Biochemical and Biophysical Research Communications 396 (2010) 95–100

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mitochondrial regulation of cell death: Processing of apoptosis-inducing factor (AIF)

Erik Norberg, Sten Orrenius *, Boris Zhivotovsky

Institute of Environmental Medicine, Division of Toxicology Karolinska Institutet, SE-171 77 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 25 January 2010

Keywords:
AIF
Apoptosis
Mitochondria
Calpain
Cathepsin
Reactive oxygen species (ROS)

ABSTRACT

Apoptosis might proceed through the activation of both caspase-dependent and -independent pathways. Apoptosis-inducing factor (AIF) was discovered as the first protein that mediated caspase-independent cell death. Initially, it was regarded as a soluble protein residing in the intermembrane space of mitochondria, from where it could be exported to the nucleus to participate in large-scale DNA fragmentation and chromatin condensation. However, later it was demonstrated that AIF is N-terminally anchored to the inner mitochondrial membrane. Hence, AIF must be liberated from its membrane anchor prior to being released into the cytosol. The current knowledge about the molecular mechanisms regulating the processing and release of AIF from the mitochondria will be summarized and discussed in this review.

1. Introduction

Since more than 25 years our research has focused on the regulation and mechanisms of apoptotic cell death and its possible implications for human disease. Much of our recent work has been concerned with mitochondrial regulation of cell death by the release of cytochrome *c* and other pro-apoptotic proteins, which was early found to represent an important pathway of apoptosis regulation [1,2]. We first studied Ca^{2+} signaling as a component of the apoptotic program and demonstrated that release of pro-apoptotic proteins from mitochondria can occur via both Ca^{2+}-dependent and Ca^{2+}-independent mechanisms [3]. We also found that the export of cytochrome *c* from mitochondria during apoptosis proceeds by a two-step process, involving the detachment of the hemoprotein from its binding to cardiolipin in the inner mitochondrial membrane (IMM) followed by its translocation into the cytosol [4]. We observed that microinjection of cytochrome *c* results in the induction of apoptosis in a number of different cell types [5], and found that aberrant mitochondrial iron distribution is responsible for spontaneous cytochrome *c* release from mitochondria in early erythroid precursor cells in myelodysplastic syndrome [6]. Detailed analysis of the mechanisms of resistance of lung cancer cells to radio- and chemotherapy revealed a deficiency in caspase-8 expression in a majority of small cell lung cancer cells and clinical samples, and suggested that mitochondrial dysfunction is an essential step for killing non-small cell lung carcinoma (NSCLC) cells resistant to conventional treatment [7].

Hence, investigations of the role of mitochondria in cell death have long been in the focus of our research. This intracellular organelle is functioning as a switchboard in cell death signaling. In particular, the permeabilization of the outer mitochondrial membrane (OMM) and the subsequent release of pro-apoptotic proteins from the intermembrane space (IMS) of mitochondria are key events in both caspase-dependent and caspase-independent pathways [8]. Apoptosis-inducing factor (AIF) is one of the mitochondrial proteins to be released into the cytosol during apoptosis, and it was discovered as the first protein that regulates caspase-independent apoptosis [9].

2. Apoptosis-inducing factor (AIF)

AIF is encoded as a 67 kDa protein that contains a mitochondrial localization signal (MLS) in the N-terminus. Upon import into the mitochondria, the MLS is removed by a mitochondrial peptidase generating the mature 62 kDa protein [10]. Initially, AIF was regarded as a soluble protein localized in the IMS [9]. However, later it became clear that AIF is N-terminally anchored to the IMM [10], where it exerts NADH oxidase activity [11]. The crystal structure of mouse and human AIF (which share 92% identity) does indeed reveal an oxidoreductase-like folding of the protein [12,13].

Based on findings with different knockout/knockdown models it was proposed that AIF might play a role in oxidative phosphorylation, mainly by modulating the structure and function of complex I of the respiratory chain [14,15]. Muscle- and liver-specific AIF ablation in mice resulted in deficient oxidative phosphorylation [16]. Genetic inactivation of AIF abolished cavitation, an

* Corresponding author. Fax: +46 8 32 90 41.
E-mail address: sten.orrenius@ki.se (S. Orrenius).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.02.163

apoptosis-regulated process essential for the early embryonic morphogenesis, prior to gastrulation [17]. Therefore, complete deficiency of AIF is embryonically lethal.

