
JVK
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SBS-922 Membrane Proteins

Mitochondria and respiratory chains

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Presentations
and
supplementary information

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Lectures in Membrane Proteins

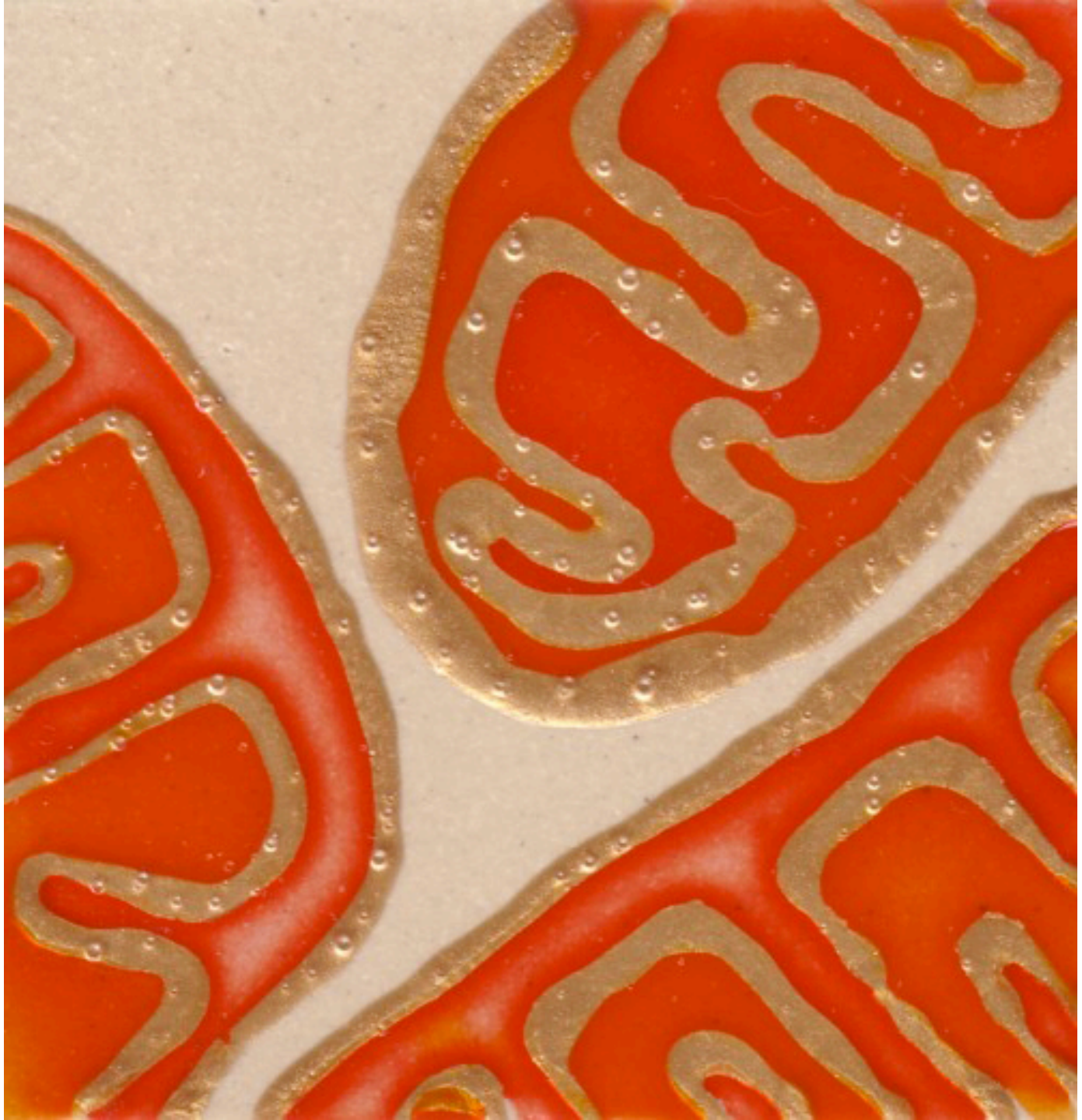
- Lecture 1. Mitochondrial membranes and chemiosmotic coupling. (Acrobat - .pdf)
- Lecture 2. Redox carriers. (Acrobat - .pdf)
- Lecture 3. Complex I. Structure and Function. Part 1 (Acrobat - .pdf)
- Lecture 4. Complex I. Part 2. Complete structure, including the hydrophobic domain. (Acrobat - .pdf)
- Lecture 5. Complex II and complex III Part 1. Structure and Function. (Acrobat - .pdf)
- Lecture 6. Complex III. The Q-cycle. (Acrobat - .pdf)
- Lecture 7. ATP Synthase. (Acrobat - .pdf)

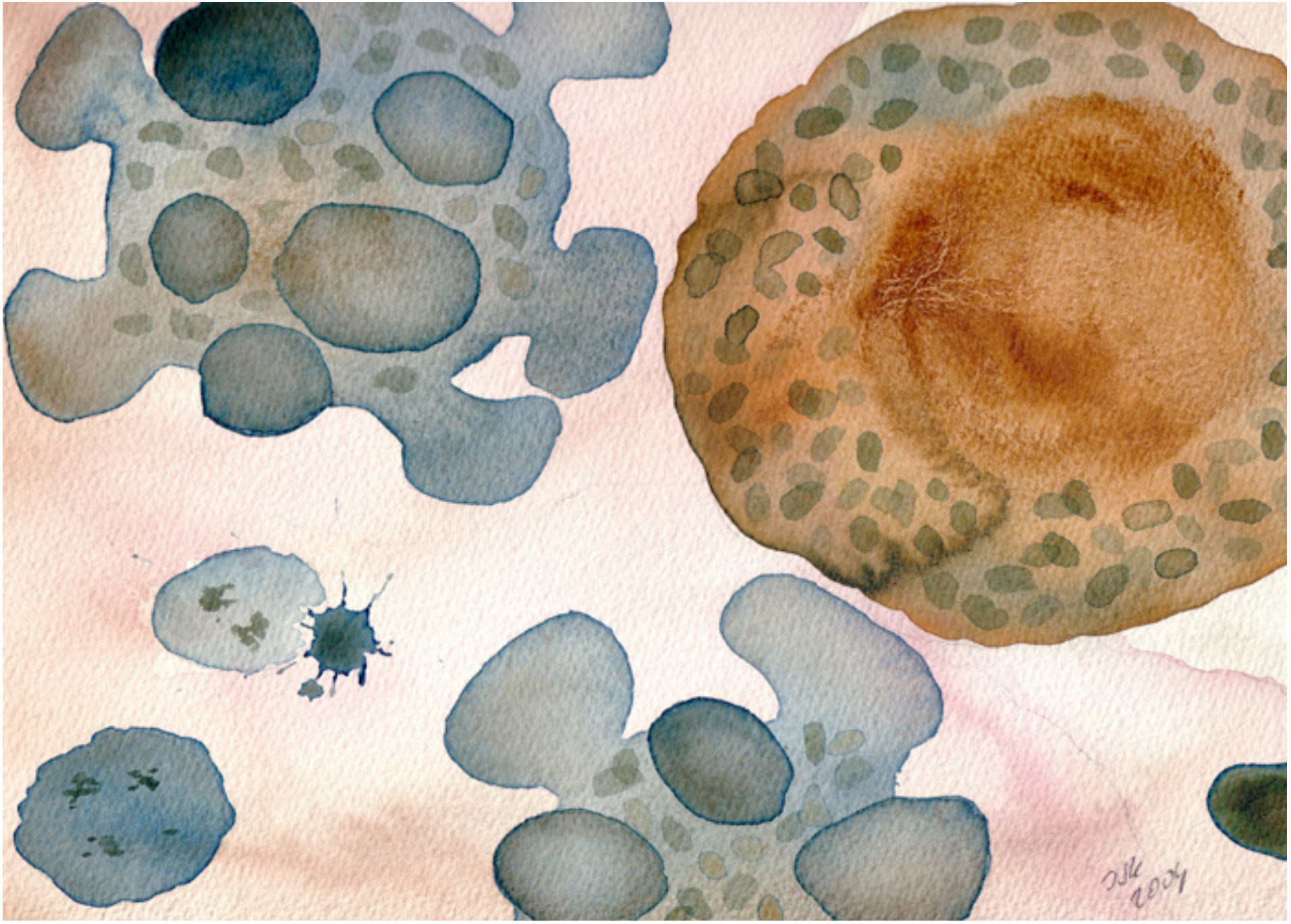
Membrane Proteins

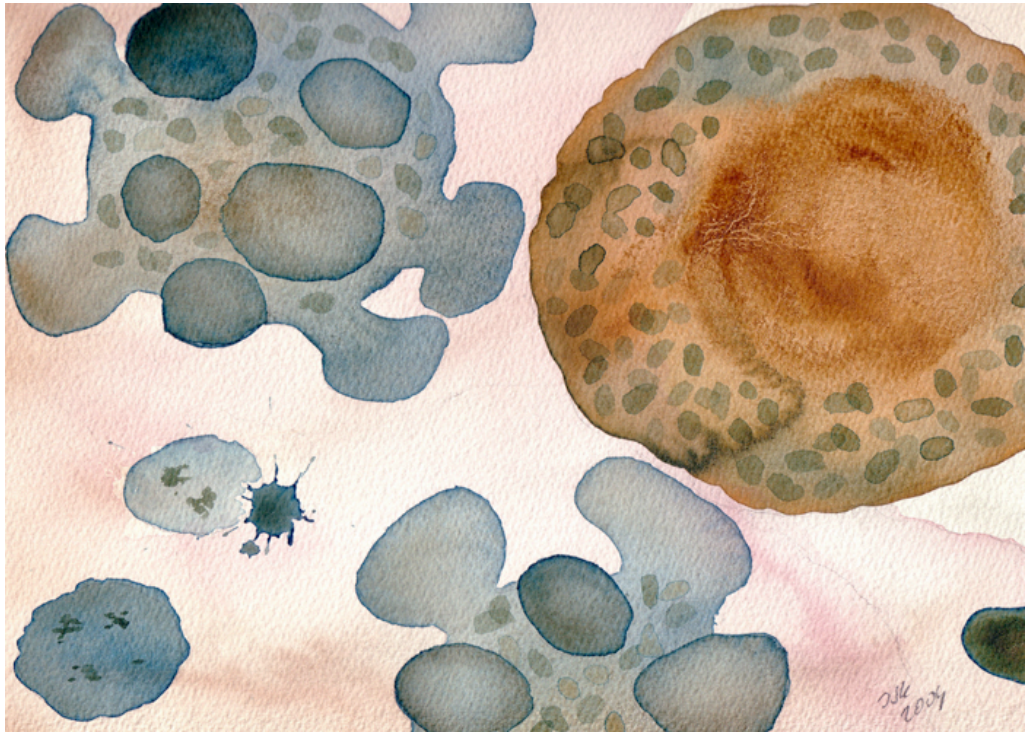
- Membrane Proteins course web page

References

- Nicholls DJ, Ferguson, SJ. Bioenergetics3. Academic Press/Elsevier Science 2002
- Molecular Biology of the Cell. Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. 5th edition. 2007. Garland Science. 4th Edition online at NCBI...







Death by apoptosis—mitochondria determine whether a cell lives or dies by enforced suicide

When cells in the body become worn out or damaged, they die by enforced suicide, or apoptosis. The cell blebs, is packaged up, and reabsorbed. If the mechanisms controlling apoptosis fail, the result is cancer, a conflict of interest between cells and the body as a whole. Apoptosis seems to be necessary for the integrity and cohesion of multicellular individuals, but how did once-independent cells come to accept death for the greater good? Today apoptosis is policed by mitochondria, and the machinery of death was inherited from their bacterial ancestors, suggesting a history of murder. So was the cohesion of the individual forged in deadly conflict.

