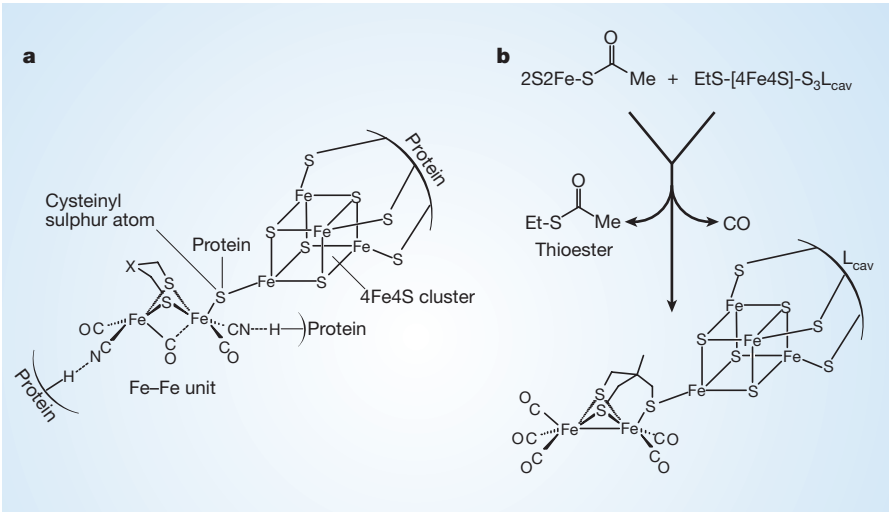

Figure 1 **Synthesis in action.** a, **The active site, or 'H-cluster', of the all-iron hydrogenase, a hydrogen-metabolizing enzyme. It consists of a cube-shaped 4Fe4S cluster bridged to an Fe–Fe organometallic unit by a cysteinyl sulphur atom.** b, **Pickett and colleagues[3] have made a close synthetic analogue of the H-cluster, using sulphur-containing precursors. The 4Fe4S and Fe–Fe units are linked up during a reaction in which a small thioester molecule and a carbon monoxide ligand are eliminated. L_{cav} is a large, bowl-shaped (cavitand) ligand[4,5], used to prevent expansion of the 4Fe4S cluster.**

cysteinyl-like sulphur donors in the cavity that bind to three of the cubane iron atoms, leaving only one sulphur donor that can react with the suitably modified Fe–Fe subsite[10]. And there was a chemical bonus as the elimination reaction occurred: a carbon monoxide ligand was simultaneously lost from the Fe–Fe subsite, allowing a cysteinyl-like sulphur bridge to form between two iron atoms, and thus further 'hard-wiring' the two subunits, precisely as in the H-cluster.

Pickett and colleagues[3] show that the physical properties of the synthetic analogue are a good match for those of the enzyme, and that the Fe–Fe organometallic unit drains electron density from the 4Fe4S cluster. Most importantly, the chemical properties of the synthetic H-cluster indicate electrocatalysis of hydrogen production, consistent with the assumption that the Fe–Fe unit is a hydrogen-producing moiety. This process is the basis for the hydrogen-catalysing action of hydrogenase and is of great biotechnical interest: unlike today's commercial fuel cells, it does not require platinum — an expensive metal of limited availability — as the electrode. Can we imitate nature and construct platinum-free fuel cells? Pickett and colleagues have demonstrated a promising first step towards this goal.

But their work is not only of technological interest. Protein crystallography is a powerful technique — allowing chemists to recognize familiar patterns of atom–atom connections and overall geometry — but it produces only a snapshot of a factory in action. A close synthetic analogue of a biological molecule such as that reported by Pickett and colleagues, one that is structurally similar to its original, will allow researchers to assess the function of individual components that have evolved naturally to give efficiency and control. When chemists build an intricate molecule from known components through established pathways, it enhances their understanding of both its structure and its function. The synthesis method described by Pickett and colleagues resolves the 'black box' spectral signals of the active centre of hydrogenase, and points towards a next generation of bio-inspired catalysts. ■

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Cancer

Catalyst of a catalyst

Stephen C. Kowalczykowski

Breast cancers arise when the BRCA2 protein is defective, but what does the normal enzyme do? Studies of a relative of BRCA2 reveal a capacity to initiate the repair of broken DNA by loading a repair protein.

