establish synchroneity. Glacial records from two areas in the central Andes, where deposits are well dated, demonstrate that glacial advances once thought to be of possible YD age are, in fact, older than the YD by several centuries; glaciers in these regions were rapidly retreating during the YD (*8*).

Bennett *et al.* make a compelling case for the absence of a YD cooling based on sediment cores from four lakes on the Taitao peninsula and Chonchos archipelago, southern Chile. Each core, dated by radiocarbon, spans the last deglaciation and includes the critical YD interval. They note the progressive southward migration of decreasingly cold-tolerant trees through the last deglaciation with no evidence of a reversal at any time. The steady temperature rise through this climatic transition implied by these data is consistent with results from several other paleobotanical studies from southern South America (*9*) yet is in striking contrast with records of Southern Hemisphere glacier advances (*6*, *8*), isotope paleotemperature data from ice cores from maritime Antarctica and the central Andes (*10*), and other paleobotanical data from elsewhere in South America (*11*).

These apparent discrepancies cannot easily be explained. It is possible that the aforementioned glacier advances reflect episodic increases in precipitation and only small decreases in temperature—too small to impact the southward spread of cold-intolerant tree species on the landscape. On the other hand, if the location of the Bennett *et al.* study area was not close to a boundary between trees of different cold tolerance at the time of the onset of a cool event, such as the YD, one would not expect to see a strong pollen signature of the cooling. One thing is certain: Much remains to be learned about the timing, geographic extent, and causes of millennial-scale climatic variability.

References

1. J. Mangerud *et al.*, *Boreas* **3**, 109 (1974); K. Jensen, *Acta Archaeol.* **5**, 185 (1935).
2. S. J. Lehman and L. D. Keigwin, *Nature* **356**, 757 (1992); R. B. Alley *et al.*, *Nature* **362**, 527 (1993).
3. K. D. Bennett, S. A. Haberle, S. H. Lumley, *Science* **290**, 325 (2000).
4. W. S. Broecker *et al.*, *Nature* **341**, 318 (1989).
5. W. Dansgaard *et al.*, *Nature* **364**, 218 (1993); G. Bond *et al.*, *Nature* **365**, 143 (1993); G. Bond and R. Lotti, *Science* **267**, 1005 (1995).
6. T. V. Lowell *et al.*, *Science* **269**, 1541 (1995); G. H. Denton and C. H. Hendy, *Science* **264**, 1434 (1994).
7. N. Roberts *et al.*, *Nature* **366**, 146 (1993); Z. S. An *et al.*, *Quat. Res.* **39**, 45 (1993).
8. D. T. Rodbell and G. O. Seltzer, *Quat. Res.*, in press.
9. A. C. Ashworth and V. Markgraf, *Rev. Chil. Hist. Nat.* **62**, 61 (1989); V. Markgraf, *Boreas* **20**, 63 (1991); *Quat. Sci. Rev.* **12**, 351 (1993).
10. E. J. Steig *et al.*, *Science* **282**, 92 (1998); L. G. Thompson *et al.*, *Science* **282**, 1858 (1998); L. G. Thompson *et al.*, *Science* **269**, 46 (1995).
11. B. C. S. Hansen and D. T. Rodbell, *Quat. Res.* **44**, 216 (1995); T. Van der Hammen and H. Hooghiemstra, *Quat. Sci. Rev.* **14**, 841 (1995); P. I. Moreno *et al.*, *Geograf. Ann.* **81A**, 285 (1999).

PERSPECTIVES: PHOTOCHEMISTRY

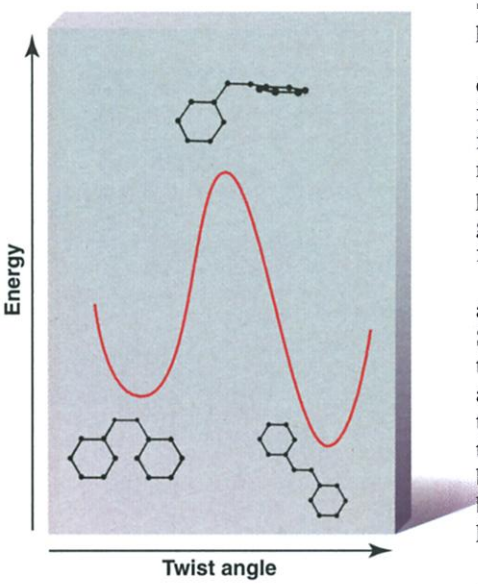
Twist and Fluoresce

John I. Brauman

The immune system is an immensely powerful apparatus capable of making proteins that bind other molecules with great strength and selectivity. Its normal function is to make proteins (antibodies) that sequester "foreign" substances. This molecular recognition has recently been used to create proteins that act as catalysts (catalytic antibodies) by making them bind to molecules with structures similar to that of the transition state of the reaction. The underlying view is that of enzyme catalysis as transition state binding. Combined with the ability to make monoclonal antibodies, it is now possible to use the immune system to engineer new catalysts (*1*).

