to perform the decarboxylation reaction has been quite puzzling. Both enzymes are present in a wide array of prokaryotes (bacterial and archaeal microorganisms), and many other microbes, including fungi, rely on homologous enzymes — proteins that share ancestry with UbiD and UbiX — to decarboxylate various acids. White *et al.* show that prenylated $FMNH_2$ is first oxidized by Fdc1, a fungal homologue of UbiD, and then used to decarboxylate cinnamic acid (see Fig. 1a of the paper[1]).

So how does prenylated $FMNH_2$ form? White *et al.* reveal that when UbiX is supplied with dimethylallyl monophosphate (a precursor of prenyl groups), it prenylates riboflavin's three-ring system. This results in the formation of a fourth ring to generate prenylated $FMNH_2$ (Fig. 1). The authors show that this previously unknown cofactor enables Fdc1 to decarboxylate substrates *in vitro*, in the absence of UbiX. These results explain the findings of other studies[4,5] that showed that an unknown small molecule — but not UbiX itself — is required to activate the ability of UbiD (and Fdc1) to decarboxylate substrates.

Payne *et al.*[2] show that apo-Fdc1 (an apoenzyme is one without its bound cofactor) catalyses unusual biochemistry only when in the presence of prenylated $FMNH_2$. They find that Fdc1 first oxidizes prenylated $FMNH_2$ to generate an 'iminium' form of the cofactor. This activates and prepares Fdc1 to decarboxylate a wide assortment of substrates known as α,β-unsaturated aromatic carboxylic acids. Many of these derive from the microbial breakdown of lignin, the structural component of the secondary cell walls of plants[6]. The authors propose a mechanism for these decarboxylation reactions known as 1,3-dipolar cycloaddition (see Fig. 4d of the paper[2]). Although this mechanism is well known to organic chemists, its use by enzymes has until now been speculative[7].

The findings raise several questions about the biological use of prenylated riboflavin cofactors. First, there is no obvious amino-acid sequence for binding prenylated $FMNH_2$: UbiX and UbiD (or Fdc1) do not share similar amino-acid sequences or structures, and the interactions of these proteins with the prenyl group seem to be restricted to providing an appropriately shaped binding site. This complicates the identification of other enzymes that might make use of this cofactor.

It is also surprising that UbiX uses dimethylallyl monophosphate as a source of prenyl groups, rather than the commonly used source dimethylallyl diphosphate. It will be important to determine the metabolic origin of dimethylallyl monophosphate and to explore whether it is used in other enzyme reactions. Dimethylallyl diphosphate is generated either through a series of biochemical reactions known as the mevalonate pathway, or through the methylerythritol phosphate pathway. In both pathways, dimethylallyl diphosphate is reversibly converted to another compound, isopentenyl diphosphate. A third, related pathway has recently been characterized[8] in the archaeon *Thermoplasma acidophilum*. This alternative route directly produces isopentenyl monophosphate — could it be that this compound interconverts with dimethylallyl monophosphate, in the same way that dimethylallyl diphosphate and isopentenyl diphosphate interconvert?

Can Pad1 (the fungal homologue of UbiX) and Fdc1 also mediate decarboxylation steps in the biosynthesis of coenzyme Q? UbiX from *E. coli* and Pad1 from yeast have been shown to perform the same function: a mutant form of *E. coli* in which the *ubiX* gene is deleted cannot synthesize coenzyme Q, but the synthesis is restored[9] if the mutant is engineered to express yeast Pad1. The two current papers now identify both Pad1 and UbiX as FMN prenyltransferases, enzymes that synthesize prenylated $FMNH_2$ as a diffusible small molecule.

