band gap energy. This is very efficient if one traps the electron and hole in a narrow quantum well of a material such as gallium arsenide (GaAs), with short radiative recombination lifetimes of typically 10^{-9} s.

Lundstrom *et al.* trick nature by developing an efficient handle on the recombination lifetime of an electron-hole pair. Previous schemes to control recombination have spatially separated and trapped both electrons and holes in tilted double quantum wells (*4*), in the piezoelectric potential pockets of a surface acoustic wave travelling along a quantum well (*5*), or in potential pockets of a quantum well created electrostatically by small electrodes (*6*) (see panel C of the figure). These devices can store photogenerated electron-hole pairs for tens of microseconds, more than four orders of magnitude longer than their natural lifetime. Lundstrom *et al.* combine coupled quantum dots with a tiny electrostatic seesaw to trap photogenerated electron-hole pairs in fully confining cages (see panel D in the figure), increasing their storage time to seconds at low temperature before photon emission is induced.

Such devices may potentially be fabricated into large pixel arrays capable of high-resolution image processing. However, quite a few obstacles must be overcome before this intriguingly simple concept becomes a useful and competitive device. Reversible and effective storage of electron-hole pairs at room temperature remains to be demonstrated; similar to a CCD, this appears to be essentially a matter of proper choice and cleanliness of materials. Long storage time as realized by Lundstrom *et al.* in enclosing electron and holes in the cages of quantum dots should ideally be combined with fast and efficient release of photons. Here, the electrostatic devices (see panel C of the figure) appear to have an advantage over the seesaw-like devices (see panel D of the figure), because they do not need fast tunneling of carriers for both the recording and the emitting mode. A third problem is how to efficiently reemit the radiation into a preferential direction. Here the combination of electron-hole pair storage with the already well-established concepts of optical microcavities (*7*) and photonic band gap structures (*8*) appears to be the most promising route to a solution.

Most likely it will still take several years to transfer the elegant concept of reversible storage of photogenerated electron-hole pairs into practical photonic processing devices. At present, one can only speculate whether such devices will eventually be able to compete with conventional digital image processing or other smart pixel arrays. But the potential of doing so makes them an attractive subject for further investigations and improvements.

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PERSPECTIVES: MOLECULAR BIOLOGY

DNA Methylation de Novo

Adrian Bird

In an ideal world, biological processes would be understood at the molecular level by first identifying all the participating components and then by deducing their roles in the system through experiment. In reality, knowledge of each component is hard-won, and the temptation to assume that the key players are those that are currently known, ever-present. Fortunately, as human cDNA sequencing approaches saturation, candidate components in mammalian systems are becoming easier to find. One beneficiary is the field of DNA methylation in which researchers study the shutting down of gene expression through the addition of methyl groups to cytosine (C) bases in the DNA. Few players are more important in this arena than DNA methyltransferases (Dnmts), the enzymes responsible for methylating DNA. Thanks to DNA sequence databases, a clutch of new Dnmts as well as proteins that bind methylated C bases have recently been uncovered (*1*). Three recent papers (*2*–*4*) make clear that several of these newly recognized Dnmts are capable of de novo DNA methylation (that is, addition of methyl groups to DNA that has not been methylated before). The new work demonstrates the crucial importance of an appropriately methylated genome for successful embryonic development.

In vertebrates, the DNA targets for modification through methylation are C bases adjacent to guanine (G) bases. These sites can be methylated to very high levels. In most human somatic cells, for example, about 80% of CGs are methylated, with introns, exons, satellite DNAs, transposons (plus their inert relics), and other "nongenic" DNA all being affected. The distribution of methylated and nonmethylated CGs is not random, but conforms to a pattern. The most obvious features of the pattern are CpG islands at the promoters of many genes. These are clusters of nonmethylated CGs that form gaps between long methylated domains. Both the pattern of methylation and its overall level can sometimes vary. In early mouse embryos, for example, the amount of global methylation plummets, only to be restored at implantation of the blastocyst in the uterine wall. Even the resistance of CpG islands to methylation is not universal. They become methylated in large numbers on the inactive X chromosome (Xi), whereas equivalent CpG islands on the active X chromosome remain methylation-free. What determines whether a specific CG is methylated or not? Does specificity lie in the selective addition of methyl groups to certain chromosomal regions? Or, is methylation applied indiscriminately and patterned by judicious demethylation? The new work promises some answers to these important questions.

