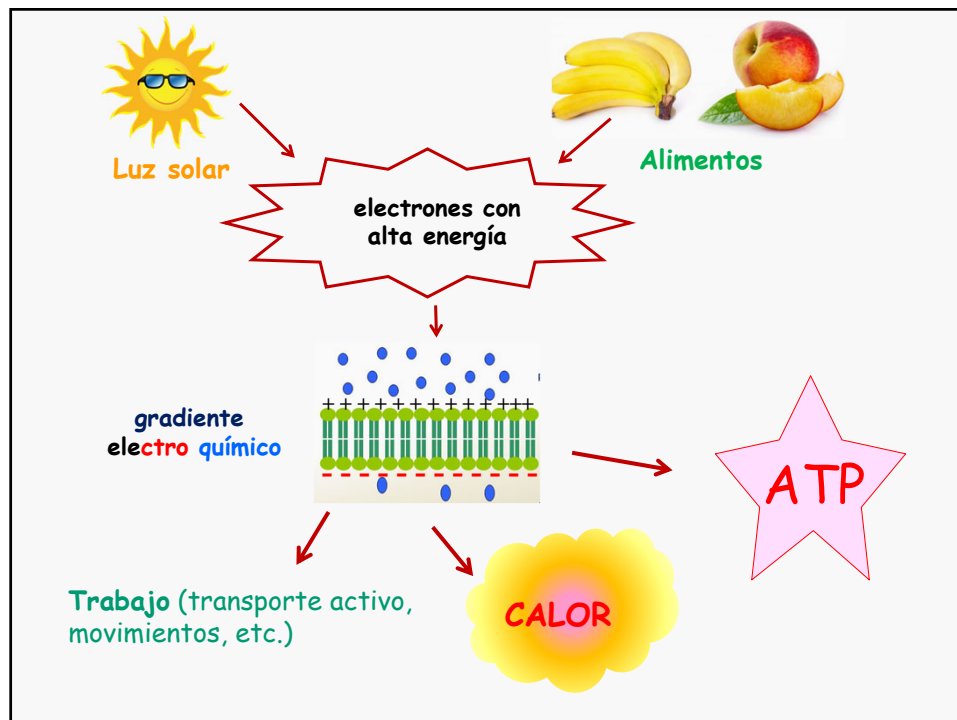


Como las células fabrican ATP

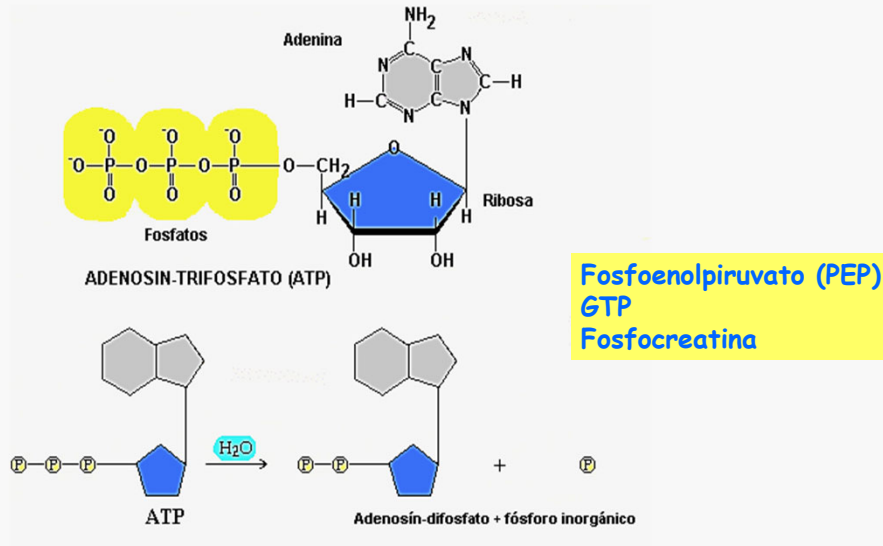
"electrones"
(Bioenergética)

1



2

ATP: "molécula de intercambio de energía del metabolismo celular"



3

	Concentración (mM)		
	ATP	ADP	Fosfocreatina
Hepatocitos (rata)	3.38	1.32	0
Miocitos (rata)	8.05	0.93	28
Neuronas (rata)	2.59	0.73	4.7
Eritrocitos (humanos)	2.25	0.25	0

De la relación ATP/ADP se decide hacia donde se va a dirigir el metabolismo !!!

4

¿De dónde se obtiene el ATP?

- Fosforilación a nivel de sustrato
- Fosforilación oxidativa

Mitocondria

5

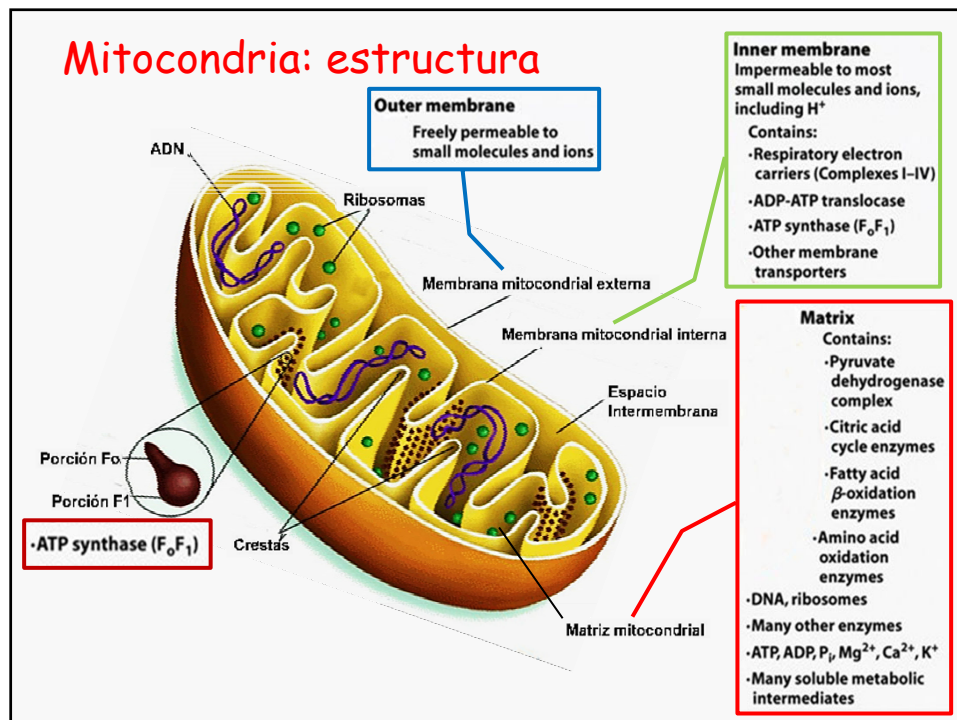
Mitocondrias

- Estructura
- Función (síntesis de ATP)
- Integración con el citosol
- Origen y duplicación
- Herencia