In a Harlequin (Hq) mouse model the AIF expression is suppressed to 10–20% of normal level due to a retroviral insertion into the AIF gene. In contrast to AIF-deficient embryos, these mice are viable but develop blindness, ataxia and progressive loss of retinal and cerebellar neurons. Based on the observation that Hq mice are sensitive to oxidative stress, it was hypothesized that AIF functions as a free radical scavenger [15,18]. This idea was challenged by findings in different cancer cell lines that a reduction (by knockout or knockdown) of the AIF expression level could lead to either an elevation or a suppression of the production of reactive oxygen species (ROS) [19,20]. More recently, using Hq mouse brain mitochondria it was shown that AIF does not directly regulate ROS production in the mitochondria [21]. Thus, the potential connection between AIF function and ROS generation/elimination remains elusive.

Upon treatment with certain apoptotic stimuli, AIF can be cleaved from its membrane anchor by proteases, generating a soluble 57 kDa AIF fragment. This fragment can be released from the IMS into the cytosol upon permeabilization of the OMM. AIF harbors two nuclear localization signals (NLS) and, once released into the cytosol, it translocates into the nucleus (Fig. 1). Here, it contributes to large-scale DNA fragmentation and chromatin condensation, although the precise mechanism(s) of the nuclear function of AIF are unknown [10]. Since nuclear translocation of AIF was not observed in cyclophilin A-depleted $(CypA)^{-/-}$ neurons exposed to hypoxia–ischemia, it was suggested that the translocation and DNA binding activities of AIF require CypA [22]. If AIF cannot enter the nucleus, it does not induce cell death. Hence, the loss of AIF from mitochondria *per se* is not sufficient to explain its function in the cell death process [23]. Finally, it is also clear that the AIF-mediated pathway is not necessarily involved in all forms of apoptosis, but appears to be an important cell death mechanism only in certain cell types. The significance of AIF in cell death signaling will be discussed in detail later in this review.

The AIF protein consists of two distinct domains, a redox active domain and another domain of importance for its DNA binding and apoptotic activities [18]. Recently, it was reported that a small fraction of full-length AIF is also localized on the cytosolic side of the OMM of brain mitochondria [24]. This fraction of AIF does not seem to require processing prior to its dissociation from the mitochondria. However, the vast majority of publications report that mitochondrial AIF has to be cleaved prior to being released into the cytosol. In this review, we will summarize current knowledge about the mechanisms of AIF processing required for its release from the mitochondria.

3. Enzymes involved in AIF processing

Several years ago it was shown that AIF and Endonuclease G are not released from permeabilized mitochondria simultaneously with cytochrome *c* and Smac/DIABLO, suggesting that, apart from OMM permeabilization, some additional signals are required for their appearance in the cytosol [25]. It was also reported that recombinant calpain-I could cleave and release AIF from Bid-permeabilized mitochondria [26]. In another study, detailed biochemical analysis revealed the presence of three forms of AIF (67, 62 and 57 kDa) in the mitochondria of apoptotic cells [10]. Thus, it appeared that AIF must be liberated from its membrane anchor in order to be released from the mitochondria. Subsequently, several studies were performed aiming to identify the enzyme(s) responsible for the release of AIF in cultured cells and *in vivo*, and it was shown that cysteine proteases, namely calpains and cathepsins, play a prominent role in AIF proteolysis. Importantly, these proteases were found to be able to truncate AIF in the same position, Gly102/Leu103 [27].

Calpains are a family of calcium-dependent, non-lysosomal cysteine proteases expressed ubiquitously in mammals and many other

