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Topical Review

The ADP-ATP Translocation in Mitochondria, a Membrane Potential Controlled Transport

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Introduction

In eukaryotic aerobic cells, mitochondria supply the major portion of the ATP consumed in the cytosol for the multitude of energy-consuming processes. For this symbiosis mitochondria are equipped with a transport system for adenine nucleotides which shuttle ADP and ATP through the inner mitochondrial membrane (Fig. 1)[1]. Phosphate is transported by a separate system which can be differentiated from the ADP-ATP carrier by inhibitors. The properties of the transport have first been elucidated on isolated mitochondria with a still largely intact inner membrane. These mitochondria contain an endogenous pool of adenine nucleotides which interacts directly with the ATP-synthase of oxidative phosphorylation and other phosphate transferring enzymes. Added extramitochondrial ADP or ATP enters this pool with the simultaneous release of equimolar amount of endogenous nucleotides (Klingenberg & Pfaff, 1966). This transport corresponds therefore to a 1:1 exchange across the inner mitochondrial membrane. The counter exchange fulfills the task of the mitochondria in oxidative phosphorylation to take up ADP and to release ATP.

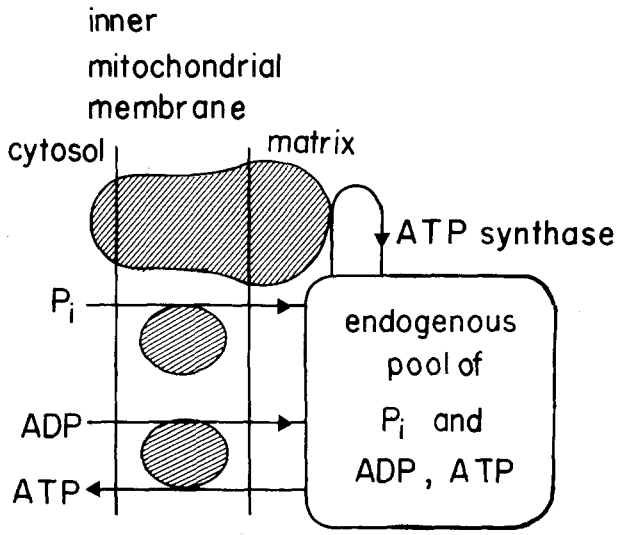

Fig. 1. The role of the ADP-ATP exchange system in the inner mitochondrial membrane

As a result of the relatively tightly coupled exchange there is no net uptake or release of ADP and ATP from mitochondria, except for a hundredfold slower rate of release under particular conditions (Meisner & Klingenberg, 1968). Therefore, it is possible to isolate the mitochondria with their native adenine nucleotide pool. All studies on the exchange are performed by measuring the rate of radioactive exchange between endogenous and exogenous nucleotides. Since the pool of endogenous nucleotides remains constant, the kinetic measurements are limited by the size of this pool. The half time of equilibration with this pool may be quite short, less than 1 sec, and measurements of the exchange require more advanced technique of starting, stopping the exchange, and separating mitochondria from the medium.

The research on the adenine nucleotide translocation has advanced all the way from first defining the exchange on the mitochondria (Klingenberg & Pfaff, 1966; Pfaff & Klingenberg, 1968), describing its property, identifying the carrier sites on the membrane (Weidemann, Erdelt & Klingenberg, 1970), defining the reorientation of the carrier sites between the two membrane surfaces (Erdelt et al., 1972), isolation of the carrier in an undenatured state (Riccio, Aquila & Klingenberg, 1975), characterizing two conformational states (Aquila, Eiermann & Klingenberg, 1976), and reconstitution of the various functions and characteristics with the isolated carrier in artificial

[1] For previous reviews *see:* Klingenberg, 1970, 1976*a*, 1979*a*; Vignais, 1976; LaNoue & Schoolwerth, 1979; Scarpa, 1979.

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liposomes (Krämer & Klingenberg, 1979). In the present short review we shall concentrate on the *energy dependency of the ADP-ATP translocation,* its phenomenology, analysis in mitochondria and in the reconstituted system. Only with this property of the ADP-ATP translocation, can its function in intracellular oxidative phosphorylation be rationalized. A large amount of data has been accumulated since the discovery in 1964, and we shall endeavor to give a concise review of the essential features, without claiming a complete coverage of all the evidence.

General Characteristics of the ADP-ATP Translocation

All mitochondria tested so far have an ADP-ATP translocation system. We actually postulate now that the ADP-ATP translocator is the most characteristic feature of mitochondria and at present the only well-defined component which distinguishes mitochondria from aerobic bacteria. Most studies on the ADP-ATP translocation are performed with mitochondria from rat liver and beef heart. The data on the translocation obtained from these mitochondria are summarized in Table 1 (Klingenberg, 1976*a*). The ADP-ATP exchange is distinguished by high specificity; it excludes AMP from transport and also all other nucleotides with bases different than adenine, with the exception of the close analogue formycin, di- and triphosphate (Pfaff & Klingenberg, 1968).

