Brain Mitochondria Are Primed by Moderate Ca\textsuperscript{2+} Rise upon Hypoxia/Reoxygenation for Functional Breakdown and Morphological Disintegration\textsuperscript{*}

Lorenz Schild‡§, Jens Huppelsberg‡, Stefan Kahler†, Gerburg Keilhoff§, and Georg Reiser¶

From the ‡Institut für Klinische Chemie und Pathologische Biochemie, ¶Institut für Neurobiochemie, and §Institut für Medizinische Neurobiologie, Medizinische Fakultät, Otto-von-Guericke-Universität Magdeburg, Leipziger Strasse 44, 39120 Magdeburg, Germany

Received for publication, March 18, 2003, and in revised form, April 16, 2003
Published, JBC Papers in Press, April 17, 2003, DOI 10.1074/jbc.M302743200

In animal models, brain ischemia causes changes in respiratory capacity, mitochondrial morphology, and cytochrome c release from mitochondria as well as a rise in cytosolic Ca\textsuperscript{2+} concentration. However, the causal relationship of the cellular processes leading to mitochondrial deterioration in brain has not yet been clarified. Here, by applying various techniques, we used isolated rat brain mitochondria to investigate how hypoxia/reoxygenation and nonphysiological Ca\textsuperscript{2+} concentrations in the low micromolar range affect active (state 3) respiration, membrane permeability, swelling, and morphology of mitochondria. Either transient hypoxia or a micromolar rise in extramitochondrial Ca\textsuperscript{2+} concentration, given as a single insult alone, slightly decreased active respiration. However, the combination of both insults caused devastating effects. These implied almost complete loss of active respiration, release of both NADH and cytochrome c, and rupture of mitochondria, as shown by electron microscopy. Mitochondrial respiration deteriorated even in the presence of cyclosporin A, documenting that membrane permeabilization occurred independent of mitochondrial permeability transition pore. Ca\textsuperscript{2+} has to enter the mitochondrial matrix in order to mediate this mitochondrial injury, because blockade of the mitochondrial Ca\textsuperscript{2+}-transport system by ruthenium red in combination with CGP37157 completely prevented damage. Furthermore, protection of respiration from Ca\textsuperscript{2+}-mediated damage by the adenine nucleotide ADP, but not by AMP, during hypoxia/reoxygenation is consistent with the delayed susceptibility of brain mitochondria to prolonged hypoxia, which is observed in vivo.

\textsuperscript{*} This work was supported by grants from Bundesministerium für Bildung und Forschung (01ZZ0107), Medizinische Fakultät (“Neuroverband”), Land Sachsen-Anhalt (2923A), and Fonds der Chemischen Industrie. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{‡} To whom correspondence should be addressed: Dept. für Pathologische Biochemie, Institut für Klinische Chemie und Pathologische Biochemie, Medizinische Fakultät, Otto-von-Guericke-Universität Magdeburg, Leipziger Str. 44, D-39120 Magdeburg, Germany. E-mail: lorenz.schild@medizin.uni-magdeburg.de.
reoxygengation and elevated extramitochondrial Ca\(^{2+}\) concentration as signals for permeabilization of the mitochondrial membrane for matrix and intermembrane space proteins. Therefore, we subjected isolated rat brain mitochondria to hypoxia/reoxygengation and/or elevated extramitochondrial Ca\(^{2+}\) concentration and determined respiration, mitochondrial morphology, cytochrome c release, and membrane permeability by applying various complementary techniques. We found that in hypoxia/reoxygengation an elevated extramitochondrial Ca\(^{2+}\) concentration dramatically enhanced inhibition of active respiration. Moreover, Ca\(^{2+}\) had to enter the mitochondrial matrix to mediate this effect. Under these conditions there was a complete loss of mitochondrial integrity. The presence of ADP, but not AMP, during hypoxia/reoxygengation completely prevented mitochondrial damage. Permeabilization of the mitochondrial membrane did not depend on cyclosporin A, a compound known to keep the mitochondrial permeability transition pore in the closed state.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cyclosporin A was purchased from Sigma, cytochrome c from Boehringer, mouse monoclonal cytochrome c antibody from Pharmingen, and anti-mouse Ig and horseradish peroxidase from Roche Diagnostics. CGP37157 was from Toeris (Cologne, Germany). All other chemicals were of analytical grade.

