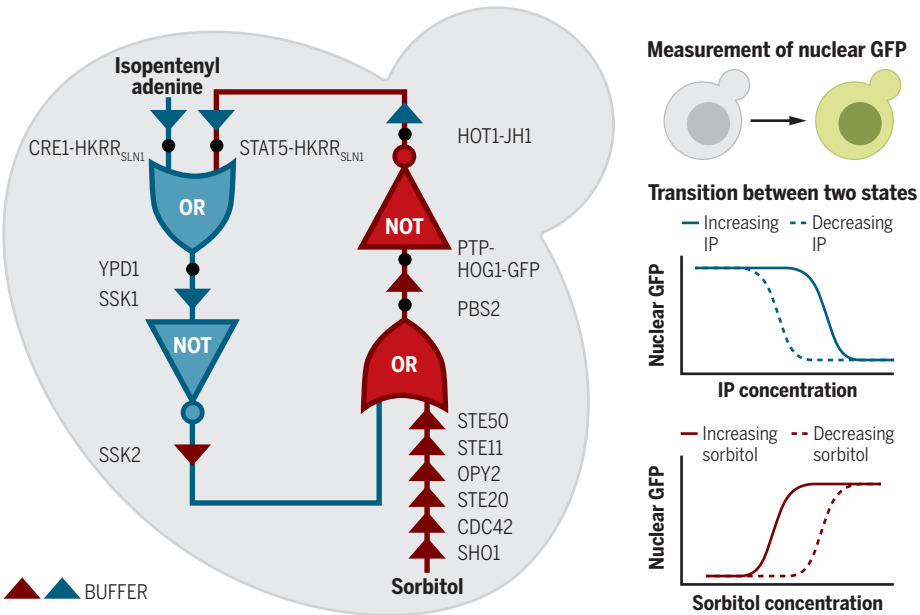
A fast, protein-only synthetic toggle network

A toggle bistable switch that contains two OR, BUFFER, or NOT gates creates a mutual repression topology in yeast cells (left). Changes in the concentration of input signals (sorbitol and isopentenyl adenine) rapidly control switches between the two stable states, which is monitored by the nuclear translocation of a fusion green fluorescent protein (right).



CDC42, cell division control protein 42; CRE1-HKRR$_{SLN1}$, *Arabidopsis thaliana* cytokinin receptor fused to histidine kinase response regulator fused to the intracellular effector domain SLN1; HOG1, high osmolarity glycerol; HOT1-JH1, high osmolarity–induced transcription protein 1 fused to the catalytic domain of Janus kinase JAK 2; OPY2, overproduction-induced pheromone-resistant protein 2; PBS2, polymyxin B resistance protein 2; PTP-HOG1-GFP, PTP domain fused to HOG1 and GFP; GFP, green fluorescent protein; SHO1, high osmolarity signaling protein 1; SSK1, osmolarity two-component system protein 1; SSK2, osmolarity two-component system protein 2; STAT5-HKRR$_{SLN1}$, signal transducer and activator of transcription 5 (STAT5)–histidine kinase response regulator fused to the intracellular effector domains; SLN1 (HKRR$_{SLN1}$) protein; STE11, serine-threonine protein kinase STE11; STE20, serine-threonine protein kinase; STE50, protein Ste50; YPD1, osmotic stress–responsive phosphorelay intermediate sensor protein 1.

Why does the engineered toggle switch have such a sophisticated design of paired OR, NOT, and BUFFER gates? Increasing the number of elements in a circuit enables rapid and robust responses. An amplifier increases the speed of a logic gate, whereas an element with a sigmoidal input-output characteristic, implemented as a BUFFER gate, filters noise and increases robustness to internal and external noise. Synthetic biochemical devices are noisier than electronic circuits because of internal noise in transcription-translation and cell heterogeneity. Future work will assess how speed and robustness of protein logic gates depend on the number and characteristics of individual elements (*13*). This will open possibilities for engineering gate combinations in complex integrative circuits.

