it possible to resolve and quantify such details.

The ability to use optical and X-ray sum-frequency generation to probe light–matter interactions at a microscopic scale will potentially advance the fields of optics, energy research and materials science. If optical lasers that emit pulses at high repetition rates (on the megahertz regime) are available at X-ray synchrotron facilities, it may be possible to explore the effect using these more accessible X-ray sources instead of an XFEL. This would allow extensive studies of stationary, microscopic optical properties. But to resolve dynamic light-induced microscopic processes in real time — a challenge that still lies ahead of us — the power of XFELs is indispensable. ■

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1. Franken, P. A., Hill, A. E., Peters, C. W. & Weinreich, G. *Phys. Rev. Lett.* **7,** 118–119 (1961).
2. Emma, P. *et al. Nature Photon.* **4,** 641–647 (2010).
3. Ishikawa, T. *et al. Nature Photon.* **6,** 540–544 (2012).
4. Glover, T. E. *et al. Nature* **488,** 603–608 (2012).
5. Eisenberger, P. M. & McCall, S. L. *Phys. Rev. A* **3,** 1145–1151 (1971).
6. Freund, I. & Levine, B. F. *Phys. Rev. Lett.* **25,** 1241–1245 (1970).

EPIGENETICS

Actors in the cell reprogramming drama

The transformation of skin cells into stem cells is a fascinating but poorly understood process. At last, the molecular characters underlying the initial steps have been revealed. SEE LETTER P.652

KYLE M. LOH & BING LIM

Coercing cells into adopting new fates — for example, turning skin into muscle[1], brain[2] or heart[3] — is one of the most impressive achievements of modern biology. Prominent among these feats is the transformation of skin cells into induced pluripotent stem cells (iPSCs)[4]. In contrast to skin cells (fibroblasts) and other differentiated cells, which cannot generate any other cell type except their own, iPSCs have the remarkable ability to give rise to any kind of cell in the body[4]. Yet the molecular changes that drive this cellular metamorphosis have remained a mystery. For the first time, Doege *et al.*[5] (page 652) provide a mechanistic explanation for how the earliest phases of such a dramatic change in cell fate might be executed.

Cellular identities were once thought immutable[6]: when a cell became differentiated, it could not return to a stem-cell state. In differentiated cells, genes typically expressed in other cell types are repressed, thereby consigning the cells to their present fate and preventing excursions into other lineages[7,8]. Likewise, stem-cell pluripotency genes are often inactive in differentiated cells[4]. However, the artificial introduction of several transcription factors (such as Oct4, Sox2 and Klf4)[4] can reverse such lineage restrictions, inducing many differentiated cell types to become iPSCs.

Gene expression is jointly controlled by two classes of protein: transcription factors and epigenetic regulators. Transcription factors act by binding directly to DNA, whereas epigenetic regulators can influence gene expression in various ways, for example by altering histones[9] (proteins that package DNA). Doege *et al.* identify two epigenetic regulators — Parp1 and Tet2 — that stimulate the expression of dormant pluripotency genes in fibroblasts, thereby initiating the reprogramming of these cells into iPSCs. The authors show that, during cell reprogramming, Parp1 and Tet2 induce the removal of a repressing mark (known as H3K27me3) from histones associated with pluripotency genes, as well as the addition of a different mark (H3K4me2) that is thought to have an activating effect[10]. In particular, they find that this occurs in the pluripotency genes *Nanog* and *Esrrb*, days before the genes become active (Fig. 1).

Doege and colleagues show that these epigenetic changes presage future increased expression of *Nanog* and *Esrrb* and correlate with enhanced binding of Oct4 to these genes, which presumably further stimulates their expression. Strikingly, both endogenous Parp1 and Tet2 are essential for reprogramming — if either is abrogated, iPSC formation fails totally[5,11]. This finding suggests that the reprogramming transcription factors cannot efficiently bind to and reawaken their target genes unless they collaborate with endogenous epigenetic regulators, which preconfigure the repressed genes for activation days before their expression is increased.

