IUBMB *Life*, 63(3): 160–165, March 2011

*Critical Review*

# Cardiolipin Drives Cytochrome *c* Proapoptotic and Antiapoptotic Actions

**Paolo Ascenzi[1], Fabio Polticelli[2], Maria Marino[2], Roberto Santucci[3] and Massimo Coletta[3,4]**

[1]*Interdepartmental Laboratory for Electron Microscopy, Via della Vasca Navale 79, Roma, Italy*

[2]*Department of Biology, University Roma Tre, Viale Guglielmo Marconi 446, Roma, Italy*

[3]*Department of Experimental Medicine and Biochemical Sciences, University of Roma "Tor Vergata," Via Montpellier 1, Roma, Italy*

[4]*Interuniversity Consortium for the Research on the Chemistry of Metals in Biological Systems (CIRCMSB), Piazza Umberto I 1, Bari, Italy*

*Summary*

**Cytochrome *c* (cyt*c*) is pivotal in mitochondrial respiration and apoptosis. The heme-Fe-atom of native hexacoordinated horse heart cyt*c* (hhcyt*c*) displays a very low reactivity toward ligands and does not exhibit catalytic properties. However, on interaction with cardiolipin (CL), hhcyt*c* changes its tertiary structure disrupting the heme-Fe-Met80 distal bond. The CL-hhcyt*c* complex displays a very low midpoint potential, out of the range required for its physiological role, binds CO and NO with high affinity, facilitates peroxynitrite isomerization to $NO_3^-$, and displays peroxidase activity. As a whole, the CL-hhcyt*c* complex could play either proapoptotic effects, catalyzing lipid peroxidation and the subsequent hhcyt*c* release into the cytoplasm, or antiapoptotic actions, such as scavenging peroxynitrite (*i.e.*, protecting the mitochondrion from reactive nitrogen and oxygen species), and binding of CO and NO (*i.e.*, inhibiting lipid peroxidation and hhcyt*c* traslocation). Here, the CL-driven allosteric modulation of hhcyt*c* properties is reviewed, highlighting proapoptotic and antiapoptotic actions.** 

IUBMB *Life*, 63(3): 160–165, 2011

**Keywords** cytochrome *c*; cardiolipin; CO binding; NO binding; peroxynitrite isomerization; lipid peroxidation; apoptosis; allostery.

**Abbreviations** CL, cardiolipin; cyt*c*, cytochrome *c*; cyt*c*-Fe(II), ferrous cytochrome *c*; CL-cyt*c*, CL-bound cytochrome *c*; hhcyt*c*, horse heart cytochrome *c*; hhcyt*c*-Fe(III), ferric horse heart cytochrome *c*; hhcyt*c*-Fe(II), ferrous horse heart cytochrome *c*; CL-hhcyt*c*, CL-bound horse heart cytochrome *c*; CL-hhcyt*c*-Fe(III), cardiolipin-ferric horse heart cytochrome *c* complex; CL-hhcyt*c*-Fe(II), cardiolipin-bound ferric horse heart cytochrome *c* complex; Mb, myoglobin; Mb-Fe(II), ferrous myoglobin.

Received 24 January 2011; accepted 2 February 2011

Address correspondence to: Paolo Ascenzi, Interdepartmental Laboratory for Electron Microscopy, Via della Vasca Navale 79, Roma I-00146, Italy. Tel: +39-06-5733-3200(2); Fax: +39-06-5733-6321. E-mail: ascenzi@uniroma3.it

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.440

Eukaryotic cytochromes *c* (cyt*c*) are small water-soluble globular heme-proteins that are located within the compartment delimited by the inner and outer mitochondrial membranes playing a pivotal role in mitochondrial respiration and apoptosis (*1–5*).

In mitochondria, cyt*c* is located between the inner and the outer membrane and functions to transfer electrons from Complex III (UQH2-cyt*c* reductase) to Complex IV (cyt*c* oxidase) of the respiratory chain. It mediates electron transfer through the heme group, which switches between the reduced ferrous form (Fe(II)) and the oxidized ferric (Fe(III)) state. Moreover, in healthy cells, cyt*c* inhibits reactive oxygen species formation, thus preventing cell oxidative stress (*3, 6–8*).

Cyt*c* displays also a central apoptotic role. Cyt*c* release into the cytosol is particularly associated with activation of the intrinsic pathway, which responds to intracellular stimuli such as DNA damage and oncogene activation. Multiple cytosolic and mitochondrial proteins regulate the mitochondrial pathway of cell death. Members of the Bcl-2 family regulate events upstream of mitochondria, weighing the prosurvival signals against the stress/damage signals. If the latter prevail, the mitochondrial membrane is permeabilized, leading to deterioration of the bioenergetic functions of mitochondria, overproduction of reactive oxygen species, as well as to the release of cyt*c* into the cytosol. Once in the cytosol, in the presence of ATP (and more efficiently in the presence of deoxyATP), cyt*c* mediates the allosteric activation and hepta-oligomerization of the adaptor molecule apoptosis-protease activating factor-1, generating the complex known as apoptosome. Each apoptosome can