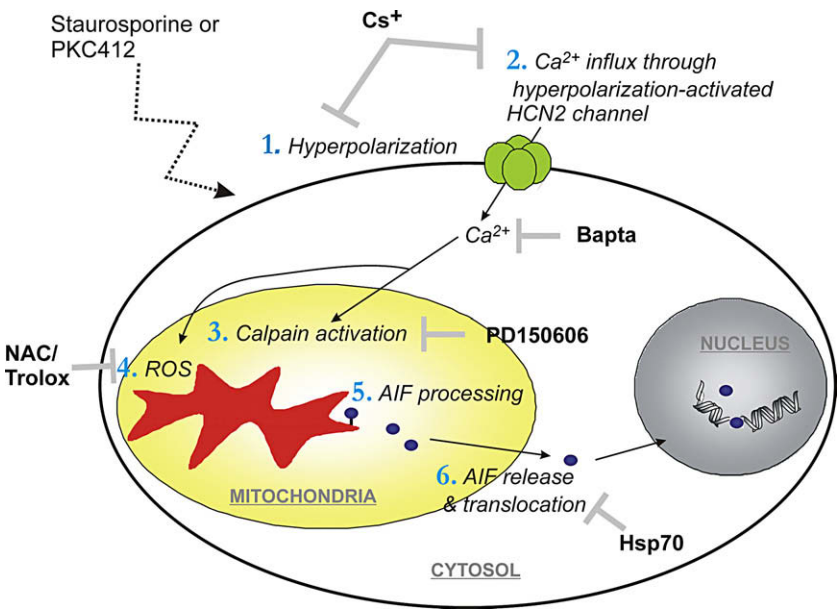

Fig. 1. Hypothetical model of the mechanisms involved in AIF processing and release. Exposure of NSCLC cells to the protein kinase C inhibitors, staurosporine or PKC412, results in a hyperpolarization of the plasma membrane. As a consequence, the hyperpolarization-activated HCN2 channel opens and permits Ca^{2+} to enter the cell. Both plasma membrane hyperpolarization and the activation of HCN2 channel are inhibited by Cs^{+}. The resulting Ca^{2+} elevation in the cytosol also translocates to the intermembrane space of the mitochondria and results in the activation of calpain as well as enhanced ROS formation. The calcium chelator, BAPTA is able to inhibit both calpain activation and ROS accumulation, whereas only the latter is inhibited by NAC and Trolox. AIF is cleaved by mitochondrial calpain-I. This cleavage is prevented by PD150606, a selective calpain inhibitor. Cleaved AIF is released into the cytosol and translocates to the nucleus, where it contributes to chromatin condensation and high-molecular weight DNA fragmentation. Nuclear translocation of AIF can be inhibited by binding to Hsp70 in the cytosol.

organisms. There are two main isoforms, calpain-I (μ-calpain) and calpain-II (m-calpain), which co-exist with the endogenous calpain-specific inhibitor, calpastatin. The interaction with calpastatin prevents both the activation and catalytic activity of calpains. Calpain I and II differ in the amount of Ca^{2+} (μM and mM, respectively), that they require to become activated. Upon activation, calpains can cleave and destroy their endogenous inhibitor, calpastatin [8]. Calpains are generally considered to be cytosolic enzymes, although calpain-I has been shown to be enriched in the mitochondrial fraction. More recently, calpain-I was found to contain a MLS and thus was suggested to be present also in the mitochondrial IMS [28]. Accordingly, we were able to detect calpain-I in a highly purified mitochondrial fraction and could further show that Ca^{2+} addition to the mitochondria was sufficient to stimulate AIF cleavage [29]. In fact, the majority of studies investigating the possible role of calpain in the AIF-mediated pathway have concluded that calpain-I is the most important enzyme involved in the processing of AIF [26,30–32]. In addition, siRNA downregulation of either one of the two calpains revealed that only deletion of calpain-I affected AIF truncation [32]. Moreover, calpain-I was shown to be critical for the release of AIF from mitochondria in neuronal cells exposed to oxygen–glucose deprivation *in vivo* [31]. Ca^{2+} is probably the most versatile cellular messenger that controls physiological processes, such as the release of neurotransmitters and muscle contraction. Physiological responses are normally governed by intracellular Ca^{2+} transients. In contrast, toxic perturbation of the intracellular Ca^{2+} homeostasis is often mediated either via an influx of extracellular Ca^{2+} into the cell, or by a redistribution of Ca^{2+} between different intracellular compartments, e.g. between the ER and the mitochondria. Toxic Ca^{2+} elevation is also more pronounced and sustained when compared to physiological Ca^{2+} signaling and lacks the oscillatory pattern typical of the latter. For example, activation of calpain-mediated AIF release from the mitochondria during retinal degeneration was reported to require a sustained elevation of the intracellular Ca^{2+} level [33]. Similarly, staurosporine or PKC412 treatment of NSCLC cell lines led to an early import of extracellular Ca^{2+} through the hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN2) (Fig. 1) (Norberg et al. unpublished observations). This resulted in a sustained (>10 min) elevation of the cytosolic Ca^{2+} level sufficient to cause calpain activation and subsequent release of AIF from mitochondria [29]. In addition to activating calpain, the imported Ca^{2+} was found to stimulate mitochondrial ROS production, which, in turn, led to oxidative modification of AIF. Importantly, calpain-I was observed to cleave oxidatively modified AIF more efficiently than non-modified AIF (Fig. 2) [34].