With ADP and ATP as the only substrates, differences in the translocation between these two substrates by an energy-dependent control will be the main features of the present review. Of great experimental importance for the studies is the existence of two groups of highly specific and effective inhibitors, the atractylate group and the bongkrekate group. They are used for differentiating specific exchange and binding, for measuring the amount of binding sites (Vignais et al., 1973; Klingenberg, Grebe & Scherer, 1975; Klingenberg, 1976*b*; Lauquin & Vignais, 1976), and, most important, for elucidating the translocation mechanism (Klingenberg et al., 1976).

The exchange activity measured in the isolated mitochondria is commensurate with the rates of oxidative phosphorylation. The amount of carrier in the membrane can be measured by the binding capacity for carboxyatractylate (Vignais et al., 1973; Klingenberg et al., 1975). The turnover rate of the carrier sites is approximately the same for both heart and liver mitochondria, but surprisingly low. In order to make up for the high translocation activity required in oxidative phosphorylation, the carrier sites are inserted at a high density in the membrane. In fact, the mitochondrial ADP-ATP exchange is the most active transport in most eukaryotic aerobic cells. The notwithstanding low efficiency of the ADP-ATP carrier can be rationalized by the unusually large size of the substrates to be translocated. ADP and ATP are considerably larger molecules than glucose, phosphate, or Na^+ and Ca^{++}.

Table 1. Some data on ADP-ATP translocation

Specificity:	Base only adenine, formycin, no other bases. Ribose 5 to 10× as active as deoxyribose. Phosphate only in 5′ position, P_n $n=2$ to 4. No activity in 3′, 2′ positions
Inhibitors ($\simeq K_i$):	Atractylate (10^{-7}), carboxyatractylate (10^{-9}), epiatractylate (10^{-7}), bongkrekate (10^{-8}), isobongkrekate (10^{-6})
Kinetics:	1 mol: 1 mol exchange
Translocation activity, 18°:	200 (RLM), 600 (BHM) (μmol/min/g prot)
Activation energy:	(RLM) (−8 to 14 °C) 125 kJ, (14–37 °C) 45 kJ
Carrier amount:	(μmol/g prot) 0.3 (RLM), 1.2 (BHM)
Turnover, 18°:	(min^{-1}) 600 (RLM), 500 (BHM)
Carrier density of inner membrane:	5 pmol/cm^2
Flux rate per membrane area:	2.5 nmol/min/cm^2

Regulation of the ADP-ATP Transport

A most important aspect of the mitochondrial ADP-ATP transport is the differentiation between ADP and ATP under the influence of the energization of the mitochondrial membrane (Pfaff & Klingenberg, 1968; Klingenberg et al., 1969*a*; Klingenberg, Heldt & Pfaff, 1969*b*). The various phenomena that result from this energy-dependent mechanism are summarized in Table 2. These include kinetic, stoichiometric, thermodynamic aspects, as well as problems of energy balance in oxidative phosphorylation, interaction of cytosol and mitochondria in the eukaryotic cell. These items are discussed in more detail below.

Basically the ADP-ATP exchange in mitochondria is not energy dependent. It proceeds with high activity when the mitochondrial membrane is completely depolarized by the addition of uncouplers, and ADP and ATP are translocated in both directions with about equal activity. It is clear that under this condi-

Table 2. Regulation of ADP-ATP transport in mitochondria

Influx rates:	$v_T^{\rightarrow}/v_D^{\rightarrow} \simeq 0.3$ (single), (T=ATP, D=ADP) $\simeq 7$ (competition $\simeq 1.3$ (+FCCP), $\simeq 0.6$ (Val.+K^+)
Efflux rates:	$v_T^{\leftarrow}/v_D^{\leftarrow} \simeq 12$ (competition, corrected for Mg^{++}) $\simeq 1.0$ (+FCCP)
Influx $K_m^{\rightarrow}$ (external nucleotide):	$\simeq 8+2.5$ μM (ATP); 2 μM ADP $\simeq 5$ μM (ATP+FCCP)
ATP analogues:	$v^{\rightarrow}$(+FCCP)/$v^{\rightarrow}$ =3 (ATP), =6 (dATP) =10 (AMPPCP), =8 (AMPPNP)
Stoichiometry of cation cotransport:	for $ATP_e \rightleftharpoons ADP_i$: H^+/ATP=1 (+FCCP), K^+/ATP=1 (+Val) for $ADP_e \rightleftharpoons ATP_i$: H^+/ATP=−0.8 (+FCCP), K^+/ATP=−1 (+Val)
Membrane potential and (ATP/ADP) ratio:	$\Delta \log (ATP/ADP)_e/(ATP/ADP)_i = 0.8 \times \Delta\Psi F/2.3\ RT - 0.3$

tion the carrier has a purely catalytic function in allowing equilibration between intra- and extramitochondrial nucleotides by the exchange. The exchange should be symmetric such that the ratio of the rates for ATP to ADP is identical for uptake and efflux, $v_{ATP}^{\rightarrow}/v_{ADP}^{\rightarrow} = v_{ATP}^{\leftarrow}/v_{ADP}^{\leftarrow}$.

In mitochondria energized by coupled electron transport, a dramatic change takes place. ADP and ATP are transported at different rates and in a contrary manner in efflux and influx (Klingenberg & Pfaff, 1966; Klingenberg, 1975). A few examples may serve to illustrate the energy-linked differentiation between ADP and ATP. The effect is most drastic when ADP and ATP are offered simultaneously (Klingenberg, 1972). By varying the relative share of either ADP and ATP, the contribution of each species to the total exchange is measured (Fig. 2). It clearly shows that the uptake of ATP is strongly suppressed in favor of that of ADP. Both contribute equally when the added ADP shares only 8%. The uptake of ADP is about ten times larger than that of ATP when both are offered at equal proportions.