**Preparation of Brain Mitochondria**—Mitochondria were prepared from the brains of 220–240-g male Wistar rats in ice-cold medium containing 250 mM mannitol, 20 mM Tris, 1 mM EGTA, 1 mM EDTA, and 0.3% (w/v) bovine serum albumin at pH 7.4 (isolation medium) using a standard procedure (17). After the initial isolation, Percoll was used for purification of mitochondria from a fraction containing some endoplasmic reticulum, Golgi apparatus, and plasma membranes. The mitochondria were well coupled, as indicated by a respiratory control index greater than 4 with glutamate plus malate as substrates. Protein concentration and determined respiration, mitochondrial morphology, cytochrome c release, and membrane permeability by applying various complementary techniques. Under these conditions there was a complete loss of mitochondrial integrity. The presence of ADP, but not AMP, during hypoxia/reoxygengation completely prevented mitochondrial damage. Permeabilization of the mitochondrial membrane did not depend on cyclosporin A, a compound known to keep the mitochondrial permeability transition pore in the closed state.

**Influence of Extramitochondrial Ca\(^{2+}\) and Hypoxia/Reoxygengation on Mitochondrial Respiration**—To investigate whether elevated extramitochondrial Ca\(^{2+}\) concentration plays a role in the impairment of mitochondrial function during ischemia/reperfusion, respiration of isolated rat brain mitochondria was analyzed. The influence of extramitochondrial Ca\(^{2+}\) alone and of the combination of a rise in Ca\(^{2+}\) with hypoxia/reoxygengation was tested (Fig. 1). First, at three different extramitochondrial Ca\(^{2+}\) concentrations (0, 1.5, and 3.5 \(\mu\)M) isolated rat brain mitochondria were subjected to 10 min of hypoxia and 5 min of reoxygengation in the absence of substrates. In general, during hypoxia/reoxygengation no substrates were present. After the addition of 5 mM glutamate plus 5 mM malate, active respiration (state 3) was induced by the addition of 200 \(\mu\)M ADP. At 3.5 \(\mu\)M extramitochondrial Ca\(^{2+}\), a decrease of about 30\% in comparison with Ca\(^{2+}\)-free incubation, was measured (Fig. 1A, black bars). The rate of active respiration of freshly isolated mitochondria was determined to be 71.0 \pm 4.9 (n = 18) nmol of O\(_2\) min \(^{-1}\) mg of mitochondrial protein. In Ca\(^{2+}\)-free incubation, 10 min of hypoxia followed by 5 min of reoxygengation caused a significant decrease in active respiration amounting to about 50\%. The Ca\(^{2+}\) rise enhanced the inhibition of respiration by hypoxia/reoxygengation in a dose-dependent manner. In the case of 3.5 \(\mu\)M extramitochondrial Ca\(^{2+}\), active respiration finally decreased down to about 15\% of active respiration of freshly isolated mitochondria (Fig. 1A, gray bars).

In the next series of experiments we investigated the influence of the duration of hypoxia on active respiration (state 3) of isolated rat brain mitochondria. Therefore, the time of hypoxia was varied in the absence of extramitochondrial Ca\(^{2+}\) or in the presence of 3.5 \(\mu\)M Ca\(^{2+}\), and then active respiration was measured immediately after reoxygengation. In Ca\(^{2+}\)-free incubation, active respiration decreased within a hypoxic period of 15 min to 58\% of initial state 3 respiration (Fig. 1B, black bars). At any hypoxic period, 3.5 \(\mu\)M extramitochondrial Ca\(^{2+}\) caused a substantial additional reduction of respiration (Fig. 1B, gray bars).