Cathepsins are lysosomal peptidases belonging to three classes of enzymes, namely, serine, aspartic acid and cysteine proteases. They are synthesized as inactive pro-enzymes, but are subsequently activated within the acidic environment of the lysosome and are finally released from the lysosomes as active enzymes. However, it is unclear how much of the enzymatic activity of the cathepsins is retained at physiological pH in the cytosol, since they are known to be stable only at pH-values below 6.0. Like for the calpains, there are endogenous cathepsin inhibitors, including cystatins and certain serpins. However, in contrast to the calpains, cathepsins are Ca^{2+}-independent enzymes [35].

Lysosomes are critically involved in autophagy but their role in apoptosis is still a matter of debate. Hence, it is unclear whether lysosomal permeabilization is an early step in the apoptotic process, or if it occurs as a consequence of caspase activation and contributes mainly to the amplification and acceleration of apoptotic cell demise [35]. Lysosomal permeabilization has been detected during both apoptosis and necrosis using fluorescence-based methods. Nevertheless, during the early apoptotic process the lysosomal structure seems to be kept intact when analyzed by transmission electron microscopy. Despite this, examples exist where cathepsins seem to be involved in the initiation of the apoptotic process. In particular, this appears to be the case with cells exposed to lysosomotropic agents, such as *O*-methylserine dodecylamide hydrochloride, detergent-like sphingosine, or Leu-Leu-Ome and the antibiotic ciprofloxacin [36]. Hence, current evidence for an involvement of lysosomes in the initiation of apoptosis originates from studies using compounds that directly disrupt the lysosomal integrity. Further, it appears that cathepsin-mediated AIF release would require an early lysosomal permeabilization to allow cathepsins to enter the mitochondria and cleave AIF. Only the cysteine cathepsins (cathepsin B, L and S) have been reported to be able to cleave AIF *in vitro* [27]. However, knockout of cathepsins B or L does not correlate with impaired cell death, as seen after deletion of caspases. Instead, cathepsins B and L deficiency leads to neurodegeneration within certain areas of the brain.

Thus, there is little evidence for the involvement of cathepsins in the cleavage and release of AIF *in vivo*. In contrast, most observations suggest that calpain-I is the critical enzyme regulating AIF processing in experimental systems in which the AIF-mediated pathway is important for cell death.

4. Other factors modulating the cleavage of AIF

Determination of the crystal structure of both the murine and the human AIF protein has revealed that the calpain/cathepsin cleavage site in AIF is not exposed on the surface of the protein, but rather embedded in its structure. Therefore, one possible limiting step in AIF processing might be the accessibility of the cleavage site to the protease. Indeed, it was proposed that redox-regulated conformational changes of the AIF structure, affecting its binding to pyridine nucleotides, might influence the ability of calpain to cleave AIF in isolated mitochondria [37]. However, using a similar approach we could not detect any protective effect of pyridine nucleotides on AIF processing in isolated mitochondria [34]. Hence, binding of pyridine nucleotides to AIF does not protect AIF from calpain-mediated cleavage in all experimental systems and therefore does not seem to be a general phenomenon.