In recent years by introducing new techniques, it became possible to follow simultaneously the uptake and extrusion of both, ADP and ATP (Klingenberg, 1975, 1977). An example for the extrusion of endogenous nucleotides during exchange against external ADP shows that in the energized state ATP is released preferentially to ADP, just opposite to the uptake (Fig. 3). In the uncoupled state, however, ADP leaves more rapidly than ATP, although equal rates should be expected from a thermodynamic viewpoint, just as they are found for the uptake. This difficulty is readily explained considering that in the mitochondria nucleotides are present mostly as Mg^{++} complexes (Heldt, Klingenberg & Milovancev, 1972; Duszynski, Savina & Woitczak, 1978), which are not transported, and thus the concentration of free ATP is decreased about ten times more than that of free ADP, as a result of the different complex binding constants. After correction for this effect, the ratio for the efflux rates in the de-energized state is $v_{ATP}^{\leftarrow}/v_{ADP}^{\leftarrow} \simeq 1$ and in the energized state $\simeq 20$.

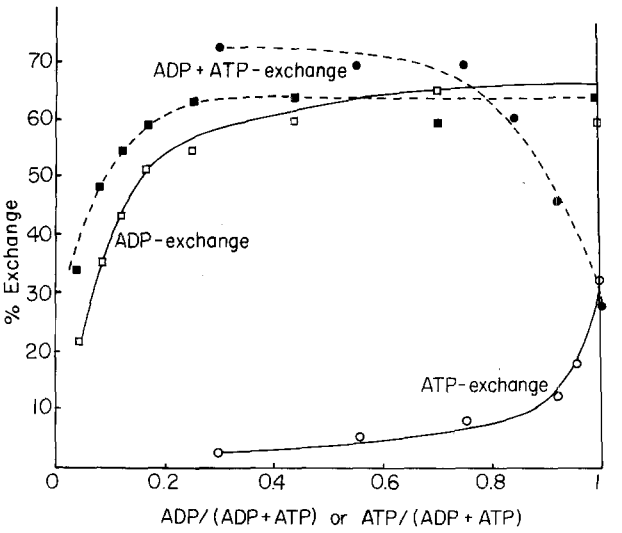

Fig. 2. The preference of the uptake of ADP as compared to ATP under the influence of "energization" of the mitochondria. Rat liver mitochondria are incubated in the presence of respiratory substrates, oligomycin at different proportions of ADP to ATP. Two series of experiments performed in parallel, one with ^{14}C-ADP, the other with ^{14}C-ATP. Before the experiments endogenous nucleotides have been labeled with ^{3}H. Thus both uptake and total efflux can be measured by the movements of ^{14}C and ^{3}H in and out of the mitochondria (Klingenberg, 1972)

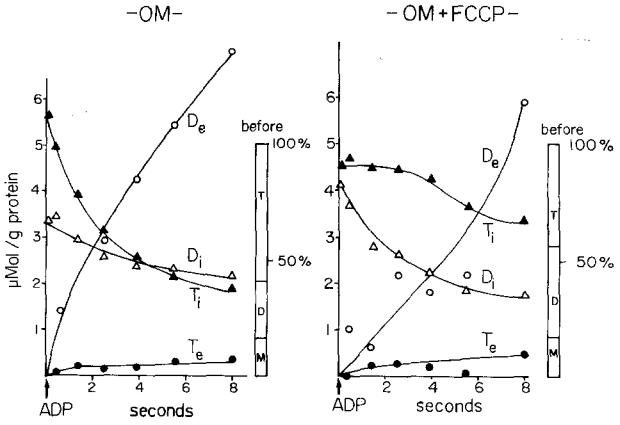

Fig. 3. The regulation of efflux of ADP *vs.* ATP by energization of the mitochondria. Experiment with rapid-mixing-pressure filtration equipment (RAMPRESA). The endogenous adenine nucleotides of rat liver mitochondria are prelabeled with ^{3}H. The exchange is started by addition of 50 μM ^{14}C-ADP at 12 °C. Experiments with coupled (+oligomycin) and uncoupled (oligomycin+FCCP) mitochondria are shown (Klingenberg, 1977). M=AMP, D=ADP, T=ATP

By preferring uptake of ADP and release of ATP, the energization of the membrane modulates the carrier such as to operate in a manner required for oxidative phosphorylation (Fig. 1). The total activity of the carrier can be divided up into four combinations (ADP_e-ADP_i, ATP_e-ATP_i, ADP_e-ATP_i, ATP_e-ADP_i), out of which the ADP_e-ATP_i combination is productive, the ATP_e-ADP_i mode counterproductive, and the ADP_e-ADP_i and ATP_e-ATP_i exchanges are unproductive. These modes must be regarded as statistical combinations since the ADP-ATP carrier is considered to operate in a sequential manner rather than by synchronous exchange (Klingenberg et al., 1976). As shown in Fig. 4, the share of the productive combination has been evaluated to amount to about 50% of the carrier activity in the energized state and surpasses the counter-productive mode about 15-fold. In accordance with expectations, in the uncoupled state all four combinations have an about equal share.

