

programmed antibodies in which antigen recognition is modified by the insertion of different ligands into the antibody binding site via a common reactive group (7). The beauty of the two-in-one molecule created by Bostrom *et al.* is its simplicity. For the first time, dual specificity has been engineered into a naturally occurring and stable antibody isotype that should pose no obstacles for manufacturing and that has been well validated for clinical use.

Two-in-one antibodies may replace combination therapies such as treatment of cancer with both bevacizumab and trastuzumab, which is currently in clinical trials. A practical limitation to this approach may be an inflexibility of dosing where optimal doses are discordant for the individual antigens targeted. A strong caveat comes from two recent studies that investigated the use of bevacizumab and chemotherapy in combination with either cetuximab (Erbix) or panitumumab (Vectibix) [antibodies that inhibit epidermal growth factor receptor (EGFR)] for treating metastatic colorectal cancer (8, 9). The studies showed that adding either of these antibodies to bevacizumab (plus chemotherapy) worsened clinical outcomes. These effects were unexpected because the antibody combinations had shown promise in the preclinical setting.

Two-in-one antibodies could also be used to target two nonoverlapping epitopes on the same antigen. Such antibodies would have a

greater potential for aggregating targets than classical antibodies. For combinations of either EGFR or HER2 monoclonal antibodies (10, 11), for example, such aggregation increases anti-tumor effects. The presence of two or more binding sites against distinct epitopes on a soluble antigen furthermore has the potential to increase binding avidity and *in vivo* potency (12).

The ability of antibodies to bind multiple antigens is, in itself, not a novel finding and has been described, for example, for the low-affinity binding of dissimilar peptides to distinct regions in a single antibody binding site (13). Indeed, by harboring multiple, spatially separated, binding sites in a single structure, antibodies may exploit a mechanism that has been recognized as a major source for multi-specificity of proteins (14, 15). The uniqueness of the work of Bostrom *et al.* is to show that promiscuous binding of antibodies is compatible with the high-affinity, pharmacologically relevant, binding of very different antigens. Promiscuous binding may even extend to natural immunity where it would represent a mechanism to maximally cover binding space by a given repertoire of antibodies. Cross-reactive antibodies, when isolated, are generally considered a nuisance and two-in-one antibodies may therefore have been overlooked. The increased availability of technologies for rapid and large-scale screen-

ing of antibody-antigen interactions should help identify promiscuous antibodies. The potential for high-affinity antibody binding of more than one antigen is intriguing and poses opportunities for future basic research and perhaps clinical development of antibody combination therapy.

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MOLECULAR BIOLOGY

Dynamic DNA Methylation

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The silencing of gene expression through the methylation of cytosine nucleotide bases in DNA is observed in a wide variety of eukaryotic organisms. It occurs mainly at repetitive elements of genomes, and plays a critical role in silencing transposable elements (transposons). Its heritability is a key aspect of DNA methylation as a stable epigenetic mark of gene repression. However, two studies, by Teixeira *et al.* on page 1600 in this issue (1) and Slotkin *et al.* (2), show that DNA methylation and gene silencing can be much more dynamic than previously thought.

In the model plant *Arabidopsis thaliana*, three different methylation systems maintain

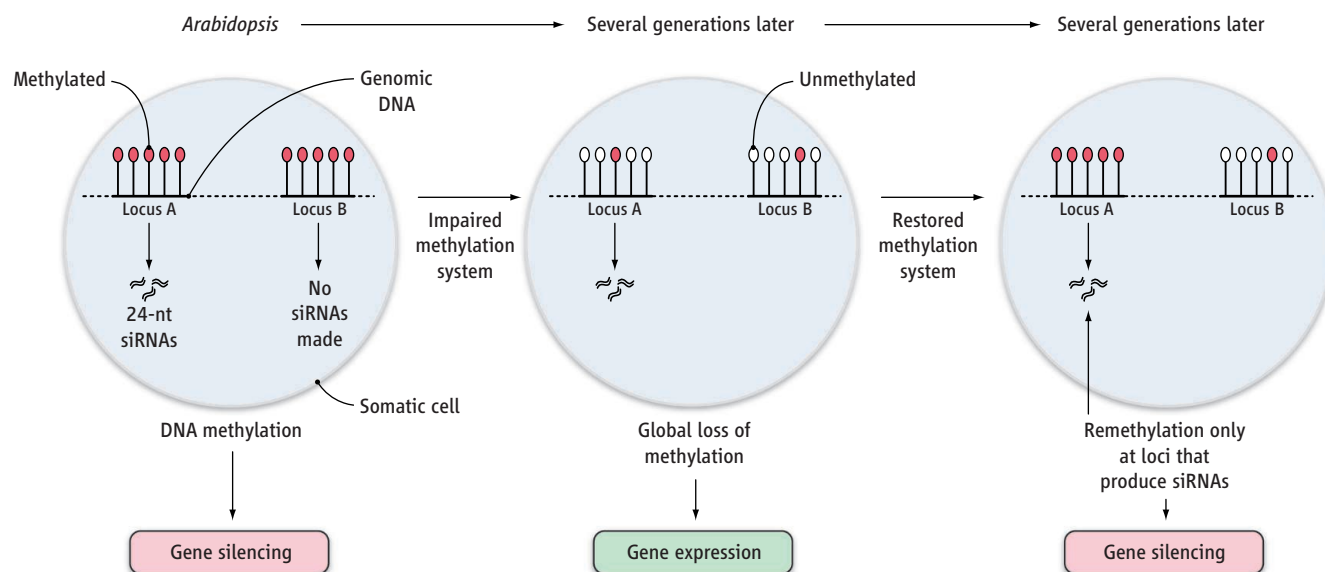
cytosine methylation in three different sequence contexts: CG [cytosine (C); guanine (G)], CHG [H is adenine (A), thymine (T), or cytosine (C)], and CHH (3). CG methylation is controlled by DNA METHYLTRANSFERASE 1 (MET1) and VARIANT IN METHYLATION 1 (VIM1) (4). The mammalian homolog of VIMI (UHRF1) recognizes hemimethylated CG DNA and facilitates its restoration to the fully methylated state (5, 6). Another critical factor is the chromatin-remodeling protein DECREASED DNA METHYLATION 1 (DDM1), whose mutation causes massive losses of methylation (7), and reactivates transposons (8). CHG methylation is maintained by the plant-specific CHROMOMETHYLASE 3 (CMT3), and KRYPTONITE (SUVH4), a histone protein methyltransferase. CMT3 binds to methylated histones (chromatin-associated proteins)

