High temperatures, above 400°C, are typically needed to crystallize silicon and germanium to synthesize nanocrystals and nanowires of interest in many optoelectronic devices. Relatively monodisperse gold nanocrystals stabilized with dodecanethiol ligands were used to template the growth of one-dimensional silicon nanowires (*5*). Whereas liquid solvents boil away at these elevated temperatures, supercritical fluids solvate the ligands on the gold catalyst crystals. The first crystalline silicon nanowires with diameters smaller than 5 nm and lengths greater than 1 μm made by any technique were produced in supercritical hexane. The process is particularly well-suited for scale-up. Mesoporous silica hosts have also been used as rigid templates to synthesize ultrahigh-density three-dimensional arrays of metal and semiconducting nanowires including silicon (*6*). The lack of surface tension in supercritical fluids accelerates the penetration of the precursors into the nanoscale pores relative to liquid solvents, which in turn reduces reaction times from days to minutes. Fukushima and Wakayama have made high–surface area platinum catalytic converters for automobiles as well as other metals and metal oxides by a similar technique in porous activated carbon (*7*).

The tunability and excellent transport and interfacial properties of supercritical fluids are also highly beneficial for processing polymer films. The low viscosity and surface tension may be exploited to generate extremely uniform thin films of photoresists on silicon wafers by spin coating (*2*). However, most polymers are not soluble in carbon dioxide, making them ideal candidates as matrices or templates for materials processing. Because carbon dioxide is small and linear, it diffuses into polymers faster than nearly any other swelling agent or plasticizer. The swelling, which controls the mobility of the polymer chains, may be fine-tuned by adjusting carbon dioxide pressure. As glassy polymers become rubbery, diffusion coefficients for organic molecules in the polymer increase by many orders of magnitude. Adding carbon dioxide by raising the pressure decreases the glass transition temperature (T_g) nearly linearly with the solubility of carbon dioxide in the films (*8*). A reduction of the T_g by 70°C at 100 atm pressure is common. Once plasticized with carbon dioxide, the polymer matrix become accessible and may be impregnated with dyes, pharmaceuticals, or organometallic precursors (*9*). Releasing the carbon dioxide pressure traps the impregnated agent inside the rigid glassy matrix. The effect of carbon dioxide on diffusion and polymer mobility in polymer thin films is also of interest in lithographic processing of microelectronic devices with sub–100-nm features, and in membrane separations.

Over the past decade, a large number of mesoporous materials have been synthesized in aqueous solution with surfactants or block copolymers (that is, polymers composed of two or three blocks linked together). The structure of the highly fluid template evolves during the reaction, making it challenging to predict and control the final morphology. To form templates, Pai *et al.* (*1*) instead used "solvent-free" block copolymer micelles, which are much more rigid and thus resistant to structural change during reaction. However, this rigidity prevents reactants from entering the template. A solvent was needed to swell the rigid polymer template film just enough to open the gates to let the reactants in without deforming the film during reaction and solvent removal (see the figure). Pai *et al.* fine-tuned this swelling as well as the partitioning of the precursor and catalyst between phases by adjusting carbon dioxide pressure. As in biomineralization in nature, the product replicated the preordered template, resulting in well-ordered pores over an exceptionally large length scale. The approach is very general, because the morphology of the templates may be designed over a wide range simply by varying the lengths and compositions of the polymer blocks. The porous mesoporous silicate films may be utilized as insulators in next-generation microelectronics devices due to their very low dielectric constants and excellent mechanical strength. The robust and yet highly accessible ordered templates are also of interest in making sensors, catalysts, biomedical materials, and materials for separations and photonics.

References

1. R. A. Pai *et al.*, *Science* **303**, 507 (2004).
2. J. M. DeSimone *et al.*, *Science* **265**, 356 (1994).
3. J. M. Blackburn *et al.*, *Science* **294**, 141 (2001).
4. P. S. Shah *et al.*, *Nano Lett.* **3**, 1671 (2003).
5. J. D. Holmes *et al.*, *Science* **287**, 1471 (2000).
6. K. M. Ryan *et al.*, *J. Am. Chem. Soc.* **125**, 6284 (2003).
7. Y. Fukushima, H. Wakayama, *J. Phys. Chem. B* **103**, 3062 (1999).
8. J. Q. Pham, S. M. Sirard, K. P Johnston, P. F. Green, *Phys. Rev. Lett.* **91**, 175503/1 (2003).
9. H. Liu, M. Z. Yates, *Langmuir* **18**, 6066 (2002).

