Bioenergetics (1972) **3**, 129–136

On Energy Conservation and Transfer in Mitochondria*

Y. Hatefi and W. G. Hanstein†

Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, California 92037, U.S.A.

Abstract

Structural and functional features of energy conservation and transfer in mitochondria have been examined in the light of recent developments, and hypothetical schemes for energy coupling and transfer have been presented.

The following is in response to the invitation of this journal for a conceptual paper in the field of bioenergetics. The ideas presented were elicited by the significant findings of several laboratories (see references). Since this article is intended mainly for workers in the field, we have considered it proper to refrain from detailed explanations and enumeration of conclusions, which we feel will be rather obvious to our colleagues.

In mitochondria, electron transfer complexes I, III and IV include the three sites of energy conservation.[1–5] Each complex appears to contain an electron carrier, which undergoes a redox potential change upon energization of mitochondria by ATP,[5–9] plus other carriers whose redox potentials remain unaltered. Furthermore, each complex is located between two redox pools as shown in Table I. These pools make it possible for each complex to function independently of the others. In addition, the cytochrome *c* pool solves the problem of the unstoichiometric relationship of *a* and a_3 with the components of the other complexes.‡ The Q and *c* pools, along with most of the electron carriers, comprise two redox plateaus of short

* Supported by USPHS grant AM-08126 to Y.H. and San Diego County Heart Association Grant-in-Aid 97-71 to W.G.H.

† Recipient of USPHS career Development Award 1-K4-GM38291.

‡ Abbreviations: *a*, a_3, *b*, c_1, *c*; cytochromes *a*, a_3, *b*, c_1 and *c* respectively; b_T, transducing cytochrome *b* (see ref. 9); $b_{559.5}$, cytochrome *b* of complex III with *α* band at 559·5 nm at 77°K; FeS2, iron–sulfur center 2 of complex I;[18] NADH deh., NADH dehydrogenase; Q, ubiquinone (coenzyme Q); OSCP, oligomycin sensitivity conferring protein; DOCA, deoxycholate.

TABLE I.

Correlations Among Coupling Regions I, II and III

Electron carriers	ΔE_m (mV)		Redox pool	
	Non-energized	Energized	Electron donor	Electron acceptor
FeS2-NADH deh.	+, large	±, small	NADH	Q
$c_1 - b_T$	+260	−15	Q	c
$a_3 - a$	+130	+35	c	O_2

potential spans. The former encompasses a span of not more than 150 mv, and includes, in the nonenergized state of mitochondria, the *b* cytochromes, iron–sulfur center 2 of complex I and Q.* The latter covers a span of only about 100 mv, and includes c_1, *c*, *a*, Cu, and the iron–sulfur protein of complex III (Fig. 1).

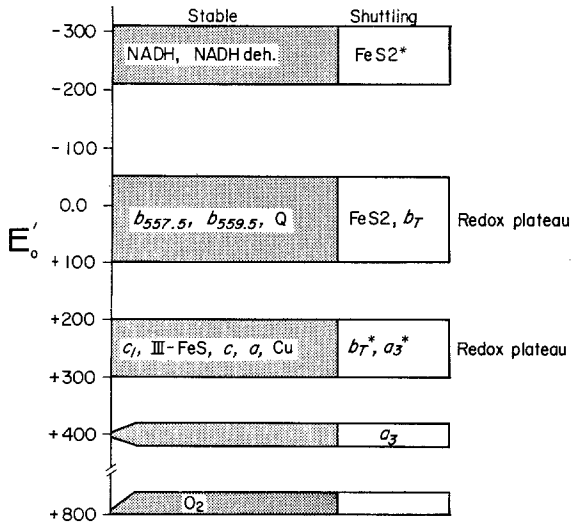

Figure 1. Approximate redox levels of the mitochondrial electron carriers. The redox-stable carriers in the shaded areas are those which do not appear to undergo potential change upon energization of mitochondria by ATP. The redox-shuttling carriers are those which appear to undergo potential change upon such treatment. These carriers in the energized state of mitochondria are identified with asterisks. For the redox potentials of Cu and III-FeS (complex III iron–sulfur protein) see refs. 14 and 15. On the basis of the data of refs. 16–18, the redox potential of FeS2 in the nonenergized state is assumed to be in the region shown. Bovine heart mitochondria contain 3 *b*-type cytochromes with 77°K α peaks at 557·5, 559·5 (b_K?) and 562·5 (b_T) nm. Cytochrome $b_{557.5}$ is located in complex II, and the other two are located in complex III.[50]

