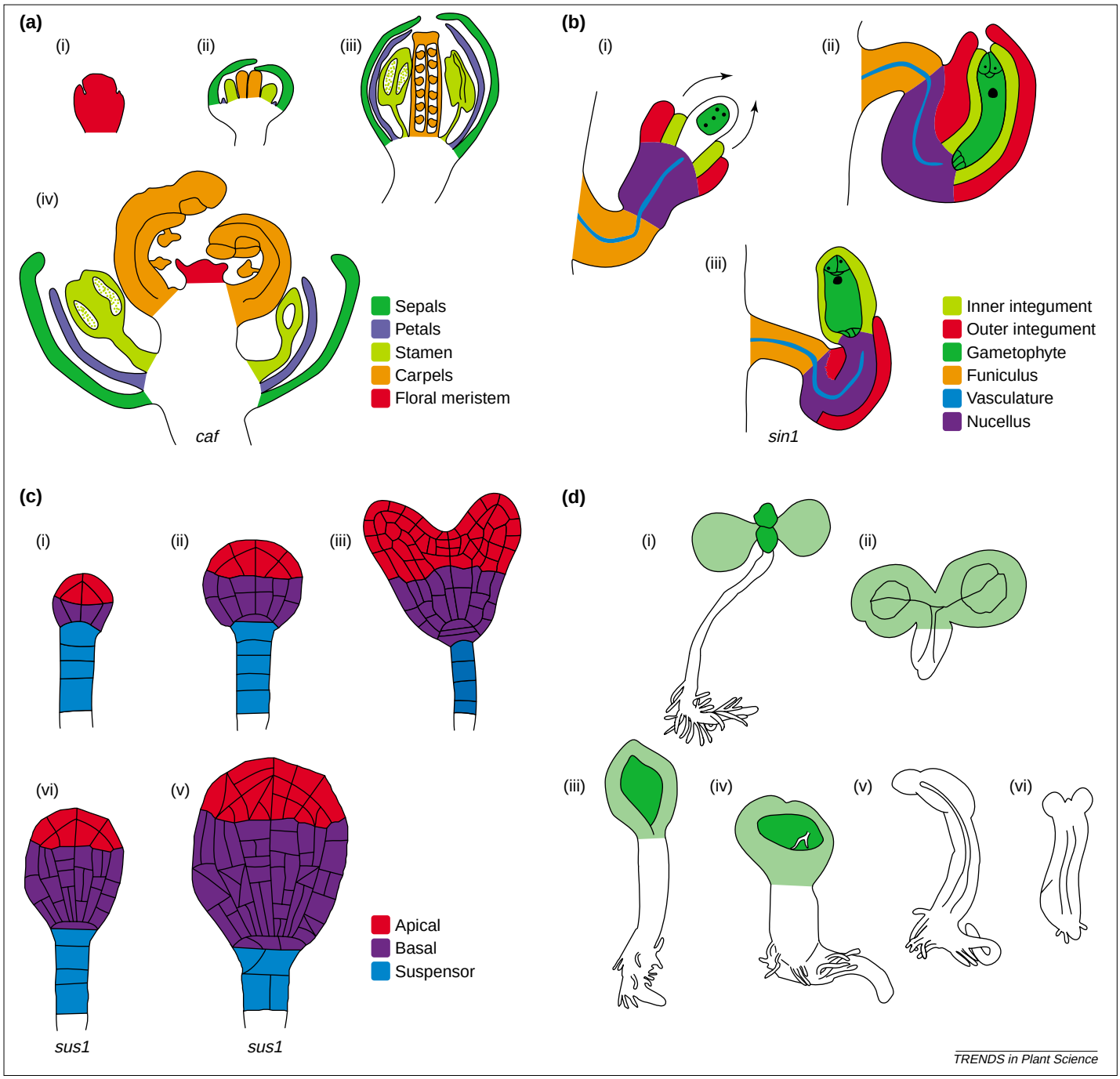

Fig. 1. Phenotypic defects in various *dcl1* mutants. (a) Effects on flower development. (i–iii) Three stages of normal flower development. (iv) Typical abnormal flower in a *caf* (*dcl1-9*) mutant, with unfused carpels, abnormal stamen and ectopic floral meristem cells in the center [15]. (b) Effects on ovule development. (i, ii) Normal development. By floral stage 12, the inner (yellow) and outer (red) integuments of the ovule (i) have initiated off the ovule primordia [8]. The black arrows depict the direction in which both integuments will expand around the developing gametophyte (or germ cells; green). In the Landsberg (*erecta*) background, the *sin1*⁻ ovules fail to expand inner and outer integuments, arresting the ovule in stage i [8,9]. The failure to expand ovule integuments is due to the combined effects of *sin1* (*dcl1-7* or *dcl1-8*) and *erecta*. (ii) At floral stage 13, the ovule is fully mature [8]. Both integuments have expanded to cover the gametophyte. The funiculus, which connects the ovule to the rest of the plant, is shown in orange and the vasculature that runs through the ovule, supplying nutrients and possibly small-RNA signals to the developing embryo, is shown in blue. (iii) A 'mature' *sin1*⁻ ovule in a wild-type *ERECTA* background, in which both integuments expand but fail to coordinate their growths, such that the inner integument covering the embryo sac remains exposed. Nucellar cells (purple) divide excessively and the outer integument cells expand just barely enough to cover the nucellus. (c) Embryo defects in *sus1* mutants. (i–iii) Normal (wild type) early embryonic development from (i) early globular through (ii) middle globular to (iii) the early heart stages. (iv, v) Terminal embryo-arrest phenotypes in homozygous *sus1* mutants at (iv) middle globular and (v) early heart stages [3–7]. Initially there are ectopic cell divisions in the basal half of the embryo, and the mutant embryo continues to grow without further morphogenesis. Suspensor cells can show a variable degree of abnormal cell-division planes. (d) Maternally affected *sin1-2* (*dcl1-8*) seedling defects. (i) A normal (wild type) seedling. (ii–vi) Maternally affected *dcl1-8/dcl1-8* or *dcl1-8/+* seedlings [12] with: (ii) no meristem; (iii) no meristem and a single cotyledon; (iv) fused cotyledon; (v, vi) no cotyledon.

