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BIOCHEMISTRY

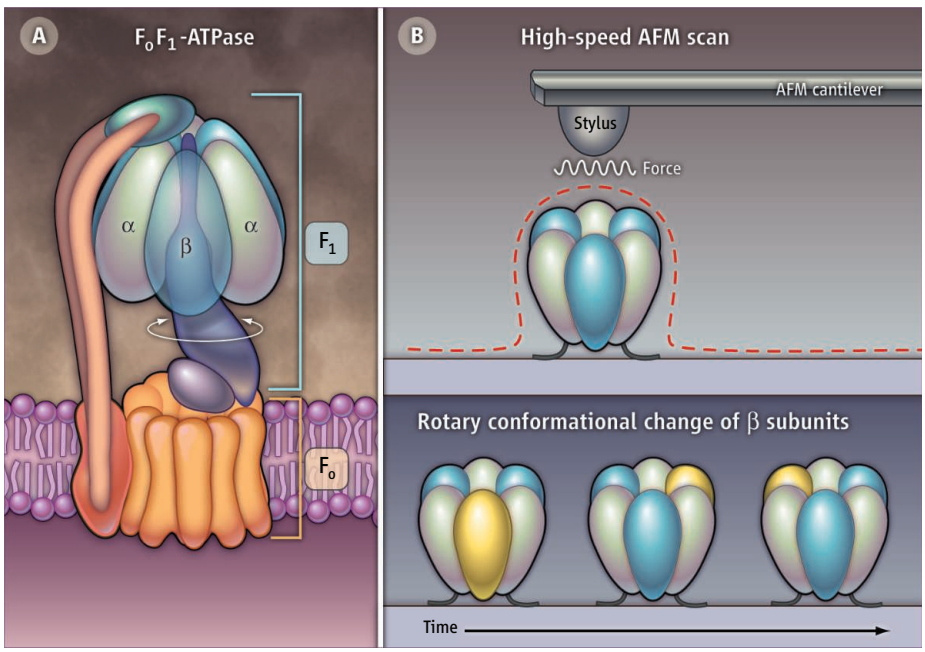
Seeing a Molecular Motor at Work

Atomic force microscopy reveals conformational changes in a rotary molecular motor.

Wolfgang Junge[1] and Daniel J. Müller[2]

The molecular machinery of life can be visualized by crystal structure analysis, which provides still pictures at atomic resolution (~0.1 nm), as well as by pulsed-laser methods that can yield recordings of events ranging from vibronic relaxation (10^{-14} s) to catalysis (10^{-6} to 10^{-3} s). The ultimate goal has been to merge high-resolution space and time recordings and advance from still pictures to movies of molecules at work. High-speed scanning probe microscopy provides excellent spatial resolution and has been extended into the time domain of chemical activity (*1*, *2*). On page 755 of this issue, Uchihashi *et al*. (*3*) use this technique to examine the F_OF_1-ATPase, the rotary enzyme that synthesizes adenosine triphosphate (ATP), the universal fuel of cells. The study demonstrates the power of atomic force microscopy (AFM) in observing the cellular machinery at nanometer and millisecond resolution.

The F_OF_1-ATPase is a membrane-associated enzyme composed of two rotary motors that are elastically coupled by a central γ shaft and stabilized by a peripheral stator-stalk (*4*) (see the figure). Its catalytic headpiece, F_1, lies outside the membrane and produces ATP by using the torque provided by the membrane-embedded electromotor, F_O. The conversion of electrochemical into chemical energy is reversible; when running backward, the enzyme hydrolyzes ATP and generates torque to pump ions in the reverse direction. Nearly 15 years ago, fluorescent probes and video microscopy were used to directly observe the rotation of the central shaft in F_1 as driven by ATP hydrolysis (*5*). Uchihashi *et al*. used high-speed AFM to observe very small conformational changes in the catalytic hexameric ring (called the stator ring, composed of three α and three β subunits) of F_1 in the absence of the central shaft and the stator-stalk. The authors asked whether the directionality of ATP hydrolysis by the hexameric ring is dictated by the chirality of the central shaft or by the chirality of the catalytic hexagon. This question is of general relevance considering nature's wide use of hexagonal nucleoside triphosphatases (NTPases): for ion translocation by F- and V-ATPases; for protein, DNA, or RNA translocation; and for unwinding DNA or RNA by helicases. The hexameric ring in F-ATPases, and in particular the construction of the catalytic site in the hexameric ring, is structurally homologous to that of DNA helicases (*6*) and in a DNA-transporting bacterial conjugation protein (*7*). This homology, together with the dissimilarity of the structural element in the center of the hexagon (protein, DNA, or RNA), have led to a proposed scenario in which ion-translocating F- and V-ATPases evolved from DNA helicases through intermediate ancestors, protein- or DNA- or RNA-translocases (*8*).



Watching the details. (**A**) F_OF_1-ATP synthase complex with the catalytic hexagon (α and β subunits) of the F_1 headpiece. The F_1 headpiece and F_O electromotor are coupled by a central γ shaft (dark blue) and stabilized by a peripheral stator-stalk (rose). (**B**) High-speed AFM imaging of the catalytic hexagon (covalently tethered to a support). In the presence of ATP, the catalytic hexagon starts hydrolyzing. Conformational changes of single subunits (yellow) in the hexagon are observed by AFM.