* Succinate dehydrogenase and other electron tributaries of the respiratory chain will not be discussed in this article, because their function is not germane to the mechanism of energy conservation.

In the energized state, the composition of these plateaus is altered (Fig. 1). The carriers which change potential in complexes I, III and IV are considered to be respectively iron–sulfur center 2, b_T and a_3 (see, however, ref. 7). Upon energization, the potential of iron–sulfur center 2 approaches that of NADH dehydrogenase, which is an iron–sulfur flavoprotein,[10] and the potentials of b_T and a_3 approach those of c_1 and a respectively.* Thus, nearly all of the electron carriers on the oxygen side of Q congregate in the redox plateau at the level of cytochrome c, and iron–sulfur center 2 forms another region of low potential gradient with NADH and NADH dehydrogenase.† Figure 2 depicts the potential transitions of iron–sulfur center 2, b_T and a_3 in complexes I, III and IV respectively. It is seen that each complex

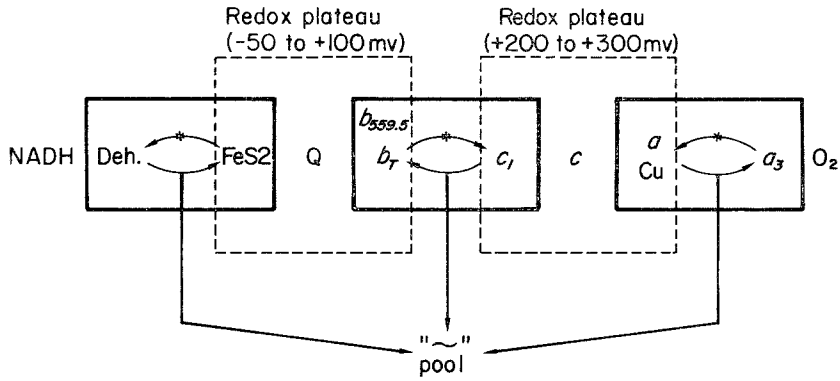

Figure 2. Energy conservation associated with the potential change of the shuttling carriers iron–sulfur center 2, cytochrome b_T and cytochrome a_3 in complexes I, III and IV respectively. Curved arrows show the redox potential changes of the above carriers; asterisks indicate energization; and the dashed squares show the redox plateaus at the pool levels of Q and c.

contains at least one component with a fixed potential at the level of the redox pool on its substrate side, and one component which is capable of potential change and redox-shuttling between the two pools which surround that complex.

The redox changes of iron–sulfur center 2, b_T and a_3 suggest that they participate in energy conservation when their potential is altered, and that they return to their original potential when the conserved energy is either transferred away from the respiratory chain or dissipated under uncoupling conditions. Thus, the redox potential shuttling property of these carriers and the closing of potential gaps as

* See, however, ref. 11 for critical comments regarding the redox potential measurements of refs. 6 and 8.

† The redox potential of NADH dehydrogenase appears to be close to those of NAD/NADH and their acetylpyridine analogues, because this enzyme catalyzes a transhydrogenation from NADH to acetylpyridine adenine dinucleotide.[10] The difference between the E_0' of the two nucleotides is 72 mv.[13]

$$a''' \overset{+e^-}{\rightleftharpoons} a'' \tag{1A}$$

$$a'' + a_3''' \rightleftharpoons a''' + a_3'' \tag{2A}$$

$$a''' + a_3'' \overset{-e^-}{\rightleftharpoons} a'''{-}a_3''' \quad \text{(low potential } a_3\text{)} \tag{3A}$$