It has been early proposed (Pfaff & Klingenberg, 1968; Klingenberg et al., 1969*a*) that the force for the regulation of the exchange is derived from the membrane potential and that the charge difference between ATP^{4-} and ADP^{3-} is utilized for the membrane potential driven exchange. As a result the heteroreaction: $ATP_e^{4-} \rightleftharpoons ADP_i^{3-}$ and $ADP_e^{3-} \rightleftharpoons ATP_i^{4-}$ is electrically unbalanced, i.e., corresponds to the transfer of one e^- charge. The homologous exchange reactions $ATP_e^{4-} \rightleftharpoons ATP_i^{4-}$ and $ADP_e^{3-} \rightleftharpoons ADP_i^{3-}$ are electroneutral and are not influenced by the regulatory electrical forces, as will be shown below.

For the carrier it can be visualized that ATP^{4-} forms a complex ($C^{3+} \cdot ATP^{4-}$) with three positive charges of the carrier binding center which has one net negative charge

$$ATP^{4-} + C^{3+} \rightleftharpoons (ATP \cdot C)^-$$

whereas ADP forms an electroneutral binding center

$$ADP^{3-} + C^{3+} \rightleftharpoons C \cdot ADP.$$

Under the influence of the membrane potential positive outside, the $(ATP \cdot C)^-$ complex will be pulled to the outside in an electrophoretic manner and repulsed in the opposite direction, whereas the $(ADP \cdot C)$ complex does not sense the potential. This conforms with the experimental observations on the influx and efflux. Moreover, this translocation is to follow the gated-pore-mechanism, as depicted in Fig. 5, by which the translocation step consists of a transition of the binding center such as to open up either to the outer or inner face of the membrane. The effect of membrane potential will be discussed below.

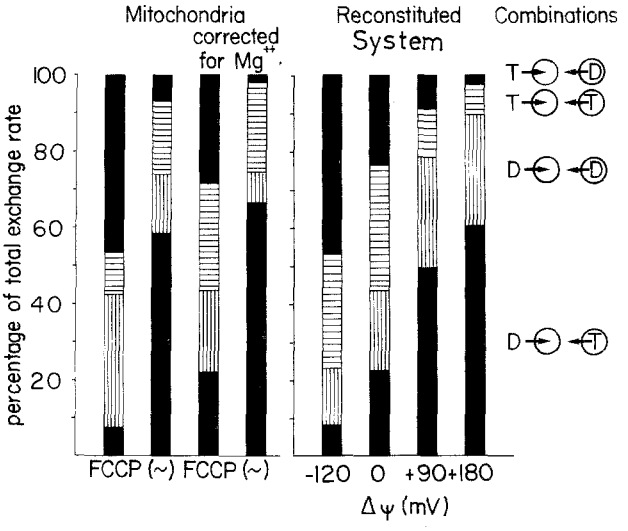

Fig. 4. Evaluation of the four combinations of exchange in competitive experiments. For mitochondria the four combinations are also given after correcting for the binding of endogenous nucleotides to excess Mg^{++}. For the reconstituted system purified translocator preparations are incorporated into egg yolk lecithin liposomes which are loaded with both ADP and ATP. Membrane potential is generated by K^+ gradient + valinomycin. The four combinations are evaluated from appropriate combinations of ^{14}C and ^3H-labeled ADP and ATP

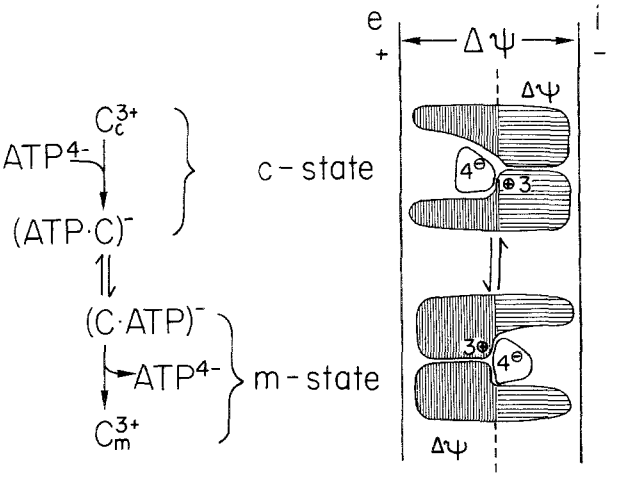

Fig. 5. Mechanism of the energy control on the ADP-ATP exchange; membrane potential electrophoretic effect on the ATP carrier complex. Reorientation of the carrier sites such that with the membrane potential positive outside the ATP carrier complex is enriched outside. The accommodation of the electrophoretic effect in the gated-pore mechanism is illustrated

The proposal of electrophoretic influence was first not accepted by other groups which, e.g., believed to have shown that the exchange is electroneutral (Mitchell & Moyle, 1968) or that there is evidence for an energy-dependent conformation change of the carrier which differentiates between ADP and ATP (Kemp & Out, 1975). The energy transduction was

also thought to be linked to a direct interaction in the membrane of the ATP synthase with the carrier (Vignais, Vignais & Doussiere, 1975).

