Chemical Shackles for Genes?

In both plants and animals, methyl group addition to DNA is proving to be necessary for normal development, apparently because it helps shut down genes

The more researchers learn about the way genomes work, the more genes seem like books in a library: Just a few are read at any one time, while the rest gather dust. Still unclear, however, is what kind of "librarian" runs the genetic library. Does the librarian actively pick out the right genes to be read, or does it maintain order by restricting the reading of all but a key few? Now, after decades of debate, researchers are converging on at least part of the answer.

In studies performed on the small plant *Arabidopsis thaliana*, three separate groups, one of which reports its results on page 654, have found evidence that the librarian does its job by placing certain genes off-limits. When the researchers genetically modified the plants to reduce the level of a natural chemical modification called methylation, in which a methyl group is attached to specific bits of DNA, genes ordinarily turned off in the course of development stayed active, and the plants developed abnormally. They made more leaves and took longer to start flowering than usual, and then produced abnormal flowers. "We're saying that methylation is required for plant development," says Yale University's Stephen Dellaporta, a coauthor of the *Science* report. "What you see is a cause-and-effect relationship, not just an association."

The finding doesn't rule out mechanisms that selectively activate certain genes, but it does support the long-standing suspicion that methylation is one of nature's ways of shackling the activity of genes that become unnecessary as development proceeds. Four years ago, for example, Timothy Bestor, then at Harvard Medical School, found the gene for a methylating enzyme in mice and then, working with Rudolf Jaenisch's team at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, crippled it. The resulting "knockout" mice produced embryos that died after only about 9 days. But because the embryos were so short-lived, it was not possible to see how lack of methylation affected their development.

And other species that naturally lack DNA methylation, including yeast and the fruit fly *Drosophila melanogaster*, do just fine without it. So even though researchers have generally found that heavily methylated genes tend to be inactive, doubts remained about whether methylation is in fact critical for gene regulation or the activities, including development, that depend on it. "There was a lot of hand-waving [about methylation]," but no direct evidence of its importance, notes developmental geneticist R. Scott Poethig of the University of Pennsylvania, Philadelphia.

The current work should change that. "It shows that methylation is critically important. It's a milestone in the field," says Aharon Razin, a molecular geneticist at Hebrew University Medical School in Jerusalem, Israel, who has been doing methylation studies for decades. Poethig agrees: "Clearly, this [new information] is a wake-up call for us to think more carefully about what methylation might be doing."

RONEMUS *ET AL.*

E. J. FINNEGAN

Petal power. Severe reduction in methylation causes the *Arabidopsis* flower to make excess petals and no stamens. The inset photo is normal.

For their experiments, Dellaporta, Michael Ronemus, also of Yale, and their colleagues worked with Jychian Chen from Academia Sinica in Taipei, Taiwan, to first block expression of the gene that codes for cytosine DNA methyltransferase, the enzyme that methylates DNA in *Arabidopsis*. They did this by introducing into *Arabidopsis* plants a reversed copy of the enzyme's gene. That copy produces a so-called antisense RNA that can bind to the normal RNA made by the methyltransferase gene and prevent it from directing synthesis of the enzyme. The researchers expected that synthesis of the enzyme would decrease in proportion to how much antisense was produced, and while they did not measure synthesis itself, the effects observed indicate that this is what happened.

In the modified plants, the researchers saw striking changes in the developmental patterns. Normally, *Arabidopsis* takes about 26 days to reach sexual maturity, passing through several distinct growth stages along the way. In the first or vegetative stage, leaves appear and the stem elongates. After an individual plant has produced eight leaves comes the transition to the inflorescence, in which flower stalks sprout. And finally, there is a shift to the reproductive stage, when flowers appear.

