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

1. Cavalli, G. & Heard, E. *Nature* **571**, 489–499 (2019).
2. Fitz-James, M. H. & Cavalli, G. *Nature Rev. Genet.* **23**, 325–341 (2022).
3. Takahashi, Y. *et al. Cell* **186**, 715–731 (2023).
4. Tang, W. W. C., Kobayashi, T., Irie, N., Dietmann, S. & Surani, M. A. *Nature Rev. Genet.* **17**, 585–600 (2016).
5. Rakyan, V. K. *et al. Proc. Natl Acad. Sci. USA* **100**, 2538–2543 (2003).
6 Morgan, H. D., Sutherland, H. G. E., Martin, D. I. K. & Whitelaw, E. *Nature Genet.* **23**, 314–318 (1999).
7. Greer, E. L. *et al. Cell Rep.* **7**, 113–126 (2014).
8. Siklenka, K. *et al. Science* **350**, aab2006 (2015).
9. Takahashi, Y. *et al. Science* **356**, 503–508 (2017).
10. Fueyo, R., Judd, J., Feschotte, C. & Wysocka, J. *Nature Rev. Mol. Cell Biol.* **23**, 481–497 (2022).

The authors declare no competing interests.
This article was published online on 21 March 2023.

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

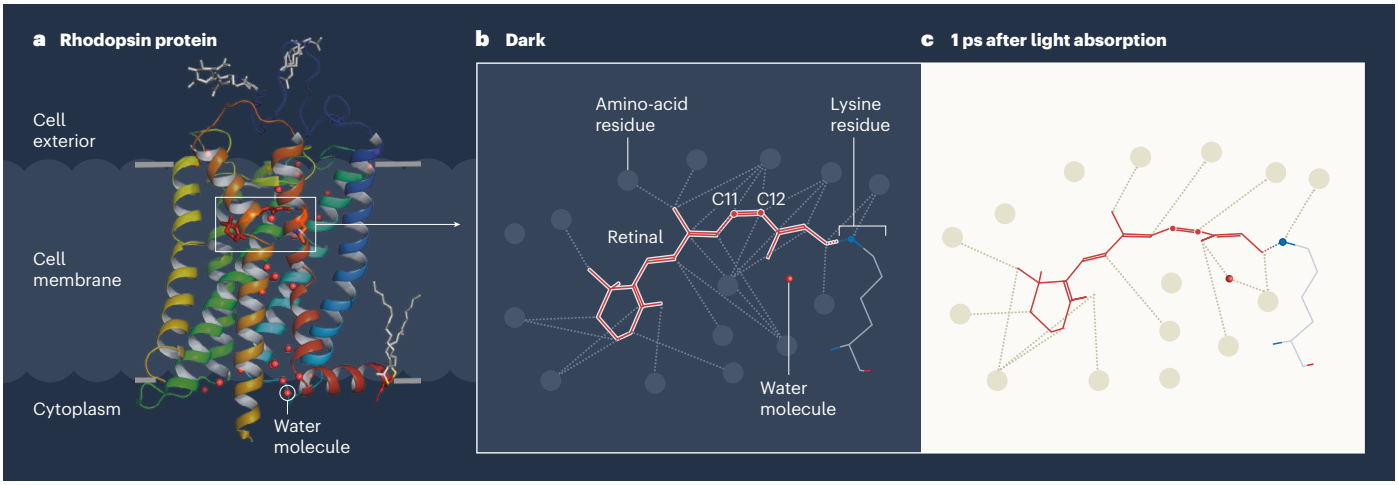

Figure 1 | Switching on the light-sensitive rhodopsin protein. In the vertebrate eye, the transmembrane protein rhodopsin initiates the cellular processes that lead to vision. It consists of a pigment molecule (retinal) covalently bound to an opsin protein. Gruhl *et al.*[3] report a time series of structures of bovine rhodopsin taken before and after the retinal molecule has absorbed light. **a**, This structure shows rhodopsin in the dark, before exposure of the protein to light; ribbons represent α-helices. The structures in grey at the cell exterior represent attached sugars; those in the cell membrane are groups that anchor the protein to the membrane. **b**, The dark-state retinal (red molecule) forms interactions (dotted lines) with many amino-acid residues, and thereby prevents the protein from generating signals to the brain. The lysine residue to which retinal is covalently bound is also shown, with its side chain. **c**, This structure shows retinal 1 picosecond (1 ps is 10^{-12} seconds) after it has absorbed light. The molecule has isomerized at the bond between carbons 11 and 12, causing the conformation of the molecule to twist. This reduces the number of interactions with surrounding residues, and triggers a series of conformational changes in the protein (not shown) that lead to rhodopsin activation and vision. (**a**, Paul Scherrer Institut; **b** and **c** adapted, respectively, from Fig. 3b and 3d of ref. 3.)

to an intense laser pulse, and then probed by a single X-ray pulse from the XFEL. In this way, a time-resolved crystallographic data set can be assembled from tens of thousands of diffraction patterns, each obtained from a different microcrystal. Reversible and irreversible reactions can be studied by TR-SFX on the same footing, because each microcrystal is discarded after exposure to the single X-ray pulse and replaced by a new, pristine one.