Complex II structure....

and location of genes

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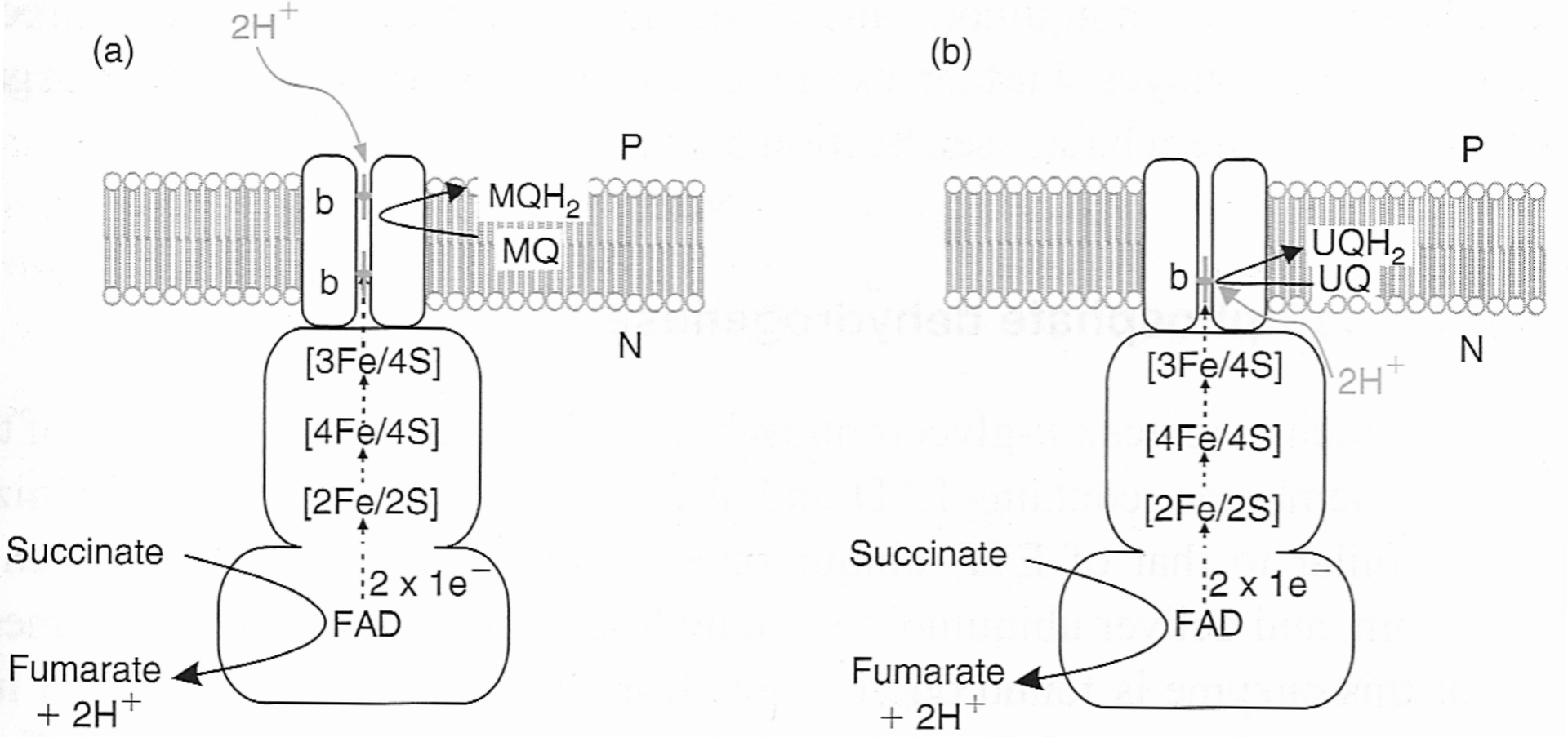
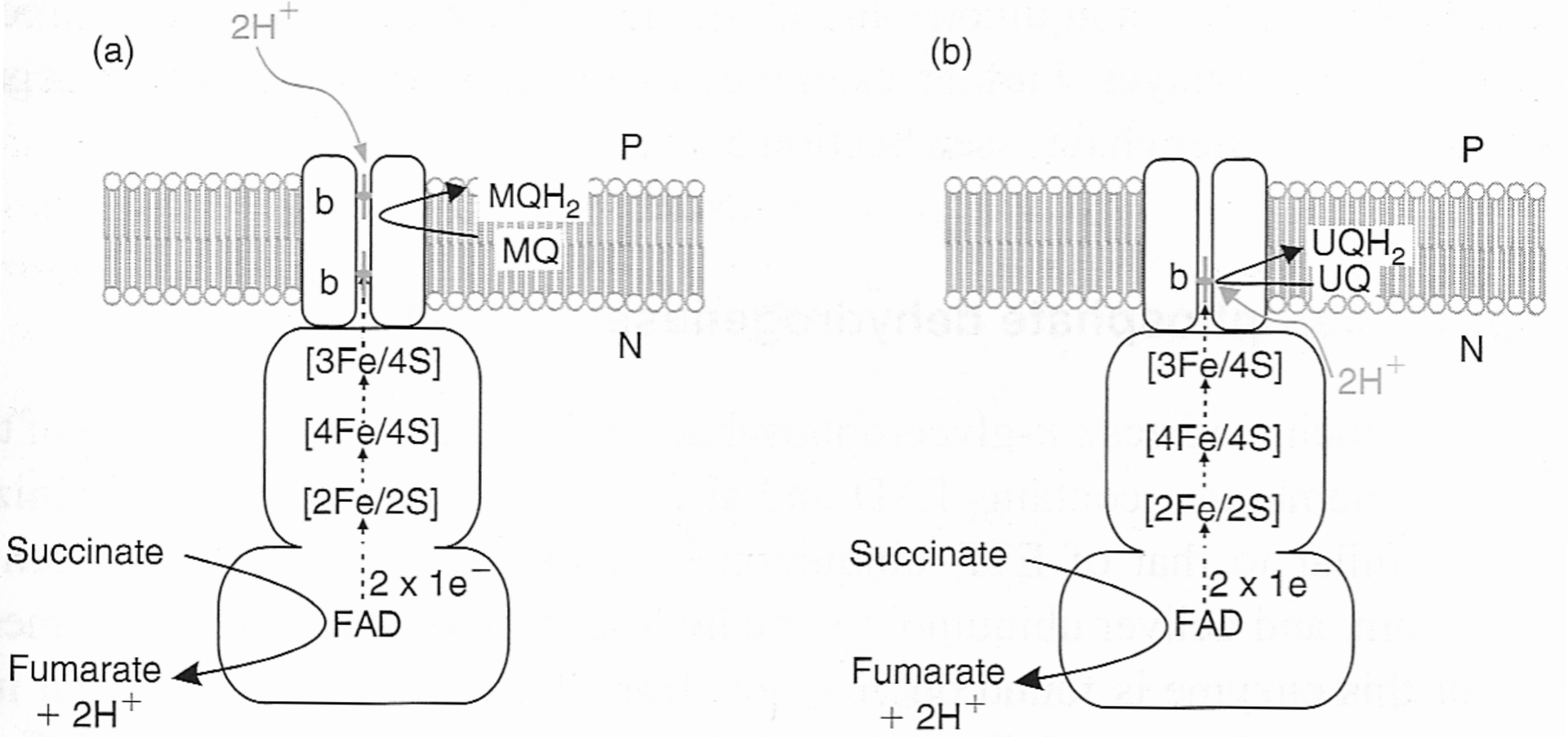


Figure 5.13 Schematic models for the structural organizations of mitochondrial (a) and *B. subtilis* (b) succinate dehydrogenases based on the crystal structures of the closely related fumarate reductases of *E. coli* and *W. succinogenes*.



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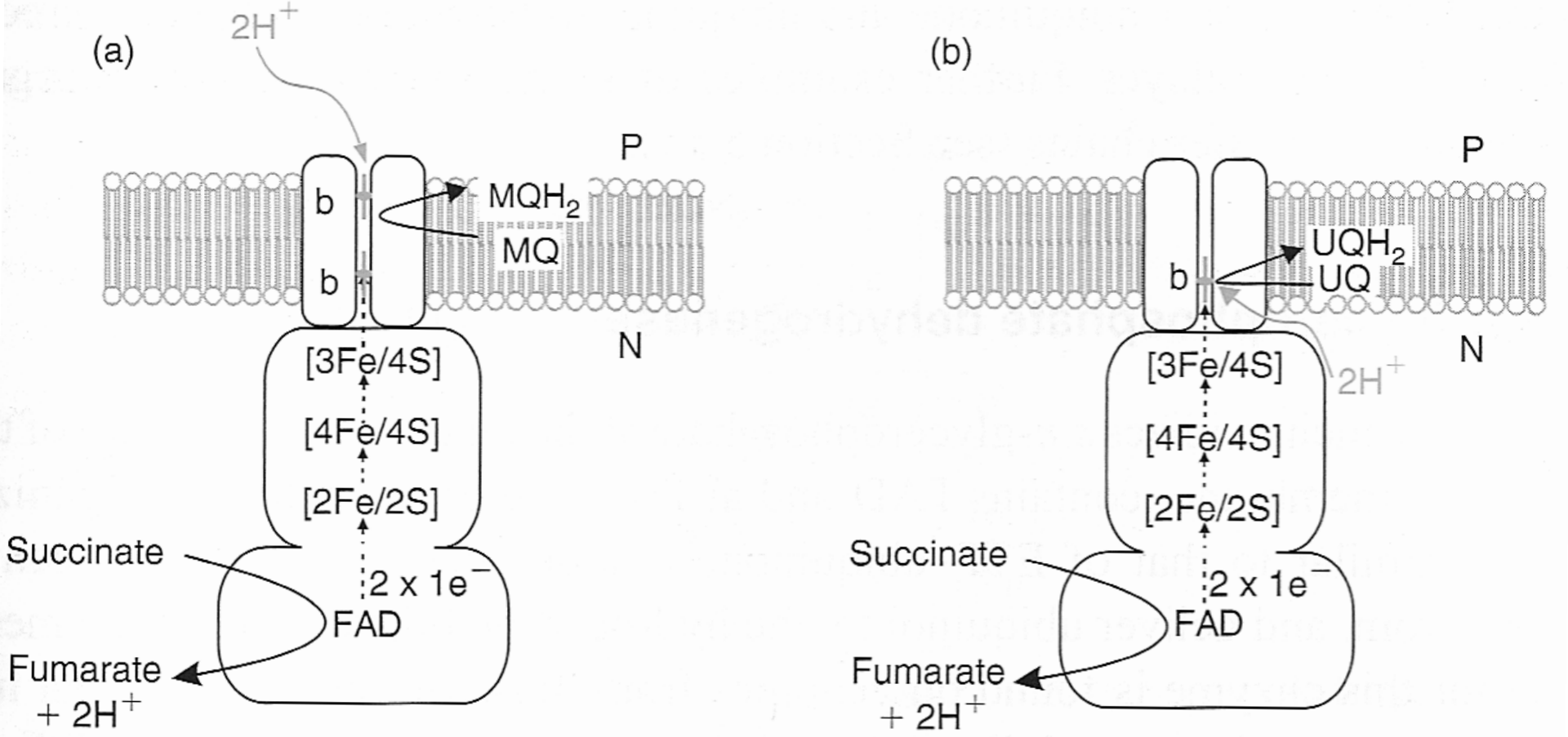

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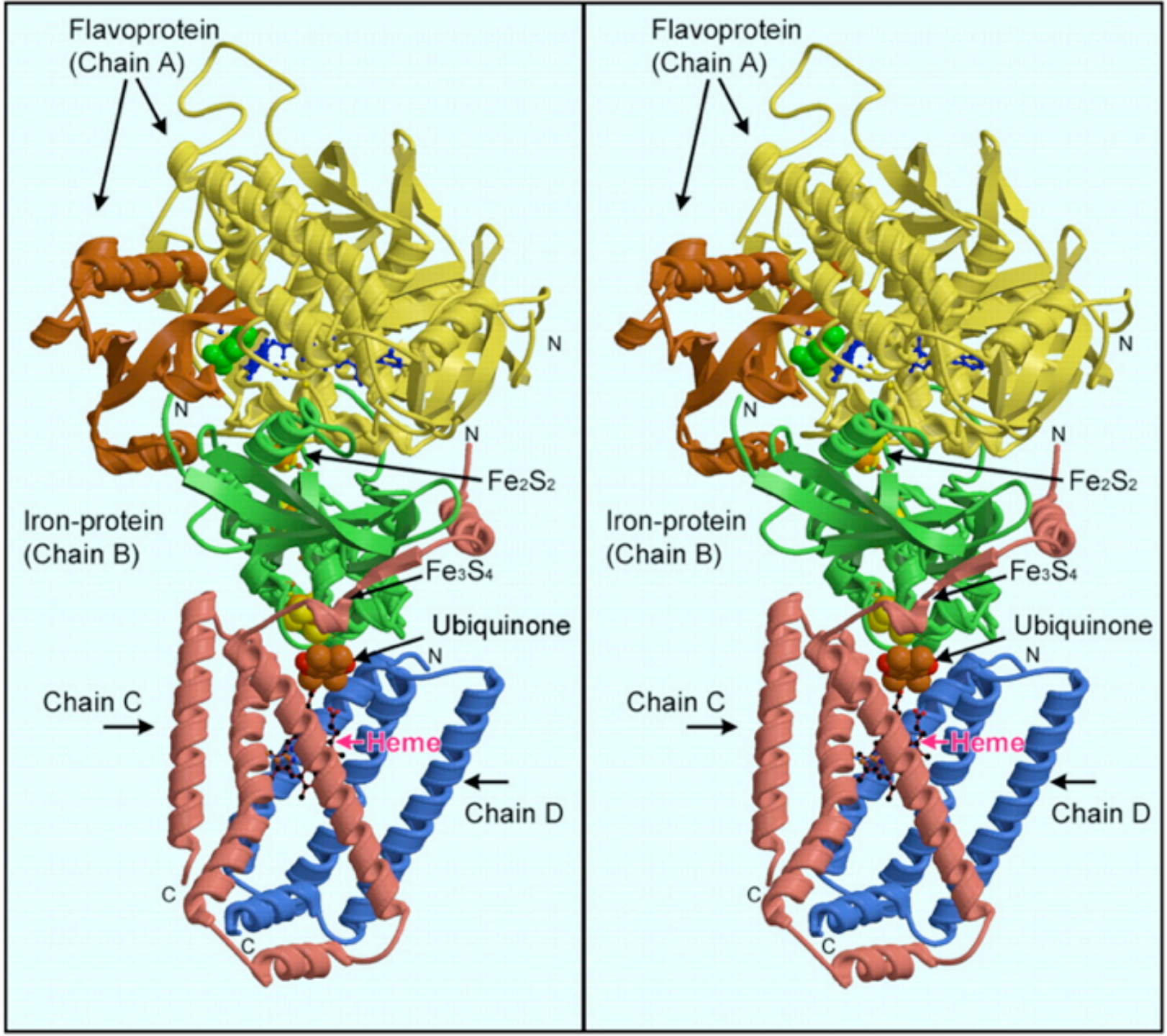

