chemical synthesis (*3*). This technology was recently commercialized by Thomas Swan & Co., Ltd., in a chemical plant designed for multipurpose synthesis. Together with ionic liquids (*4*–*6*), these alternative solvent strategies (sometimes referred to as alternative reaction media or green solvents) provide a range of options to industrialists looking to minimize the environmental impact of their chemical processes.

What are the advantages of using a room-temperature ionic liquid in an industrially relevant catalytic process? As noted above, ionic liquids have no detectable vapor pressure, and therefore contribute no VOCs to the atmosphere. But this is not the only reason for using ionic liquids. Another is that at least a million binary ionic liquids, and 10^{18} ternary ionic liquids, are potentially possible (*7*). (For comparison, about 600 molecular solvents are in use today.)

This diversity enables the solvent to be designed and tuned (*2*) to optimize yield, selectivity, substrate solubility, product separation, and even enantioselectivity. Ionic liquids can be highly conducting (*8*), dissolve enzymes (*9*), form versatile biphasic systems for separations (*10*), can form both polymers and gels for device applications (*8*), are media for a wide range of organic and inorganic reactions (*4*–*6*), and are the basis for at least one industrial process, called the BASIL process (see the figure) (*11*).

The BASIL process was developed and is operated by BASF. At the meeting, Matthias Maase (BASF) revealed that use of the BASIL process increases the productivity of their alkoxyphenylphosphine formation process by a factor of 80,000 compared with the conventional process. Other companies are also pursuing the use of ionic liquids. Bernd Weyershausen (Degussa) presented an ionic liquid–based process for the synthesis of organosilicon compounds. Use of an ionic liquid solvent enabled the catalyst to be easily recycled and reused without further treatment after separation from the product at the end of the reaction. Christian Mehnert (ExxonMobil) described biphasic hydroformylation with rhodium catalysts in ionic liquids.

Because research into ionic liquids is at an early stage, many of their properties remain to be elucidated. Nonetheless, ionic liquids have already provided access to new chemical processes. Recent papers describe their potential application as embalming fluids (*12*), in ion drives for space travel (*13*), for desulfurization of fuels (*14*), and as lubricants (*15*).

Ionic liquids have already found many laboratory applications in synthesis, catalysis, batteries, and fuel cells (*4*–*6*, *8*, *16*), and numerous new combinations of ionic liquid solvent properties are available or predicted. The next decade should see ionic liquids being used in many applications where conventional organic solvents are used today. Furthermore, ionic liquids will enable new applications that are not possible with conventional solvents. In the future, solvents will be designed to control chemistry, rather than the chemistry being dictated by the more limited range of molecular solvents currently used.

References and Notes

1. ACS Fall Meeting, 7 to 11 September 2003, New York. The Ionic Liquids symposium was sponsored by the ACS Division of Industrial and Engineering Chemistry, the Green Chemistry and Engineering Subdivision, the Separation Science and Technology Subdivision, and the Green Chemistry Institute.
2. M. Freemantle, *Chem. Eng. News* **76**, 32 (30 March 1998).
3. M. Poliakoff, J. M. Fitzpatrick, T. R. Farren, P. T. Anastas, *Science* **297**, 807 (2002).
4. R. D. Rogers, K. R. Seddon, Eds., *Ionic Liquids as Green Solvents: Progress and Prospects* (ACS Symp. Ser. 856, American Chemical Society, Washington, DC, 2003).
5. R. D. Rogers, K. R. Seddon, Eds., *Ionic Liquids: Industrial Applications for Green Chemistry* (ACS Symp. Ser. 818, American Chemical Society, Washington, DC, 2002).
6. P. Wasserscheid, T. Welton, Eds., *Ionic Liquids in Synthesis* (Wiley-VCH, Weinheim, Germany, 2003).
7. K. R. Seddon, in *The International George Papatheodorou Symposium: Proceedings*, S. Boghosian *et al.*, Eds. (Institute of Chemical Engineering and High Temperature Chemical Processes, Patras, Greece, 1999), pp. 131–135.
8. H. Ohno, Ed., *Ionic Liquids: The Front and Future of Material Developments* (CMC, Tokyo, 2003).
9. R. A. Sheldon, R. M. Lau, M. J. Sorgedrager, F. van Rantwijk, K. R. Seddon, *Green Chem.* **4**, 147 (2002).
10. K. E. Gutowski *et al.*, *J. Am. Chem. Soc.* **125**, 6632 (2003).
11. K. R. Seddon, *Nature Mater.* **2**, 363 (2003).
12. P. Majewski, A. Pernak, M. Grzymislawski, K. Iwanik, J. Pernak, *Acta Histochem.* **105**, 135 (2003).
13. M. Gamero-Castano, V. Hruby, *J. Propulsion Power* **17**, 977 (2001).
14. A. Bosmann *et al.*, *Chem. Commun.*, 2494 (2001).
15. W. M. Liu, C. F. Ye, Q. Y. Gong, H. Z. Wang, P. Wang, *Tribol. Lett.* **13**, 81 (2002).
16. R. D. Rogers, K. R. Seddon, S. Volkov, Eds., *Green Industrial Applications of Ionic Liquids* (Kluwer, Dordrecht, Netherlands, 2002), vol. 92.
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MOLECULAR BIOLOGY

