STRUCTURAL BIOLOGY

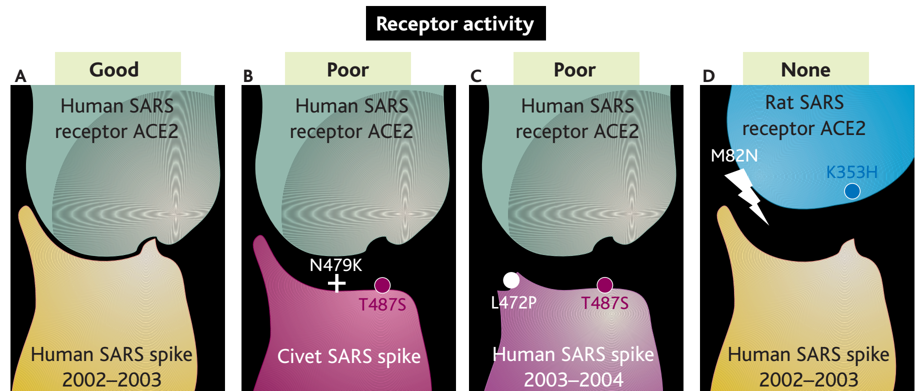
Adaptation of SARS Coronavirus to Humans

Kathryn V. Holmes

The 2002–2003 epidemic of SARS (severe acute respiratory syndrome) that killed nearly 10% of the more than 8000 infected people is probably the most thoroughly studied example of an animal virus "jumping" into humans. SARS coronavirus was caught in the act of adapting to humans, acquiring mutations in several genes that allowed it to be transmitted from person to person and cause lethal disease. Coronaviruses closely related to the human epidemic strains of SARS coronavirus were discovered in several wild animal species, including the Himalayan masked palm civet, in exotic meat markets in Southern China (*1*). By sequencing hundreds of SARS viral RNA genomes from humans and animals during and after the epidemic, mutations were identified that distinguish the species-specific strains (*2*). Which of these mutations account for the explosive and virulent SARS epidemic? Strong evidence implicates the viral spike glycoprotein as one major determinant of the species specificity of coronavirus infection (*3*). Infection is initiated by trimers of the ~200-kD spike glycoprotein on the coronavirus envelope. The trimers bind SARS virus particles to their specific receptor glycoprotein, angiotensin-converting enzyme 2 (ACE2), on the surface of host cells (*4*).

In the spike protein of SARS coronavirus, the ~220–amino acid receptor-binding domain was identified by mutational analysis and binding of neutralizing monoclonal antibodies (*5*, *6*). Only four amino acids in the receptor-binding domain differ between the human epidemic and civet strains, but they cause more than a 1000-fold difference in binding affinity to human ACE2 (*7*). The landmark paper by Li *et al.* on page 1864 of this issue characterizes the structure of the receptor-binding domain of human SARS coronavirus spike protein bound to its receptor, human ACE2 (*8*). Together with previous elegant mutational analyses (*7*, *9*), this structural study identifies critical molecular determinants that allow SARS coronavirus to adapt to humans. The host cell receptor is bound by an extended loop in the spike protein that projects from a compact core within the receptor-binding domain. Of the 14 residues on the loop that contact 18 residues on human ACE2, only two differ between human and animal virus strains. The intimate interface between the loop of a spike protein from the 2002–2003 SARS coronavirus and its human receptor mediates efficient virus binding and infection (see the figure, panel A). In particular, a methyl group from a threonine residue at position 487 of the spike protein at the interface extends into a hydrophobic pocket in ACE2 that contains a lysine residue at position 353. The two amino acid residues that differ in the spike protein of a civet virus strain would strongly reduce binding to human ACE2 due to absence of the methyl group (a serine residue is present at position 487) and the introduction of a charged lysine residue at position 479 (see the figure, panel B). The spike protein from a coronavirus that caused a sporadic and mild SARS case in 2003–2004 (see the figure, panel C) resembles civet virus spike protein in that it has a serine residue at position 487 as well. This spike protein also has a proline substitution for a leucine residue at position 472 that reduces the total binding surface to human ACE2. These amino acid substitutions may account for the reduced virulence and transmission of the virus in humans.

The ACE2 protein is highly conserved among mammals and surprisingly few amino acid substitutions at the virus-binding site can strongly affect its receptor activity for SARS coronavirus (*7*–*9*). Rat ACE2, which does not serve as a receptor for SARS coronavirus, contains a large N-linked glycan at an asparagine residue at position 82 in the binding interface that likely inhibits binding to the human SARS coronavirus spike protein. It also lacks the lysine-containing hydrophobic pocket critical for binding the key methyl group of threonine 487 [see the figure, panel D; (*8*)].



Key amino acids in the SARS coronavirus spike protein and the receptor protein that determine the host range of the virus. (A) There is a large binding interface between a loop structure in the spike protein of the human SARS coronavirus of 2002–2003 and its human receptor ACE2. **(B)** Two amino acid substitutions in the spike protein of a civet SARS virus reduce receptor activity of human ACE2 by adding a charge to the binding surface (N479K) and deleting a key methyl group (T487S) that fits into a hydrophobic pocket in the receptor. **(C)** In the spike protein from coronavirus of a mild SARS case from 2003–2004, the key methyl group is also missing and a proline residue (L472P) reduces the binding surface. **(D)** Rat ACE2 contains a large glycan at M82N and lacks the hydrophobic pocket (K353H).