Gruhl *et al.* now report the first direct visualization of the retinal isomerization in a mammalian rhodopsin, using the state-of-the-art XFELs at SwissFEL in Villigen, Switzerland, and at the SACLA facility in Harima Science Garden City, Japan. The authors isolated rhodopsin from cow retinas and crystallized it in a viscous medium known as a lipidic cubic phase (LCP), which strongly supports the crystallization of membrane proteins. The resulting LCP paste containing rhodopsin microcrystals was extruded as a thin jet for TR-SFX analysis.

One potential issue with this approach is that the retinal might absorb more than one photon from the optical laser pulse, thereby becoming damaged. To alleviate this concern, the authors carefully measured the heat released after the retinal had absorbed light from the laser pulse, and concluded that the amount released is commensurate with absorption of a single photon.

Gruhl and colleagues took a series of 'snapshots' of retinal isomerization, and the resulting structural changes of the rhodopsin using XFEL pulses produced 1 picosecond (1 ps is 10^{-12} seconds), 10 ps and 100 ps after the optical photon was absorbed by retinal in the rhodopsin microcrystals. The snapshots reveal that structural rearrangements of the retinal molecule at 1 ps are compatible with isomerization occurring through the abortion of a previously proposed 'bicycle pedal' mechanism[11]. The upshot of this is that, at 1 ps, the isomerized all-*trans* retinal fills the same volume as the 11-*cis* retinal does in the dark, but no longer forms the interactions (hydrogen bonds and van der Waals interactions) with the protein that prevent rhodopsin from generating a signal (Fig. 1b,c). After isomerization, the protein disperses excess energy not needed for isomerization through an evolutionarily conserved pathway — a sequence of structural changes — that has been identified[12] in various other GPCRs. The snapshots show that this process is almost complete by 100 ps.

The light-induced structural changes observed around the retinal are similar to those observed in structurally related, light-activated retinal-binding proteins from bacteria and archaea[13]. The findings suggest that retinal-binding proteins, regardless of their evolutionary origin and types of isomerization, disengage the retinal from their central transmembrane helix before further activation processes occur. The authors observed other structural rearrangements occurring elsewhere in rhodopsin, which are also similar to those seen in TR-SFX studies on related proteins in bacteria and archaea[13].

Events happening within less than 1 ps in rhodopsin should now be characterized. Such structural changes need to be related to conformational changes on longer, nanosecond-to-millisecond timescales, to reveal the complete mechanism of rhodopsin's response to light. It might also be possible to investigate the activation and enzymology of transducin — the G protein that is bound to and activated by rhodopsin in the cytoplasm of rod cells — by using time-resolved crystallography to study the rhodopsin–transducin complex. These investigations will cast light on the activation mechanisms for all GPCRs related to rhodopsins.

Marius Schmidt is in the Department of Physics, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin 53201, USA.
Emina A. Stojković is in the Department of Biology, Northeastern Illinois University, Chicago, Illinois 60625, USA.
e-mails: smarius@uwm.edu; e-stojkovic@neiu.edu

1. Ernst, O. P. *et al. Chem. Rev.* **114**, 126–163 (2014).
2. Wald, G. *Science* **162**, 230–239 (1968).
3. Gruhl, T. *et al. Nature* **615**, 939–944 (2023).
4. Dryja, T. P. *et al. Nature* **343**, 364–366 (1990).
5. Rao, V. R., Cohen, G. B. & Oprian, D. D. *Nature* **367**, 639–642 (1994).
6. Ovchinnikov, Y. *et al. Bioorg. Khim.* **8**, 1011–1014 (1982).
7. Schertler, G. F. X., Villa, C. & Henderson, R. *Nature* **362**, 770–772 (1993).
8. Palczewski, K. *et al. Science* **289**, 739–745 (2000).
9. Tenboer, J. *et al. Science* **346**, 1242–1246 (2014).
10. Chapman, H. N. *et al. Nature* **470**, 73–77 (2011).
11. Warshel, A. *Nature* **260**, 678–683 (1976).
12. Zhou, Q. *et al. eLife* **8**, e50279 (2019).
13. Nogly, P. *et al. Science* **361**, 145–148 (2018).

The authors declare no competing interests.
This article was published online on 22 March 2023.