MeCP2 Repression Goes Nonglobal

Robert Klose and Adrian Bird

After replication, mammalian DNA becomes marked by the addition of methyl groups to certain cytosine bases, almost exclusively those in the sequence 5′CpG. Just how the resulting pattern of methylated and nonmethylated cytosines is converted into biological outcomes is now starting to become clear. Methyl-CpG has emerged as a gene silencing signal that usually ensures the long-term shutdown of gene expression. Likely mediators of this effect are the methyl-CpG binding domain (MBD) proteins that recruit transcriptional silencing machinery to the DNA. One of these proteins, MeCP2, is of particular interest because about 80% of patients with a profound neurological condition called Rett syndrome carry a mutation in their *MECP2* gene (*1*). Mice lacking the *Mecp2* gene exhibit several features of Rett syndrome. Furthermore, targeted deletion of *Mecp2* in mouse brain causes a Rett-like phenotype that is virtually indistinguishable from the phenotype of mice in which every tissue lacks MeCP2. Global microarray analyses designed to search for target genes that are derepressed in the brains of MeCP2-deficient mice, and thus might be causally implicated in Rett syndrome, have not yielded any clear candidates (*2*). The existence of a genuine target gene is now highlighted on pages 885 (*3*) and 889 (*4*) of this issue. Chen *et al*. (*3*) and Martinowich *et al*. (*4*) describe their discovery that normal MeCP2 regulates expression of the gene encoding brain-derived neurotrophic factor (BDNF), a secreted protein that is essential for neural plasticity, learning, and memory. Their new findings, together with recent work in the amphibian *Xenopus laevis* (*5*), confirm that MeCP2 is a methyl-CpG–dependent transcriptional repressor and reveal its unexpected role in the induction of gene expression in the nervous system.

Belonging to a set of proteins synthesized in response to neuronal activity, BDNF is thought to be essential for converting transient stimuli into long-term changes in brain activity (*6*). Understanding how BDNF is regulated in neurons is important if we are to comprehend brain development, learning, and memory. There are four BDNF promoters, one of which (promoter III) responds

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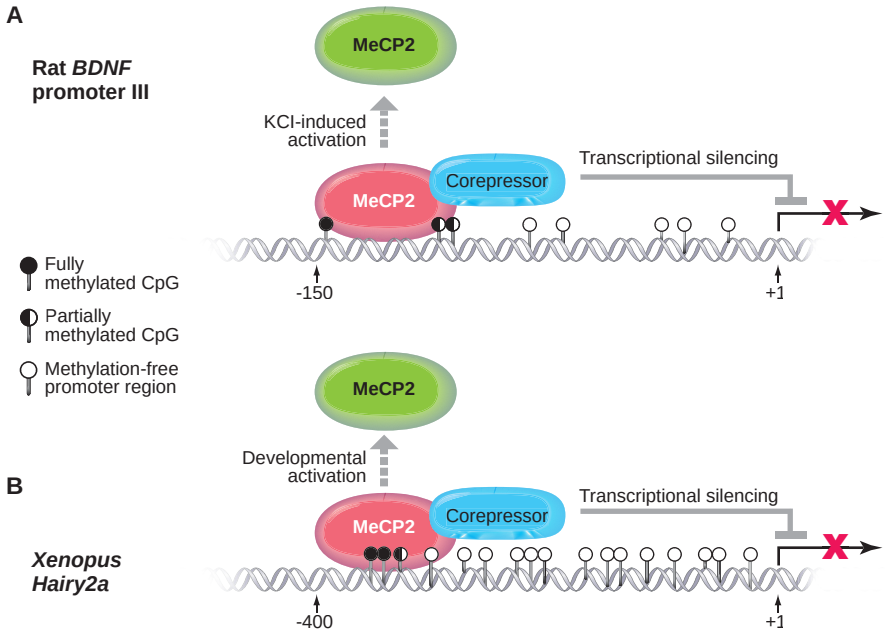

