volume of ice that would melt owing to warming, and by ignoring potential feedbacks that might occur if the meltwater were put back into the ocean.

Li *et al.* are not the first to address this shortcoming in a modelling study (see, for example, refs 12 and 13). However, they are the first to examine whether meltwater from Antarctica is directly or indirectly causing the observed temperature increase at the bottom of the ocean (Fig. 1).

The authors used a model that accurately simulates ocean–ice interactions, and that reproduces the observed volume and characteristics of AABW along the edge of the Antarctic in the correct geographical locations[14]. They first imposed changes in the wind, heat and meltwater that were measured[15] between 1991 and 2019, to see how the formation of AABW and the circulation of the abyssal ocean would respond. They then modelled the response to changes that are projected to occur between 2020 and 2050 according to the current global climate models.

Two hypotheses have been proposed previously to explain some or all of the observed deep warming. The first holds that AABW forms at the same rate as it did previously, but at a slightly higher temperature[2]. The second suggests that production of AABW has decreased, allowing other (warmer) water to intrude on its former territory[3]. These two mechanisms affect climate in different ways — the first actively sequesters 'new' heat (from the atmosphere) in the deep ocean, whereas the second repartitions 'old' heat in the deep ocean.

Li and colleagues' study supports the latter hypothesis: increased meltwater inhibits the formation of cold AABW, which reduces its volume, thereby warming the abyssal ocean and decreasing its ventilation. The authors' simulations suggest that this trend will continue, and that the combination of wind and warming perturbations have little effect on the abyssal ocean.

Studying the deep waters of the ocean might seem remote from everyday concerns, but these waters are crucial for distinguishing between transient and equilibrium climate change. The former relates to the temperature change that results from increases in atmospheric heat and carbon dioxide before the deep oceans have had time to equilibrate[16] (as well as heat, oceans sequester around 25% of anthropogenic CO_2 emissions[17]). The depth at which atmospheric heat and CO_2 are stored influences the time it will take for the ocean to come into equilibrium with the 'new' atmosphere, and therefore defines the timescale of transient climate change.

Taken together with the results of other studies[12,13], Li and colleagues' simulations indicate that atmospheric heat is not making it down to the deepest ocean and that only intermediate depths are currently available to buffer the anthropogenic effects on climate. The timescale associated with transient climate change will probably be shorter rather than longer, which is bad news for humans in this century.

The convergence of models of global climate, the Earth system and weather enhances scientists' ability to make accurate predictions[18]. Such predictions are essential to better prepare society to withstand extreme events such as droughts and floods, heatwaves and wildfires[19]. Li and colleagues' study takes a step in the right direction by highlighting the ocean's influence, from top to bottom, on the global climate.

Scientists at Australian institutions — including the authors, as well as many others — have long been acclaimed for their expeditionary oceanography and climate research. It is our good fortune that they live on the doorstep of the Southern Ocean, the most influential and least-understood player in the Earth system's response to anthropogenic climate change.

Joellen L. Russell is in the Department of Geosciences, University of Arizona, Tucson, Arizona 85721, USA.
e-mail: jrussell@arizona.edu

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Molecular biology

Inheritance of epigenetic marks scrutinized

Serge McGraw & Sarah Kimmins

There is debate about how epigenetic marks, such as methyl groups on DNA, can be passed down from parent to offspring. A mouse model involving targeted DNA methylation will better equip researchers to study this process.

Parental obesity, nutrient-poor diets or stress can compromise the health of children, and potentially grandchildren, through inheritance of 'epigenetic modifications'[1]. These biochemical changes to DNA or associated histone proteins — the addition of methyl or acetyl groups, for instance — affect gene expression, cell type and more, without altering DNA sequence. Epigenetic modifications can arise in response to environmental cues and are passed down to subsequent generations in eggs and sperm[2]. Writing in *Cell*, Takahashi *et al.*[3] present a mouse model in which methylation is induced at targeted sites in DNA, and demonstrate that this approach can be used to address pressing mechanistic questions about such transgenerational epigenetic inheritance (TGI).