Mishra *et al.* also searched the Kyoto Encyclopedia of Genes and Genomes for all known signaling pathways in the yeast *Saccharomyces cerevisiae* and identified several toggled network motifs. Their analysis suggests that biological networks might inherently enable logical operations and computations. This emphasizes the importance of reconstructing causal, directional regulatory networks from "-omics" data; current statistical methods mostly infer correlative nondirectional networks (*14*). When network biology moves from correlation to causation., it will be possible to deconstruct intracellular signaling networks into smaller parts to analyze and, more ambitiously, to reverse-engineer them by creating artificial cells with preprogrammed properties. It may not be long before the precise picture of signaling network abnormalities that cause diseases and the logic of potential treatments are identified from synthetic biology approaches. ■

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PLANT SCIENCE

Small RNAs on the move in male germ cells

DNA in plant male meiocytes is methylated by intercellular small interfering RNAs

By **Rebecca A. Mosher**

Plants produce 24-nucleotide (nt) small interfering RNAs (siRNAs) to maintain asymmetric DNA methylation in cis—that is, an siRNA will methylate DNA in the same region from which it is produced (*1*). However, 24-nt siRNAs also direct the methylation of DNA in trans to homologous loci (see the figure) and can move intercellularly to guide methylation in recipient cells (*2*). On page 76 of this issue, Long *et al.* (*3*) describe siRNAs from hundreds of loci that function both intercellularly and at nonallelic sites. Specifically, 24-nt siRNAs move from somatic cells to adjacent male germline cells (meiocytes). This joins a growing list of siRNA movements during plant reproductive development.

Through small RNA directed DNA methylation, the 24-nt RNAs recruit DNA methylation machinery to target loci. Although these siRNAs act in trans, observations of this activity have been limited to specific experimental conditions or rare allelic combinations. Cell-autonomous trans activity of 24-nt siRNAs includes their production from viral RNA, which can trigger DNA methylation of related sequences in the host genome (*4*), or their production from a methylated allele, which can cause the methylation of a related naïve allele (*5*).

By contrast, the siRNAs observed by Long *et al.* are produced from hundreds of locations in the tapetum, a layer of diploid somatic cells that surrounds the developing male germline. The authors demonstrate that these siRNAs move into male meiocytes (cells that eventually differentiate into gametes) and induce DNA methylation at their cis locus as well as

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in trans at protein-coding genes. Tapetal siRNAs also appeared sufficient for asymmetric methylation in sperm cells, indicating that they play a role in establishing the epigenome of the germline. Long *et al.* further show that intercellular methylation is not limited to the cis and trans targets of tapetum-enriched siRNAs but is found at thousands of 24-nt siRNA loci that are not abundantly expressed in the tapetum. This indicates that there is widespread intercellular movement of siRNAs from the tapetum to the germline.

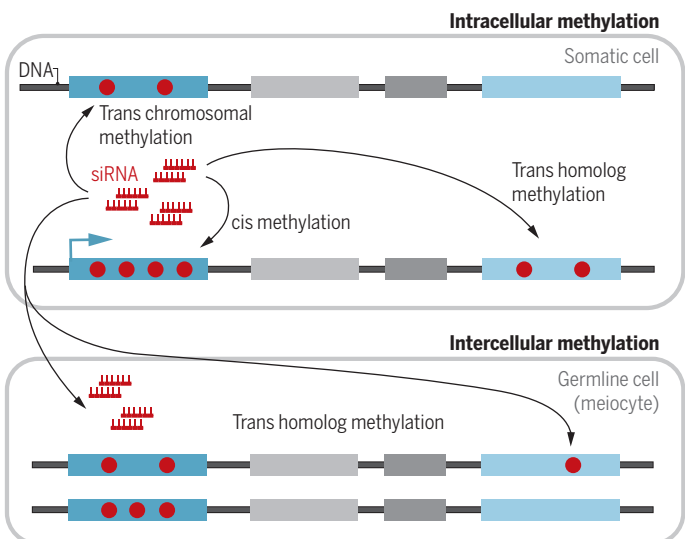
Long *et al.* also show that tapetal siRNAs transcriptionally silence transposons, selfish genetic elements that insert copies of themselves in the genome, causing mutations. Such mutations in germ cells can be passed to the next generation. Thus, movement of siRNAs from tapetum to germline presents a means for somatic cells to protect the next generation from harmful mutations. The ability of these siRNAs to trigger methylation despite mismatches means that transposons will be recognized and silenced even in the face of sequence evolution, whereas the production of siRNAs from diploid somatic cells avoids potential segregation of the siRNA-generating locus away from the transposon during meiosis.

