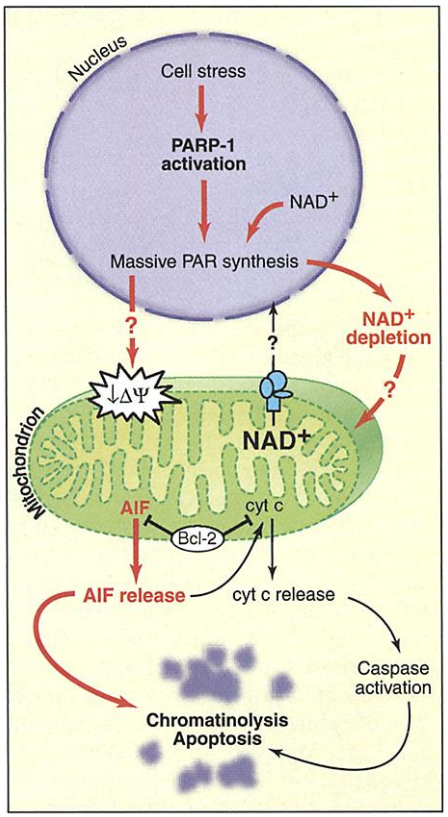
PERSPECTIVES: CELL BIOLOGY

PARP-1—a Perpetrator of Apoptotic Cell Death?

Alberto Chiarugi and Michael A. Moskowitz

About 2 billion years ago, anaerobic cells evolved into eukaryotes, in part by integrating into their cytoplasm an antecedent of the modern mitochondrion. Despite the triumph of this symbiotic step, mitochondria retain a repertoire of molecules that trigger cell suicide or apoptosis. These molecules were perhaps at one time essential for survival of the mitochondrial ancestor, the protomitochondrion (*1*). Learning the identity of these death-inducing players and how they are activated is an urgent challenge for biologists, and should promote the discovery of new therapeutic approaches for treating certain human diseases (*2, 3*). On page 259 of this issue, Yu *et al.* disclose that release of a powerful mitochondrial cytotoxin that promotes apoptotic cell death is triggered by activation of the nuclear enzyme poly(ADP-ribose) polymerase–1 (PARP-1) (*4*).

PARP-1 is highly expressed in the nucleus, with approximately one molecule per 1000 DNA base pairs. PARP-1 catalyzes the transformation of β-nicotinamide adenine dinucleotide (NAD^+) into nicotinamide and poly(ADP-ribose). Under homeostatic conditions, PARP-1 participates in genome repair, DNA replication, and the regulation of transcription (*5*). In response to stresses that are toxic to the genome, PARP-1 activity increases substantially, an event that appears crucial for maintaining genomic integrity (*6*). Massive PARP-1 activation, however, can deplete the cell of NAD^+ and ATP, ultimately leading to energy failure and cell death (*7*). The discovery that cell death may be suppressed by PARP-1 inhibitors or by deletion of the *parp-1* gene prompted an explosion of interest in the process of poly(ADP-ribosyl)ation. In particular, much excitement accompanied the finding that PARP-1 suppression could protect against ischemia, inflammation, diabetes, and septic shock in animal models of these diseases (*8*). That PARP-1 might directly trigger apoptosis is supported by the observations of bursts of poly(ADP-ribose) production during the early stages of apoptotic cell death, and the enhancement of cell survival after deletion of the *parp-1* gene (*9–11*).



PARP-1–dependent signaling in apoptosis. Insults to cells (for example, oxidative stress and DNA damage) activate PARP-1 in the nucleus (violet), leading to the formation of poly(ADP-ribose) (PAR) in large amounts and NAD^+ depletion. Through unknown mechanisms, PAR formation and NAD^+ depletion may trigger a cascade of events (orange arrows), such as mitochondrial depolarization (↓ΔΨ) and release of mitochondrial AIF. Released AIF, in turn, migrates to the nucleus where it induces lysis of chromatin and cell death. AIF also promotes cytochrome c (cyt c) release from mitochondria and subsequent caspase activation, the downstream effects of which are not primarily responsible for cell death (at least regarding the execution of this apoptotic program). The antiapoptotic protein Bcl-2 delays PARP-1–dependent apoptosis by reducing release of AIF and cytochrome c. The possible exit of NAD^+ from the mitochondria via the mitochondrial permeability transition pore (blue) is shown.

A family of cysteine proteases called the caspases are the executioners of apoptosis, often becoming activated after release of cytochrome c from mitochondria in the presence of ATP. Although caspase activation independent of cytochrome c is well documented, conditions under which ATP is severely depleted are usually less favorable for caspase-dependent apoptosis. Low ATP levels encourage cells to die by necrosis by inducing ion pump failure, swelling of the cell, and rupture of the plasma membrane (*12*). How then can apoptosis be a consequence of massive PARP-1 activation, NAD^+ depletion, and putative energy failure? Yu *et al.* (*4*) provide evidence that PARP-1 activity triggers release of a mitochondrial proapoptotic protein called apoptosis-inducing factor (AIF) that promotes programmed cell death through a caspase-independent pathway (see the figure).

AIF is a flavoprotein residing in the mitochondrial intermembrane space (like cytochrome c and other apoptotic modulators) and is among the most powerful, albeit incompletely understood, triggers of apoptosis. Once released from mitochondria, AIF rapidly translocates to the cytoplasm and nucleus, where it induces chromatin condensation and movement of phosphatidylserine into the outer leaflet of the plasma membrane—acknowledged phenotypic markers of apoptosis (*13*). Subsequent to its own expulsion from mitochondria, AIF also triggers release of mitochondrial cytochrome c and caspase activation. Blockade of caspase activation does not abrogate AIF-dependent apoptotic cell death in experimental models tested to date (*4, 13*). Consistent with this, Yu *et al.* show that PARP-1–dependent AIF-mediated apoptotic cell death is heralded by the very early production of poly(ADP-ribose) and depletion of NAD^+. These authors could block apoptosis with AIF-neutralizing antibodies but not with caspase inhibitors, despite the presence of features indicative of caspase-dependent cell death (such as cytochrome c release, caspase activation, and PARP-1 cleavage). Notably, overexpression of Bcl-2, one of the most powerful antiapoptotic proteins identified so far (*14*), delayed (but did not prevent) PARP-1–dependent apoptosis (*4*). The AIF results provide one possible explanation for why blockade of apoptotic cell death is incomplete after caspase inhibition or caspase deletion in some in vitro and in vivo models of apoptosis (*15*).

Yu *et al.* observe very rapid signaling by nuclear PARP-1 to the mitochondria,

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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

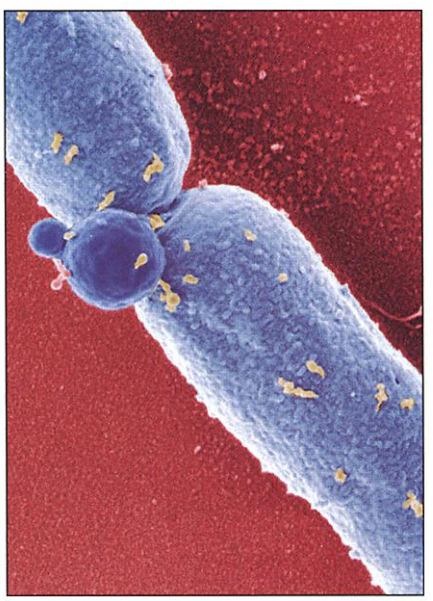
A Binding Contract for Anthrax

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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