The methylation of DNA during plant development is a much more dynamic process than previously assumed.

and KRYPTONITE binds to methylated CHG sites, thereby creating a feedforward loop for maintaining CHG methylation (9). CHH methylation is controlled by a third DNA methyltransferase, DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2). DRM2 is guided to its DNA targets by 24-nucleotide small interfering RNAs (siRNAs) in a pathway called RNA-directed DNA methylation (9, 10). In addition to maintaining CHH methylation, the RNA-directed DNA methylation pathway also controls the establishment of DNA methylation in all sequence contexts (11).

Although the details of these methylation systems are being quickly fleshed out, much less is known about the extent to which they are acting throughout plant development. Teixeira *et al.* show that some regions of the *Arabidopsis* genome can be efficiently

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Silenced, again. DNA methylation that is lost in previous generations (for example, through mutation of a gene required for methylation) can be restored in subsequent generations when a gene encoding the wild-type version of the protein is

reintroduced. However, remethylation is restricted to loci that produce siRNAs, and depends on the RNA-directed DNA methylation pathway. SiRNAs can thus selectively correct methylation defects to enforce silencing.

remethylated if methylation was lost in previous generations. Using *ddm1* mutants, which display a global reduction in DNA methylation, the authors investigated whether DNA methylation can be restored after a wild-type *DDM1* is reintroduced. Roughly half the sequences they examined regained methylation, thus reestablishing gene silencing. Complete remethylation was observed only after several generations, consistent with the multigenerational nature of transgene silencing known for plants.

The loci that became remethylated were characterized by the presence of high amounts of siRNAs, whereas loci that remained unmethylated lacked siRNAs (see the figure). Furthermore, reestablishing methylation required RNA-DEPENDENT RNA POLYMERASE 2, a key component of the RNA-directed DNA methylation pathway. Most siRNAs correspond to transposons and other highly repetitive DNA, which if expressed could lead to genome instability. Thus, the ability to specifically remethylate these sequences is likely beneficial in a multigenerational manner to reinforce silencing and to correct defects in methylation patterning that might otherwise lead to transposon activation.

In mammals, DNA methylation is dynamic during development, and examples include gene-specific imprinting as well as genome-wide changes in some cell types (12). *Arabidopsis* and other flowering plants imprint specific genes by selective demethylation of promoters in the endosperm (nutritive tissue in seeds of plants) (9), but whether methylation patterns are altered globally in

different plant tissues or cell types has been unclear. Slotkin *et al.* (2) report that the vegetative nucleus of *Arabidopsis* pollen cells shows a global loss of gene silencing, coupled with reactivation of transposon expression. Pollen contain three nuclei: the vegetative nucleus, which powers the cell; a sperm nucleus, which fertilizes the egg to form the zygote; and a second sperm nucleus, which fertilizes the central cell in the ovule to form the endosperm.

By comparing data from pollen with that of isolated sperm nuclei, Slotkin *et al.* (2) deduced that the vegetative nucleus was the location of transposon activation. Further, although new transposition events were detected in pollen, they were not inherited, again suggesting that transposon reactivation occurs in the vegetative nucleus, which does not contribute DNA to the zygote. Transposon reactivation was coupled with decreased expression of *DDM1*, and several genes that control RNA-directed DNA methylation, as well as reduced numbers of 24-nucleotide siRNAs. Interestingly however, a different class of transposon-related siRNAs (21 nucleotides in length) accumulates in pollen. The authors propose that these 21-nucleotide siRNAs, originating in the vegetative nucleus, may travel to the adjacent sperm cells to reinforce silencing, perhaps in a manner akin to that shown by Teixeira *et al.* for the remethylation of hypomethylated DNA in somatic tissue. Thus, only those transposons with the potential to be expressed (because they were expressed in the vegetative nucleus) would be targeted by siRNAs in sperm nuclei.

The results of Slotkin *et al.* raise the question of whether similar processes occur in the *Arabidopsis* female gametophyte—for instance, if loss of silencing in the central cell might cause reinforcement of silencing in the egg cell. There are also interesting parallels with the siRNA-mediated communication between nuclei seen in *Tetrahymena thermophila*, where small RNAs generated from the micronucleus target chromatin modifications (and eventually DNA deletion) to homologous genomic DNA sequences in the developing new macronucleus (13). In the future, it will be important to assess the extent to which the dynamic processes uncovered by these recent findings are utilized in other aspects of eukaryotic development.

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