But what about Fdc1 and UbiD? Do these enzymes both recognize 3-polyprenyl-4-hydroxybenzoic acid (the substrate that is decarboxylated in the biosynthesis of coenzyme Q)? Unfortunately, 3-polyprenyl-4-hydroxybenzoic acid is not commercially available, and so the authors of the current papers could not perform direct assays of Fdc1 or UbiD with this substrate. But the synthesis of coenzyme Q is not impaired when the genes that express Pad1 and Fdc1 are both deleted from two species of yeast[9,10]. The enzyme responsible for the decarboxylation step of coenzyme Q biosynthesis in eukaryotes (organisms that include fungi, plants and animals) therefore remains an open question. ■

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1. White, M. D. *et al. Nature* **522,** 502–506 (2015).
2. Payne, K. A. P. *et al. Nature* **522,** 497–501 (2015).
3. Crane, F. L. *Mitochondrion* **7,** S2–S7 (2007).
4. Leppik, R. A., Young, I. G. & Gibson, F. *Biochim. Biophys. Acta* **436,** 800–810 (1976).
5. Lin, F., Ferguson, K. L., Boyer, D. R., Lin, X. N. & Marsh, E. N. *ACS Chem. Biol.* **10,** 1137–1144 (2015).
6. Weng, J. K. & Chapple, C. *New Phytol.* **187,** 273–285 (2010).
7. Krenske, E. H., Patel, A. & Houk, K. N. *J. Am. Chem. Soc.* **135,** 17638–17642 (2013).
8. Vinokur, J. M., Korman, T. P., Cao, Z. & Bowie, J. U. *Biochemistry* **53,** 4161–4168 (2015).
9. Gulmezian, M., Hyman, K. R., Marbois, B. N., Clarke, C. F. & Javor, G. T. *Arch. Biochem. Biophys.* **467,** 144–153 (2007).
10. Mukai, N., Masaki, K., Fujii, T., Kawamukai, M. & Iefuji, H. *J. Biosci. Bioeng.* **109,** 564–569 (2010).

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CELL METABOLISM

Sugar for sight

Retinitis pigmentosa causes the death of cone cells, leading to blindness. A factor secreted from rod cells, RdCVF, promotes cone survival in a mouse model of the disease. It now emerges that RdCVF works by increasing glucose uptake in cones.

CONNIE CEPKO & CLAUDIO PUNZO

In daylight, light-sensing photoreceptor cells called cones endow us with rich, high-acuity colour vision. By contrast, their night-time counterparts, rod cells, are so light-sensitive that they enable us to see when walking in the woods under starlight. Sadly, a mutation in any one of more than 200 genes can cause diseases such as retinitis pigmentosa that bring about photoreceptor degeneration and lead to blindness[1,2]. Many of these disease-causing genes are rod-specific, affecting only night vision, but when rods die, the malfunction and death of cones soon follows. Writing in *Cell*, Aït-Ali *et al.*[3] provide evidence for an interaction between these two cell types that might explain why cones depend on rods for their survival, and that might eventually lead to a therapy for such diseases.

Retinitis pigmentosa affects 1 in 3,000 people[2], and effective treatments are sorely needed. The large number of disease-causing mutations means that these treatments should be gene-independent, focusing instead on targeting the biological pathways that cause the cones' death. The reasons for their death are unclear. However, there is evidence that several factors can contribute to cone death in retinitis pigmentosa. These include hyperoxia (an excess of oxygen), which causes oxidative damage by increasing the levels of free radicals[4–6]; a lack of energy[7]; and a lack of intermediates in the anabolic processes by which large molecules are constructed from smaller ones[7].

In agreement with a role for such factors, hyperactivation of the protein complex mTOR1, which controls cell metabolism by balancing demand with supply, increases cone survival[8]. This protein complex probably acts by promoting the expression of genes that improve glucose uptake and use, raising levels of anabolic intermediates and of an anabolic cofactor molecule called NADPH. Adequate

