Both groups observed genome rearrangements in diploids that involved the deletion of one copy of essential genes. The presence of such rearrangements in improved diploid strains shows that, compared to haploids, diploids are more robust to deleterious deletions during SCRaMbLE. This in turn allows a greater number of beneficial rearrangements to be manifested. Although it is premature to claim that SCRaMbLE is a universal tool for engineering yeast, taken together, the various findings[3-7] certainly show that it has great potential for generating yeasts for a wide range of purposes.

Wu *et al.*[8] have taken SCRaMbLE out of cells and used it *in vitro* with purified Cre recombinase to generate different genetic arrangements of the β-carotene biosynthetic pathway. They thus discovered arrangements that increase β-carotene production compared with the original pathway. By contrast, Liu *et al.*[9] used an *in vitro* method involving recombinase enzymes separate from the SCRaMbLE system, to rapidly generate different versions of β-carotene- and violacein-producing pathways and to identify highly productive ones. They then flanked the DNA sequences of the best pathways with *loxPsym*, and used SCRaMbLE to randomly incorporate the pathways at *loxPsym* sites in the synthetic yeast genome. SCRaMbLE concurrently rearranged the resulting genomes, allowing yeast strains to be optimized for the production of the desired compounds. These two papers illustrate the versatility of the basic SCRaMbLE concept and how it can be used in innovative ways.

So where next for Sc 2.0? So far, six synthetic chromosomes of Sc2.0 have been completed[13], and consortium members are working full-time to construct the remaining ten. The seven new papers show that researchers are eager to work with the newly available synthetic chromosomes to see how SCRaMbLE techniques can generate useful yeast variants and improve our understanding of the fundamental processes and properties of yeast. Thousands of *loxPsym* sites will be present in the fully assembled Sc 2.0 genome, and so the number of genomic structures that can be generated by SCRaMbLE is immense — which suggests that it should be possible to produce a yeast variant that displays any desired set of characteristics.

Nevertheless, SCRaMbLE systems are still in their infancy. Further improvements are needed, along with tools that maximize the potential of SCRaMbLE-based techniques. For example, the screening of SCRaMbLE-modified yeast has generally relied on visible cues, such as growth rate and colour (both β-carotene and violacein are pigments that colour the yeast cells). Luo and colleagues' reporter offers a useful new screening tool, but high-throughput methods are also needed that can identify yeast strains that produce large amounts of colourless chemicals. Crucially, the characterization of genetic rearrangements relies heavily on whole-genome sequencing. The development of more-efficient, cheaper sequencing techniques would allow more strains to be sequenced than is currently possible, to work out and study changes in the genome. Given the promising early results and synergy among the members of the Sc2.0 consortium, the establishment of SCRaMbLE as a staple tool for engineering yeast is highly anticipated. ■

Jee Loon Foo *and* **Matthew Wook Chang**
are in the Department of Biochemistry, Yong Loo Lin School of Medicine, and in the NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI), Centre for Life Sciences, National University of Singapore, Singapore 117456.
e-ma il: bchcmw@nus.edu.sg

1. Burgess, D. J. *Nature. Rev. Genet.* **18**, 274 (2017).
2. Enyeart, P. J. & Ellington, A. D. *Nature* **477**, 413–414 (2011).
3. Shen, M. J. *et al. Nature Commun.* https://doi.org/10.1038/s41467-018-04157-0 (2018).
4. Jia, B. *et al. Nature Commun.* https://doi.org/10.1038/s41467-018-03084-4 (2018).
5. Hochrein, L., Mitchell, L. A., Schulz, K., Messerschmidt, K. & Mueller-Roeber, B. *Nature Commun.* https://doi.org/10.1038/s41467-017-02208-6 (2018).
6. Luo, Z. *et al. Nature Commun.* https://doi.org/10.1038/s41467-017-00806-y (2018).
7. Blount, B. A. *et al. Nature Commun.* https://doi.org/10.1038/s41467-018-03143-w (2018).
8. Wu, Y. *et al. Nature Commun.* https://doi.org/10.1038/s41467-018-03743-6 (2018).
9. Liu, W. *et al. Nature Commun.* https://doi.org/10.1038/s41467-018-04254-0 (2018).
10. Dymond, J. S. *et al. Nature* **477**, 471–476 (2011).
11. Annaluru, N. *et al. Science* **344**, 55–58 (2014).
12. Shen, Y. *et al. Genome Res.* **26**, 36–49 (2016).
13. van der Sloot, A. & Tyers, M. *Mol. Cell* **66**, 441–443 (2017).

This article was published online on 22 May 2018.

In retrospect

A catalyst for 50 years of cancer research

In 1968, a defect in DNA repair was found to underlie a disorder that makes people extremely sensitive to sunlight. This finding continues to influence research into the origins, diagnosis and treatment of cancer.

RICHARD D. WOOD

Some people are born with exceptional sensitivity to sunlight. Fifty years ago, writing in *Nature*, the biologist James Cleaver[1] reported a study of one such condition, and concluded that a failure of DNA repair was related to the extreme susceptibility of affected individuals to skin cancer. This was the first description of defective DNA repair in a genetically inherited disorder that makes people prone to cancer. The concepts that developed from this work now permeate research into the genetic origins of cancer and its treatment.