Another possibility that might involve changes in the tertiary/quaternary conformation of AIF of importance for its processing and release from the mitochondria is oxidative modification(s) triggered by ROS (Fig. 2). Indeed, there are several observations linking ROS and AIF, although the precise mechanism(s) of such interaction are still unclear [15,18–20]. For instance, deletion of AIF can result in either an increase or a decrease in mitochondrial ROS production. It was proposed that AIF can function as a free radical scavenger and thereby prevent apoptosis [15]. This was based on the observation that Hq mice were more susceptible to hydrogen peroxide treatment than the wild-type mice. In contrast, it was reported that mitochondria from both wild-type and Hq mice have the same capability to scavenge ROS [21]. Another study demonstrated that glutathione peroxidase-4 can sense and translate oxidative stress into AIF-mediated cell death [38]. Manganese superoxide dismutase (MnSOD) was observed to protect from cell death in a mouse model of permanent focal cerebral ischemia. In this case, antioxidant-treated mice exhibited significantly less mitochondria/nuclear AIF translocation and large-scale DNA fragmentation [39]. Further, an AIF-mediated mechanism was shown to be critical for myocardial cell death upon ischemia–reperfusion. In this experimental model, Ca^{2+} and ROS signals were assigned a role as key mediators in regulating the release of AIF from mitochondria [40]. More recently, several studies have demonstrated that antioxidants can suppress AIF-mediated cell death in neurons as well as in NSCLC and other cancer cell lines [41–43]. In this scenario, ROS were able to positively regulate AIF cleavage and release. In line with these observations, we found that oxidative modification of AIF markedly (5-fold) increased its susceptibility

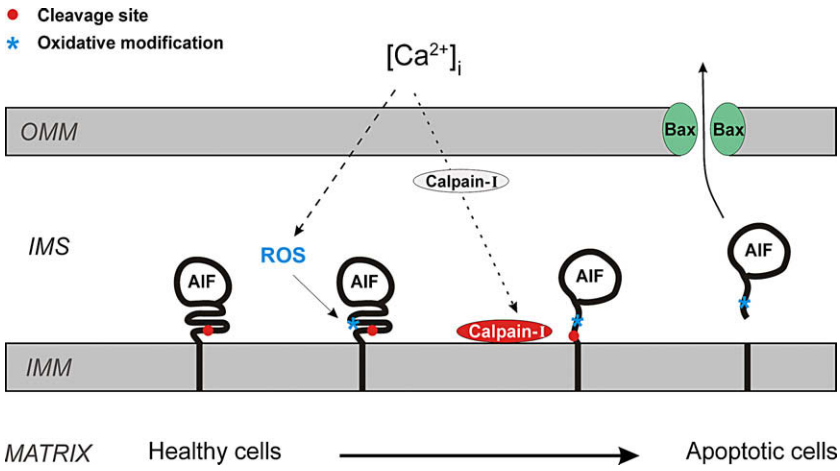

Fig. 2. AIF processing is sequentially regulated by Ca^{2+} and ROS. The import of Ca^{2+} from the extracellular milieu in staurosporine-treated cells leads to an elevation of the intracellular Ca^{2+} level for several minutes (>10 min). Subsequently, the mitochondria sequester part of the Ca^{2+} increase during the normalization phase. The Ca^{2+} elevation has at least two effects, first, a mitochondrial calpain located in the intermembrane space is activated by Ca^{2+} and second, Ca^{2+} uptake into mitochondria stimulates ROS production. This leads to oxidative modification (carbonylation) of AIF, which presumably is associated with increased accessibility of the calpain cleavage site to proteolytic activity. Hence, this conformational change makes AIF more susceptible to calpain-mediated processing. The soluble 57 kDa AIF is then released through Bax/Bak pores in the OMM.

to calpain-I-mediated processing (Fig. 2) [34]. Furthermore, time-lapse analysis revealed that in cells exposed to antimycin A prior to STS treatment, the release and nuclear translocation of AIF-GFP occurred more rapidly as compared to cells treated with STS alone. Accordingly, pretreatment of the cells with antioxidants prevented the STS-induced oxidative modification of AIF as well as its subsequent cleavage and release from the mitochondria into the cytosol. This led us to propose that oxidative modification might expose a normally hidden calpain cleavage site in AIF [34], in accordance with what has been reported for other calpain substrates (Fig. 2) [44–50].

