Indian scenario

In the Indian context, it is realized that ‘stand-alone supercomputers are passé. The decade-old massively parallel processing systems are increasingly considered old hat. Cluster computing is “in” and grids are “hot’.” C-DAC, a scientific society under Ministry of Information and Communications Technology, was set-up in 1988 to design and develop high-performance computing systems. C-DAC has been developing the PARAM series of computers for this purpose from Pune. It has begun work on the India-Grid or I-Grid to create 10 supercomputing sites in the country, contributing totally ten teraflops of computing power. The I-Grid is expected to help Indian scientists initiate/ carry out a variety of scientific and engineering applications like N-body simulations, finite element and finite difference algorithms on large-scale problems at a faster rate. The applications may cover various disciplines like computational- atmospheres, -chemistry, -structural mechanics, -fluid dynamics, evolutionary computing and seismic data processing. The I-Grid project is expected to be fully operational by 2007, with Pune and Bangalore scheduled as the hubs of grid activity. It may eventually become a part of the Global Grid initiated during SC2003.

To conclude, ‘The vision for the future using grid computing is that we will never have to concern ourselves again about where our files are stored, or on which continent the computer processing our data is located. The grid will transparently give us seamless access to the globally distributed computational resources at our disposal, so that we can get our work done in the most efficient manner possible’. However, some opine that ‘a great many challenges have to be overcome before grid computing may be a common reality’.

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RESEARCH NEWS

ATP synthase and the torsional mechanism: Resolving a 50-year-old mystery

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As the energy currency and regulator of cellular metabolism, adenosine triphosphate (ATP) participates in all the pathways of metabolism. ATP is synthesized from adenosine diphosphate (ADP) and inorganic phosphate (P) by the enzyme $F_{0}F_{1}$-ATP synthase which is located in the cytoplasmic membranes of prokaryotes and in the membranes of mitochondria and chloroplasts of eukaryotes. ATP synthase has two domains, the $F_{0}$ domain is a transmembrane domain and the $F_{1}$ domain, which is joined to the $F_{0}$ portion by the $\gamma$-subunit of the enzyme, is the site of ATP synthesis (Figure 1). The $F_{1}$ part of ATP synthase was first isolated in 1960 by Ephraim Racker and colleagues and the first high-resolution structure of the $F_{1}$ portion of the enzyme was solved by Abrahams et al. in 1994.

The mechanism of ATP synthesis was a major question even before the structure was known. The chemiosmotic hypothesis of oxidative phosphorylation, proposed by Peter Mitchell in the 1960s, postulated a delocalized, protonotive force, with both its components ($\Delta\phi$ and $A\phi$) being created by a proton gradient, as the driving force for ATP synthesis. That is, the organelle was seen as a delocalized electric cell that used a proton current as the sole energy source for ATP synthesis. Mitchell was awarded the Nobel Prize in Chemistry in 1978. The binding change mechanism was postulated by Paul Boyer in 1973, to explain how the proton current and ATP synthesis were coupled. In the binding change mechanism, energy stored as ion gradients across the membrane containing the $F_{0}$ domain is used for free rotation of the c-rotor, the $\gamma$-shaft and the e-subunit attached to the rotor. This free rotation gets translated into binding changes in the catalytic sites in the $\beta$-subunits of the $F_{1}$ domain causing ADP and P to combine spontaneously to form ATP, followed by endergonic release of the product. Boyer shared the Chemistry Nobel Prize for this work in 1997.

Both the chemiosmotic and binding change mechanisms have been modified over time in efforts to make them consistent with more recent experimental data. However, there are basic flaws in both the Mitchell and Boyer mechanisms that cannot be corrected by minor modifications. A fundamental problem that is not addressed correctly by either mechanism is the thermodynamics of energy coupling between transmembrane ion currents and ATP synthesis. Both mechanisms were developed from a static equilibrium viewpoint, and both can be shown to violate the laws of thermodynamics for a dynamic system (Box 1). The novel element in Boyer’s mechanism is that the energy of the proton gradient is not used in the synthesis step, but only to release the ATP from the ATP synthase enzyme. At no step in the binding change mechanism does energy get locked into the
ATP. This mechanism thus postulates it is the binding energy released when ATP is bound to an enzyme doing external work and not the hydrolysis of ATP that is the energy source for the work. Thermodynamic analysis of such an isothermal cycle shows that it violates both the first and second laws.