To investigate whether the deleterious effect on active respiration found after hypoxia/reoxygengation by Ca\(^{2+}\) is exerted from the inside or outside of mitochondria, we repeated the experiments in the presence of ruthenium red in combination with CGP37157. Under these conditions both Ca\(^{2+}\) uptake by the electrogenic uniporter and Ca\(^{2+}\) influx by the Na\(^{+}\)-Ca\(^{2+}\) exchanger is blocked. In Fig. 2, oxygen traces after 10 min of hypoxia and 5 min of reoxygengation (trace a), after 10 min of hypoxia and 5 min of reoxygengation in the presence of 3.5 \(\mu\)M Ca\(^{2+}\) (trace b), and in the additional presence of 2 \(\mu\)M ruthenium red and 25 \(\mu\)M CGP37157 (trace c) are presented. 3.5 \(\mu\)M extramitochondrial Ca\(^{2+}\) caused a decrease in active respiration (14.6 \pm 3.4 (n = 4) versus 21.9 \pm 2.8 (n = 4) nmol of O\(_2\) min/mg of mitochondrial protein). The block of the mitochondrial Ca\(^{2+}\) transport system not only prevented Ca\(^{2+}\)-induced decrease in active respiration but also partially protected mitochondria from hypoxia/reoxygengation-mediated damage of active respiration (32.0 \pm 4.4 (n = 4) versus 21.9 \pm 2.3 (n = 4) nmol of O\(_2\) min/mg of mitochondrial protein). Thus, protection could be partial because small amounts of endogenous Ca\(^{2+}\) are present during exposure of mitochondria to
hypoxia/reoxygenation. In conclusion, Ca\textsuperscript{2+} exerts its deleterious effect in the mitochondrial matrix.

Adenine nucleotides are present commonly within mammalian cells. In the ischemic phase, ATP is converted first into ADP and subsequently into AMP to maintain energy-consuming processes for as long as possible. In a separate series of experiments, we tested whether extramitochondrial ADP or extramitochondrial AMP affects the decrease in active respiration by hypoxia/reoxygenation. Fig. 3 shows the oxygen traces of a normoxic control (trace a), of mitochondria after 10 min of hypoxia followed by 5 min of reoxygenation in the presence of 3.5 mM extramitochondrial Ca\textsuperscript{2+} (trace b) and in the additional presence of 5 mM ADP (trace c) or 5 mM AMP (trace d). The presence of ADP during hypoxia/reoxygenation almost completely protected brain mitochondria from decrease in active respiration (62.0 ± 5.7 (n = 5) versus 19.2 ± 3.6 (n = 5) and 73.0 ± 4.4 (n = 5) nmol of O\textsubscript{2}/min/mg of mitochondrial protein). Under this condition, the addition of 200 mM ADP did not result in any further stimulation of respiration because the ADP added previously was not completely consumed by the mitochondria. In contrast, AMP had no protective effect (11.7 ± 2.1 nmol of O\textsubscript{2}/min/mg of mitochondrial protein).

**Mitochondrial Morphology Is Influenced by Extramitochondrial Ca\textsuperscript{2+} and Hypoxia/Reoxygenation**—To elucidate the role of hypoxia/reoxygenation and elevated extramitochondrial Ca\textsuperscript{2+} concentration on mitochondrial morphology, we performed electron microscopy analyses. The electron micrographs were inspected by two independent investigators blinded to the treatment group. Typical structures corresponding to distinct incubation conditions are presented at a magnification of 1:20,000 in Fig. 4. In each image in Fig. 4 (A–D) an inset illustrates detailed structures at the magnification of 1:30,000. Increasing the extramitochondrial Ca\textsuperscript{2+} concentration from zero up to 3.5 mM did not significantly modify the mitochondrial structure (Fig. 4, B versus A). We were not able to detect changes in the cristae structure. Moreover, intact outer membrane structures were clearly maintained.

Hypoxia/reoxygenation by itself (Fig. 4C) led to several changes in mitochondrial morphology. Besides a population of mitochondria with normal morphology, mitochondria with dented (bleb-like) outer membranes and another population of mitochondria with loss of cristae were found. The combination of 3.5 mM extramitochondrial Ca\textsuperscript{2+} with hypoxia/reoxygenation...
Ca$^{2+}$-induced swelling, could not prevent decrease in active respiration. Respiration measurements (Fig. 5B) show that for active respiration of brain mitochondria subjected to substrate-free 10 min of hypoxia and 5 min of reoxygenation in the presence of 3.5 $\mu$M Ca$^{2+}$, no significant difference was found between the values either with or without 2 $\mu$M cyclosporin A. Thus, we conclude that mitochondrial rupture did not require the opening of the mitochondrial permeability transition pore.

