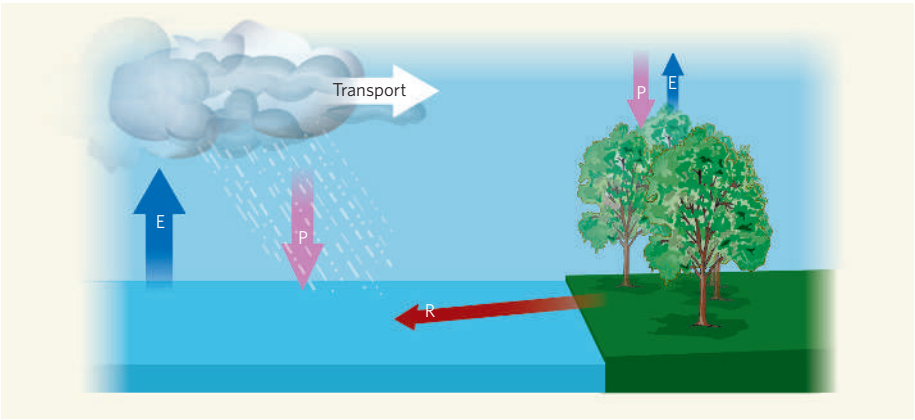

Figure 1 | Plants, CO_2 and the global water cycle. The balance between precipitation (P) and evaporation (E) over land determines the surface runoff (R), which returns water from the continents to the oceans. Plant photosynthesis plays an integral role in the global water cycle, by mediating the transfer of water from the land surface to the atmosphere. Elevated CO_2 can lead to closure of leaf stomata, which reduces leaf water loss and thereby decreases overall continental evaporation. Gedney *et al.*[2] show that this process, initiated by increased atmospheric CO_2, can account for the increases in surface runoff observed over the past century.

used in climate science to attribute observed climate trends to both natural and anthropogenic causes[5]. Recent increases in surface temperature have been successfully attributed to increases in CO_2 and other greenhouse gases[6], as have changes in other climate variables such as ocean heat content[7] and sea-level pressure[8]. Gedney and colleagues' research represents the first time that detection-and-attribution techniques have been used to successfully link changes in the functioning of terrestrial ecosystems to human influences on the atmosphere.

As with any statistical analysis, these results are only as sound as the model used, the experimental design and the quality of observations. As our understanding of the terrestrial biosphere and our ability to model and monitor it improves, contributors to observed runoff trends that were not considered in this study may well be identified. Gedney and colleagues' findings are nonetheless an important step forward in our understanding of the diverse and complex ways in which human activities are affecting the global climate system. The findings have implications for future surface warming and freshwater availability, both of which could increase if CO_2 continues to affect surface-water fluxes as demonstrated here. This research also opens some fascinating avenues of investigation — such as the possibility of using records of river runoff to monitor the functioning of terrestrial ecosystems in response to climate change, or of studying how changes in runoff induced by elevated CO_2 might affect ocean circulation. ■

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1. Labat, D., Godderis, Y., Probst, J. L. & Guyot, J. L. *Adv. Wat. Res.* **27,** 631–642 (2004).
2. Gedney, N. *et al. Nature* **439,** 835–838 (2006).
3. Medlyn, B. E. *et al. New Phytol.* **149,** 247–264 (2001).
4. Field, C., Jackson, R. & Mooney, H. *Plant Cell Environ.* **18,** 1214–1255 (1995).
5. International Ad Hoc Detection and Attribution Group *J. Clim.* **18,** 1291–1314 (2005).
6. Tett, S. F. B. *et al. J. Geophys. Res.* **107,** 4306 (2002).
7. Reichert, K. B., Schnur, R. & Bengtsson, L. *Geophys. Res. Lett.* **29,** 1525 (2002).
8. Gillett, N., Zwiers, F. W., Weaver, A. J. & Stott, P. A. *Nature* **422,** 292–294 (2003).

