the flicker floor, this correlation provides a means of determining *g* with a precision of better than 25% — between two and three times better than the precision obtained from conventional observations.

As Bastien *et al.* note, the general dependence of brightness variations on stellar properties can be described in terms of stellar evolution. Young stars tend to have stronger magnetic activity, with many starspots, and hence display a large total range of variations. With increasing age, this activity diminishes and the stars settle on the flicker floor. As the stars grow older their radii increase, leading to a lower *g* and a higher flicker.

Bastien and colleagues demonstrate how flicker can be used to measure *g*, but do not provide a detailed analysis of the physical nature of the flicker. The stars for which the investigation was carried out have outer convection zones, in which energy is transported to the surface through the motion of gas. In the Sun, this transport is visible in granulation — a time-varying pattern of small-scale brighter and dimmer regions on the solar surface that reflects hot, rising and cooler, sinking gas pockets. Granulation also leads to minute variations in the total solar brightness.

The authors' study indicates that stellar granulation is a contributor to flicker. Indeed, the spatial scale and other properties of granulation depend on *g* (ref. 3), with lower *g* resulting in a larger scale and thus probably causing larger brightness variations on timescales relevant to flicker, in agreement with the correlation that the authors found. Further support for the relationship between granulation and flicker comes from other Kepler observations and modelling of red-giant stars[4]. Brightness variations caused by granulation are expected in all the stars considered by the authors, hence defining a lower limit to the variations — the flicker floor. A better physical understanding of the origin of flicker might allow the observed brightness variations to be used to probe the dynamics of the outermost stellar layers. The resulting improved stellar modelling could, in turn, improve the accuracy with which *g* can be determined.

"Studying the twinkling of stars does indeed help us to understand what they are."

The very small amplitude of flicker makes it essentially unobservable using ground-based telescopes, owing to the effect of Earth's atmosphere. However, the ability to measure flicker with Kepler observations will be valuable in the continuing analysis of Kepler data on exoplanets, which are detected through the slight dimming of a star's light as a planet transits, or passes in front of it. An accurate determination of *g* from flicker greatly aids the analysis of spectroscopic observations used to infer the chemical composition of planet-hosting stars, and so advances our understanding of planet formation[5]. Furthermore, planetary transit observations provide a measurement of only planetary radius relative to stellar radius, and uncertain information about stellar radii hampers the characterization of the planets. With knowledge of *g* from flicker, as well as of the surface temperature and composition of the star, fits of stellar models to these quantities can be used to obtain a more precise value of the stellar radius, and hence of the planetary radius.

Beyond Kepler, the authors' technique will be valuable for NASA's planned Transiting Exoplanet Survey Satellite (TESS), which is slated for launch in 2017. TESS will carry out an all-sky survey for extrasolar planetary systems by monitoring at least half a million stars, and will require efficient methods to characterize the target stars. The same applies to the European Space Agency's Planetary Transits and Oscillations of Stars (PLATO) exoplanet mission, should it be selected for launch in 2022–24.

Therefore, Bastien and colleagues' analysis holds great promise for measuring stellar properties and understanding the complex dynamics of the outermost layers of stars. Studying the twinkling of stars does indeed help us to understand what they are. ■

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1. Bastien, F. A., Stassun, K. G., Basri, G. & Pepper, J. *Nature* **500,** 427–430 (2013).
2. Chaplin, W. J. *et al. Science* **332,** 213–216 (2011).
3. Trampedach, R., Asplund, M., Collet, R., Nordlund, Å & Stein, R. F. *Astrophys. J.* **769,** 18 (2013).
4. Mathur, S. *et al. Astrophys. J.* **741,** 119 (2011).
5. Buchhave, L. A. *et al. Nature* **486,** 375–377 (2012).

