assimilation by ecosystems[6,12]. It would therefore be helpful to include alternative estimates of GPP in future analyses.

Another limitation is that the authors use a single metric[13] to quantify and characterize droughts. But drought is difficult to assess properly[3,14,15] — particularly when assessing changes relevant to ecosystems, which relate more to changes in soil-moisture storage[12] than to changes in precipitation. The metric used by Schwalm *et al.* partly accounts for evapotranspiration (the sum of evaporation and plant transpiration from Earth's surface, a factor that affects soil-moisture storage), in addition to precipitation deficits. But a lack of global-scale measurements means that the effects of evapotranspiration factored into the metric are based mainly on estimates of a quantity known as potential evaporation[13], which is the maximum possible evaporation and thus tends to overestimate drought[16] when used instead of evapotranspiration. Moreover, potential evaporation is highly sensitive to air temperature. But under strong drought conditions, air temperature is increased by soil-moisture limitation (which reduces evaporative cooling[12]), and doesn't lead to further drying because plants reduce evapotranspiration during drought. Using a different drought metric in the analysis could produce smaller trends in drought recovery. It would thus be beneficial to expand the authors' analysis to include other data sets that describe drought.

Limitations aside, Schwalm and colleagues' study is highly valuable because it points to an under-appreciated dimension of drought impacts: the timescale of recovery and its relationship to the occurrence of drought events. The work offers crucial perspectives that might help in the development of new indices[17] of extreme climate that are more directly relevant to ecosystem impacts than existing metrics. Given that current models of the Earth system and climate do not simulate the complex processes required to properly address ecosystem drought recovery[5], projected changes in land carbon uptake may be biased, potentially affecting climate-change projections. Schwalm and co-workers bring attention to that issue, thus allowing better plans to be made to adapt to or mitigate the effects of climate change. ■

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STEM CELLS

The cost of perpetual youth

The ability to become nearly any cell type is restricted to eggs, sperm and primitive stem cells in very early embryos. Two studies reveal that maintaining this pluripotent state *in vitro* comes at a cost. SEE LETTERS P.219 & P.224

THOMAS P. ZWAKA

Very early in embryonic development, cells that eventually give rise to the fetus are contained in a structure known as the inner cell mass. These cells are pluripotent stem cells (PSCs): they have virtually unlimited potential to become any cell type[1]. The pluripotent stage is short (lasting just a few hours in mice), but it can be maintained *in vitro* for weeks or months. These laboratory conditions have been used for years to explore the inner workings of PSCs, and there has been much interest in using them to create populations of healthy cells that can replace those that have succumbed to disease. But what is the effect of artificially extending the pluripotent state? Two papers[2,3] in this issue find that certain *in vitro* conditions can cause mouse PSCs to undergo profound changes.

"MEK inhibitors should be used with caution when studying developmental processes."

Naive pluripotency, the most primitive pluripotent state, is maintained *in vitro* by protecting PSCs from the influence of kinase enzymes that stimulate them to proliferate and take on particular identities. Typically, this is achieved using a system called 2i (ref. 4), which involves treating PSCs with two small molecules that act as kinase inhibitors: one inhibits the kinases MEK1 and MEK2, and the other inhibits the kinase GSK3. In the current studies, Choi *et al.*[2] (page 219) and Yagi *et al.*[3] (page 224) found that preventing the natural progression of naive PSCs, even for only a few weeks in culture, affected gene expression (Fig. 1).

One of the ways in which cells adopt specific identities is by activating particular genes while keeping others transcriptionally silent through the addition of methyl groups to the DNA. Patterns of DNA methylation are fairly predictable across different cell types, including naive PSCs. In the 2i-treated cells, however, the expected methylation pattern was lost across some genomic features, including repetitive DNA sequences and genes that are imprinted (those for which only one of two copies is expressed; for each copy, this is determined by whether it is inherited from the mother or the father). Loss of methylation would be expected to lead to expression of genes that should normally be silent in these cells.

Misexpression of imprinted genes is troubling, because it is strongly associated with developmental disorders such as Angelman syndrome and Prader–Willi syndrome[5]. Choi *et al.* also found another abnormality strongly associated with developmental disorders — 2i caused some cells to gain or lose whole chromosomes.

