Beyond the Chemiosmotic Theory: Analysis of Key Fundamental Aspects of Energy Coupling in Oxidative Phosphorylation in the Light of a Torsional Mechanism of Energy Transduction and ATP Synthesis— Invited Review Part 2

Sunil Nath

Abstract The core of this second article shows how logical errors and inconsistencies in previous theories of energy coupling in oxidative phosphorylation are overcome by use of a torsional mechanism and the unified theory of ATP synthesis/hydrolysis. The torsional mechanism is shown to satisfy the pioneering and verified features of previous mechanisms. A considerable amount of data is identified that is incompatible with older theories but is now explained in a logically consistent and unified way. Key deficiencies in older theories are pinpointed and their resolution elucidated. Finally, major differences between old and new approaches are tabulated. The new theory now provides the elusive details of energy coupling and transduction, and allows several novel and experimentally verifiable predictions to be made and a considerable number of applications in nanotechnology, energy conversion, systems biology, and in health and disease are foreseen.

Keywords Bioenergetics · F_1F_O-ATP synthase · Oxidative Phosphorylation · Photosynthesis and photophosphorylation · Mitochondria · Energy transduction · Chemiosmotic theory · Torsional mechanism · Coupling · Membrane and ion transport · Electrogenic · Electroneutral · Valinomycin · Uncoupler · Unified theory of ATP synthesis and hydrolysis

S. Nath (✉)
Department of Biochemical Engineering and Biotechnology,
Indian Institute of Technology, Delhi,
Hauz Khas,
New Delhi 110016, India
e-mail: sunath@dbeb.iitd.ernet.in
e-mail: sunil_nath_iit@yahoo.com

Introduction

Part 1 of this Invited Review has explained how the quest for a comprehensive understanding of the link between energy-generating and energy-utilizing processes in the cell has generated an immense amount of research. In Part 2 the complex issues are further investigated and it is shown how the inconsistencies in previous theories of energy coupling are satisfactorily removed by a torsional mechanism of energy transduction and ATP synthesis, and the subsequent unified theory.

An alternative molecular mechanism of ATP synthesis, named the torsional mechanism of energy transduction and ATP synthesis has been formulated and developed in detail during the past 15 years (Nath 2008 and see Part 1 (Nath 2010) for a comprehensive list of relevant publications). The new mechanism is based on sound theoretical principles (that include those of nonequilibrium energy transduction and storage, nonequilibrium thermodynamics and kinetics, the occurrence of conservative nonequilibrium processes by a mode of ion translocation and dynamics involving displacement from and regaining of electrical neutrality in condensed phases, consistency with the laws of thermodynamics, a direct and local energy transduction, a fast mechanical mode of operation of the molecular machine quantifiable by the theory of elasticity, a key role of membrane elements in energy coupling and a lack of equilibration or presence of slow exchange of the mechanical degrees of freedom of the molecular machine with the thermal degrees of freedom of the surrounding reservoir and the central importance of timescales, the pioneering and innovative application of engineering principles to biology at the molecular and sub-molecular level, and the consid-

eration together of ATP generation and utilization in a unified theory).

The original proposal and its logical development resolve fundamental issues in biological energy transduction and offer new ways of analyzing and interpreting the plethora of experimental data. Recently, using a novel systems biology/engineering approach, the rationale has been advanced as a powerful unifying concept in bioenergetics and motility through the formulation in detail of the unified theory of ATP synthesis and hydrolysis (Nath 2008 and references therein; Nath and Nath 2009). This has resulted in an extension to other related energy transductions such as those that occur in the fundamental processes of muscle contraction and in intracellular transport by diverse cargo-carrying processive motors.

Explanation of the overall coupling concept postulated by the new theory, removal of inconsistencies, and solution of longstanding problems in biological energy coupling by the torsional mechanism of energy transduction and ATP synthesis

The observations of massive, valinomycin-induced uptake of K^+ in mitochondria first noted over four decades ago (Pressman 1965; Pressman et al. 1967; Brierley 1970), a phenomenon taken advantage of by Mitchell and Moyle in their experiments analyzed in Part 1, and the transport of dicarboxylic acids such as succinate and malate (Quagliariello et al. 1969) are *inconsistent* with a key postulate of the chemiosmotic theory, maintained throughout from inception, that of the low permeability of the coupling membrane to solutes and ions (Mitchell 1966; Mitchell 1979; Nath 2002; Nath 2003; Nath 2004). This phenomenon of large uptake/translocation of these ions necessitates modification of the fundamental postulates of chemiosmosis or requires the proposal of a new theory of energy coupling that can accommodate these longstanding observations, phenomena that have been reconfirmed by researchers from subsequent generations (Kaim and Dimroth 1999; Nath 2004; Nath 2006; Nath 2008) in various experimental systems and must now be regarded as facts. However, the torsional mechanism of energy transduction and ATP synthesis (Nath 2008 and references therein) and the unified theory of ATP synthesis/hydrolysis (Nath 2008; Nath and Nath 2009) is the only mechanism to address these fundamental aspects and propose a novel solution to the problem that is consistent with these facts.