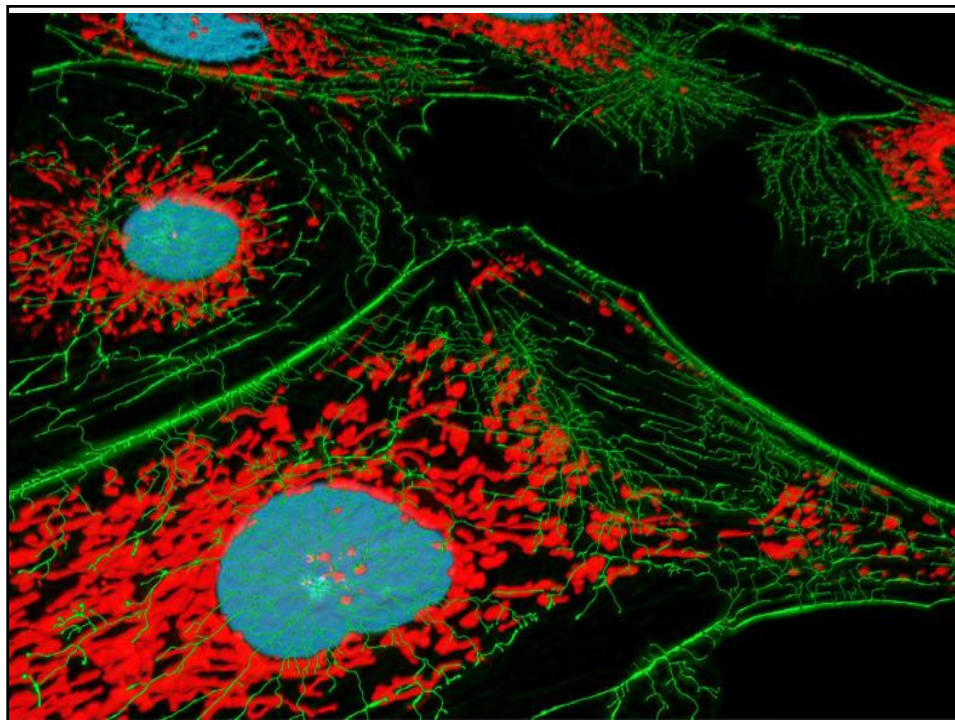
Peroxisoma

- Estructura
- Función

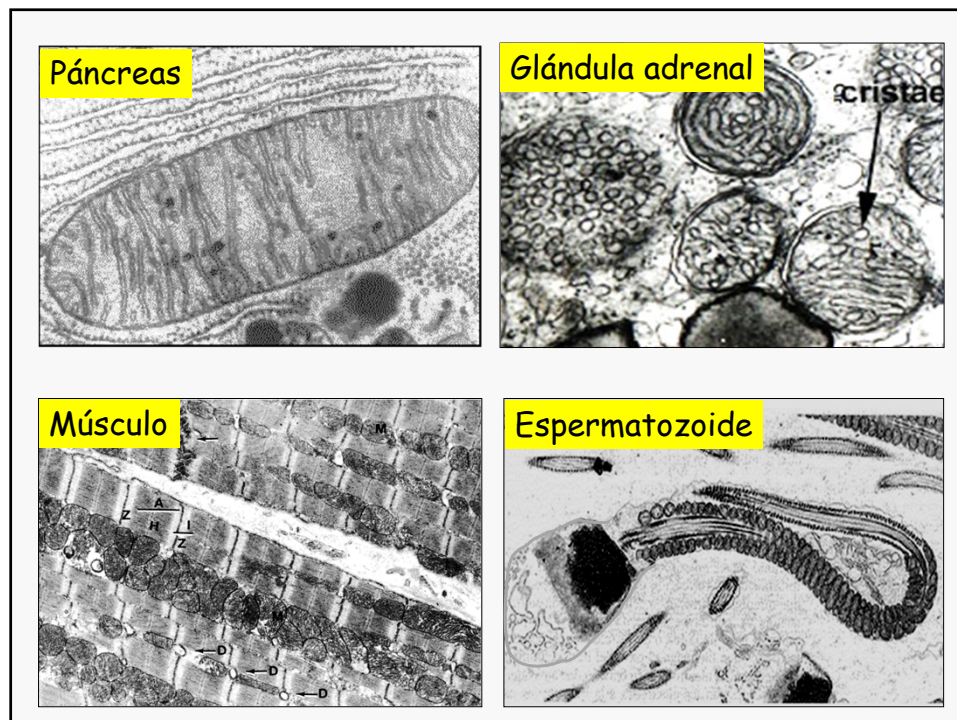
6



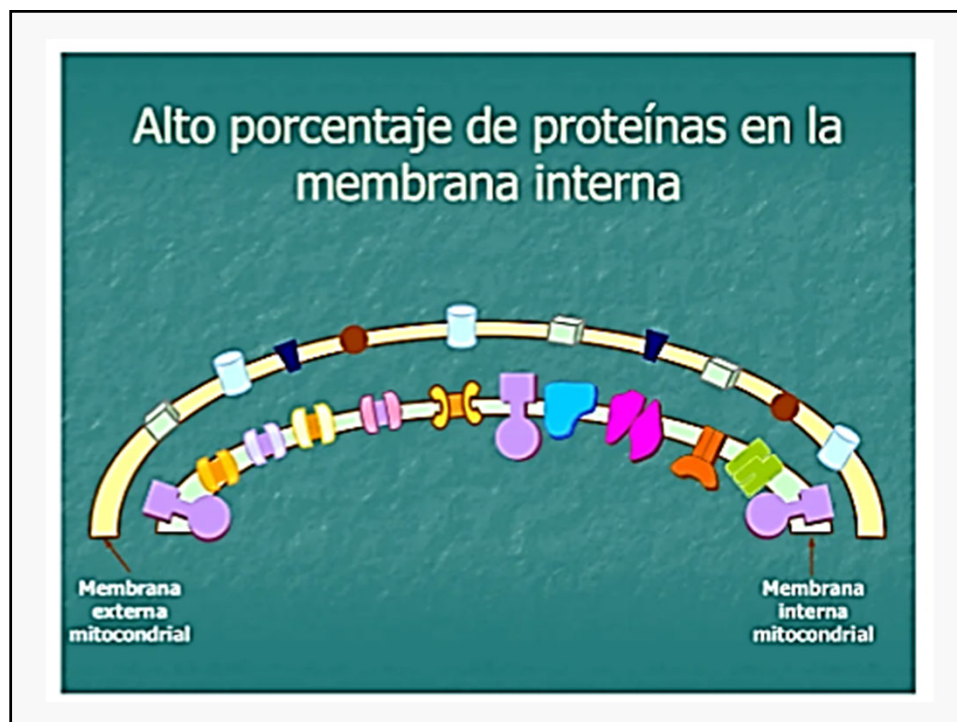
7



8



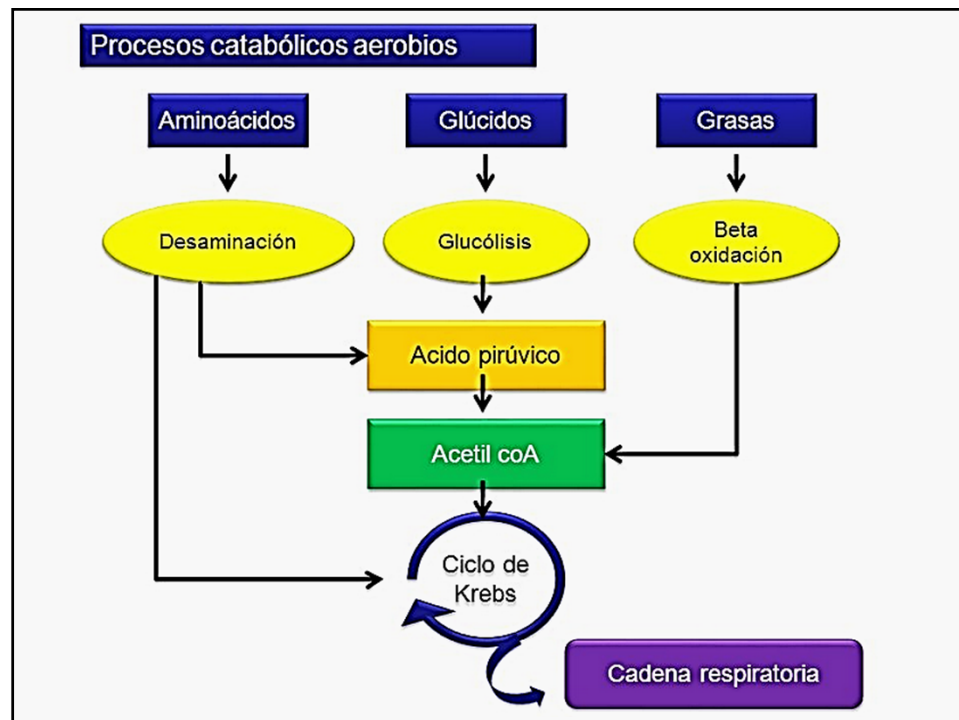
9



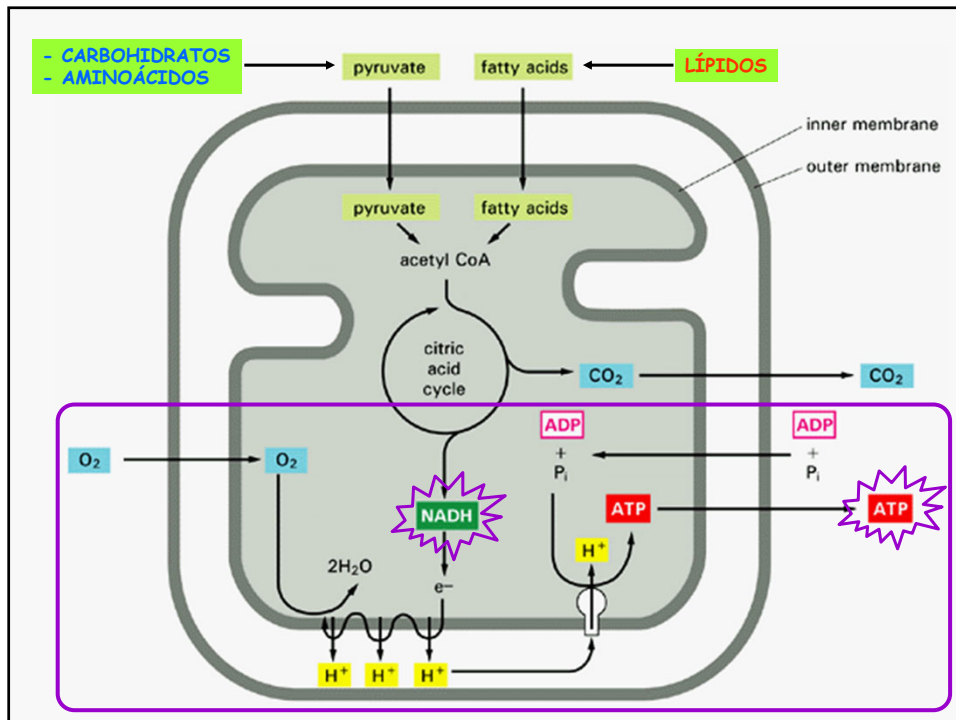
10

Las mitocondrias como integradores metabólicos

11



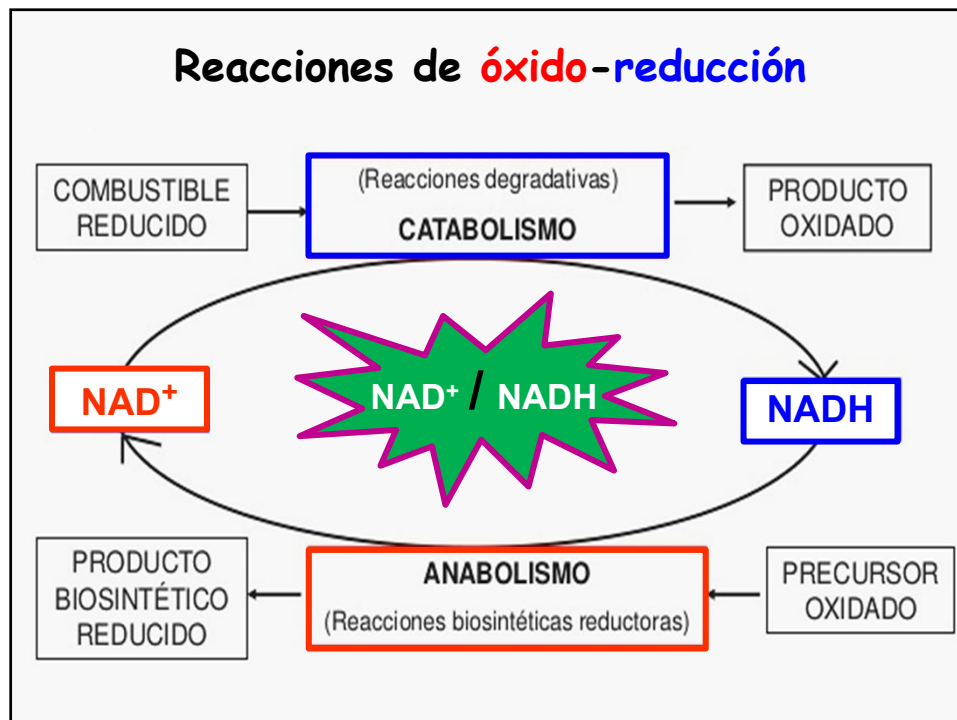
12



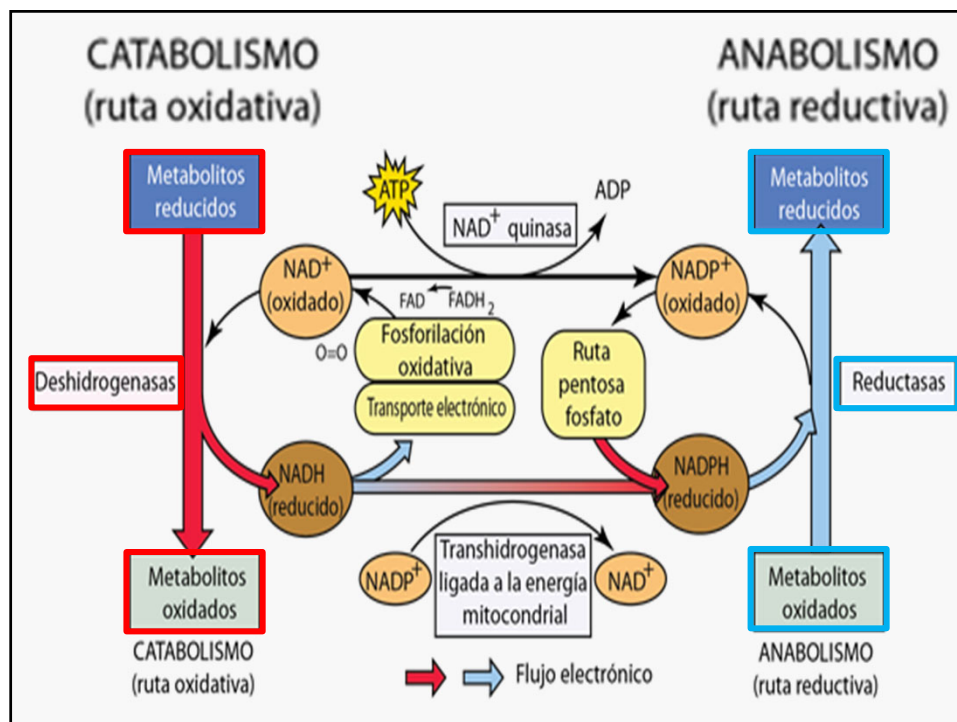
13



14



15

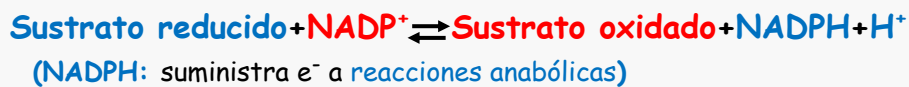
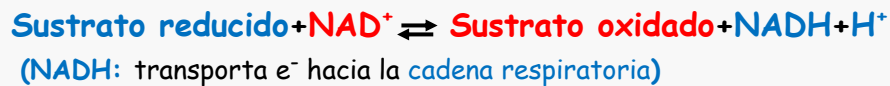


16

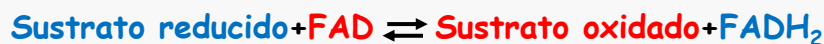
La importancia de las deshidrogenasas

Captan e^- de alta energía de las vías **catabólicas** y los ceden a **aceptores universales (co-factores)**: NAD^+ o $NADP^+$, FAD o FMN

- ✓ **Deshidrogenasas** asociadas a nucleótidos de nicotina catalizan **reacciones reversibles**:



- ✓ **Flavoproteínas**: unen fuertemente FAD o FMN (se unen al **centro activo**)



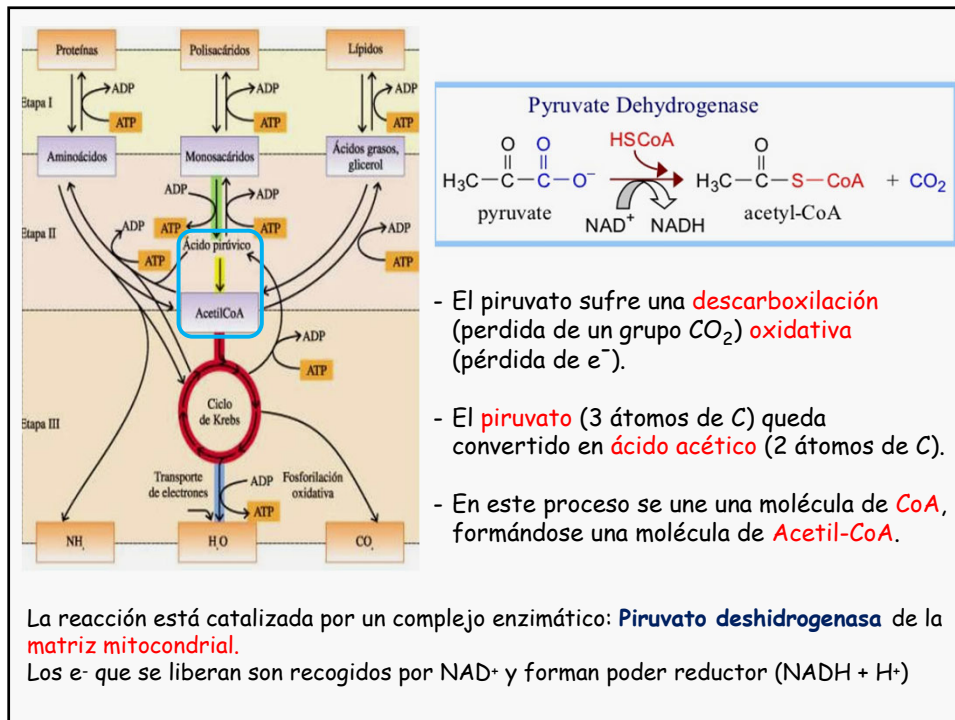
17

TABLA 19-1 Algunas reacciones importantes catalizadas por deshidrogenasas ligadas a NAD(P)H