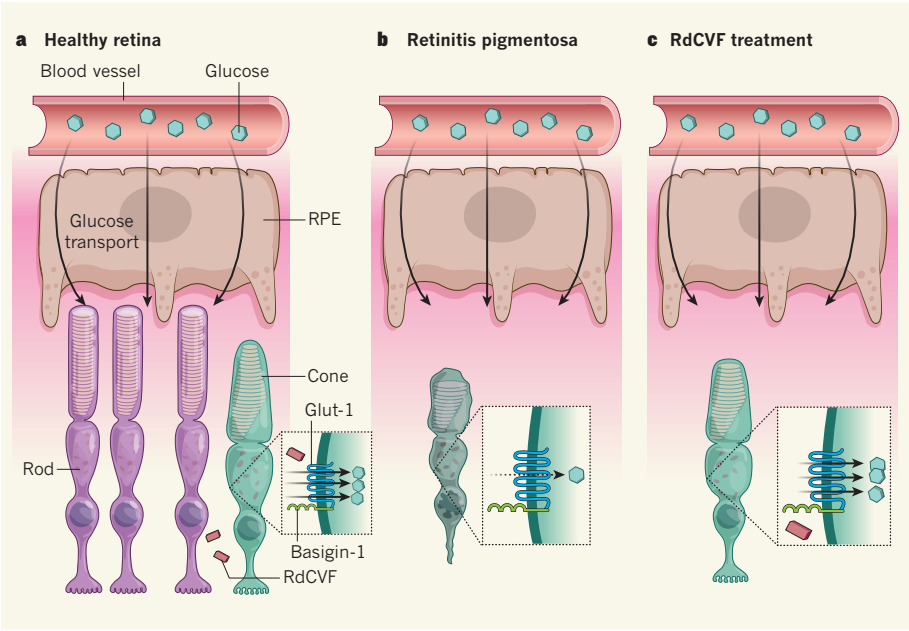

Figure 1 | Glucose uptake in cone cells. **a**, Intermingled rod and cone photoreceptor cells in the outer portion of the retina receive glucose from blood vessels, through retinal pigmented epithelial (RPE) cells. Glucose uptake in photoreceptors is mediated by the transporter protein Glut-1, which interacts with another protein, Basigin-1. Aït-Ali *et al.*[3] demonstrate that a protein secreted from rods, called rod-derived cone viability factor (RdCVF), associates with Basigin-1 to improve glucose uptake and the production of energy from glucose by inducing the formation of a more active form of Glut-1. **b**, In diseases such as retinitis pigmentosa, in which rods die, cones seem to be unable to take up enough glucose to fuel their metabolism, and so also die. **c**, The authors show that this defect can be prevented by addition of RdCVF.

levels of NADPH are likely to be crucial to cone survival because, in addition to its role in anabolic processes, it is needed for pathways that detoxify free radicals in hyperoxic retinas. Injection of antioxidants[4] or viral-vector delivery of genes that fight oxidation[6] prolong cone survival in mouse models of retinitis pigmentosa, supporting the theory that oxidation is a cause of cone death.

Healthy photoreceptors are metabolically very active[9], and so require high levels of glucose, which is delivered from the bloodstream through retinal pigmented epithelial cells. Because NADPH is produced by the oxidation of glucose, the demand for glucose in hyperoxic conditions is likely to be exceptionally high. A glucose transporter protein called Glut-1, located on the cell surface, mediates glucose uptake by photoreceptors, and evidence suggests[7] that a failure to take up sufficient glucose might contribute to cone death in retinitis pigmentosa. But there is a puzzling aspect to this model — glucose is delivered to the retina at a high rate and, after the death of rods, cones should have access to higher than normal levels of glucose. This suggests that there must be an added level of complexity underlying glucose uptake in cones.

Another factor that supports cone-cell survival[10] is a protein secreted from rods, called rod-derived cone viability factor (RdCVF)[11], that may have antioxidant activity. The cones of mice lacking RdCVF are more susceptible to oxidative damage than those of controls, and these mice show reduced photoreceptor activity with ageing[12]. Aït-Ali *et al.* therefore set out to explore the mechanism by which RdCVF promotes cone-cell survival. Using mass spectrometry, they identified a protein, called Basigin-1, that binds to RdCVF. Basigin-1 is found on the surface of cones and is known[13] to cause retinitis pigmentosa when mutated in mice. The authors also identified Glut-1 as a Basigin-1-binding protein. But a previous study showed that, contrary to what might have been expected, loss of Basigin-1 did not affect the expression of Glut-1 or, for the most part, its distribution in the retina[14].