The torsional mechanism solves the conundrum by postulating the existence of a specific, regulated anion access channel (constructed as an anion binding pocket in the a-subunit of the F_O portion of ATP synthase at the a-c lipid-water interface) in the vicinity of the proton access channel (with its binding site in the c-subunit of F_O at the a-c interface). Both proton and anion (for example, succinate monoanion) translocate through their respective access pathways in the direction of their electrochemical potentials and provide ~50% of the energy to produce torque on the c-oligomer and eventually synthesize ATP (Nath 2008 and references therein). Thus in the *overall* sense, the sum of the electrochemical potentials of proton and anion, i.e., $(\Delta\mu_H^+ + \Delta\mu_A^-)$ provides the energy and driving force to synthesize ATP. However, according to the postulates of the new paradigm, the translocations of anion and proton are not simultaneous but rather, *ordered and sequential*. On the ATPase side, A^- translocation is primary and precedes H^+ translocation (and vice-versa on the redox or light side). New terminology [e.g. symsequenceport (for the case of anion translocation in the same direction as the proton) and antisequenceport (for the case of cation translocation, for instance, K^+ translocation in the presence of valinomycin, opposite to the direction of proton movement)] was coined to illustrate these new concepts and avoid confusion with older terms. In the new framework, a *local* electrical potential, $\Delta\psi$, is created in the access channels in the vicinity of the binding sites due to discrete acts of ion translocation and ion binding and unbinding events, to be distinguished from the very different character and location of the *delocalized* $\Delta\phi$ of the chemiosmotic theory across bulk aqueous phases. This local electrical potential is transient, created first by the translocation of the primary ion $(\Delta\psi_{A^-/C^+})$ by the process of ion-protein interactions in each half-access channel, and measures ~45 mV. The translocation of the secondary ion contributes its own potential $(\Delta\psi_H^+)$ of approximately the same magnitude (~45 mV in its half-access channel that lies contiguous to the anion access pathway at the a-c interface) and provides its own energy and further, it also destroys, through destructive interference, the electrical field created by the primary translocation. This change in electrical potential $\Delta(\Delta\psi)$ (~90 mV in all in each entry and exit half-access channel) is transduced to a torque of the c-oligomer and subsequently to torsional energy in the γ-subunit in the F_1 portion of ATP synthase as described in consummate detail by the torsional mechanism (Nath 2008; Nath 2010). The same process of energy transduction occurs for each ion binding and unbinding event in each entry and exit access channel for the two ions, donating a total of ~90 +~90= ~180 meV of energy per translocation of anion (or countercation) and proton. Ten such ion translocations provide the energy to make three molecules of ATP in mitochondrial ATP synthase, as explained in great detail in the unified theory of ATP synthesis (Nath 2008), i.e. ~600 meV per ATP. For mitochondria and chloroplasts, the in vivo case is considered by the torsional mechanism and the unified theory to be that of anion and proton translocation in sequence. However, monovalent countercation movement

(of K^+ or Na^+) for example in certain bacterial systems, or in vitro in the presence of an ionophore such as valinomycin—instead of monoanion movement—is electrically equivalent to the monoanion case, and both alternatives have been clearly described in the new theory (Nath 2002; Nath 2003; Nath 2004). The detailed molecular mechanism has been proved to work for both symmetry and symmetry-mismatch cases and for various stoichiometries in oxidative phosphorylation (Nath 1998; Nath et al. 1999; Nath 2008). [The interested reader is referred to the original papers on the torsional mechanism of energy transduction and ATP synthesis and the unified theory of ATP synthesis/hydrolysis for a detailed qualitative as well as quantitative description of the molecular mechanism, thermodynamics and kinetics of ATP synthesis. The description and approach of each paper as well as of this work is original and different from one another and the reader can decide for himself or herself which type of verbal or mathematical perspective/commentary appeals to him or her.]

This section has attempted to summarize how the new conceptual thinking on energy coupling through the formulation of the torsional mechanism of energy transduction and ATP synthesis and the unified theory of ATP synthesis/hydrolysis remove the inconsistencies and lacunae present in previous theories by including transport of a second ionic species. In other words, the flow of both anion/countercation and proton are *coupled* to each other, and each provides approximately half the energy to synthesize ATP. This implies that the proton gradient/protonmotive force is not the *sole* high-energy intermediate or the *sole* driving force in the process of oxidative phosphorylation and calls into question Mitchell's view of proticity and uncompensated electrogenic translocation of an ion (e.g. H^+) from one bulk phase to another until a delocalized electrical field of a large magnitude is created in the entire organelle. According to an earlier estimate (in agreement with our own calculations), an electrogenic (i.e., uncompensated by any other ion transport) charge transfer (efflux) of ~1×10^{-6} mol H^+/g protein is required to establish a delocalized $\Delta\phi$ of ~200 mV by the chemiosmotic process in rat liver mitochondria (Mitchell 1966). Such a massive uncompensated H^+ efflux in the absence of transport of other ions has never been experimentally demonstrated, though one would have expected such a demonstration to be fairly routine using today's advanced instrumentation. Using classical morphological data that 1 g protein contains 7.2×10^{12} mitochondria, this translates into an electrogenic transfer of $\{1 \times 10^{-6} \times 6 \times 10^{23}\}/7.2 \times 10^{12}$ protons (83,333 H^+) per mitochondrion in order to generate a delocalized $\Delta\phi$ of ~200 mV (Nath 2002), corresponding to a huge electrical field at every point in the organelle of ~4.5×10^5 V cm^{-1}. Translocation of a single H^+ ion will generate only a negligible bulk-to-bulk