A further series of experiments was performed to test whether mitochondrial constituents are lost during hypoxia/reoxygenation in the presence of low micromolar Ca$^{2+}$, to substantiate our conclusion of mitochondrial disintegration under these conditions. It is well known that the active respiration of intact brain mitochondria is insensitive to extramitochondrial cytochrome c and NADH, because neither of these compounds can permeate through the mitochondrial membrane system. Therefore, we analyzed whether active respiration after 10 min of hypoxia and 5 min of reoxygenation in the presence of 3.5 $\mu$M Ca$^{2+}$ becomes sensitive to extramitochondrial cytochrome c and NADH. This approach was used to demonstrate membrane permeabilization. Respiration of intact mitochondria is shown in Fig. 6A. The oxygen consumption after hypoxia/reoxygenation is depicted in Fig. 6B. In intact mitochondria, ADP caused a 4.6-fold increase in respiration (stimulation of active respiration) to 87 ± 5.8 (n = 5) nmol of O$_2$/min/mg of mitochondrial protein, whereas after hypoxia/reoxygenation the rate of active respiration was as low as 15.2 ± 1.9 (n = 5) nmol of O$_2$/min/mg of mitochondrial protein. The application of 30 $\mu$M cyclosporin A after hypoxia/reoxygenation in the presence of 3.5 $\mu$M Ca$^{2+}$ only very moderately accelerated state 3 respiration (19.6 ± 2.2 (n = 5) versus 15.2 ± 1.9 (n = 5) nmol of O$_2$/min/mg of mitochondrial protein). In contrast, a nearly 4-fold increase in the rate of oxygen consumption was found in the presence of 5 mM NADH in the incubation medium (55.3 ± 3.3 (n = 5) versus 15.2 ± 1.9 (n = 5) nmol of O$_2$/min/mg of mitochondrial protein). This oxygen consumption was not associated with ATP synthesis, because oligomycin was without influence. In intact mitochondria, however, the inhibition of ATP synthesis by oligomycin slowed down the rate of active respiration to resting (state 4) level (18.7 ± 2.3 (n = 5) versus 87 ± 5.8 (n = 5) nmol of O$_2$/min/mg of mitochondrial protein).

Stimulation of oxygen consumption by extramitochondrial NADH clearly demonstrates that the inner mitochondrial membrane becomes permeabilized, because under physiological conditions the mitochondrial membrane is impermeable to NADH. The moderate increase in respiration after cytochrome c addition proves that most of the cytochrome c was still associated with parts of the respiratory chain. In fact, Western blot analysis revealed that only 15% of the cytochrome c pool was released into the incubation medium during 10 min of hypoxia and 5 min of reoxygenation in the presence of 3.5 $\mu$M Ca$^{2+}$ (data not shown).

**DISCUSSION**

**Impairment of Mitochondria upon Ischemia/Reperfusion**—Animal models of stroke have revealed that mitochondria are impaired upon ischemia/reperfusion in brain tissue. Decrease

![Figure 3. Influence of ADP and AMP on the impairment of mitochondrial respiration by hypoxia/reoxygenation (hypoxia/reoxygenation) in the presence of elevated Ca$^{2+}$ concentration. Rat brain mitochondria (about 0.5 mg of protein/ml) were incubated at 30 °C in the incubation medium. Active (state 3) respiration was achieved by the addition of 200 $\mu$M ADP in the presence of 5 mM glutamate and 5 mM malate in freshly isolated rat brain mitochondria in the absence of external Ca$^{2+}$ (a); 10 min of hypoxia was followed by 5 min of reoxygenation in the presence of 3.5 $\mu$M external Ca$^{2+}$ (b); 10 min of hypoxia was followed by 5 min of reoxygenation in the presence of external Ca$^{2+}$ and 5 mM ADP (c); and 10 min of hypoxia was followed by 5 min of reoxygenation in the presence of external Ca$^{2+}$ and 5 mM AMP (d). When mitochondria were subjected to hypoxia/reoxygenation, the substrates were added after 5 min of reoxygenation, and oxygen concentration was monitored. In trace e, the addition of 200 $\mu$M ADP did not result in stimulation of respiration, because the mitochondria had already respired under state 3 conditions. The numbers beside the traces represent the rates of active respiration in nmol of O$_2$/min/mg of mitochondrial protein. The experiment shown is typical for five preparations of mitochondria. Mean values ± S.E. for the example shown here are given in the text.](image-url)
in respiratory capacity (1), change in mitochondrial morphology (3), and permeabilization of the mitochondrial membrane system have been reported (4). A further hallmark of ischemic neuronal insults is the disturbance of cellular Ca$^{2+}$/H$^{+}$ homeostasis, characterized by increased cytosolic Ca$^{2+}$/H$^{+}$ concentration. Elevation of the cytosolic Ca$^{2+}$/H$^{+}$ concentration is a well known trigger of mitochondrial damage (6).