The prevailing view has been that the orientation of the γ shaft relative to the three β subunits in the catalytic hexameric ring dictates the next step and therewith the direction of rotary catalysis. The important result of the time-resolved AFM study of Uchihashi *et al*. is that the three β subunits in the bare catalytic hexagon of F_1 undergo cyclic conformational changes (upon binding and hydrolyzing an ATP molecule) in the same rotary direction (counterclockwise) as in the presence of the central γ shaft. Although the rate and precision of forward rotation is lower without, rather than with, the shaft attached, it is obvious that the cooperativity between the catalytic sites in the three β subunits is intrinsic to the hexagon. Because the contrast of tapping-mode AFM images can result from structural, mechanical, and electrostatic features of the object, the future use of different AFM imaging modes will reveal detailed origins underlying the conformational changes described. It will also be interesting to extend this type of work to other hexagonal NTPases that are rotating protein, DNA, or RNA.

Time-resolved AFM is one example among the various technological developments that turns the technique into a multifunctional nanoscopic toolbox (*9*, *10*). The claim that the AFM-based toolbox "opened the door to the nanoworld" (*9*) awaits development for biological applications. Besides using AFM to image the molecular machinery of the cell at work, AFM is widely applied to detect biomolecular interaction forces (*10*–*12*). In the force spectroscopy mode, the AFM stylus can detect interactions within a bio-

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logical sample to molecular sensitivity. Functionalizing the AFM stylus through chemical (functional groups, charged or hydrophobic moieties), biological (oligopeptides, oligosaccharides, oligonucleotides, lipids), and physical (pH sensing, ion selective, magnetic) modification allows detection of specific biomolecular interactions within and among native proteins at work. Measuring the force at a fixed position (i.e., one pixel) over distance and time allows quantification of the vertical (>0.1 nm) and temporal (>1 μs) (*9*) dependencies of such interactions, as well as analysis of how they are established and guide biomolecular processes. In the scanning mode, over a field of 100 × 100 pixels, imaging rates of 100 frames per second appear within reach. Recent examples quantify and structurally localize the interactions and energies established upon substrate binding of membrane transporters and enzymes (*10*, *11*).

Approaches that merge topographic imaging with a qualitative and quantitative analysis of biological interactions (*10*) enable multifunctional imaging that allows observations such as the dynamic reassembly of cell surface receptors in living cells and the mechanical properties of membrane proteins from extracted membranes (*12*). So far, the resolution of multifunctional images has been insufficient (>>2 nm) to observe structural details of protein complexes. Over the past few years, robust AFM technologies have been established that, in principle, could be extended to obtain such multifunctional images at high resolution. Now, it is up to the AFM users to establish useful protocols to reveal such images of native proteins and observe them at work—how individual domains of these machines hydrolyze and synthesize ATP, translocate electric charges, change affinity, change their elastic stiffness, or undergo transient structural changes and chemical modifications. It should also allow the quantification of interactions that guide individual machines to dynamically assemble for new functional tasks at nanometer and millisecond resolution.

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MICROBIOLOGY

Exploiting Malaria Drug Resistance to Our Advantage

High-throughput chemical and gene analyses help identify promising pairs of antimalarial compounds that could prevent resistance.

Nick Cammack

In the world of developing anti-infective drugs, resistance comes with the territory. It is literally in the DNA (or RNA) of the viruses and pathogens targeted for treatment. Because resistance has far-reaching consequences for human health, researchers have studied the resistance of infectious agents such as human immunodeficiency virus (HIV) type 1 with unprecedented intensity and documented in excruciating detail the genetic determinants of resistance to FDA-approved drugs (*1*). However, understanding drug resistance in a complex eukaryotic parasite, such as the *Plasmodium* parasite that causes malaria, is a very different challenge. On page 724 of this issue, Yuan *et al*. (*2*) confront the issue head-on. Using high-throughput chemical and gene analysis methods, they not only identify potential new antimalarial drugs that could be used in combination to suppress the development of drug resistance but also characterize a common set of genetic loci and genes affected by these molecules.

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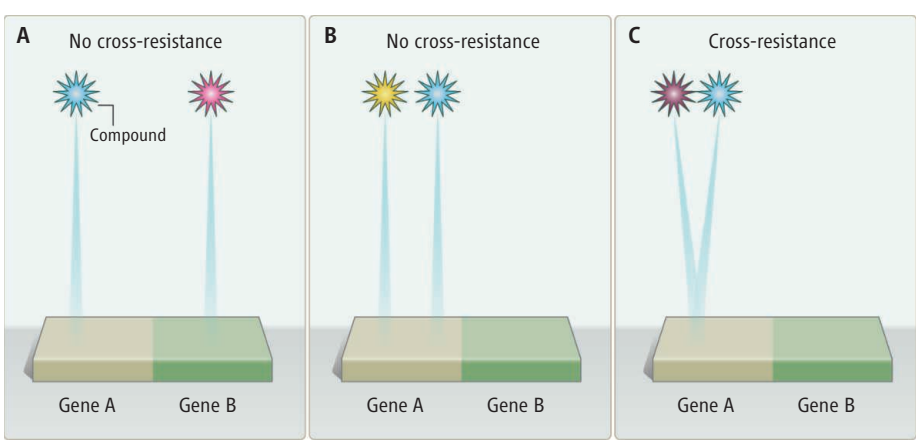
In the context of infectious pathogens, drug resistance is the response of the organism to a new environment. In the case of malaria, resistance reflects the parasite's ability to survive and replicate despite the presence of a drug, reducing the drug's effectiveness. Many mechanisms can confer resistance. The parasite can decrease drug accumulation, for example, by actively pumping the drug out of its cell membrane. It could also modify a target site on a specific protein, interfere with a metabolic pathway, and per-



Combining to fight malaria. A simple model of cross-resistance between different compounds. (**A**) Two compounds targeting the products of different genes are not cross-resistant and are negatively correlated. (**B**) Two compounds targeting distinct sites on the same gene product are not cross-resistant, but two compounds targeting the same site on a gene product (**C**) are cross-resistant and would show a strong positive pairwise comparison. These two compounds could be good candidates for combination treatment of malaria.

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