mass produce these treatments for use in low-income countries at a cost close to the cost of production, with a small royalty paid back to the pharmaceutical companies. This is the same mechanism used to sell vaccines in low-income countries. In some countries, reductions in the price of HIV-1 treatments were only achieved after long legal battles with pharmaceutical companies. In some cases, countries overruled company patents on drugs and started importing generic drugs at lower costs—so-called compulsory licensing—which is permitted in cases of national medical emergencies.

Creating a new funding mechanism for poor nations is difficult in the current economic climate, but HIV has left a legacy of structures ready to adapt to hepatitis C to complement government and private-sector efforts. UNITAID, the United Nations agency created in 2006 to overcome market barriers for treatments of HIV, tuberculosis, and malaria, recently announced its first funding for hepatitis C, with an aim of reducing treatment costs to $500 to $1000 per patient (*14*). It plans to scale up treatment through a multinational group of HIV programs run by Médicins Sans Frontières, the international medical humanitarian organization. The Global Fund, which addresses HIV/AIDS, tuberculosis, and malaria, has funded treatment programs with old-generation HCV drugs in several developing countries for the past 3 years.

If we can learn from the lessons of HIV/AIDS, mass production of generics can save millions of lives. This has been an inspiring medical success story which need not stand alone but can be repeated, even more rapidly, for hepatitis C. ■

REFERENCES AND NOTES

1. K. Mohd Hanafiah, J. Groeger, A. D. Flaxman, S. T. Wiersma, *Hepatology* **57**, 1333 (2013).
2. R. Lozano *et al.*, *Lancet* **380**, 2095 (2012).
3. K. N. Ly *et al.*, *Ann. Intern. Med.* **156**, 271 (2012).
4. G. S. Cooke *et al.*, *J. Viral Hepat.* **20**, 600 (2013).
5. A. Hill, S. Khoo, J. Fortunak, B. Simmons, N. Ford, *Clin. Infect. Dis.* **58**, 928 (2014).
6. Fair Pricing Coalition, Activists condemn Gilead for exorbitant price of its new hepatitis C drug; www.thebody.com/content/73462/activists-condemn-gilead-for-exorbitant-price-of-t.html (2013).
7. N. Ford *et al.*, *Clin. Infect. Dis.* **54**, 1465 (2012).
8. Medivir, 2012 Annual Report, Stockholm (2012), p. 26.
9. Bristol-Myers Squibb Company, Form 10-K: Annual report pursuant to section 13 or 15(d) of the securities exchange act of 1934. Fiscal year ending Dec 31, 2011, p. 9.
10. Pharmasset, Inc. Form 10-K: Annual report pursuant to section 13 or 15(d) of the securities exchange act of 1934. Fiscal year ending Sep 30, 2011, p. 26.
11. MSF Drug Access Team, Untangling the web of antiretroviral price reductions: 16th edition (Geneva, 2012); www.msfaccess.org/reports.
12. B. Schwartländer, I. Grubb, J. Perriëns, *Lancet* **368**, 541 (2006).
13. M. S. Sulkowski *et al.*, *N. Engl. J. Med.* **370**, 211 (2014).
14. www.unitaid.org/en/resources/press-centre/releases/1352-unitaid-approves-grants-of-160-million

10.1126/science.1257737

BIOCHEMISTRY

Fishing for peroxidase protons

Where are the protons in heme protein catalysis?

By **John T. Groves** *and* **Nicholas C. Boaz**

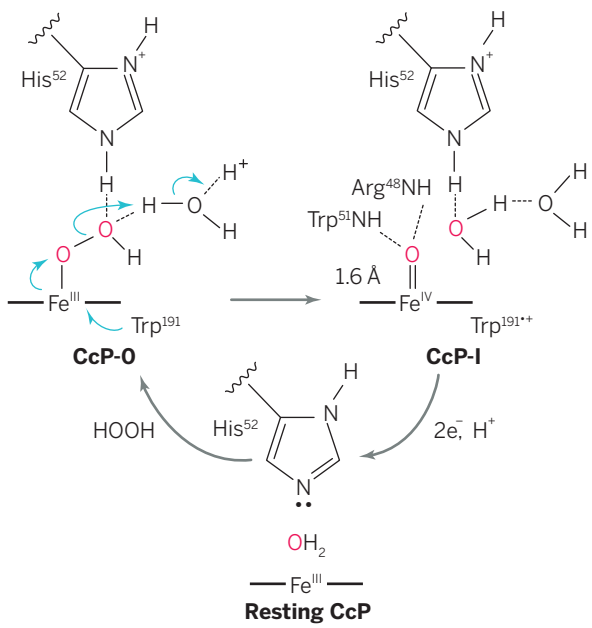
Cytochrome c peroxidase (CcP) consumes hydrogen peroxide in mitochondria, using electrons derived from reduced cytochrome c. This and a related enzyme, horseradish peroxidase (HRP), have played key roles in the development of structural and mechanistic biochemistry and are used in biocatalysis and chemiluminescent bioassays (*1*). On page 193 of this issue, Casadei *et al.* (*2*) use neutron diffraction to reveal the role and origin of protons in heme oxidation by hydrogen peroxide, a key step in this essential enzymatic reaction.