To see whether these abnormalities *in vitro* would actually affect the developing organism *in vivo*, both groups injected naive PSCs back into mouse embryos. Healthy PSCs readily developed alongside resident cells to form a mixed-lineage organism, and could even be coaxed to form entire embryos under particular conditions. By contrast, 2i-treated cells lost this ability over time and the embryos into which they were injected showed developmental abnormalities such as fetal overgrowth. Even

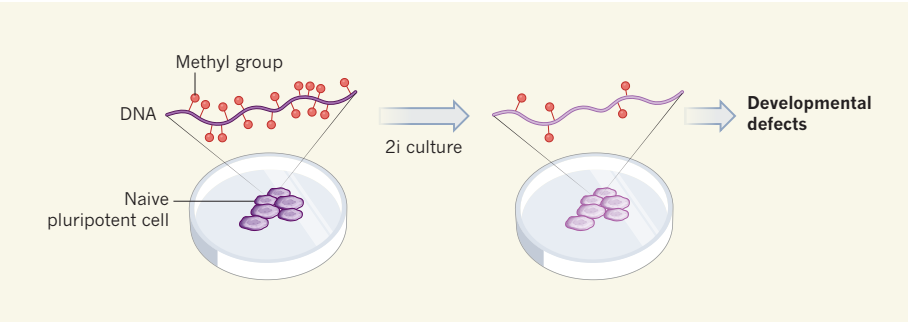

Figure 1 | Effects of maintaining pluripotency long-term *in vitro*. Naive pluripotent stem cells in early-stage embryos can give rise to any cell type. The DNA of these cells bears a pattern of molecular modifications that ensures the proper genes are expressed while others, which are tagged with methyl groups, are kept silent at this early embryonic stage. Such methylation modulates gene expression in both embryonic and adult cells. A drug cocktail called 2i can be used to prolong the naive pluripotent state in cells cultured *in vitro*, but two groups[2,3] report that 2i treatment over a few weeks causes loss of methylation, leading to the expression of genes that would normally be silent. Injecting these treated cells into developing embryos causes developmental defects. Thus, culturing stem cells in 2i has consequences for both research and clinical applications.

when transferring the nuclear material from a 2i-treated pluripotent cell into a healthy egg whose nucleus had been removed (a technique called somatic cell nuclear transfer that should, in principle, restore all methylation), Yagi *et al.* could not prevent imprinting-associated growth defects in the embryo grown from that egg.

Choi and colleagues demonstrated that all the adverse effects were mediated primarily by the MEK inhibitor, rather than the GSK3 inhibitor. Clearly, then, MEK inhibitors should be used with caution when studying developmental processes or using naive cells to model or treat disease. Both groups tried replacing the MEK inhibitor with related kinase blockers, with some limited success.

Arguably more interesting, however, are the biological questions that arise from these studies. For instance, what exactly is the role of the MEK pathway in early embryonic development? What are its targets and how does protection from this signalling pathway, which is a vital pathway in almost all other cell types, help stem cells to remain fully naive? Answers to these questions will help us to better understand not only pluripotency, but also why the MEK pathway is among the most frequently mutated signalling cascades in cancer[6].

The loss of methylation caused by 2i treatment resembles the demethylation that occurs naturally in all DNA sequences in the early embryo apart from imprinted regions[7]. But why is the early embryonic genome demethylated in the first place? Perhaps repetitive elements that are normally heavily methylated, such as transposons (virus-like mobile DNA sequences that invaded the genome millions of years ago), need to be transiently activated during this early developmental period[8]. If so, this would suggest a deep symbiosis between our genome and certain foreign elements.

A strength of the current studies is that they analyse both male and female PSCs. Strikingly, the loss of methylation in 2i-treated cells of either sex resembles not only the state in the inner cell mass *in vivo*, but also the state in female PSCs cultured without 2i (ref. 9). These cells become unstable in culture. By contrast, male PSCs propagated without 2i always progress to a slightly more advanced developmental state that is stable and marked by well-established changes in molecular profile. But they can revert back to a naive state if 2i is added later.

It has been suggested that female embryos grow more slowly and show a fundamentally different biology than that of males[10]. If this is true, perhaps other stem cells — during development or in the adult — also show major sex differences. This possibility is something that almost no study currently takes into consideration.

Finally, the current studies act as a reminder that the highly artificial environment of the laboratory does have consequences. They are a useful check on the hubris of our quest to dominate nature. ■

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ECOLOGY

Contests between species aid biodiversity

A modelling approach used to investigate competition between different species provides insight into how contests that have multiple players can help to maintain biodiversity. SEE LETTER P.210

JAMES P. O'DWYER

How and why do distinct species coexist? Even in the case of closely related organisms competing for a set of common resources — such as different tree species competing for space, light and nutrients — we still don't have a complete answer to this fundamental question. On page 210, Grilli *et al.*[1] report their analysis of a theoretical framework that might illuminate this debate.

The authors developed a combination of analytical and numerical results for species competing for resources in a series of tournaments. This provides a standard and intuitive picture of how competition might unfold in a community — for example, how seedlings of different tree species might compete to fill a gap that suddenly opens up on the forest floor when a tree falls. But Grilli and colleagues' model introduces a few tweaks to this conventional scenario.

The first is that their model functions like a modified version of the children's game rock–paper–scissors (in which players simultaneously signal one of these three options, and each option wins, loses or draws depending on which of the three options the other player signals), but with tens or hundreds of different moves rather than just three. The idea is