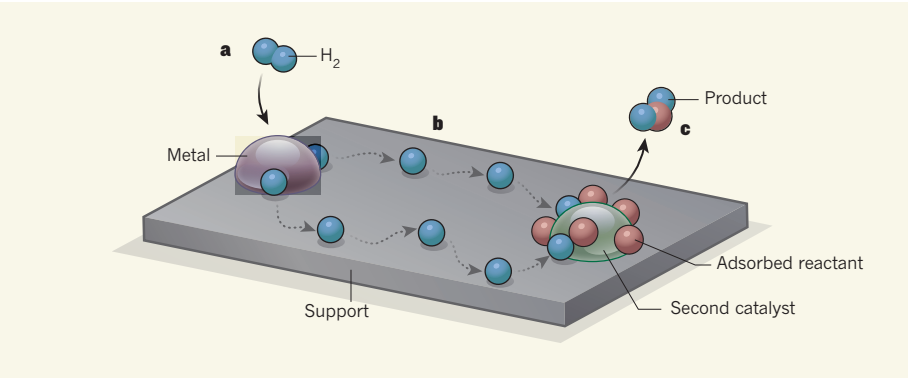

Figure 1 | Mechanism for hydrogenation reactions that involve spillover. Catalytic hydrogenations are reactions of hydrogen gas (H_2) with other atoms or molecules, and sometimes require two catalytic sites on a support. **a**, Gaseous H_2 molecules first adsorb onto a metal nanoparticle. The metal catalyses H_2 dissociation (break-up), forming adsorbed hydrogen atoms. **b**, The hydrogen atoms migrate along the surface of the oxide support (each curved arrow represents a 'step' taken along the surface), until they reach the second catalyst — a process called spillover. **c**, The second catalyst mediates the reaction of the hydrogen atoms with other adsorbed atoms or molecules, yielding the final product. Karim *et al.*[1] have studied spillover in a reaction in which the metal is platinum and the second catalyst is iron oxide. The iron oxide also acts as the reactant in their system; it is reduced to metallic iron and produces water on reaction with hydrogen atoms.

iron. The catalysts consisted of a support — a thin film of either titanium oxide or aluminium oxide — onto which the researchers deposited 15 pairs of nanoparticles. Each pair consisted of a platinum nanoparticle and an iron oxide nanoparticle (30 and 60 nanometres in diameter, respectively) separated by defined distances that ranged from 0 to 45 nm.

The authors then exposed these model catalysts to a hydrogen atmosphere under set conditions (1×10^{-5} millibars, 343 kelvin), and recorded X-ray absorption spectra before and after exposure to determine the degree to which each iron oxide nanoparticle was converted to metallic iron. They observed only slight conversion for systems on aluminium oxide, and only when the platinum and iron oxide nanoparticles were less than 15 nm apart. (Maximum reduction was observed for the pair of nanoparticles that overlapped on aluminium oxide, presumably because no migration of hydrogen atoms between catalysts is needed.)

By contrast, maximum iron oxide reduction occurred for all the iron oxide nanoparticles on titanium oxide, regardless of the distance between the paired particles. The authors also used X-ray absorption to show that, when reactions were performed on titanium oxide, titanium ions in the support are reduced during the process, from Ti^{4+} to Ti^{3+}. Therefore, they concluded that spillover requires the catalyst support to be a reducible oxide.

This better understanding of spillover should aid the design of catalytic hydrogenation processes. It may also help to explain the mechanisms of other important chemical reactions. For example, in the light-induced production of H_2 from water using semiconductor catalysts, the conventional wisdom has been that metal additives catalyse the required reduction of water by trapping excited electrons generated from light absorption. However, my co-workers and I have argued[8] that, instead, the role of the metal is to promote H_2 formation through the recombination of hydrogen atoms produced from the reduction of water at semiconductor sites. Our explanation hinges on the ability of hydrogen atoms to travel from the surface of the semiconductor to the metal — a reverse spillover effect[9]. Karim and colleagues' approach could potentially be adapted to test this hypothesis directly. Their experiments could also be expanded to quantify the kinetics of the spillover effect and to assess its contribution to the rates of many other hydrogenation reactions.