Until 2 years ago we knew of only one Dnmt (Dnmt1), and this seemed to be enough (*5*). Dnmt1 can "maintain" CG methylation after DNA replication by methylating the daughter DNA strand using the methylation pattern of the parental strand as a template (see the figure). Also, at least in vitro, Dnmt1 can methylate unmodified DNA de novo. The first evidence that Dnmt1 by itself could not explain de novo methylation in vivo arose when mouse embryonic stem (ES) cells lacking the enzyme nevertheless proved capable of methylating a retroviral provirus (*6*). Trawls of the EST (expressed sequence tag) database (which contains large numbers of unidentified cDNA sequences) revealed two novel proteins (called Dnmt3a and 3b) that looked like Dnmts and could transfer methyl groups to DNA in vitro (*7*). Were these the missing de novo methyltransferases?

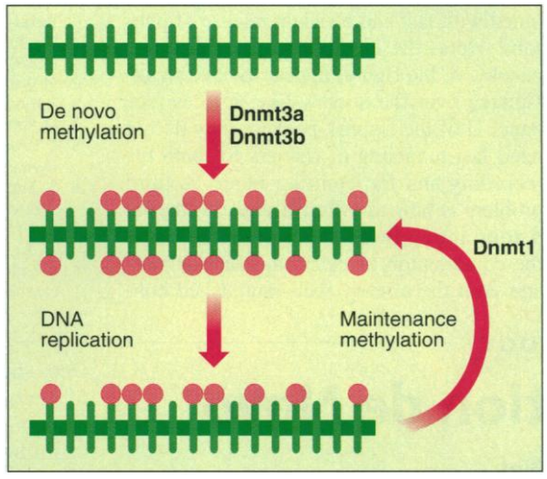
To answer this question, Okano *et al.* disrupted the mouse genes and examined the consequences (*2*). Mutations in either gene that resulted in nonfunctioning proteins prevented normal development. Mouse embryos that lacked Dnmt3a were runts and died 3 to 4 weeks after birth; those deficient in Dnmt3b did not develop further than 9.5 days after fertilization. Animals with mutations in both genes (double mutants) were

The author is at the Institute of Cell and Molecular Biology, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK. E-mail: a.bird@ed.ac.uk

much more severely affected, failing to develop beyond gastrulation. This result implies that the two gene products interact, and this notion was supported by analysis of methylation in mutant ES cells and mouse embryos. Various repetitive sequences were severely undermethylated in double-mutant mice, and single mutant animals often showed a partial reduction in methylation at specific sequence families. The provirus assay revealed that double-mutant ES cells failed to methylate retroviral provirus de novo, although each single mutant was partially active. The results indicate that Dnmt3a and 3b collaborate to bring about de novo methylation, and that Dnmt1, which is present normally in these cells, is unable to accomplish this task. The Dnmt3a/3b combination also appears to be crucial for successful global de novo methylation in embryos, as DNA sequences in the double mutants have the same low level of methylation at 9.5 days as at the minimally methylated blastula stage.

The credentials of Dnmt3a as a de novo methyltransferase have been established independently by Lyko *et al.* (*3*) using a very different approach. They introduced inducible *Dnmt3a* and *Dnmt1* gene constructs into *Drosophila* embryos, which normally lack detectable methylated cytosines. Induced expression of Dnmt3a in third-instar larvae caused measurable methylation of fly DNA, and this was lethal at all but very low levels. Dnmt1, by contrast, failed to modify fly DNA when expressed, although active enzyme could be detected in embryo extracts. Only when both Dnmt3a and Dnmt1 were active together did Dnmt1 activity become apparent. The kinetics of pupal mortality were now significantly accelerated, implying that methylation reached toxic levels more rapidly in the presence of Dnmt1, the maintenance methyltransferase. Like the mouse knockout studies, these experiments suggest that Dnmt3a can methylate DNA de novo, whereas Dnmt1 cannot (see the figure). Dnmt1 may be exclusively a maintenance enzyme. The idea that de novo and maintenance activities reside in different enzymes, which dates back more than two decades (*8*, *9*), is strikingly confirmed by these findings.