How MeCP2 lets go. Despite the existence of methylated CpGs throughout the vertebrate genome, loss of MeCP2 (a methyl-CpG binding domain protein) does not cause global deregulation of gene expression. MeCP2 binds to and represses the promoters of two specific genes: promoter III of the rat *BDNF* gene (**A**) and the *Hairy2a* gene promoter of *Xenopus* (**B**). In both cases, MeCP2 binds to the upstream fully methylated (black dot) or partially methylated (black and white dot) CpGs, but not to the downstream methylation-free (white dot) promoter region. MeCP2 recruits corepressor complexes, maintaining and solidifying a state of gene repression (red cross). Induction of gene expression leads to complete loss of MeCP2 from the upstream promoter region (dashed arrow), enabling transcription to proceed.

to artificial stimulation of cultured rodent primary cortical neurons treated with potassium chloride (KCl). By immunoprecipitating chromatin fragments containing MeCP2, Chen *et al.* and Martinowich *et al.* discovered that MeCP2 is bound to methylated CpG sites near promoter III of *BDNF* in resting neurons. But when the neurons were exposed to KCl—which causes membrane depolarization, calcium influx, and BDNF activation—MeCP2 dissociated from the *BDNF* gene promoter. Martinowich *et al.* further demonstrated that Sin3a—a transcriptional corepressor that forms complexes with histone deacetylases and associates with MeCP2 (*7*)—is also displaced after KCl treatment. Accordingly, loss of MeCP2 is accompanied by changes in histone modification (*8*), resulting in a transcriptionally repressive chromatin state being replaced by a permissive one.

How is MeCP2 displaced from promoter III when the *BDNF* gene is activated? One possibility is that the CpG methylation holding MeCP2 at the promoter is abruptly lost. In line with this hypothesis, Martinowich *et al.* found somewhat reduced CpG methylation in the relevant region of the activated promoter. An alternative mechanism for MeCP2 loss could involve modification of MeCP2 during the induction of gene expression such that it loses its affinity for the methylated promoter site. In support of this possibility, Chen *et al.* observed a time-dependent increase in phosphorylation of MeCP2 when neurons were stimulated with KCl. Moreover, Southwestern blot analysis indicated that the phosphorylated form of MeCP2 had a lower affinity for methylated DNA, although its presence at an unrelated methylated promoter—that of the imprinted *H19* gene—was not diminished by KCl treatment. Conceivably, displacement of MeCP2 is caused by a combination of cytosine demethylation and MeCP2 phosphorylation.

If MeCP2 is required for repression of promoter III before and after induction of *BDNF* gene expression, its absence should disrupt this process. Chen *et al.* tested this in vivo by examining inducible *BDNF* gene expression in cultured neurons from MeCP2-deficient mice. They found that *BDNF* transcription doubled in resting cells, whereas under stimulatory conditions there was no difference in *BDNF* transcription between wild-type and MeCP2-deficient neurons. While supporting the idea that MeCP2 helps to repress basal levels of BDNF expression, the effects of deleting the *Mecp2* gene appear to be subtle; uninduced *BDNF* gene expression increased from ~1% to ~2% of the induced level. It is perhaps not surprising that microarrays failed to detect such an effect.