Reconstituted System

The integration of the ADP-ATP carrier in the mitochondrial membrane with other components allows for a certain latitude of the interpretations of the energy transfer, due to experimental complications resulting from the rapid interconversion of the nucleotides by phosphate transferring enzymes and the problems of assessing the Mg^{++} influence on the nucleotide concentrations. A great advantage affords therefore the isolation of the ADP-ATP carrier (Riccio et al., 1975) and reconstitution of transport with the purified ADP-ATP in artificial liposomes, which also permits one to study the regulation (Krämer & Klingenberg, 1979).

Not only the intramitochondrial Mg^{++} but also the rapid interconversions of ADP-ATP and AMP by phosphate transferring enzymes complicate accurate evaluation of the nucleotide concentration. An unequivocal demonstration of the regulation of ADP-ATP transport has been possible in a reconsituted system where the purified ADP-ATP carrier has been inserted into phospholipid membranes (Krämer & Klingenberg, 1977, 1979). This achievement proved that the isolated carrier, consisting of two identical subunits of 32,000 mol wt, represents the complete transport system including also the regulatory functions. It also eliminated the complications and interferences from other proteins in the mitochondria as presumed by some authors. Another advantage, particularly for analyzing the regulation, is the possibility in the liposomal system to generate membrane (diffusion) potentials of various sizes and polarities (Krämer & Klingenberg, 1980).

Also in the reconstituted system the differentiation between ADP and ATP is most strongly expressed under competitive conditions. The replacement of the oxidative energization in mitochondria by a simple diffusion potential causes the same shifts in the ATP *vs.* ADP influx and efflux. This is again demonstrated by calculating the four exchange combinations from the corresponding measurements of both the influx and efflux (Fig. 4). With $\Delta\Psi = 180$ mV the combination ADP influx *vs.* ATP efflux (ADP$\rightleftharpoons$ATP) is the predominant one and about 20 times larger than the reversed combination. With a potential $\Delta\Psi = 0$, all four combinations are about equally active. In the artificial system it is possible, in contrast to mitochondria, to reverse the membrane potential. Thereby also the shares of the possible exchange combinations are

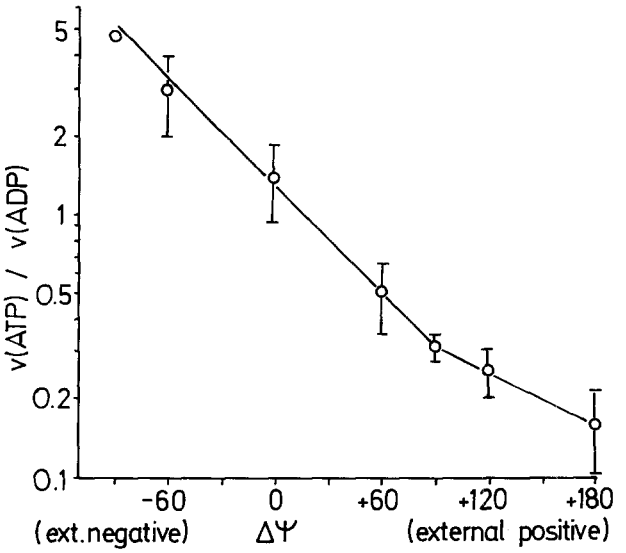

Fig. 6. Symmetric control of the membrane potential in the reconstituted system of the uptake rates ATP *vs.* ADP. The linear relation of log v_{ATP}/v_{ADP} to $\Delta\Psi$ can be described by the factor $e^{nF\Delta\Psi/RU}$ ($n = 0.6$). The factor n is smaller than 1, as explained by partial leakiness of the reconstituted system (Krämer & Klingenberg, 1980)

reversed, i.e., ATP is entering more rapidly than ADP.

The differentiation between ATP and ADP can be quantitatively appraised as a function of the membrane potential, as shown in Fig. 6. From -90 to about $+90$ mV the ratio of ATP to ADP uptake rates is linearly dependent on $\Delta\Psi$ and is also reversed around $\Delta\Psi = 0$. The ADP-ATP transport therefore follows a simple electrophoretic pattern in both directions of the membrane potential. This is the more remarkable, as these carrier molecules, which are active in transport, face with their *c*-side to the outside of the vesicles. Despite the unidirectional embedment of the carrier molecules in the membrane, they do not act as a valve which clips the current when the membrane potential alternates its direction but rather behave analogous to an ohmic resistor.

Stoichiometry of Charge Transfer and the Energy Balance in Oxidative Phosphorylation

The electrical nature of the ADP-ATP exchange was further investigated by determining the charge compensating cations which follow the ATP current (Klingenberg et al., 1969*a*; Wulf, Kaltstein & Klingenberg, 1978; LaNoue, Mizani & Klingenberg, 1978). In mitochondria, made permeable to K^+ by valinomycin, a parallel movement of 1 mol K^+ per 1 mol ATP exchange against ADP was measured. Also in the presence of uncoupler, the ratio $H^+/ATP \simeq 1$ was determined under similar exchange conditions. In the

absence of uncoupler, H^+ cotransport with ATP was negligible if corrected for H^+ leakage. A H^+ cotransport would be postulated for an electroneutral ATP-ADP exchange; e.g., according to $H_e^+ + ATP_e^{4-} = ATPH_e^{3-} \rightleftharpoons ATPH_i^{3-} = ATP_i^{4-} + H_i^+$. There is no evidence that in the absence of added ionophores other cations such as Na^+, Ca^{++} or Mg^{++} are moved with the ADP-ATP exchange, contrary to proposals by Mitchell (1979). All these results give convincing evidence that one negative charge is transferred in the ATP-ADP exchange. With membrane potential probes, it has been possible to observe the opposite case on the electrogenic effect of ATP by the generation of membrane potential when ATP is added to completely depolarized nonrespiring mitochondria (Laris, 1977; Klingenberg, 1979*b*).