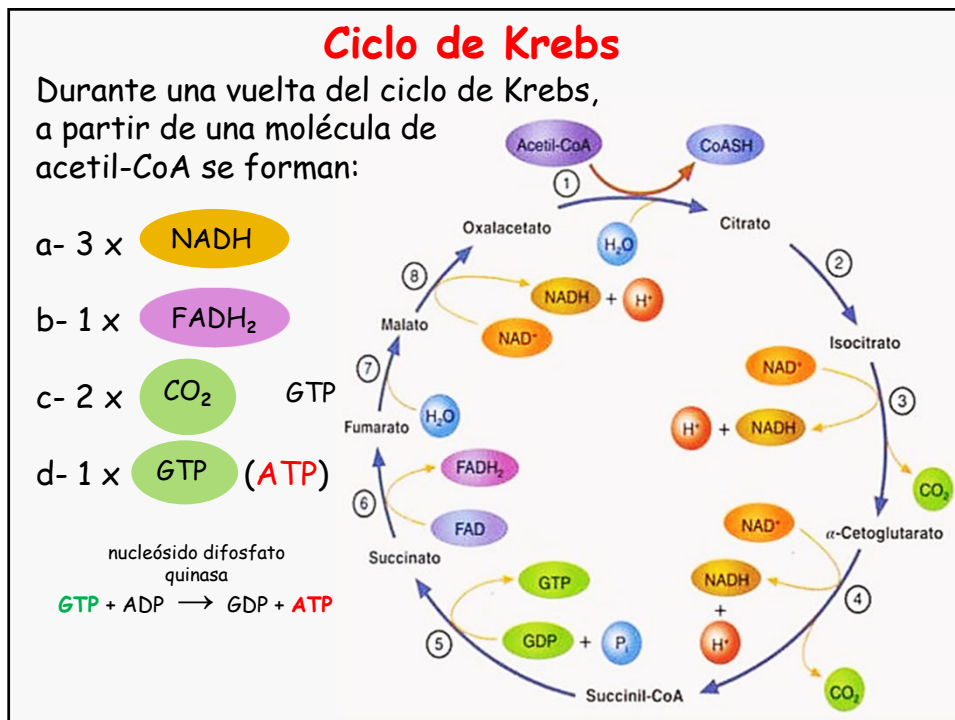
Reacción*	Localización†
Ligadas a NAD	
α -Cetoglutarato + CoA + NAD^+ $\rightleftharpoons$ succinil-CoA + CO_2 + NADH + H^+	M
L-Malato + NAD^+ $\rightleftharpoons$ oxalacetato + NADH + H^+	M y C
Piruvato + CoA + NAD^+ $\rightleftharpoons$ acetil-CoA + CO_2 + NADH + H^+	M
Gliceraldehído 3-fosfato + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisfosfoglicerato + NADH + H^+	C
Lactato + NAD^+ $\rightleftharpoons$ piruvato + NADH + H^+	C
β -Hidroxiacil-CoA + NAD^+ $\rightleftharpoons$ β -cetoacil-CoA + NADH + H^+	M
Ligadas a NADP	
Glucosa 6-fosfato + $NADP^+$ $\rightleftharpoons$ 6-fosfogluconato + NADPH + H^+	C
Ligadas a NAD o NADP	
L-Glutamato + H_2O + $NAD(P)^+$ $\rightleftharpoons$ α -cetoglutarato + NH_4^+ + NAD(P)H	M
Isocitrato + $NAD(P)^+$ $\rightleftharpoons$ α -cetoglutarato + CO_2 + NAD(P)H + H^+	M y C

Todas estas reacciones y sus enzimas se tratan en los Capítulos 14 a 18.
M indica mitocondria; C, citosol.

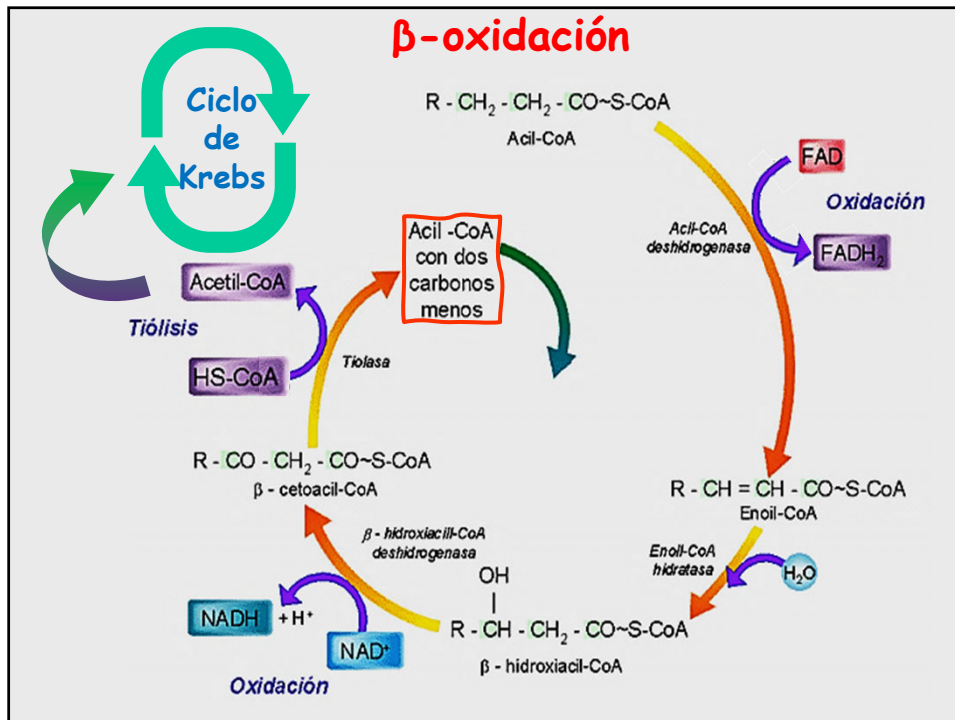
18



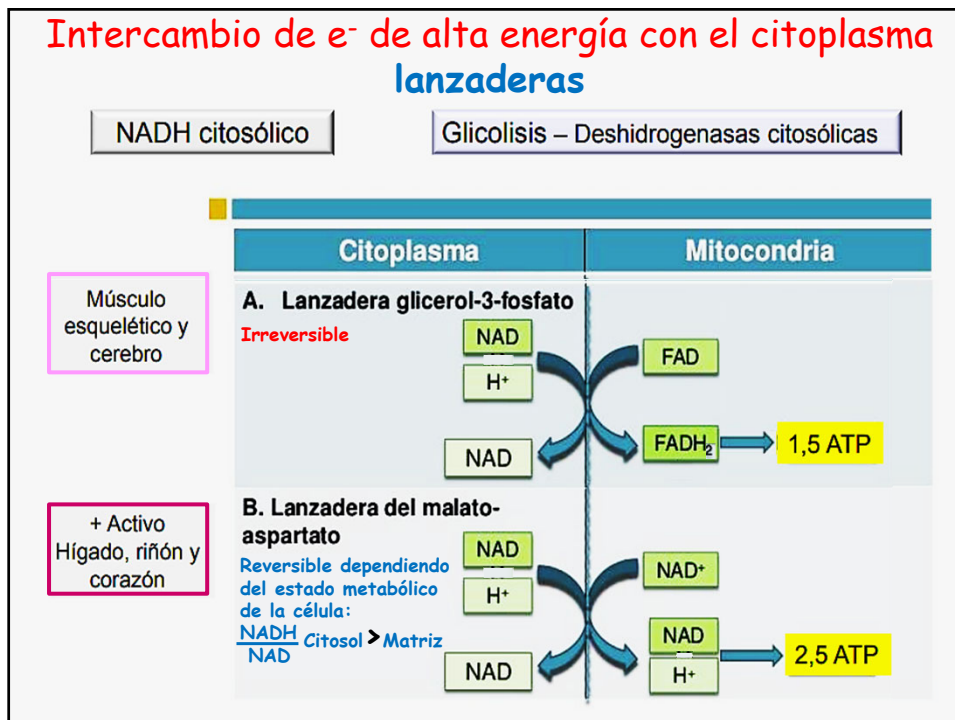
19



20

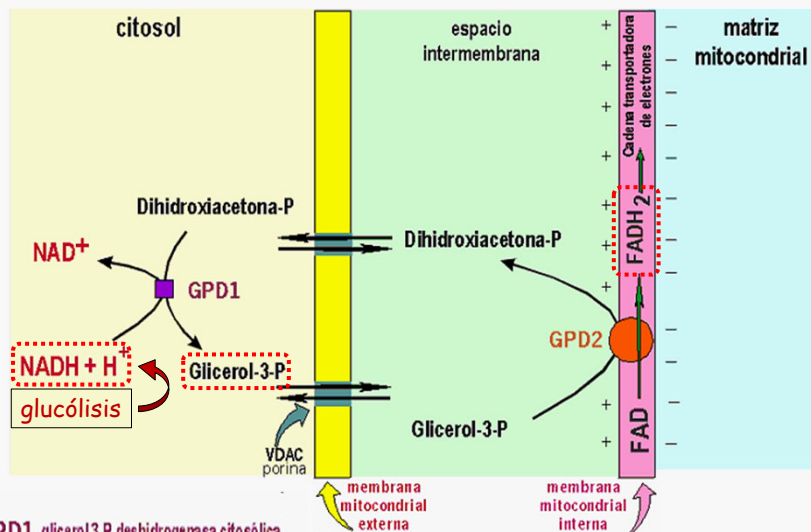


21



22

Lanzadera dihidroxiacetona fosfato/glicerol-3-fosfato



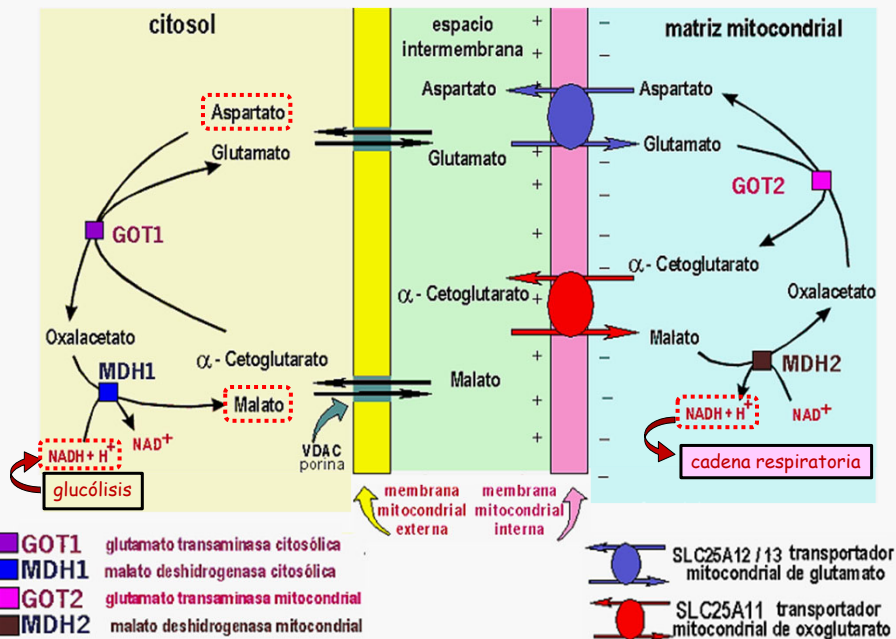
■ GPD1 glicerol 3 P deshidrogenasa citosólica

● GPD2 glicerol 3 P deshidrogenasa mitocondrial

La glucólisis puede proveer FADH_2 para la cadena respiratoria

23

Lanzadera del malato-aspartato



■ GOT1 glutamato transaminasa citosólica

■ MDH1 malato deshidrogenasa citosólica

■ GOT2 glutamato transaminasa mitocondrial

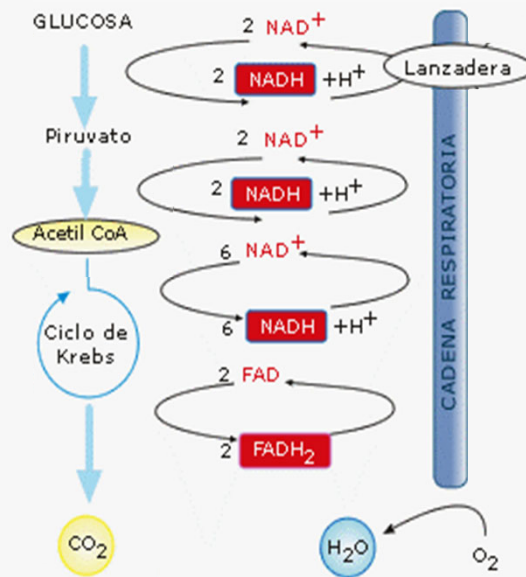
■ MDH2 malato deshidrogenasa mitocondrial

■ SLC25A12 / 13 transportador mitocondrial de glutamato

■ SLC25A11 transportador mitocondrial de oxoglutarato

24

¿Qué reacciones proveen de NADH y FADH₂ (electrones) a la cadena respiratoria?