$$a'''{-}a_3''' \overset{+e^-}{\rightleftharpoons} a''{-}a_3''' \tag{4A}$$

$$a'' + a_3''' \rightleftharpoons a'''{-}a_3'' \tag{5A}$$

$$a'''{-}a_3'' \overset{-e^-}{\rightleftharpoons} a''' \sim a_3''' \tag{6A}$$

$$a''' \sim a_3''' + x \rightleftharpoons a''' + a_3''' \sim x \tag{7A}$$

$$a''' + a_3''' \sim x \rightleftharpoons a''' + a_3''' + {\sim}x \quad \text{(high potential } a_3\text{)} \tag{8A}$$

Scheme A. Hypothetical mechanism for energy coupling at site III. A variation of reactions 3A and 4A might be as follows:

$$a''' + a_3'' \overset{+e^-}{\rightleftharpoons} a'' + a_3''$$

$$a'' + a_3'' \overset{-e^-}{\rightleftharpoons} a'' - a'''$$

The high energy state formally represented by ~ in this scheme could be with respect to hydrolysis, or coulombic or conformational strain.

$$b'''_{559\cdot5} \overset{+e^-}{\rightleftharpoons} b''_{559\cdot5} \tag{1B}$$

$$b''_{559\cdot5} + b'''_{\mathrm{T}} + c_1''' \rightleftharpoons b'''_{559\cdot5} + b'''_{\mathrm{T}} - c_1'' \quad \text{(high potential } b_{\mathrm{T}}\text{)} \tag{2B}$$

$$b'''_{\mathrm{T}} - c_1'' \overset{-e^-}{\rightleftharpoons} b'''_{\mathrm{T}} - c_1''' \tag{3B}$$

$$b'''_{\mathrm{T}} - c_1''' \overset{+e^-}{\rightleftharpoons} b''_{\mathrm{T}} \sim c_1''' \tag{4B}$$

$$b''_{\mathrm{T}} \sim c_1''' \rightleftharpoons b'''_{\mathrm{T}} \sim c_1'' \tag{5B}$$

$$b'''_{\mathrm{T}} \sim c_1'' \overset{-e^-}{\rightleftharpoons} b'''_{\mathrm{T}} \sim c_1''' \tag{6B}$$

$$b'''_{\mathrm{T}} \sim c_1''' + x \rightleftharpoons c_1''' + b'''_{\mathrm{T}} \sim x \tag{7B}$$

$$b'''_{\mathrm{T}} \sim x + c_1''' \rightleftharpoons b'''_{\mathrm{T}} + c_1''' + {\sim}x \quad \text{(low potential } b_{\mathrm{T}}\text{)} \tag{8B}$$

Scheme B. Hypothetical mechanism for energy coupling at Site II. A possible mechanism of electron transfer in reaction 2B, which would conform with a single site of antimycin inhibitiin, would be electron transfer from $b_{559\cdot5}$ to c_1 through low-potential b_{T} as an electron siphon.

shown in Table I appear to be phenomena which are intimately related to energy conservation.[8, 9, 19–21] These characteristics are summarized for better visualization in schemes A and B, which depict a unified hypothetical mechanism for energy conservation at sites III and II.* The main features of these mechanisms are:

1. Stepwise energy conservation, as a result of two cycles of single electron transfer, in carrier pairs, each of which is composed of a redox-stable and a redox-shuttling carrier.
2. Redox potential change of the shuttling carrier upon an energy yielding reduction or oxidation reaction, or upon energization by ATP.
3. Controlled respiration in state 4 because electron transfer through the coupled carriers could be slow.
4. Rapid electron transfer (characteristic of state 3) through the wide potential gap of each complex after transfer (or dissipation by uncouplers) of the "high energy" state and concomitant return of the shuttling carrier to its noncoupled state.

