within 5 min of cell injury and release of mitochondrial AIF (*4*). NAD^+ depletion may be part of this signaling pathway, although this has yet to be confirmed. Intriguingly, mitochondria contain most of the cell's NAD^+ stores (*16*), yet nuclear PARP-1 activation depletes total cell NAD^+ to undetectable levels. How are mitochondrial NAD^+ stores modified by nuclear PARP-1 activity? Multiple mechanisms may contribute to this modification, for example, mitochondrial NADase activity, or a shift in NAD^+ compartmentation (perhaps through early opening of the mitochondrial permeability transition pore) may render NAD^+ more accessible to PARP-1 (*4, 16, 17*). Further clarification of how NAD^+ levels fluctuate in response to shifts in cell compartmentation, as well as a more thorough understanding of NAD^+ metabolism and its place in cell signaling, are required. The impact of such events on cellular ATP levels and energy dynamics will be one determinant of the mechanisms by which stressed cells die.

The Yu *et al.* findings suggest many other fruitful areas for investigation. For example, is there a threshold for PARP-1 activation that discriminates between the dual role of this enzyme in apoptosis and necrosis, or are the boundaries between events in cell suicide more complex? Is the first step in bidirectional signaling between the nucleus and mitochondria mediated by release of poly(ADP-ribosyl)ated molecules? How does neutralization of AIF activity suffice to protect NAD^+-depleted cells from undergoing apoptosis for 24 hours (*4*)—dissecting this pathway in wild-type and AIF-deficient cells might help—and how can this information be harnessed therapeutically? What are the downstream mediators of AIF? Also, does AIF stimulate a specific pathway that renders cells resistant to caspase inhibitors? Answers to such questions may not only clarify linkages between PARP-1 activation and apoptosis, but may even elucidate the compromise negotiated 2 billion years ago between the ancestors of mitochondria and those of eukaryotic cells.

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PERSPECTIVES: MICROBIOLOGY

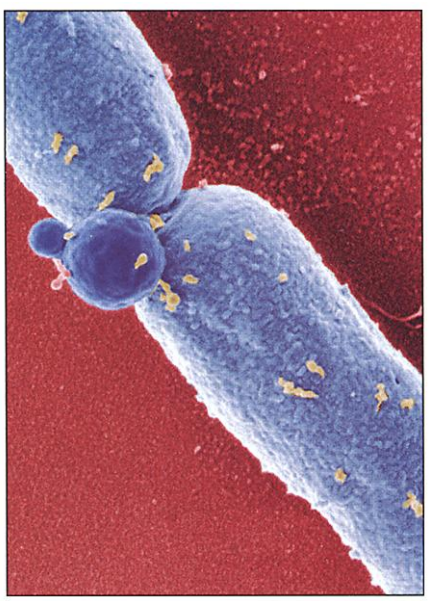
A Binding Contract for Anthrax

James J. Bull and Colin R. Parrish

Antibiotics are typically so successful at combatting bacteria that we do not easily comprehend how a person can walk into a hospital with flu-like symptoms, and yet die of a bacterial infection after days of aggressive drug treatment even though the bacterium is not resistant to antibiotics. Yet this is what happened during last year's bioterrorism incident in the United States when five people died after inhaling spores of *Bacillus anthracis*, the bacterium that causes anthrax. One of the reasons that this bacterium is so difficult to treat is that symptoms appear after *B. anthracis* has already multiplied inside its human host and started to produce large amounts of the tripartite toxin. Thus, although antibiotics may kill or suppress growth of the bacteria, it is the toxin that will eventually kill the human patient. Clearly, a two-pronged counterattack is required: killing of *B. anthracis* with antibiotics and neutralization of the toxin. One possible solution is to passively immunize infected patients with an antibody against the toxin while also aggressively treating them with antibiotics (*1*). Enter Maynard and colleagues (*2*) with their recent paper in *Nature Biotechnology* that reports the isolation of a potent antibody against the protective antigen (PA) subunit of anthrax toxin.

The construction of an anti-PA antibody by Maynard and co-workers is a poster child for the budding field of directed evolution. The biochemical processes by which the human body creates and perfects antibodies is a miniature form of accelerated evolution, with selection of the B cell clone secreting the specific antibody, hypermutation of immunoglobulin (Ig) genes, and recombination of different Ig gene segments. To recapitulate these processes artificially, Maynard *et al.* used in vitro DNA manipulation and an *Escherichia coli* expression system to create an antibody library displayed in phage. The phage display library was screened by "panning" with the PA subunit of anthrax toxin, which selected the antibodies that bound with highest affinity to PA. This approach yielded a few candidate antibodies among the several million in the library. Error-prone PCR and gene shuffling generated further variation in the candidate antibodies, which were then subjected to subsequent rounds of selection. The antibody with the highest affinity for PA, 1H, prevented anthrax toxin from binding to its receptor on cultured alveolar macrophages and also protected rats against a lethal challenge with the anthrax toxin.

There are several properties of an antibody that are required if it is to be an effective antitoxin: it must not be cleared too rapidly from serum, it must bind with high affinity to its toxin target, and the antibody-toxin complex must be quickly removed from serum before the complex dissociates. Panning of the phage display antibody library selected antibodies according to how tightly they bound to PA. However, such antibodies may have to be further modified to ensure that, for example, they are not cleared too rapidly from serum. Fortunately, Maynard *et al.*'s most promising antibody with the



A deadly assassin. The bacterium *Bacillus anthracis*. When spores of this bacterium are inhaled they cause anthrax in herbivores and humans.

