rather that the forests dominated throughout (*7*). So, for low-latitude environments, isolation in refugia may not have occurred in the manner originally anticipated, if at all (*8*). Indeed, the emerging picture from the Neotropics—South America, the West Indies, and Central America, south of the Mexican plateau—as a whole is that the effectiveness of cold-stage aridity in rolling back rain forest has been greatly overestimated. For instance, a recent study of the genetic diversity among widely separated populations of the lowland canopy tree *Poulsenai armata* in Central America has demonstrated that they have greater within-population diversity than would be consistent with a postglacial expansion from distant South American refugia (*9*). Rather, the evidence points to a persistence of this and other lowland forest species in Central America during full-glacial periods.

The latest twist in the Amazonian refugial debate, however, is the suggestion (*10*) that a substantial part of the patterning of endemic species within lowland Amazonia is the product of rises in sea level of about 100 meters that took place during warm phases (that is, not during glacials) of the Quaternary and late Tertiary periods (10 to 2 million years ago). Such transgressions would have fragmented the region into two large islands and a series of smaller archipelagos, encouraging speciation through geographic isolation during warm stages rather than cold stages.

The picture now emerging depicts refugia as a crucial influence in shaping mid- and high-latitude biodiversity. However, this view is probably no longer valid for low-latitude tropical regions. For these zones, it would appear that the refugial hypothesis is far too simplistic (*8*). Clearly, a much more complex model is needed to explain the biotic patterns in these regions and, in particular, the pockets of endemicity for which full-glacial refugia were originally thought to be the cause (*2*).

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PERSPECTIVES: SURFACE SCIENCE

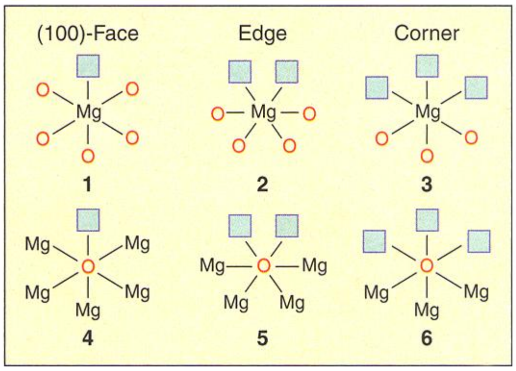
Catalysis on Oxide Surfaces

Helmut Knözinger

Free coordination sites play a major role in catalysis by metal complexes, because reactants may bind to these sites and become activated for catalytic conversion (*1*). Similar considerations apply to the surfaces of solid catalysts in general and of metal oxides in particular, because the surface atoms are characterized by a "ligand" sphere that differs from that in the bulk. The surface atoms generally have lower coordination numbers than those characteristic for the bulk: They are cus (*2*). Their coordination sphere may be completed by adsorbed molecules, and these may be activated for catalytic transformations, in close analogy to processes occurring on metal complexes. On page 1474 of this issue, Over *et al.* (*3*) succeed in verifying this concept coordinatively unsaturated sites (cus) on the atomic scale.

The coordination numbers of surface atoms in real catalysts may vary over wide ranges because different crystallographic faces, edges, steps, point defects, and dislocations may be exposed, resulting in an often substantial energetic heterogeneity (*4*). Oxide surfaces typically expose cus cations and cus oxygen anions, and chemisorption—adsorption involving chemical bond formation—frequently involves both simultaneously. For a specific catalytic transformation, certain geometric and energetic requirements must be fulfilled, so that frequently only a small percentage of all surface atoms may act as active sites.

The cus surface sites on real, high–surface area catalysts can only be characterized indirectly, with the use of probe molecules that can fill the free coordination sites. For example, the carbonyl infrared spectra of CO adsorbed on microcrystalline α-Cr_2O_3 (*5*, *6*) and on epitaxially grown chromium oxide films (*7*) show a complex pattern of bands of carbonyl surface complexes characterizing the heterogeneity of chromium sites.



Site-specific catalysis. Schematic representation of cus Mg^{2+} and cus O^{2-} ions on the surface of microcrystalline MgO. Sites on the (100) plane, on edges, and at corners are shown.

In another example, infrared spectroscopy has shown that methane is adsorbed on MgO in a C_{3v} symmetry at low temperature (*8*). Density-functional calculations (*8*) suggest that CH_4 does not bind to any of the cus Mg^{2+} sites **1** through **3** (see the figure), which have coordination numbers of 5, 4, and 3, respectively. Interaction with the cus O^{2-} sites **4** and **5** is also repulsive. A finite binding energy was found only for the three-coordinate cus O^{2-} corner site **6**, characterizing an H-bonding interaction of the type $O \cdots H–CH_3$.

This last example clearly demonstrates that very specific geometric and energetic requirements must be fulfilled by the adsorption sites for methane. An interesting example of reactive chemisorption is the adsorption of CO on MgO. CO binds to the Mg species **1**, **2**, and **3** as suggested on the basis of the infrared spectra of adsorbed CO (*9*). The simultaneous formation of chemisorbed CO_2^- species coordinated to Mg^{2+} sites is indicative of the concerted action of cus Mg^{2+} and cus O^{2-} sites (cation-anion pairs) (*8*).

For a more detailed understanding of the nature and action of chemisorption and catalytically active sites, their structure and reactivity must be characterized directly on an atomic scale. Modern surface analysis techniques, including low-energy electron diffraction (LEED) and scanning tunneling microscopy (STM), permit this type of observation, provided that the substrate is sufficiently electrically conductive and crystallographically well-defined (*6*, *10*). Monocrystals of many oxides are available today, but their poor conductivity often prevents their investigation

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by surface science techniques. To overcome this limitation, model oxide catalysts consisting of sufficiently thin, crystalline oxide films have been prepared by epitaxial growth on metallic substrates (*7*).