Can studies of Dnmt3a and 3b also shed light on the origin of DNA methylation patterns? It can, thanks to pioneering work on a rare human condition called ICF (Immunodeficiency, Centromeric instability, and Facial anomalies) syndrome. Extreme chromosomal abnormalities found in lymphocytes of ICF patients include frequent chromosomal fusions and pronounced extensions of the densely packed DNA (heterochromatin) in the centromeres (the constricted regions in the middle of chromosomes). A crucial insight into the disease came with the realization that these abnormalities resemble those seen when the drug 5-azacytidine was first used on human cells (*10*, *11*). Since those early observations, it has become clear that many of the effects of 5-azacytidine are due to its ability to dramatically demethylate DNA (*12*). Putting two and two together, French investigators were able to show that ICF patients are deficient in genomic methylation at satellite DNA sequences on either side of the centromeres but apparently not elsewhere (*10*). The ICF mutation turns out to map within the same region of chromosome 20 as the *DNMT3B* gene (*13*). Three laboratories now report that genomic DNA from a total of nine ICF patients carry mutations in both alleles of *DNMT3B* (the ortholog of mouse *Dnmt3b*) (*2*, *4*, *14*). All of the mutations affect highly conserved regions of the enzyme's catalytic domain, and in one case, a mutant enzyme was found to be unable to methylate DNA in vivo (*4*). A reasonable deduction is that ICF syndrome is caused by specific deficiencies in genomic DNA methylation.



Decorating DNA. A stretch of genomic DNA is shown as a line with self-complementary 5′-CG-3′ pairs marked as vertical strokes extending above and below the line. Unmethylated DNA at the top of the figure becomes methylated "de novo" by the DNA methyltransferase enzymes, Dnmt3a and Dnmt3b, to give symmetrical methylation at certain CG pairs. Upon semiconservative DNA replication, a progeny DNA strand is base-paired with one of the methylated parental strands (the other replication product is not shown). Symmetry is restored by the maintenance DNA methyltransferase Dnmt1, which completes half-methylated sites, but does not methylate unmodified CG pairs.

Mouse and human *DNMT3B* orthologs resemble one another because both show a strong preference for methylating pericentromeric satellite DNA. In other respects, however, the difference between the phenotypes of Dnmt3b-deficient mice and ICF patients is puzzling. Why does loss of DNMT3B activity cause embryonic death in mice, but comparatively mild symptoms in humans? Is it because ICF patients retain minimal levels of DNMT3B activity, whereas the mice are completely deficient in this DNA methyltransferase? Or, does the amino terminus of the protein—which is missing in the mouse mutants, but escapes mutation in ICF patients—perform an essential function that is unrelated to the transfer of methyl groups? There again, is it simply that mice and humans respond differently to methylation deficiency? Future work will doubtless distinguish between these possibilities, but there are also other pressing questions to be answered. Why is Dnmt3b activity preferentially targeted to specific regions of the genome? Is some feature of the primary DNA sequence recognized, or is sequence repetition—so often invoked as the raison d'être of mammalian de novo methylation—the real trigger? An intriguing feature of ICF syndrome raises an altogether different possibility. A CpG island on the Xi chromosome also turns out to be significantly undermethylated in ICF patients (*15*) although global methylation levels are not significantly reduced. "Silenced" CpG islands and pericentromeric satellite DNA share a chromosomal environment that can be described as heterochromatic. Could it be that Dnmt3b is attracted by specific "flavors" of heterochromatin, causing the DNA to become methylated and chromatin to condense stably? If so, DNA modification would not be causing heterochromatin to form anew, but would be sensing a preexisting heterochromatic state and ensuring its transmission to succeeding cell generations. In other words, CG methylation would be providing cellular memory.

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