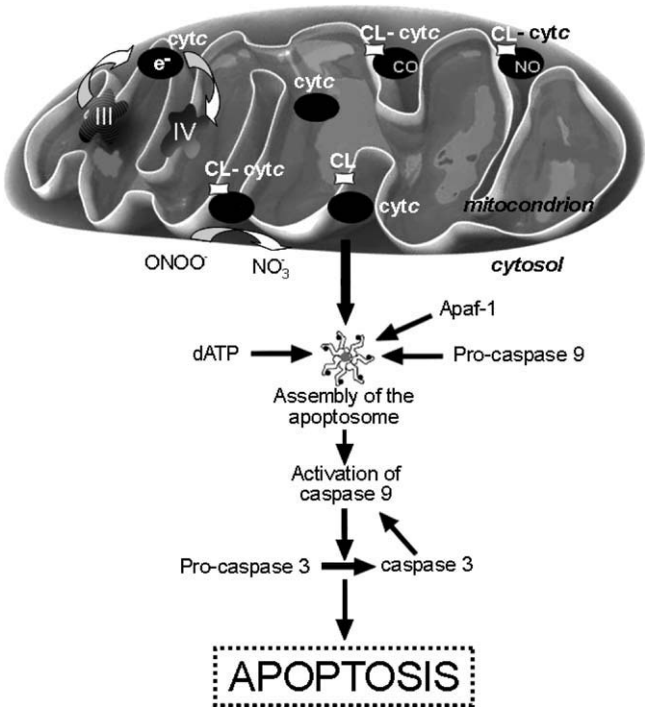


**Figure 1.** The multiple functions of cyt*c*. In the mitochondria, cyt*c* mediates electron transfer from Complex III (III) to Complex IV (IV). Mitochondrial cyt*c* is placed within the compartment delimited by the inner and the outer membranes or localized into the mitochondrial inner membranes bound to cardiolipin (CL). CL-cyt*c* binds CO and NO with high affinity and facilitates peroxynitrite conversion to $NO_3^-$ (antiapoptotic action). CL-cyt*c* displays peroxidase activity, and CL being a favorable substrate, this pathway allows cyt*c* release into the cytoplasm. Once in the cytosol, in the presence of deoxyATP (dATP), cyt*c* mediates the activation of the adaptor molecule apoptosis-protease activating factor-1 (Apaf-1), generating the apoptosome. Apoptosome can recruit caspase 9 favoring proteinase activation. These events induce the catalytic maturation of caspase 3, which mediates the biochemical and morphological features of apoptosis.

recruit seven dimers of caspase 9 favoring proteinase activation. These events, tightly regulated by several heat shock proteins (Fig. 1), allow for the catalytic maturation of caspase 3 and other caspases, which eventually mediate the biochemical and morphological features of apoptosis (*9–13*).

At least 15% of mitochondrial cyt*c* is bound to cardiolipin (CL), an unusual lipid largely confined to the inner mitochondrial membrane (*3, 4, 13–20*). The interaction with CL is pivotal for switching cyt*c* function(s) from mitochondrial respiration to apoptosis. In fact, upon CL binding, cyt*c* has been shown (i) to change its tertiary structure disrupting the heme-Fe-Met80 distal bond (the proximal axial ligand being His) and, in some cases, to vary the spin state of the metal (*4, 14, 18–22*), (ii) to reduce drastically the midpoint potential out of the range required for its role in the respiratory chain (*17*), (iii) to display peroxidase activity, using CL as a favorable substrate (*15, 23–26*), (iv) to bind CO and NO with high affinity (*27, 28*), and (v) to facilitate peroxynitrite scavenging (*i.e.*, conversion to $NO_3^-$) (*29*). All these effects suggest that CL-bound cyt*c* (CL-cyt*c*) could play either proapoptotic effects, catalyzing lipid peroxidation, and cyt*c* release into the cytoplasm, or antiapoptotic actions, scavenging peroxynitrite (*i.e.*, protecting the mitochondrion from reactive nitrogen and oxygen species), and binding CO and NO (*i.e.*, inhibiting lipid peroxidation and cyt*c* traslocation). Here, the CL-driven cyt*c* proapoptotic and antiapoptotic actions are reviewed.

## HOW CARDIOLIPIN BINDS TO HORSE HEART CYTOCHROME *c*?

CL, which constitutes about 20% of total lipids of the mitochondrial membrane, is synthesized in the mitochondrion and possesses a unique structure, being composed of four (instead of two, as in most lipids) acyl chains (*20, 30*).

To the authors' knowledge, no crystal structures are available for the CL-cyt*c* complex, thus the proposed binding modes are based on available experimental data and molecular modeling investigations. Although CL/cyt*c* recognition is uncertain and still matter of controversy, both regions considered by molecular modeling studies provide suitable sites for CL binding (*18, 20, 21*).