BIOTECHNOLOGY

Programming genomes with light

The combination of two techniques — optogenetics and genome editing using engineered nucleases — now provides a general means for the light-controlled regulation of any gene of interest. SEE LETTER P.472

ANDREAS MÖGLICH & PETER HEGEMANN

In this issue, Konermann *et al.*[1] combine two sparkling biological technologies developed over the past decade. The first is optogenetics[2], the process by which light-responsive proteins are engineered into target cells and used to regulate their activity. The second is the use of sequence-targeted DNA-cleaving enzymes to specifically alter the genome. By uniting these techniques, the authors present a versatile method for targeted control of gene transcription and genomic modifications.

Optogenetics can be used in cells and in living organisms, and allows cellular regulation using light of different colours, intensities and duration in a graded, non-invasive, reversible and spatiotemporally precise fashion. Most optogenetic applications so far have relied on the use of light-sensitive ion channels and ion pumps to modulate the voltage dynamically across biological membranes, in particular to elicit action potentials in neurons.

Nature offers a plethora of other processes that are regulated by light, such as those controlled by photoreceptors in plants and microorganisms. With only a few exceptions, these processes are intricately tied to their organism of origin, and their deployment in others is challenging. However, their existence suggests that optogenetics could be extended to regulating enzyme activity or could be used to induce more persistent effects by targeting DNA. Indeed, natural photoreceptors have provided design blueprints for the engineering of several biological systems with customized light responses[3].

A particularly versatile strategy uses photoreceptors that associate with other proteins in a light-regulated process. In terms of performance, robustness, response time and ease of use, the blue-light-responsive protein cryptochrome 2 and its light-induced interaction with its partner protein CIB1 (ref. 4) currently have the edge over alternative photodimerizers such as the red-light-responsive phytochrome–PIF pair[5]. Several laboratories have successfully modulated gene transcription using photodimerizing proteins[4–8]. Initially, these optogenetic systems were directed to specific DNA sites by coupling to the DNA-binding part of the transcriptional-activator

protein Gal4 (ref. 5). Light exposure recruited an interacting protein that was coupled to the activation domain of Gal4, thus initiating transcription. Although these systems are powerful[7,8], they are inherently limited because they use DNA-binding domains with fixed target-sequence specificity, and because target genes have to be introduced into the host genome as exogenous DNA templates.

In parallel with the introduction of optogenetics, DNA-engineering strategies have been developed that can target unique sites among the billions of nucleotides in a genome. Early versions of such approaches[9] were based on zinc-finger and transcription-activator-like effector (TALE) proteins, which contain repetitive amino-acid sequences that recognize single DNA nucleotides or nucleotide triplets, and introduce double-stranded DNA breaks on binding to these sequences. The DNA-repair process that is activated in response to this damage can be used to introduce novel genetic elements at the site. However, adjusting the sequence specificity of zinc-finger and TALE proteins entails the laborious production of customized proteins.

A more recently developed approach, called the CRISPR–Cas system[10,11], overcomes this limitation. In this system, an endonuclease enzyme that induces a double-strand break is used with a sequence-specific guide RNA molecule — simply replacing the guide RNA is sufficient for sequence adaptation. The CRISPR–Cas technology stands to make engineering of zinc-finger and TALE proteins obsolete and to render genome engineering fast, efficient and inexpensive.

Capitalizing on their expertise in both optogenetics and genome engineering, Konermann *et al.* have overcome the sequence-restriction problem of earlier light-activated transcription-modulation approaches in their light-inducible transcriptional effector (LITE) system (Fig. 1a). The system uses a TALE protein coupled to cryptochrome 2, and CIB1 coupled to the transcriptional-activator protein VP64. This combination results in the cellular transcriptional machinery being recruited to the genomic site defined by the TALE protein when blue light is absorbed. The authors showed *in vitro* that, following light exposure, site-specific gene expression was enhanced by 10–20 times compared with darkness, and they convincingly validated the technology in mouse neurons and in the brains of conscious mice by monitoring light-mediated transcription of the genes *Grm2* and *Neurog2*.

The LITE approach has several favourable characteristics. First, the light-responsive molecules of cryptochrome 2 are the chromophores flavin-adenine dinucleotide and methyltetrahydrofolate, which are universally abundant. Second, induction of transcription occurs within minutes of light exposure.