The identification of the human *BRCA2* gene was a landmark discovery in the field of breast cancer[1]. Mutations in this gene result in a dramatically increased predisposition to breast cancer, as well as to other tumour types. The BRCA2 protein is known to be necessary for DNA repair, but its precise role has been unclear. Work from Pavletich and colleagues (Yang *et al.*[2], page 653 of this issue) now shows that a fungal counterpart of BRCA2 can promote the loading of a key DNA-repair protein, Rad51, onto broken DNA. DNA breaks are a chronic problem for cells, arising from endogenous sources such as free radicals and the replication of damaged DNA, as well as from exogenous sources such as chemical mutagens and ionizing radiation. If not repaired correctly, these breaks cause chromosomal rearrangements that can result in cancer.

Initially, the sequence of the *BRCA2* gene[1] was both revealing and puzzling: the predicted protein was huge — 3,418 amino acids — but gave few hints to its function. However, it is now known that the protein comprises eight consecutive repeats of an approximately 30-amino-acid sequence ('BRC repeats'), as well as a DNA-binding domain and a nuclear-localization signal. The first evidence that BRCA2 might be involved in DNA repair came from the finding that it interacts physically with the repair protein Rad51, and that it is needed to form foci of Rad51 at sites of DNA breaks[3,4]. The eight BRC repeats are particularly intriguing in this regard: they interact with Rad51, both *in vivo* and *in vitro*[3–6], but in isolation they have the puzzling property of inhibiting Rad51 activity[4,5]. Thus, although it was thought that BRCA2 would be involved in Rad51-induced DNA repair, its precise role remained enigmatic.

Nonetheless, the discovery of the interaction with Rad51 did provide an important clue to exactly how BRCA2 might function. Rad51 is essential for the repair of DNA breaks by homologous recombination. In this process, Rad51 needs to assemble into a

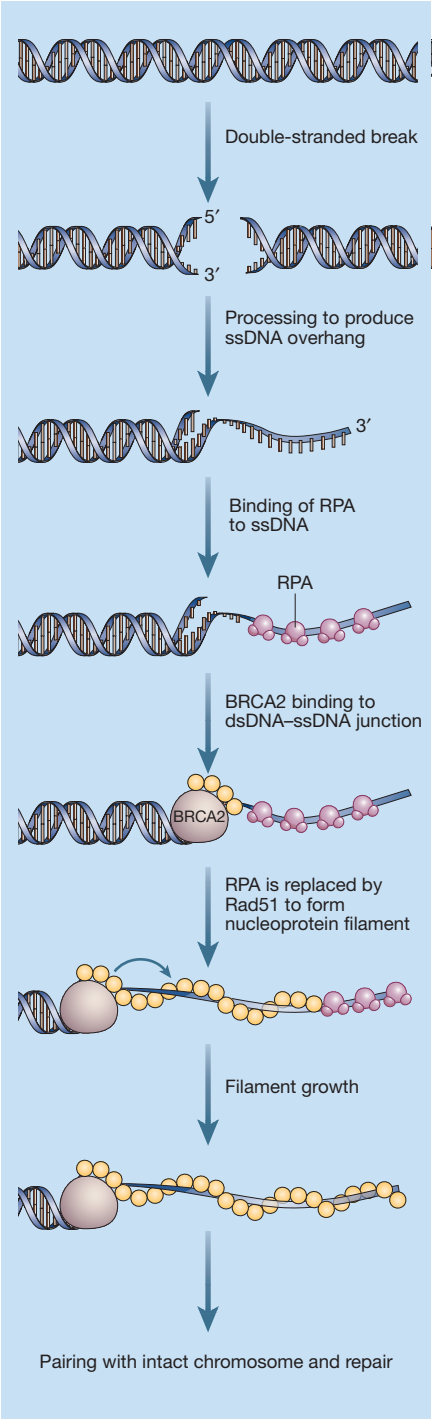

Figure 1 **Model for the function of BRCA2, based on the findings of Yang *et al.*[2]. When a chromosome breaks, its DNA must be repaired. The first step in one repair process, homologous recombination, involves the processing of the DNA break to produce a single-stranded (ss) region with a 3′ overhang. Replication protein A (RPA) binds to the single-stranded region. Yang *et al.* propose that BRCA2 then binds to the junction between the double-stranded (ds) and single-stranded regions, and recruits the Rad51 protein, which ousts the RPA, forming a Rad51 'nucleoprotein filament'. Pairing with the matching DNA region on the intact chromosome copy then enables the break to be repaired by homologous recombination.**