The first model to be proposed suggests that on CL binding to horse heart cyt*c* (hhcyt*c*), one acyl chain of CL protrudes into the protein interior through the hydrophobic channel located close to the Asn52 residue. The insertion of the acyl chain into hhcyt*c* is assumed to be favored by H-bonding between Asn52 and the protonated phosphate group of CL (Fig. 2) (*21*).

An alternative model asserts that the binding of CL to hhcyt*c* occurs in the region of the Met80-containing loop, and that the acyl chain protrudes into the protein between the hydrophobic strands formed by residues 67–71 and 82–85 after anchoring of the phospholipid to the protein via electrostatic interactions between the deprotonated phosphate group of CL and the Lys72 residue of hhcyt*c* (Fig. 2) (*18*).

Very recently, it has been hypothesized that CL binds to hhcyt*c* at two distinct regions (*20*), which are characterized by different affinity for phospholipids (*14, 19*). According to this model, the acyl chains of CL could be easily accommodated both in the hydrophobic channel in the vicinity of Asn52 and in the region of the Met80-containing loop (Fig. 2). In the latter case, the deprotonated phosphate group of CL could electrostatically interact with the Lys72 and Lys73 residues. The view that two acyl chains bind to hhcyt*c* at distinct sites is in agreement with the observed two-state transition binding process and with the biphasic character of CL-binding kinetics. This hypothesis is very realistic from a stereochemical viewpoint, in that the insertion of only one acyl chain into hhcyt*c* would cause the

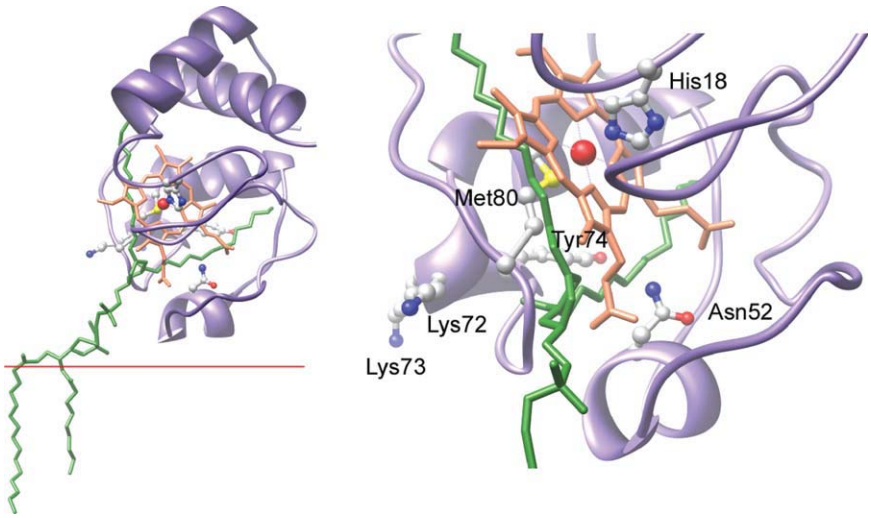


**Figure 2.** Molecular model of the putative CL-hhcyt*c* complex. The CL molecule is shown in green, and the membrane plane is indicated by a red line. The enlarged view of the CL-hhcyt*c* complex (right panel) shows CL binding to hhcyt*c* by insertion of two of the four acyl chains in the vicinity of Asn52 and Met80. The proximal ligand of the heme-Fe-atom His18 and residues Lys72, Lys73, and Tyr74 are also shown. This CL-binding mode allows the tethering of hhcyt*c* to the membrane as CL can remain membrane-bound through the other two acyl chains. The insertion of a single acyl chain into the hhcyt*c* molecule is unlikely due to stereochemical constrains, which would force the adjacent CL acyl chain to be solvent exposed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

partial exposure to the solvent of (at least) one of the adjacent acyl chains in CL, the situation disfavored from a solvation energy viewpoint (Fig. 2) (*20*).

As a whole, CL binding to hhcyt*c* seems to induce a gross conformational change(s) on both the proximal and distal side of the heme, causing the loss of the electron transfer properties of hhcyt*c* and its transformation into a globin- and peroxidase-like heme-protein (*15, 17, 27–29*).

## THE CL-BOUND HORSE HEART CYTOCHROME *c* DISPLAYS PROAPOPTOTIC AND ANTIAPOPTOTIC PROPERTIES

The CL-hhcyt*c* interaction plays an important role in modulating the heme-protein functions. CL-bound cyt*c* shows a non-native tertiary structure and a disrupted heme-Fe-Met80 distal bond, with Lys79 as the likely sixth axial ligand of the heme-Fe-atom at pH $>$ 9 (*14, 18–22*). CL binding to hhcyt*c* induces a drastically reduced midpoint potential, which falls out of the range required for its physiological role in mitochondrial respiration (*17*). Further, the cleavage of the distal Fe-Met80 bond endows hhcyt*c* with proapoptotic activity, due to the achievement of peroxidase action (*15, 23–26, 31, 32*), and anti-apoptotic functions, increasing the affinity for CO and NO (*27, 28*) and inducing peroxynitrite detoxification properties (*29*).