(*sin1-1*) [8,9]. A second allele (*sin1-2*) with a similar but less-pronounced ovule phenotype was also identified in Gasser's laboratory. Prophetically, this name played a role in the establishment of a curator for mutant gene symbols in *Arabidopsis*, because there was confusion over the simultaneous description

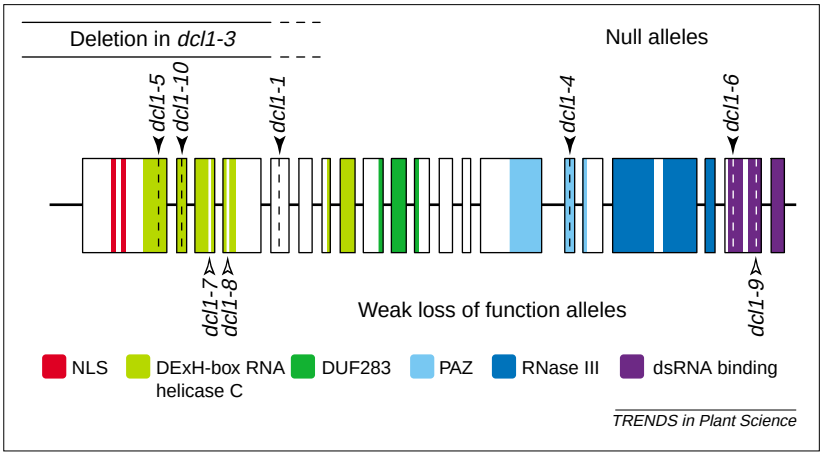

Fig. 2. The genomic region containing *DCL1* showing intron–exon boundaries, color-coded predicted protein domains and mutation sites in *DCL1*. There are two regions of homology found in most *Dicer* gene-family members but with no function yet described: the DUF283 domain (dark green) and the Piwi/Argonaute/Zwille (PAZ) domain (light blue). Null alleles are indicated by black arrows above the exons and black broken lines. Reduction-of-function mutations are indicated by white arrows below the exons and white broken lines. Mutant alleles are described in Table 1. Transcription is from left to right. Abbreviations: dsRNA, double-stranded RNA; NLS, predicted nuclear localization signal; RNase III, ribonuclease III.

of an unrelated *sin1* mutation involved in sinapic-acid biosynthesis [10,11].

The weak *sin1-2* allele revealed an important role for the maternal sporophyte in embryo pattern formation [12]. Partial introgression of *sin1-2* into the Columbia background produced mutant plants with morphologically normal ovules. These subsequently formed seeds that had embryos with a loss of apical, basal or radial symmetry elements. Most of the embryos were nonviable, lacking differentiation of the apical meristem. Strikingly, the zygote was defective even when heterozygous for the recessive mutant allele, as long as it developed within a homozygous-mutant maternal sporophyte. These results suggested that *DCL1* activity is essential in diploid maternal cells for normal embryogenesis, and that genetic contribution of the pollen cannot overcome this requirement. The only other known gene with a similar role in *Arabidopsis* is *SERRATE*, which encodes a zinc-finger protein believed to be involved in chromatin remodeling [13].