It was early recognized (Klingenberg et al., 1969*a*) that the energy for the membrane potential-driven ADP-ATP exchange comes from the electrochemical H^+ potential at the inner mitochondrial membrane. It was proposed that the negative charge moved outside in the ATP release *vs.* ADP uptake, i.e., the exchange mode effective in oxidative phosphorylation is compensated by corresponding H^+ pumped outwards, as illustrated in Fig. 7 and that the ADP-ATP exchange withdraws energy from the same source as the ATP synthesis in oxidative phosphorylation. In other words, ADP-ATP exchange will compete with the same electrochemical H^+ as used by the ATP synthesis. The extrusion of ATP from the mitochondria requires 1 H^+, in addition to 2 H^+ used for ATP synthesis according to chemiosmosis (Mitchell, 1966). This contention was opposed by Mitchell and Moyle (1968), who maintained that the ATP-ADP exchange is electroneutral. However, their measurements could not be confirmed.

With 2 H^+ generated per coupling site at the respiratory chain, according to the chemiosmotic theory, a discrepancy between the hitherto reported ATP/$2e^-$ (*P/O*) in oxidative phosphorylation and the arithmetics of H^+ generation and consumption became apparent. It was therefore pointed out that the *P/O* of mitochondria might be lower than 3, e.g., 2, due to the use of 1/3 of the available H^+ by the ATP extrusion. In fact, recently Hinkle and Yu (1979) reassessed this question on the basis of new experiments and of the literature arriving at the conclusion that too high values for *P/O* have been claimed and the approximate values are at 1.33 and 2 for substrate oxidized via 2 and 3 coupling sites, respectively. In this view also 1/3 of energy equivalents are reserved for the ATP translocation.

Surprisingly little attention has been paid for some years by other laboratories to these questions until they were again raised more recently by findings that

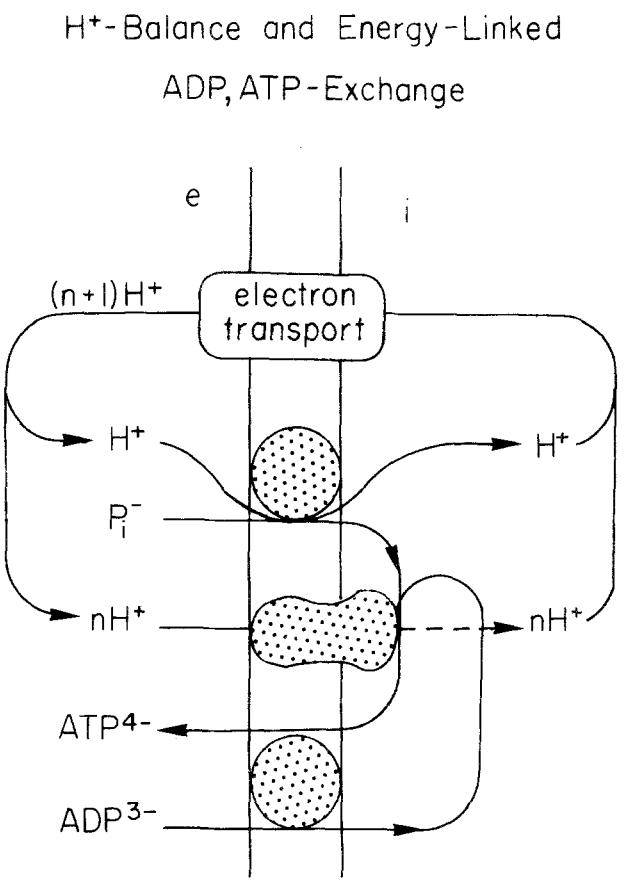

Fig. 7. The H^+ movements during the formation of extramitochondrial ATP. The stoichiometric balances of H^+ extruded by respiratory chain, and H^+ consumed by ATP synthesis, and ADP-ATP transport is indicated

in the respiratory chain per coupling site 3 or 4 H^+ are generated, in contrast to the tenets of the chemiosmotic hypothesis (Wikström & Saari, 1977; Brand, Chen & Lehninger, 1976; Alexandre, Reynafarje & Lehninger, 1978; Sigel & Carafoli, 1978; Pozzan et al., 1979; Krab & Wikström, 1979). In fact, most data converge on a ratio of 2 H^+/e^- which equals 4 H^+/coupling site. With these numbers there could be enough "extra" H^+ available to compensate the requirement of the ATP/ADP transport, provided the H^+/ATP ratio in the synthesis remains at 2. The "share" of energy used in transport out of the total energy for synthesis of extramitochondrial ATP would then be 25%, an amount more in line with other data on the free energy of ATP as shown in the following.