### *Proapoptotic Activity of CL-bound Horse Heart Cytochrome c*

At the first stage of apoptosis, cyt*c* released into the cytosol binds to apoptosis-protease activating factor-1. This event starts the process leading to the cleavage of protein substrates and subsequent cell death. Hhcyt*c* release is induced by the dissociation of the CL-hhcyt*c* complex; hhcyt*c*-mediated CL peroxidation is indicated as the process responsible for such an event, although the exact mechanism governing it is not yet fully understood. CL peroxidation is responsible for hhcyt*c* detachment from the mitochondrial membrane. This supported by several evidences, such as the observation that the oxidative degradation of CL occurs in the p53-induced apoptosis (*33*) and that CL hydroperoxides show a decreased affinity for hhcyt*c* with respect to CL (*4, 31*). CL oxidation leads to structural changes of hhcyt*c*, such as partial protein unfolding, weak axial binding to the heme iron, and enhanced access of the heme catalytic site to small molecules (*e.g.*, hydrogen peroxide), which result in a modification of the hhcyt*c* catalytic properties, transforming it into a peroxidase (*8, 34*).

### *Antiapoptotic Activity of CL-bound Horse Heart Cytochrome c*

CL binding to hhcyt*c* facilitates CO and NO binding to the heme-Fe-atom and peroxynitrite isomerization (*27–29*). While native ferrous hhcyt*c* (hhcyt*c*-Fe(II)) is unable to bind CO (*35*), ferrous CL-hhcyt*c* (CL-hhcyt*c*-Fe(II)), like mammalian myoglobins (Mbs) (*36*), interacts with CO by a simple second-order process (*27*). The value of the apparent dissociation equilibrium constant for CL-hhcyt*c*-Fe(II) carbonylation ($\sim 3 \times 10^{-8}$ M) (*27*) corresponds to that reported for ferrous horse heart Mb (Mb-Fe(II)) generally taken as a molecular model ($\sim 3 \times 10^{-8}$ M) (*36*). However, both combination and dissociation rate

constants for (de)carbonylation of CL-hhcyt*c*-Fe(II) ($\sim 1 \times 10^7$ $M^{-1}$ $s^{-1}$ and 0.18 $s^{-1}$, respectively) (*27*) are larger than those reported for horse heart Mb-Fe(II) (de)carbonylation, ($=5 \times 10^5$ $M^{-1}$ $s^{-1}$ and $1.7 \times 10^{-2}$ $s^{-1}$, respectively) (*36*). This suggests a very open heme crevice in CL-hhcyt*c* and a weak proximal His18-Fe bond (*27*), these characteristics seem to be confirmed also by the very fast dissociation rate of the His18-Fe bond on NO binding to the sixth coordination position of the heme-Fe-atom (*28*).

The value of the dissociation equilibrium constant for NO binding to hhcyt*c*-Fe(II) ($=8.2 \times 10^{-6}$ M) (*37*) is less favorable than that for CL-hhcyt*c*-Fe(II) nitrosylation ($=2.5 \times 10^{-11}$ M) (*28*), which is similar to that reported for horse heart Mb-Fe(II) ($=7.1 \times 10^{-12}$ M) (*38*).

NO binds to the sixth coordination position of the heme-Fe-atom of hhcyt*c*-Fe(II) by a simple second-order process as Mb (*37*). Nevertheless, the nitrosylated CL-hhcyt*c*-Fe(II) complex is pentacoordinated with NO as the proximal fifth ligand of the heme-Fe-atom (*28*).

In CL-hhcyt*c*-Fe(II), NO replaces the proximal His18 residue via an unusually complex kinetic mechanism. Indeed, the nitrosylation of CL-hhcyt*c*-Fe(II) involves seven species: (i) the initial NO-unbound pentacoordinated species (the proximal fifth axial ligand is His18), (ii) the transient ferrous nitrosylated hexacoordinated species (the proximal fifth axial ligand is His18 and the distal sixth axial ligand is NO), (iii) the transient ferrous nitrosylated pentacoordinated species (the proximal His18-Fe bond is cleaved and the distal sixth axial ligand is NO), (iv) the transient ferrous nitrosylated hexacoordinated species (the proximal fifth axial ligand is a weak-field ligand, possibly an intrinsic amino acid residue or a water molecule), (v) the transient ferrous bis-nitrosylated hexacoordinated species (two NO molecules are the fifth and the sixth axial ligands of the heme-Fe-atom), (vi) the transient ferrous nitrosylated pentacoordinated species (the proximal sixth axial ligand is NO) not identical to the final species, and (vii) the final ferrous nitrosylated pentacoordinated species (the proximal sixth axial ligand is NO). The denitrosylation of CL-hhcyt*c*-NO is characterized by: (i) the transient tetracoordinated species following the cleavage of the proximal NO-Fe-atom bond, but retaining NO in the protein matrix, (ii) the pentacoordinated species (the proximal fifth axial ligand is His18), but retaining NO in the protein matrix, and (iii) the stable NO-unbound pentacoordinated species (the proximal fifth axial ligand is His18). This suggests a remarkable mobility of the heme environment of hhcyt*c*-Fe(II) induced by CL (*28*).

