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.

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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).

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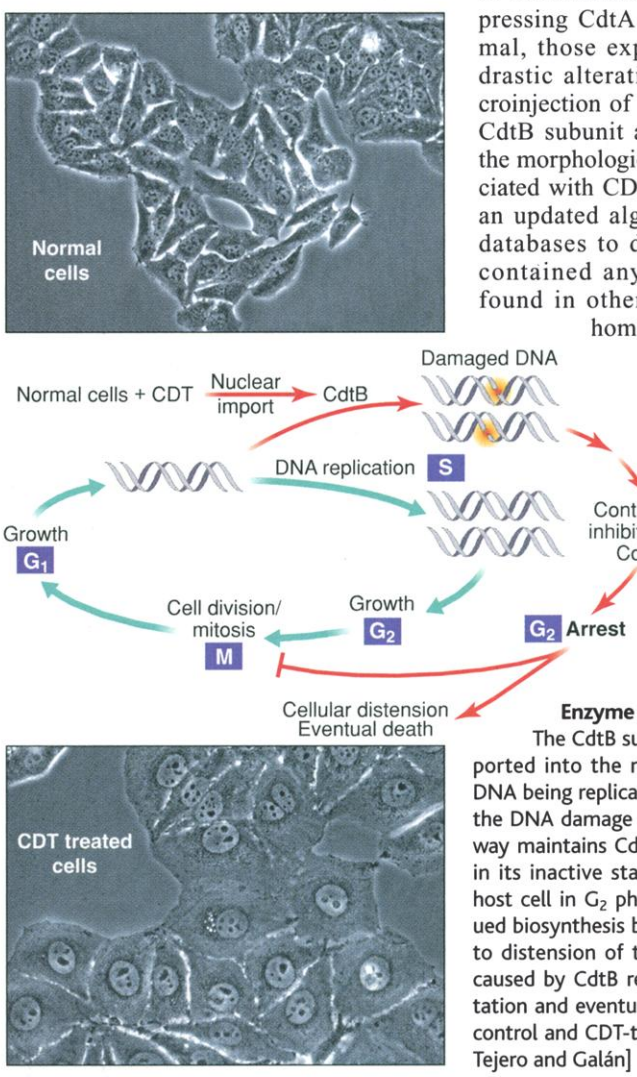

The CDTs are multisubunit toxins composed of three proteins—CdtA, CdtB, and CdtC. To determine which of the three subunits is able to halt the cell cycle, Lara-Tejero and Galán (*1*) expressed each of the three CDT genes from the intestinal pathogen *Campylobacter jejuni* in mammalian cells. Whereas 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]

malian DNase I and *Escherichia coli* CdtB.

DNase I proteins—enzymes that cut DNA into smaller pieces—share conserved amino acid residues that are important for enzyme activity. Both groups (*1*, *2*) tested mutant CdtB proteins containing altered conserved residues to see whether the toxins were still potent. Each mutation resulted in a substantial decrease in CdtB toxicity. Elwell and Dreyfus (*2*) correlated the decrease in toxicity with a concomitant decrease in DNase I activity in vitro. Meanwhile, Lara-Tejero and Galán (*1*) showed that CdtB became localized in the nucleus of toxin-treated cells, consistent with its proposed role as a DNase (*1*).

Identification of CDT as a DNase immediately suggests a model for how the toxin arrests the host cell in G_2. Damage to the DNA induces cell cycle arrest by triggering signaling cascades that keep Cdc2, a key regulatory protein, in an inactive (phosphorylated) form (*5*) (see the figure). Damage to the DNA inflicted by CDT results in activation of a damage response pathway, and accumulation of inactive Cdc2 resulting in arrest of cells in G_2 (*4*). Continued biosynthesis by these arrested cells may result in the characteristic distension of the cytoplasm associated with CDTs. DNA damage inflicted by CdtB results in chromatin fragmentation and eventual cell death.