Flavoprotein
(Chain A)
N
N
N
Fe2S2
Iron-protein
(Chain B)
Fe3S4
Ubiquinone
N
Chain C
Heme
Chain D
C
C
Flavoprotein
(Chain A)
N
N
N
Fe2S2
Iron-protein
(Chain B)
Fe3S4
Ubiquinone
N
Chain C
Heme
Chain D
C
C

(By Richard Wheeler (Zephyris) 2006. {{PDBl1YQ3}} ==Licensing== {{GFDL-self}} Category:Protein images)

http://en.wikipedia.org/wiki/Image:Succinate_Dehydrogenase_1YQ3_Electron_Carriers_Labeled.png

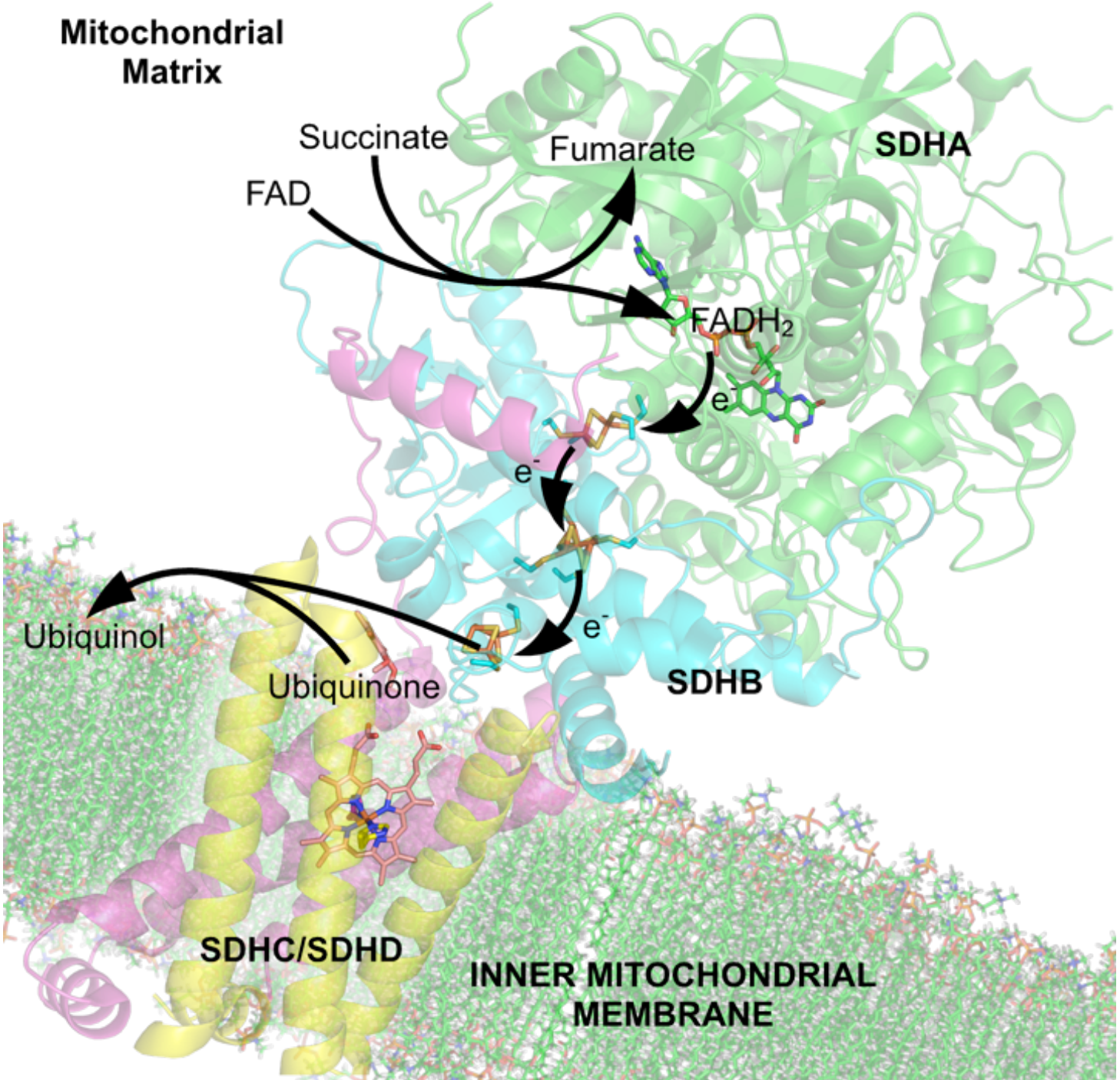
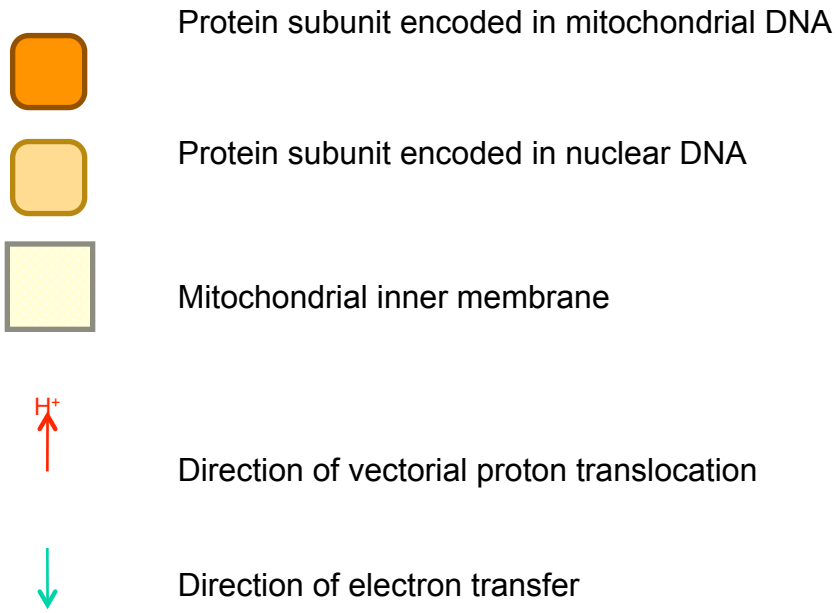
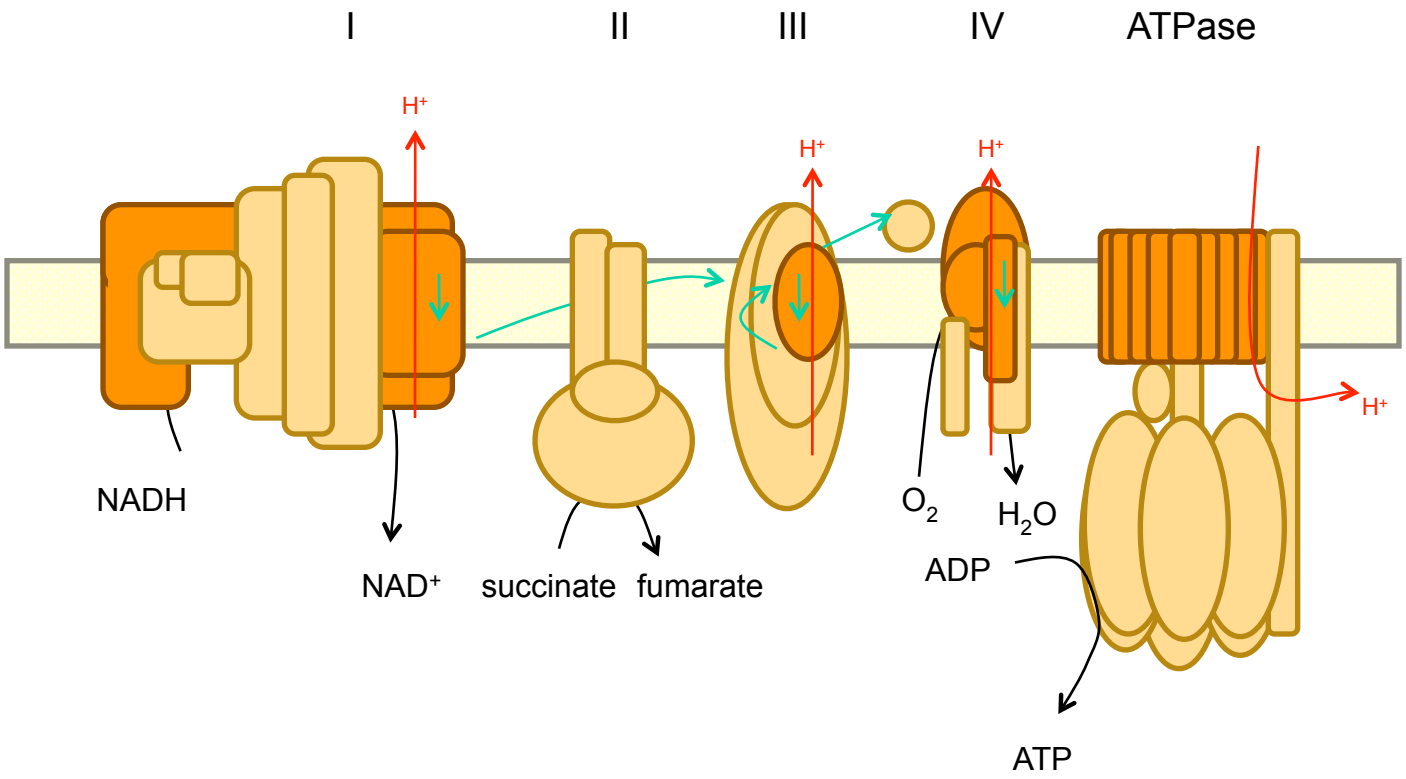

Mitochondrial Matrix
Succinate
Fumarate
SDHA
FAD
FADH2
e-
e-
e-
Ubiquinol
Ubiquinone
SDHB
SDHC/SDHD
INNER MITOCHONDRIAL MEMBRANE

TABLE 1
Tabulation of some x-ray structures available for members of the SQR/FRD family

Enzyme	Source	Reference	Protein Data Bank code	Resolution	Relevance
SQR	Chicken		1YQ3	2.2	Crystallized with OAA
			1YQ4	2.4	Crystallized with 3-NP
			2FBW	2.1	Crystal soaked with carboxin
	Pig	24	1ZOY	2.4	Chain A Arg^{298} out of active site
			1ZP0	3.5	Crystallized with 3-NP and TTFA
	E. coli	23	1NEK	2.6	Chain A Arg^{286} out of active site
FCc	*Shewanella*	38	1QJD	1.8	Dicarboxylate site like SQR
FRD	*Wolinella*	49	1QLA/B	2.2	Open CAP domain
		46	1QO8	3.0	Chain A Arg^{301} disordered
FRD	*E. coli*	22	1KF6	2.7	CAP domain slightly open


Protein subunit encoded in mitochondrial DNA
Protein subunit encoded in nuclear DNA
Mitochondrial inner membrane
H+
Direction of vectorial proton translocation
Direction of electron transfer


Inter-membrane space
I
II
III
IV
ATPase
H+
H+
H+
H+
H+
NADH
NAD+
succinate
fumarate
O2
H2O
ADP
ATP
Mitochondrial matrix

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 2328–2332, March 1996
Evolution

Genes encoding the same three subunits of respiratory complex II are present in the mitochondrial DNA of two phylogenetically distant eukaryotes

(mitochondrion/succinate:ubiquinone oxidoreductase/succinate dehydrogenase/*Porphyra purpurea*/*Reclinomonas americana*)

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Communicated by Charles S. Levings III, North Carolina State University, Raleigh, NC, November 8, 1995 (received for review July 28, 1995)

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ABSTRACT **Although mitochondrial DNA is known to encode a limited number (<20) of the polypeptide components of respiratory complexes I, III, IV, and V, genes for components of complex II [succinate dehydrogenase (ubiquinone); succinate:ubiquinone oxidoreductase, EC 1.3.5.1] are conspicuously lacking in mitochondrial genomes so far characterized. Here we show that the same three subunits of complex II are encoded in the mitochondrial DNA of two phylogenetically distant eukaryotes, *Porphyra purpurea* (a photosynthetic red alga) and *Reclinomonas americana* (a heterotrophic zooflagellate). These complex II genes, *sdh2*, *sdh3*, and *sdh4*, are homologs, respectively, of *Escherichia coli sdhB*, *sdhC*, and *sdhD*. In *E. coli*, *sdhB* encodes the iron-sulfur subunit of succinate dehydrogenase (SDH), whereas *sdhC* and *sdhD* specify, respectively, apocytochrome b_{558} and a hydrophobic 13-kDa polypeptide, which together anchor SDH to the inner mitochondrial membrane. Amino acid sequence similarities indicate that *sdh2*, *sdh3*, and *sdh4* were originally encoded in the protomitochondrial genome and have subsequently been transferred to the nuclear genome in most eukaryotes. The data presented here are consistent with the view that mitochondria constitute a monophyletic lineage.**

