(Fig. 1). In control conditions, the complex synaptic response would consist of both the electrical response and a transient calcium signal. Activity-induced insertion of GluR2-containing AMPA receptors into the postsynaptic membrane would switch the synapse to a second mode, in which the calcium signal is suppressed because of the reduced calcium entry. Another plausible, but equally speculative, role for the switch in subunit composition is that it serves as a mechanism to scale transient changes in postsynaptic calcium levels[8]. Or it could have a purely neuroprotective function; calcium entry through AMPA receptors has been implicated in the neurodegeneration associated with ischaemia (reduced blood flow) in the brain and epilepsy.

Before we can completely understand this process and its implications, it will be important to determine the calcium-dependent events that lead to the insertion of a GluR2-containing receptor complex into the postsynaptic membrane. Also interesting is how GluR2 might be removed to reset the system and to re-establish the functional properties found in control conditions. The answers to these questions may come with analysis of the amplitude and kinetics of the transient changes in intracellular calcium that induce these effects. In fact, different patterns of such calcium transients may either upregulate or downregulate synaptic function through activation of calcium-permeable AMPA receptors in other neurons[9,10]. The self-regulation of AMPA receptors revealed by Liu and Cull-Candy[1] has added yet another piece to the puzzle of synaptic plasticity. ■

Christine R. Rose and Arthur Konnerth are at the Institute of Physiology, Technical University of Munich, Biedersteiner Strasse 29, 80802 Munich, Germany.
e-mail: konnerth@physiol.med.tu-muenchen.de

1. Liu, S.-Q. & Cull-Candy, S. G. *Nature* **405,** 454–458 (2000).
2. Malenka, R. C. & Nicol, R. A. *Science* **285,** 1870–1874 (1999).
3. Benke, T. A. *et al. Nature* **393,** 793–797 (1998).
4. Durand, G. M., Kovalchuk, Y. & Konnerth, A. *Nature* **381,** 71–75 (1996).
5. Hayashi, Y. *et al. Science* **287,** 2262–2267 (2000).
6. Song, I. *et al. Neuron* **21,** 393–400 (1998).
7. Burnashev, N. *et al. Neuron* **8,** 189–198 (1992).
8. Turrigiano, G. G. *et al. Nature* **391,** 892–896 (1998).
9. Gu, J. G. *et al. Nature* **381,** 793–796 (1996).
10. Laezza, F., Doherty, J. J. & Dingledine, R. *Science* **285,** 1411–1414 (1999).

DNA repair

The bases for Cockayne syndrome

Philip C. Hanawalt

Cockayne syndrome is a rare human hereditary disease, characterized by growth failure, deficient neurological development and severe sensitivity to sunlight. It can arise from mutations in any one of five genes. The protein products of these genes are involved in different aspects of the repair of damaged DNA, and it has been far from clear how all these different mutations result in the same syndrome. Le Page and colleagues[1], writing in *Cell*, now provide important clues to the answer. It seems that the common problem in cells from patients with Cockayne syndrome is a failure to repair oxidation-induced damage to DNA bases, specifically in the strands of DNA that are being transcribed into RNA.

Different DNA-repair pathways operate on different types of DNA lesions. Nucleotide-excision repair (NER), for example, is a ubiquitous cellular process by which short, single-stranded DNA segments, containing damaged nucleotides, are removed from duplex DNA. The gaps are then filled in by repair DNA synthesis, using the intact strand as a template (see ref. 2 for a review). Defects in NER underlie the hereditary disease xeroderma pigmentosum. This disease — like Cockayne syndrome — is characterized by severe sensitivity to sunlight. However, patients with xeroderma pigmentosum are several thousand times more likely than Cockayne syndrome patients to develop cancer in exposed areas of skin. Otherwise, mutations in most of the seven *XP* (xeroderma pigmentosum) genes needed for NER of photoproducts in DNA do not usually pose serious health problems. Another pathway, termed base-excision repair (BER), operates on the damage to bases produced by reactive oxygen species, ionizing radiation and some alkylating agents, as well as certain inappropriate bases (such as uracil) in DNA.

Yet another process, transcription-coupled repair (TCR), deals with a variety of DNA lesions that are thought to arrest the transcription of genes[3]. This process has been considered for some time to be a type of NER. If the ultraviolet wavelengths of sunlight cause damage to the strand of a DNA duplex that is being transcribed into RNA, TCR solves the problem. By contrast, lesions throughout the genome — including ultraviolet-light-induced damage to the non-transcribed strands of expressed genes — are repaired by global genomic NER.

Mutations in either of two non-essential genes — *CSA* or *CSB* — result in defective TCR, and are the genetic defect in over 90% of Cockayne syndrome patients. Certain mutations in *XP* genes also underlie a small number of Cockayne syndrome cases. Two of these genes, *XPB* and *XPD*, encode components of the general transcription factor TFIIH. This complex is needed to open up the DNA strands in preparation for the enzyme RNA polymerase II to begin transcription. It also opens up regions that include a DNA lesion, allowing NER to take place. The third *XP* gene so involved is *XPG*, which encodes a protein required to make the first of the two incisions in the DNA strand needed for NER.