delocalized $\Delta\phi$ in the chemiosmotic model. However, transfer of 83,333 protons requires an astronomical energy input of ~10^{10} meV in order to violate electrical neutrality of bulk aqueous media to this extraordinarily large extent, as proved from first principles (Nath 2004; Jain et al. 2004). Succinctly, this is because the electrical field generated due to the translocated ions will oppose the continued movement of more ions of the same charged species, and each subsequent act of discrete ion translocation will require a greater and greater expenditure of energy, and very soon the electron transport chain does not have the competence to supply this large amount of energy, and the transport will cease. In other words, such large-scale departure from electrical neutrality of bulk aqueous phases cannot be sustained for a long time, and hence the delocalized driving force postulated by chemiosmosis cannot be built up in the first place.

As discussed above, the fundamental difficulty for chemiosmosis then is that to separate charge to the extremely large extent postulated by it in order to create the presumed delocalized driving force requires an enormous amount of energy in violation of the first law of thermodynamics (Nath 2004) and the principle of electrical neutrality of a condensed phase. A small amount of charge separation (e.g. by one quantum or a few quanta of H^+ charge) can readily occur, but then we have a huge shortfall in the driving force postulated by chemiosmosis because of the creation of only a negligible delocalized $\Delta\phi$. This energetic shortfall can only be met by the provision of thermal energy, but since the process of biological energy transduction takes place under isothermal conditions, the second law of thermodynamics would also be violated, and the situation is similar to the one already treated elsewhere (Nath 2008). Hence the dilemma is: either create sufficient driving force but break the universal laws of science, or save the laws but create only a grossly inadequate driving force for ATP synthesis. On the contrary, in the dynamically electrogenic but overall electroneutral mode of ion translocation postulated by the torsional mechanism and the unified theory, electroneutrality is violated only locally and transiently, and that too by the smallest possible quantum (e.g., one ionic charge). Further, we can perform useful work with small $\Delta\mu$ values, and we do not need to work against large heads, which should lead to far greater efficiencies of energy transfer than in chemiosmosis. Moreover, in the new paradigm, the driving force is produced in situ and useful work is done at the site where it is needed. It also enables us to satisfy the principle of electrical neutrality of bulk aqueous phases and maintain consistency with both the first and second laws of thermodynamics. By defining the driving forces to act at a molecular level in the access half-channels rather than macroscopically in bulk aqueous phases across the "insu-

lator" of the coupling membrane as conceived by chemiosmosis, and by incorporating the essential role of the anion/countercation in energy coupling, these new developments (Nath 2002; Nath 2003; Nath 2004; Nath 2008) also go beyond the concept of coupling proposed by the chemiosmotic theory at the physiological level (Mitchell 1979). The new coupling concept at the overall physiological level according to the torsional mechanism of energy transduction and ATP synthesis for the process of oxidative phosphorylation is illustrated diagrammatically in Fig. 1.

Another major assumption of the chemiosmotic theory, that the coupling membrane is a mere barrier that undergoes no conformational change and does not itself take part in energy coupling has been questioned earlier (Williams 1979; Nath and Jain 2002; Nath 2006). The assumption runs contrary to our current knowledge of the dynamics of membranes. The assumption of an inert membrane permitted biological energy coupling to be treated using *equilibrium* thermodynamics and to formulate the problem using ΔpH and $\Delta \phi$, with the symbol Δ referring to differences
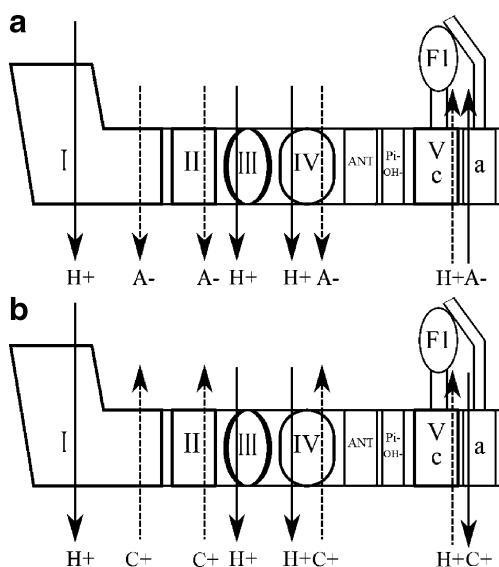