BDNF is an important player in neuronal development, raising the possibility that misregulation of its gene because of the absence of MeCP2 may contribute to the symptoms of Rett syndrome. Other inducible promoters in neurons may also rely on repression by MeCP2. Recently, another bona fide MeCP2 target gene was discovered, this time in *Xenopus*. Stancheva and colleagues (*5*) found that reducing production of MeCP2 during early *Xenopus* embryogenesis by injecting antisense oligonucleotides caused gross defects in frog neurogenesis. By screening candidate genes known to be involved in Notch-Delta signaling cascades, they found that a transcriptional repressor protein, Hairy2a, is abnormally up-regulated in the absence of MeCP2. Hairy2a represses expression of proneuronal genes in non-neuronal cells surrounding developing neurons. Neurogenesis defects and gene expression changes caused by depletion of MeCP2 could be completely rescued by reexpressing wild-type human MeCP2, but not mutant forms of MeCP2 from Rett syndrome patients. The behavior of MeCP2 at the Hairy2a promoter has striking parallels with the BDNF story. Once again, MeCP2 is localized to the methylated upstream flank of the promoter (see the figure) and vacates the promoter upon activation of the gene (although CpG methylation in this case apparently remains unchanged). A deficiency of *Xenopus* MeCP2 leads to inappropriate activation of the *Hairy2a* gene, with severe consequences for the embryonic nervous system.

DNA methylation is often thought of as a "global" parameter in gene regulation. It is pervasive, being distributed throughout the genome. It is also an essential process—mice lacking the DNA methyltransferase enzyme, DNMT1, die during embryogenesis (*9*). Furthermore, mouse cells lacking DNMT1 display genomic instability (*10*), implying that methylation has wide-ranging effects on global genome function and integrity. Whether individual MBD proteins qualify as global gene regulators through their interpretation of methylation marks is less certain (*11*). Mice lacking MBD2, for example, are viable and fertile, but show deregulated expression of the interleukin-4 gene in T helper cells, indicating a gene-specific requirement for MBD2 (*12*). The papers discussed here establish for the first time that MeCP2 itself has specific gene targets and that it may work in concert with other factors as part of a multiprotein promoter complex. More unexpected still is its dynamic association with DNA, perhaps regulated by phosphorylation. This scenario contrasts with the conventional view that DNA methylation and MBD proteins create an almost immovable repressive environment. The new findings reveal unexpected plasticity in the biological interpretation of methylated DNA.

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Questions inevitably remain. Is the dynamic behavior of MeCP2 associated with the *BDNF* or *Hairy2a* gene promoters the exception or the rule? What other genes are induced when MeCP2 becomes phosphorylated? Which are the genes whose misregulation causes Rett syndrome? The explosion of knowledge about DNA methylation and the brain is at last making these questions experimentally accessible.

References

1. R. E. Amir *et al.*, *Nat. Genet.* **23**, 185 (1999).
2. M. Tudor *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 15536 (2002).
3. W. G. Chen *et al.*, *Science* **302**, 885 (2003).
4. K. Martinowich *et al.*, *Science* **302**, 889 (2003).
5. I. Stancheva *et al.*, *Mol. Cell* **12**, 425 (2003).
6. A. E. West *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 11024 (2001).
7. X. Nan *et al.*, *Nature* **393**, 386 (1998).
8. T. Jenuwein, C. D. Allis, *Science* **293**, 1074 (2001).
9. E. Li *et al.*, *Cell* **69**, 915 (1992).
10. A. Eden *et al.*, *Science* **300**, 455 (2003).
11. S. Kriaucionis, A. Bird, *Hum. Mol. Genet.* **12**, R221 (2003).
12. A. S. Hutchins *et al.*, *Mol. Cell* **10**, 81 (2002).

PHYSICS

Searching for Gravity's Hidden Strength

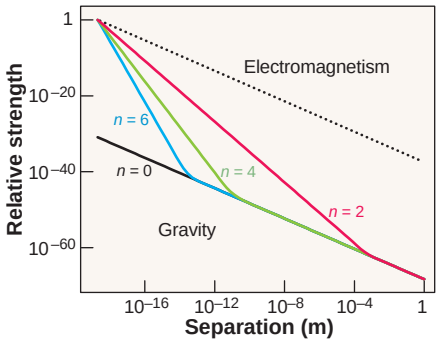
Jonathan L. Feng

Of the four known fundamental forces—gravity, electromagnetism, and the weak and strong forces—gravity is by far the weakest. The reasons for this weakness have long remained enigmatic. Recent proposals suggest, however, that the weakness of gravity may be evidence for extra spatial dimensions. Experiments ranging from tabletop tests of Newtonian gravity to searches for microscopic black holes in kilometer-scale detectors are now putting these ideas to the test.