25

Formas de transferencia de e⁻ para la fosforilación oxidativa

➤ **Transferencia directa de e⁻**

- **Citocromos (Fe³⁺ → Fe²⁺)**

➤ **Transferencia de e⁻ + H⁺**

- **CoQ, FAD (FMN) → formas reducidas**

➤ **Transferencia de ion hidruro :H⁻ (e⁻)**

- **NAD → formas reducidas**

26

Componentes de la cadena de transporte electrónico

FLAVOPROTEINAS: FMN o FAD: Transportan $2 e^-$ y $2 H^+$

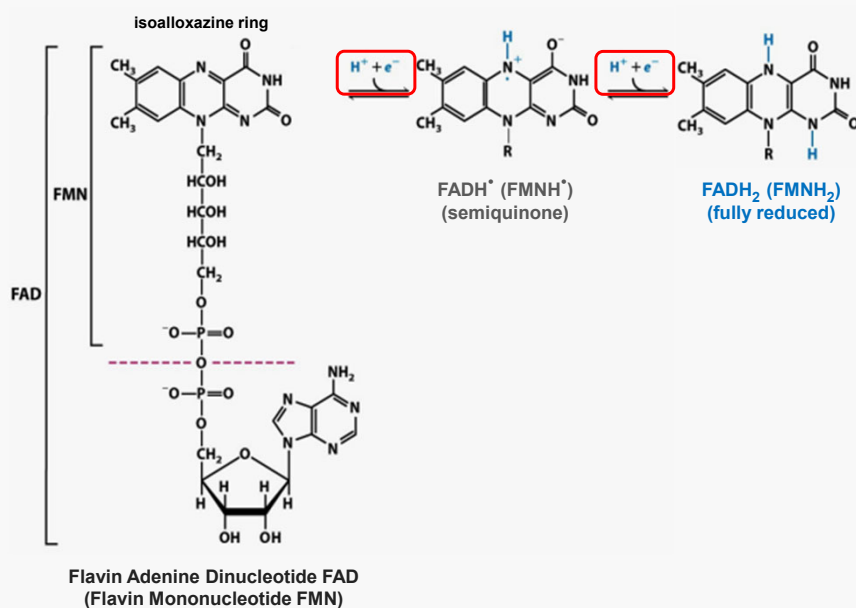
PROTEINAS FERROSULFURADAS: Transportan e^- ($Fe^{+++} \rightarrow Fe^{++}$)

COENZIMA Q o UBIQUINONA: Quinona isoprenoide **no** proteica. Transporta $1 e^-$ y libera $2 H^+$.

CITOCROMOS b, c, c_1 , a, a_3 : **Proteínas** que contienen un grupo hemo. Transportan $1 e^-$

27

Flavoproteínas: tiene flavina como grupo prostético



28

Citocromos

➤ Hemoproteínas

➤ Fe^{3+} capta e^-

➤ tipos:

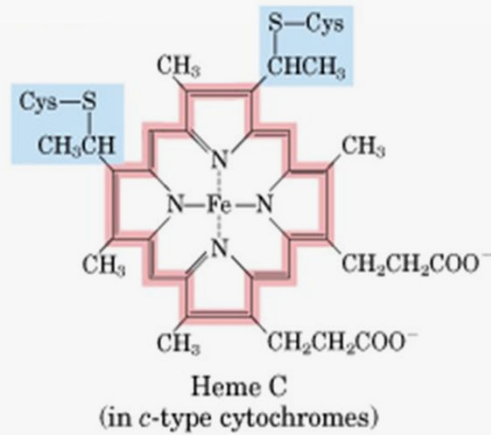
a: a y a_3

b: b_{566} y b_{562}

c: c y c1

➤ Potencial de reducción creciente:

$b_{566} > b_{562} > c1 > c > a > a_3$

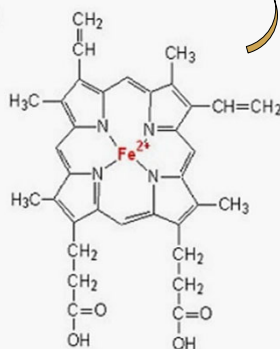


29

Tipos de Citocromos

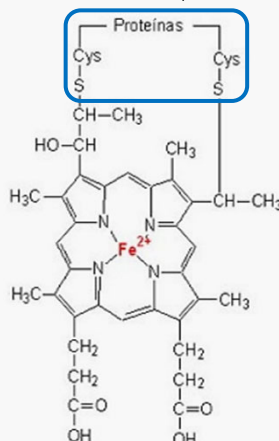
unión covalente
a sus proteínas

unión **no** covalente
a sus proteínas



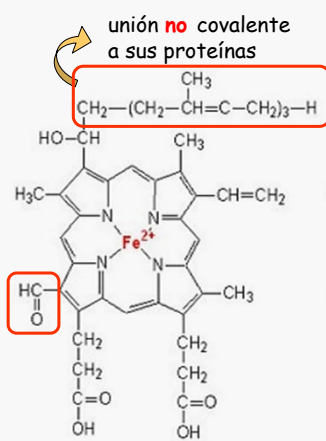
citocromo b

- b_{566} y b_{562}
- **complejo III**
Proteínas integrales en la MMI



citocromo c

Asociada la MMI por interacciones electrostáticas

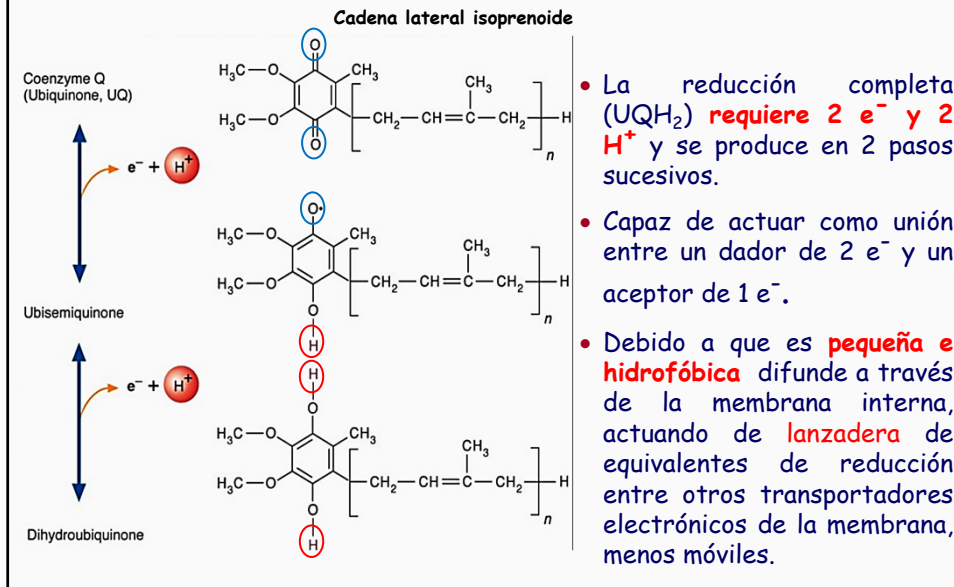


citocromo a

- a y a_3
- **complejo IV**
Proteínas integrales en la MMI

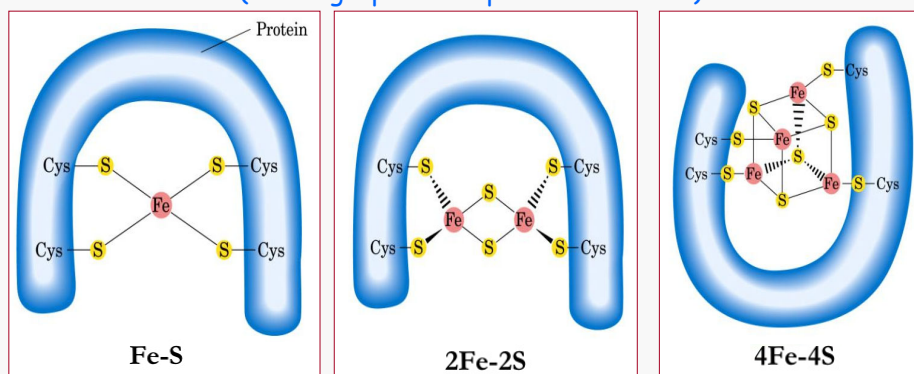
30

Ubiquinona o Coenzima Q (Otros grupos transportadores de e^-)



31

Proteínas ferrosulfuradas (Otros grupos transportadores de e^-)



- En las proteínas ferrosulfuradas, el **Fe** está en asociación con átomos de **S inorgánico**, con **S** de residuos **Cys** de la proteína o con ambos.
- Participan en reacciones de transferencia **de 1 e^- por vez** en la que se oxida o reduce uno de los átomos de Fe
- Centros (Fe-S): estructuras sencilla a complejas
- Al menos, **8 proteínas Fe-S** intervienen en la cadena de transporte de e^-

32

Mitocondrias

Estructura

Función (síntesis de ATP)

Cadena respiratoria

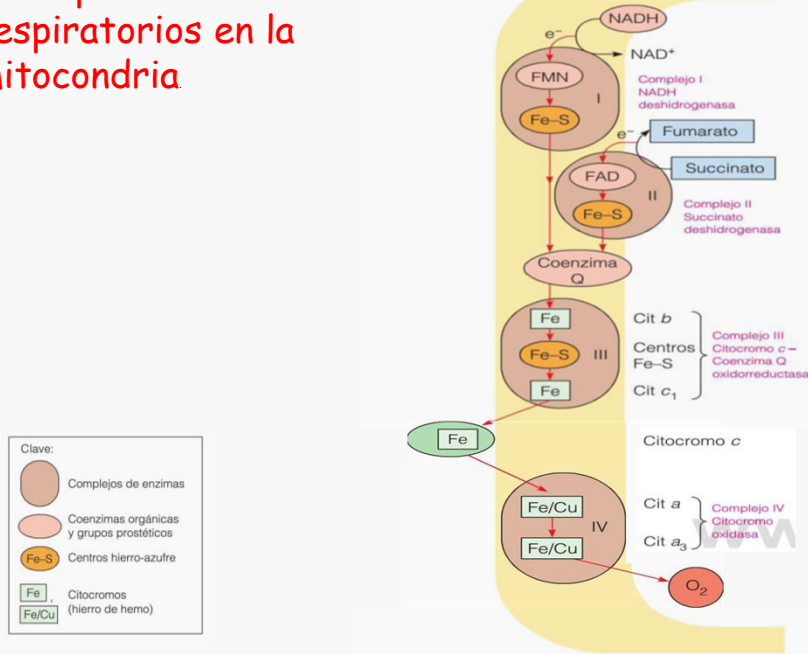
33

TABLE 14.1 Characteristics of protein complexes of the mitochondrial respiratory electron-transport chain in bovine heart

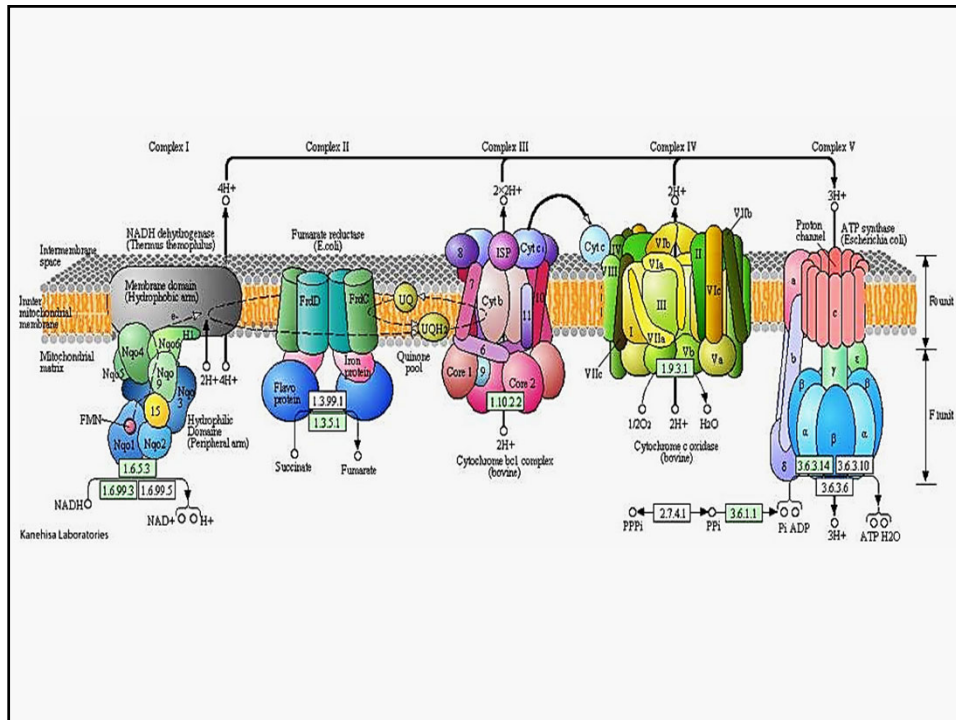
Complex	Subunits	Molecular weight	Oxidation-reduction components
I. NADH-ubiquinone oxidoreductase (NADH deshidrogenase)	42 or 43	> 900 000	1 FMN 22–24 Fe–S in 7 or 8 clusters
II. Succinate-ubiquinone oxidoreductase (Succinate deshidrogenase)	4	125 000	1 FAD 3 Fe–S clusters Cytochrome b_{560}
Coenzima Q o Ubiquinona			
III. Ubiquinol–cytochrome c oxidoreductase (Complex cytochrome bc_1)	2	~250 000 (dimer of 11-chain subunits)	1 Fe–S cluster Cytochrome b Cytochrome c_1
Citocromo c			
IV. Cytochrome c oxidase (Cytochrome oxidase)	2	420 000 (dimer of 13-chain subunits)	Cytochrome a Cytochrome a_3 2 Copper ions