Starting in the 1870s, the Viennese dermatologist Moritz Kaposi performed pioneering work that defined a rare disorder characterized by high sensitivity to sunlight. Young patients were severely burned by brief exposure to the sun and acquired frequent skin lesions, and some had a high incidence of skin tumours. Kaposi dubbed the condition xeroderma pigmentosum[2] (XP), using the Greek words for dry, pigmented skin — one of the symptoms of the disease. He recognized that this was a hereditary syndrome, but the underlying cause was not obvious.

Little research into XP was then done until the 1960s, when a process called nucleotide excision repair was discovered in bacteria[3–5]. In this process, enzymes clip out segments of DNA that have been damaged by light and replace them with fresh, undamaged DNA. Mutant bacterial strains were isolated that could be killed by low doses of ultraviolet radiation, and some of these were found to be unable to carry out excision repair[4,5].

These concepts of DNA repair were then extended to human cells. By 1964, the biologists Robert Painter and Ronald Rasmussen had discovered that UV irradiation of mammalian cells led to a phenomenon that they interpreted as excision repair[6]. In their experiments, cultured human cells were supplied with radioactive molecules (bases) that could be incorporated into DNA. The cells were observed to incorporate new bases after UV irradiation, even when they were not duplicating their genomes, indicating that UV-damaged DNA was being replaced.

In 1967, Cleaver joined Painter's laboratory in San Francisco as a postdoctoral fellow. Cleaver had obtained his PhD at the University of Cambridge, UK, where he had been using radioactive bases to label DNA in human cells. In April of that year, Cleaver read a newspaper article in the *San Francisco Chronicle* that mentioned research showing that skin cells grown from patients with XP were extraordinarily sensitive to UV radiation[7]. Cleaver raised with Painter the idea that XP might involve a mutation that causes DNA repair to be defective, and suggested investigating this

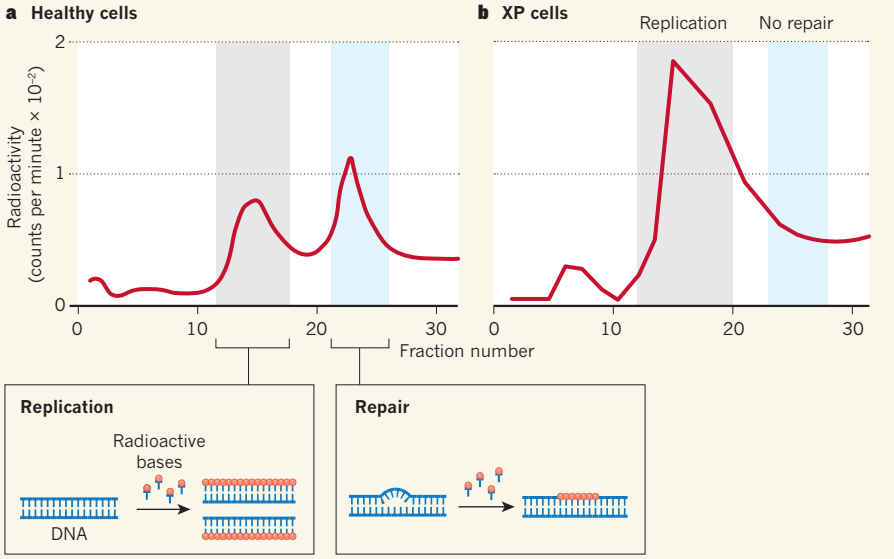

Figure 1 | Evidence of defective DNA repair in cells from people with xeroderma pigmentosum. People born with the condition known as xeroderma pigmentosum (XP) are extremely sensitive to sunlight and are prone to skin cancer. In 1968, Cleaver[1] reported experiments in which cultured cells from a healthy individual and from people with XP were irradiated with ultraviolet light to cause DNA damage and then analysed to see whether the cells incorporated radioactive molecules (bases) into their DNA. **a**, For the healthy cells, plots of measured radioactivity for different fractions of DNA revealed distinct peaks associated with DNA replication and DNA repair. **b**, By contrast, the XP cells lacked the repair-associated peak. This was the first evidence that defective DNA repair underpins a genetically inherited disorder that makes people susceptible to cancer.

possibility. Painter replied: "It's a crazy idea, but at your stage what have you got to lose!"[8]

Cleaver acquired cultures of growing skin cells from people with XP, and applied newly developed techniques[3,6] to determine whether the cells were capable of excision repair. The results clearly showed that DNA repair was defective in XP cells that had been damaged by UV irradiation (Fig. 1). Painter was a generous mentor, and encouraged his junior colleague to pursue this major discovery independently. Cleaver's results were published in *Nature* on 18 May 1968.

The paper's conclusions were strong. Cleaver used two completely different methods to show that DNA repair in XP cells is defective, using cells from three patients clinically verified to have XP, and control cells taken from a patient with an unrelated hereditary disorder and from a healthy individual. The results suggested that XP is not a homogeneous disease, because cell lines from different individuals exhibited different levels of DNA repair. There was no indication, however, of which step was affected in the repair process, or which genes might be altered. Cleaver estimated that about 70 DNA bases were incorporated in each repair event — not far from the actual number of about 30 bases per repair event obtained later using more-precise methods[9,10].