But plants making enough antisense to reduce the methylation of their genomes by 71% took about 47 days to start making flower stalks, during which time each plant produced about 27 extra leaves. They then went on to generate five times the usual number of flower stalks. In addition, the transitions between these developmental stages were sloppier, says Dellaporta. Leaf-making genes were still going strong in some cells even after others had shifted into a flower-production mode. These changes indicated that without the proper amount of methylation, developmental stages were prolonged, so that the actively growing tip of the plant retained its ability to differentiate into a wider variety of tissues for a longer time.

The second team studying methylation and *Arabidopsis* development has come up with results that show "remarkable" parallels to the Yale findings, says one of the researchers, E. Jean Finnegan at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Canberra, Australia. In work that will appear in the 6 August issue of *Proceedings of the National Academy of Sciences*, Finnegan and her colleagues genetically engineered *Arabidopsis* plants to make the same antisense as in the Yale work. The result was the same pattern of developmental abnormalities, together with some alterations not seen by the Yale group: The flowers of some plants developed extra petals instead of stamens and carpels, and in a few plants, genes for flower development were active in leaves.

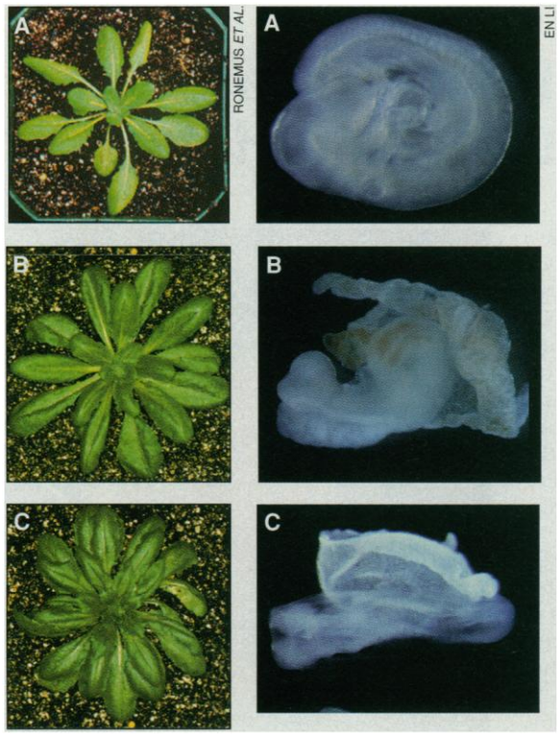
Taking a different tack, molecular biologist Eric Richards, of Washington University in St. Louis, also found that methylation is important for proper *Arabidopsis* development. Richards works with a mutant called *ddm1*, for "*decrease in DNA methylation*." When he first made the mutation, the resulting plants grew almost normally, even though their DNA was poorly methylated. As a result, some researchers had viewed the mutants as evidence against methylation's importance. But that is not the case, Richards says.

In still unpublished work, his team found that when the *ddm1* mutants are interbred, the progeny plants eventually show the same sorts of abnormalities as the antisense plants studied by the Yale and CSIRO groups. The original *ddm1* mutants looked relatively normal, he says, because in that first generation, methylation is lost mainly from repetitive, noncoding DNA, which does not make the proteins needed for forming leaves or flowers. Richards has not yet identified the specific biochemical defect causing low DNA methylation in *ddm1* plants—it apparently does not affect the methyltransferase—but suggests that as the defect is passed from generation to generation, it may lead to ever less methylation, with the result that developmentally important genes are eventually affected.

Indeed, the Yale and CSIRO groups found that the more severe the loss of methylation, the greater the effect on development. Studies of their original antisense plants showed, for example, that those making small amounts of antisense have much more normal growth patterns than those with lots of antisense. Furthermore, crossing two antisense plants yields some progeny with two copies of the antisense gene, one more than the parents. Consequently, their genes undergo even less methylation than those of their parents and, like Richards's *ddm1* mutants, become more abnormal with each passing generation, taking longer to mature and developing extra leaves and flower stalks. In contrast, the progeny of antisense plants and normal parents were still abnormal—as if the antisense parents had passed on their altered methylation patterns—but the progeny's abnormalities were less severe, presumably because the methylating enzyme is back in action.