Thus all the mutations that cause Cockayne syndrome have in common the property that they eliminate TCR of ultraviolet-damaged DNA. This explains the sensitivity to sunlight, but what about the developmental defects, which are unlikely to result from ultraviolet damage to DNA? Might the basis for these defects lie in the defective NER of similar damage caused by other agents? This seems unlikely, as mutations in the *XPA* gene (which is involved in lesion recognition) that totally eliminate both global genomic NER and the TCR of such damage do not result in Cockayne syndrome. A second hypothesis is that the disease is a 'transcription syndrome', in which certain groups of genes are deficiently expressed[4]. In this model, the mutations in *CSA* and *CSB*, like those in *XPB* and *XPD*, are envisaged to have direct effects on transcription itself. But it is hard to explain how the mutations in *XPG* affect transcription.

So what is the basis for Cockayne syndrome? Getting to the answer requires rethinking the relationship between TCR and NER. It seems that, far from being a subpathway of NER, TCR may in fact act upstream of both nucleotide- and base-excision repair.

A first step along the way to this answer was provided by the report[5] that DNA damage produced by ionizing radiation (not ultraviolet radiation) is subject to TCR in normal human cells and in *XPA* mutant cells, but not in *CSB* mutant cells. DNA damage produced by ionizing radiation is generally thought to be remedied by BER, not NER. In addition, TCR of an oxidized base, thymine glycol, has been shown to be defective as a result of *XPG* mutations that result in Cockayne syndrome[6], but not of other *XPG* mutations that result only in the symptoms of xeroderma pigmentosum[7]. Repair of thymine glycol is also achieved by BER. So, TCR can be linked to BER as well as to NER. These results are further evidence that Cockayne syndrome might result from defective TCR of oxidative lesions.

Le Page *et al.*[1] have now finished testing the hypothesis that all patients with Cockayne syndrome — no matter which gene is mutated — should be deficient in the TCR of oxidative lesions, whatever their nature. Their results firmly establish that both the XPG protein and TFIIH have

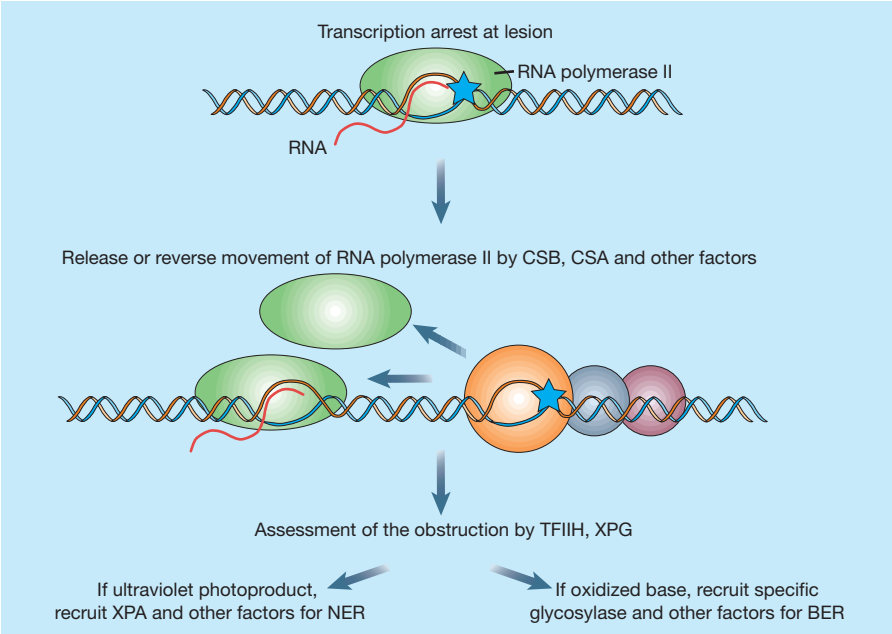

Figure 1 **The DNA-repair pathways meet. The results of Le Page *et al.*[1] lead to the following model for the repair of lesions in the transcribed strands of expressed genes. Top, RNA polymerase II transcribes DNA into RNA. If RNA polymerase II meets a lesion (asterisk) in the DNA strand, it will stop. Centre, repair of the lesion and further transcription is blocked until the polymerase is either released from the DNA or backed away from the lesion. Both TFIIH and XPG are then required before the lesions are repaired, perhaps to determine the nature of the obstruction. Bottom, enzymes of either the nucleotide-excision-repair (NER) or the base-excision-repair (BER) pathways are recruited, to remove the offending lesion and allow transcription to resume. The genes that can be mutated in Cockayne syndrome are required to remove the stalled polymerase, and to assess the nature of the damage.**

different roles in the TCR of oxidative damage and in NER.