During the 1990s, the torsional mechanism of energy transduction and ATP synthesis, which is consistent with all of the available experimental results and all the known laws of nature, was developed by Sunil Nath, IIT Delhi. The torsional mechanism gives a complete picture of

**Box 1. Summary of Nath’s arguments for violations of thermodynamic laws by existing mechanisms of ATP synthesis**

In his trilogy tome on ATP synthesis, Nath has carried out a fundamental analysis that proves the violations of thermodynamic laws by currently accepted mechanisms of ATP synthesis. Since classical equilibrium thermodynamics forms the basis of the chemiosmotic theory, Nath has calculated the concentration ratio of an equivalent concentration cell by considering the mid-point potential of the NADH → NAD⁺ redox couple and use of the Nernst equation. He obtains a gigantic \( \frac{c_{out}}{c_{in}} \) ratio of \( 3.5 \times 10^{18} \). Hence, if a typical physiological value of an ion/solute concentration in the aqueous phase on one side of the membrane is taken \( (c_{out} \sim 10 \text{ mM}) \), the ion/solute concentration in the aqueous phase on the other side of the membrane \( (c_{in}) \) works out to be only \( 3.0 \times 10^{-27} \text{ M} \), a value well below the detection limit of the most sophisticated chemical measurement technique. Hence, Nath concludes it is impossible for the biological system to achieve the observed steady-state rates of metabolism with these below-detection levels of species concentration predicted by the chemiosmotic theory.

Further, Nath has performed a first principles energetic analysis for generation of a delocalized \( \Delta \psi \) of 120 mV by electrogenic proton translocation. He shows that each successive proton translocation act has to work against a higher electric field in the overall electrogenic mode of ion translocation postulated by the chemiosmotic theory. Assuming an ideal reversible translocation with no losses, he elegantly expresses the (reversible) redox energy expenditure as a mathematical series. Summing the series yields a redox energy requirement of \( 4.8 \times 10^{10} \text{ meV} \), an astronomical number, considering that the available redox energy per two electrons in mitochondria measures 2280 meV only. He emphasizes that since the calculation assumes perfect coupling at the sites, neglects all pump losses, and further neglects the extra energy consumption required to work against the increasing concentration gradient of protons with each successive translocation event, the energy estimate is a conservative one. He also points out that chemiosmosis postulates an even larger delocalized \( \Delta \psi \) (\( \sim 200 \text{ mV} \)) than assumed in the analysis. Hence Nath concludes that it is energetically impossible for the respiratory chain to violate electroneutrality of bulk aqueous phases to such a large extent as conceived in the chemiosmotic theory. A corollary of this analysis is that proton translocation must be accompanied in strict time sequence by anion translocation (in the same direction) or counter-cation translocation (in the opposite direction) to ensure steady-state functioning of the cellular organelle. New terminology (symsequence and antisquence) has been coined to describe the new concepts. The powerful unifying theme emerges from the torsional mechanism that a direct, local energy transduction takes place by structural/functional association of transporters/channels in the membrane for addition and joint utilization of energy, and for regulation of transport and cell metabolism.

Nath has also tested the consistency of proposed mechanisms in \( F_1 \) with the laws of thermodynamics. He considers the thermodynamic cycle for the performance of useful work by an ATP-hydrolysing molecular machine. He defines the molecular machine itself as the system, and carries out an overall energy balance. In such a process, free ATP enters the system, free \( (\text{ADP} + \text{P}_i) \) leave it, and the difference between their enthalpies equals \( W \) (neglecting losses), or \( (Q + W) \), in general, i.e., heat released from the system plus work done by the system. This is the most general statement of the first law of thermodynamics for open, steady state systems. The problem is that the binding energy does not appear anywhere in these equations: the free ATP and the free \( (\text{ADP} + \text{P}_i) \) have no binding energy associated with them! In fact, Nath shows that for the isothermal, cyclic process mediated by the enzyme, all thermodynamic property changes are necessarily zero, and the binding energy changes (e.g., those occurring during the \( \text{E} + \text{ATP} \rightarrow \text{E} \cdot \text{ATP} \) binding step) are internal to the system, as defined. Hence, he concludes that binding energy release due to the binding step cannot perform useful external work, \( W \), contradicting the fundamental tenet of the binding change mechanism. These contradictions of the first law of thermodynamics would not have appeared in an energy balance at a node or for a sub-system. By judiciously choosing the system (the entire molecular machine) and by performing the appropriate type of energy balance for his purposes (the overall balance), Nath has proved the violations of the laws of thermodynamics by extant mechanisms, which can now only be regarded as akin to scientific dogma.