Karim *et al.* end their report with a molecular-level theory to explain why spillover takes place on titanium oxide, but not on aluminium oxide, on the basis of computational modelling. However, that modelling did not provide a direct comparison of the two systems, because it started at different points in the reaction pathway for each of the oxide films. Moreover, the energy diagram derived from their calculations suggests the existence of a viable, low-energy pathway for hydrogen spillover on aluminium oxide, even though this is not observed experimentally. Finally, the authors acknowledge, but do not fully resolve, the role that water may have in the spillover effect, which is crucial in many catalytic systems. These issues should be topics for future work. Nevertheless, Karim and co-workers' study reveals an innovative way to probe spillover, and opens fresh avenues of investigation to better understand and use this effect in catalysis. ■

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SYSTEMS BIOLOGY

Molecular memoirs of a cellular family

A system that introduces random modifications to barcode sequences embedded in cells' DNA allows lineage relationships between cells to be discerned, while preserving the cells' spatial relationships. SEE LETTER P.107

LAUREN E. BECK & ARJUN RAJ

Determining how large, multicellular organisms emerge from a single cell is a major goal for biologists, and a key to understanding many dynamic processes and diseases. A fundamental component of this goal is the mapping of cellular lineages, which is crucial for understanding not only embryonic development, but also processes such as the growth of cancers and stem-cell differentiation. Frieda *et al.*[1] describe a technique on page 107 that uses genetic engineering to embed lineage information in a cell's DNA. The results can then be read out by direct visualization — an advance that stands to transform our ability to build spatio-temporal cell lineages.

One of the landmark achievements in the field of lineage tracing has been the ability to resolve the complete developmental

cell-lineage tree for the roundworm *Caenorhabditis elegans*[2]. This tree revealed a great deal about how development progresses, and most notably uncovered an essential process called programmed cell death[3]. These successes were a result of, in equal parts, elegant manipulation and elbow grease. The worm's relatively small and invariant lineage tree, the optical transparency of *C. elegans* and the sheer determination of the researchers involved were crucial to mapping the embryonic lineages of this organism.

When it comes to more-complex organisms, how can we tell, just from looking, which cells are sisters, cousins, aunts or uncles? An ability to read out and compare the DNA-sequence variation between individuals and species has revolutionized studies of ancestry, and the same strategy can be applied to cells. However, whereas species have had time to accumulate many DNA mutations, individual cells accumulate natural mutations rarely and randomly, making them difficult to measure.

To get around this problem, Frieda *et al.* used a genome-editing system called CRISPR–Cas9, which can precisely delete specific DNA sequences. The authors constructed a series of identical DNA sequences that they dubbed scratchpads. They then attached each one to a unique sequence called a barcode, and introduced all of these barcoded scratchpads into the genome of an individual cell. Over time, the Cas9 enzyme randomly removes scratchpads, leaving some barcodes without an associated scratchpad. The cell transcribes its barcoded scratchpads into RNA, which the researchers can detect using fluorescent probes — one set of probes that binds to the scratchpads and another set that binds to each barcode. In this way, the researchers can tell which barcodes had their corresponding scratchpads removed[4,5].

The cell's progeny inherit the deleted scratchpads and then go on to accumulate other deleted scratchpads, allowing the researchers to read out lineage information by determining (through fluorescent imaging) the subset of edited barcodes that differ between any two cells (Fig. 1). Frieda and colleagues call this technique 'memory by engineered mutagenesis with optical *in situ* readout' (MEMOIR).

As a proof of principle, the authors applied MEMOIR to mouse embryonic stem cells grown in culture. As a reference against which to compare MEMOIR's results, they used time-lapse microscopy to track lineage development over three or four cell divisions. Impressively, despite all the random events required for the method to work, they were still able to reconstruct even these relatively fine-grained family trees up to about 72% of the time. These results were squarely within the range obtained when they used computer simulations to predict the accuracy of an idealized MEMOIR experiment, showing that the method performs optimally from a technical perspective.