There are two windows in a mammal's life during which most epigenetic marks are removed, then reset to ensure proper cell regulation — a process called epigenome reprogramming. The first is in developing sperm and eggs, beginning with precursors called primordial germ cells (PGCs), and the second is in embryos immediately after fertilization[4]. For TGI to occur, genes must 'remember' the epigenetic marks they previously had, enabling them to escape complete erasure during epigenome reprogramming. So far, only a handful of studies[5–8] have provided evidence for TGI.

DNA sequences at which cytosine and guanine bases are found side by side (known as CpG sites) are common sites of DNA methylation. These can be clustered

together in genomic regions called CpG islands – these islands are typically found in non-protein-coding sequences called promoters that drive gene expression. Unlike individual CpG sites, CpG islands are typically unmethylated. The group that performed the current study previously developed a technique to introduce a DNA fragment lacking CpG sites into a CpG island in human stem cells[9]. They found that the fragment induced methylation of the entire region. Remarkably, the methylation marks were retained after the CpG-free DNA fragment was removed, and throughout subsequent cell division and differentiation.

In the current study[3], Takahashi *et al.* expanded this *in vitro* approach to study TGI in mice. They started in culture, using mouse embryonic stem cells, which are isolated from early embryos and can give rise to all the cells of the body. They targeted CpG islands in promoters for either the *Ankrd26* or *Ldlr* genes, both of which regulate aspects of metabolism. They then injected the edited cells into eight-cell embryos, to give rise to 'mosaic mice' made up of both normal and methylation-edited cells (Fig. 1a).

Methylation of promoters is associated with repression (silencing) of gene expression. Takahashi and colleagues found that the induced DNA methylation and associated silencing of either *Ankrd26* or *Ldlr* expression was stably maintained during embryonic development, and resulted in adult mice that had abnormal metabolic traits – obesity in the *Ankrd26*-edited mice and high cholesterol levels in the *Ldlr*-edited mice.

Next, the authors showed that the acquired DNA methylation could be transmitted from parents to offspring across at least four generations. The marks were preserved in somatic cells (from tissues other than eggs, sperm and their precursors), in the DNA inherited from both mothers and fathers. The researchers demonstrated that repression of *Ankrd26* expression was associated with other epigenetic modifications indicative of inactive chromatin (the complex that packages DNA around histones in the nucleus, which, when inactive, is associated with gene silencing). Descendants of these animals, like the first generation, were obese.

Unexpectedly, the methylated CpG islands in each promoter were altered differently during epigenome reprogramming. The island in the *Ldlr* promoter was demethylated in PGCs, and this lack of methylation was maintained in mature eggs and sperm and through the second window of reprogramming after fertilization. The inherited pattern of promoter methylation was finally re-established in the early embryo. By contrast, the *Ankrd26* CpG island was only partially demethylated in PGCs, eggs and post-fertilization embryos. The pattern was more variable in sperm, with some *Ankrd26*-edited mice showing partial demethylation in sperm, and others showing high DNA methylation levels in about half of sperm and near-total demethylation in the other half. Regardless of the situation in sperm, the pattern of methylation became re-established in the early embryo. Thus, rather than the methyl marks themselves being inherited, this mouse model indicates that a memory of DNA methylation enables TGI (Fig. 1b).