Fig. 1 Energy coupling in oxidative phosphorylation. **a** The overall coupling process in oxidative phosphorylation in mitochondria and the elementary anion (A^-) and proton (H^+) translocation events at the redox and ATPase coupling sites according to the torsional mechanism of energy transduction and ATP synthesis. Primary translocations are represented by bold arrows, and secondary translocations by dashed arrows. The stoichiometries of the oxidative phosphorylation complexes I–V and the involvement of supercomplex formation do not alter, in principle, the overall mechanistic picture of coupling depicted in the diagram, as long as electrons move unidirectionally down the respiratory chain. **b** In other systems such as certain bacteria, the anion translocation (A^-) event is replaced by countercation (C^+) translocation of Na^+ or K^+ in a direction opposite to the direction of proton (H^+) translocation. Primary translocations are denoted by bold arrows, and secondary translocations by dashed arrows

across bulk aqueous phases, and to experimentally measure these quantities across the membrane. However, the aqueous pathways of a transporter such as the F_1F_O-ATP synthase are such that the two bulk phases are not in communication with each other at any instant of time; rather, the inner bulk phase communicates with the ion binding site at one instant of time, while the outer bulk phase interacts with the binding site at another instant of time. The two snapshots are separated by the time it takes the c-rotor to rotate by 18° (taking the number of c-subunits equal to ten for animal mitochondria) in such an alternating access-type system. The kinetics of such a system are characterized by two independent variables (any two among pH_{in}, pH_{out} and ΔpH can be selected), as shown by the development of a detailed kinetic model (Jain and Nath 2000), and not just a single variable such as the ΔpH across bulk aqueous phases, as envisaged by chemiosmosis. Characterizing such a system by the ΔpH has no physical meaning, since there is no aqueous pathway directly connecting the two bulk phases. Moreover, there is a concentration gradient of protons and anions along which the transport of these species takes place and hence the situation is inherently *nonequilibrium* in nature, and simple equilibrium relations of the type used earlier do not hold. Further, according to the torsional mechanism, the α-helical elements of the c-oligomer exhibit nonequilibrium conformational states that store energy as twist due to the phenomenon of ion-protein interactions and participate in a dynamical conformational cycle that is central to energy coupling (Nath 2002; Nath and Jain 2002; Nath 2004; Nath 2008). Kinetics, mechanics and nonequilibrium thermodynamics are the appropriate frameworks to model such processes (Nath 1998; Nath 2002; Nath 2003).

Finally, it should be emphasized that in the new paradigm, the energy transduction is *direct and local* in the access channels at the aqueous-lipid interfaces of F_O, whose structure itself contains the transducers for addition and collaborative utilization of energies of discrete anion (or countercation) and proton translocations, and for regulation of transport and cell metabolism. Thus, the so-called "protonmotive force" as an *indirect, long-range messenger* conceived in the chemiosmotic theory is not needed, and in fact, such an entity, as defined by Eq. (1) (Nath 2010; Invited Review Part 1) and obtained by incessant electrogenic translocation solely of a single ion (e.g., H^+) until a *delocalized* bulk-to-bulk electrical potential of ~200 mV is created simply does not exist. The detailed explanations contained in the comprehensive theory of the torsional mechanism of the role of membrane elements in a lipophilic region (Nath 2002; Nath 2008) lead to deeper understanding and offer a more realistic and complete picture of biological energy transduction and coupling than considering the bilayer as "mere insulation".

Consideration of the source of the chief logical deficiences in chemiosmotic dogma in general in the light of the torsional mechanism

The above sections have discussed a number of problems associated with chemiosmosis and their resolution in the light of the torsional mechanism of energy transduction and ATP synthesis. It will be instructive to understand the source of these in chemiosmotic dogma in general. To an extent, they arise because the electrical potential, $\Delta\phi$, is presumed to be delocalized due to ion distribution (e.g., K^+ in the presence of valinomycin) across bulk aqueous media, while in reality, the electrical potential is localized in the aqueous access channel, due to a single ion that binds to its site in the channel (e.g. at the a-c interface in the F_O portion of ATP synthase) or unbinds from it. This localized electrical potential, $\Delta\psi$, has no relationship with the K^+ ions remaining in the outer bulk aqueous phase: the value of this localized potential is the *same* irrespective of whether more K^+ ions remain in the outer bulk aqueous phase, or fewer remain. If the magnitude of the presumed delocalized $\Delta\phi$ is calculated from the values of K^+ concentration in bulk aqueous phases, as in chemiosmosis, then it is clear that the calculated value of $\Delta\phi$ will change with the K^+ concentration outside, as analyzed and discussed at length in Part 1 of this work (Nath 2010). (As shown there, one can obtain whatever value one wishes for $\Delta\phi$ by changing the K_{out}^+ concentration because Eq. (2) in Part 1 of the Invited Review (Nath 2010) has infinite solutions). However, this has no correspondence with the real physical situation, what is actually happening, as clarified by the torsional mechanism. The torsional mechanism explains the in vivo case (in the absence of the antibiotic) in mitochondria as that of anion translocation; the in vitro case of K^+ in the presence of valinomycin is somewhat harder to explain, but it is quite possible that the a-c interfaces in the F_O portion of Complex V ATP synthase transporter (or at the analogue of such interfaces in Complex I or Complex IV transporter in mitochondria in the 1969 experiments of Mitchell and Moyle) catalyze the antisequenceport of K^+/H^+ (in the absence of the true permeant anion, e.g., succinate, in the assays) and valinomycin increases the accessibility of such transporters. A similar explanation has been suggested before by Massari and Azzone (1970), and recently, by Tedeschi (2005). However, going beyond the previous suggestions, it is proposed here that the identities of these antisequenceporters are the very complexes involved in mitochondrial oxidative phosphorylation (for example, Complexes I, IV, and V), since symsequenceport or antisequenceport is the intrinsic function of these transporters according to the torsional mechanism (Fig. 1).