Aït-Ali and colleagues observed that addition of RdCVF increased glucose uptake, lactate release and ATP production in photoreceptor cells *in vitro* — three cellular responses suggesting that RdCVF increases metabolic flux. Furthermore, the authors found that a decrease in levels of Basigin-1 and Glut-1 eliminated the ability of RdCVF to promote photoreceptor survival. The authors propose that RdCVF, Basigin-1 and Glut-1 form a complex at the cell surface that increases glucose uptake (Fig. 1). However, the level of Glut-1 on the cell surface did not increase after RdCVF addition, leading the researchers to suggest that this complex instead acts to increase levels of the active form of Glut-1. Future work should test this model. It will also be of interest to study the potential antioxidant role of RdCVF, together with the formation and activity of this three-protein complex, which might, as Aït-Ali *et al.* suggest, depend on a redox-sensitive interaction between RdCVF and Basigin-1.

A study published earlier this year showed that the delivery of RdCVF in mice with retinitis pigmentosa using an adeno-associated virus (AAV) prolonged cone survival and function[15]. AAV is a safe and effective vector that is used for ocular gene therapy in humans[16], and this, together with Aït-Ali and colleagues' finding that Basigin-1 is expressed in human retinas, suggests that AAV–RdCVF might be an effective way to treat many types of photoreceptor disease. Owing to the large number of disease genes that cause blindness in humans, a treatment that could promote the survival of cones in a gene-independent manner would be a welcome prospect. ■

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1. https://sph.uth.edu/retnet
2. Hartong, D. T., Berson, E. L. & Dryja, T. P. *Lancet* **368,** 1795–1809 (2006).
3. Aït-Ali, N. *et al. Cell* **161,** 817–832 (2015).
4. Komeima, K., Rogers, B. S., Lu, L. & Campochiaro, P. A. *Proc. Natl Acad. Sci. USA* **103,** 11300–11305 (2006).
5. Yu, D.-Y., Cringle, S. J., Su, E.-N. & Yu, P. K. *Invest. Ophthalmol. Vis. Sci.* **41,** 3999–4006 (2000).
6. Xiong, W., MacColl Garfinkel, A. E., Li, Y., Benowitz, L. I. & Cepko, C. L. *J. Clin. Invest.* **125,** 1433–1445 (2015).
7. Punzo, C., Kornacker, K. & Cepko, C. L. *Nature Neurosci.* **12,** 44–52 (2009).
8. Venkatesh, A. *et al. J. Clin. Invest.* **125,** 1446–1458 (2015).
9. Graymore, C. *Br. J. Ophthalmol.* **43,** 34–39 (1959).
10. Léveillard, T. S. *et al. Nature Genet.* **36,** 755–759 (2004).
11. Holmgren, A. *Annu. Rev. Biochem.* **54,** 237–271 (1985).
12. Cronin, T. *et al. Cell Death Differ.* **17,** 1199–1210 (2010).
13. Hori, K. *et al. Invest. Ophthalmol. Vis. Sci.* **41,** 3128–3133 (2000).
14. Philp, N. J., Ochrietor, J. D., Rudoy, C., Muramatsu, T. & Linser, P. J. *Invest. Ophthalmol. Vis. Sci.* **44,** 1305–1311 (2003).
15. Byrne, L. C. *et al. J. Clin. Invest.* **125,** 105–116 (2015).
16. Trapani, I., Banfi, S., Simonelli, F., Surace, E. M. & Auricchio, A. *Hum. Gene Ther.* **26,** 193–200 (2015).

ADDENDUM

The News & Views article 'Alzheimer's disease: From big data to mechanism' by Vivek Swarup and Daniel H. Geschwind (*Nature* **500,** 34–35; 2013) commented on the paper 'Integrative genomics identifies APOE ε4 effectors in Alzheimer's disease' by H. Rhinn *et al.* (*Nature* **500,** 45–50; 2013). This paper has now been retracted. For further information, see http://dx.doi.org/10.1038/nature14591