The structural data suggest that at the start of the process (Fig. 1b), the carboxy-terminal domain (CTD) region of RecA binds to double-stranded DNA and guides it towards the primary DNA-binding site near the centre of the RecA-bound DNA filament. A small protein loop there helps to open up the double-stranded DNA. Once open, the displaced non-complementary strand binds to a region along the surface of the RecA–DNA filament called the secondary DNA-binding site. This site was already known to exist, but its location on the protein surface had not been fully defined. The non-displaced complementary DNA strand is positioned in a way that enables it to pair with the presynaptic single-stranded DNA.

The continued opening of the double-stranded DNA then proceeds preferentially in a 3′-to-5′ direction with respect to the orientation of the presynaptic DNA. However, if the newly interacting strands don't match, the probability of further opening of the double-stranded DNA at each successive RecA protein is substantially reduced. When the interacting strands match, the D-loop continues to be extended. In this case, the structure is further stabilized by binding of the displaced strand to the secondary DNA-binding site, as well as by base-pairing interactions between the complementary strand and the presynaptic single-stranded DNA.

Yang and colleagues also discovered that this process has a surprising feature – there is an asymmetry between the DNA interactions in the primary DNA-binding site and the secondary DNA-binding site. Three nucleotides of the stretched single-stranded DNA are bound to each RecA protein within the primary site, whereas each RecA protein binds to five nucleotides of DNA in its secondary site.

The functional purpose of this binding asymmetry is unknown. One possible benefit would be to enable some flexibility within the complementary single-stranded DNA such that if five of these bases unwind (as opposed to just three), then this unwound stretch of single-stranded DNA could, in principle, be positioned in three different registers in the primary binding site to test for different base-pairing interactions with the presynaptic DNA. This might help to ensure the quick formation of optimal base-pairing interactions in the primary DNA-binding site. In addition, once a correct sequence match is found, the binding asymmetry might help to propagate strand invasion by further opening up enough double-stranded DNA to help establish the base-pairing associated with the next RecA protein in the filament. These findings considerably advance our knowledge of the mechanism that underlies homologous recombination.

Even with the remarkable insights provided by Yang and colleagues' work, many key questions remain. How quickly can non-homologous sequences that don't match the presynaptic DNA strand be tested and rejected? Non-homologous sequences vastly outnumber homologous ones, and homologous recombination could not succeed if too much time were spent sampling the wrong sequences. How much complementarity between sequences of nucleotides is necessary for stable pairing interactions to occur, and what happens when sequences are not perfectly matched? Understanding the exact nature of these interactions could shed light on what determines whether a sequence is sufficiently homologous to support RecA-mediated recombination.

The version of RecA found in eukaryotes (organisms that have a nucleus in their cells) is known as Rad51. Understanding recombination in eukaryotes will require determination of the structures and mechanisms used by other accessory proteins that are also involved in recombination, because Rad51 is highly dependent on such protein co-factors[9,10]. The advent of cryo-electron microscopy as a tool for defining high-resolution structures of complex recombination intermediates offers the potential to address these and many other intriguing puzzles about homologous recombination.

Upasana Roy and **Eric C. Greene** are in the Department of Biochemistry and Molecular Biophysics, Columbia University Irving Medical Center, New York, New York 10032, USA.
e-mails: ur2129@cumc.columbia.edu; ecg2108@cumc.columbia.edu

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Catalysis

Titanium atoms pair up in industrial catalyst

Bert M. Weckhuysen

A study of the industrial catalyst titanium silicalite-1 suggests that the conventional view of the structure of its active sites is wrong. The findings might enable further optimization of related industrial catalysts. **See p.708**

Metal ions trapped in crystalline microporous solids known as zeolites are promising solid-state catalysts for a wide variety of oxidation reactions[1–3]. In the past few decades, there has been intense research into the structure and nuclearity (the number of metal ions) of the active sites in these zeolite-based catalysts[4,5]. Despite these efforts, there is still no agreement on the nuclearity: the proposed number of metal ions in the active sites ranges from one to three[1,2,4,5]. On page 708, Gordon *et al.*[6] propose that there are two titanium ions in the active sites of a well-characterized industrial zeolite catalyst called titanium silicalite-1 (TS-1), challenging the widely accepted idea that there is only one. Their work has implications not only for TS-1, but also for other metal-containing zeolites for which the structure of the active sites is not yet fully established.

TS-1 has a rich scientific and industrial history[3,7]. It started with the seminal work[3] of industrial researchers in the early 1980s, who prepared it by partially replacing silicon atoms with titanium atoms in a zeolite that has a particular type of porous structure (the MFI structure). They found that TS-1 catalyses several oxidation reactions, most notably the epoxidation of propylene ($H_3CCH{=}CH_2$) – a reaction in which an oxygen atom in hydrogen peroxide (H_2O_2) is added to propylene's carbon–carbon double bond (Fig. 1a). The product of this reaction is propylene oxide, a compound widely used to manufacture the building blocks of polyurethane plastics. This initial work spurred further industrial interest, and led to the use of TS-1 as a catalyst for the

commercial production of propylene oxide[8].

The structure, composition and performance of TS-1 have been investigated in great detail, with the aim of making catalysts that are more active and stable than existing ones for a range of oxidation reactions, and which promote the highly selective formation of targeted products. On the basis of a wide variety of characterization methods, there has been a general consensus about the type of active site needed to activate propylene for epoxidation, and about the reaction intermediates involved in the process[9,10]. More specifically, the active site has been thought to be mononuclear — that is, to contain a single titanium ion. This ion is presumed to activate hydrogen peroxide, leading to the formation of an intermediate peroxo (O–O) or hydroperoxo (HO–O) species (Fig. 1b), which then reacts with propylene to form propylene oxide.