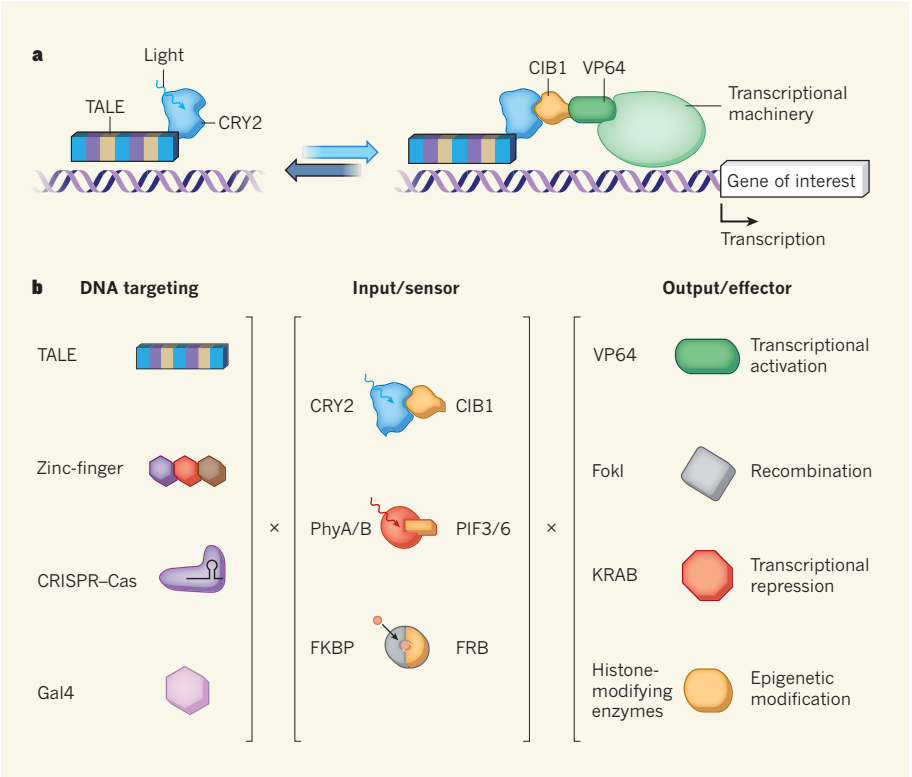

Figure 1 | Modular control of genome function. **a**, Konermann and colleagues' LITE system[1] uses transcription-activator-like effector (TALE) proteins — which specifically bind to unique DNA sequences — coupled to the light-sensitive photoreceptor cryptochrome 2 (CRY2). On light exposure, a complex of CRY2's interaction partner CIB1 coupled to the transcriptional-activator protein VP64 is attracted to CRY2, and VP64, in turn, attracts the cellular transcriptional machinery, initiating transcription at the target site. This reaction is reversible. **b**, The system is highly versatile because the various components can be interchanged. DNA targeting can be achieved using Gal4, zinc-finger proteins or the CRISPR–Cas system. Proteins that respond to light of different colours (such as red light for the phytochromes A/B (PhyA/B) and their interaction partners PIF3/6) or small molecules (such as rapamycin for the FKBP–FRB interaction pair) can be used as the sensor and recruitment molecules. Also, different output molecules can be used for various effects, including recombination (using endonucleases such as FokI), transcriptional repression (through the protein KRAB[11]) or histone modification (using enzymes that elicit epigenetic effects).

Third, the response can be graded with light dose and is fully reversible after light retraction. Finally, because light can be applied non-invasively, its use is not restricted to cultured cells but extends to freely moving animals, as established for conventional optogenetic tools[2].

Great power lies in the modularity and resultant versatility of this technique (Fig. 1b). By replacing constituent modules of LITE, the system can be tuned to be sensitive to light of different colours or to have different effector outputs. The authors impressively demonstrated this second possibility by interfacing LITE with various molecules that modify histones — the proteins around which DNA is wrapped. They show that their system can be used to site-specifically enhance histone methylation and acetylation — two epigenetic modifications that regulate the rate of gene transcription. The LITE approach thus enriches the optogenetic arsenal with novel applications.