PLANT SCIENCES

Imprinting—a Green Variation

Frederic Berger

The conquest of the terrestrial environment by plants and mammals is linked to the parallel evolution of a predominantly maternal control over embryogenesis. This evolution of maternal control spurred the development of an intriguing epigenetic mechanism, called imprinting, for controlling gene expression. Imprinted genes are those in which expression depends on their parental origin. For example, imprinted genes are expressed only from the maternal allele, the paternal allele having been silenced by methylation of cytosines in the gene sequence. On page 521 of this issue, Kinoshita and colleagues (*1*) propose a mechanism for establishing one-way control of imprinting in plants that is distinct from imprinting in mammals.

The nutrition and protection of the embryo by the mother requires the invention of a specific interface between the two. In mammals, this interface derives from extraembryonic tissues, which form the placenta. In plants, the embryo is connected to the maternal tissues by the endosperm, the product of a second fertilization event (see the figure). The plant endosperm and the mammalian placenta are both subjected to imprinting, resulting in the preferential expression of maternal copies of genes, most notably those involved in the control of growth (*2*, *3*, *4*). In the mouse, disruption of the imprinting of specific genes, such as *Igfr2r*, leads to aberrant development of the trophoblast (which contributes to the placenta) (*5*, *6*). In plants, the polycomb-group gene *MEDEA*, a master regulator of endosperm development, is known to be imprinted in the endosperm but not in the embryo (*7*, *8*). Kinoshita *et al*. now report that a second plant gene, *FWA*, which encodes a homeodomain transcription factor, is imprinted in the endosperm of the model plant *Arabidopsis* and is expressed only from the maternal allele (*1*). Expression of *FWA* in endosperm coincides with an overall reduction in the amount of methylated cytosine residues in the direct repeat sequences of the 5′ region of this gene. The level of *FWA* methylation remains high in other