Gordon *et al.* now bring fresh evidence to the table. They have studied the active site of TS-1 using a spectroscopic technique called solid-state oxygen-17 nuclear magnetic resonance (^{17}O NMR), in combination with a battery of other analytical techniques and computational modelling. The authors synthesized a set of catalytically active TS-1 materials that had different amounts of titanium incorporated, and reacted them with hydrogen peroxide in which both of the oxygen atoms were oxygen-17 (a rare, heavy isotope of oxygen). Interestingly, the spectroscopic signature of the reaction intermediates observed using ^{17}O NMR was similar to that seen for the Berkessel–Katsuki catalyst — a soluble titanium catalyst that also promotes epoxidation reactions, and which has a dinuclear active site (that is, the active site contains two titanium atoms)[11,12]. This observation suggested that the active sites in TS-1 are also dinuclear.

The authors used computational modelling to calculate the ^{17}O NMR spectra of titanium species that could form in TS-1 on reaction with hydrogen peroxide. This confirmed that the observed ^{17}O NMR spectrum is probably produced by a dinuclear species. Taken together with experimental evidence obtained using two other spectroscopy techniques (ultraviolet–visible diffuse reflectance spectroscopy and Raman spectroscopy), the researchers conclude that the active sites in TS-1 consist of a peroxo species sandwiched between two titanium ions (Fig. 1c).

Gordon and colleagues also used computational modelling to assess whether dinuclear titanium sites in TS-1 can activate propylene for epoxidation in the presence of hydrogen peroxide. This showed that the modelled dinuclear site does indeed promote a favourable reaction pathway for propylene epoxidation, thereby confirming that such sites are plausible candidates for the catalytic centres in TS-1.

The authors' data are convincing, but it should be noted that metal-containing zeolites are highly complex and intrinsically heterogeneous: TS-1 could contain several types of titanium species, depending on the exact conditions used to synthesize it. Such species could either be defects in the molecular framework of the zeolite, or be extra-framework species. And they might be present in minute amounts that would escape detection by some analytical methods. Furthermore, TS-1 could contain small clusters of titanium oxides, which could complicate spectroscopic analysis.

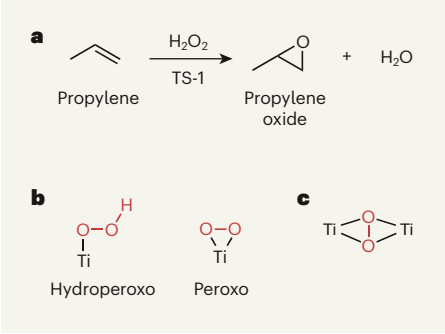

Figure 1 | Active sites in an industrial catalyst. **a**, The solid-state titanium-containing catalyst titanium silicalite-1 (TS-1) is used industrially to promote the reaction of propylene with hydrogen peroxide (H_2O_2), which yields propylene oxide as the product. **b**, The catalytic sites of TS-1 were thought to contain single titanium (Ti) atoms. It was presumed that a variety of titanium species could potentially form on reaction of TS-1 with hydrogen peroxide, such as hydroperoxo and peroxo species (hydroperoxo and peroxo groups are shown in red). **c**, Gordon *et al.*[6] now report strong evidence that TS-1 instead produces a species in which a peroxo group is sandwiched between two titanium atoms. Note that the titanium atoms in the solid-state catalyst will be bound to other atoms, which are not shown here.

"The researchers conclude that the active sites consist of a peroxo species sandwiched between two titanium ions."

More experimental and theoretical evidence is therefore needed to confirm that the active sites in TS-1 are dinuclear, rather than mononuclear, as had been widely accepted. This is especially important given that TS-1 active sites have previously been proposed to be dinuclear[13,14]. That proposal was based on a study of TS-1 carried out using a spectroscopic technique called extended X-ray absorption fine structure (EXAFS), but was eventually rejected on the grounds that a peak in the EXAFS data had been interpreted erroneously[15]. In view of Gordon and colleagues' findings, the EXAFS data for TS-1 require proper re-evaluation, and even further exploration using more-advanced analytical methods. Another technique that could be used for further studies is atom-probe tomography, which could identify specific pairs of titanium atoms in TS-1 (ref. 16).

It should also be noted that the conditions used in Gordon and co-workers' analytical experiments differ from those used in epoxidation reactions with TS-1. This raises the possibility that the active site reorganizes during reactions. Experiments that characterize the active sites *in situ* during epoxidation reactions might therefore help to settle the debate about their structures.

Nevertheless, the current work is exciting and has several broad implications. For example, it shows that chemists studying solid-state catalysts can learn much from studies of homogeneous (soluble) catalysts — an adjacent field that has its own debates about the molecular structure and nuclearity of active sites. Gordon *et al.* also introduce the use of ^{17}O NMR as a powerful method that could be used to characterize a wide variety of industrial zeolite catalysts. More specifically, it would be advantageous to use ^{17}O NMR to investigate the nuclearity of active sites in zeolite ZSM-5 (refs 1, 2). This is a catalyst that has the same MFI structure as TS-1, but which contains aluminium instead of titanium atoms, and is loaded with copper or iron to produce catalysts for the selective activation of methane or benzene.

Bert M. Weckhuysen is in the Inorganic Chemistry and Catalysis Group, Debye Institute for Nanomaterials Science, Utrecht University, 3584 CG Utrecht, the Netherlands.
e-mail: b.m.weckhuysen@uu.nl

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