Hypoxia/reoxygenation caused impairment of mitochondrial function in cultured astrocytes (22) and isolated mitochondria (23). Here we provide evidence that hypoxia/reoxygenation induces decrease in active respiration and changes in mitochondrial structure, which is characterized by a subpopulation of mitochondria with dented (bleb-like) outer membranes and another subpopulation of mitochondria with diminished number of cristae.

Mitochondria isolated from brain display high resistance to relatively high extramitochondrial Ca$^{2+}$ concentrations in comparison with mitochondria from other tissues such as liver (13). Also, recently published data from our own studies (12) have shown that Ca$^{2+}$ in the micromolar range induces only a moderate decrease in active respiration and the release of a small amount of cytochrome c through the outer membrane from brain mitochondria with still intact morphology. Ca$^{2+}$ has to enter the mitochondrial matrix via the mitochondrial Ca$^{2+}$ transport system in order to affect the permeability of the mitochondrial membrane. It is known that alterations in the Ca$^{2+}$ concentration can modify the number of contact sites between the inner and outer membrane, possibly by unmasking nonspecific channels of the mitochondrial outer membrane such as the voltage dependent anion channel (24–26). This might explain the Ca$^{2+}$-mediated change in the permeability of the mitochondrial membrane system. At the cellular level, an increase in cytosolic Ca$^{2+}$ concentration has been identified as the major cause for mitochondrial impairment, such as in striatal neurons (27) and in hippocampal astrocytes (28).

Here we demonstrate that the application of hypoxia/reoxygenation sensitizes mitochondria to moderately elevated extramitochondrial Ca$^{2+}$ concentration. Then, even low micromolar Ca$^{2+}$ causes dramatic functional and morphological changes in isolated brain mitochondria. These include permeabilization and breakdown of the mitochondrial membrane. Again, Ca$^{2+}$ has to enter the mitochondrial matrix to exert the deleterious
effect, because blocking the Ca\(^{2+}\) transport system completely protected mitochondria from Ca\(^{2+}\)-mediated damage. Transient hypoxia in combination with Ca\(^{2+}\) elevated into the low micromolar range most appropriately mimics the in vivo situation during ischemia/reperfusion. Interestingly, in our experiments, the impairment of mitochondria was not sensitive to cyclosporin A. In vivo studies, however, using animal models of stroke indicate the involvement of the mitochondrial permeability transition pore (10). Remarkably, the latter seems to be true only for a part of the brain cells localized within the infarct area, because cyclosporin A only diminished the size of the infarct but could not completely prevent necrosis (10, 29, 30). It still remains unclear which Ca\(^{2+}\)-mediated mechanism is responsible for the cyclosporin A-independent rupture of the mitochondrial membrane.

Investigations on isolated mitochondria reveal that elevated Ca\(^{2+}\) concentration, hypoxia/reoxygenation, or the combination of both treatments clearly induces distinct effects. These observations suggest that, depending on the degree of hypoxia and the level of the cytosolic Ca\(^{2+}\), mechanisms other than the opening of the mitochondrial permeability transition pore may be involved in the process of mitochondrial damage during ischemia/reperfusion.