Analysis of *sin1* alleles in a wild-type *ERECTA* background also revealed an influence on meristem fate determination and the control of flowering time [14]. Homozygous-mutant plants are late flowering and make more rosette leaves and inflorescence axes than their wild-type siblings. Both *sin1* alleles enhance the conversion of floral meristems to an indeterminate inflorescence state and enhance the late-flowering phenotypes of *apetala1* and *leafy* mutations [14] (T.A. Golden, unpublished). Conversely, *sin1* alleles suppress the conversion of inflorescence meristem to a determined floral state and the early-flowering phenotype of both *terminal flower1* loss-of-function mutations and ectopic *APETALA1* expression [14] (T.A. Golden, unpublished). These results suggest that *DCL1* plays an important role in signaling the developmental transition of a vegetative meristem to a floral meristem.

The caf-1 *allele*

A mutant screen of T-DNA-induced alleles undertaken by Steven E. Jacobsen for floral pattering defects yielded the *carpel factory* (*caf-1*) allele, which causes excessive proliferation of the third and fourth whorls of floral organs (Fig. 1) [15]. In *caf-1*-mutant flowers, the central region of the floral meristem remains in an indeterminate state, generally producing extra organs, and the two carpels do not fuse to form a complete pistil. Double-mutant analysis showed that the function of *DCL1* in the flower is partially redundant with those of the *CLAVATA* genes, which encode meristem signaling molecules [16–18], and *SUPERMAN* (*SUP*), which encode a zinc-finger protein [19], in determining organ identity and cell fate in the center of the flower [15]. Intriguingly, *SUP* is necessary for normal ovule development [20], and *caf-1*-mutant plants (like the *sin1*-mutant plants) produce defective ovules [21]. Abnormal proliferation of shoot meristem cells and the loss of axillary meristems occur in both *caf-1* and *sin1* mutants [14,15].

Thus, like the wise blind men of Indostan [1], each mutation described only a subset of the functions of *DCL1*, and only through molecular analysis was the whole elephant revealed.

DCL1 encodes a complex RNA-processing enzyme

The *DCL1* gene (At1g01040) was independently cloned by virtue of a T-DNA insertion in the *caf-1* allele [15], and by mapping of the *sin1* and *sus1* alleles [21], and additional knockout alleles were identified through border recovery [7] (Fig. 2). *DCL1* has homology to the *Drosophila* gene *Dicer* [22]. Dicer-related proteins generally have the following predicted domain structure (in order): N-terminal DExH-box RNA-helicase-C motifs, a DUF283 domain (domain of unknown function), a PAZ (Piwi/Argonaute/Zwille) domain, two ribonuclease-III motifs and at least one double-stranded-RNA (dsRNA)-binding domain at the C-terminus (Fig. 3). The 1909 amino acid DCL1 protein differs from the canonical Dicer proteins by the presence of an N-terminal domain with highly charged residues and a bipartite nuclear localization signal (NLS), and a second dsRNA-binding domain at the C-terminus [15,21]. There are three additional Dicer family members in *Arabidopsis*: T17B22.28 (At3g03300); T15B3.60 (At3g43920); and F5024.210 (At5g20320) [21]. We have named these loci *DCL2*, *DCL3* and *DCL4*, respectively (Fig. 3). The original *sus1* alleles are all presumed to be nulls, ranging from a >20 kb deletion to T-DNA disruption of essential protein domains (Table 1, Fig. 2). Both *sin1* point mutations lie within the RNA-helicase domain of DCL1, producing subtle changes in the predicted substrate-binding face [21], whereas the *caf-1* product presumably lacks a dsRNA-binding domain at the C-terminus [15]. Interestingly, one of the embryo-lethal alleles (*sus1-6*) should lack both dsRNA-binding domains, suggesting that at least one functional dsRNA-binding domain is required for proper embryogenesis [7]. Thus, the RNA-helicase domain and both dsRNA-binding domains are important for DCL1 function *in vivo*.

How does *DCL1* function?