Another major concern in the chemiosmotic approach (Mitchell and Moyle 1967; Mitchell and Moyle 1969) is the central assumption (which is what was required to be proved or disproved by it) that the translocation of H^+ in mitochondria is electrogenic and without other parallel anion or cation movements. However, the classical work of Chance and Mela (1966) shows that H^+ efflux occurs only in the presence of cation uptake. Further, the addition of oxygen pulses to anaerobic mitochondria leads to H^+ efflux of lower than the actual stoichiometry (Mitchell and Moyle 1967; Pozzan and Azzone 1976; Gould and Cramer 1977). In fact, the minimum amount of proton extrusion cannot be experimentally detected without a co-transport of permeant anions or a counter-transport of permeant cations. Hence, there is no experimental support for a steady state, electrogenic, unbalanced movement of charges as assumed in the chemiosmotic theory. Tedeschi (2005) also makes this important point, but asserts that no $\Delta\psi$ is created, probably because his measurements with microelectrodes inserted into giant mitochondria detected almost zero potential (Tupper and Tedeschi 1969). A transient, local $\Delta\psi$ will be created by the discrete translocation of the primary ion, which can then be collapsed by movement of the secondary ion by the dynamically electrogenic but overall electroneutral mode of ion translocation favored by the torsional mechanism. However, this does not mean that there is no $\Delta\psi$, as claimed by Tedeschi (2005). In fact, with an oppositely-charged secondary ion compared to H^+, or with a similarly-charged secondary ion as H^+ but moving in the opposite direction serving as a measuring "electrode" (a fast measurement), this local $\Delta\psi$ can be detected because the secondary ion, if permeant (e.g., K^+ in the presence of valinomycin), can move and destroy the local potential created by the primary ion (e.g., H^+). However, there will be no electrical potential detected in the steady state or after both the ions have been translocated (e.g., if the time resolution of the measurement is low, as in measurements with microelectrodes), because the molecular mechanism is overall electroneutral. Hence both the poles of experimental data that have caused considerable disagreement historically in bioenergetics are readily explained by the torsional mechanism.

Finally, we return to the paper of Mitchell and Moyle (1969). The authors themselves state that, "The quantities of K^+ and H^+ ion passing across the M phase were equal within experimental error," (p. 477), and again on p. 478, "The entry of K^+ ions through the M phase is almost exactly equivalent to the outward translocation of H^+ ions." In other words, a 1:1 H^+–K^+ exchange was detected in the original work. The logical error or fallacy made, as explained above, is the assumption that in their experimental system, only a single ion (H^+) moves electrogenically, and that the movement of K^+ in the presence of valinomycin is only a *means* to measure this sole electrogenic H^+ flow and quantify it, and that in the real

situation in mitochondria, the counter-flow of K^+ (or the co-flow of a permeant anion) does not occur. In conclusion, the fact that a second ion, in addition to H^+, may also be translocated in the real energy-transducing system, donate energy, and participate in energy coupling was not realized by the proponents of the chemiosmotic theory. Thus, the theory postulates a role in coupling and provision of driving force to make ATP by a single ion (H^+) only, and in fact, the proton is conceived as the *sole* link between oxidation and phosphorylation; yet, experimental demonstration of this role has always required a second ion (e.g., K^+). Hence the possibility that the second ion also plays a key role in energy transduction and coupling was failed to be given its due recognition and, in fact, could not have been ruled out from the experiments. The fundamental advances made in the new paradigm take us beyond the chemiosmotic theory. In our opinion, the aspects dealt with by the torsional mechanism of energy transduction and ATP synthesis and the unified theory of ATP synthesis/hydrolysis constitute the key elements whose lack of detailed consideration has held back the progress of research in the important interdisciplinary and multidisciplinary field of bioenergetics.

