nism for fullerene ring rearrangement at high temperatures, and the lack of a plausible mechanism represents a major roadblock in our understanding of the formation of these materials. On the other hand, just a decade ago no one expected the relative ease with which this family of carbon-based nanostructures would form. The next decade should see the evolution and maturation of this exciting new branch of organic chemistry and materials science along with the answers to some of these fundamental questions.

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New Lessons for Rotavirus Vaccines

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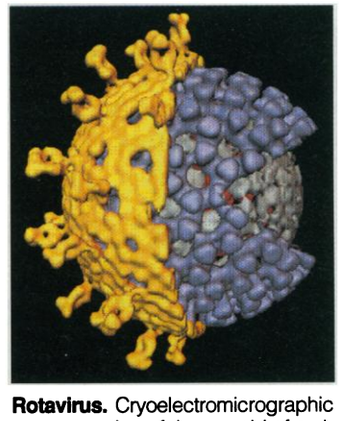
Two decades ago, Bishop discovered human rotaviruses, the most common cause of severe dehydrating diarrhea in children worldwide (*1*). Although most rotavirus infections are mild, field studies from more than 50 countries document tremendous morbidity and mortality. Rotaviruses are detected in 20 to 70% of fecal specimens from children hospitalized with acute diarrhea, and in developing countries they cause about 870,000 deaths each year (*2*). Even in the United States, rotavirus is associated with 3% of all hospitalizations of children younger than 5 years old, which translates to a staggering 55,000 to 70,000 hospitalizations per year, with medical and indirect costs in excess of $1 billion (*3*). Efforts to prevent disease by improving water or sanitation seem unlikely to succeed, because all children in developed and developing countries become infected with rotavirus in the first 3 to 5 years of life. A high priority has therefore been placed on the generation of a safe and effective vaccine.

Development of a vaccine for rotavirus has progressed rapidly, aided by several key breakthroughs. Most important was the recognition that the primary infection induces natural immunity to rotavirus and protects a child from subsequent episodes of severe disease (*4*). Once the virus could be propagated by cell culture, vaccine seeds could be prepared (*5*). Finally, cross protection is induced between human and animal strains, so that animal strains hypothesized to be naturally attenuated for humans could be tested as first-generation candidates for heterotypic "Jennerian vaccines" (*6*). Live oral vaccines derived from animal strains of rotavirus proved to be relatively safe and very effective (>80%) in protecting children against severe rotavirus diarrhea in some populations—but they were ineffective in others. This variability was attributed in part to differences in serotypes of circulating strains and the failure of animal strains to elicit heterotypic protection in some studies.



Rotavirus. Cryoelectromicrographic reconstruction of the capsid of a virus particle. Outer capsid (yellow); inner capsid (blue). [Photo courtesy of B. V. V. Prasad]

To increase the efficacy of the Jennerian vaccine, researchers developed second-generation polyvalent reassortant vaccines. These contained neutralization antigens that could provide homotypic (serotype-specific) immunity against all four predominant human rotavirus serotypes. Of the 11 segments of double-stranded RNA in the rotavirus core, two encode the proteins of the outer capsid—the VP4 hemagglutinin spike and the VP7 glycoprotein—that are key targets for virus neutralization. Coinfection of cells under selective pressure leads to the reassortment of these gene segments, and vaccine candidates can be selected from these reassorted rotaviruses. The candidate strains contained 10 segments from the original animal rotavirus strain and a single gene encoding one of the outer capsid proteins from each of the four major human strains. Mixtures of these reassortants have been combined to form tetravalent vaccines that can be given orally to children, and they protect in the field against severe rotavirus diarrhea. These vaccines have an efficacy of >80% against severe rotavirus diarrhea (similar to the protection conferred by natural infection) and could be licensed for use within a year or two (*7*). Although these modified Jennerian vaccines were engineered to produce serotype-specific immunity, community studies and vaccine trials have not yet convincingly demonstrated that homotypic (serotype-specific) immunity is more protective than heterotypic immunity. If it is not, some basic assumptions underlying the development of this vaccine are called into question.