CcP and HRP were the first heme enzymes for which oxidized intermediates were observed (*1*). In the textbook mechanism for heme oxidation, protonated histidine N-H assists O-O bond heterolysis in an Fe(III)-OOH intermediate (CcP-0), producing CcP compound I (CcP-I) and water. The overall course of this reaction was established long ago. But where are the protons? Casadei *et al.* use neutron diffraction to reveal the positions of protons in resting CcP and CcP-I. They show that the iron(IV) of CcP-I is an unprotonated ferryl, Fe(IV)=O. The results bring new clarity to heme oxidation by hydrogen peroxide (see the figure).

Neutron diffraction has distinct advantages over x-ray diffraction techniques for the structural characterization of enzymes that contain redox-active metals. Non-ionizing neutron beams avoid the photoreduction that often plagues structural analysis with x-rays and that also occurs in the laser beams used for resonance Raman spectroscopy. Laser and x-radiation lead to ambiguities in the oxidation states of redox-active metals such as iron or manganese. By contrast, neutrons interact only with atomic nuclei and scatter much more effectively from hydrogen and, especially, deuterium atoms. Catalytic proton networks and even deuterated hydronium ions (D_3O^+) have been observed in proteins by means of neutron diffraction (*3*, *4*).

Efforts to understand the atomic and electronic structure of the oxidized intermediates in the CcP catalytic cycle have been hampered by the fact that Fe(III)/Fe(IV) redox potentials in heme proteins lie in the same range as those of the porphyrin ring and those of tryptophan and tyrosine. This "redox non-innocence" greatly increases the complexity of these systems because it increases the number of plausible sites of oxidation. In HRP-I and in model porphyrin complexes, ferryl states, Fe(IV)=O, with very short Fe-O bond lengths have been reported (*5*, *6*). The distinction between Fe(IV)=O species and their hydroxylated equivalents, Fe(IV)-OH, has taken on considerable importance with recent evidence that cytochrome P450 compound II is protonated and that the basicity of ferryl oxygen strongly affects heme protein reactivity (*7*).

To identify the positions of active-site protons in CcP and CcP-I, Casadei *et al.*



Proton-mediated mechanism. Reaction of ferric CcP with H_2O_2 first gives CcP-0, followed by O-O bond scission driven by external protonation to afford CcP-I. Casadei *et al.* now report neutron diffraction data that pinpoint the locations of the protons and elucidate the catalytic mechanism.

Department of Chemistry, Princeton University, Princeton, NJ 08544, USA. E-mail: jtgroves@princeton.edu

have compared neutron and x-ray diffraction data from both species. The authors first replaced exchangeable protons with deuterons in large (1 mm) crystals of CcP, and then treated a CcP crystal with hydrogen peroxide to produce CcP-I. The results show that the catalytic imidazole of His^{52} is not protonated in resting CcP, as expected (see the figure). In CcP-I, both nitrogen atoms in His^{52} are protonated (deuterated), which was unexpected according to the generally accepted mechanism. This means that the protons required for O-O bond cleavage in CcP-0 (see the figure) must have come from another source, such as an adjacent water molecule. In this snapshot of the catalytic cycle, the ferryl oxygen, Fe(IV)=O, of CcP-I is not protonated, and the short iron-oxygen distance expected for the ferryl is confirmed.

Visualizing the mechanistically pertinent protons has important implications for the mechanism of O-O bond scission mediated by CcP and other heme proteins. A "wet" version of the peroxidase mechanism has been proposed, in which a water molecule adjacent to His^{52} mediates O-O bond cleavage (*1*, *8*, *9*). Retention of the His^{52} proton adjacent to the ferryl heme after O-O bond heterolysis, as revealed by Casadei *et al.*, suggests that another proton, likely traveling through an aqueduct of water molecules leading to the active-site cavity, is also necessary (see the figure).

CcP-catalyzed peroxide bond heterolysis thus seems to occur via a proton relay mechanism similar to that of cytochrome P450 (*10*), with electrons arriving through Trp^{191} (see the figure). In this scenario, deprotonation of His^{52} would occur during subsequent reduction of CcP-I by another enzyme, ferrocytochrome c. This realization points to a water-mediated, acid-catalyzed process for O-O bond heterolysis, which is mechanistically satisfying because of its analogies to other proton relay mechanisms, such as that of cytochrome P450. Further, the need for a water channel and an external proton in peroxidase catalysis are highly informative for the design and construction of new heme-iron biocatalysts. ■

REFERENCES

1. T. L. Poulos, *Chem. Rev.* **114**, 3919 (2014).
2. C. M. Casadei *et al.*, *Science* **345**, 193 (2014).
3. M. G. Cuypers *et al.*, *Angew. Chem. Int. Ed.* **52**, 1022 (2013).
4. S. J. Tomanicek *et al.*, *J. Biol. Chem.* **288**, 4715 (2013).
5. J. T. Groves, *J. Inorg. Biochem.* **100**, 434 (2006).
6. J. E. Penner-Hahn *et al.*, *J. Am. Chem. Soc.* **108**, 7819 (1986).
7. T. H. Yosca *et al.*, *Science* **342**, 825 (2013).
8. P. Vidossich *et al.*, *J. Phys. Chem. B* **114**, 5161 (2010).
9. K. Sen, W. Thiel, *J. Phys. Chem. B* **118**, 2810 (2014).
10. X. Wang, S. Peter, M. Kinne, M. Hofrichter, J. T. Groves, *J. Am. Chem. Soc.* **134**, 12897 (2012).