Finally, it should be emphasized that none of the serious difficulties discussed above arise in the torsional mechanism. In fact, the torsional mechanism has been shown to pass the test of consistency with the laws of thermodynamics successfully and, it appears to us, triumphant.
how energy from discharge of proton and anion gradients across the F_0 domain is coupled with ATP synthesis in the F_1 domain. The torsional mechanism is consistent with data from biochemical, spectroscopic, microscopic imaging, structural and molecular biology experiments. The mechanism has also been analysed in detail from kinetic, thermodynamic and mechanistic perspectives. A unique aspect of the torsional mechanism is its ability to predict the dynamic roles of various components and subunits of ATP synthase.

A main feature of the torsional mechanism is the generation of torsion in the γ-subunit by rotation of the e-rotor of the F_0 domain in discrete steps of 30° (which are further divisible into rapid sub-steps of 15°), the γ-subunit being constrained from rotation on the F_1 domain end by interactions with the β-catalytic sites. The energy from discharge of the ion gradients through F_0 is thus accumulated as torsional energy in the γ-subunit of an F_0F_1-ATP synthase molecule (Figure 2). The torsion in the γ-subunit is released upon reaching the threshold strain (after four ion translocations) in a single 120° step, causing conformational changes in the β-subunits and thereby causing the binding of ADP (to form a closed site), followed by P_i binding (in loose conformation), ATP synthesis (during loose-to-tight transition), and finally release of bound ATP from the open conformation formed by interaction of the e-subunit with the tight β-subunit (Figure 3). The sequence of events in the torsional mechanism requires that all the catalytic sites have nucleotides bound during the actual catalysis step, as recently shown by the X-ray crystal structure of Menz et al. According to the torsional mechanism, all the elementary steps require energy, thus contradicting a central postulate of the binding change mechanism. A central feature in the torsional mechanism is the irreversible mode of catalysis by single ATP synthase molecules, whereas the binding change mechanism predicts reversible catalysis (with K_0 = 1 in the cata-
ytic site for the ATP synthesis step). This feature of the torsional mechanism has been validated in recent single-molecule spectroscopy experiments on F_1 and F_0 in both the hydrolysis and, recently, for the first time, in the synthesis mode. Finally, unlike the binding change mechanism, the torsional mechanism predicts product (ATP) release precedes substrate (ADP) binding, a result in agreement with the detailed kinetic analysis of the mechanism. Kinetic modelling of the F_1 portion also shows the absence of site–site cooperativity, which was one of the main tenets of the binding change mechanism.

Expatriation upon the recent X-ray single-molecule spectroscopy experiments in relation to the torsional mechanism is of interest. The torsional mechanism predicts three-site occupancy during catalysis, as discussed above, in contrast to the fundamental tenet of the binding change mechanism that postulates two-site occupancy. The recent X-ray structure solved by Walker’s group confirms this key prediction of the torsional mechanism. Moreover, in the new structure, ADP binds to the catalytic site with the least affinity, i.e. to the catalytic site that had remained unoccupied in the earlier structures. This fact, again, is in complete agreement with the central features of the torsional mechanism (Figure 3). In the single-molecule FRET studies of Diez et al. on F_1F_0 in the synthesis mode, donor and acceptor fluorophores were coupled to the bottom of γ and the β-subunit of ATP synthase, respectively. FRET efficiencies were used to compute the distance between the fluorophores. These studies showed the existence of three distinct FRET levels corresponding to three discrete positions of γ (relative to the β-subunit) in the three conformations of β, as proposed by the torsional mechanism. The torsional mechanism goes further, in that it associates and pairs the observed FRET states with the distinct catalytic site conformations of the enzyme, a goal the single-molecule experiments by themselves have not been able to achieve. The experimental studies support the idea of step-wise rotation of the γ-subunit rather than its free rotation. Moreover, broadening of the number of F_1/F_0 distributions (Figure 4 in Diez et al.) during ATP synthesis by F_1F_0-ATP synthase (compared to ATP hydrolysis by F_1F_0-ATPase) reveals the existence of sub-steps in the rotation of the bottom of γ (Figure 2), exactly as predicted by the torsional mechanism over five years ago. A future challenge for experimentalists would be to fine-tune the resolution of their techniques and validate the exact number of sub-steps predicted by the torsional mechanism. In addition, absence of major fluctuations caused by Brownian motion in the experiments rejects hypotheses that attribute a dominant driving role in the ATP synthase mechanism to thermal fluctuations; a
unidirectional force due to electrostatic interactions between rotor and stator as has been postulated by the torsional mechanism since the beginning\textsuperscript{6,8-10}, explains the observations completely. In conclusion, recent experiments appear to establish the torsional mechanism of ATP synthesis.