The progress toward an effective rotavirus vaccine is way ahead of our understanding of how rotaviruses cause disease and how immunity is generated. Studies in animals indicate that damage occurs in epithelial tissue in the proximal small intestine (*8*). The villi become shortened and slough, mitochondria swell, the cisternae of the endoplasmic reticulum become distended, and mononuclear cells infiltrate the lamina propria. This cellular damage, associated with a loss of the ability to control fluids and electrolytes, has been postulated to cause severe diarrhea. However, limited studies in humans are less clear-cut. Among 40 ill German infants biopsied by intestinal suction, only 5% had damage by histologic analysis, whereas in another small study intestinal damage was observed (*9*). Therefore, diarrhea may occur in the absence of substantial cellular damage, suggesting that the conclusions of the animal studies do not apply to humans (or that subtle epithelial damage in humans was overlooked).

Of the 11 rotavirus proteins, no single gene product has yet been clearly associated with virulence. Such an association could be

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Generation of GTP-Ran for Nuclear Protein Import

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The nuclear localization sequence (NLS) (one or two stretches of basic amino acids) marks cytoplasmic proteins for active transport through the nuclear pore (*1*). Nuclear import requires the assistance of four soluble proteins: karyopherin α/importin α (the NLS receptor), karyopherin β/importin β (which mediates binding to the nuclear pore complex), the small guanosine triphosphatase (GTPase) Ran, and p10/NTF2 (a protein with a heretofore mysterious function). In this issue, Nehrbass and Blobel (*2*) examine the interaction of p10 with the other soluble proteins and with the proteins of the nuclear pore complex and begin to dissect how these interactions result in movement of a substrate through the nuclear pore complex.

The new findings indicate that p10 can bind guanosine diphosphate (GDP)–Ran, karyopherin β, and a family of repeat-containing nucleoporins (to which karyopherin β can also bind independently). Perhaps the most important aspect of these results is the welcome light shed on a puzzling riddle about the GTPase cycle of Ran during nuclear import. This riddle is attributable to the fact that Ran's exchange factor (RCC1) is located inside the nucleus, whereas its GTPase-activating protein (Ran-GAP1 or RNA1) is cytoplasmic. Thus, RCC1 generates GTP-Ran in the nucleus, and the Ran GTPase-activating protein (Ran-GAP1) would quickly convert any cytoplasmic Ran to the GDP-bound form. Yet paradoxically, all experimental evidence has indicated that Ran must at some point be in the GTP-bound form on the cytoplasmic side of the nuclear envelope to function in nuclear import. So what is the source of this GTP-Ran?

This question has been complicated by the fact that Ran is required for mRNA export from the nucleus (movement in the opposite direction), a process that appears also to require Ran in the GTP-bound form and hydrolysis of its bound GTP in the cytoplasm (hence the yeast name for the GAP of RNA1). These observations have resulted in a critical question: Does the GTP-Ran generated by RCC1 inside the nucleus diffuse (or get transported) into the cytoplasm and survive the presence of the GAP long enough to facilitate import? Or is there another mechanism for generating Ran-GTP on the cytoplasmic side of the nuclear envelope?

The new results of Nehrbass and Blobel indicate that there is another exchange reaction on the cytoplasmic side of the nuclear envelope—at the nuclear pore complex itself—and that p10 is a major player in this reaction. Their data show that, by the cumulative effects of multiple, relatively low-affinity interactions, a transient complex is formed that consists of a repeat-containing nucleoporin, karyopherin α and β, GDP-Ran, and p10. Complex formation stimulates GDP release and GTP uptake by Ran, thereby forming GTP-Ran at the exact spot at which this "active" Ran is required for dissociation of the complex and forward movement of an import substrate into the nucleus.

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key to future treatment or vaccines, to distinguishing virulent from less virulent strains, or to understanding the mechanism of attenuation. Progress in this direction is presented in this issue of *Science* (*10*).