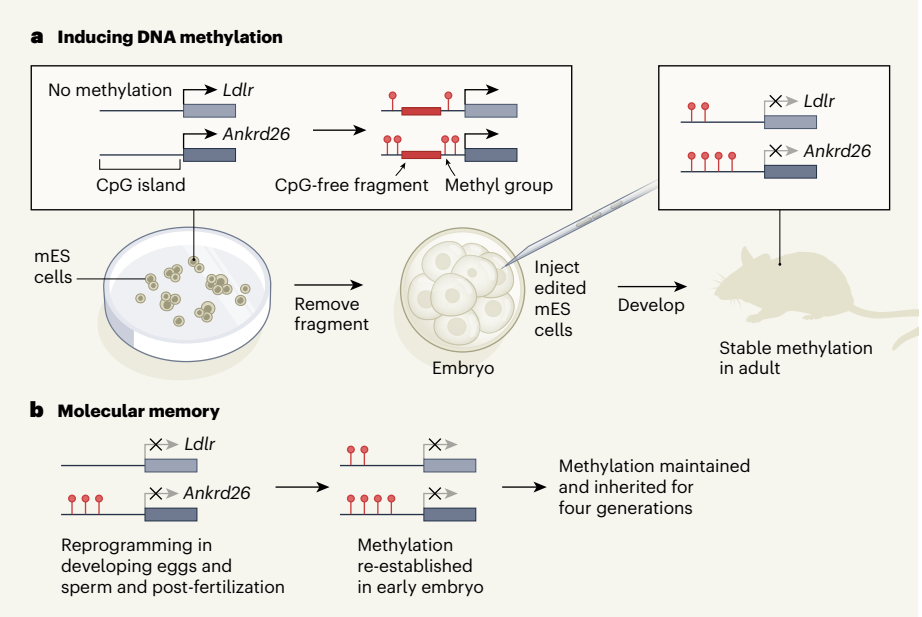

Figure 1 | Inducing DNA methylation to study transgenerational epigenetic inheritance. DNA sequences that drive gene expression often contain regions called CpG islands, in which methyl groups (which are associated with gene repression) are absent from DNA. **a**, Takahashi *et al.*[3] edited the CpG islands of two metabolic genes, *Ldlr* and *Ankrd26*, in mouse embryonic stem cells (mES cells). They added a CpG-free fragment to the island (first inset box), which induced methylation in the surrounding region. They then removed the fragment and injected the cells into embryos. This manipulation led to methylation of the CpG islands, to differing degrees for each gene. Methylation remained stable even when the embryos developed into adult mice (second inset box). **b**, Methyl groups are typically wiped from the genome during two windows of reprogramming: first, in developing eggs and sperm; and second, immediately after fertilization. However, not all methyl marks were lost from the *Ankrd26* island during reprogramming and, unlike in other regions, methyl marks were not re-established in either island between the two windows of reprogramming (not shown). Methylation was fully re-established in early embryos, indicating a molecular memory of the induced methylation, which was inherited down at least four generations of animals.

> **"The acquired DNA methylation could be transmitted from parents to offspring across at least four generations."**

Two obvious questions arise from these findings: what are the mechanisms that protect the *Ankrd26* CpG island from demethylation, and what constitutes this methylation memory? To answer these questions, the authors' mouse models should be further characterized to examine other factors that might contribute to TGI. For example, an examination of the interactions between chromatin and non-coding RNAs in key reprogramming periods – with or without the CpG-free DNA fragment – is warranted.

The mechanisms might also involve neighbouring DNA sequences. Sometimes, one copy of a gene is permanently silenced (a phenomenon known as imprinting), which involves the gene escaping epigenome reprogramming after fertilization. In some cases, the ability of imprinted genes to escape this reprogramming window depends on DNA elements called retrotransposons being nearby[5,6,10]. Moreover, imprinted genes are 'insulated' by the protein CTCF – this prevents DNA elements outside the insulated region affecting genes[10]. To explore these possibilities for this article, we examined the DNA sequences for mouse *Ankrd26* and *Ldlr*, and identified retrotransposons throughout the genes, along with promoter-adjacent binding sites for CTCF. DNA sequences known as transposable elements often harbour CTCF binding sites – these elements can influence spreading of epigenetic modifications across chromatin[10]. We posit that a molecular memory of CpG-island methylation persists, perhaps involving a unique combination of retrotransposons, transposable elements and CTCF around a given promoter that dictates

how well protected it is from epigenome reprogramming.

The effects that methylation editing have on the body in general should also be considered. For instance, the metabolic traits that arise in Takahashi and colleagues' animals can themselves lead to widespread epigenetic changes[1] – these might contribute to the observed TGI. And *Ankrd26* is normally expressed in immature sperm and in the ovaries, so it is possible that *Ankrd26* silencing itself contributes to alterations in epigenome reprogramming.

Takahashi and colleagues' approach to methylation enables targeted interrogation of genomic features that might be involved in TGI. The next steps include characterizing how the molecular landscape (epigenetic modifications, 3D chromatin organization and more) and the underlying DNA sequence together contribute to protecting edited genes from the reprogramming machinery. The authors' approach should also be used to study the heritability of epigenetic marks in disease-linked genes. Identifying the mechanisms that link a parent's accumulation of epigenetic marks to an offspring's development and health could make huge waves in our understanding of inherited disease.