Transcription factors such as Oct4 and Sox2 have long occupied the pantheon of cardinal reprogramming factors[6], but now Doege *et al.* show that epigenetic regulators are equally important. However, these regulators are not widely introduced into cells alongside transcription factors to induce reprogramming[6]. So, where in the reprogramming ensemble are the obligatory epigenetic regulators? The present report provides an elegant explanation: that the reprogramming transcription factors liaise with endogenous epigenetic regulators to

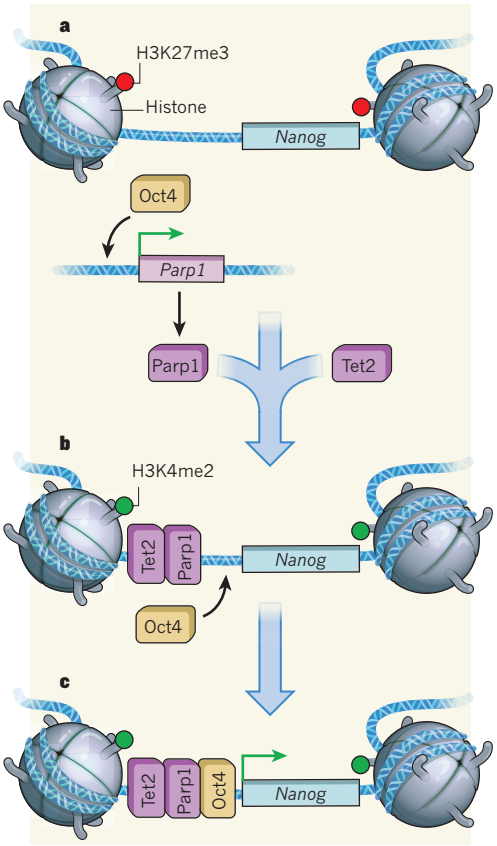

Figure 1 | Cell metamorphosis. Skin fibroblasts and other differentiated cells can be reprogrammed to become stem cells by the introduction of transcription-factor proteins such as Oct4. These reprogramming factors induce the expression of *Nanog* and other genes that are inactive in differentiated cells and active in stem cells, although the mechanisms underlying such gene reactivation have been obscure. **a**, In fibroblasts, the *Nanog* gene is surrounded by histone proteins that carry a chemical modification (H3K27me3) that is commonly associated with repressed genes. Doege *et al.*[5] report that the exogenously added Oct4 induces the expression of the protein Parp1. **b**, Early during reprogramming, this protein and another protein, Tet2, bind to the regulatory region of *Nanog*, facilitating depletion of the repressive H3K27me3 modification and the acquisition of another histone mark (H3K4me2) that is often found on genes poised for reactivation[10]. **c**, Such changes probably help Oct4 to bind strongly to, and so enhance the expression of, *Nanog*. Several days later, *Nanog* expression becomes markedly high. Subsequent cellular events, which remain unclear, lead to the production of full-fledged stem cells.

execute reprogramming. These regulators may already be present in the starting cell[12], or their expression can be stimulated by the added transcription factors — indeed, Doege *et al.* show that Oct4 induces Parp1 expression in fibroblasts (Fig. 1). However, some cell-lineage switches do explicitly require co-introduction of a particular epigenetic regulator[13]. Therefore, efforts to reprogram cells to currently unattainable lineages might benefit from the addition of relevant epigenetic modifiers.

The authors' findings provoke further questions. Although Parp1 and Tet2 reactivate pluripotency genes such as *Nanog* and *Esrrb*, what extinguishes the expression of fibroblast-specific genes? Moreover, is there some hierarchical order in which the awakened pluripotency proteins activate more of their dormant kin?

Another unresolved issue is how epigenetic regulators 'know' which genes need to be reactivated. Do they derepress gene expression broadly across the genome, or are they guided to specific genes by the reprogramming transcription factors? It is known that Oct4 binds to Parp1 in stem cells[14], which suggests that it might target Parp1 to reactivate specific pluripotency genes. Are certain epigenetic regulators (for example, Parp1) universally required to convert any cell type into any other one? Some fundamental epigenetic machinery, such as the TrxG H3K4 methyltransferase protein complex[12], might be broadly required to activate the expression of alternative-lineage genes for any reprogramming event. By contrast, cell-type-specific epigenetic regulators might direct transformation into selected lineages — for example, the regulator Baf60c specifically induces conversion into heart cells[13].