helical structure by binding to the single-stranded DNA (ssDNA) that is formed after one DNA strand is degraded (processed) at the site of a break. The resulting 'nucleoprotein filament' finds matching (homologous) DNA in the intact copy of the damaged chromosome, and then exchanges DNA strands to provide a template that is used to replicate the damaged or lost DNA. Sealing the DNA nicks completes the repair. Assembly of the Rad51 nucleoprotein filament is inhibited by replication protein A (RPA)[7,8], which binds to ssDNA, blocking Rad51 binding. To accelerate filament assembly, proteins known as mediators intervene[9,10], recruiting Rad51 to the RPA–DNA complex and replacing RPA by Rad51. Might BRCA2 also function as a mediator?

That notion was given a physical basis from two separate crystal structures. One structure[11] revealed that BRCA2 possesses a DNA-binding domain with separate regions for binding ssDNA and double-stranded DNA (dsDNA). The second[6] showed that a BRC repeat binds to the core of Rad51, and 'mimics' the structure of an adjacent Rad51 monomer. Together, these structures suggested that the intact BRCA2 protein might bind both DNA and Rad51, and help deliver Rad51 to processed DNA breaks[12].

Yang *et al.*[2] have now defined a function for BRCA2 by studying a simpler relative of the human protein. *BRCA2* genes are not unique to mammals, and homologues exist in plants and fungi[13]. The homologue from the fungus *Ustilago maydis* is *BRH2* (for 'BRCA2 homologue'), and it encodes a small (1,075-amino-acid) and relatively simple protein, comprising just one BRC repeat and a DNA-binding domain similar to that of BRCA2 (ref. 13). Cells defective in *BRH2* are sensitive to ultraviolet and ionizing radiation, are defective in the recombinational repair of DNA damage and, consequently, undergo chromosomal rearrangements — features common to cells with mutated *BRCA2*.

Yang *et al.* show convincingly that the Brh2 protein can nucleate the assembly of Rad51 onto ssDNA that is coated with RPA, efficiently producing a Rad51 nucleoprotein filament that can promote DNA strand exchange. Furthermore, even though Brh2 can promote the formation of Rad51 filaments on ssDNA, it acts preferentially at a dsDNA–ssDNA junction that has a particular type of single-stranded overhang (a 3′-terminated overhang). Brh2 binds to this junction to initiate the replacement of RPA by Rad51 in the 5′ to 3′ direction along the ssDNA (Fig. 1). Other recombination mediator proteins act by one of two broad mechanisms: there are those that act in stoichiometric complexes with RPA and Rad51, and those that act sub-stoichiometrically (as catalysts) relative to the amount of RPA and Rad51 (refs 7–10,14). Brh2 belongs to this second class. Thus, Brh2 is confirmed as a mediator of Rad51 assembly — a catalyst of a catalyst in DNA repair.

What is remarkable about these results is that Brh2 loads Rad51 onto ssDNA at the junction with dsDNA without any other factors, energy source or phosphorylation/de-phosphorylation cycle. This activity would be unique were it not for the behaviour of one other set of proteins that functions in recombination — the bacterial RecFOR protein complex[14]. RecFOR loads the bacterial homologue of Rad51 (RecA) onto complexes of ssDNA and ssDNA-binding protein, specifically at the junction with dsDNA, and with the same polarity. Moreover, only catalytic amounts of RecFOR are required. Thus, Brh2 is a precise functional homologue of the RecFOR complex.

Yang and colleagues' work[3] on Brh2 has clear implications for our understanding of BRCA2 function. First, it makes it virtually certain that BRCA2 is a catalyst of human Rad51 assembly at the sites of processed DNA breaks. This explains why Rad51 foci do not form in cells that lack functional BRCA2. Second, the inhibitory effects of BRC peptides can now be understood: such peptides can bind to Rad51 but, lacking the DNA-binding domain, cannot deliver Rad51 to the ssDNA, and therefore behave as inhibitors. And finally, the DNA-binding domain is required to bring BRCA2 — presumably with Rad51 bound to the BRC repeats — to processed DNA breaks. Once there, Rad51 can be transferred onto the ssDNA to form a filament; or, as Yang *et al.* propose, a BRC-bound Rad51 can initiate the formation of a filament without dissociating from BRCA2. Nucleation of Rad51 assembly on the ssDNA then initiates DNA repair. Further clarification of the mechanism of BRCA2 function should improve our understanding of the processes involved in tumour development and, hopefully, of how to correct the underlying defects. ■

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