DEVELOPMENTAL BIOLOGY

Two paths to silence merge

Panthea Taghavi and Maarten van Lohuizen

To maintain their identity across generations, specialized cells must heritably repress swathes of genes — keeping active only genes necessary for the cell's purpose. Now it seems two repressive pathways join forces.

Even though cells of all developmental stages carry the same DNA, they have their own identity, defined by the combination of proteins expressed in each cell. These expression patterns, although set early during development, are reproduced in each mature, specialized cell later in life, over many cell divisions. This phenomenon is referred to as 'cellular memory'. Any perturbation of this fine-tuned system is a hazard to proper development and health.

Cellular memory is thought to be regulated by two 'epigenetic' mechanisms, which heritably change the characteristics of the cell without altering its DNA sequence. These mechanisms do this by altering the chromatin (the DNA and its associated proteins), which changes the availability of the genes to be expressed. One mechanism involves a group of enzymes that attach methyl groups to the DNA, the DNA methyltransferases (DNMTs); the other involves Polycomb group (PcG) proteins, which modify the histone proteins around which the DNA is wrapped. On page 871 of this issue, Viré *et al.*[1] show a direct interaction between DNMTs and the Polycomb protein EZH2 that hints at how these complex systems might collaborate to set up cellular memory.

DNA methylation helps to reorganize chromatin into a 'silent' state in which genes are not expressed. In mammals, DNMT3A and -3B are mainly responsible for establishing methylation at previously unmethylated sites, whereas DNMT1 is the major maintenance methyltransferase, reproducing existing methylation patterns during cell division[2].

PcG proteins were originally identified for their role in maintaining the repressed state of certain developmental genes in the fruitfly *Drosophila*. EZH2 is a histone methyltransferase that can methylate lysine 27 of the tail of histone H3 (H3-K27) or lysine 26 of histone H1 (H1-K26)[3]. This enzyme functions as part of the Polycomb repressive complexes 2 and 3 (PRC2/3), and whether it methylates H1 or H3 depends on which other proteins are in the complexes[3]. These histone modifications can attract other repressive complexes, such as Polycomb repressive complex 1 (PRC1), that then propagate the silenced state[4,5].

In their study, Viré *et al.* show convincingly that EZH2 interacts with all three DNMTs *in vitro* and *in vivo* (Fig. 1). DNMTs have not been found previously in purified PcG complexes from *Drosophila* or mammals, perhaps indicating that the interactions observed by Viré *et al.* are transient. How could these interactions affect the gene-expression programme of the cell? The authors find that proper repression of a few genes targeted by EZH2 requires both EZH2 and DNMTs. Moreover, EZH2 is needed to bring DNMTs to the regulatory regions ('promoters') of the EZH2 target-genes. Depletion of EZH2 disturbs this recruitment, de-repressing the genes and allowing expression. At the same time, there is a decrease in the repressive histone H3-K27 methylation marks.

These results reveal that EZH2, as part of the PRC2/3 complexes, can physically recruit DNMTs to certain target-genes and that this process is essential for silencing the genes.

Surprisingly, reducing the levels of any of the DNMTs separately results in similar de-repression of the EZH2 target-genes. This is unexpected because the different DNMTs by and large seem to fulfil distinct roles in development and cellular viability[2]. It is possible that various EZH2-containing complexes might exist that can interact with the *de novo* DNMTs or the maintenance DNMT under different circumstances. This would allow PcG complexes to take part in initiating and maintaining transcriptional programmes depending on the chromatin environment and stimuli.

Viré *et al.* show that overexpression of EZH2 increases the DNA methylation of the promoters of its target genes, and that, remarkably, when EZH2 is downregulated the methylation decreases rapidly. This highlights the dynamic nature of DNA methylation and suggests that, just as histones are associated with separate enzymes for methylation and demethylation of their tails[6], DNA demethylases may exist to actively remove the methyl marks, although such enzymes remain elusive.