Ball and colleagues (9) provide evidence that NSP4, a nonstructural protein of rotavirus, may be the first viral enterotoxin. For the virus, NSP4 may play a role in viral assembly, although its function is not completely understood. To the host, NSP4—more specifically, a 22–amino acid peptide in the hydrophilic cytoplasmic domain of the molecule—acts as an enterotoxin and triggers a signal transduction pathway that causes an increase in intracellular Ca^{2+}, which potentiates Cl^- secretion by a Ca^{2+}-dependent signaling pathway and induces diarrhea in mice. NSP4 causes secretory diarrhea in a way similar to that of the heat-labile enterotoxin β of *Escherichia coli*, suggesting that viral and some bacterial enterotoxins may be similar, although the mechanisms by which other enteric viruses such as the caliciviruses, astroviruses, or adenoviruses cause diarrhea are not known. This virulence factor is age dependent, as are natural rotavirus infections, and its existence partially explains the cross protection conferred by some vaccines against heterologous strains of rotaviruses that differ in their outer capsid proteins associated with neutralization but may share NSP4 antigens. NSP4 may define attenuation as well. Antibodies to NSP4 also protect against disease; pups born to dams immunized with the key peptide had decreased incidence and severity of diarrhea. Perhaps NSP4 should be a target for future rotavirus vaccines.

Another murky area in rotavirology is the mechanism of immunity to rotavirus. Because rotavirus only infects the small intestine, protection against disease is believed to be associated with antibody at the mucosal surface, a mechanism important in other respiratory and enteric infections. Unfortunately, local immunity is difficult to measure in children, and antibody levels that rise after infection return to baseline in a few weeks so that static levels cannot be used to predict protection. Therefore, serum antibodies are the standard measure of immunity for most field studies and vaccine trials. Neutralization titers against the two outer capsid proteins of the virus correlate to some degree with protection, and most vaccinated children seroconvert by neutralization assay to at least one of the strains included in the vaccine (*11*). In contrast, antibodies to the inner capsid protein VP6 do not neutralize the virus and are not associated with protection in passive feeding studies.

Recently, improved mouse models for rotavirus have facilitated studies of the mechanism of immune protection against disease (*12*). In this issue, Burns and coworkers (*13*) have raised BALB/c mice with "backpack tumors" that each expressed a different monoclonal antibody against the inner (VP6) or one of the outer (VP4) capsid protein of rotavirus in an effort to identify the viral antigen critical for protection against disease. The tumors that protected the mice best against rotavirus infection were not those that produced neutralizing antibodies to the outer capsid protein VP4. Rather, the most protective backpacks unexpectedly secreted a nonneutralizing immunoglobulin A (IgA) antibody directed at VP6, the abundant inner capsid protein that is not associated with neutralization in vitro. Furthermore, this antibody was not active directly in the gut lumen where secretory IgA is believed to be protective but was active when it reached the gut epithelium from the basolateral side, where it presumably inactivates virus during transcytosis into the lumen.

The epithelial cells of the small intestine migrate from the deep valleys of the crypts to the villous tips and, although rotavirus damage is most evident in the villous tips, IgA is secreted most efficiently from the crypt cells. Where do antibody and virus meet? We do not yet know. These new results challenge our traditional thinking about the mechanism of mucosal protection, the site of virus neutralization, and the key antigens to which protective antibodies are directed.

Rotavirus vaccines nearing licensure could provide an important new weapon to decrease diarrhea morbidity and mortality in both developed and developing countries. If these were administered together with the other routine childhood vaccines, they could rapidly reach nearly 80% of the world's 130 million newborns who are already covered by the expanded program for childhood immunizations (*14*). The impact—a decrease in diarrheal hospitalizations and deaths—could be measurable almost immediately.

In spite of this optimism, the vaccines nearest licensure do not prevent all episodes of severe disease in American children, so further improvements will likely be needed. Many new approaches to immunization—the use of viruslike particles, DNA vaccines, microencapsulated viruses, and other live strains for oral delivery—are all being pursued. Each approach will benefit from a more detailed understanding of the mechanisms of pathogenesis and immunity to disease. The issues raised in the two new papers will help redefine targets for the next generation of rotavirus vaccines and give pause to reconsider basic principles in the prevention of other enteric and respiratory infections where local immunity may be at play and where the mechanisms of pathogenesis remain unclear.