Finally, NO induces the reductive nitrosylation of ferric hhcyt*c* (hhcyt*c*-Fe(III)) at pH $\geq 7$ as reported for ferric heme-proteins, including sperm whale Mb. In fact, the addition of NO to heme-Fe(III) leads to the transient formation of heme-Fe(III)-NO in equilibrium with heme-Fe(II)$^-$NO$^+$. Then, heme-Fe(II)-NO$^+$ undergoes nucleophilic attack by $OH^-$ to yield hhcyt*c*-Fe(II), which in turn reacts further with NO to give hhcyt*c*-Fe(II)-NO. This process, occurring *in vitro* under anaerobic conditions, may occur *in vivo* under hypoxia (*39*).

The unusual features of CO and NO binding to the distal and proximal sides of the heme-Fe-atom, respectively, may represent a new mechanism in the regulation of biological processes by sensing changes in the concentration of CO and NO. Remarkably, a similar situation has been reported for gas discrimination by cytochromes *c′*, a distinct family of class IIa cytochromes found in the periplasm of certain denitrifying, nitrogenfixing, photosynthetic, methanotrophic, and sulfur-oxidizing bacteria (*40*).

As reported for pentacoordinated ferric heme-proteins, including horse heart and sperm whale Mb (*41–43*), ferric CL-hhcyt*c* (CL-hhcyt*c*-Fe(III)) quickly catalyzes peroxynitrite scavenging, inducing the formation of nitrate (the process takes $10^{-3}$ to 1 s) (29). The value of the second-order rate constant for CL-hhcyt*c*-Fe(III)-mediated scavenging of peroxynitrite ($=3.2 \times 10^5$ $M^{-1}$ $s^{-1}$) (*29*) is similar to those reported for ferric heme-proteins (ranging between $1.2 \times 10^4$ $M^{-1}$ $s^{-1}$ and $4.1 \times 10^5$ $M^{-1}$ $s^{-1}$) (*41–43*). However, it has also been shown that peroxynitrite very slowly induces the nitration of the solvent-exposed Tyr74 residue of hhcyt*c* in the absence of CL (the process takes 10 to 30 min). This leads to the cleavage of the Fe-Met80 bond, which is substituted by a weak Fe-Lys72 heme distal ligation (*44*).

Kinetic and thermodynamic parameters for CO and NO binding to CL-hhcyt*c*-Fe(II) are very favorable, suggesting that the physiological levels of CO and NO may impair CL-hhcyt*c* pro-apoptotic action inhibiting the cyt*c* peroxidase activity (*27, 28*). Moreover, peroxynitrite scavenging by CL-hhcyt*c*-Fe(III) may protect the mitochondrion from reactive nitrogen and oxygen species helping cell survival (*29*).

## CONCLUSION AND PERSPECTIVES

CL binding to hhcyt*c* induces tertiary changes facilitating the cleavage of the distal heme-Fe-Met80 bond, and this favors the enzymatic activity of CL-hhcyt*c* (*40*). Data reported so far indicate that CL-hhcyt*c* could act as either a proapoptotic or an antiapoptotic factor, depending on the conditions under which it operates. In particular, CL-hhcyt*c* functions as a proapoptotic factor catalyzing the peroxidative reduction of $H_2O_2$, which leads to CL peroxidation (*31, 34*). In contrast, CL-hhcyt*c* exerts an antiapoptotic action facilitating the isomerization of peroxynitrite to nitrate with the consequent scavenging of reactive nitrogen species (*29*). Further, the reaction of CO and NO with CL-hhcyt*c*-Fe impairs the hhcyt*c* peroxidase activity (*27, 28*).

As a whole, CL-cyt*c* could act as a modulator of the apoptotic cascade depending on the levels and the type of oxidizing species present in the cellular microenvironment. This pathway appears more complicated in that cytosolic cyt*c* would bind to several (macro)molecules. Note that cyt*c* could interact with neuroglobin, a recently discovered neuroprotectant globin in neurons (*45–49*). The neuroglobin-cyt*c* interaction would be significantly enhanced by the electrostatic interactions between the two proteins, as cyt*c* is an unusually basic protein (pI =

10.2) while neuroglobin is an acidic protein (pI = 4.6). Thus, at neutral pH, neuroglobin would be highly negatively charged, whereas cyt*c* would be highly positively charged. The finding that ferrous neuroglobin and ferric cyt*c* could react leads to the hypothesis that neuroglobin can inhibit cyt*c* apoptotic activity allowing cell survival under stress conditions by reducing the heme-Fe-atom of cyt*c* (*48, 49*). Moreover, several cytosolic and mitochondrial tRNAs specifically associate with cyt*c* (*50*). Notably, tRNA blunted the ability of cyt*c* to induce apoptosis while degradation of tRNA by a RNase enhanced apoptosis via the intrinsic pathway. The cyt*c*-tRNA interaction (a previously unexpected connection between two ancient molecules) is pivotal in apoptosis, and may represent an evolutionarily conserved connection between metabolism and cell survival (*50, 51*).