How is DNA methyltransferase activity of DNMTs regulated by EZH2 histone methyltransferase activity? The authors propose a model whereby DNMTs require the EZH2-mediated histone H3-K27 methylation to methylate the DNA at target-gene promoters. This is analogous to the link proposed between the H3-K9 histone methylation system and DNA methylation in certain silent chromatin regions[7]. One possibility is that DNMTs recognize the H3-K27 methylation marks directly or indirectly through the interaction with another yet unknown partner that can regulate DNMT accordingly. Another possibility is that the H3-K27 methylation mark is not the engaging signal, but that DNMTs could be activated by EZH2 directly, perhaps by methylation.

DNMT recruitment to EZH2 target-genes not only adds another layer of repression to 'lock in' the silent state by methylating the surrounding DNA, but it might also help to recruit PRC1 proteins[8]. It is also possible that EZH2 acts independently of PRC1 by recruiting co-repressors that associate with DNMTs. In both scenarios, DNA methylation of EZH2 target-genes can lead to the recruitment of proteins that bind to the methyl-DNA marks, and the formation of more repressive complexes (Fig. 1). So are all EZH2 target-genes also silenced by DNA methylation, and are they overlapping with or separate from genes repressed by the EZH2–PRC1 interaction? Notably, the mechanism by which PcG-induced silencing is inherited is still not known. Perhaps, for a select group of target genes, EZH2-coupled DNA methylation can participate in preserving PcG-related cellular memory during DNA replication, through the maintenance DNMT (Fig. 2).

Although Viré *et al.* provide a simple and attractive model, their findings cannot easily be generalized to other PcG target-genes. For example, during X-chromosome inactivation, when one entire X chromosome is silenced in female mammals, EZH2 and another PcG protein known as EED accumulate on the inactive X chromosome at the onset of the process — well before there is any DNA methylation[9]. Moreover, paternal-specific repression of the *Kcnq1* gene domain in mouse placenta depends mainly on recruitment of EZH2 and EED with no apparent promoter DNA methylation[10,11]. These and other examples[12,13] imply that although PcG-related silencing and DNA methylation cooperate in many cases, they are not always coupled. One intriguing possibility is that DNMTs could be inactive when they are recruited by EZH2, only being activated later by some as yet unknown regulatory signal.

Finally, the core members of the PRC2 complex are evolutionarily conserved from plants to mammals[5]. Yet, the nematode *Caenorhabditis elegans* seems to lack any detectable DNA methylation or genomic sequences resembling genes encoding DNMTs[14], and fly DNA has very little methylation[5,15]. So, the connection between PcG proteins and DNA methylation must have evolved relatively recently.

Notwithstanding the questions that remain, the demonstration that the PcG protein EZH2 and DNA methylation can be directly coupled will provoke new hypotheses regarding the dynamics and heritability of epigenetic marks that can now be explored. ■

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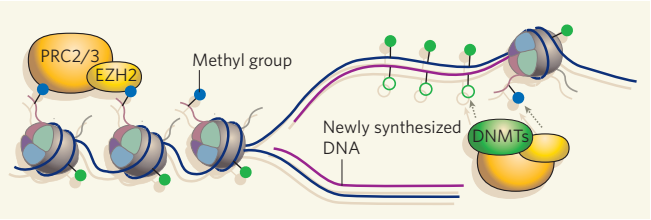

Figure 2 | Inheritance of epigenetic memory. A possible mechanism for inheritance of Polycomb-related epigenetic memory through DNA methylation during DNA replication. As the DNA is replicated, the maintenance DNA methyltransferase, DNMT1, adds a methyl mark (open green circles) to the newly replicated strand (purple), thus passing the DNA methylation mark to the progeny of the dividing cell through DNA replication. Viré *et al.*[1] report that DNMT1 interacts with the histone methyltransferase EZH2, contained in the PRC2/3 complexes, possibly recruiting the complexes to sites of newly methylated DNA, to ensure that the histones too are in a repressive state.