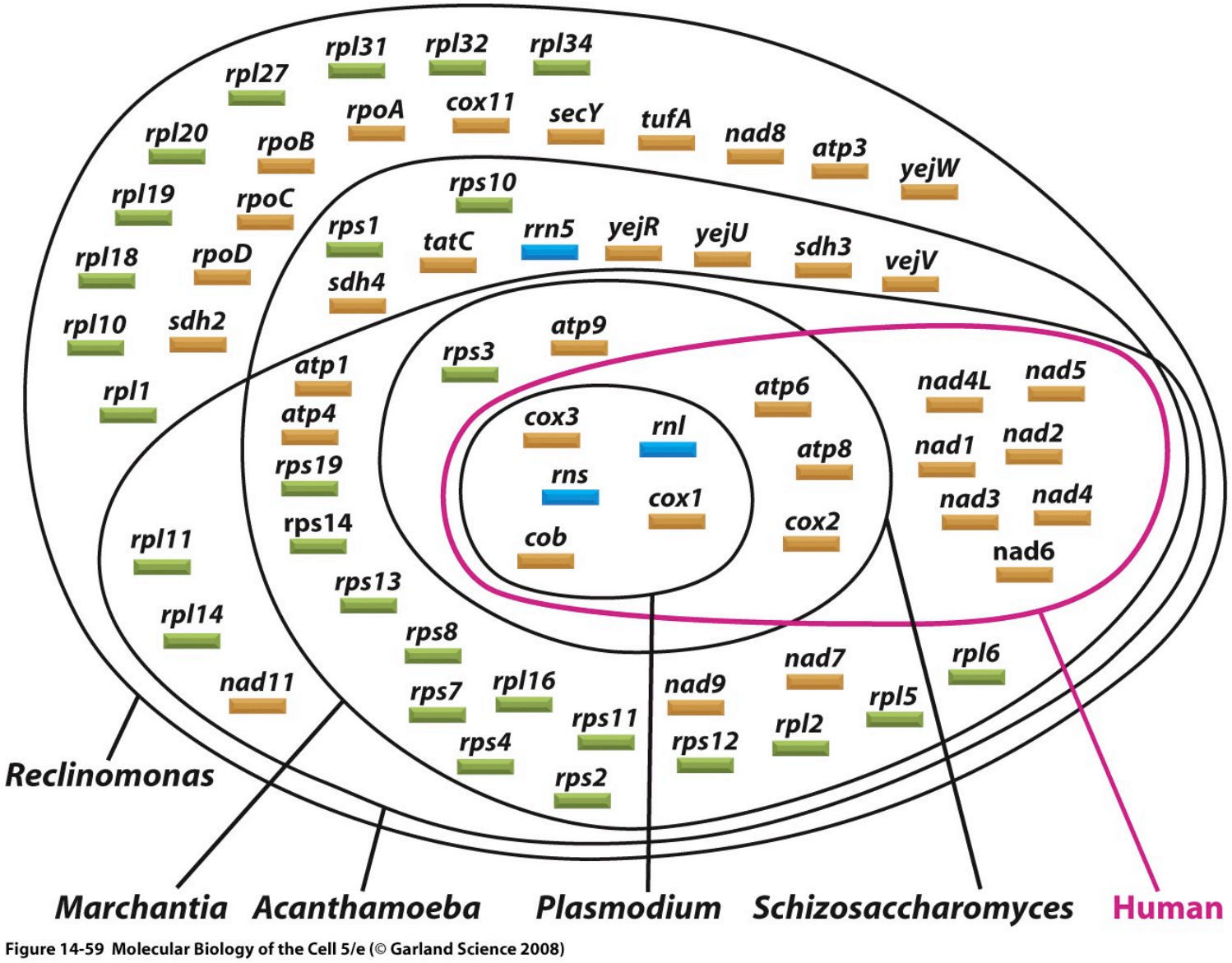


Figure 14-59 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Figure 14-59 Comparison of mitochondrial genomes.
Less complex mitochondrial genomes encode subsets of the proteins and ribosomal RNAs that are encoded by larger mitochondrial genomes. There are only four genes present in all known mitochondrial genomes; these encode ribosomal RNAs *(rns* and *rnl),* cytochrome *b (cob),* and a cytochrome oxidase subunit *(cox1).* (Adapted from M.W. Gray et al., *Science* 283:1476-1481, 1999. With permission from AAAS.)

5.11 SUPEROXIDE PRODUCTION BY COMPLEXES I AND III

Reviews Turrens 1997, Barja 1999

As discussed above, the $E_{m,7}$ values for the two-stage oxidation of UQH_2 at the Q_p site of complex III via $UQ^{\bullet -}$ to UQ are, respectively, +280 mV and −160 mV. This means that the $UQ^{\bullet -}$/UQ couple is highly reducing. Molecular oxygen appears to have access to the Q_p site, since there is a small but finite chance that $UQ^{\bullet -}$ may donate its electron, not to b_L but to O_2, with the formation of the superoxide anion, $O_2^{\bullet -}$.

$$O_2 + 1e^- = O_2^{\bullet -} \qquad E_{m,7} = -160\,\text{mV} \qquad [5.2]$$

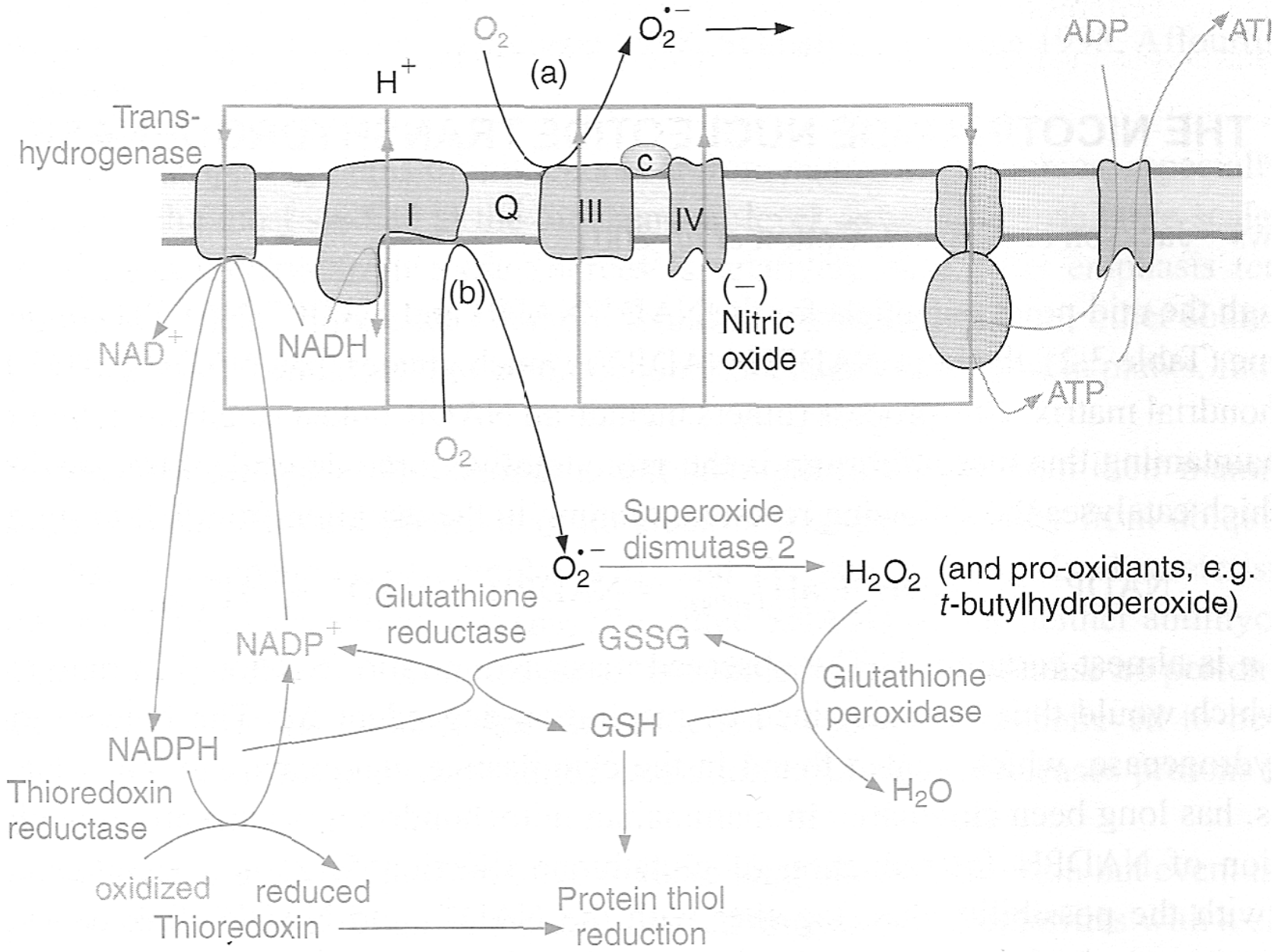
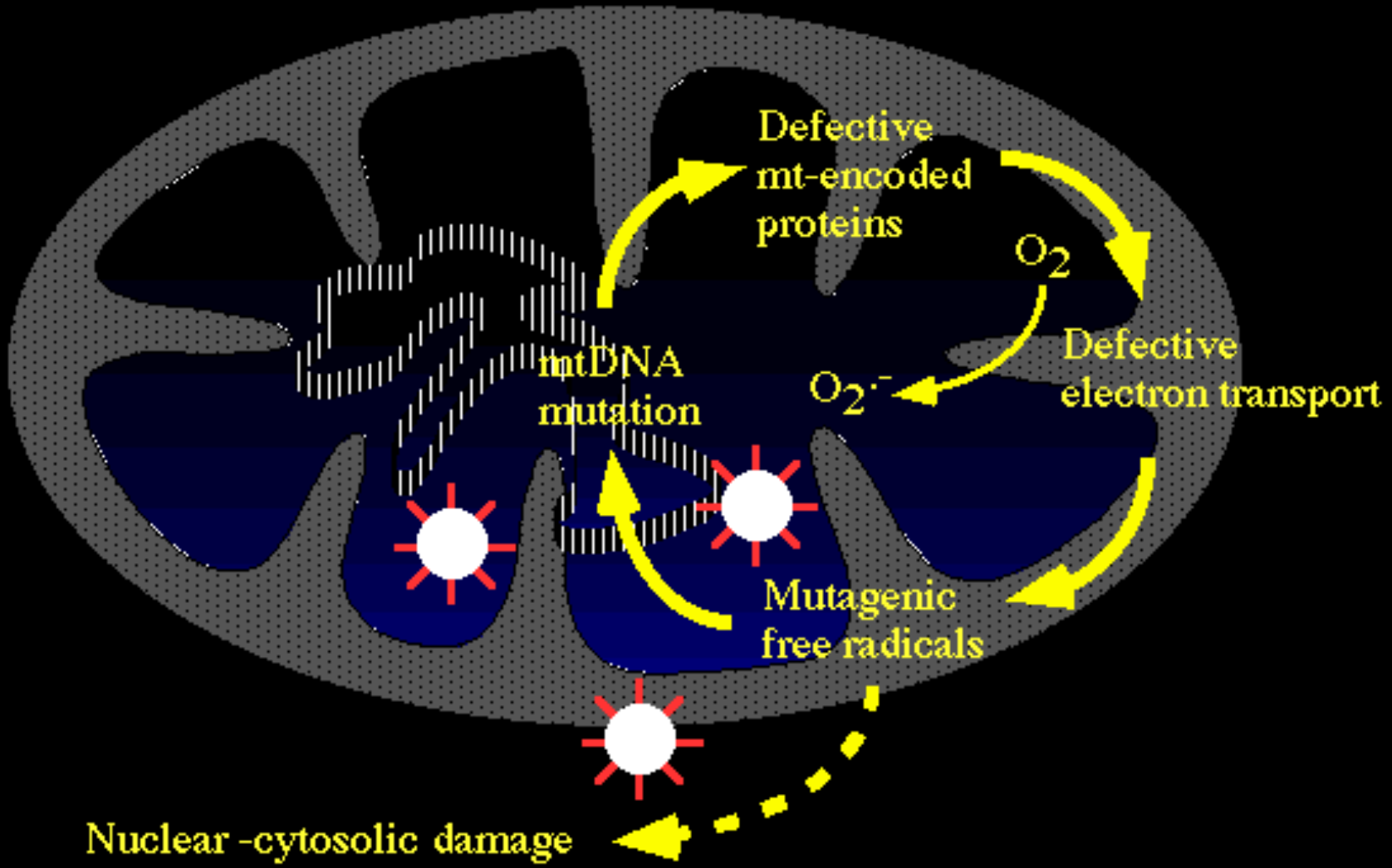


Figure 5.19 Mitochondrial 'oxidative stress': interactions between the GSSG/GSH couple and reactive oxygen species.