Kinetic modelling of the F\textsubscript{0} portion of ATP synthase shows release of a proton from the trailing c-subunit followed by binding of a proton to the leading c-subunit without involving any kind of cooperativity, as has been proposed by some researchers. Instead, the rate of ATP synthesis follows Michaelis–Menten kinetics. Furthermore, determination of the dependence of the rate of ATP synthesis on the proton concentration in the mitochondrial or chloroplast compartments shows the macroscopic driving forces (\(\Delta \Phi\) and \(\Delta \Psi\)) are not kinetically equivalent. That is, the rate of ATP synthesis is different when \(\Delta \Phi\) is replaced by a chemiosmotically equivalent \(\Delta \Psi\). In fact, there may not be any ATP synthesis in the absence of either one of the two driving forces. The kinetic inequivalence arises because \(\Delta \Phi\) comes from the proton gradient across the membrane whereas \(\Delta \Psi\) is created only in the vicinity of the ATP synthase molecule by the anion (or counterion) in specifically designed experiments, e.g. with K\textsuperscript{+}-valinomycin) gradient. The ions are transported across the membrane in a locally electrogenic but overall electroneutral mode that causes a transient, local \(\Delta \Psi\) that can be measured according to the Nernst equation with K\textsuperscript{+}-valinomycin; but the overall electrical potential across the membrane is close to zero as measured with microelectrodes by Tedeschi and colleagues\textsuperscript{44}. The variation in the K\textsuperscript{+}/ATP ratio from 0 to 4 and the requirement of membrane-permeable anions for ATP synthesis are naturally explained by this mode of ion transport. The torsional mechanism also provides insights into the rotation and conformational changes occurring in the c-rotor upon binding and unbinding of protons that disturb the electrostatic equilibrium at the a-stator–c-rotor interface, causing the c-rotor to rotate and attain a new local equilibrium position. Energy is stored during this process in the c-subunits of F\textsubscript{0} via a detailed rotation–twist–tilt mechanism. These details had proved difficult to unravel despite decades of research.

The torsional mechanism has a solid foundation in nonequilibrium thermodynamics and kinetics\textsuperscript{5,7}. Ideal, mechanistic P/O ratios of 2.4 and 1.6 predicted respectively, for 3-hydroxybutyrate and succinate agree with experimentally obtained values. The absence of large (\(\approx 200 \text{ mV}\)) transmembrane potentials and the presence of localized small potentials predicted by the torsional mechanism agree with experimental data. The number of anions and cations transported per ATP synthesized and the close coupling of anion and cation transport are further predictions in agreement with the torsional mechanism that arises from nonequilibrium thermodynamic considerations. This treatment of the torsional mechanism thus shows that macroscopic thermodynamics and kinetics of biological systems can be derived from knowledge of molecular mechanisms. Such a transition from the local to the global level in biology is a requirement in proving that a mechanism is accurate and is thus a significant achievement of the torsional mechanism.

Several other biological systems are ripe to be studied by the methods used to elucidate the torsional mechanism of ATP synthase. Innovative application of the same methods by a team of interdisciplinary researchers from biochemistry, microbiology, molecular biology, chemistry and engineering would allow deeper understanding of the mechanisms of DNA translation, protein synthesis and transport, cell division and motility, movement of kinesin on microtubules, work done by muscles, catalytic and anabolic coupling and many other cellular processes involving energy transduction by macromolecular assemblies. The cell, even though a highly complex interacting system\textsuperscript{15}, has the same chemical reactions and mechan-