tional potent agents are incorporated into these already powerful regimens.

There is little doubt that viral load determinations will become useful tools, along with CD4 lymphocyte counts, in the clinical management of HIV-1–infected patients. Both measurements provide important insights into the disease process. To borrow a crude but illustrative analogy from a prominent retrovirologist, John Coffin: The development of AIDS can be likened to an impending train wreck, where the viral load indicates the speed with which the train is headed for catastrophe and the CD4 cell count marks the distance from the site of doom. The means of slowing the train are now available, but ways of stopping and reversing the locomotive must be found.

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UPDATE

# Mammalian Cytochrome c Oxidase, a Molecular Monster Subdued

Shelagh Ferguson-Miller

The high-resolution crystal structure of mammalian cytochrome c oxidase, a key enzyme in aerobic metabloism, was recently reported in *Science* by Tsukihara *et al.* (*1*), and discussed in an accompanying Perspective (*2*). The original paper (*1*), a landmark achievement in protein structure analysis, described the structure of the six metal centers (two hemes, two copper centers, Mg, and Zn), information critical to understanding the energy-transforming activity of the enzyme. In this issue, Tsukihara *et al.* (*3*) now present the complete structure of bovine cytochrome c oxidase at ≈2.8 resolution. With a molecular weight of 200,000 and 13 different subunits, this is the largest, most complex membrane protein so far analyzed at this resolution. The atomic coordinates of the amino acids of all 13 peptides have been determined, and the refinement of the x-ray data did not alter the sites of the metal centers previously reported, which show remarkable structural identity with the bacterial version of the enzyme (*4*).

The full text of a previous Perspective on this topic (*2*) can be found at http://science-mag.aaas.org/science/scripts/display/full/269/5227/1063.htm

The number, location, and functional significance of the numerous subunits of the mammalian enzyme have been hotly debated for many years (*5*, *6*). Now, at least the number and location are resolved. As originally reported by Kadenbach and colleagues (*6*), the three largest, mitochondrially encoded subunits are associated with a total of 10 nuclear-encoded peptides. The new structural information will certainly aid efforts to define the functions of these 10 dissimilar subunits, but it is already clear that none of them directly impinges on the metal centers, supporting the idea of a regulatory or insulating role.

The dimer in the crystal structure has minimal protein contacts between monomers, with subunits VIa and VIb as the sole bridging peptides. This arrangement protects an exposed side of the active site, where the monomeric bacterial enzyme (*4*) has another subunit (IV); however, a mechanistic role for the dimer seems unlikely in view of the lack of intimate contact and the existence of functional monomeric forms of several eukaryotic enzymes (*7*, *8*).

A fundamental question regarding all membrane proteins is whether there is a requirement for specific lipids. Cytochrome oxidase has been a model system for addressing this issue, because a strong association with the anionic phospholipid, cardiolipin, has reproducibly been observed (*9*, *10*). Eight phospholipids and two cholate molecules (possibly occupying adenosine diphosphate–binding sites) are resolved in the crystal structure, but not cardiolipin. Its lack of resolution in the crystals of both mammalian and bacterial oxidases (*4*) favors the idea that cardiolipin facilitates the conformational changes necessary for activity, rather than maintaining a particular static form (*11*).

The process of energy generation in all biological systems is critically dependent on the movement of protons through proteins, yet the mechanism of this transfer is not established. In the mammalian enzyme, three proton relay pathways are postulated, for which two of the entry sites are similar to those defined in the bacterial enzyme (*4*) and by mutational analysis (*12*, *13*). However, the two proposed proton pumping pathways follow different routes than the one described by Michel and colleagues, and do not access the heme $a_3$–$Cu_B$ center where oxygen chemistry is carried out. If correct, these paths suggest an indirect coupling mechanism rather than a direct connection to the oxygen chemistry, a fundamental issue remaining to be resolved.

Pathways through the protein for water, electrons, and oxygen are also proposed, the latter taking into account the higher solubility of oxygen in hydrophobic environments and suggesting the possibility that a pool of bound lipid in subunit III could provide an oxygen reservoir. It will be a challenge for future research to determine whether specific facilitated routes are mechanistically or physiologically important for any of these key substrates and products of energy transduction. The monster is subdued, but far from tamed.

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The author is in the Department of Biochemistry, Michigan State University, East Lansing, MI 48824–1319, USA. E-mail: fergus20@pilot.msu.edu