As a whole, the ability of triggering either proapoptotic and antiapoptotic processes renders cyt*c* complexes with (macro)molecules crucial elements for the regulation of the cell fate, thus opening a new (although complex) scenario where the role of cyt*c* appears to be significantly more extensive than what thought so far.

## ACKNOWLEDGEMENTS

This work was partially supported by grants from the Ministero dell'Istruzione, dell'Università e della Ricerca of Italy (University Roma Tre, CLAR 2010, to P.A.).

## REFERENCES

1. Bayir, H., Fadeel, B., Palladino, M. J., Witasp, E., Kurnikov, I. V., Tyurina, Y. Y., Tyurin, V. A., Amoscato, A. A., Jiang, J., Kochanek, P. M., DeKosky, S. T., Greenberger, J. S., Shvedova, A. A., and Kagan, V. E. (2006) Apoptotic interactions of cytochrome *c*: redox flirting with anionic phospholipids within and outside of mitochondria. *Biochim. Biophys. Acta* **1757** 648–659.
2. Orrenius, S., Gogvadze, V., and Zhivotovsky, B. (2007) Mitochondrial oxidative stress: implications for cell death. *Annu. Rev. Pharmacol. Toxicol.* **47** 143–183.
3. Ow, Y.-L. P., Green, D. R., Hao, Z., and Mak, T. W. (2008) Cytochrome *c*: functions beyond respiration. *Nat. Rev. Mol. Cell. Biol.* **9** 532–542.
4. Caroppi, P., Sinibaldi, F., Fiorucci, L., and Santucci, R. (2009) Apoptosis and human diseases: mitochondrion damage and lethal role of cytochrome *c*as proapoptotic protein. *Curr. Med. Chem.* **16** 4058–4065.
5. Pun, P. B., Lu, J., Kan, E. M., and Moochhala, S. (2010) Gases in the mitochondria. *Mitochondrion* **10** 83–93.
6. Gnaiger, E. (2003) Oxygen conformance of cellular respiration: a perspective of mitochondrial physiology. *Adv. Exp. Med. Biol.* **543** 39–55.
7. Lenaz, G. and Genova, M. L. (2010) Structure and organization of mitochondrial respiratory complexes: a new understanding of an old subject. *Antioxid. Redox Signal.* **12** 961–1008.
8. Santucci, R., Sinibaldi, F., Patriarca, A., Santucci, D., and Fiorucci, L. (2010) Misfolded proteins and neurodegenerative diseases: mitochondrial dysfunction and role of nonnative states of cytochrome *c* in cell apoptosis. *Exp. Rev. Proteomics* **7** 507–517.
9. Zou, H., Li, Y., Liu, X., and Wang, X. (1999) An APAF-1·cytochrome *c* multimeric complex is a functional apoptosome that activates procaspase-9. *J. Biol. Chem.* **274**, 11549–11556.
10. Hengartner, M. O. (2000) The biochemistry of apoptosis. *Nature* **407** 770–776.
11. Purring-Koch, C. and McLendon, G. (2000) Cytochrome *c* binding to Apaf-1: the effects of dATP and ionic strength. *Proc. Natl. Acad. Sci. USA* **97** 11928–11931.
12. Twiddy, D., Brown, D. G., Adrain, C., Jukes, R., Martin, S. J., Cohen, G. M., MacFarlane, M., and Cain, K. (2004) Pro-apoptotic proteins released from the mitochondria regulate the protein composition and caspase-processing activity of the native Apaf-1/caspase-9 apoptosome complex. *J. Biol. Chem.* **279** 19665–19682.
13. Garrido, C., Galluzzi, L., Brunet, M., Puig, P. E., Didelot, C., and Kroemer, G. (2006) Mechanisms of cytochrome *c* release from mitochondria. *Cell Death Differ.* **13** 1423–1433.
14. Rytömaa, M. and Kinnunen, P. K. J. (1994) Evidence for two distinct acidic phospholipids-binding sites in cytochrome *c*. *J. Biol. Chem.* **269** 1770–1774.