Whereas DNA damage normally results in arrest of cells at either G_1 (just before DNA replication) or G_2 (just before cell division), CDT-treated cells invariably halt in G_2 only. Lara-Tejero and Galán propose that CdtB damages DNA only when the DNA is in a vulnerable physical state, that is, during replication in S phase. Indeed, exposure of cells to CDT during DNA replication is required for arrest at the subsequent G_2; exposure to CDT after DNA replication is complete allows cells to progress through mitosis and not to halt until the next G_2 (see the figure) (*4*).

An unusual aspect of the CDT family is that its members are made by diverse sorts of bacteria. The only common feature of all known CDT-producing bacteria is that they infect epithelial cell layers, such as those comprising the gastrointestinal or genitourinary tract. Epithelial cells would be especially sensitive to the cell cycle–arresting activity of CDTs, because they continuously proliferate and differentiate as they migrate from deeper layers toward the epithelial surface, from which they are eventually shed. Disruption of normal epithelial cell turnover could lead to breakdown of the epithelial barrier, permitting easier access of bacteria and their secreted toxins to underlying tissues. Cells of the immune system are another potential target for CDTs because they proliferate in response to antigen. Supporting this hypothesis is the finding that certain CDTs inhibit proliferation of monocytes and lymphocytes (*3*, *6*), potentially affecting both innate and acquired immunity.

Now that CDTs have been identified as DNases, many exciting avenues for investigation should open up. For example, a next step will be to identify the host cell or bacterial factors that regulate the DNase activity of CDTs or that deliver CdtB to the nucleus (perhaps with the help of the other two CDT subunits). Further amino acid mutation studies will allow rigorous analysis of the importance of CdtB's DNase activity in animal models of infection. With the finding that CDT family members are DNases, pathogenic bacteria have provided us with yet more tools to study basic biological processes in the eukaryotic cell—in this case, control of the cell cycle.

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PERSPECTIVES: EVOLUTION

The Benefits of Allocating Sex

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Evolutionary biologists have developed an excellent understanding of the selective factors that shape the way in which a given organism allocates resources to male and female offspring—a process called sex allocation (*1*). Studies of sex allocation have provided explanations for a wide range of phenomena—for example, the variation among animals in the proportion of offspring that are male (sex ratio), pollen-ovule ratios in plants, and the age of sexual transition in organisms such as certain coral reef fish that change sex during their lifetime (*1*, *2*). The strength of empirical support for the existence of sex allocation and the selective factors that shape it allows studies of sex allocation to address more detailed questions about natural selection. Furthermore, sex allocation theory can be used to elucidate the population structure and epidemiology of medically important pathogens such as the protozoan parasites that cause malaria.

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Precision of Adaptation

At a time when school boards in the United States are debating whether to include the theory of evolution by natural selection as part of the curriculum, studies of sex allocation are providing some of the best support for this theory (*2*). There are several reasons for this: (i) sex allocation can often have a clear, immediate, and direct effect on fitness; (ii) theoretical models are based on relatively simple trade-offs that often rely on only a small number of key variables; and (iii) the important variables are usually easy to measure. Moreover, relative to most other traits, sex allocation has the advantage that predictions of optimal allocation patterns can be derived from first principles that are directly linked to the most basic elements of evolutionary theory.

For example, extreme sex ratio adjustments in fig-pollinating wasps confirm many of the tenets of evolutionary theory (*3*–*5*). There are many species of fig-pollinating wasps, and in each case, female wasps pollinate and lay eggs in the enclosed fruit of their own host fig species. Mating occurs between the wasps that develop in the same fruit, before the females disperse. Typically, if only a single female lays eggs in a fruit, she produces an extremely female-biased sex ratio (only 5 to 10% of the offspring are males). As the number of females laying eggs in a fruit increases, the sex ratios in the broods become less biased (see the figures, next page, top and bottom). Although there are deviations between observed sex ratios and those predicted by theory (*6*), the fit is often very close.

The observed deviations from the predicted optimal sex ratio are not random. When different fig-pollinating wasp species are compared, the mean sex ratio of offspring produced by a given number of females laying eggs in a fruit is closest to theoretical predictions for the situations (number of females laying eggs in a fruit) that are encountered most frequently in that species (*4*). Furthermore, females show a greater ability to alter their brood sex ratios (in response to variations in the number of females laying eggs in the same fruit) in those species where the number of females enter-