Defective
mt-encoded
proteins
O_2
Defective
electron transport
$O_2^{\cdot -}$
mtDNA
mutation
Mutagenic
free radicals
Nuclear -cytosolic damage

Problem

Why Do Mitochondria and Chloroplasts Have Their Own Genetic Systems?

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Mitochondrial DNA is a relic of ancient history. It is a legacy from a single aerobic bacterium that took up residence in the cytoplasm of a primitive cell that ultimately became an ancestor of all eukaryotic cells. Most of the genes of this ancient symbiont were either lost or transferred over the course of evolution to the nucleus of the host cell, leaving only a handful of genes to encode some of the most hydrophobic proteins of the inner mitochondrial membrane.

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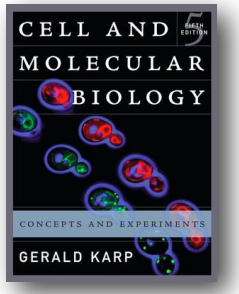
Cell and Molecular Biology Concepts and Experiments
Gerald Karp. Fifth Edition 2008. John Wiley & Sons Inc.



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Why do mitochondria and chloroplasts require their own separate genetic systems when other organelles that share the same cytoplasm, such as peroxisomes and lysosomes, do not? …. The reason for such a costly arrangement is not clear, and the hope that the nucleotide sequences of mitochondrial and chloroplast genomes would provide the answer has proved unfounded. We cannot think of compelling reasons why the proteins made in mitochondria and chloroplasts should be made there rather than in the cytosol.

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At one time, it was suggested that some proteins have to be made in the organelle because they are too hydrophobic to get to their site in the membrane from the cytosol. More recent studies, however, make this explanation implausible. In many cases, even highly hydrophobic subunits are synthesized in the cytosol.

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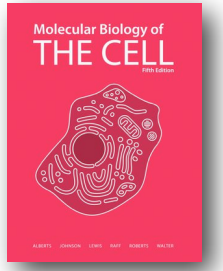
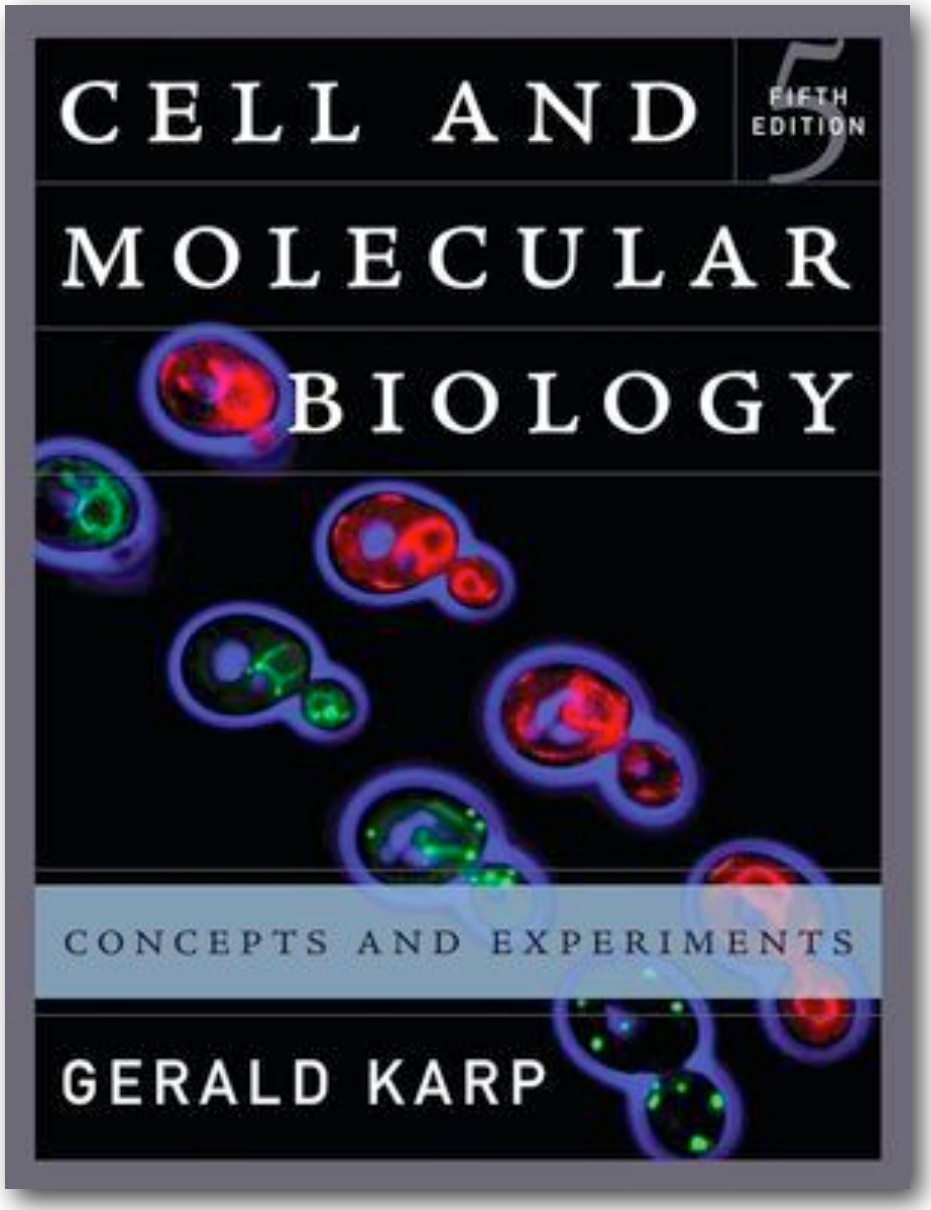
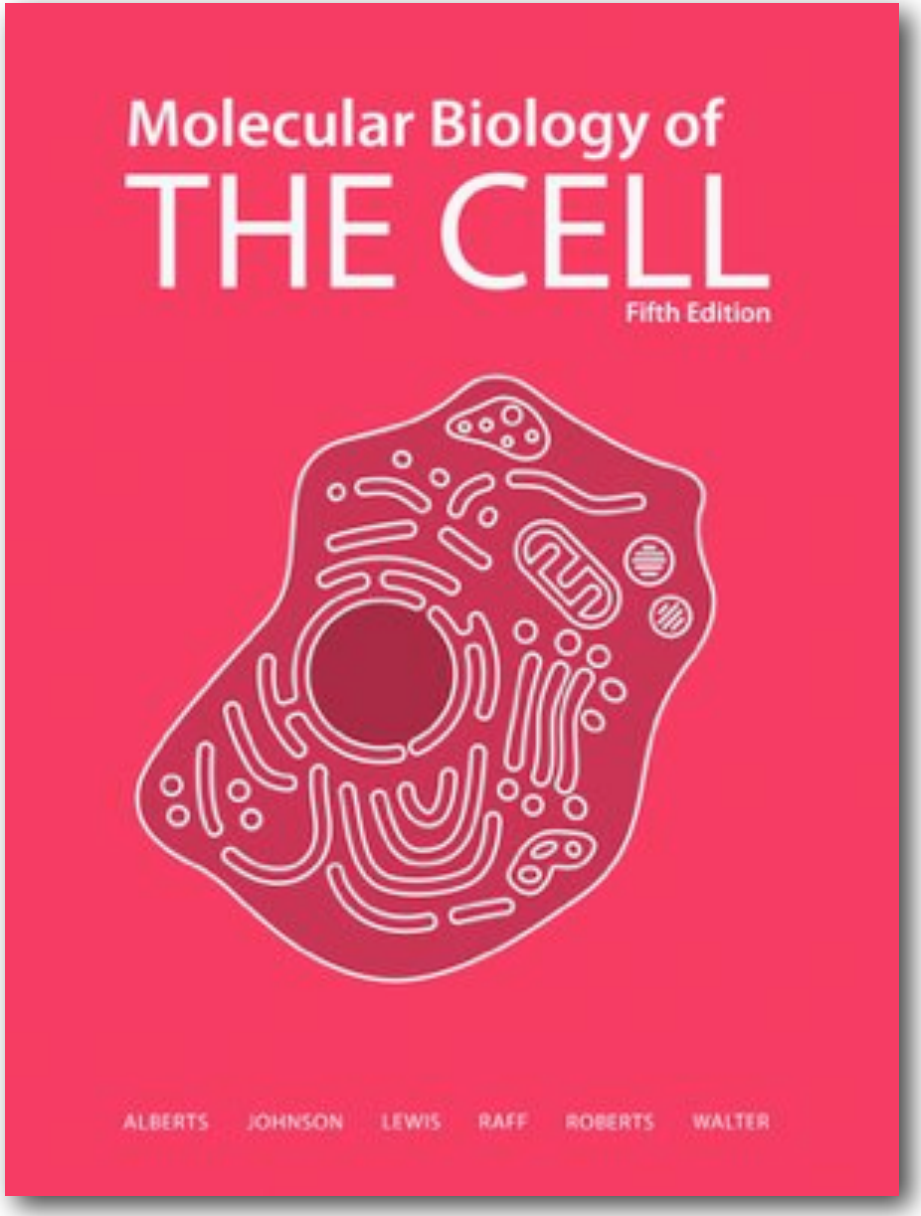


Molecular Biology of the Cell

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, and Walter P Molecular Biology of the Cell. Fifth Edition. New York and London: Garland Science; 2007


CELL AND MOLECULAR BIOLOGY
FIFTH EDITION
5
CONCEPTS AND EXPERIMENTS
GERALD KARP


Molecular Biology of
THE CELL
Fifth Edition
ALBERTS JOHNSON LEWIS RAFF ROBERTS WALTER

Even without the Mitochondrial Theory of Ageing….

Even without the Mitochondrial Theory of Ageing….

The mitochondrion is the worst imaginable place in the cell to keep genes.

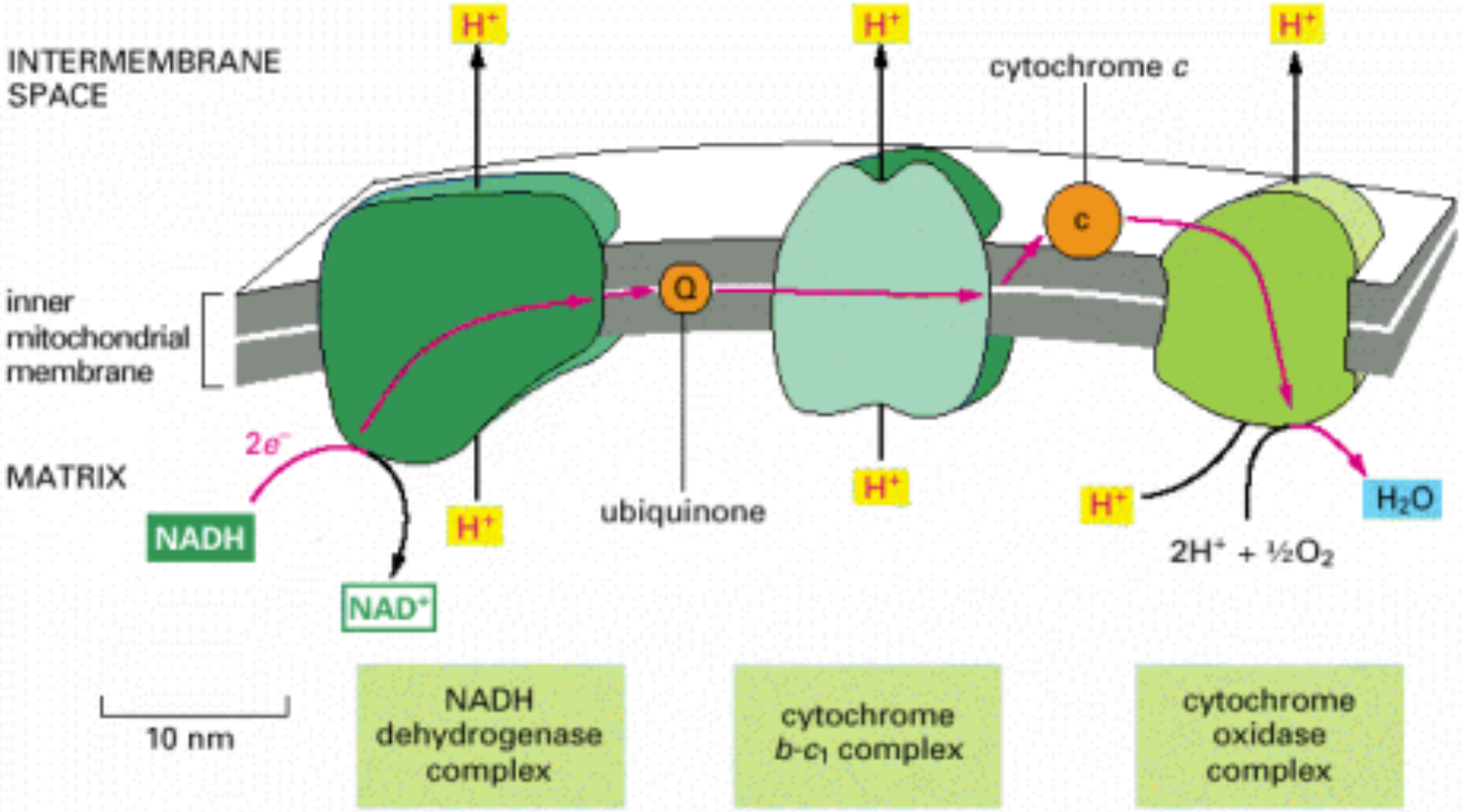
Even without the Mitochondrial Theory of Ageing….