Novel predictions and applications of the torsional mechanism of energy transduction and ATP synthesis and the unified theory

Green (1981) has leveled the serious accusation at the chemiosmotic theory of indirect coupling that it has not led to "any important predictions" or correct new insights and has criticized its "sterile predictive record" over several decades. The torsional mechanism of energy transduction and ATP synthesis cannot be the target of such an attack as it has provided a large number of experimentally verifiable predictions. In fact, fifteen novel predictions of the mechanism have been meticulously listed in a previous publication (Nath 2002). The salient differences between the torsional mechanism and the binding change mechanism have also been covered in detail (Nath 2003; Nath 2008; Nath and Nath 2009). Novel applications of the torsional mechanism to the design of second-generation machines that can directly convert energy from one form to another without an intervening thermal step, thereby leading to ultra-high efficiencies of energy conversion have been detailed (Nath 2003). Applications in nanotechnology, and in the field of mechanochemical energy conversion have been further explored, and a prototype of such a machine has been fabricated to illustrate the new design principles (Nath 2003; see also the review by the Hansen group (Jain et al. 2004)). A futuristic (but concrete) solution to the energy crisis has also been proposed (Nath 2003). Novel applications in the field of systems biology and engineering have been shown (Nath 2006). Recently, innovative application of the new mechanistic principles in the unified theory to health and disease has been made and dealt with in great detail (Nath 2008).

Conclusions

Key fundamental aspects of energy coupling in oxidative phosphorylation have been further analyzed from first principles. It has been shown in Part 1 (Nath 2010) that the equation used to calculate the values of the delocalized electrical potential, $\Delta\phi$, does not possess a unique solution, a property that places great limitations on the approach adopted by chemiosmosis (Mitchell and Moyle (1969)). It has been concluded from our analysis that re-interpretation of the nature of the ion gradients/electrical potential is necessary. The longstanding observations of massive energy-linked valinomycin-induced uptake of potassium by mitochondria, and the permeability of mitochondria to dicarboxylic acids and other anions have been shown to contradict a central postulate of the chemiosmotic theory. It has been shown in considerable detail how the new concepts in the torsional mechanism of energy transduction and ATP synthesis overcome the above difficulties in a natural way.

Other fundamental problems inherent in earlier approaches, such as the unprecedented extent of charge separation and violation of the principle of electrical neutrality of bulk aqueous media, have been solved. It has been explained in great detail how energy transduction by a dynamically electrogenic but overall electroneutral mode of ion transport involving sequential translocation of membrane-permeable succinate monoanion (or Na^+ or K^+ countercation) and proton postulated by the torsional mechanism removes fundamental difficulties. The large number of merits of the new paradigm, for instance the greater efficiencies of energy transfer, and, above all, the *transmission* of energy of the ion gradients to the ion-binding sites in the coupling device of F_O in the F_1F_O-ATP synthase have been emphasized; these advantages have been shown to be unavailable in models based on purely delocalized coupling. The equilibrium relations used in chemiosmosis and the very nature of energy transduction at equilibrium postulated in the chemiosmotic theory have been shown to be inadequate for a true understanding of biological energy coupling. The novel nonequilibrium concepts and approaches formulated in the torsional mechanism of energy transduction and the unified theory of ATP synthesis and hydrolysis, and the inclusion, in the new alternative theory, of the inherently nonequilibrium

Table 1 The major differences between the torsional mechanism of energy transduction and ATP synthesis and the chemiosmotic theory. Revised, updated and enlarged for this work from Nath (2003) with the kind permission of Springer Science