15. Belikova. N. A., Vladimirov, Y. A., Osipov, A. N., Kapralov, A. A., Tyurin, V. A., Potapovich, M. V., Basova, L. V., Peterson, J., Kurnikov, I. V., and Kagan, V. E. (2006) Peroxidase activity and structural transitions of cytochrome *c* bound to cardiolipin-containing membranes. *Biochemistry* **45** 4998–5009.
16. Vlasova, I. I., Tyurin, V. A., Kapralov, A. A., Kurnikov, I. V., Osipov, A. N., Potapovich, M. V., Stoyanovsky, D. A., and Kagan, V. E. (2006) Nitric oxide inhibits peroxidase activity of cytochrome *c*-cardiolipin complex and blocks cardiolipin oxidation. *J. Biol. Chem.* **281** 14554–14562.
17. Basova, L. V., Kurnikov, I. V., Wang, L., Ritov, V. B., Belikova, N. A., Vlasova, I. I., Pacheco, A. A., Winnica, D. E., Peterson, J., Bayir, H., Waldeck, D. H., and Kagan, V. E. (2007) Cardiolipin switch in mitochondria: shutting off the reduction of cytochrome *c* and turning on the peroxidase activity. *Biochemistry* **46** 3423–3434.
18. Kalanxhi, E., and Wallace, C. J. A. (2007) Cytochrome *c* impaled: investigation of the extended lipid anchorage of a soluble protein to mitochondrial membrane models. *Biochem. J.* **407** 179–187.
19. Sinibaldi, F., Fiorucci, L., Patriarca, A., Lauceri, R., Ferri, T., Coletta, M., and Santucci, R. (2008) Insights into cytochrome *c*-cardiolipin interaction: role played by ionic strength. *Biochemistry* **47** 6928–6935.
20. Sinibaldi, F., Howes, B. D., Piro, M. C., Polticelli, F., Bombelli, C., Ferri, T., Coletta, M., Smulevich, G., and Santucci, R. (2010) Extended cardiolipin anchorage to cytochrome *c*: a model for protein-mitochondrial membrane binding. *J. Biol. Inorg. Chem.* **15** 689–700.
21. Rytömaa, M. and Kinnunen, P. K. J. (1995) Reversibility of the binding of cytochrome *c* liposomes: implications for lipid-protein interactions. *J. Biol. Chem.* **270** 3197–3202.
22. Tuominen, E. K., Wallace, C. J., and Kinnunen, P. K. J. (2002) Phospholipid-cytochrome *c* interaction: evidence for the extended lipid anchorage. *J. Biol. Chem.* **277**, 8822–8826.
23. Chen, Y. R., Deterding, L. J., Sturgeon, B. E., Tomer, K. B., and Mason, R. P. (2002) Protein oxidation of cytochrome *c* by reactive halogen species enhances its peroxidase activity. *J. Biol. Chem.* **277** 29781–29791.
24. Diederix, R. E., Ubbink, M., and Canters, G. W. (2002) Peroxidase activity as a tool for studying the folding of *c*-type cytochromes. *Biochemistry* **41** 13067–13077.
25. Kapralov, A. A., Kurnikov, I. V., Vlasova, I. I., Belikova, N. A., Tyurin, V. A., Basova, L. V., Zhao, Q., Tyurina, Y. Y., Jiang, J., Bayir, H., Vladimirov, Y. A., and Kagan, V. E. (2007) The hierarchy of structural transitions induced in cytochrome *c* by anionic phospholipids determines its peroxidase activation and selective peroxidation during apoptosis in cells. *Biochemistry* **46** 14232–14244.
26. Patriarca, A., Eliseo, T., Sinibaldi, F., Piro, M. C., Melis, R., Paci, M., Cicero, D. O., Polticelli, F., Santucci, R., and Fiorucci, L. (2009) ATP acts as a regulatory effector in modulating structural transitions of cytochrome *c*: implications for apoptotic activity. *Biochemistry* **48** 3279–3287.
27. Kapetanaki, S. M., Silkstone, G., Husu, I., Liebl, U., Wilson, M. T., and Vos, M. H. (2009) Interaction of carbon monoxide with the apoptosis-inducing cytochrome *c*-cardiolipin complex. *Biochemistry* **48** 1613–1619.