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highest binding affinity, 1H, only needed minor modifications to improve its half-life in serum.

Although the serum half-life of the 1H antibody is slightly less than that of the anthrax toxin, the antibody's high binding affinity ensures that the antibody-toxin complex is removed from the serum before the complex dissociates. However, important questions remain about the safety and efficacy of the antibody under field conditions. If toxin has irreversibly bound to and damaged tissues once symptoms appear in infected individuals, the benefit of the antibody as a postsymptom treatment may be lessened. An additional concern is that an antibiotic-resistant form of *B. anthracis* might be used as a bioweapon. In this case, an antitoxin antibody might not be protective as bacteria would continue to proliferate unchecked and produce large amounts of toxin that would eventually overwhelm the host.

Vaccines to protect against prime biowarfare agents are often antiques. The current smallpox vaccines, for example, until recently were prepared from the skin of calves. Current anthrax vaccines were developed more than 30 years ago. Both vaccines are associated with adverse reactions and are not recommended for vaccinating the general public in the absence of an immediate threat. One big advantage of an antitoxin antibody is that it would be administered to people showing symptoms of anthrax or suspected of having been exposed to *B. anthracis*, so that the general population would not need to receive the antibody. Despite our increased understanding of the molecular pathogenesis of anthrax, smallpox, and other biowarfare agents, the old vaccines remain in place because few discoveries made in the laboratory are translated into new therapeutics or modern vaccines.

Maynard and colleagues still have to overcome some technical hurdles before their antibody can move to the next stage of development. For example, their *E. coli* expression system means that they can only produce a partial (single-chain) antibody molecule. Conversion of their partial antibody to a full antibody by expression in a mammalian system may be necessary before it can be administered to humans. Several production facilities that could produce sufficient quantities of such converted proteins already exist in the United States.

But the biggest hurdles to bringing a new anthrax therapeutic to market are likely to be financial and legal. First, there is no assurance of a market (the very existence of an effective drug might help to dissuade any future attack and contribute to a lack of demand). The anthrax bioterrorist assault last year killed five people and led several thousand others to seek treatment or at least purchase antibiotics. Such low demand is insufficient to warrant corporate investment. Were this an over-the-counter pill that people could stock in their homes, a large market based on fear might be imagined. But an antitoxin antibody would have to be injected, so stockpiling in individual households is unlikely to be an option. A further disincentive is presented by the biotechnology patents that cover methods (for example, antibody phage display) used to obtain the 1H antibody, even though such methods would not be involved in the actual production of the therapeutic antibody.

Thus, we face the daunting prospect of knowing how to make but not how to market a drug that, along with antibiotic treatment, could possibly eliminate the threat of a drug-sensitive anthrax bioweapon and diminish our dependence on vaccines whose safety is questionable. It is satisfying that biotechnology has proved itself capable of inventing a molecule tailored to public need. The big question is whether the substantial economic hurdles can be overcome so that this molecule can be quickly added to the therapeutic arsenal against bioterrorism. Perhaps public impatience over vulnerability to future bioterrorist attacks will instill an urgency to tackle this problem from all angles. The recent effort to purchase a safer genetically engineered anthrax vaccine by the National Institutes of Health and the Department of Health and Human Services is an encouraging step in this direction (*3*).

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PERSPECTIVES: PALEOCLIMATE

The Glacial Tropical Pacific—Not Just a West Side Story

David W. Lea

The tropical oceans—especially the tropical Pacific—serve as a heat engine for Earth's climate and as a vapor source for its hydrological cycle. The impact of the tropical Pacific on global climate is well known during El Niño events. By implication, tropical climate must have played a major role in ice age cycles. But until recently, researchers could not resolve the relatively subtle changes in tropical sea surface temperature (SST) that occurred during glacial episodes. The impact of tropical climate cycles has therefore remained elusive.

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Two papers on pages 222 and 226 in this issue (*1*, *2*) now report the first high-resolution records of past climate for two key regions of the tropical Pacific: the cool waters of the Galápagos region in the east, and the warm waters south of Mindanao in the Philippines in the west. The records reveal several new aspects of tropical Pacific climate during the last glaciation.

The authors combine two recent advances to derive their records. First, two new methods, alkenone unsaturation ratios (*3*) and foraminiferal Mg/Ca ratios (*4*), enable second-hand (proxy) information on past SSTs to be obtained. Second, usable, rapidly accumulating sediments have been recovered from the tropical Pacific—a difficult endeavor because of the great depth and extensive carbonate dissolution of much of the central basin.

The Galápagos data of Koutavas *et al.* (*2*) suggest that the cold tongue of the eastern Pacific cooled less than other regions (see the figure). According to sediment geochemical data, most of the warm (>26°C) tropical Pacific cooled by about 3°C (*3*–*5*), and this inference is corroborated by the new Mindanao data (*1*). In contrast, glacial cooling may have been only 1°C in the coldest part of the eastern tropical Pacific (*2*). This finding is important because the temperature of the eastern Pacific cold tongue is a direct diagnostic of trade wind strength, thermocline depth, and the upwelling of cold subsurface waters.

This result challenges the paradigm of stronger trade winds, a steeper east-west thermocline tilt, and more intensive eastern boundary cooling during glacial episodes (*6*). It further suggests that cold (La Niña) episodes, which are characterized by such conditions, are not a suitable analog for glacial conditions (*7*).

The Mindanao record of Stott *et al.* (*1*), on the other hand, mostly demonstrates the rapid response of the hydrological cycle of the western Pacific on millennial time scales. The authors exploit