Chemiosmosis	Torsional mechanism
$\Delta\mu_H$ (or Δp) is the driving force for ATP synthesis	$\Delta\mu_H$ (or Δp) is not the true driving force for ATP synthesis. ΔpH and ΔpA are the overall driving forces for the oxidative phosphorylation process. The anion/countercation gradient is converted to a $\Delta\psi$; hence, at this level, ΔpH and $\Delta\psi$ are the driving forces for ATP synthesis
$\Delta\phi$ and $\Delta\mu_H$ are delocalized and in bulk aqueous phases	ΔpH and ΔpA are delocalized but $\Delta\psi$ is localized to a-c access channels in F_O
ΔpH and $\Delta\phi$ are equivalent and additive	ΔpH and $\Delta\psi$ are kinetically inequivalent driving forces that each affect the rate of ATP synthesis independently of the other
A decrease in ΔpH is compensated exactly by an increase in $\Delta\phi$ and vice-versa	Need not be so because each is a separate entity created by an independent source
Ion-well; $\Delta\phi$ is converted to ΔpH	Not so; Δp(anion/countercation) is converted to $\Delta\psi$ and then both $\Delta\psi$ and ΔpH create a $\Delta(\Delta\psi)$ by ion-protein interactions
H^+ is primary and generates $\Delta\phi$	During ATP synthesis, anion/countercation generates $\Delta\psi$ and precedes H^+ translocation and is primary in that sense. Both proton as well as anion/countercation contribute ~half the energy required for ATP synthesis
Energy flow is confined to protons; no role of anions/countercations in energy coupling	Role of anions/countercations in energy coupling explained
Counterion gradients always dissipate $\Delta\phi$	Not necessarily so; counterion gradients can even generate $\Delta\psi$
K^+ distributes passively in response to $\Delta\phi$ created by H^+ transport	K^+-valinomycin creates a transient, local $\Delta\psi$ that is utilized by H^+ antisequenceport
Electrogenic and violates electroneutrality in the bulk aqueous phases	Dynamically electrogenic but overall electroneutral; does not violate overall electroneutrality
Interchangeability between $\Delta\phi$ and ΔpH	Lack of interchangeability between $\Delta\psi$ and ΔpH
Indirect coupling. $\Delta\mu_H$ or $\Delta\phi$ serves as an indirect long-range messenger	Coupling is direct and local with no need for an indirect, long-range messenger such as the so-called "protonmotive force" Δp, or the delocalized potential, $\Delta\phi$
$\Delta\phi$ is ~180 mV in state 4	The calculation of $\Delta\phi$ must be interpreted with caution as the equation used has multiple solutions, as shown in Nath (2010). No substantial local $\Delta\psi$ in state 4
Membrane is just an insulator	Cyclical dynamic changes take place in membrane constituents during energy transduction; the membrane plays a key mechanical, electrical and chemical role and participates in ion-protein interactions
Chemiosmotic	Mechano(electro)chemical
Equilibrium theory	Nonequilibrium theory capturing energy transduction dynamics
Macroscopic	Energy is stored as macroscopic ion gradients, but molecular interactions between ion and protein-in-the-membrane are key to energy transduction and utilization. Torque generation in the c-rotor of F_O is a result of change in local electrostatic potential, $\Delta(\Delta\psi)$ brought about by the ion gradients
Redox loop; H^+/O per site = 2; H^+/ATP = 2	Ion pumps; In mitochondria, H^+/O for sites I, III, IV=4, 2, and 4 respectively, i.e., H^+/O = 10 overall; H^+/ATP=3.333. Similar numbers hold for A^-/O and A^-/ATP in mitochondria (Nath 2008).
Role of various uncouplers explained only as dissipators of $\Delta\mu_H$	Anionic uncouplers compete with substrates for entry into the a-subunit access channel in F_O but due to its lipid solubility the U^- anion recombines with the H^+ in the vicinity of the binding site at the a-c interface to form neutral UH. Thus, the uncoupling mechanism is more complex and subtle as detailed in this work+explained as interfering with conformational transitions in F_O
Does not explain the occurrence of uncoupler-resistant mutants	Satisfactorily explains the occurrence of uncoupler-resistant mutants
$\Delta\phi=[(RT/F)\ln(K_{in}^+/K_{out}^+)]$	The equation is only a measure of macroscopic energy; increase in $\Delta\phi$ does not mean greater driving force per molecule. The $\Delta\psi$ per ATP synthase molecule still remains the same. At higher $\Delta\phi$, more enzyme molecules are capable of synthesis and diffusion potential is created in the vicinity of more enzyme molecules that can then be utilized by proton translocation, among other possibilities
Protons participate directly in ATP synthesis	Conformational; protons do not participate directly at the F_1 catalytic site in synthesis
No real molecular mechanism coupling $\Delta\mu_H$ and ATP synthesis presented	Detailed molecular mechanism coupling ion gradients to ATP synthesis proposed
Analogy with a fuel cell	Analogy with an enthalpic nonequilibrium molecular machine

concentration gradients and nonequilibrium conformational states of membrane and extra-membrane elements of the ATP synthase that arise from ion-protein interactions in the F_O portion have been shown to be essential for a complete understanding of these fundamental energy transduction processes.

As mentioned above, the detailed explanations contained in the comprehensive theory of the torsional mechanism of the role of membrane elements in a lipophilic region have been shown to lead to deeper understanding and to offer a more realistic and complete picture of biological energy transduction and coupling than considering the bilayer as "mere insulation". The transduction process itself has been emphasized in the new paradigm to be direct and local, and hence it has been shown that there is no need for an indirect, long-range delocalized messenger such as Δp, the protonmotive force, created by uncompensated, electrogenic proton translocation. It has been summarized how the new insights question the veracity of the chemiosmotic concept of coupling at the physiological-cum-biochemical level and reveal the physical unsoundness of proticity as the sole coupling agent and driving force. The new coupling concept at the overall physiological-cum-biochemical level according to the torsional mechanism of energy transduction and ATP synthesis for the process of mitochondrial oxidative phosphorylation has been depicted diagrammatically (Fig. 1).

Finally, the fundamental source of the logical deficiences in the old paradigms of energy transduction has been explored. The conclusion has been reached that demonstration of the coupling role of the proton has always required a *second* ion (e.g. K^+, or a membrane-permeable anion like succinate) in the experimental design; hence the possibility that the second ion is also translocated and contributes to energy coupling cannot be logically ruled out from the experiments.