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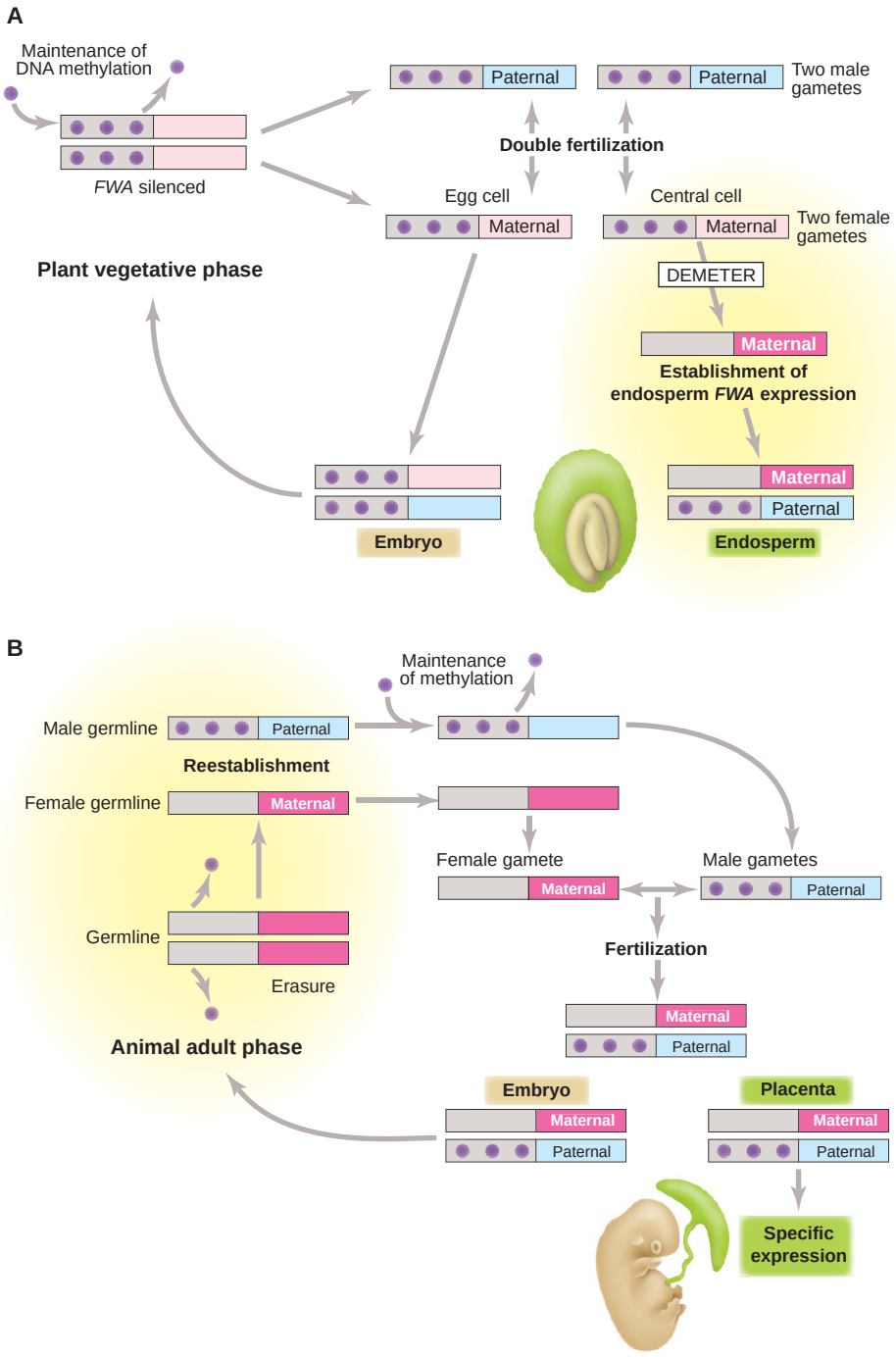

The control of imprinting. Maternal imprints in the extraembryonic tissues of plants and mammals. (**A**) In plants, two identical male gametes are delivered by the pollen tube to two distinct female gametes, the egg cell and the central cell. A double fertilization process leads to the development of the embryo (yellow) surrounded by the nurturing endosperm (green). Genes specifically expressed in the endosperm, such as *FWA*, are subjected to release of constitutive repression (caused by methylation of cytosines) of the maternal but not the paternal allele (possibly through the action of *DEMETER*). Repression of *FWA* expression through DNA methylation of 5′ repeats is maintained throughout the plant vegetative phase by the methyltransferase MET1. A more complex cycle of imprinting must exist for the *MEDEA* gene, which is expressed during vegetative development. (**B**) In mammals, maintenance of imprinting relies on the activity of the methyltransferase DNMT1 and is very complex. Imprinting is initially erased during global DNA demethylation in the germline; it is then reestablished during gametogenesis through unknown mechanisms. For example, in the case of the *Igfr2r* gene, the paternal allele is silenced by a mechanism involving DNA methylation, a noncoding RNA, and the PcG gene *Eed*. In female gametogenesis, methylation of the ICR of *Igfr2r* is prevented and expression of the maternal allele of *Igfr2r* is possible (*19*). After fertilization, methylation imprints in mammals are fully resistant to waves of DNA demethylation in both the extraembryonic and embryonic lineages. Expression of the imprints in the placenta requires a specific transcription mechanism, which is not active in the embryo.

tissues of the plant, preventing transcription of *FWA* in these tissues.