The publication generated immediate excitement. DNA repair had previously been considered a somewhat obscure topic, but Cleaver showed that it had a key role in human health. The Nobel-prizewinning molecular biologist Joshua Lederberg penned an editorial in *The Washington Post* highlighting this important example of fundamental research that turned out to be relevant to disease[11]. J. Michael Bishop, who won a Nobel prize in 1989 for his work on oncogenes, which have the potential to cause cancer, was also influenced by the finding. He wrote[8]: "While I was still in medical school, James Cleaver recognized xeroderma pigmentosum as a deficiency in the repair of DNA damage caused by ultraviolet light… I have been a believer in the somatic mutation hypothesis of cancer ever since". Somatic mutations are caused by DNA damage and copying errors in the genes of tumour cells as cancer progresses. Cleaver's paper helped to stimulate the worldwide explosion of DNA-repair research that started in the 1970s[8].

Cleaver's results were soon confirmed and extended by laboratories around the world. In 1972, it was reported that XP is a genetically complex disease[12], and it is now known that alterations in eight different genes can give rise to it[13,14]. Seven of these genes encode components of the molecular machinery that performs excision repair; this machinery was biochemically reconstituted *in vitro* in the 1990s[15,16]. One form of XP, however, is caused by abnormal DNA synthesis after UV irradiation[13], rather than by a problem in excision repair.

Specific defects in DNA repair are now known to be associated with major neurological and developmental abnormalities in other UV-sensitivity disorders, including Cockayne syndrome[13,14]. More broadly, it has become clear that many of the XP-associated genes have functions in addition to excision repair, and several are essential for life[13,14]. This means that only mild disablement of the functions of some XP genes can be tolerated.

Although XP is a rare disease (fewer than 1 person in 250,000 is affected in the United States and Western Europe)[13], the consequences of mutations in XP genes are being explored widely. For example, a recent analysis found that mutations in the *XPD* gene (also known as *ERCC2*) are fairly frequent in cancer and might modulate individual responses to treatment[17]. There is also active research aimed at suppressing the action of XP proteins in tumour cells, to improve the effectiveness of chemotherapies that damage DNA[18].

There is still no cure for XP, but intensive research into the disease means that an early diagnosis can be made. People with XP can then be protected rigorously from sunlight, allowing them a greater quality of life and longer life expectancy than was previously possible. XP societies in the United States and Europe provide support for affected children, with retreats such as Camp Sundown and Owl Patrol. Retinoid compounds can reduce the incidence of skin tumours[14], and dietary interventions might improve the prospects for people with XP and related disorders[19]. More broadly, Cleaver's discovery of the DNA-repair defect in XP continues to spawn vigorous research into responses to environmental DNA damage that applies not only to humans, but to every organism on the planet. ■

Richard D. Wood *is in the Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, Texas 78957, USA.*
e-mail: rwood@mdanderson.org

1. Cleaver, J. E. *Nature* **218**, 652–656 (1968).
2. Kaposi, M. *Wien. Med. Jb.* (October 1882).
3. Pettijohn, D. & Hanawalt, P. *J. Mol. Biol.* **9**, 395–410 (1964).
4. Boyce, R. P. & Howard-Flanders, P. *Proc. Natl Acad. Sci. USA* **51**, 293–300 (1964).
5. Setlow, R. B. & Carrier, W. L. *Proc. Natl Acad. Sci. USA* **51**, 226–231 (1964).
6. Rasmussen, R. E. & Painter, R. B. *Nature* **203**, 1360–1362 (1964).
7. Perlman, D. *San Francisco Chronicle* 12 April p.4 (1967).
8. Cleaver, J. E. *DNA Repair* **10**, 906–914 (2011).
9. Wood, R. D. *Annu. Rev. Biochem.* **65**, 135–167 (1996).
10. Sancar, A. *Angew. Chem. Int. Edn* **55**, 8502–8527 (2016).
11. Lederberg, J. *The Washington Post and Times-Herald* 8 June p.A13 (1968).
12. de Weerd-Kastelein, E. A., Keijzer, W. & Bootsma, D. *Nature N. Biol.* **238**, 80–83 (1972).
13. Lehmann, A. R., McGibbon, D. & Stefanini, M. *Orphanet J. Rare Dis.* **6**, 70 (2011).
14. DiGiovanna, J. J. & Kraemer, K. H. *J. Invest. Dermatol.* **132**, 785–796 (2012).
15. Aboussekhra, A. *et al. Cell* **80**, 859–868 (1995).
16. Mu, D., Hsu, D. S. & Sancar, A. *J. Biol. Chem.* **271**, 8285–8294 (1996).
17. Knijnenburg, T. A. *et al. Cell Rep.* **23**, 239–254 (2018).
18. Gavande, N. S. *et al. Pharmacol. Ther.* **160**, 65–83 (2016).
19. Vermeij, W. P. *et al. Nature* **537**, 427–431 (2016).