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No "End of History" for Photolyases

Aziz Sancar

After the fall of communism in 1989, Francis Fukuyama wrote a best seller titled *The End of History and the Last Man* (*1*). He argued that the absence of competing ideologies in the world would result in no more large-scale conflicts, the stuff of which history is made. Unfortunately, events since publication of the book have proven that humans are capable of conflict even in the absence of the Cold War. Hence, there is still history to be written.

A similar situation has occurred in the scientific subspecialty of photoreactivation research. Photoreactivation reverses the effect of ultraviolet (UV) light when the organism is either concomitantly or subsequently exposed to blue light. In DNA UV light induces two major types of damage—cyclobutane pyrimidine dimers (CPDs) and (6-4)photoproducts. In 1958 an enzyme was discovered that repaired UV-damaged DNA. Later work revealed that the enzyme, called photoreactivating enzyme or photolyase, binds to UV-damaged DNA and on absorbing a blue-light photon (of wavelength 350 to 450 nm) splits the cyclobutane ring of a CPD, restoring the bases to their native form (*2*). The gene for photolyase was later cloned from several organisms (*3*), and the enzyme was purified and extensively characterized (*4*).

The reaction mechanism of CPD photolyase has been elucidated in considerable detail (see figure). The enzyme is a 50- to 65-kD polypeptide with two chromophores. One of the chromophores [which can be either methenyltetrahydrofolate (MTHF) or deazariboflavin] is the photoantenna that absorbs the blue-light photon (step 1) and transfers excitation energy (step 2) to the active site cofactor, which is invariably two-electron–reduced flavin-adenine dinucleotide ($FADH^-$). Flavin in the excited state then donates an electron to the CPD (step 3), splitting the cyclobutane ring, and the electron is transferred back to flavin concomitant with the generation of the two canonical bases (step 4). The general outline of this mechanism was worked out in 1987 and was considered by some to be "the end of history" for photolyase. In the Keystone meetings of 1988 and 1995 on DNA repair there were no presentations on DNA photolyase. Similarly, for many repair scientists, the recent solution of the crystal structure of DNA photolyase from *Escherichia coli* (*5*) was considered an epilogue to a story completed long ago. However, as with sociopolitical history, events that have unfolded during the last 3 years have revealed that there are many more chapters to write on photolyase.

The first unexpected and exciting discovery in the "New Age" history of photolyase was the detection of a photolyase specific for (6-4)photoproduct [(6-4)photolyase] in *Drosophila* cell-free extracts (*6*). The classic photolyase—now referred to as CPD photolyase—cannot repair (6-4)photoproducts. Furthermore, it was thought that photochemical (photoenzymatic) reversal of (6-4)photoproducts was very unlikely for the following reason: The formation of (6-4)photoproducts involves the transfer of the group at the C-4 position (–NH or –OH) of the 3′ base of the dinucleotide to the C-5 position of the 5′ base concomitant with the formation of the σ bond between the C-6 of the 5′ base and the C-4 of the 3′ base (see figure). Even if an enzyme breaks the σ bond joining the two adjacent pyrimidines (as occurs with CPD photolyase), the bases would not be restored to their original forms. However, Todo *et al.* (*6*) previously demonstrated that the (6-4)photolyase restores biological activity to UV-irradiated DNA, and Kim *et al.* (*7*) obtained direct evidence that the (6-4)photolyase converted this photoproduct to unmodified bases. Kim *et al.* (*7*) also proposed a chemical mechanism that involves an oxetane intermediate and an electron transfer reaction for repair by (6-4)photolyase. At the time there were no suspicions that the two enzymes are related, simply because CPD and the (6-4)photoproduct are structurally very dissimilar (*8*).

In contrast, two papers published shortly after the (6-4)photolyase paper revealed that

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