Model of Dicer function in non-plant systems

Dicer-related proteins have been identified as key enzymes in the process of RNA silencing (for recent

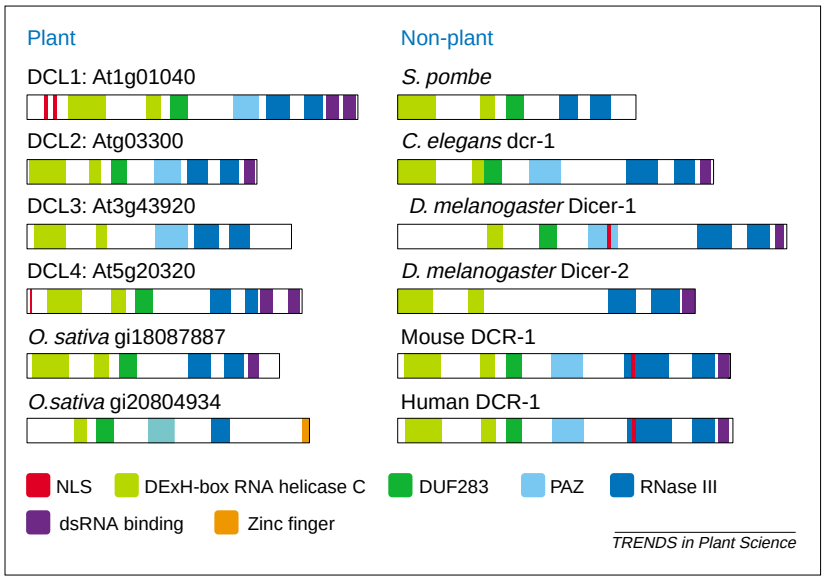

Fig. 3. The known Dicer family members. Computer searches [21] have identified six plant *Dicer* gene-family members: *DCL1* (At1g01040); *DCL2* or T17B22.1 (Atg03300); *DCL3* or T15B3.60 (At3g43920); *DCL4* or F5024.210 (At5g20320); *Oryza sativa* gi18087887; and *O. sativa* gi20804934. In addition, six *Dicer* gene-family members have been identified in non-plant species: *Schizosaccharomyces pombe* CAB37423 (gi2130449); *Caenorhabditis elegans dcr-1* (gi630692); *Drosophila melanogaster Dicer-1* (gi17738129); *D. melanogaster Dicer-2* (gi16215719); *Mus musculus mDCR-1* (gi20385913); and human *Dicer-1* (gi14748177). The domain structures for Dicer-like proteins were predicted using Pfam and Prosite searches. Abbreviations: dsRNA, double- stranded RNA; DUF283, a domain of unknown function; NLS, nuclear localization signal; PAZ, Piwi/Argonaute/Zwille domain; RNase III, ribonuclease III.

reviews, see Refs [23,24]). The *Drosophila* Dicer protein was first identified as a factor necessary for the cleavage of large dsRNA molecules into smaller fragments of 21–25 nucleotides [23,25]. These small RNAs, termed short interfering RNAs (siRNAs), are integral components of post-transcriptional gene-silencing systems, targeting homologous RNAs for destruction. Another Dicer-dependent post-transcriptional regulatory mechanism has been characterized that, in animals, operates on small non-coding RNA hairpins (<100 bp) [26]. In this pathway, Dicer cleaves these small hairpins, resulting in the production of 21–25-nucleotide single-stranded RNA products. These small RNAs, termed microRNAs (miRNAs) or small temporal RNAs (stRNAs), are thought to anneal to target transcripts, thereby directly inhibiting translation [27,28].

This pathway is crucial for normal temporal transitions during *C. elegans* development [28–30]. *C. elegans* strains with the *dicer* (*dcr-1*) mutation phenocopy double mutants of two closely related *ARGONAUTE* family members, *alg1* and *alg2*, in which the viscera burst through the vulval wall, owing to a lack of adult alge or seam cells, and trail behind the adult hermaphrodite [30]. This suggests that Dicer and Argonaute-related proteins act in the same genetic pathway. Members of the *ARGONAUTE* gene family have been shown to be involved in RNA silencing [31] and to interact physically *in vitro* with Dicer-related proteins [32], and are predicted to bind RNA and/or to control translation (e.g. rabbit translation-initiation factor eIF2C is an Argonaute-related protein [33]). It is possible that Dicer-related proteins and Argonaute-related proteins work together for RNA silencing in animal systems, perhaps in a multiprotein complex.