The main import of Doege and colleagues' article is that it begins to clarify, at surprisingly early stages of the process and in unprecedented detail, the mechanisms by which cell types can be switched from one to another. This supersedes previous phenomenological descriptions of the reprogramming process and provides insight into its underlying mechanisms. If we can ascertain the fundamental principles underlying such changes in cell identity, the terra incognita of cellular reprogramming may at last be mapped, expanding the ways in which diverse cell types can be generated for cell-based therapies. ■

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1. Davis, R. L., Weintraub, H. & Lassar, A. B. *Cell* **51,** 987–1000 (1987).
2. Vierbuchen, T. *et al. Nature* **463,** 1035–1041 (2010).
3. Ieda, M. *et al. Cell* **142,** 375–386 (2010).
4. Takahashi, K. & Yamanaka, S. *Cell* **126,** 663–676 (2006).
5. Doege, C. A. *et al. Nature* **488,** 652–655 (2012).
6. Graf, T. *Cell Stem Cell* **9,** 504–516 (2011).
7. Enver, T. & Greaves, M. *Cell* **94,** 9–12 (1998).
8. Loh, K. M. & Lim, B. *Cell Stem Cell* **8,** 363–369 (2011).
9. Rosenfeld, M. G., Lunyak, V. V. & Glass, C. K. *Genes Dev.* **20,** 1405–1428 (2006).
10. Koche, R. P. *et al. Cell Stem Cell* **8,** 96–105 (2011).
11. Lai, Y.-S. *et al. Proc. Natl Acad. Sci. USA* **109,** 3772–3777 (2012).
12. Ang, Y.-S. *et al. Cell* **145,** 183–197 (2011).
13. Takeuchi, J. K. & Bruneau, B. G. *Nature* **459,** 708–711 (2009).
14. Pardo, M. *et al. Cell Stem Cell* **6,** 382–395 (2010).

ASTRONOMY

Collision course

Four billion years from now, the Andromeda galaxy will have a close encounter with the Milky Way. The two galaxies will commence a dance of disruption that will, over the course of another two billion years, lead to their complete union.

R. BRENT TULLY

In a series of three papers published in *The Astrophysical Journal*, van der Marel and collaborators[1–3] discuss the timing and dynamics of the 'imminent' — or at least inevitable — collision between the Milky Way and the neighbouring Andromeda galaxy*. The inevitable aspect of this situation has been known for a long time. In 1959, Kahn and Woltjer estimated[4] the mass of the Milky Way–Andromeda system through what has come to be called the 'timing argument', which invoked the assumption that the two galaxies would ultimately collide.

The argument posits that the two galaxies — or rather, their current contents — started in the Big Bang in close proximity, flew apart with cosmic expansion but were arrested by their mutual attraction, and are now falling back towards each other. On the basis of the simplistic assumption that the two are moving directly towards each other, Kahn and Woltjer estimated a mass for the system that is in good agreement with current projections.

Getting down to details, there is the possibility that the galaxies orbit one another with angular momenta caused by tidal forces from such prominent players as the Maffei, Messier 81 and Centaurus A galaxies. The question since 1959 has been whether the two galaxies would collide on first return or fly past each other. The answer lies in the relative translational velocities of the two systems. How fast is Andromeda's position displacing on the sky? We have accurate knowledge of how fast it is moving in the line of sight, and we know well enough its distance from Earth. If we know its displacement rate on the sky (proper motion), then we can calculate its orbit.

We are only just beginning to measure the proper motions of galaxies. They are relatively well determined for the Milky Way's immediate neighbours, such as the Large and Small Magellanic Clouds. It turns out that the proper motions have been measured for two neighbours of the Andromeda galaxy, Messier 33 and Index Catalogue 10, because they emit water masers[5,6] — radio signals associated with the excitation of water molecules in star-forming regions. The observations of these masers are made using radio interferometers with baselines (extending from Hawaii to Maine) that afford angular resolution at the level of 10 microarcseconds. But masers in Andromeda have been found only recently, so a measurement of its proper motion had to come from the displacement of the galaxy's stars in the manner obtained by van der Marel and colleagues.

Here is the challenge. At a good observation site on the ground, a star is blurred to a disk with a diameter of about 1 arcsecond. The expected annual proper motion of Andromeda is only a few parts in 100,000 of this blurry dimension. Van der Marel and his team[1–3] helped themselves by making observations with the Hubble Space Telescope, which allowed them to obtain a resolution of 0.1 arcseconds. Given typical galaxy orbits, the authors predict displacements of Andromeda's star centres at the level of one part in 1,000–10,000 of the measured star diameters per year. It does not help that the stars they examined were in crowded regions (the brightest stars among the billions superimposed on each other in the Andromeda galaxy) and that the background references for measuring the motions are galaxies of irregular shapes that therefore have ill-defined centres; these background reference galaxies are so far away that they are effectively motionless.

To improve the statistics, the authors were able to make measurements of several thousand stars against a static background of several hundred distant galaxies. To look for changes in star positions, they made observations of three fields in Andromeda at separations of 5 to 7 years. The final accuracy of the measured proper motion of the Andromeda galaxy on the sky was 12 microarcseconds

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