Mice show a similar correlation between the extent of their methylation problems and the resulting developmental abnormalities. In still unpublished work, Jaenisch's group genetically engineered mice that contain a totally inactivated form of the methylating enzyme, rather than a crippled one. The embryos of these mice, with even more severely lowered gene methylation than the original knockouts, died within 8 days, rather than 9.5 or so, Jaenisch says. While that difference seems small, Bestor notes, "a day and a half [in the life of these embryos] reflects enormous changes."

Just how lack of methylation might disrupt development is less certain, but the Yale and CSIRO teams have come up with a clue. In contrast to Richards's finding that *ddm1* plants primarily lack methyl groups on their noncoding DNA, at least initially, they find that their antisense plants lack methyl groups on coding sequences, including DNA bits called promoters, which help control expression of actual genes, as well as on some noncoding sequences.



Proportional problems. The more drastic the drop in methylation (A to C), the more rosette leaves *Arabidopsis (left)* produces, and the less development mouse embryos *(right)* undergo.

Because experiments with cultured plant cells by other workers had shown that the addition of methyl groups to a gene's promoter can inactivate the gene, Dellaporta and Finnegan propose that in the whole plants, normal methylation of particular promoters turns off genes important to one stage of development, say, leaf formation, opening the way for genes for the next—flower stalk formation—to be expressed until they, too, are turned off by methylation. "[Methylation] is sort of an endogenous clock, a higher order type of regulation," Dellaporta suggests. "It can serve as a signal for the transitions that take place." This idea is consistent with other teams' observations that the DNA of leaves produced early in the plant's life is less methylated than that of later leaves.

Not everyone is ready to embrace that proposal, however. Bestor, now at Columbia University College of Physicians and Surgeons, thinks that methylation is not an endogenous clock at all, but a host defense mechanism. Normally, he argues, methyl groups serve to silence foreign genes that have inserted themselves into the plant genome sometime during its evolutionary history. With the methylating enzyme gone, these genes are able to become active, and their uncontrolled activity could cause the developmental abnormalities seen, he suggests. It will take determining the exact nature of methylated DNA to decide between these hypotheses.

There is debate, too, about whether methylation causes gene inactivation in the first place, or simply reinforces that state. And if methylation does both, then how many enzymes are involved? Jaenisch insists that there are two, one that inactivates the genes and another that keeps adding methyl groups at that spot each time the chromosome is replicated to keep the gene quiet. In support of this, he cites evidence from his knockout mice, in which some methylation goes on even without the one known methyltransferase.

For their part, Dellaporta and Bestor predict that there is one methylating enzyme that plays both roles in gene activation. As evidence, they cite the fact that so far no one has been able to find a second methylating enzyme. What's more, the plant and animal methyl transferases are very similar. So, if the plant enzyme can both maintain methylation patterns and initiate new ones, as it seems to be able to in tests with cultured plant cells, the animal enzyme ought to be able to do the same. "There may be others [methylating enzymes], but they may be very minor," Bestor says.

Israel's Razin thinks that no matter how many enzymes are involved, methylation works with other control mechanisms. He points to evidence from his group and others suggesting that protein factors that bind to DNA are what really shut down the gene, not methylation per se. These factors are known to help regulate gene expression, although exactly how is uncertain. It could be that the protein attaches wherever methylation has occurred and shuts down the gene, or that methylation alters the three-dimensional structure of a chromosome to make the target DNA accessible to these factors. Or the protein may be what enables methylation (and deactivation) to occur at all. Thus, Razin argues, "methylation is not the whole story."

But even if a methylating enzyme is just the genome's assistant librarian, and not the chief, the new results are boosting researchers' appreciation for methylation's role. This simple chemical modification alters how genetic information will be used, without changing the DNA sequence, and that, says Dellaporta, is quite impressive: "It's a beautiful way of changing the gene expression pattern in a stable but reversible fashion."

–Elizabeth Pennisi