Serge McGraw is in the Department of Obstetrics and Gynecology, Faculty of Medicine, University of Montreal, Quebec H3C 3J7, Canada, and in the CHU Sainte-Justine Research Centre, Montreal. **Sarah Kimmins** is in the Department of Pathology and Cell Biology, Faculty of Medicine, University of Montreal, Quebec H3C 3J7, Canada, and in the CHUM Research Centre, Montreal.
e-mail: sarah.kimmins@umontreal.ca

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Structural biology

Earliest molecular events of vision revealed

Marius Schmidt & Emina A. Stojković

Light-sensitive proteins called rhodopsins in the vertebrate eye initiate the cellular processes of vision. Leading-edge crystallography experiments have revealed the molecular mechanism by which light activates these proteins. **See p.939**

Most vertebrate animals depend on vision to navigate their environment and avoid predators. In the vertebrate eye, light is converted into electrical signals by a receptor protein known as rhodopsin, which spans the membranes of rod cells in the retina; the electrical signals are then processed in the brain to generate a mental image. The 'master switch' that responds to light and activates rhodopsin is a pigment called retinal[1,2] – an organic cofactor and derivative of vitamin A. On page 939, Gruhl *et al.*[3] report ultrafast, time-resolved crystallography experiments that show how this switch is flipped, finally revealing the molecular mechanism of rhodopsin activation.

"This is the first direct visualization of retinal isomerization in a mammalian rhodopsin protein."

Rhodopsins are found in all three domains of life, and consist of an opsin protein covalently linked to retinal. Animal rhodopsins are a specialized subset of G-protein-coupled receptors (GPCRs), a large family of proteins that mediate cell responses to external stimuli. Defects in the gene that encodes human rhodopsin cause eye diseases such as retinitis pigmentosa[4] and congenital stationary night blindness[5].

The first amino-acid sequence for a rhodopsin was reported in 1982, for the bovine version of the protein[6]. In 1993, the first glimpse of the 3D structure of an animal rhodopsin was revealed by 2D electron crystallography[7], and a high-resolution (2.8-ångström) structure became available[8] in 2000. The structures show that rhodopsins consist of seven transmembrane α-helices, with the amino terminus on the outside of the cell membrane, and the carboxy terminus inside the cell[1] (Fig. 1a). Retinal is attached to an evolutionarily conserved lysine amino-acid residue.

When rhodopsin is exposed to light, the retinal cofactor absorbs a photon and uses the energy to isomerize from one form (known as the 11-*cis* isomer) to another (the all-*trans* isomer). The resulting change of molecular configuration causes the opsin protein to undergo a series of conformational changes. Despite decades of attempts, no one had directly observed this isomerization reaction in real time.

In the eye, all-*trans* retinal is ejected from the opsin protein and enzymatically recycled back to the functional 11-*cis* retinal; this is then combined with another rhodopsin molecule for further use. Such reconstitution of the retinal is not possible in the rhodopsin crystals used for structural studies, and the isomerization reaction is therefore irreversible. This has been the biggest challenge to time-resolved crystallographic investigations of the reaction: once a rhodopsin crystal has been exposed to a single pulse of laser light, irreversible retinal isomerization occurs and a new crystal has to be mounted on a goniometer (a device that orients the crystal in the X-ray beam) for study, which is experimentally impractical.

The problem has been solved[9] for another type of light-activated protein using a technique called time-resolved serial femtosecond crystallography (TR-SFX), which requires X-ray free-electron lasers (XFELs). These facilities produce ultra-short, highly intense X-ray pulses, only one of which is required to generate a diffraction pattern from a protein microcrystal; each pulse lasts just femtoseconds, where 1 fs is 10^{-15} seconds. Any radiation damage caused by the intense X-ray pulse is negligible, because the ultrashort pulses terminate before damage can be done to the crystal[10].

In TR-SFX, a large number of microcrystals are delivered for analysis by a liquid jet, one by one and in random orientations, avoiding the need for macroscopic crystals to be mounted on a goniometer. For studies of light-sensitive proteins, each microcrystal is first subjected