Similarly, the TALE module of LITE can be exchanged for other DNA-binding modules, including ones based on the CRISPR–Cas system, as Konermann *et al.* demonstrate. Because the CRISPR–Cas system can be rapidly directed to different DNA sites, this will allow faster fine-tuning of the efficacy of any LITE experiments. Thus, the combination of CRISPR–Cas and LITE may truly usher in a new era of systems biology, in which gene expression and epigenetic modifications can be manipulated at the genome level with supreme sequence specificity, exquisite temporal resolution and full reversibility.

As with any new technology, there is room for improvement. In particular, it would be desirable to increase the degree of transcriptional activation by LITE. There is also the question of where the LITE system should be positioned in the genome to achieve maximum effect, but this can be easily addressed with the rapid manipulation offered by the CRISPR–Cas system. Even in its present implementation, LITE represents a powerful approach to light-controlled genome programming. Given its versatility, ease of use, performance and potential for automation, we expect this

technology to be widely and rapidly taken up across many biological disciplines. ■

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1. Konermann, S. *et al. Nature* **500,** 472–476 (2013).
2. Yizhar, O., Fenno, L. E., Davidson, T. J., Mogri, M. & Deisseroth, K. *Neuron* **71,** 9–34 (2011).
3. Moffat, K., Zhang, F., Hahn, K. M. & Möglich, A. in *Optogenetics* (eds Hegemann, P. & Sigrist, S.) 7–22 (Walter de Gruyter, 2013).
4. Kennedy, M. J. *et al. Nature Meth.* **7,** 973–975 (2010).
5. Shimizu-Sato, S., Huq, E., Tepperman, J. M. & Quail, P. H. *Nature Biotechnol.* **20,** 1041–1044 (2002).
6. Wang, X., Chen, X. & Yang, Y. *Nature Meth.* **9,** 266–269 (2012).
7. Polstein, L. R. & Gersbach, C. A. *J. Am. Chem. Soc.* **134,** 16480–16483 (2012).
8. Müller, K. *et al. Nucleic Acids Res.* **41,** e124 (2013).
9. Boch, J. *et al. Science* **326,** 1509–1512 (2009).
10. Charpentier, E. & Doudna, J. A. *Nature* **495,** 50–51 (2013).
11. Gilbert, L. A. *et al. Cell* **154,** 442–451 (2013).

CONDENSED-MATTER PHYSICS

A solid triple point

The observation of a triple point of coexistence between two insulating phases and a conducting phase in vanadium dioxide reveals physics that may help to unravel the role of electronic correlations in this material. SEE LETTER P.431

DOUGLAS NATELSON

Much of condensed-matter physics is concerned with thermodynamic phases, their properties and their transitions. In correlated materials, the electron–electron and electron–lattice interactions result in a competition between various electronic, magnetic and structural phases. The transitions between competing phases can reveal information about the underlying states that is otherwise difficult to obtain. On page 431 of this issue, Park *et al.*[1] use a micromechanical device and single-crystal nanobeams to determine with high precision the tensile stress–temperature phase diagram of vanadium dioxide (VO_2), an archetypal correlated oxide. Their experiment reveals a surprising and interesting fact: the metal–insulator phase transition for which VO_2 is famous is in fact a triple point, a rare circumstance in which three phases (here two insulators and a metal) can coexist. The experiment also determines the entropy differences between the various phases — information crucial to a complete understanding of the transition.

When a large amount of a substance (such as water) is brought together, it may exist in distinct phases (such as solid, liquid and gas). At given conditions, for example at a particular pressure and temperature, the thermodynamically stable phase is the one with the lowest free energy, which is determined by the arrangement, motion and interactions of the constituents. A phase diagram is a map of the stable phases as a function of parameters such as pressure and temperature.