The mitochondrion is the worst imaginable place in the cell to keep genes.

Whatever the reason for the persistence of mitochondrial genomes, it had better be a good one.

Complex III. Structure and Function.

jfallen.org/lectures/



The Respiratory Chain Includes Three Large Enzyme Complexes Embedded in the Inner Membrane

Molecular Biology of the Cell Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. 2007

Complex III

The cytochrome bc_1 complex

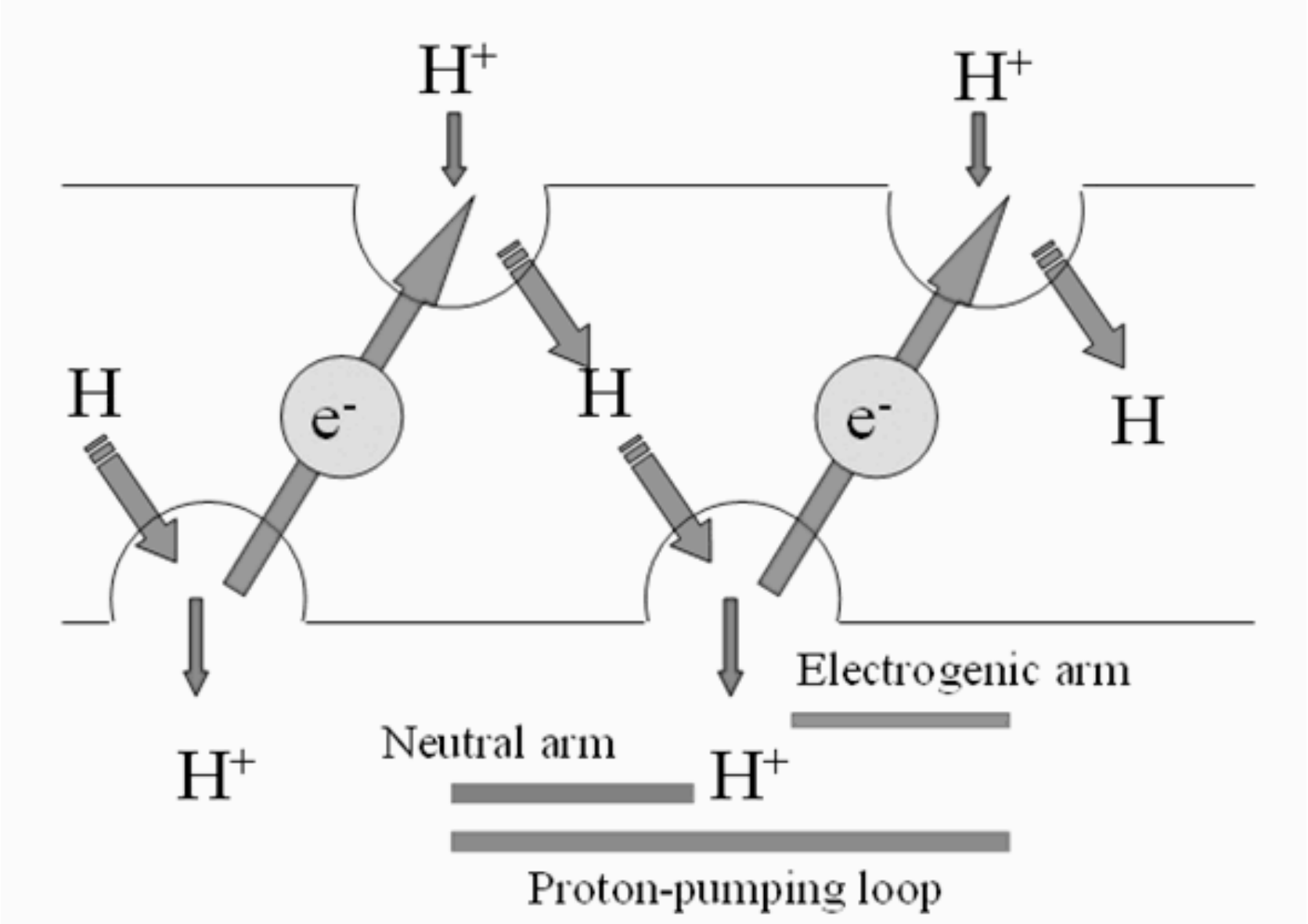
The cytochrome bc_1-complex

Complex III site

http://www.life.uiuc.edu/crofts/bc-complex_site/

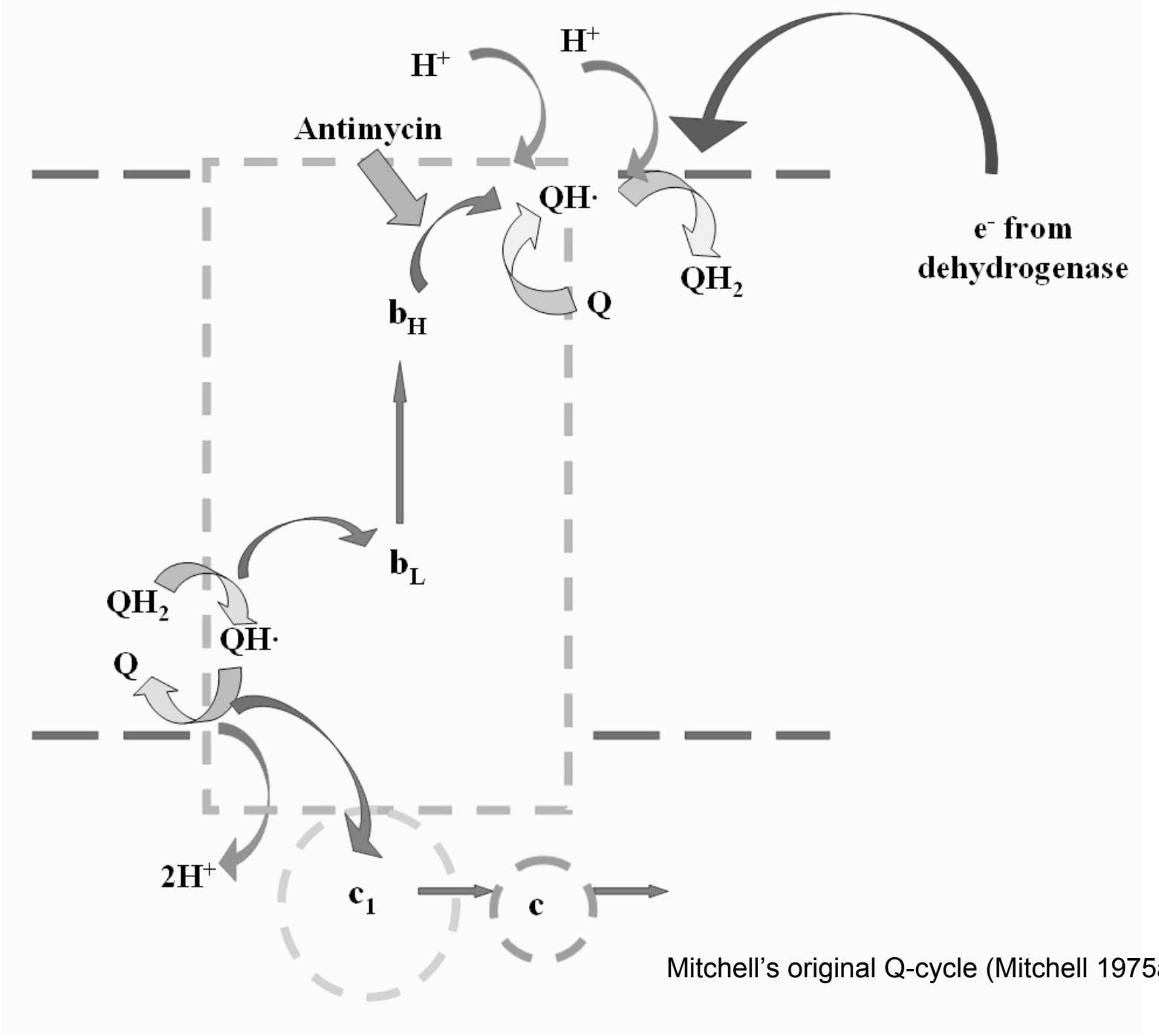
Peter Mitchell c. 1943 (adapted from Mitchell 1981). A young Peter Mitchell in the Department of Biochemistry at Cambridge. Left to right are Joan Keilin, Jim Danielli, Peter Mitchell, Mary Danielli. The ideas of David Keilin on the cytochromes and Jim Danielli on the lipid bilayer were seminal in the development of Mitchell's views on chemiosmosis and vectorial metabolism.

Crofts, A. R. (2004) The Q-cycle, - a personal perspective. Photosynth. Res.

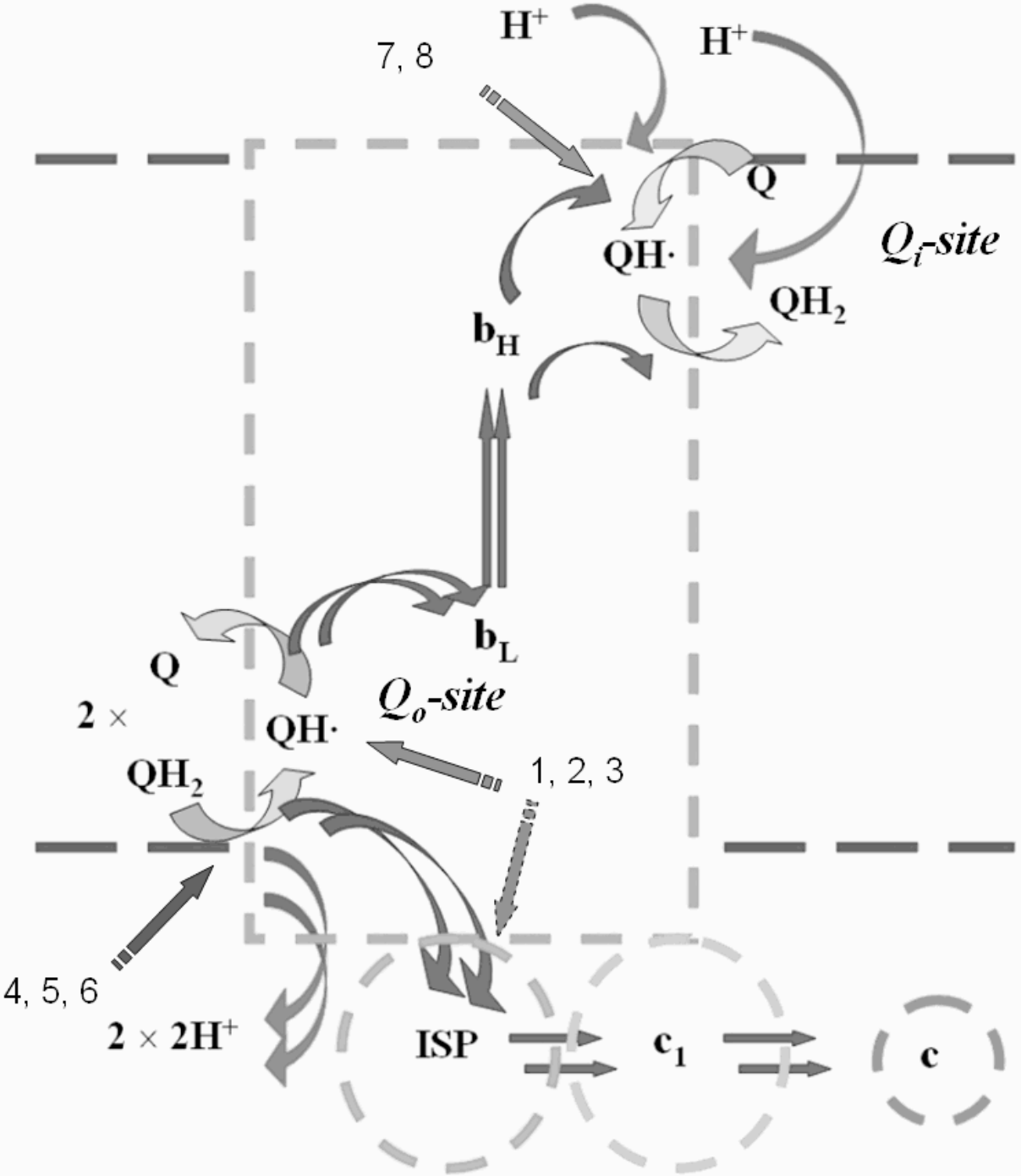


Mitchell's proton pumping loops (Mitchell 1961, 1966).