34

Transportadores electrónicos respiratorios en la mitocondria

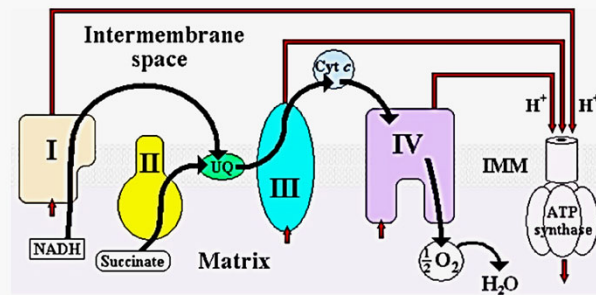


35



36

Transporte de e⁻: 4 complejos enzimáticos y una coenzima (UQ)



Complejo I: NADH Deshidrogenasa

Complejo II: Succinato Deshidrogenasa

Ubiquinona: Acepta electrones del complejo I y II

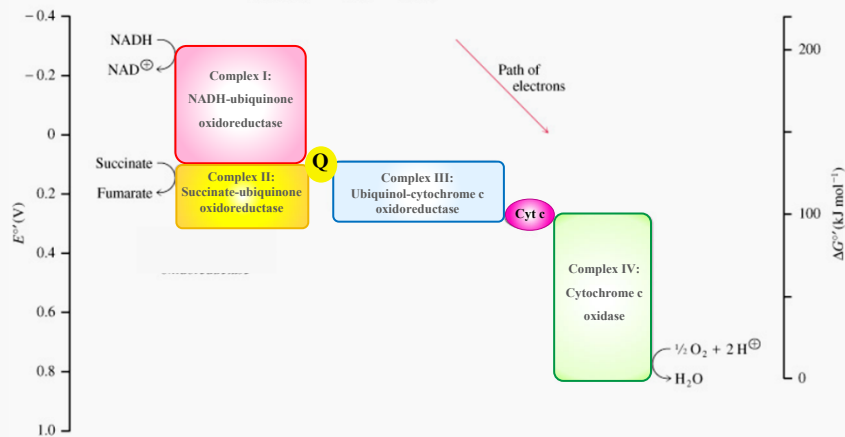
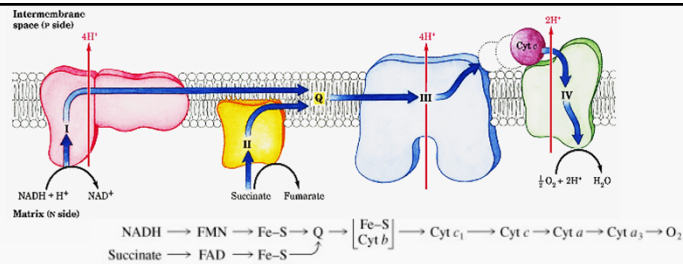
Complejo III: citocromo bc1

Citocromo c: espacio intermembranal

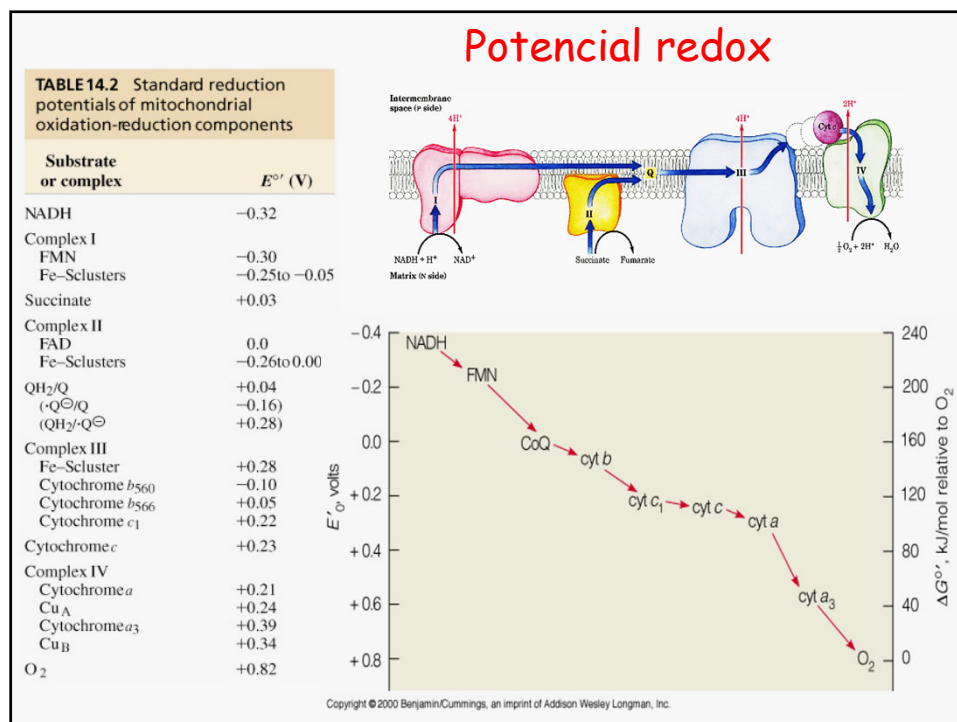
Complejo IV: Citocromo c oxidasa

37

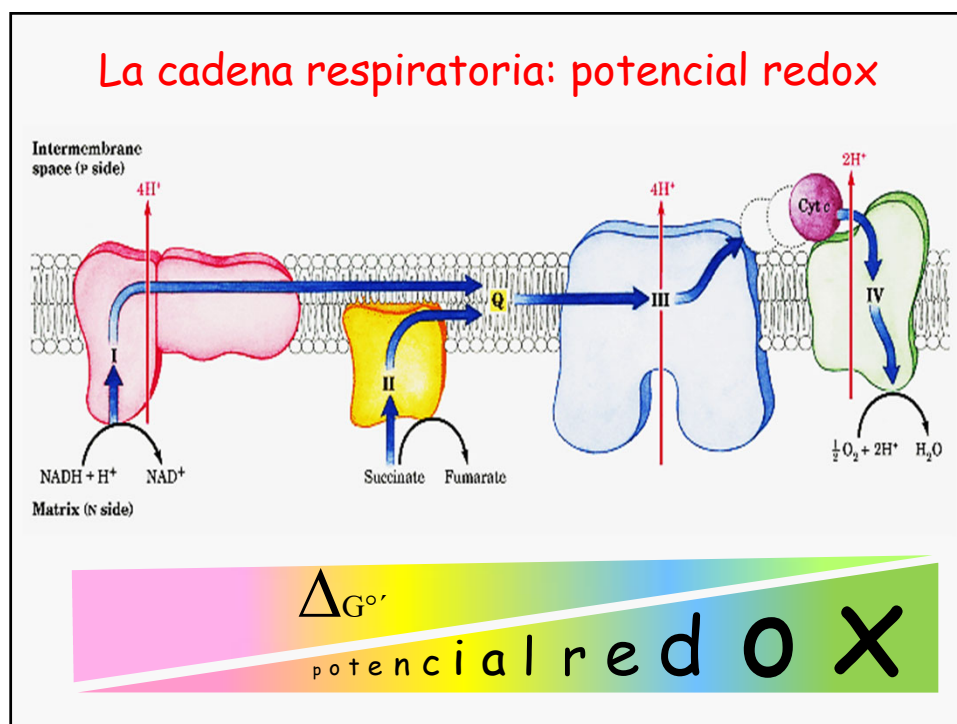
Potencial redox



38



39

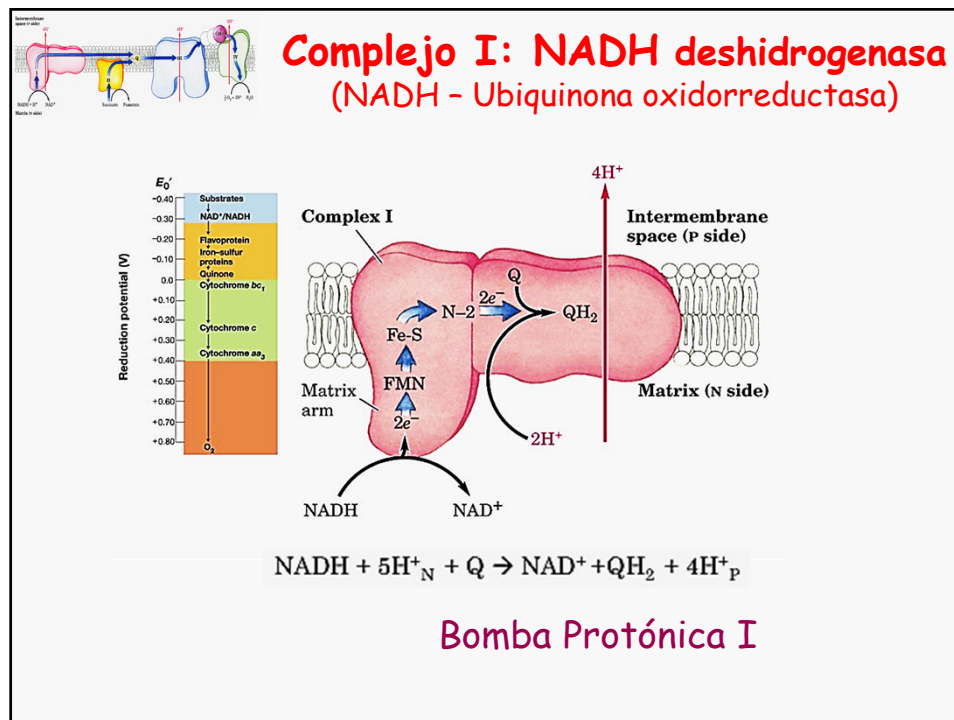


40

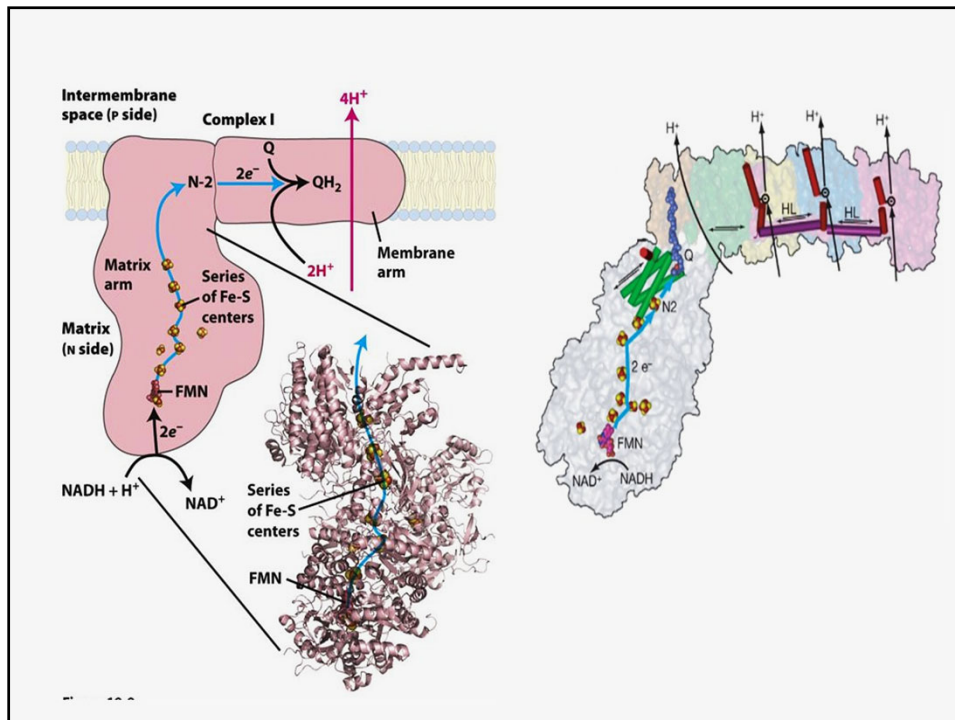
TABLE 14.1 Characteristics of protein complexes of the mitochondrial respiratory electron-transport chain in bovine heart