When two phases coexist stably, their free energies must be equal, and for a single species, this condition leads to a coexistence 'line' for the two phases in the phase diagram. For example, ice and liquid water coexist in equilibrium at 0 °C and atmospheric pressure, and increasing the pressure decreases the melting point. Similarly, liquid water and water vapour coexist stably at 100 °C and atmospheric pressure, with decreasing pressure reducing that temperature (hence water boils at a lower temperature on top of a mountain than at sea level). These two coexistence curves can intersect only at a single value of pressure and temperature — a triple point (Fig. 1a). For water, this happens at 0.01 °C and 612 pascals. This particular triple coexistence defines the Kelvin temperature scale[2].

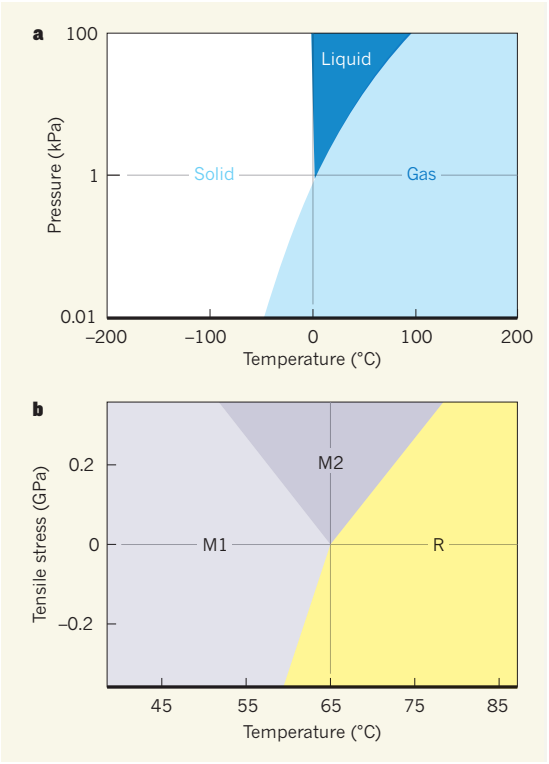

Figure 1 | Phase transitions with triple points. a, The phase diagram of water. At only one pressure and temperature can solid, liquid and gaseous water coexist in equilibrium. This triple point defines the Kelvin temperature scale. **b**, Park *et al.*[1] have mapped the phase diagram of vanadium dioxide. The triple point at zero tensile stress and the slopes of the phase boundaries greatly constrain theories that seek to understand the transitions from metal (R) to insulator (M1 or M2) in this material. Part **b** is based on Fig. 4b of the paper.

In VO_2, the competing phases of interest are all solids, albeit with different lattice structures and electronic properties: a high-temperature metallic phase (with a rutile lattice structure, R), and two insulating phases (with monoclinic structures, M1 and M2). The competition between these phases is of great interest because of the marked change in electronic and optical properties that occurs at the metal–insulator transition, the proximity of the transitions to room temperature, and the need to better understand the underlying physics. The relative importance of electron–electron interactions (Mott physics) and lattice distortion (Peierls physics) in stabilizing the M1 phase has been debated for decades. In addition to temperature, the intensive quantity relevant to VO_2 is the tensile stress rather than the pressure. Controlling this stress makes measurements in bulk crystals and thin films challenging.

Single-crystal VO_2 nanobeams[3] with a well-defined tensile-stress profile along the beam have been a boon to those trying to understand the intrinsic physics of this material. Park *et al.* attached an individual single-crystal VO_2 nanobeam to bridge a notched silicon structure, and used a piezo actuator to apply a controlled longitudinal deformation to the nanobeam, and so vary its length. Through polarized optical microscopy, Raman microscopy and electrical measurements, they identified regions of the suspended beam in the M1, M2 and R phases. Because the entire system was mounted on a temperature-controlled stage, the authors were able to determine the tensile stress–temperature phase diagram (Fig. 1b) by performing measurements of phase composition as a function of temperature at fixed length (which they can relate to the stress) and as a function of length at fixed temperature. To obtain measurements at zero stress, they broke