Crofts, A. R. (2004) The Q-cycle, - a personal perspective. Photosynth. Res.



Mitchell's original Q-cycle (Mitchell 1975a).



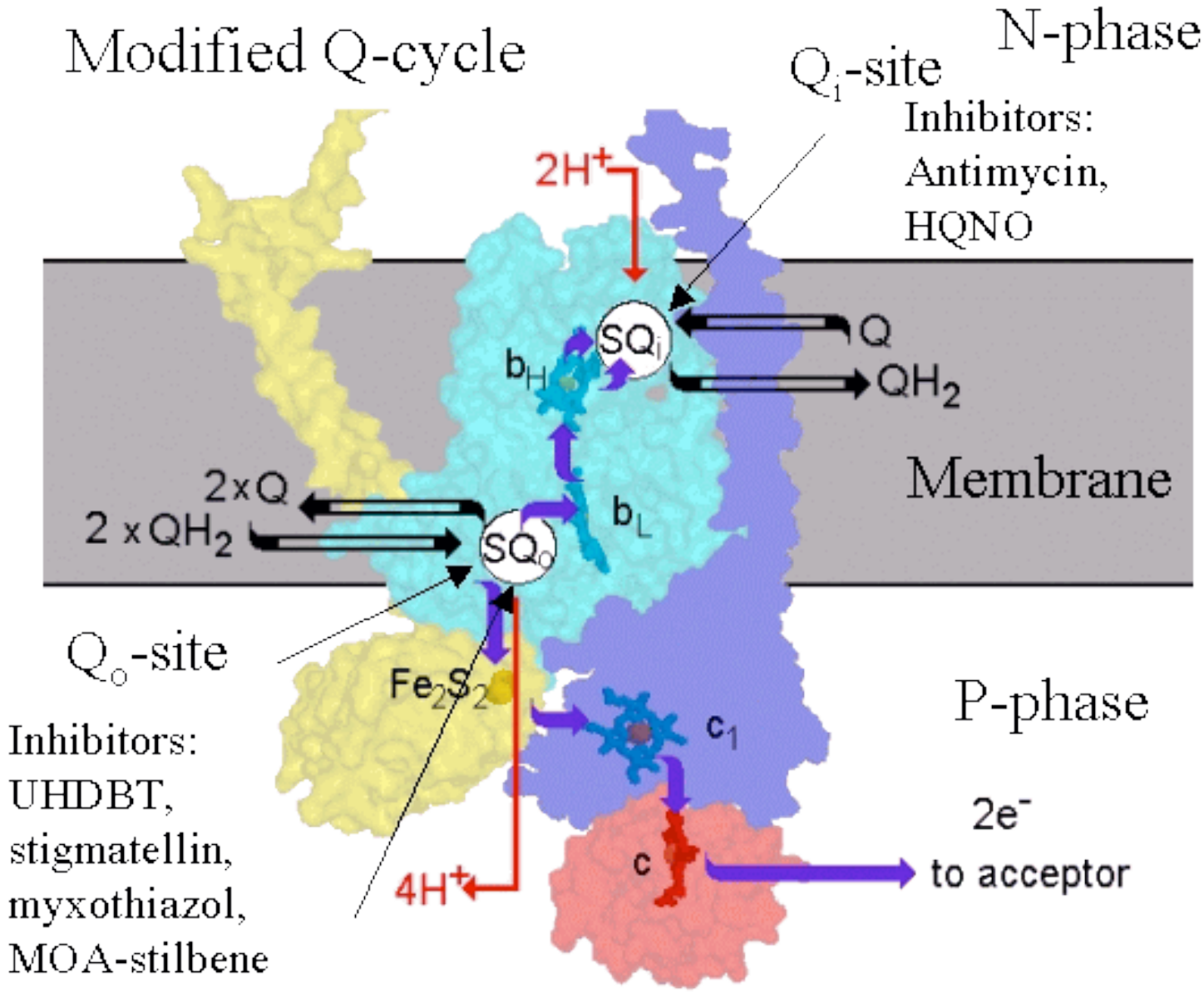
The Modified Q-cycle. The experiments from the Crofts lab in the early 1980's provided severe constraints that limited the types of plausible Q-cycle model. This version is essentially the same as that proposed by Crofts et al 1982, and reviewed by Crofts (1986)

Crofts, A. R. (2004) The Q-cycle, - a personal perspective. Photosynth. Res.

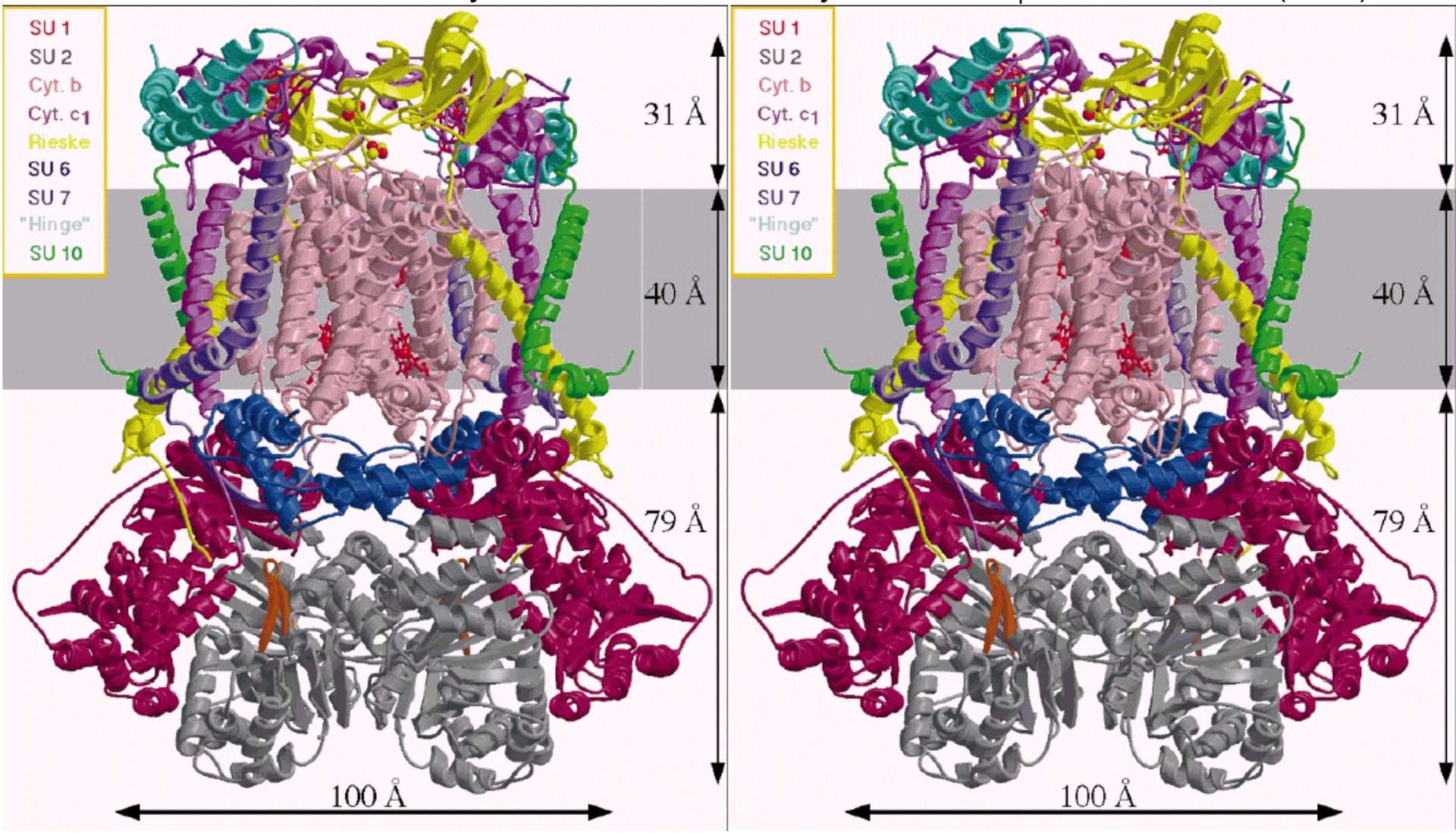
Subunit composition

The bc_1-complex from beef heart mitochondria

Subunit (no.)	Redox centers	M_r(beef)	Function
Core I	none	53.6	No catalytic, protein transport
Core II	none	46.5	No catalytic, protein transport
Cyt b (III)	heme b_H	42.6	donor to Q_i-site
	heme b_L		acceptor from SQ at Q_o-site
			Transmembrane electron transfer
Cyt c_1(IV)	heme c_1	27.3	donor to cyt c
Rieske (V)	2Fe.2S center	21.6	acceptor from Q_oH_2
			donor to cyt c_1
Subunit VI	none	13.3	none known
Subunit VII	none	9.5	none known
Subunit VIII	none	9.2	hinge protein (interacts with c_1)
Subunit IX	none	8.0	none known
Subunit X	none	7.2	none known
Subunit XI	none	6.4	none known (not present in chicken)


Modified Q-cycle
N-phase
Q_i-site
Inhibitors:
Antimycin,
HQNO
$2H^+$
SQ_i
b_H
Q
QH_2
Membrane
2xQ
2 x QH_2
b_L
SQ_o
Q_o-site
Fe_2S_2
P-phase
c_1
Inhibitors:
UHDBT,
stigmatellin,
myxothiazol,
MOA-stilbene
$2e^-$
c
to acceptor
$4H^+$

Z. Zhang, L. Huang, V. M. Shulmeister, Y.-I. Chi, K.-K. Kim, L.-W. Hung, A. R. Crofts, E. A. Berry & S.-H. Kim. Electron transfer by domain movement in cytochrome bc_1 Nature 392, 677 (1998)