Complex	Subunits	Molecular weight	Oxidation-reduction components
I. NADH-ubiquinone oxidoreductase (NADH deshidrogenase)	42 or 43	> 900 000	1 FMN 22–24 Fe–S in 7 or 8 clusters
II. Succinate-ubiquinone oxidoreductase (Succinate deshidrogenase)	4	125 000	1 FAD 3 Fe–S clusters Cytochrome b_{560}
Coenzima Q o Ubiquinona			
III. Ubiquinol–cytochrome c oxidoreductase (Complex cytochrome bc_1)	2	~250 000 (dimer of 11-chain subunits)	1 Fe–S cluster Cytochrome b Cytochrome c_1
Citocromo c			
IV. Cytochrome c oxidase (Cytochrome oxidase)	2	420 000 (dimer of 13-chain subunits)	Cytochrome a Cytochrome a_3 2 Copper ions

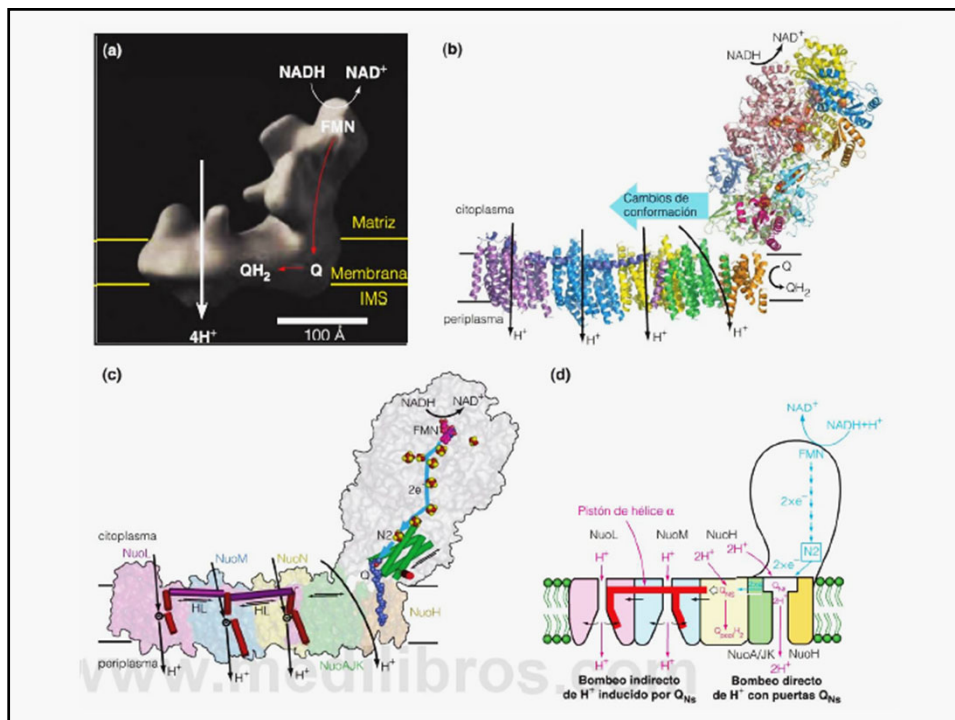
41



42

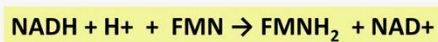
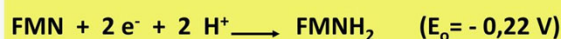
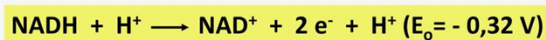


43

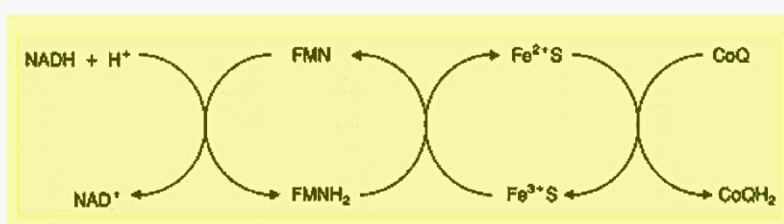


44

Reacciones del Complejo I



Camino de los equivalentes de reducción en el Complejo I

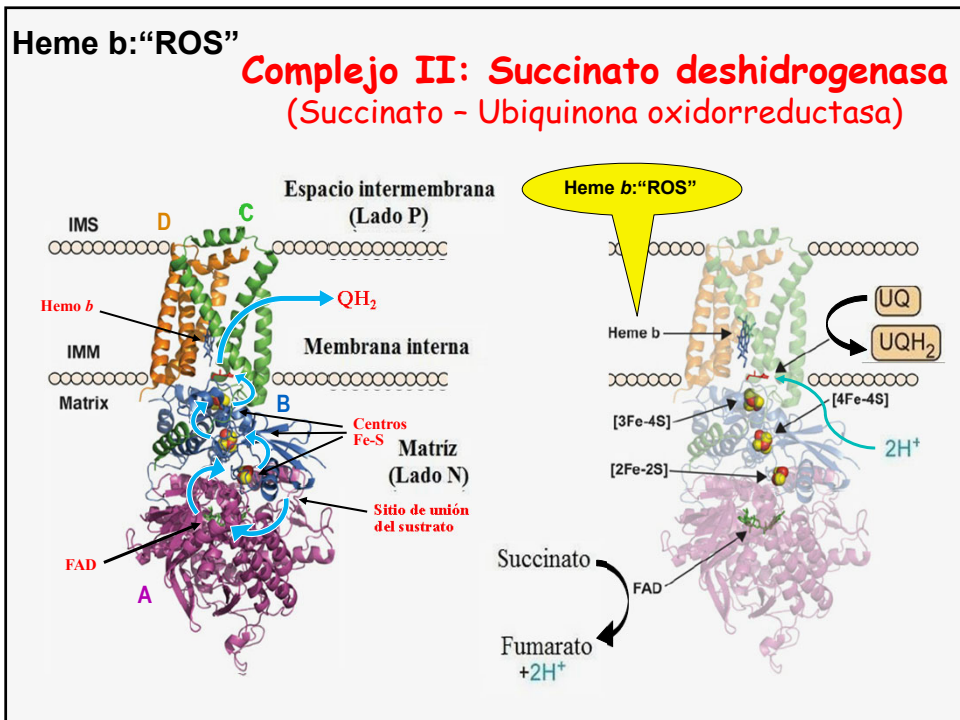


45

TABLE 14.1 Characteristics of protein complexes of the mitochondrial respiratory electron-transport chain in bovine heart

Complex	Subunits	Molecular weight	Oxidation-reduction components
I. NADH-ubiquinone oxidoreductase (NADH deshidrogenase)	42 or 43	> 900 000	1 FMN 22–24 Fe–S in 7 or 8 clusters
II. Succinate-ubiquinone oxidoreductase (Succinate deshidrogenase)	4	125 000	1 FAD 3 Fe–S clusters Cytochrome <i>b</i> ₅₆₀
Coenzima Q o Ubiquinona			
III. Ubiquinol–cytochrome <i>c</i> oxidoreductase (Complex cytochrome <i>bc</i> ₁) Citocromo <i>c</i>	2	~250 000 (dimer of 11-chain subunits)	1 Fe–S cluster Cytochrome <i>b</i> Cytochrome <i>c</i> ₁
IV. Cytochrome <i>c</i> oxidase (Cytochrome oxidase)	2	420 000 (dimer of 13-chain subunits)	Cytochrome <i>a</i> Cytochrome <i>a</i> ₃ 2 Copper ions

46



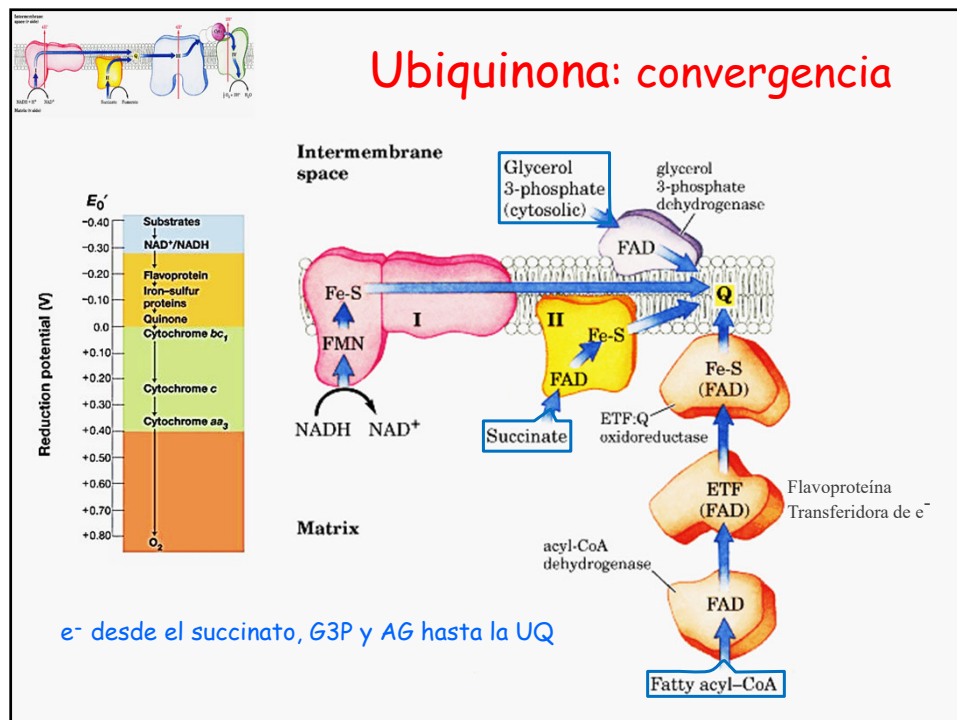
47

Complejo II

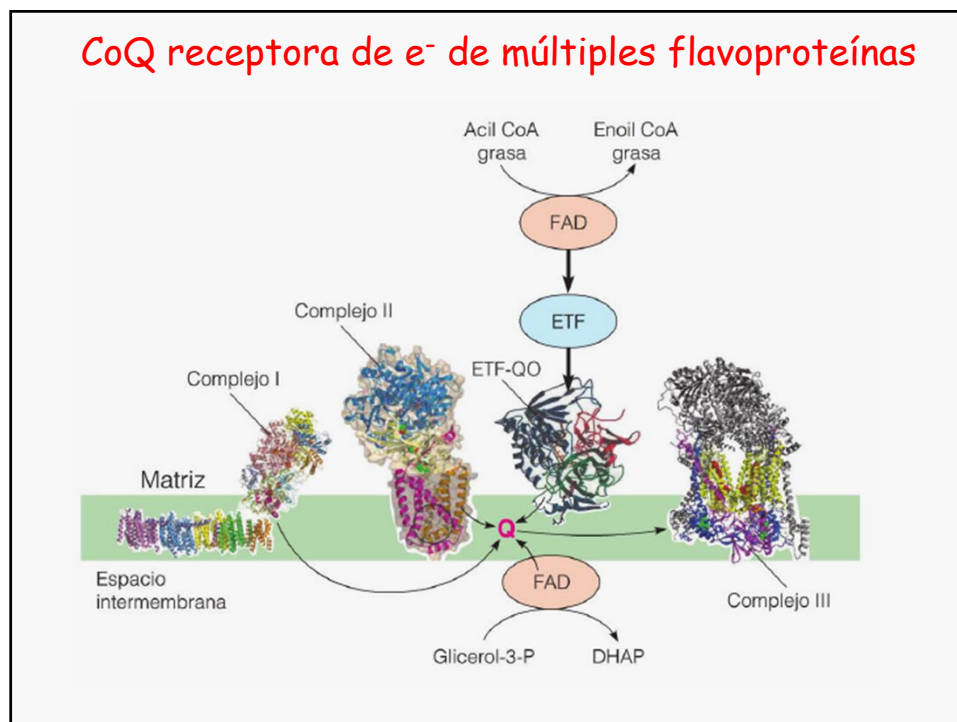
- Succinato-coenzima Q oxidorreductasa (E)
- Coenzima: FAD
- Proteínas ferrosulfuradas
- Transfiere equivalentes de reducción desde succinato a la coenzima Q

Succinato + E-FAD	→	Fumarato + E-FADH ₂
E-FADH ₂ + Prot-Fe ⁺⁺⁺	→	E-FAD + Prot-Fe ⁺⁺
Prot-Fe ⁺⁺ + CoQ	→	Prot-Fe ⁺⁺⁺ + CoQH ₂