It might be added that in these hypothetical mechanisms a number of experimental observations have also been taken into consideration. Among these are:

a. The fact that b_T is not reduced without energy input and unless c_1 is in the oxidized state.[9, 22, 23]
b. The observations that the inhibitory effects of loosely bound compounds such as amytal, 2-heptyl-4-hydroxyquinoline-*N*-oxide,† and azide, are reversed by uncouplers.[25–28] This is because uncouplers might be expected to act near the site of action of these respiratory inhibitors.
c. The fact that antimycin A treatment leads to a potential change of b_T.[22, 23] Several pieces of evidence indicate that antimycin A results in a stronger structural association of b and c_1,[29, 30] which might be analogous to the coupling of b_T and c_1 in the energized state.

Although not included in the above hypothetical schemes, iron–sulfur centers 3 and 4 of complex I[18] and the iron–sulfur protein of complex III[14] might be involved respectively in electronic communication between the carrier pairs of each complex, whereas copper in complex IV might be required, in addition, for the 4 electron reduction of oxygen through the rapid sequential oxidation of a_3'', 2 Cu′, a''.[31, 32]

The work of Lee and Ernster[33] indicates that the three coupling

* The mechanism for site I could be analogous to scheme A with the replacement of a and a_3 respectively by NADH dehydrogenase and iron–sulfur center 2 of complex I.

† Relative to the binding of antimycin A.[24]

sites communicate through a "high energy" pool. Other studies have shown that this pool is, in turn, in communication with the adenine nucleotide (ATP/ADP) pool through a set of factors (see reviews 34 and 35) capable of isoenergetic energy transfer. Thus, the mitochondrial energy conservation-transfer apparatus may be conceived of as 3 energy "transducing" systems (complexes I, III and IV) and one energy transfer system, each of which communicates with its neighbor by way of an energy (potential or "high-energy") pool (Figure 3).

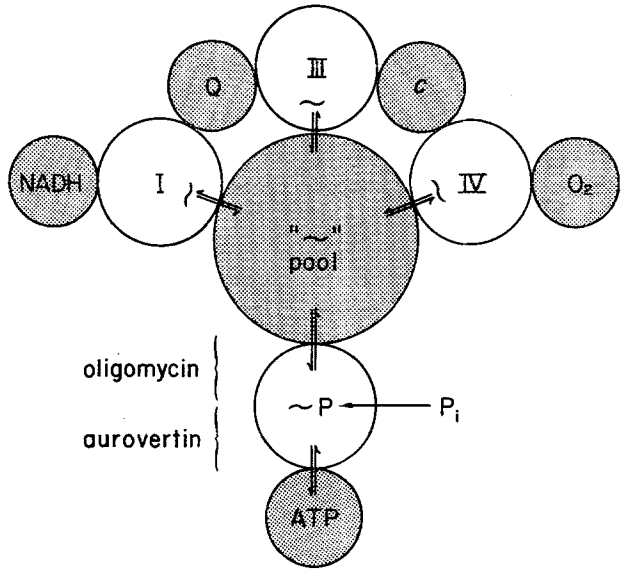

Figure 3. Schematic representation of the "transducing" complexes I, III and IV, the "high-energy" transfer complex (unshaded circles), and the energy pools (shaded circles).

The "high-energy" pool of Lee and Ernster suggests the possible existence of a "high-energy" carrier operating at this level. This carrier might be identical with component X in schemes A and B. Sanadi's factor B appears to be a possible candidate for this "high-energy" carrier. It is water soluble, has a small molecular weight (29,200), and is believed to operate near the respiratory chain.[36, 37] The "high-energy" transfer system appears to involve the sites of inhibition by oligomycin and aurovertin.[38, 39] The differential effect of these inhibitors on stimulation of state 4 respiration by arsenate has suggested that they act at separate sites, and that phosphate enters the phosphorylation sequence prior to ADP.[40] Wang and his colleagues have used the differential effect of these two inhibitors to trap membrane bound phosphate.[49] These results suggest that the energy

transfer system is composed of at least 2 components, OSCP and F_1 or factor A (see review 34).