The importance of gravity in everyday life results not from its strength but from its universality: Objects cannot be gravitationally neutral, and all bodies with mass attract. Yet as an interaction between elementary particles, gravity is extremely weak. For example, the gravitational attraction between two protons is 35 orders of magnitude weaker than their electromagnetic repulsion. This holds for protons separated by any distance r, because both gravitational and electromagnetic forces are proportional to $1/r^2$.

The observed weakness of gravity may, however, not be an intrinsic property of gravity, but may instead be an effect of extra spatial dimensions. This possibility is based on a simple consideration. Suppose that our three-dimensional (3D) world is merely a subspace of a higher-dimensional space, and that gravity propagates freely in all dimensions, but that all other forces are confined to our three dimensions. In contrast to the familiar three dimensions, the extra dimensions are curled up in small circles of circumference L. Hence, moving a distance L in the direction of any of the extra dimensions brings one back to one's starting place.

Now suppose that at some separation distance $r < L$, gravity is strong, that is, comparable to electromagnetism. As r increases, the electromagnetic force drops as $1/r^2$. However, the gravitational field spreads out in all available spatial dimensions, and the gravitational force therefore decreases much more rapidly as $1/r^{2+n}$, where n is the number of extra dimensions. This rapid drop continues until $r > L$, at which point the extra dimensions become less and less important and gravity recovers its $1/r^2$ behavior (see the figure).



Gravity in extra dimensions. The strength of gravity for various numbers of large extra dimensions n is compared to the strength of electromagnetism (dotted). Without extra dimensions, gravity is weak relative to the electromagnetic force for all separation distances. With extra dimensions, the gravitational force rises steeply for small separations and may become comparable to electromagnetism at short distances.

If this picture is correct, then gravity is not intrinsically weak: It is as strong as electromagnetism at small length scales. It appears weak at the relatively large distances of common experience only because its effects are diluted by propagation in extra dimensions. The distance at which the gravitational and electromagnetic forces might have equal strength is unknown, but a particularly interesting possibility is that it is 10^{-19} m, the distance at which the electromagnetic and weak forces are known to unify to form the electroweak force (*1*).

A priori, the size of the extra dimensions L and their number n are independent parameters. However, to achieve equality of gravitation and electromagnetic forces at 10^{-19} m, they become constrained by the relation

$$L \approx 10^{(32/n)-19}\ \text{m} \qquad (1)$$

For large n, the strength of gravity grows very rapidly at microscopic length scales. Gravity may then deviate from its $1/r^2$ behavior only at very small distances and still be comparable to electromagnetism at 10^{-19} m.

This scenario, called "large extra dimensions" because the length L of Eq. 1 is large relative to typical length scales in particle physics, raises many more questions than it answers. When first proposed, perhaps its most surprising aspect was that such a bold modification of Newtonian gravity was not immediately excluded by data. Now, however, a wide variety of experiments are reaching the sensitivity required to test these speculative ideas. In combination, they probe all possible values for the number of extra dimensions, placing the entire scenario on the threshold of detailed investigation.

The possibility of one large extra dimension is untenable. It requires the extra dimension to be of size $L \approx 10^{13}$ m, a length scale where the $1/r^2$ gravitational force law is clearly still valid. For two extra dimensions, each extra dimension would have $L \approx 1$ mm. Sensitive tests of gravity are notoriously difficult at such length scales. Nonetheless, recent tabletop experiments with torsion pendulums have excluded significant deviations from the $1/r^2$ force law at length scales as small as 0.1 mm (*2*).

Astrophysical observations provide less direct but more stringent constraints on low numbers of extra dimensions (*3*, *4*). For two extra dimensions, for example, the gravitational force would be enhanced at large enough length scales that supernovae should release much of their energy as gravitational energy—in conflict with observations. These constraints, which were noted immediately after the proposal of large extra dimensions, exclude scenarios with few extra dimensions.

The challenge, then, has been to explore large numbers of extra dimensions, such as the six or seven favored by string theory. In such cases, tabletop and astrophysical constraints are ineffective, because the predicted deviations from Newtonian gravity oc-

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