On page 307 of this issue, Simeonov *et al.* (*2*) report the application of this approach to a new area, namely photochemical processes and reactions. Antibodies have been made that exhibit extraordinary photochemistry. They bind *trans*-stilbene, and, when irradiated, the stilbene does not isomerize but instead exhibits intense fluorescence.

To understand why this is remarkable, we have to look at the usual photochemistry of stilbene, which has been studied extensively (*3*). Stilbene readily undergoes photochemical trans → cis isomerization and shows only weak fluorescence. This behavior can be understood in the context of the ground- and excited-state potential energy surfaces. In the ground state, a 90° rotation around the C-C double bond costs roughly 45 kcal/mol (the energy of the π bond that has been broken). In contrast, the orbital to which the electron is photoexcited has antibonding character in the double bond, and the excited state has a minimum at 90°. When *trans*-stilbene absorbs a photon, the excited state rapidly twists to 90° and then undergoes a radiationless transition to the ground-state surface, ending up somewhere near the ground-state potential energy maximum. Subsequent rotation produces the isomeric product, *cis*-stilbene.



Ground-state potential surface for *cis*- (left) and *trans*-stilbene (right).

The time required for twisting in the excited state is short compared with the fluorescence lifetime, so relatively little fluorescence emission is observed. If the rate of rotation is slowed, for example, by placing stilbene in a viscous medium or by geometrically preventing the rotation, then fluorescence is observed (*4*).

Some of the features of the stilbene-antibody photochemistry observed by Simeonov *et al.* (*2*) can be explained on the basis of these previous studies. For example, it is not particularly surprising that the antibodies inhibit stilbene isomerization. As Simeonov *et al.* show for one stilbene-antibody complex (*2*), the antibody binds the *cis*-stilbene only weakly and inhibits isomerization.

Nevertheless, much of the observed photochemistry is still unexpected. Stilbenes that are constrained from rotational isomerization are known to fluoresce, but the spectra of the stilbene-antibody complexes are clearly different from that of stilbene itself. Moreover, a study of the time and temperature dependence of the fluorescence emission reveals that the process leading to the fluorescence is rather complex. Much of the fluorescence is quenched at low temperature, but at temperatures above 250 K, the fluorescence occurs with two separate time scales: a very fast one characteristic of isolated stilbene, followed

The author is in the Department of Chemistry, Stanford University, Stanford, CA 94305–5080, USA. E-mail: brauman@stanford.edu

by a slower, redshifted emission. This long-wavelength fluorescence is characteristic of a complex between the initial excited state and another molecule. This exciplex (excited-state complex) is lower in energy than the initial excited state, and because it is weakly bound in its ground state, it fluoresces at a longer wavelength.

The time and temperature dependence of the fluorescence emission can thus be understood as follows. After the initial photoexcitation of the stilbene, there are two competing processes. There is a weak initial fluorescence, but some motion of the stilbene and the adjacent protein also occurs. The excited stilbene finds itself close enough to an electronic partner, presumably the indole ring of a tryptophan (*2*), with which it forms an exciplex. This intermediate fluoresces at longer wavelength and on a longer time scale. When the temperature is lowered below 250 K, the motions required for forming the exciplex are too slow to compete with the normal relaxation processes (fluorescence and radiationless decay), and the exciplex does not form.

The rate at which the long-wavelength fluorescence appears acts as a clock that measures the dynamics of molecular motion in the antibody-stilbene complex. The antibody-stilbene complex thus not only produces an unexpected interaction that has electronic consequences, it also provides a way of measuring internal molecular motion within the antibody on a very fast time scale. Fluorescent antibodies may find applications in immunochemistry, histological assays, and genomic studies.

References

1. P. G. Schultz and R. A. Lerner, *Science* **269**, 1835 (1995); P. Wentworth Jr. and K. D. Janda, *Curr. Opin. Chem. Biol.* **2**, 138 (1998).
2. A. Simeonov *et al.*, *Science* **290**, 307 (2000).
3. J. Saltiel and Y.-P. Sun, in *Photochromism: Molecules and Systems*, H. Dürr and H. Bouas-Laurent, Eds. (Elsevier, New York, 1990), pp. 64–162; D. H. Waldeck, *Chem. Rev.* **91**, 415 (1991); H. Görner and H. J. Kuhn, *Adv. Photochem.* **19**, 1 (1995).
4. S. Malkin and E. Fischer, *J. Phys. Chem.* **68**, 1153 (1964); J. Saltiel and J. T. D'Agostino, *J. Am Chem. Soc.* **94**, 6445 (1972); J. Saltiel, A. Marinari, D. W. L. Chang, J. C. Mitchener, E. D. Megarity, *J. Am Chem. Soc.* **101**, 2982 (1979).