48



49

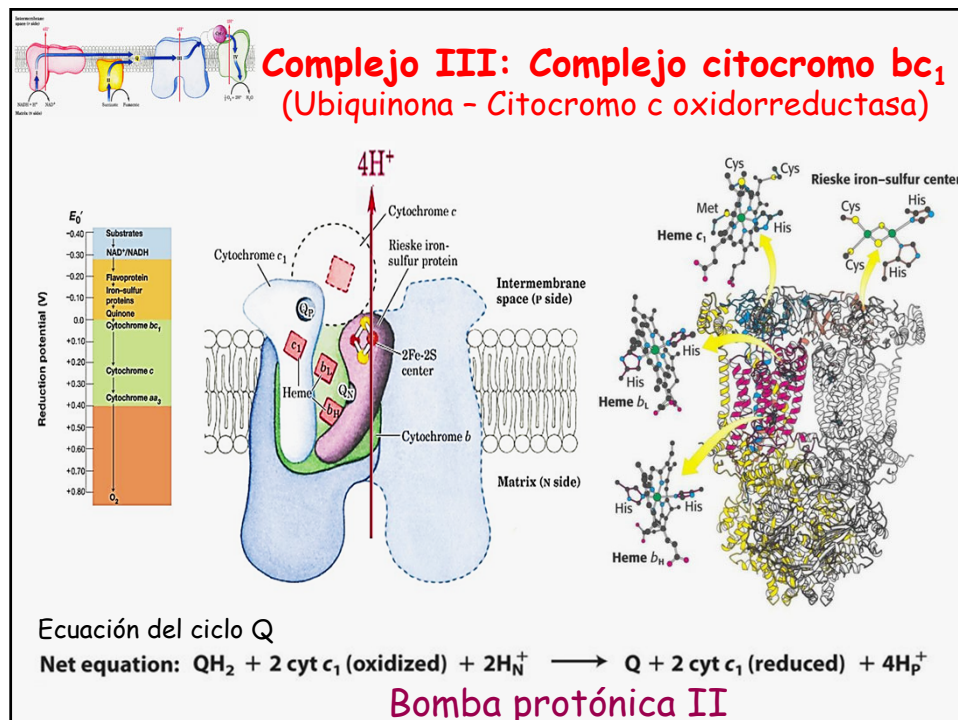


50

TABLE 14.1 Characteristics of protein complexes of the mitochondrial respiratory electron-transport chain in bovine heart

Complex	Subunits	Molecular weight	Oxidation-reduction components
I. NADH-ubiquinone oxidoreductase (NADH deshidrogenase)	42 or 43	> 900 000	1 FMN 22–24 Fe–S in 7 or 8 clusters
II. Succinate-ubiquinone oxidoreductase (Succinate deshidrogenase)	4	125 000	1 FAD 3 Fe–S clusters Cytochrome b_{560}
Coenzima Q o Ubiquinona			
III. Ubiquinol–cytochrome c oxidoreductase (Complex cytochrome bc_1)	2	~250 000 (dimer of 11-chain subunits)	1 Fe–S cluster Cytochrome b Cytochrome c_1
Citocromo c			
IV. Cytochrome c oxidase (Cytochrome oxidase)	2	420 000 (dimer of 13-chain subunits)	Cytochrome a Cytochrome a_3 2 Copper ions

51



52

Complejo III: estructura (dímero)

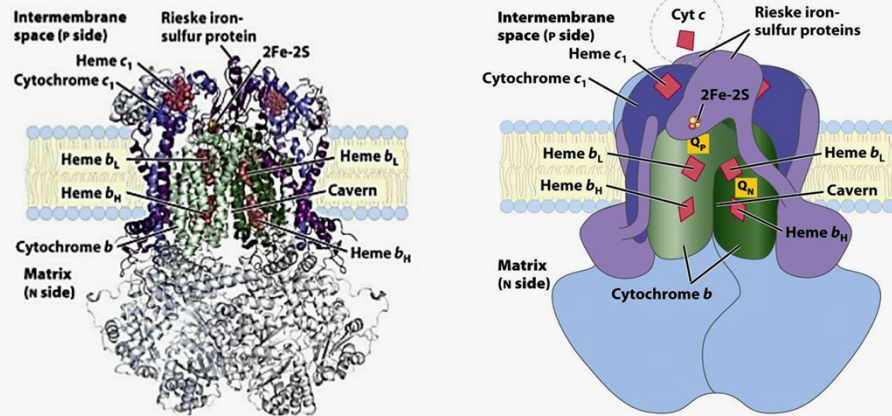


Figure 19-11b
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

53

ciclo Q: complejo citocromo bc1

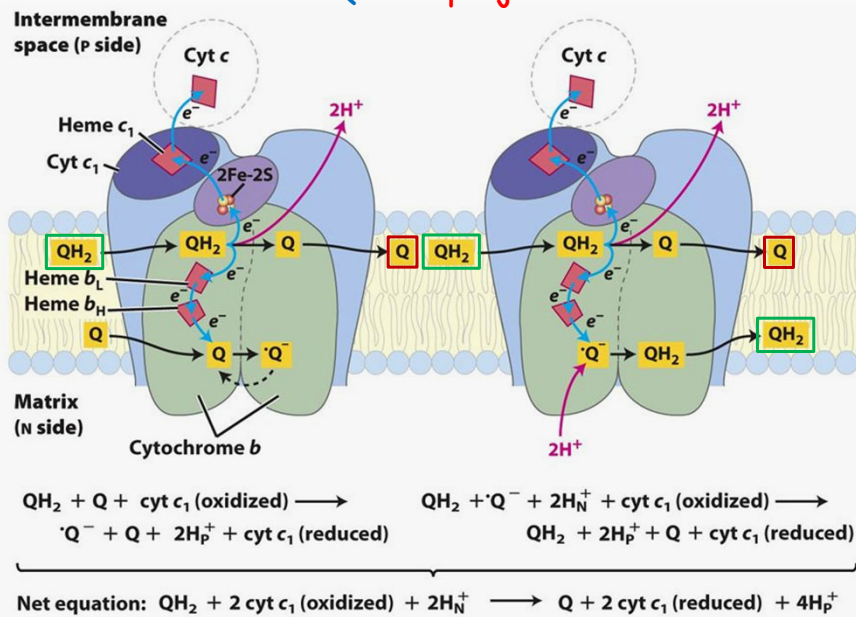
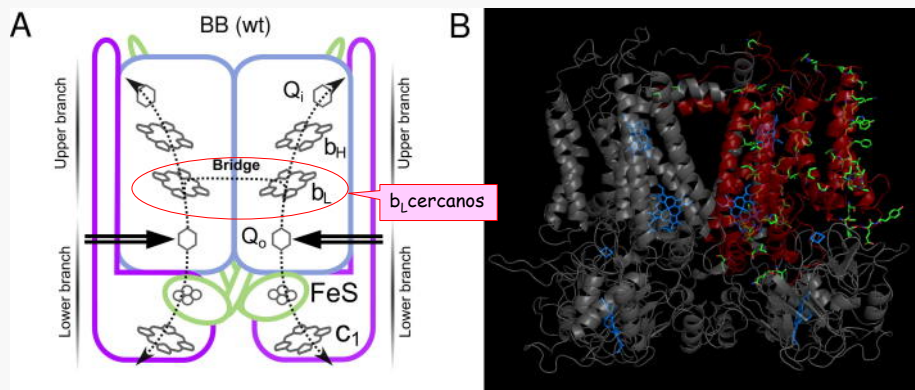


Figure 19-12
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

54

Intra- and inter-monomer electron transfers

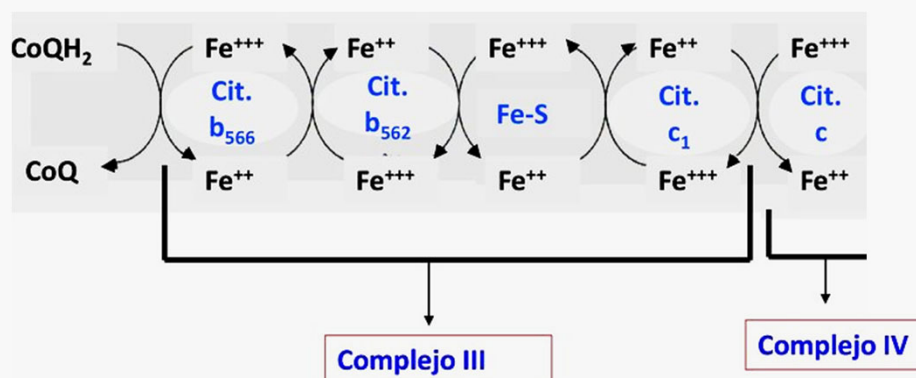


Catalytic core of cytochrome bc_1 . (A) H-shaped electron transfer system formed by cofactor chains of cytochrome b (blue), cytochrome c_1 (purple) and FeS subunits (green). Electron entry sites (double arrow), electron paths (dotted lines). (B) Crystal structure of *R. capsulatus* cytochrome bc_1 dimer (PDB: 1ZRI). Heme and FeS cofactors are blue. Cytochrome b subunit of one monomer is shown in red and depicts those amino acids that are different in *R. sphaeroides* (green sticks) to illustrate the heterogeneity of hybrid fusion protein used in this study.

Hybrid fusions show that inter-monomer electron transfer robustly supports cytochrome bc_1 function in vivo. Robert Ekiert, Monika Czapl, Marcin Sarewicz, Artur Osyczka. Biochemical and Biophysical Research Communications 451 (2014) 270-275.

55

Paso de e^- por el Complejo III

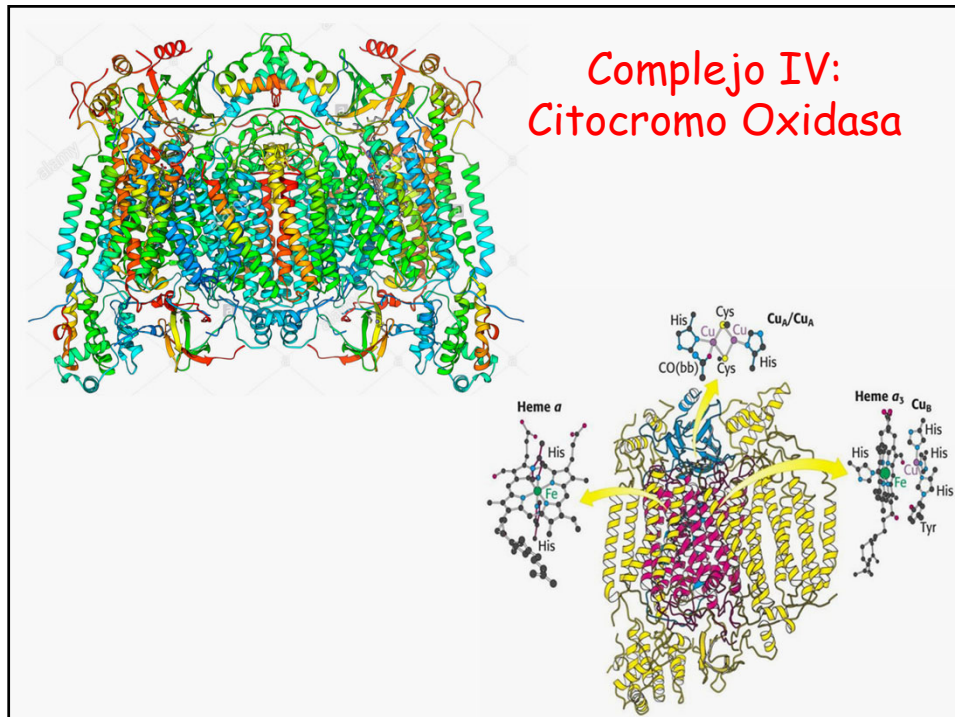


56

TABLE 14.1 Characteristics of protein complexes of the mitochondrial respiratory electron-transport chain in bovine heart

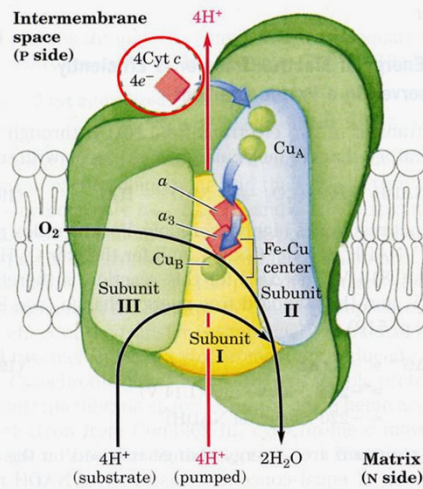
Complex	Subunits	Molecular weight	Oxidation-reduction components
I. NADH-ubiquinone oxidoreductase (NADH deshidrogenase)	42 or 43	> 900 000	1 FMN 22–24 Fe–S in 7 or 8 clusters
II. Succinate-ubiquinone oxidoreductase (Succinate deshidrogenase)	4	125 000	1 FAD 3 Fe–S clusters Cytochrome b_{560}
Coenzima Q o Ubiquinona			
III. Ubiquinol–cytochrome c oxidoreductase (Complex cytochrome bc_1)	2	~250 000 (dimer of 11-chain subunits)	1 Fe–S cluster Cytochrome b Cytochrome c_1
Citocromo c			
IV. Cytochrome c oxidase (Cytochrome oxidase)	2	420 000 (dimer of 13-chain subunits)	Cytochrome a Cytochrome a_3 2 Copper ions