Model of DICER-LIKE1 *function in plants*

We propose that a small RNA-mediated pathway involving *DCL1* operates during plant development to regulate a range of important genes. In *Arabidopsis*, double mutants disrupted in two closely related *ARGONAUTE* gene-family members (*argonaute1* and *pinhead/zwille*) phenocopy *dcl1* null mutants, arresting differentiation at the globular stage and causing an overproliferation of cells [34]. In addition, there are phenotypic similarities between weak *dcl1* loss-of-function mutants and *argonaute1* or *pinhead/zwill*e single mutants – thin sepals and petals, antherless stamens, tube-shaped leaves, loss of axillary meristems, and filaments on the stems [15,21,34,35]. This parallels the homology between the synthetic phenotype of *alg1 alg2* double mutants and the *dcr-1* phenotype in *C. elegans*, suggesting that, in both systems, these gene products work together in a complex to control the translation of certain developmentally important mRNAs [21,30].

Table 1. Mutant alleles of *dcl1*

Mutant allele		Mutagen[a]	Lesion	Position	Ecotype[a]	Refs
New name	**Old names**					
dcl1-1	*emb76-1*, *sus1-1*	T-DNA	T-DNA insert	RNA helicase domain (exon 5)	Ws	[3–7,21]
dcl1-2	*emb76-2*, *sus1-2*	T-DNA	Unknown	Unlinked T-DNA insert	Ws	[3–7]
dcl1-3	*emb60*, *sus1-3*	X-ray	>20 kb deletion	Removes 5′ end of the ORF	Co	[3–7,21]
dcl1-4	*sus1-4*	T-DNA	T-DNA insert	PAZ domain (exon 15)	Co	[7][b]
dcl1-5	*sus1-5*	T-DNA	T-DNA insert	RNA-helicase domain (exon 1)	Co	[7][b]
dcl1-6	*sus1-6*	T-DNA	T-DNA insert	First dsRNA-binding domain (exon 19)	Co	[7][b]
dcl1-7	*sin1-1*	EMS	P415S	RNA-helicase domain (exon 3)	La-*er* (Co-*gl*)	[8,9,14,21]
dcl1-8	*sin1-2*	EMS	I431K	RNA-helicase domain (exon 4)	La-*er* (Co-*gl*)	[12,14,21]
dcl1-9	*caf-1*	T-DNA	T-DNA insert	Second dsRNA-binding domain (exon 19)	Ws (La-*er*)	[15]
dcl1-10	sus1-7	T-DNA	T-DNA insert	RNA-helicase domain (exon 2)	Co	[7][b]

[a]Introgressed alleles are noted with the introgressed ecotype in brackets.
[b]http://www.seedgenes.org/
Abbreviations: Co, Columbia; Co-*gl*, Columbia-*glabrous*; dsRNA, double-stranded RNA; EMS, ethyl methane sulfonate; La-*er*, Landsberg-*erecta*; ORF, open reading frame; PAZ, Piwi/Argonaute/Zwille domain; Ws, Wassilewskija.

This suggests that small RNAs might have been used for developmental gene regulation since the evolution of early eukaryotes. There have also been observations suggesting that dsRNA fragments can induce the methylation of promoter regulatory elements [36], which is intriguing because DCL1 and DCL4 are predicted to contain nuclear localization signals, and might function in the nucleus. Recently, over 100 miRNAs (from 60 to 4000 nucleotides long) were isolated from *Arabidopsis* and rice (*Oryza sativa*) [37,38], and the processing of some of them was dependent on *DCL1* activity [38]. Louise Jones describes these significant findings in more detail in a Research News article in this issue of *Trends in Plant Science*.

Small RNAs as developmental signals?

The pleiotropic developmental abnormalities observed in *dcl1*, *argonaute1* and *pinhead/zwille* mutants clearly indicate that small RNAs are crucial for plant development. Furthermore, studies on the *sin1* alleles demonstrated that *DCL1* could act at a distance, from the maternal sporophyte into the embryo [12,21]. It has been proposed that the siRNAs created by the RNA-silencing machinery can act as signals by traveling through the plasmodesmata to neighboring cells and silencing homologous RNA [39]. This suggests that the small RNAs or their precursors (acting through a *DCL1*-dependent signaling pathway) could help to coordinate development at the whole-plant level in a non-cell-autonomous manner. It has not escaped our attention that small RNA signaling through *DCL1* might non-autonomously signal the meristem to progress to a floral fate, which could help to resolve some outstanding questions in plant development [21,40]. Although this hypothesis remains to be tested, one wonders whether there are other mutant alleles that, like the wise blind men of Indostan [1], will reveal more of the elephant and, ultimately, the role of small RNAs in development.

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