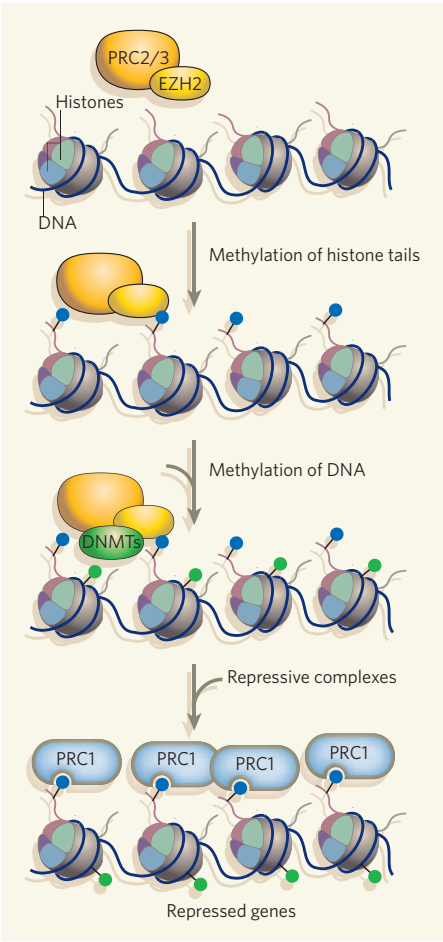

Figure 1 | Making epigenetic methylation marks. The Polycomb group protein EZH2 acts as part of the Polycomb repressive complexes 2 and 3 (PRC2/3), which add methyl groups to histone proteins, primarily to lysine 27 of histone H3 (small blue circles). Viré *et al.*[1] show that EZH2 interacts with DNA methyltransferases (DNMTs) to recruit them to the regulatory region of certain target genes. The DNMTs methylate DNA (small green circles), either setting up new methyl marks during development, or maintaining the marks after each round of cell division. These marks are correlated with gene repression, and they could have a role in recruiting further repressive complexes, such as PRC1 or histone deacetylase co-repressors, through methyl-binding domain proteins.

1. Viré, E. *et al. Nature* **439,** 871–874 (2006).
2. Li, E. *Nature Rev. Genet.* **3,** 662–673 (2002).
3. Kuzmichev, A., Jenuwein, T., Tempst, P. & Reinberg, D. *Mol. Cell* **14,** 183–193 (2004).
4. Lund, A. H. & van Lohuizen, M. *Curr. Opin. Cell Biol.* **16,** 239–246 (2004).
5. Ringrose, L. & Paro, R. *Annu. Rev. Genet.* **25,** 413–434 (2004).
6. Yu-ichi, T. *et al. Nature* advance online publication doi:10.1038/nature04433 (2005).
7. Lehnertz, B. *et al. Curr. Biol.* **13,** 1192–1200 (2003).
8. Hernández-Muñoz, I., Taghavi, P., Kuijl, C., Neefjes, J. & van Lohuizen, M. *Mol. Cell. Biol.* **25,** 11047–11058 (2005).
9. Heard, E. *Curr. Opin. Cell Biol.* **16,** 1–9 (2004).
10. Umlauf, D. *et al. Nature Genet.* **12,** 1296–1300 (2004).
11. Lewis, A. *et al. Nature Genet.* **12,** 1291–1295 (2004).
12. Yu-Wei, L. *et al. Cancer Res.* **64,** 8184–8192 (2004).
13. Feldman, N. *et al. Nature Cell Biol.* advance online publication doi:10.1038/ncb1353 (2006).
14. Grace Goll, M. & Bestor, T. H. *Annu. Rev. Biochem.* **74,** 481–514 (2005).
15. Marhold, J., Kramer, K., Kremmer, E. & Lyko, F. *Development* **131,** 6033–6039 (2004).