The 5′ direct repeats were originally identified in a study of epigenetic mutations in the *FWA* gene. The late flowering mutant *fwa-1* of *Arabidopsis* is characterized by hypomethylation of the 5′ direct repeats associated with ectopic expression of *FWA* in vegetative tissues (*9*). Kinoshita *et al*. investigated the origin of methylation of the 5′ repeats in the *Arabidopsis* mutant. In plants, DNA methylation is maintained on cytosine residues by methyltransferase 1 (MET1), which is similar to mammalian DNMT1 (*10*), and by the plant-specific chromomethylase 3 (CMT3) (*11*). Domain-rearranged methyltransferases (DRMs) 1 and 2, with activities similar to the mammalian de novo methylase Dnmt3, are involved in reestablishing methylation patterns (*12*). MET1 targets CpG sites, whereas CMT3 and DRMs target other sites. The imprint of the *FWA* paternal allele is affected by reduced methylation induced by loss-of-function mutations in MET1, but not in CMT3 or in DRMs. This finding demonstrates that maintenance of the imprint relies on MET1 activity throughout the plant life cycle. Similarly, in mammals, DNMT1, which has strong homology to MET1, is involved in the maintenance of methylation linked to imprinted genes (*13*).

As the default status of *FWA* is to be silenced, differential expression of the maternal and paternal alleles (the basis of imprinting) relies on the release of maternal allele silencing. The DNA glycosylase DEMETER is specifically expressed in the central cell, the female gamete that gives rise to the endosperm (*14*). Loss of DEMETER activity results in the absence of *FWA* expression in endosperm (*1*). A similar result has been reported for the endosperm-specific expression of *MEDEA* (*14*). DEMETER activity may result in removal of methyl groups from cytosine residues in the 5′ region of the maternal allele of *MEDEA*,

and may have a similar effect on the maternal allele of *FWA*. A study reported in a recent issue of *Developmental Cell* (*15*) proposes that imprinting of *MEDEA* is controlled by antagonism between the two DNA-modifying enzymes, MET1 and DEMETER (*15*). Thus, imprinting of *MEDEA* and *FWA* in endosperm may rely on the same mechanism. Initially, both parental copies are silenced, then DEMETER removes methylation of *FWA* 5′ repeats but only for the maternal allele of the central cell, triggering endosperm-specific expression of *FWA*. Unlike *FWA*, which is expressed only during formation of the female gametes, *MEDEA* is expressed during the vegetative phase of the plant life cycle (*16*). It is not yet clear when and how *MEDEA* expression is silenced before female gametogenesis begins. Mutation of *MEDEA* causes a marked phenotype in endosperm (*17*), but only when the mutation is maternally inherited. The maternal effect is currently presumed to rely on the imprinted, silenced status of the paternal allele. In contrast to *MEDEA*, the function of *FWA* in endosperm remains unknown (*18*).

Imprinting in *Arabidopsis* apparently relies on a different mechanism for controlling DNA methylation compared with imprinting in mammals. Imprinting in mammals is linked to DNA methylation of large (up to 100 kb) specific intergenic regions, called imprinting control centers (ICRs), that regulate the expression of a group of genes (*19*). Mammalian DNA methylation undergoes a cycle where it is removed globally in the germline. Imprints are erased in primordial germ cells and are then reestablished during gametogenesis. In plants, no such global demethylation has been detected during the plant life cycle (*20*). Imprinting results from the removal of the methylation mark from one of the parental alleles. Unlike the situation in mammals, the imprinted status of plants is not inherited and appears to be confined to the endosperm, which does not contribute to the next generation.

References

1. T. Kinoshita *et al.*, *Science* **303**, 521 (2004); published online 20 November 2003 (10.1126/science.1089835).
2. F. Berger, V. Gaudin, *Chromosome Res.* **11**, 277 (2003).
3. C. Baroux, C. Spillane, U. Grossniklaus, *Adv. Genet.* **46**, 165 (2002).
4. M. Spielman, R. Vinkenoog, H. G. Dickinson, R. J. Scott, *Trends Genet.* **17**, 705 (2001).
5. M. Constancia *et al.*, *Nature* **417**, 945 (2002).
6. M. Charalambous *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8292 (2003).
7. J.-P. Vielle-Calzada *et al.*, *Genes Dev.* **13**, 2971 (1999).
8. T. Kinoshita *et al.*, *Plant Cell* **11**, 1945 (1999).
9. W. J. Soppe *et al.*, *Mol. Cell* **6**, 791 (2000).
10. E. J. Finnegan, W. J. Peacock, E. S. Dennis, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8449 (1996).
11. A. M. Lindroth *et al.*, *Science* **292**, 2077 (2001).
12. X. Cao *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4979 (2000).
13. E. Li, C. Beard, R. Jaenisch, *Nature* **366**, 362 (1993).
14. Y. Choi *et al.*, *Cell* **110**, 33 (2002).
15. W. Xiao *et al.*, *Dev. Cell* **5**, 891 (2003).
16. U. Grossniklaus, J.-P. Vielle-Calzada, M. A. Hoeppner, W. B. Gagliano, *Science* **280**, 446 (1998).
17. F. Berger, *Curr. Opin. Plant Biol.* **6**, 42 (2003).
18. T. Kinoshita, personal communication.
19. K. Delaval, R. Feil, *Curr. Opin. Genet. Dev.* **14**, in press.
20. H. Saze *et al.*, *Nature Genet.* **34**, 65 (2003).