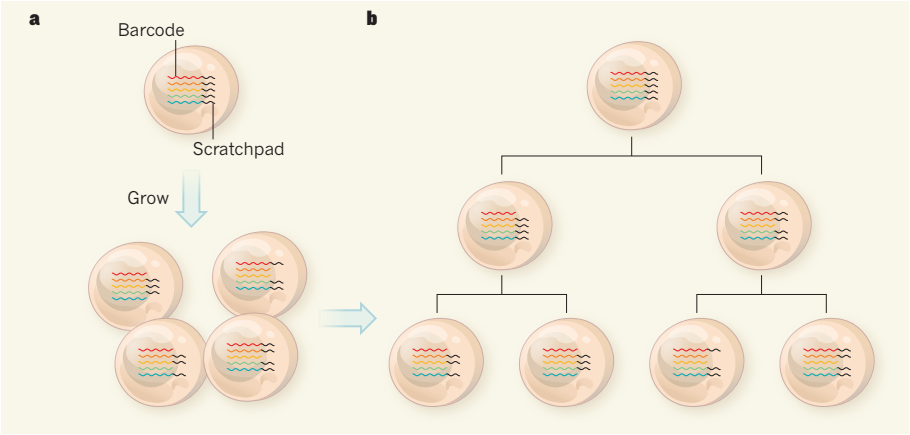

Figure 1 | Lineage tracing finds its place. **a**, Frieda *et al.*[1] use genetic engineering to introduce a series of identical DNA sequences called scratchpads, each attached to a unique DNA sequence called a barcode, into the genome of a cell. As the cell proliferates, scratchpads are stochastically and permanently deleted. Microscopy, combined with RNA-dectection techniques, can be used identify the RNA transcripts produced from each barcode in each cell — in this way, the barcodes attached to deleted scratchpads can be identified. **b**, This information can be used to reconstruct a complete cell-lineage tree. In this example, the tree reveals that the two cells on the left in **a** are sisters, and that the two on the right are their cousins.

The real beauty of the system is that, because the ultimate measurement occurs *in situ* (in intact cells in their native positions), it both preserves the spatial relationships of cells and allows for measurements of other features, such as gene expression. To demonstrate the power of this approach, Frieda *et al.* studied *Esrrb*, a gene that has highly variable expression in embryonic stem cells, at the same time as measuring lineages using MEMOIR.

If *Esrrb* expression slowly switches between on and off, one would expect the progeny of a cell that has 'on' expression also to have 'on' expression (and the same would be true for 'off' expression). However, if *Esrrb* expression rapidly switches between on and off, one would expect the expression state of a daughter cell to be largely independent of that of its parent. In their test, the authors inferred expression dynamics for *Esrrb* — the probabilities that expression would flip from off-to-on or on-to-off were 0.09 and 0.04 per generation, respectively. Although the current iteration of MEMOIR serves mainly to prove its efficacy for lineage tracing, it is remarkable that these temporal quantities can be inferred without having to make dynamic measurements.

As the number of barcodes increases, the fidelity and complexity of the relationships that MEMOIR can read out stands to increase. Frieda and colleagues used tens of barcodes, but advances in *in situ* RNA-detection methods[6–9] should enable the use of hundreds or thousands, vastly increasing the power of MEMOIR to discern family trees with high fidelity. Moreover, the methods for RNA detection used by the authors can be applied in animal tissues, in theory allowing family trees to be directly visualized on top of an image of, say, an organ or potentially an entire organism. Combining MEMOIR with techniques for imaging in tissues may facilitate such developments.

Notably, a study[10] this year outlined a complementary approach to lineage tracing, which also used CRISPR–Cas9 to induce genomic mutations, but that read out the results with DNA or RNA sequencing, rather than imaging. Sequencing comes at the expense of spatial information, but does enable hundreds of thousands of cells to be interrogated. Developments in spatial genomics might help to circumvent the spatial limitations of sequencing-based lineage strategies[11]. These techniques, complemented by methods such as MEMOIR, stand to reinvigorate the field of lineage tracing in the coming years. ■

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