During isolation of the electron transfer complexes I, II, III and IV from submitochondrial particles, OSCP and F_1 accumulate in a fifth fraction, which is also particulate[42] (see also refs. 43 and 44) (Fig. 4). As in the case of the components of electron transfer complexes,

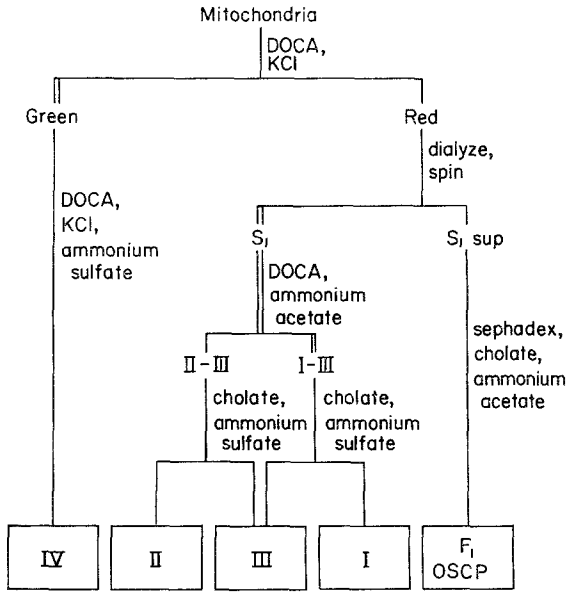

Figure 4. Resolution of mitochondria into the four electron transfer complexes and a fifth particulate fraction containing F_1 and OSCP.

F_1 and OSCP can be separated from one another by chaotropes.[29, 44–47] Therefore, it is possible that, similar to the "transducing" complexes, the "high-energy" transfer components of mitochondria also form a hydrophobic complex capable of protecting "~" from hydrolysis. In addition to OSCP and factor A, such a complex might contain the Pullman-Monroy inhibitor[48] and embody the properties of Sanadi's factor A.D (ATPase synthetase).[49]

References

1. Y. Hatefi, *Comprehensive Biochemistry*, **14** (1966) 199.
2. B. Chance and G. R. Williams, *Advances in Enzymology*, **17** (1956) 65.
3. B. Chance and M. Erecińska, *Arch. Biochem. Biophys.*, **143** (1971) 675.
4. P. C. Hinkle, R. A. Butow and E. Racker, *J. Biol. Chem.*, **242** (1967) 5169.
5. M. Gutman, T. P. Singer and H. Beinert, *Biochem. Biophys. Res. Commun.*, **44** (1971) 1572.
6. D. F. Wilson and P. L. Dutton, *Arch. Biochem. Biophys.*, **136** (1970) 583.
7. P. Hinkle and P. Mitchell, *J. Bioenergetics*, **1** (1970) 45.