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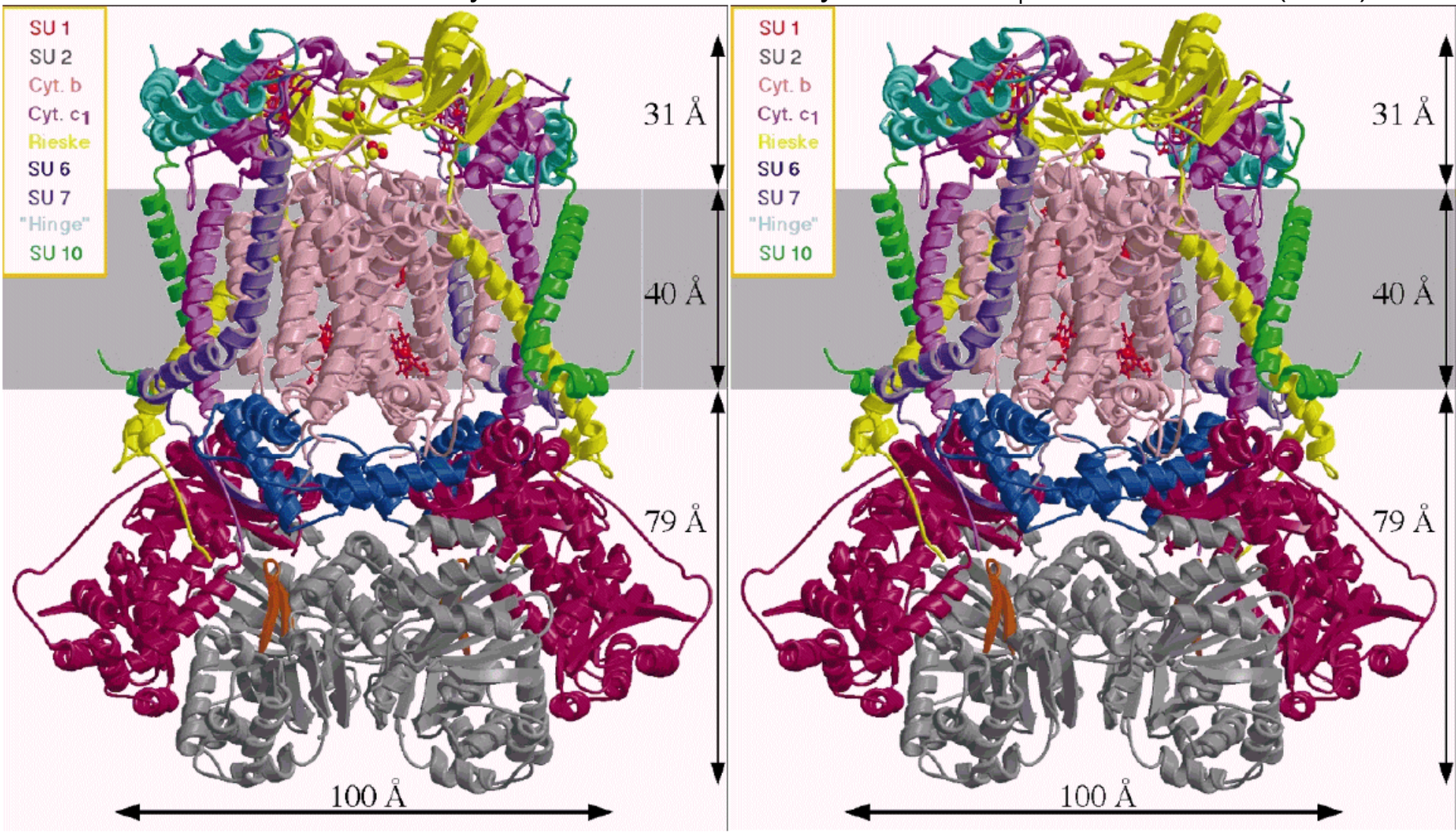
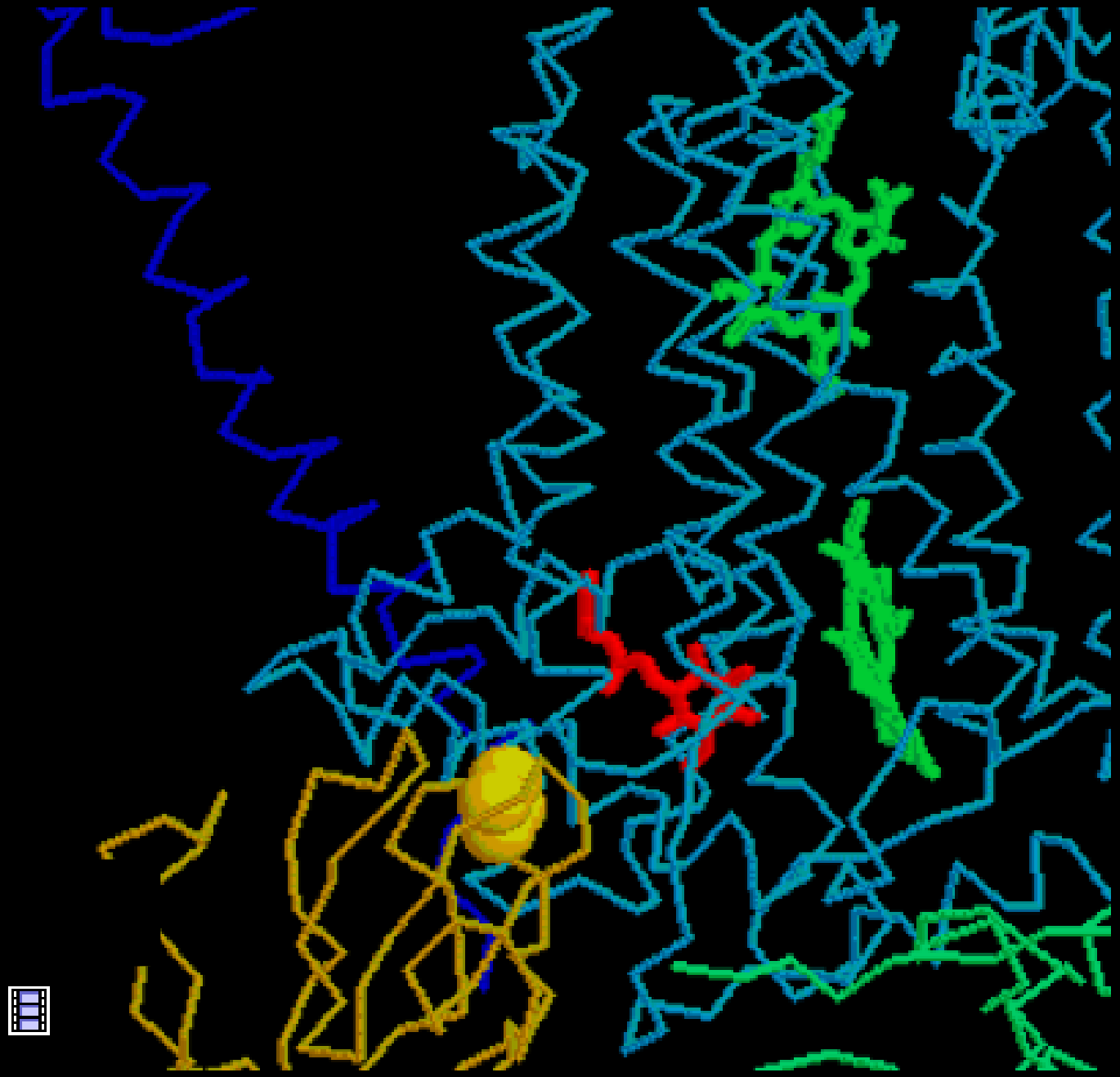
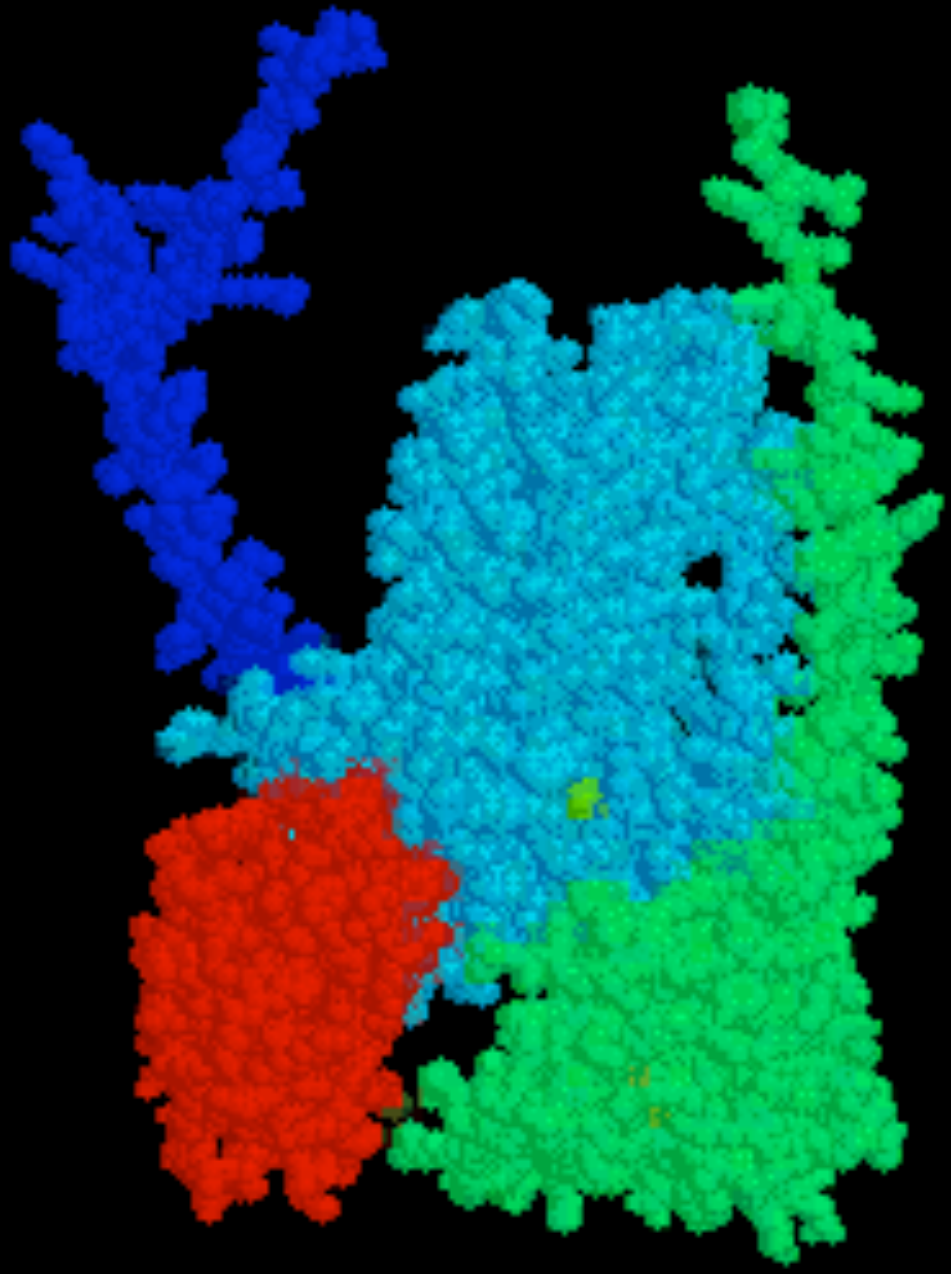


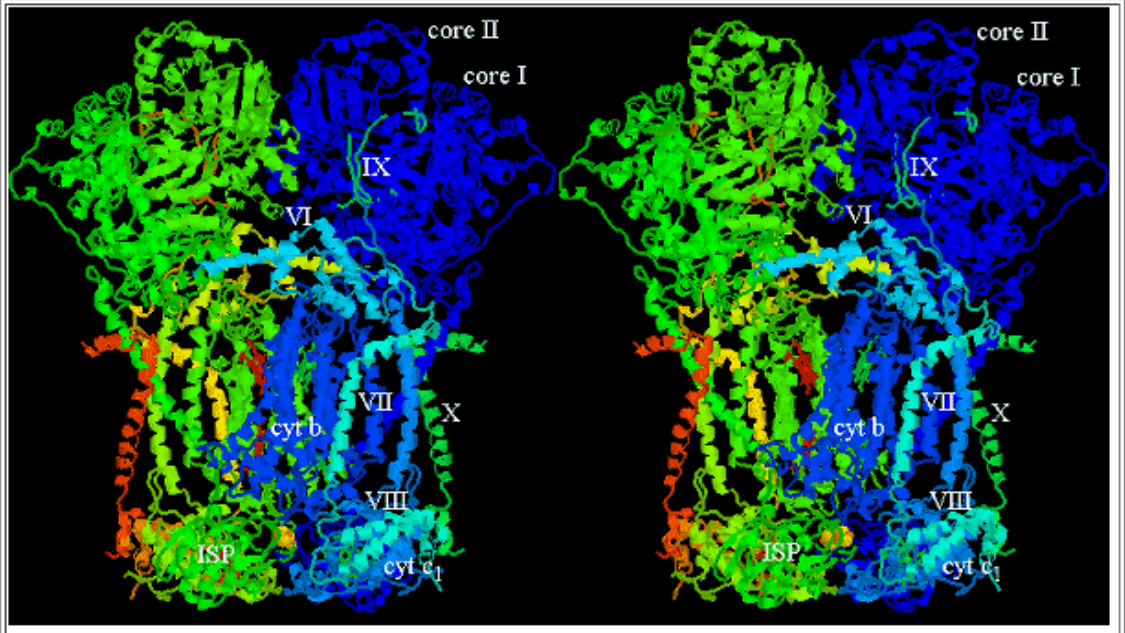
The general features of the protein are as expected from previous biochemical and structural studies, with a large fraction corresponding to the "core" proteins (subunits I and II) on the N-side (bottom in the Fig. above), and cyt c_1 and the FeS protein on the P-side. The dimensions of the dimer are about 130 Å in diameter and 151 Å in height, with the inter-membrane space region, the transmembrane region, and the matrix region contributing about 41 Å, 35 Å and 75 Å respectively.

Two different structures from X-ray crystallography

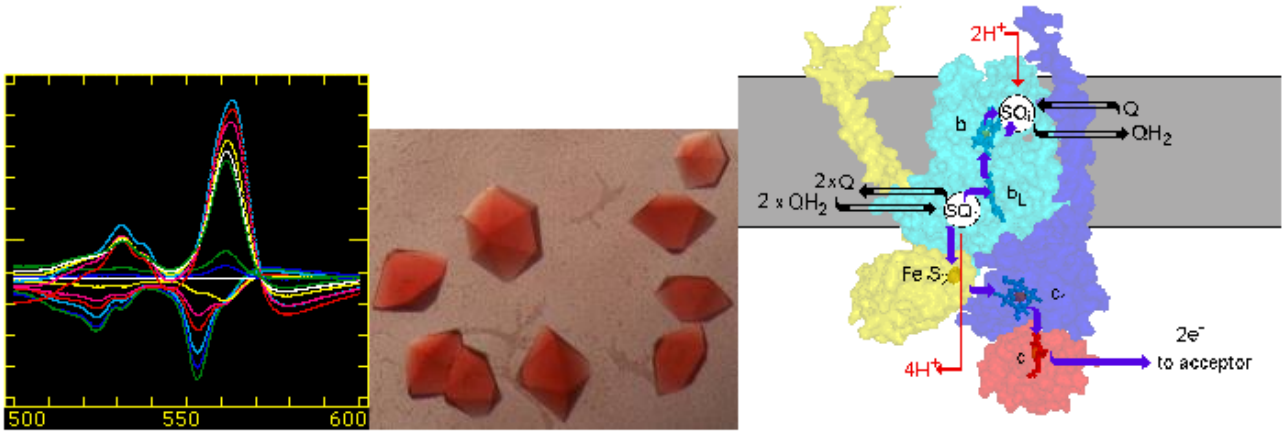
- suggesting two different positions for the head of the Rieske Fe-S protein







Ed Berry's structure of the bc_1-complex
(Chicken heart mitochondria)



Spectra and Crystals of the bc_1-complex from Ed Berry, and the Q-cycle mechanism.

Next lecture...

Complex III Part 2.

The Q-cycle and the cytochrome b_6f complex

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JVK
2004