Le Page *et al.* also turned their attention to another prominent oxidative lesion, 8-oxo-guanine. Using a shuttle vector, they introduced DNA containing 8-oxo-guanine residues into several human cell strains. They found that 8-oxo-guanine, like thymine glycol, is subject to TCR, but not significantly to NER — so it must be subject to BER instead. They also showed that 8-oxo-guanine (or 8-oxo-guanine plus a ligand) is an obstacle to the movement of RNA polymerase II, and that the arrested polymerase must be released to give the repair enzymes access to the site. So, not only is TCR missing in Cockayne syndrome, but the blocked RNA polymerase II prevents lesion recognition and repair by the global BER pathway as well.

Instead of being considered a subpathway of NER, it now seems that TCR is a process needed before either the BER or the NER pathway can repair oxidative damage to genes that are being transcribed, in which RNA polymerase is stalled at a lesion. TCR might be considered an 'obstacle-recognition' step, in which TFIIH and XPG may work together to assess the nature of the obstruction (Fig. 1). This obstruction might be removed only when the arrested RNA polymerase II has been moved away, which may be achieved by CSA and CSB, amongst other factors. TFIIH and XPG might then recruit the enzymes needed for BER or NER, as appropriate.

It is clear that an impasse provided by an arrested RNA polymerase II would severely affect transcription. In that sense, Cockayne syndrome could, indeed, be considered a 'transcription syndrome'. But the arrest of transcription also provides a strong signal for a cell-death (apoptosis) pathway[8]. So, Cockayne syndrome could also be characterized as a disease of excessive cell death by apoptosis — a disease that would affect rapidly metabolizing cells, such as neurons, that generate high levels of reactive oxygen species. The apoptosis model could also, in principle, explain the problems of stunted growth and neurological deterioration. It might also explain why Cockayne syndrome patients are not prone to skin cancer, even in the face of severe sunlight sensitivity — after all, dead cells do not form tumours. ■

Philip C. Hanawalt is in the Department of Biological Sciences, Stanford University, Stanford, California 94305-5020, USA.
e-mail: hanawalt@stanford.edu

1. Le Page, F. *et al. Cell* **101,** 159–171 (2000).
2. Lindahl, T. & Wood, R. D. *Science* **285,** 1897–1905 (1999).
3. Tornaletti, S. & Hanawalt, P. C. *Biochimie* **81,** 139–148 (1999).
4. Bootsma, D. & Hoeijmakers, J. H. J. *Nature* **363,** 114–115 (1993).
5. Leadon, S. A. & Cooper, P. K. *Proc. Natl Acad. Sci. USA* **90,** 10499–10503 (1993).
6. Cooper, P. K. *et al. Science* **275,** 990–993 (1999).
7. Nouspikel, T. *et al. Proc. Natl Acad. Sci. USA* **94,** 3116–3121 (1997).
8. Ljungman, M. & Zhang, F. *Oncogene* **13,** 823–831 (1996).

Daedalus

The depths of madness

Protein molecules, folded so subtly, work all the enzymatic wonders of biochemistry. Heat can unfold and denature them — as all cooks know. But so can pressure. Raw egg white is opacified and hardened by a few thousand atmospheres (though it still tastes raw). Daedalus is now pondering the implications for deep-sea creatures.

A crucial problem for such creatures is to know how deep they are. Some rely on their eyes — daylight from the surface fades out with depth. Others have a swim-bladder, whose gas is compressed by the rising hydrostatic pressure as its owner sinks. But below 1 km or more, neither scheme works well. Daedalus reckons that whales, squid and other creatures who repeatedly patrol a wide vertical range exploit pressure-dependent protein folding. Some 'barometric enzyme' in their make-up changes its reactivity reversibly with pressure to register their depth.

So DREADCO trawlers are now lowering pressurized traps into the oceans, to snare abyssal creatures and haul them to the surface still under pressure. They will then be transferred to a pressurized aquarium for study. They, or perhaps their symbiotic and gut organisms, should harbour novel barometric enzymes which could throw new light on the protein-folding problem.

But this costly research programme has a greater goal. Daedalus argues that all the proteins of an abyssal creature must unfold somewhat in the deeps. And even if they fold again when their owner rises, very likely at least one molecule will do so wrongly. It will form a prion — and once one has formed, it will catalyse production of more prions the next time the creature dives. In the brain, proliferating prions pose a lethal threat. What protects a squid from 'mad squid disease'?

Whatever it is, Daedalus wants to know. For we also are vulnerable to malfolded proteins, not only as the brain plaques of Alzheimer's disease and CJD, but in the slow advance of wrinkled skin and hardened eye lenses and arteries. Somewhere in abyssal biochemistry, he hopes, is the crucial refolding reagent that prevents a protein from prionizing or forming a plaque, or even hauls it back from that doom. It could be an elixir of health and youth for all mankind. Daedalus cannot guess how it may work. But his team is on the look-out for any separated fraction that can unboil an egg. **David Jones**

***The Further Inventions of Daedalus* is published by Oxford University Press.**