8. D. F. Wilson and P. L. Dutton, *Biochem. Biophys. Res. Commun.*, **39** (1970) 59.
9. B. Chance, D. F. Wilson, P. L. Dutton and M. Erecińska, *Proc. Nat. Acad. Sci. U.S.A.*, **66** (1970) 1175.
10. Y. Hatefi and K. E. Stempel, *J. Biol. Chem.*, **244** (1969) 2350.
11. A. H. Caswell, *Arch. Biochem. Biophys.*, **144** (1971) 445.
12. M. Gutman, T. P. Singer and H. Beinert, *J. Biol. Chem.*, **240** (1965) 475.
13. B. M. Anderson, C. J. Ciotti and N. O. Kaplan, *J. Biol. Chem.*, **221** (1956) 823.
14. J. S. Rieske, *Non-Heme Iron Proteins*, A. San Pietro (ed.), The Antioch Press, 1965, p. 461.
15. M. Erecińska, B. Chance and D. F. Wilson, *FEBS Letters*, **16** (1971) 284.
16. Y. Hatefi and K. E. Stempel, *Biochem. Biophys. Res. Commun.*, **26** (1967) 301.
17. C. I. Ragan and P. B. Garland, *European J. Biochem.*, **10** (1969) 399.
18. N. R. Orme-Johnson, W. H. Orme-Johnson, R. E. Hansen, H. Beinert and Y. Hatefi, *Biochem. Biophys. Res. Commun.*, **44** (1971) 446.
19. J. H. Wang, *Science*, **167** (1970) 25.
20. E. C. Slater, C.-P. Lee, J. A. Berden and H. J. Wegdam, *Biochim. Biophys. Acta*, **223** (1970) 354.
21. D. DeVault, *Biochim. Biophys. Acta*, **226** (1971) 193.
22. D. F. Wilson, M. Koppelman, M. Eercińska and P. L. Dutton, *Biochem. Biophys. Res. Commun.*, **44** (1971) 759.
23. J. S. Rieske, *Arch. Biochem. Biophys.*, **145** (1971) 179.
24. Y. Hatefi, A. G. Haavik and P. Jurtshuk, *Biochim. Biophys. Acta*, **52** (1961) 106.
25. B. Chance and G. Hollunger, *J. Biol. Chem.*, **238** (1963) 418, 432.
26. D. F. Wilson and B. Chance, *Biochem. Biophys. Res. Commun.*, **23** (1966) 751.
27. D. F. Wilson and B. Chance, *Biochim. Biophys. Acta*, **131** (1967) 421.
28. J. L. Howland, *Biochim. Biophys. Acta*, **73** (1963) 665.
29. J. S. Rieske, H. Baum, C. D. Stoner and S. H. Lipton, *J. Biol. Chem.* ,**242** (1967) 4854.
30. Y. Hatefi and W. G. Hanstein, *Arch. Biochem. Biophys.*, **138** (1970) 73.
31. Q. H. Gibson and C. Greenwood, *J. Biol. Chem.*, **240** (1965) 2694.
32. C. Greenwood and Q. H. Gibson, *J. Biol. Chem.*, **242** (1967) 1782.
33. C.-P. Lee and L. Ernster, *European J. Biochem.*, **3** (1968) 385.
34. H. A. Lardy and S. M. Ferguson, *Ann. Rev. Biochem.*, **38** (1969) 991.
35. K. van Dam and A. J. Meyer, *Ann. Rev. Biochem.*, **40** (1971) 115.
36. D. R. Sanadi, K. W. Lam and C. K. R. Kurup, *Proc. Nat. Acad. Sci. U.S.A.*, **61** (1968) 277.
37. D. R. Sanadi, K. W. Lam, B. P. Sani and J. C. Chen, *8th Int. Congress of Biochemistry, Switzerland, Abstracts*, p. 143.
38. H. A. Lardy, D. Johnson and W. C. McMurray, *Arch. Biochem. Biophys.*, **78** (1958) 587.
39. H. A. Lardy, J. L. Connelly and D. Johnson, *Biochemistry*, **3** (1964) 1961.
40. R. L. Cross and J. H. Wang, *Biochem. Biophys. Res. Commun.*, **38** (1970) 848.
41. R. L. Cross, B. A. Cross and J. H. Wang, *Biochem. Biophys. Res. Commun.*, **40** (1970) 1155.
42. W. G. Hanstein and Y. Hatefi (unpublished).
43. A. Tzagoloff, K. H. Byington and D. H. MacLennan, *J. Biol. Chem.*, **243** (1968) 2405.
44. D. H. MacLennan and A. Tzagoloff, *Biochemistry*, **7** (1968) 1603.
45. Y. Hatefi and W. G. Hanstein, *Proc. Nat. Acad. Sci. U.S.A.*, **62** (1969) 1129.
46. K. A. Davis and Y. Hatefi, *Biochemistry*, **8** (1969) 3355.
47. K. A. Davis and Y. Hatefi, *Biochemistry*, **10** (1971) 2509.
48. K. Asami, K. Juntti and L. Ernster, *Biochim. Biophys. Acta*, **205** (1970) 307.
49. R. J. Fisher, J. C. Chen, B. P. Sani, S. S. Kaplay and D. R. Sanadi, *Proc. Nat. Acad. Sci. U.S.A.*, **68** (1971) 2181.
50. K. A. Davis, Y. Hatefi, K. L. Pofl and W. L. Butler, *Biochem. Biophys. Res. Commun.*, in press.