PERSPECTIVES: MICROBIOLOGY

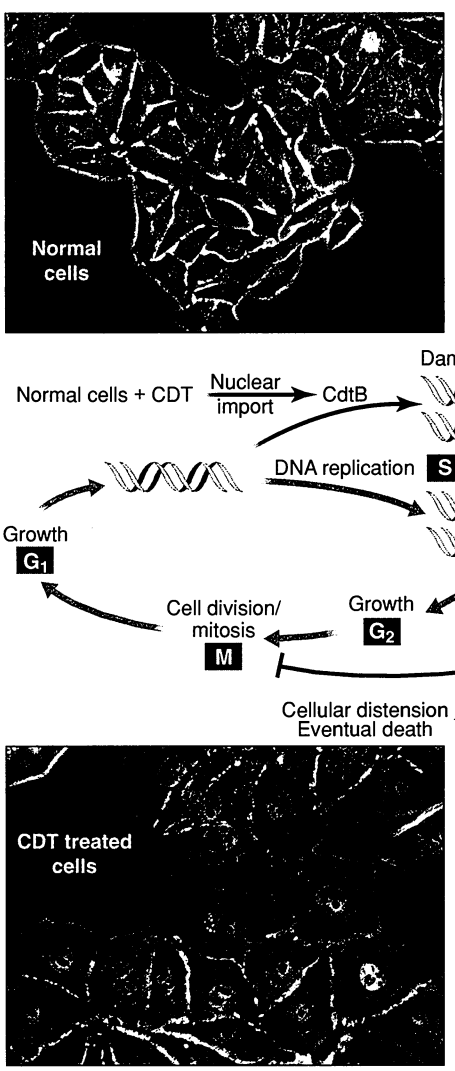
Arresting Features of Bacterial Toxins

Jenifer Coburn and John M. Leong

Many bacterial pathogens make protein toxins that work in fascinating ways to disrupt the normal processes of host cells. These bacterial toxins are key factors in determining the outcome of infection and are among the most potent poisons known to humankind. Two recent reports, one by Lara-Tejero and Galán (*1*) on page 354 of this issue and the other by Elwell and Dreyfus (*2*) in a recent issue of *Molecular Microbiology*, provide another example of how these powerful weapons disrupt the host cell. The two studies demonstrate that a family of bacterial toxins called the cytolethal distending toxins (CDTs) are enzymes that attack DNA in the host cell nucleus. In contrast, almost all bacterial toxins that act inside host cells either destroy or modify host cell proteins.

The CDTs are secreted by a diverse group of bacterial pathogens, including several that are important causes of gastrointestinal illness (*3*). As the name implies, these toxins cause marked swelling and eventual death of many types of cultured mammalian cells. The two new studies now suggest how the CDTs cause arrest of host cells in G_2 phase of the cell cycle—that is, after DNA replication in S phase but before the cell divides into two daughters during mitosis (*4*)—leading to cell destruction (see the figure).

J. Coburn is in the Division of Rheumatology and Immunology, Department of Medicine, Tufts–New England Medical Center, Boston, MA 02111, USA. J. M. Leong is in the Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655, USA.



The CDTs are multisubunit toxins composed of three proteins—CdtA, CdtB, and CdtC. To determine which of the three subunits is able to halt the cell cycle, Lara-Tejero and Galán (*1*) expressed each of the three CDT genes from the intestinal pathogen *Campylobacter jejuni* in mammalian cells. Whereas cells expressing CdtA and CdtC appeared normal, those expressing CdtB displayed drastic alterations in their nuclei. Microinjection of small amounts of purified CdtB subunit alone recapitulated all of the morphological changes typically associated with CDTs. The investigators used an updated algorithm to search protein databases to determine whether CdtB contained any motifs that have been found in other proteins. They spotted homology between the amino acid sequences of *C. jejuni* CDT and mammalian deoxyribonuclease I (DNase I). This finding was noted independently by Elwell and Dreyfus (*2*), who pointed out the similarity between the amino acid sequences of mam-

Enzyme activities of toxin terrorists. The CdtB subunit of CDT, a DNase, is imported into the nucleus where it attacks the DNA being replicated during S phase, activating the DNA damage response pathway. This pathway maintains Cdc2 (a key regulatory protein) in its inactive state, resulting in arrest of the host cell in G_2 phase of the cell cycle. Continued biosynthesis by the arrested host cell leads to distension of the cytoplasm. DNA damage caused by CdtB results in chromatin fragmentation and eventual cell death. [Photographs of control and CDT-treated cells courtesy of Lara-Tejero and Galán]