Phosphorylation Potential Difference across the Mitochondrial Membrane

Closely related to the problems of distribution of energy equivalents between synthesis and transport of

ATP is the free energy of ATP, i.e., the phosphorylation potential and its change by the electrophoretic ATP export. In a near equilibrium situation, as a result of an energy-driven preference of ATP efflux to ADP influx, the concentration ratio ATP/ADP is expected to be higher in the cytosol than in the matrix. This prediction, which at first appeared to be paradox because ATP is generated inside the mitochondria, was fully confirmed (Klingenberg et al., 1969*b*; Heldt et al., 1972; Davis & Lumeng, 1975) with respiring mitochondria. The external ATP/ADP ratio can be up to 50 times higher than the matrix ratio. The inner ATP/ADP ratio increased on de-energization by anaerobiosis or uncoupling, and when ATPase is inhibited by oligomycin. The gradient of the ATP/ADP ratio can be quantitatively correlated to the membrane potential (Klingenberg & Rottenberg, 1977). A linear relation between the log $(ATP/ADP)_e/(ATP/ADP)_i$ and the $\Delta\Psi$ is obtained with a slope corresponding to nearly one electrical charge (Fig. 8).

The cell physiological consequences may be described by an early quotation (Klingenberg et al., 1969*b*): "The cell may have developed a mechanism by which the number of energy equivalents is sacrified for the generation of less energy equivalents of a higher potential. The higher energy potential appears to be preferred to more equivalents at lower potential". It was an obvious consequence of the electrophoretic ATP-ADP exchange that the free energy of extramitochondrial ATP is increased substantially as compared to the intramitochondrial ATP. The phosphorylation potential is increased by about 12 kJ, as a result of the increased ATP/ADP ratio.

Also in intact organs such as liver, heart or other cells correspondingly large gradients of the ATP/ADP ratio between the cytosol and mitochondria have been demonstrated (Elbers et al., 1974). The magnitude of this gradient correlates to the energetic activity in these cells. This gradient signifies the difference of the phosphorylation potentials between the extra- and intramitochondrial space such that ΔG of extramitochondrial ATP may be higher by about 12 kJ. It is clear that this energy is transduced on the ATP by the membrane potential driven ATP translocation.

Mechanism of Membrane Potential Influence

It can be concluded that the ADP-ATP exchange is a uniquely simple and straightforward example for understanding energy-dependent transport as an electrophoretic process. Nevertheless, the energy transduction mechanism has been described in terms of conformational changes (Vignais et al., 1975), or affinity changes to the substrate (Kemp & Out, 1975), disregarding the basic principle of such an electrophoretic process. A more logical interpretation, which also better satisfies the data, places the energy transduction directly on the translocation step (Klingenberg, 1972) (Figs. 5 and 9). This explanation has been based particularly on the competition between ADP and ATP in mitochondria and is more precisely supported by studies in the reconstituted system (*unpublished data*).

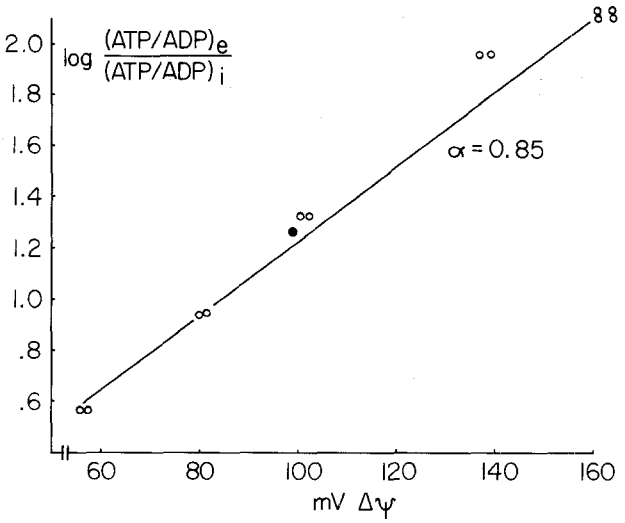

Fig. 8. Relation of the ratio of external to internal (ATP/ADP) to membrane potential $\Delta\Psi$ in mitochondria. $\Delta\Psi$ varied by changing external K^+ concentration in the presence of valinomycin (Klingenberg & Rottenberg, 1977)

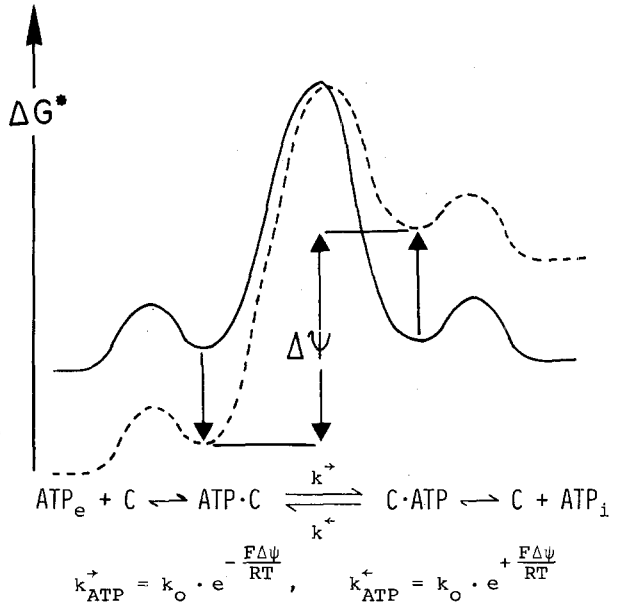

Fig. 9. Activation energy profile for describing the electrophoretic influence reorientation of the ATP-carrier complex. As a result the distribution of the carrier-ATP complex in the two states is influenced and no change of the affinity (K_d) by the membrane potential is considered (Klingenberg, 1972)