In a model study, Over *et al.* (*3*) verify the concept of coordinatively unsaturated sites on oxides on an atomic scale. The authors prepared an epitaxially grown RuO_2 (110) film on a Ru single crystal by oxidation of the metal at 700 K. The film thickness was typically about 1 to 2 nm. The films were fully structurally characterized by quantitative LEED and STM measurements. The atomic positions in the RuO_2 (110) surface were fully consistent within experimental error with those known for the (110) plane of bulk RuO_2. The electronic structure of the surface oxide phase was elucidated by density-functional theory (DFT) calculations.

The RuO_2 (110) surface is characterized by rows of bridging oxygen atoms that are running along the [110] direction. These rows were clearly imaged by STM, and the plane of the bridging O atoms was found to be located 0.115 nm above the topmost plane of Ru atoms. This surface also exposes Ru atoms not capped by oxygen atoms, in rows parallel to those of the bridging oxygens. The coordination number of these Ru atoms is 5, and they were therefore identified as cus Ru atoms. According to DFT calculations, these cus Ru atoms maintain their bulk hybridization, and they are therefore expected to expose dangling bonds at the surface and to create high reactivity. Adsorbed CO molecules, which could be imaged by STM because of their low mobility on the oxide surface, were shown to be bonded to the Ru atoms, consistent with their coordinative unsaturation. The pronounced localization of the chemisorption at dangling bond sites distinguishes oxide surfaces from metal surfaces because of the different nature of neighboring surface atoms.

Over *et al.* (*3*) also showed that the chemisorbed CO molecules reacted with bridging oxygen atoms upon brief heating to 600 K, leading to the formation of CO_2 that readily desorbs into the gas phase. The resulting oxygen vacancies were also imaged by STM; reoxidation with O_2 led to healing of the surface. Obviously, in this case, the CO molecule undergoes bonding interactions with both cus Ru atoms and surface O atoms (pair sites), in close similarity to the formation of CO_2^- on the MgO surface mentioned above, the crucial difference being that the latter species cannot easily be desorbed as CO_2.

The work of Over *et al.* (*3*) represents a key step toward an understanding of the nature of chemisorption and active sites on oxide surfaces. The results provide direct evidence for the existence of cus surface sites on oxides. The imaging of chemisorbed CO, and of the O defects created by the oxidation of the former, permits an interpretation of elementary steps of an important catalytic reaction at an atomic scale. Such studies are key for driving surface science research further toward investigations into dynamic processes occurring on oxide surfaces.

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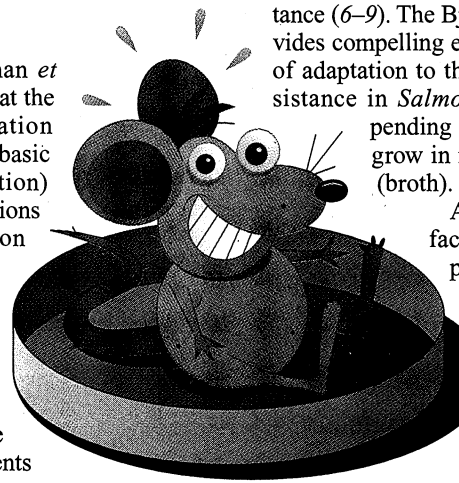
PERSPECTIVES: MICROBIOLOGY

Mice Are Not Furry Petri Dishes

James Bull and Bruce Levin



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It is not as though microbiologists really believe that what is true in vitro is also true in vivo. Like most other scientists, they realize that progress depends on developing model systems that, although not faithful replicas of the in vivo environment, enable the easy and repeatable study of biological phenomena. Microbial culture methods have facilitated the isolation and selective propagation of microbes and control of their genetics and metabolism. Unfortunately, it has become clear that interactions between microbes and the complex environment of their hosts cannot be gleaned from in vitro studies alone. The genes expressed by microbes inhabiting a mammalian host are different from those expressed by microbes living in a petri dish (*1*). On page 1479 of this issue, Björkman *et al.* (*2*) now show that the processes of mutation and selection (the basic elements of evolution) in bacterial populations differ depending on whether the bacteria grow in vivo or in vitro.

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Mutations that enable bacteria and other microbes to grow in the presence of antimicrobial agents commonly engender a cost that is manifest as a reduced growth rate (competitive disadvantage) in environments where the drug is absent. The cost incurred by drug resistance has been touted as a route to combatting the ever-increasing numbers of drug-resistant pathogenic microbes. The rationale is that because resistance incurs a cost, its incidence will wane if we administer antimicrobial drugs more prudently (*3–5*). Unfortunately, bacteria and viruses adapt to the cost of drug resistance through secondary mutations that compensate for the loss of fitness but usually do not reduce the level of resistance (*6–9*). The Björkman *et al.* study provides compelling evidence that the process of adaptation to the costs of antibiotic resistance in *Salmonella* are different depending on whether the bacteria grow in mice or culture medium (broth).

A mutation in elongation factor G (which decreases protein synthesis and slows growth) confers resistance to fusidic acid on *Salmonella*. The authors recovered 26 independent compensatory mutations and two revertants when *Salmonella* with this drug-resistance mutation were grown in culture broth. (Revertants are bacteria in which the drug-resistance mutation has been lost and the original wild-type DNA sequence has been restored.) In contrast, 11 compensatory mutations and 14 revertants were obtained when bacteria with the drug-resistance mutation were grown in mice. The amino acid substitutions in the 11 mouse-derived compensatory mutations differed from those in the

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