In analyzing siRNA-mediated trans-methylation, Long *et al.* allowed up to three mismatches between the siRNA and the target locus. Although such mismatches are known for 21-nt microRNAs, it was only recently reported that 24-nt siRNAs can target methylation without 100% sequence complementarity (*4*). It is unclear how much complementarity is required for function and whether a specific siRNA-binding protein is associated with trans-methylation. There also may be tissue-specific or chromatin-based limitations to this activity. Long *et al.* observed that trans-methylation does not occur within the tapetum despite high expression of siRNAs in those cells, indicating that trans-methylation is not universal.

Analysis of 24-nt siRNA function at allelic sites revealed that siRNAs can be diluted by acting at multiple locations, falling below a threshold needed to maintain asymmetric methylation (*6*). This suggests that robust trans-methylation might be limited to highly expressed siRNA loci. The tapetal-enriched siRNA loci described by Long *et al.* are expressed 100 to 1000 times more than other 24-nt siRNA loci. This extreme expression requires the Snf2 domain-containing protein CLASSY 3 (CLSY3), which Long *et al.* found to be expressed in the tapetum but not in other cells of the anther. A recent preprint reports that CLSY3 may be required for extreme expression of siRNAs in somatic tissue surrounding the female germline (*7*), suggesting that CLSY3 might be a key determinant of tissue-specific siRNA expression.

Mobile small RNAs

Small RNAs (24 nucleotides) produced in plant somatic (tapetal) cells can guide DNA methylation intracellularly or in adjacent male germ cells (meiocytes). Methylation of both transposons (a source of genome mutations) and of protein-coding genes persists in sperm cells. siRNA, small interfering RNA.



Abundant 24-nt siRNAs produced from maternal somatic cells are also proposed to move into adjacent tissues (*8*). Parallel movement of distinct 24-nt siRNAs into the male and female germlines evokes questions of parental conflict and imprinted gene expression after fertilization (*9*). Consistent with this, loss of 24-nt siRNAs in tetraploid pollen donors overcomes the endosperm failure typically induced by paternal genome excess (*10*). It may be that 24-nt siRNAs balance maternal and paternal influence after fertilization. However, it remains to be determined whether paternal influence is through heritable methylation marks placed in the male germline by tapetal siRNAs or through retention of tapetal siRNAs in sperm cells for delivery during fertilization. The ability of these siRNAs to target methylation despite mismatches with the target locus indicates that paternally derived siRNAs could trans-methylate maternal alleles.

Although the tapetal siRNAs described by Long *et al.* are synthesized differently, they are reminiscent of the 24-nt phased secondary siRNAs (phasiRNAs) present in many plant species. Both classes of siRNA are abundantly expressed in tapetum during meiosis and are proposed to load into meiocytes (*11*). Asymmetric DNA methylation is induced by 24-nt phasiRNAs (*11*), although whether they function in trans at imperfectly complementary regions is not known; 24-nt phasiRNAs are broadly distributed in angiosperms but are not found in Brassicaceae (such as *Arabidopsis*) (*12*), raising the possibility that CLSY3-dependent tapetal siRNAs might substitute for the phasiRNAs that *Arabidopsis* lacks.

Small RNA movement is a key aspect of seed development. During female germline development, 21-nt trans-acting siRNAs move within the developing ovary to restrict germline specification to a single cell (*13*). In gametophytes, 21-nt siRNAs produced in the pollen vegetative nucleus can move in the sperm cells, whereas 24-nt siRNAs from the central cell are proposed to move into the egg cell (*14*). And after fertilization, siRNAs are proposed to move from the endosperm to embryo and from the maternal sporophytic integuments to the endosperm (*8*, *15*). A better understanding of how mobile siRNAs function at trans sites should further illuminate the roles of siRNAs during plant reproduction. ■

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