The following simple reaction sequence consists of a binding and dissociation step on each side of the membrane and the translocation step between the substrate carrier complexes which either face the outer or inner side of the membrane.

$$ATP_e + C_e \overset{K_D^T}{\rightleftharpoons} (ATP \cdot C)_e \overset{K^T}{\rightleftharpoons} (C \cdot ATP)_i \overset{K_D^T}{\rightleftharpoons} C_i + ATP_i$$

with $K^T = \vec{k}/\vec{k}$.

The effect of energization can be visualized to affect the binding step or/and the translocation step. The relation between $\Delta\Psi$ and the ATP-carrier complex as viewed from the reorienting carrier mechanism is depicted in Fig. 5. ATP remains at the central binding site which opens up either to out- or inside. Thus the total or the major part of $\Delta\Psi$ can be visualized to be extended alternatingly from the center to the right or left surface, thus exerting its full effect on the translocation step as reflected in the redistribution of the sites. In contrast, a major affected $\Delta\Psi$ in the diffusion path to the binding center should rather result in a change of the K_d or K_m.

The analysis of the competition between ADP and ATP influx under the influence of membrane potential in mitochondria let us propose that $\Delta\Psi$ does not influence the competition at the binding site but changes primarily the distribution of the carrier complex when loaded with ATP (Klingenberg, 1972). Thus the membrane potential causes an enrichment of the carrier-ATP complex on the outside, whereas the carrier-ADP complex is influenced only to a minor extent. The asymmetric distribution would result from $\Delta\Psi$ decreasing $v_{ADP}^{\rightarrow}$ and increasing $v_{ATP}^{\leftarrow}$, i.e., an electrophoretic influence on the $(ATP \cdot C)$ complex. This explains the strong, $\Delta\Psi$-conditioned competition of ADP with ATP and is in agreement with the fact that $\Delta\Psi$ increases the K_m for ATP only slightly in our hands.

More compelling evidence in favor of the carrier distribution mechanism in the regulation of adenine nucleotide exchange is provided by the data of the reconstituted system which are in clear discrepancy to a model where the membrane potential prevailingly influences the K_d. As mentioned above, with the liposomal system it is possible to set up defined exchange conditions in both compartments of the system, i.e., with only one nucleotide species or with both, ADP and ATP, in a constant ratio on each side of the membrane. Observed changes in the apparent K_m and V_{max} for ADP and ATP exchange caused by membrane potential in these experiments cannot be rationalized by K_d changes. They can, however, easily be explained with an influence of the membrane potential on the influx and efflux velocity constants of the translocation step $k^{\rightarrow}$ and $k^{\leftarrow}$.

An influence on the distribution reflects an accelerating or decelerating effect of the membrane potential on the translocation step. Thus the carrier ATP complex with a negative charge excess is attracted by the positive potential outside and repulsed from the negative potential inside. It would be in agreement that predominantly the ATP-carrier complex is affected. There are, however, also some smaller effects on the ADP-carrier complex in the reconstituted system which are not explained in this manner.

Here the observation also deserves attention that energization has a relatively stronger effect on the much slower transport of ATP analogues (Pfaff & Klingenberg, 1968). It was proposed that the rate limitation on the analogues resides in the less efficient translocation rather than in an impaired binding. Some analogues were found which only bind and are not transported at all (Schlimme et al., 1973; Boos et al., 1975). The dramatic effect of the membrane potential on the analogues would consequently indicate the translocation step as its target site.

An activation energy diagram may illustrate our understanding of the membrane potential effect, whereas in the de-energized state a symmetrical activation profile must be postulated that will be distorted by the membrane potential in such a manner that the energy level of the ATP carrier complex is raised on the inside and lowered on the outside. The chemical potentials of the free ATP are, of course, also correspondingly changed, so that there is no effect on the binding activities. According to this simple model the release rate should be increased by the same factor, by the reciprocal of which the uptake rate is decreased.

Conclusion

The energy-dependent ADP-ATP exchange on mitochondria appears to be an example where the energy transduction on transport through a biomembrane will be first understood. The system is considerably simpler than ATP-driven pumps, the carrier molecules are relatively small, they have no chemical function and appear to be well manageable in artificial systems.

This energy control is vital for the symbiosis of mitochondria with the cytosol in the eukaryotic cell since the ATP system on both compartments is to be maintained on different levels of phosphorylation potentials. The mitochondria, probably originating from prokaryotic ancestors (John & Whatley, 1975), which were operating at a lower phosphorylation po-

tential, had to adapt to the higher phosphorylation potential of the cytoplasm of the host cell. The membrane potential generated for oxidative phosphorylation and the charge differences between ADP and ATP were utilized for counterbalancing the phosphorylation potential differences, however, not without a certain cost of energy.

The further investigation of this quantitatively most active transport process in eukaryotic cells and its energy dependency still remains an important challenge.

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