Impact of Mitochondrial Deterioration after Ischemia/Reperfusion on Brain Cell Fate—A complete breakdown of mitochondrial ATP production is a prerequisite of necrotic cell death in brain (31). ATP is required for the maintenance of cellular morphology, ion homeostasis, protein synthesis, and many other cellular functions. Moreover, ATP is even necessary to perform the apoptotic cell death program (32). Another factor in inducing neuronal demise is the change of the permeability transi-
of the mitochondrial membrane. Depending on the mode of activation, permeabilization can cause either apoptosis or necrosis. Distinct permeabilization of the mitochondrial outer membrane can be achieved in brain mitochondria by increased cytosolic Ca\(^{2+}\) concentration or by members of the Bcl-2 family in cooperation with cardiolipin (33). This type of permeabilization causes a partial release of proapoptotic factors such as cytochrome c (12, 13). The members of the Bcl-2 family also interact with the mitochondrial permeability transition pore (34). Reversible permeabilization of the mitochondrial membrane by opening of the permeability transition pore also causes the release of proapoptotic factors from mitochondria. If sufficient ATP is available within the cell, apoptosis is initiated. In fact, signs of apoptosis and cyclosporin A sensitivity in brain injury have been demonstrated in animal models of stroke (35). In contrast, the disruption of the mitochondrial membrane causes necrotic cell death (36), which can be induced by permanent opening of the permeability transition pore by Ca\(^{2+}\) overload (37, 38) or by massive lipid peroxidation (39). Our experiments demonstrate that extramitochondrial Ca\(^{2+}\) concentration in the low micromolar range leads to cyclosporin A-insensitive (e.g., permeability transition pore-independent) disruption of the mitochondrial membrane.

Isolated mitochondria can be maintained under conditions of almost complete anoxia (23), whereas in vivo, some oxygen may diffuse from the environment into the infarct area. Both the in vivo studies of stroke and the cell culture investigation on hypoxia/reoxygenation require a relatively long period of hypoxia to reach significant injury (2). However, in isolated brain mitochondria only a few minutes of hypoxia are sufficient to cause dramatic damage. Differences in local oxygen concentration may be the reason for this apparent discrepancy between in vivo and isolated mitochondria experiments in the time required to reach injury. We have found that at elevated extramitochondrial Ca\(^{2+}\) concentrations, ADP at physiological concentration (5 mM) protects mitochondria from hypoxia/reoxygenation-induced damage. Only when all of the ADP is converted into AMP, mitochondrial damage occurs. This finding may contribute further to the fact that longer periods of ischemia are required to achieve tissue damage in comparison with isolated mitochondria that have to be exposed only for a short period of time to hypoxia in order to induce mitochondrial damage.

Pathological Consequences in Brain Mitochondria Caused by Ischemia/Reperfusion—From both the present data and the observations obtained with animal models of stroke, we conclude that three qualitatively different situations of stroke injury have to be distinguished. (i) Increased cytosolic Ca\(^{2+}\) concentrations into the low micromolar range induce permeabilization of the mitochondrial outer membrane and release of proapoptotic factors such as cytochrome c, resulting in the induction of apoptosis. (ii) Short periods of hypoxia or moderate elevation of the extramitochondrial Ca\(^{2+}\) concentration cause reversible opening of the mitochondrial permeability transition pore followed by the release of proapoptotic factors from morphologically intact mitochondria, also resulting in the induction of apoptosis. (iii) Long-lasting hypoxia, followed by reoxygenation at moderately elevated cytosolic Ca\(^{2+}\) concentration, leads to permanent opening of the permeability transition pore or the permeability transition pore-independent disruption of the mitochondrial membrane. The resulting failure of mitochondrial energy metabolism causes necrotic cell death.

In conclusion, we provide evidence that isolated rat brain mitochondria are highly vulnerable to hypoxia/reoxygenation applied in combination with the rise of the extramitochondrial Ca\(^{2+}\) concentration into the low micromolar range. However, each stimulus, Ca\(^{2+}\) or hypoxia/reoxygenation, applied individually can be well tolerated by brain mitochondria. Thus, our data explain the apparent discrepancy between in vivo and in vitro data concerning the harm exerted by cytosolic Ca\(^{2+}\) concentrations in the low micromolar range. When observed in vitro, high resistance of mitochondrial membrane is seen, whereas in vivo, upon ischemia/reperfusion cell death occurs. Therefore, in cells of the nervous tissue therapeutic concepts aimed at preventing neural damage after ischemia (stroke) should focus on the prevention of pathological elevations of cytosolic Ca\(^{2+}\).
Brain Mitochondria Are Primed by Moderate Ca$^{2+}$ Rise upon Hypoxia/Reoxygenation for Functional Breakdown and Morphological Disintegration
Lorenz Schild, Jens Huppelsberg, Stefan Kahlert, Gerburg Keilhoff and Georg Reiser

J. Biol. Chem. 2003, 278:25454-25460.
doi: 10.1074/jbc.M302743200 originally published online April 17, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M302743200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 6 of which can be accessed free at
http://www.jbc.org/content/278/28/25454.full.html#ref-list-1