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Many coronaviruses cause disease in mammals and birds, and specific receptor glycoproteins have been identified for coronaviruses of humans, cats, pigs, and mice (*3*). In addition to SARS coronavirus, only the newly discovered human coronavirus NL63 uses human ACE2 as its receptor (*10*). As shown by Li *et al.* (*8*), the extended loop on the SARS virus spike protein that binds human ACE2 has no homolog among spike proteins of other coronaviruses. Perhaps the large (~90 kD) amino-terminal domain of coronavirus spike proteins share a conserved structure from which virus-specific domains project that can bind to different host cell receptors. Will the NL63 spike protein, which lacks a tyrosine-rich receptor-binding loop like that on the SARS virus spike protein (*11*), bind to the same site on human ACE2 as does the SARS virus spike? How

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could a unique receptor-binding domain be introduced into a spike protein? Coronavirus replication includes frequent RNA recombination events that can insert or delete long RNA sequences in the genome. Large deletions that occur spontaneously in the porcine transmissible gastroenteritis coronavirus eliminate binding to a carbohydrate moiety and change the tissue tropism and virulence of the virus (*3*). Coronaviruses can hijack foreign genes, such as the hemagglutinin esterase glycoprotein gene from influenza C virus (*12*). Genes of unknown origin that encode the virus-specific, nonstructural proteins are also acquired and inserted between the essential genes on the coronavirus genome (*3*). Thus, coronaviruses might change receptor specificity by mutation or by RNA recombination in the genes that encode their spike glycoproteins.

The rather alarming conclusion from the structural studies of the SARS virus spike-ACE2 interface (*8*) is that adaptation of a virus to a homologous receptor of a new host species may require very few amino acid substitutions at the large receptor-binding interface. This is true not only for SARS coronavirus, but also for other viruses including influenza A virus and parvoviruses (*13*, *14*). Why, then, don't viruses constantly jump from one host species to another? Probably because successful adaptation to a new host not only requires mutations to optimize receptor binding and entry, but also mutations in other viral genes that optimize virus replication and transmission in the new host. Only when a constellation of mutations allows a virus to replicate and transmit moderately well in the new host can infection in a new species become established.

Can we predict whether another human SARS epidemic will occur? So far, extensive epidemiological surveillance has not found the 2002–2003 epidemic strains of SARS coronavirus in humans or animals since the epidemic ended in July 2003 (*2*). However, SARS coronaviruses continue to circulate in civets and perhaps other animals and to cause sporadic, mild human cases (*2*, *15*). Fortunately, if new mutants of SARS coronavirus from animals do initiate another SARS epidemic in humans, the disease could promptly be recognized with new diagnostic tests. The outbreak could be stopped by the stringent isolation procedures that controlled the first SARS epidemic of 2002–2003. This could perhaps be supplemented with promising new candidate vaccines and antiviral drugs that are currently being developed. The structure of the interface between the spike protein and receptor shown by Li *et al*. (*8*) suggests novel strategies for developing an improved SARS vaccine and receptor-targeted drugs to block virus entry into host cells.

Can the next emerging virus epidemic, other than SARS, be predicted? Probably not. All viruses mutate, and an unfortunate combination of mutations could occur and be selected at any time. The inherent unpredictability of emerging viral diseases is the best reason for further characterization of viruses in wildlife that could jump to humans and for global surveillance for new epidemic diseases in humans and animals.

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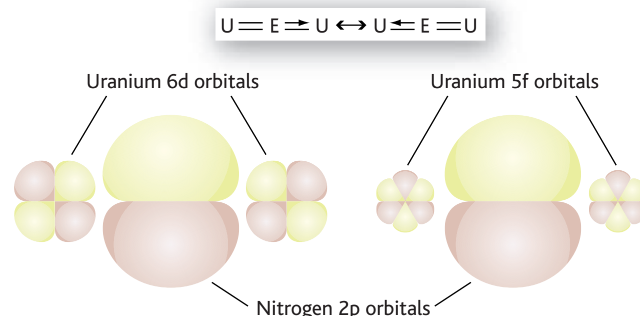
CHEMISTRY

Bridging a Gap in Actinide Chemistry

Carol J. Burns

Understanding the behavior of the actinide elements such as uranium and plutonium is central to predicting nuclear weapons performance, advanced nuclear fuel cycles, radioactive waste management, and environmental remediation. During much of the past century, knowledge of the chemical behavior of these elements was derived principally from investigations designed to develop processes for efficient large-scale separation and recovery. Although this has provided models to describe the coordination and redox behavior of the early actinides in acidic aqueous media, we still lack a comprehensive picture of the behavior of elements in this part of the periodic table. It has been particularly difficult to reconcile descriptions of the fascinating structural and electronic behavior of f-series metals and compounds in condensed-matter systems [including those displaying f-electron itinerancy (*1*)] with the solution molecular behavior of these elements.

Recently, there have been suggestions in the literature that the behavior of solid-state actinide oxides has previously unappreciated similarities to that of molecular systems (*2*). The chemistry of individual metal sites tends to be dominated by strong (presumably relatively covalent) metal-oxygen multiple bonding; discrete terminal metal-oxo units with short metal-oxo bonds are common structural elements. One vital aspect in understanding the electronic structure and thermodynamic stability of these systems is assessment of the type and strength of bonding found in the molecular metal-ligand bonds (particularly the stability of bridging versus terminal bonds; see the figure). Unfortunately, the molecular chemistry of AnE moieties (A, actinide; E, first-row element) has been largely restricted to date to metal-oxo complexes. Evans *et al.* report on page 1835 of this issue the first example of a molecular actinide complex containing a



A bridge just right. First-row elements (E) such as nitrogen have the capacity to bridge between two actinide metal centers. The nitrogen 2p orbitals are of the appropriate symmetry to overlap with both uranium (U) 6d and 5f orbitals. The bridging mode in the nitride complex reported by Evans *et al.* suggests delocalized metal-ligand multiple bonding, as illustrated schematically by the resonance structures (box at top).

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