57

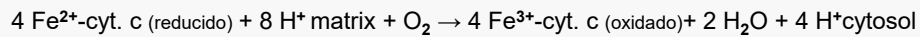


58

Complejo IV: Citocromo Oxidasa

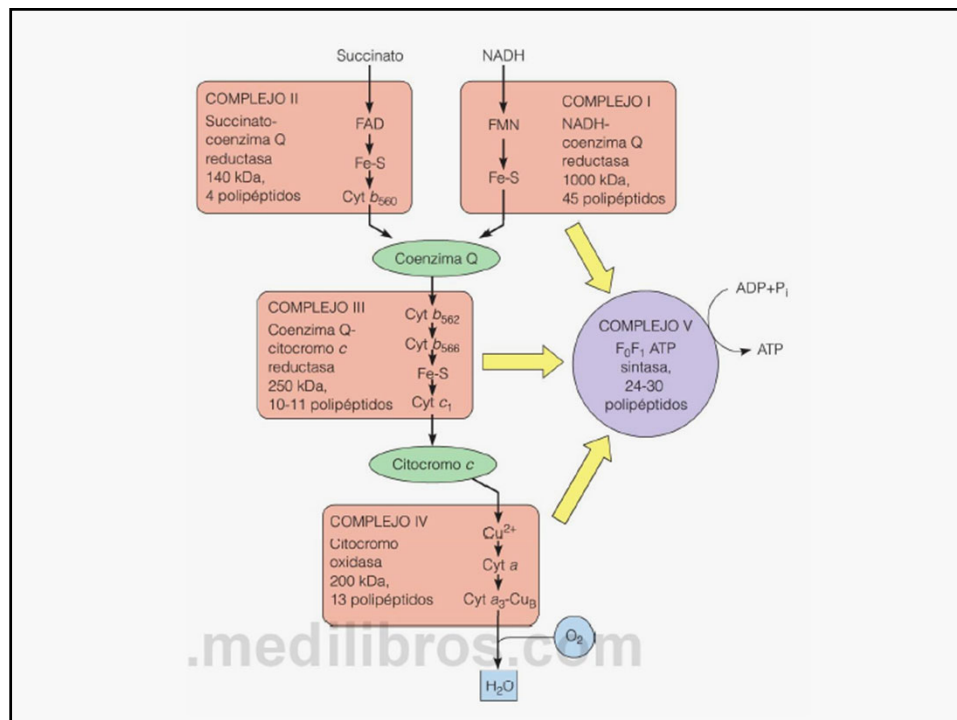


Complejo IV: Citocromo Oxidasa

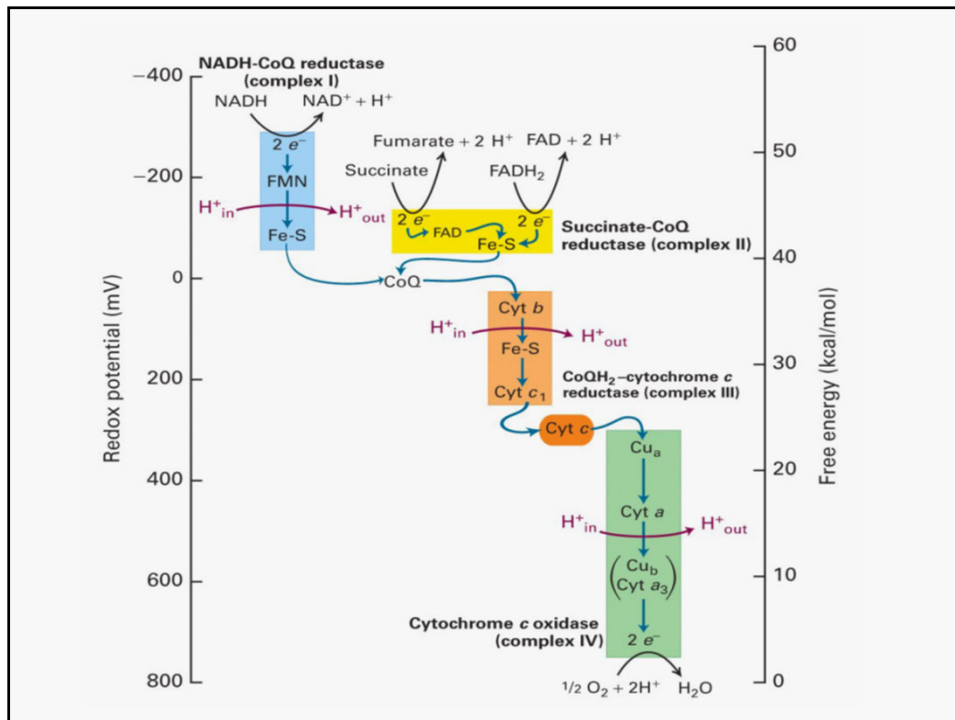


Bomba protónica III

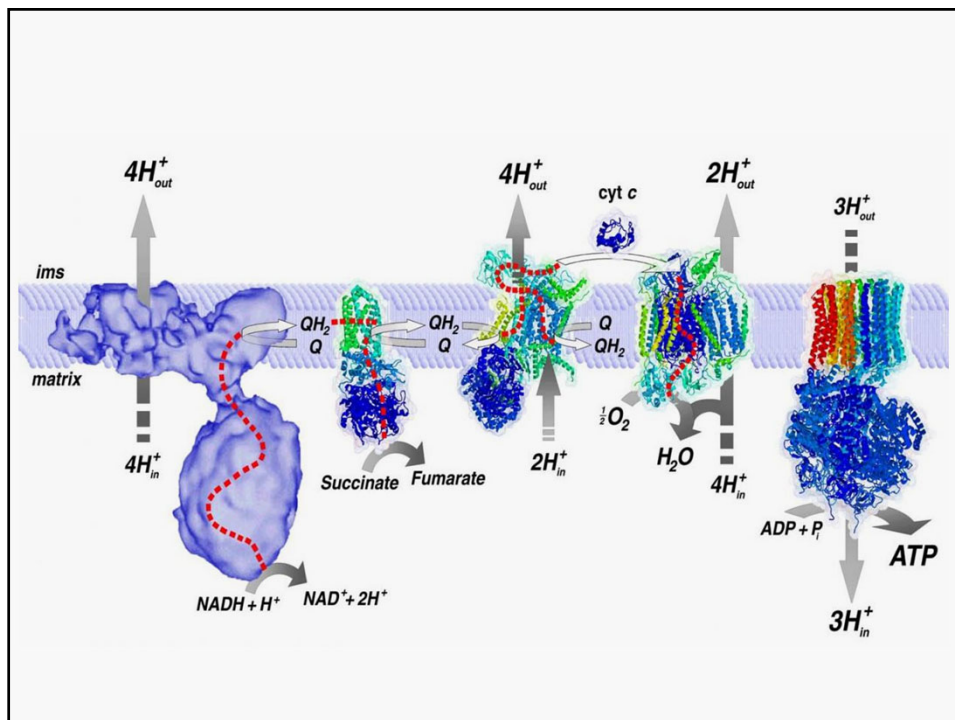
59



60

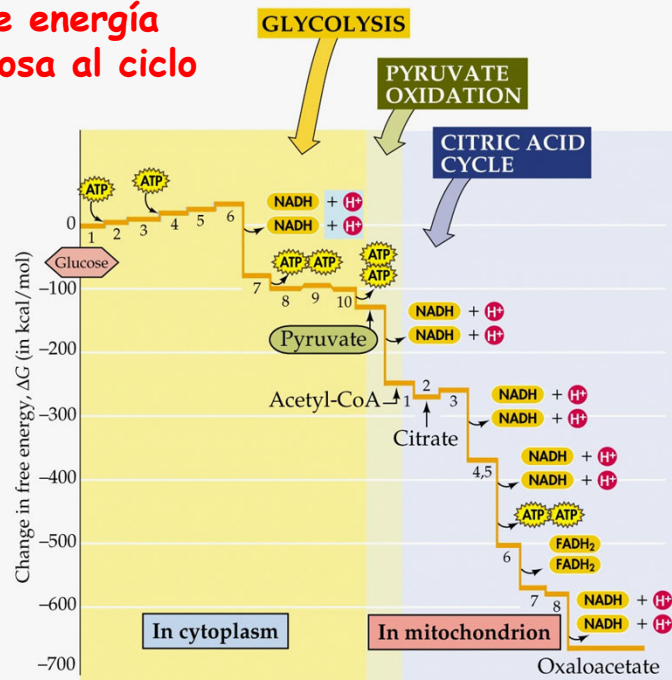


61



62

Los niveles de energía desde la glucosa al ciclo de Krebs

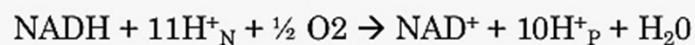


63

Conclusiones

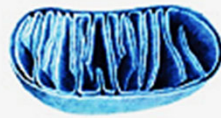
- La energía de la transferencia de electrones se conserva eficientemente en un gradiente de protones.
- La transferencia de dos electrones desde el NADH hasta el O₂ molecular se puede expresar así:

$$\text{NADH} + \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O}$$
- Por cada par de electrones transferidos al O₂:
 el complejo I bombea: 4 protones
 el complejo III bombea: 4 protones
 el complejo IV bombea: 2 protones.

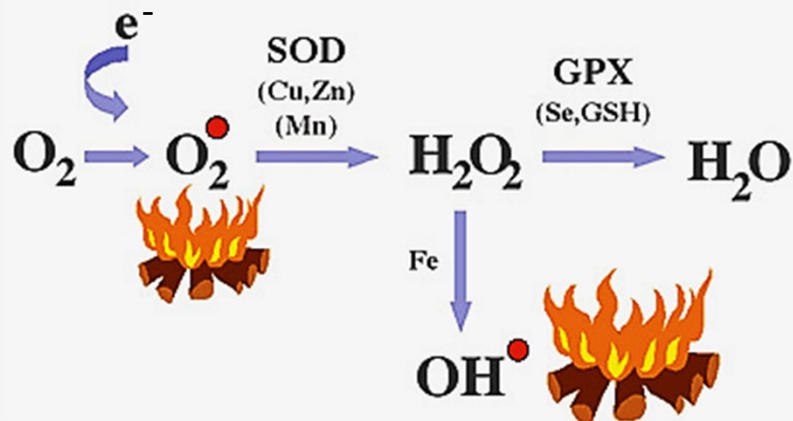


64

Mitocondria: Radicales Libres

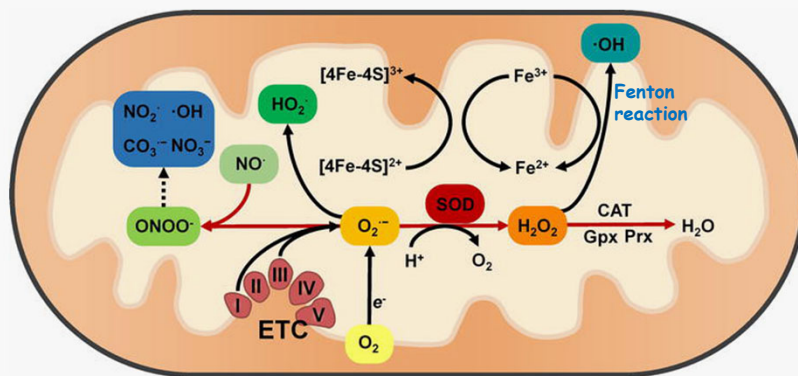


Cerca de 5% de los electrones de alta energía caen de la cadena de los transportistas



65

Reacciones y transformaciones de los ROS mitocondriales



Reactions and transformations of mitochondrial ROS. SOD enzymes catalyse the dismutation of superoxide ($\text{O}_2^{\cdot -}$), generating hydrogen peroxide (H_2O_2). The catalase (CAT), glutathione peroxidases (Gpxs) and peroxiredoxins (Prxs) convert H_2O_2 into water. H_2O_2 reacts with redox-active iron to generate the hydroxyl radical ($\text{OH}^{\cdot}$) through the Fenton reaction. The reaction between $\text{O}_2^{\cdot -}$ and nitric oxide ($\text{NO}^{\cdot}$) produces peroxynitrite ($\text{ONOO}^{\cdot}$), whose decomposition in turn gives rise to some highly oxidizing intermediates including $\text{NO}_2^{\cdot}$, $\text{OH}^{\cdot}$, $\text{CO}_3^{\cdot -}$ as well as, finally, stable NO_3^- . Thus, increased $\text{O}_2^{\cdot -}$ levels can also reduce $\text{NO}^{\cdot}$ and generate $\text{ONOO}^{\cdot}$ toxicity. $\text{O}_2^{\cdot -}$ by itself can reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in iron-sulphur centres of proteins, leading to enzyme inactivation and concomitant loss of Fe^{2+} from the enzymes. The protonation of $\text{O}_2^{\cdot -}$ can form the more reactive hydroperoxyl radical ($\text{HO}_2^{\cdot}$).

66