IMMUNOLOGY

The Robin Hood of Antigen Presentation

Gennaro De Libero

T lymphocytes recognize a wide variety of antigens including peptides, glycolipids, and phosphorylated metabolites. The diversity of this antigenic repertoire, analogous to that of B lymphocytes, has been appreciated only recently. On pages 523 and 527 of this issue, two reports provide new information about how lipid antigens are presented to and activate T cells (*1*, *2*). In their study, Zhou *et al.* (*1*) reveal how lipid antigens are processed inside antigen-presenting cells before presentation to T cells. In complementary work, Moody *et al.* (*2*) identify lipopeptides as a new class of mycobacterial antigens that activate T cells and may be important in host defense.

Lipids, like peptides, are T cell immunogens if they fulfill three important prerequisites: (i) They must efficiently bind to the appropriate antigen-presenting molecule; (ii) they must form complexes with the presenting molecule that persist long enough to interact with the T cell receptors of a specific T cell population; and (iii) they must be efficiently loaded onto the presenting molecule at low concentrations.

Regarding the first requirement, glycolipid antigens bind via their hydrophobic regions to antigen-presenting molecules of the CD1 family. Resolving the structures of CD1d, CD1b, and CD1a has revealed the structural constraints of CD1 binding to lipid antigens (*3*–*5*). CD1 molecules contain a deep hydrophobic pocket responsible for binding the acyl chains of glycolipid antigens. The hydrophobic pockets of CD1d, CD1a, and CD1b have different shapes, conferring unique lipid-binding capacities.

The second requirement, the prolonged persistence of CD1-glycolipid complexes, is necessary if glycolipids are to be efficient T cell immunogens. Although the half-life of CD1-glycolipid complexes in vivo is unknown, the half-life in living cells in vitro is about 24 hours or longer and varies according to which CD1 molecule is involved (*6*). This period is sufficient to allow interaction of the glycolipid antigen with specific T lymphocytes.

The last requirement, the way in which glycolipid antigens are loaded onto CD1 molecules, is still poorly defined. The Zhou *et al.* work now reveals that endosomal lipid transfer proteins (LTPs) including saposins and GM2-activator proteins are important for loading glycolipid antigens onto CD1d molecules in late endosomes (see the figure). Their study initiates a new chapter in the field of antigen presentation to T cells.

Saposins comprise four different proteins that are found predominantly in the late endosomes of antigen-presenting cells and behave as lipid chaperones (*7*, *8*). They are able to pull glycosphingolipids such as gangliosides out of the endosomal membrane and offer them to hydrolases, which initiate glycolipid degradation. Zhou *et al.* convincingly show that saposins are required for loading recombinant CD1d with sulfatides and phosphatidylserine, two types of self-lipid antigens that are generated inside cells. Saposins optimally execute this function at pH 5.0 (the pH of late endosomes) and at 37°C. Lipid transfer occurs at equimolar ratios of CD1d and saposin, suggesting a direct intermolecular interaction rather than an enzyme-like mechanism.

On the basis of these results, the authors suggest a tug-of-war model in which LTPs and CD1d bind to and thus compete for the same lipid antigen. In this model, LTPs are envisaged as pulling glycolipid antigens from the membranes of late endosomes in antigen-presenting cells, as well as from endosomal CD1d molecules (see the figure). This renders endosomal

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