28. Silkstone, G., Kapetanaki, S. M., Husu, I., Vos, M. H., and Wilson, M. T. (2010) Nitric oxide binds to the proximal heme coordination site of the ferrocytochrome *c*/cardiolipin complex: formation mechanism and dynamics. *J. Biol. Chem.* **285** 19785–19792.
29. Ascenzi, P., Ciaccio, C., Sinibaldi, F., Santucci, R., and Coletta, M. (2011) Cardiolipin modulates allosterically peroxynitrite detoxification by horse heart cytochrome *c*. *Biochem. Biophys. Res. Commun.* **404** 190–194.
30. Lewis, R. N. and McElhaney, R. N. (2009) The physicochemical properties of cardiolipin bilayers and cardiolipin-containing lipid membranes. *Biochim. Biophys. Acta* **1788** 2069–2079.
31. Kagan, V. E., Tyurin, V. A., Jiang, J., Tyurina, Y. Y., Ritov, V. B., Amoscato, A. A., Osipov, A. N., Belikova, N. A., Kapralov, A. A., Kini, V., Vlasova, I. I., Zhao, Q., Zou, M., Di, P., Svistunenko, D. A., Kurnikov, I. V., and Borisenko, G. G. (2005) Cytochrome *c* acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat. Chem. Biol.* **1** 223–232.
32. Belikova, N. A., Tyurina, Y. Y., Borisenko, G., Tyurin, V., Samhan Arias, A. K., Yanamala, N., Furtmüller, P. G., Klein-Seetharaman, J., Obinger, C., and Kagan, V. E. (2009) Heterolytic reduction of fatty acid hydroperoxides by cytochrome *c*/cardiolipin complexes: antioxidant function in mitochondria. *J. Am. Chem. Soc.* **131** 11288–11289.
33. Hoye, A. T., Davoren, J. E., Wipf, P., Fink, M. P., and Kagan, V. E. (2008) Targeting mitochondria. *Acc. Chem. Res.* **41** 87–97.
34. Kagan, V. E., Bayir, H. A., Belikova, N. A., Tyurina, Y. Y., Tyurin, V. A., Jiang, J., Stoyanovsky, D. A., Wipf, P., Kochanek, P. M., Greenberger, J. S., Pitt, B., Shvedova, A. A., and Borisenko, G. (2009) Cytochrome *c*/cardiolipin relations in mitochondria. *Free Radic. Biol. Med.* **46** 1439–1453.
35. Wilson, M. T., Brunori, M., Rotilio, G. C., and Antonini, E. (1973) Properties of modified cytochromes. II. Ligand binding to reduced carboxymethyl cytochrome *c*. *J. Biol. Chem.* **248** 8162–8169.
36. Antonini, E. and Brunori, M. (1971) *Hemoglobin and Myoglobin in their Reactions with Ligands*. North Holland Publishing Co., Amsterdam.
37. Ascenzi, P., Coletta, M., Santucci, R., Polizio, F., and Desideri, A. (1994) Nitric oxide binding to ferrous native horse heart cytochrome *c* and to its carboxymethylated derivative: a spectroscopic and thermodynamic study. *J. Inorg. Biochem.* **53** 273–280.
38. Copeland, D. M., West, A. H., and Richter- Addo, G. B. (2003) Crystal structures of ferrous horse heart myoglobin complexed with nitric oxide and nitrosoethane. *Proteins* **53** 182–192.
39. Ascenzi, P., Cao, Y., di Masi, A., Gullotta, F., De Sanctis, G., Fanali, G., Fasano, M., and Coletta, M. (2009) Reductive nitrosylation of ferric human serum heme-albumin. *FEBS J.* **277** 2474–2485.
40. Ascenzi, P., Santucci, R., Coletta, M., and Polticelli, F. (2010) Cytochromes: reactivity of the "dark side" of the heme. *Biophys. Chem.* **152** 21–27.
41. Herold, S. and Kalinga, S. (2003) Metmyoglobin and methemoglobin catalyze the isomerization of peroxynitrite to nitrate. *Biochemistry* **42** 14036–14046.
42. Herold, S., Fago, A., Weber, R. E., Dewilde, S., and Moens, L. (2004) Reactivity studies of the Fe(III) and Fe(II)NO forms of human neuroglobin reveal a potential role against oxidative stress. *J. Biol. Chem.* **279** 22841–22847.
43. Ascenzi, P., di Masi, A., Coletta, M., Ciaccio, C., Fanali, G., Nicoletti, F. P., Smulevich, G., and Fasano, M. (2009) Ibuprofen impairs allosterically peroxynitrite isomerization by ferric human serum heme-albumin. *J. Biol. Chem.* **284** 31006–31017.
44. Abriata, L. A., Cassina, A., Tortora, V., Marin, M., Souza, J. M., Castro, L., Vila, A. J., and Radi, R. (2009) Nitration of solvent-exposed Tyrosine 74 on cytochrome *c* triggers heme iron-Methionine 80 bond disruption: nuclear magnetic resonance and optical spectroscopy studies. *J. Biol. Chem.* **284** 17–26.
45. Fago, A., Mathews, A. J., Moens, L., Dewilde, S., and Brittain, T. (2006) The reaction of neuroglobin with potential redox protein partners cytochrome $b_5$ and cytochrome *c*. *FEBS Lett.* **580** 4884–4888.
46. Bønding, S. H., Henty, K., Dingley, A. J., and Brittain, T. (2008) The binding of cytochrome *c* to neuroglobin: a docking and surface plasmon resonance study. *Int. J. Biol. Macromol.* **43** 295–299.
47. Fago, A., Mathews, A. J., and Brittain, T. (2008) A role for neuroglobin: resetting the trigger level for apoptosis in neuronal and retinal cells. *IUBMB Life* **60** 398–401.
48. Brittain, T., Skommer, J., Raychaudhuri, S., and Birch, N. (2010) An antiapoptotic neuroprotective role for neuroglobin. *Int. J. Mol. Sci.* **11** 2306–2321.
49. Raychaudhuri, S., Skommer, J., Henty, K., Birch, N., and Brittain, T. (2010) Neuroglobin protects nerve cells from apoptosis by inhibiting the intrinsic pathway of cell death. *Apoptosis* **15** 401–411.
50. Mei, Y., Yong, J., Liu, H., Shi, Y., Meinkoth, J., Dreyfuss, G., and Yang, X. (2010) tRNA binds to cytochrome *c* and inhibits caspase activation. *Mol. Cell* **37**, 668–678.
51. Mei, Y., Yong, J., Stonestrom, A., and Yang, X. (2010) tRNA and cytochrome *c* in cell death and beyond. *Cell Cycle* **9** 2936–2939.