10.1126/science.1256754

ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*

HIV/AIDS

Persistence by proliferation?

Latently HIV-infected cells driven to proliferate may raise a further challenge for eradication strategies

By **David Margolis**[1] *and* **Frederic Bushman**[2]

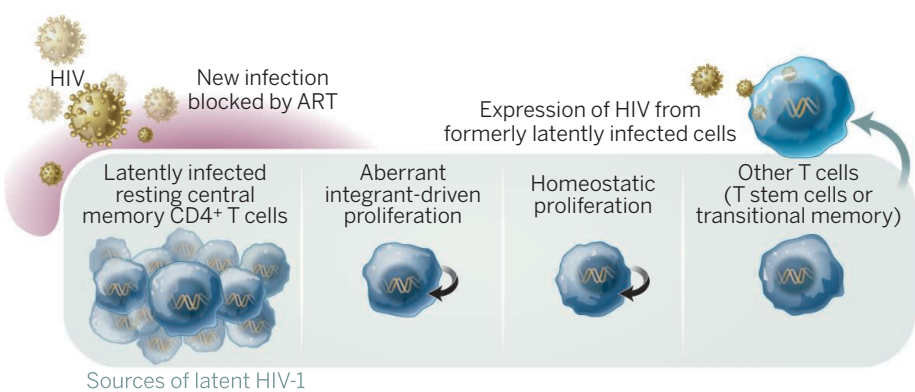
The persistence of HIV-1 infected cells in individuals on antiretroviral therapy (ART) presents an obstacle for cure of infection. ART is the best available remedy for millions of infected people, but treatment must be life-long because HIV establishes latent infection that is unaffected by antiretrovirals and is invisible to immune surveillance. Because decades of treatment may be unsustainable, there is intense interest in reversing latency. If quiescent HIV in $CD4^+$ T cells can be identified and activated without enhancing new infection, HIV-targeted immune response might be able to control or even clear infection. On page 179 in this issue and in this week's *Science* Express, Maldarelli *et al.* (*1*) and Wagner *et al.* (*2*), respectively, raise a new challenge for these efforts suggesting that proliferation of latently infected cells may be a key factor in sustaining this durable viral reservoir.

Latent HIV proviruses (viral genome integrated into the host cell DNA) are found most often in resting $CD4^+$ T cells within the central memory arm of the immune system (*3*–*5*), although other T cell subpopulations have been implicated (*6*, *7*). The latent pool shows minimal or absent decay (*8*), which is not fully understood. One possible explanation is that ongoing low-level HIV replication during ART replenishes the pool. However, viral genetic diversity does not increase over time in individuals during ART (*9*), at odds with this view.

Other evidence suggests that virus emerges from the pool of latently infected cells periodically. Even patients whose viral load is well suppressed show intermittent bursts of viremia ("blips"), and in many patients viremia is detectable in specialized assays (*10*, *11*). Given that the pool of latently infected cells must be primarily established before ART, it is difficult to understand why such periodic induction of the pool does not lead to it running dry.

Homeostatic proliferation of infected transitional memory T cells (*6*) has been proposed as a source that could maintain the pool, but this does not explain persistence in the dominant central memory reservoir. Latently infected stem cell–like memory T cells could proliferate (*7*), and it will be of great interest to compare integration patterns seen in these cells and in more differentiated cell populations.

Maldarelli *et al.* and Wagner *et al.* harvested DNA from the blood cells of HIV-infected individuals after a decade of successful ART, and analyzed the distribution of sites of proviral integration in the human genome. Typically, HIV favors integration in regions of the genome that are transcriptionally active (*12*), but a unique pattern was seen in rare proviruses from well-suppressed patients. Both groups found expanded proviral clones that were enriched for proviruses in or near a limited set of cellular genes, some of which



The persistent pool of HIV-1. Antiretroviral therapy can prevent the creation of new latently infected cells, but it does not affect cells in which latency was initially established. Intermittent bursts of viremia originate in part from this latent reservoir. Forcing these cells to exit the latent state without enhancing new infection could make the virus vulnerable to clearance by an HIV-targeted immune response. Blocking the proliferation of these latently infected T cells could deplete the pool if its stability is driven by such multiplication.

Science
AAAS

Fishing for peroxidase protons

John T. Groves and Nicholas C. Boaz

Science **345**, 142 (2014);
DOI: 10.1126/science.1256754