Hence taken together with our previous work, the major differences between the torsional mechanism and chemiosmosis can now be conveniently summarized (Table 1). The new theory has been shown to explain all the poles of experimental data and rationalize the elusive details of biological energy coupling. As such these fundamental advances take us beyond the chemiosmotic theory and provide both a mechanism of energy transduction and ATP synthesis and a unified theory of ATP synthesis/hydrolysis that constitutes the key elements whose lack of detailed consideration has held back the progress of research. It is now ideally poised to guide the design of new experiments, rationalize and interpret experimental data, and catalyze the progress of future research in bioenergetics.

References

Brierley GP (1970) Energy-linked alteration of the permeability of heart mitochondria to chloride and other anions. Biochemistry 9:697–707

Chance B, Mela L (1966) A hydrogen ion concentration gradient in a mitochondrial membrane. Nature 212:369–372

Gould JM, Cramer WA (1977) Relationship between oxygen-induced proton efflux and membrane energization in cells of *Escherichia coli*. J Biol Chem 252:5875–5882

Green DE (1981) A critique of the chemiosmotic model of energy coupling. Proc Natl Acad Sci USA 78:2240–2243

Jain S, Nath S (2000) Kinetic model of ATP synthase: pH dependence of the rate of ATP synthesis. FEBS Lett 476:113–117

Jain S, Murugavel R, Hansen LD (2004) ATP synthase and the torsional mechanism: Resolving a 50-year-old mystery. Curr Sci 87:16–19

Kaim G, Dimroth P (1999) ATP synthesis by F-type ATP synthase is obligatorily dependent on the transmembrane voltage. EMBO J 18:4118–4127

Massari S, Azzone GF (1970) The mechanism of ion translocation in mitochondria. 1. Coupling of K^+ and H^+ fluxes. Eur J Biochem 12:301–309

Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol Rev 41:445–502

Mitchell P (1979) Keilin's respiratory chain concept and its chemiosmotic consequences. Science 206:1148–1159

Mitchell P, Moyle J (1967) Respiration-driven proton translocation in rat liver translocation. Biochem J 105:1147–1162

Mitchell P, Moyle J (1969) Estimation of membrane potential and pH difference across the cristae membrane of rat liver mitochondria. Eur J Biochem 7:471–484

Nath S (1998) A thermodynamic principle for the coupled bioenergetic processes of ATP synthesis. Pure Appl Chem 70:639–644

Nath S (2002) The molecular mechanism of ATP synthesis by F_1F_0-ATP synthase: A scrutiny of the major possibilities. Adv Biochem Eng Biotechnol 74:65–98

Nath S (2003) Molecular mechanisms of energy transduction in cells: Engineering applications and biological implications. Adv Biochem Eng Biotechnol 85:125–180

Nath S (2004) The torsional mechanism of energy transduction and ATP synthesis as a breakthrough in our understanding of the mechanistic, kinetic and thermodynamic details. Thermochim Acta 422:5–17

Nath S (2006) A novel systems biology/engineering approach solves fundamental molecular mechanistic problems in bioenergetics and motility. Process Biochem 41:2218–2235

Nath S (2008) The new unified theory of ATP synthesis/hydrolysis and muscle contraction, its manifold fundamental consequences and mechanistic implications and its applications in health and disease. Int J Mol Sci 9:1784–1840

Nath S (2010) Beyond the chemiosmotic theory: Analysis of key fundamental aspects of energy coupling in oxidative phosphorylation in the light of a torsional mechanism of energy transduction and ATP synthesis—Invited Review Part 1. J Bioenerg Biomemb 42. doi:10.1007/s10863-010-9296-5

Nath S, Jain S (2002) The detailed molecular mechanism of ATP synthesis in the F_0 portion of ATP synthase reveals a non-chemiosmotic mode of energy coupling. Thermochim Acta 394:89–98

Nath SS, Nath S (2009) Energy transfer from adenosine triphosphate: Quantitative analysis and mechanistic implications. J Phys Chem B 113:1533–1537

Nath S, Rohatgi H, Saha A (1999) The torsional mechanism of energy transfer in ATP synthase. Curr Sci 77:167–169

Pozzan T, Azzone GF (1976) The coupling of electrical ion fluxes in rat liver mitochondria. FEBS Lett 72:62–66

Pressman BC (1965) Induced active transport of ions in mitochondria. Proc Natl Acad Sci USA 53:1076–1083

Pressman BC, Harris EJ, Jagger WS, Johnson JH (1967) Antibiotic-mediated transport of alkali ions across lipid barriers. Proc Natl Acad Sci USA 58:1949–1956

Quagliariello E, Palmieri F, Prezioso G, Klingenberg M (1969) Kinetics of succinate uptake by rat-liver mitochondria. FEBS Lett 4:251–254

Tedeschi H (2005) Old and new data, new issues: The mitochondrial $\Delta\Psi$. Biochim Biophys Acta 1709:195–202

Tupper JT, Tedeschi H (1969) Mitochondrial membrane potentials measured with microelectrodes: Probable ionic basis. Science 166:1539–1540

Williams RJP (1979) Some unrealistic assumptions in the theory of chemi-osmosis and their consequences. FEBS Lett 102:126–132