5. Involvement of AIF in apoptosis execution

It is currently known that the AIF-mediated pathway is not important for all cell death mechanisms. Hence, the elimination of AIF does not protect from apoptosis induced by most drugs. Similarly, not all caspases are involved in the regulation of apoptosis, although overexpression of any one of the caspases culminates in cell death. However, this does not occur upon AIF overexpression. Further, knockout of any of the caspases results in an increase in cell number. In contrast, genetic inactivation of AIF in mice was embryonically lethal but, surprisingly, no excess in cell number was observed [17]. Therefore, in this respect AIF differs from other mediators of apoptosis. Instead, available evidence strongly suggests that AIF plays a critical role in cell death only in certain cell types, such as neurons and some tumor cells [22,29–31,51–53]. Furthermore, the type of apoptotic trigger also determines whether AIF will be important in the cell death process. Preferential AIF triggers should either directly perturb intracellular Ca^{2+} homeostasis, or lead to early lysosomal permeabilization, in order to make the AIF-mediated pathway an essential mechanism of subsequent apoptosis. However, both these events are frequent components of cell death signaling, particularly in ischemia–reperfusion injury or after treatment with cytotoxic drugs.

Another feature of significance for the role of AIF in cell death is the timing of its processing and release in relation to other pro-apoptotic events. Indeed, mitochondria contain several pro-apoptotic proteins that are being released from their intermembrane space during apoptotic signaling, including Smac/DIABLO, cytochrome *c* and AIF. These three proteins are of different size and are also located differently within the IMS. Smac/DIABLO is a soluble (25 kDa) protein, the bulk of cytochrome *c* (14 kDa) is bound to cardiolipin in the IMM by both electrostatic and hydrophobic interactions [4], whereas AIF (62 kDa) is anchored to the IMM with its transmembrane region. Thus, it is reasonable to assume that the small molecular-weight and soluble proteins are released prior to AIF upon OMM permeabilization, since AIF requires an additional cleavage step before its liberation. However, if AIF cleavage would precede OMM permeabilization, solubilized AIF could well be released early upon the loss of mitochondrial integrity. One example of this is seen in STS-treated lung carcinoma cells. In this model, Ca^{2+} is imported from the extracellular milieu and further translocates into the mitochondria, where it activates calpain-I to cleave AIF prior to its liberation from the IMM and release into the cytosol [29]. Importantly, all these events occurred before any caspase activation was detected. Similar observations were made in several other studies using NSCLC cells treated with STS, or with a specific protein kinase C inhibitor, PKC412 (Fig. 1) [29,51,54].

As mentioned above, there are also many studies indicating the importance of AIF in mediating neuronal cell death. Hence, microinjection of antibodies neutralizing AIF, or siRNA downregulation of AIF, have been found to suppress glutamate-, hypoxia- and NMDA-induced cell death in primary neurons [31,52]. Further, AIF knockdown in PC12 cells reduced the neurotoxic effects evoked by MPP+(1-methyl-4-phenylpyridinium) [55]. Moreover, DNA fragmentation caused by AIF was also seen in myocardial cell death in ischemia–reperfusion injury [40].

There are also several *in vivo* observations demonstrating the importance of the AIF-mediated pathway in neuronal cell death. For instance, as compared to wild-type mice, Hq mice were protected against NMDA- and kainic acid-induced neuronal damage in the hippocampus [53]. Cell death was also found to be suppressed in Hq mice subjected to hypoxia–ischemia [56]. Embryonic stem cells lacking AIF were less susceptible to serum withdrawal-induced apoptosis, as compared to wild-type ES cells [17]. Treatment of mice with a MnSOD mimetic (MnTBAP) prevented AIF translocation and DNA fragmentation upon ischemia and resulted in neuroprotective effects [39]. Neuroprotection was also observed in different mouse models, when AIF translocation was prevented by oral administration of HIV protease inhibitors [57]. Finally, it was shown that inhibition of the nuclear translocation of AIF was required to achieve neuroprotection in a rat model of retinal degeneration [58].

6. Conclusions

Like many other pro-apoptotic proteins, AIF was initially considered to be involved only in cell death signaling. However, gradually there has been accumulating evidence that AIF fulfills several other important physiological functions, both as a redox-active protein and in mitochondrial bioenergetics. The AIF-mediated death pathway is activated in a trigger-specific manner and seems to play a more prominent role in certain cell types. As also supported by current *in vivo* data, calpain-I appears to be the most important protease involved in the cleavage and subsequent release of AIF from its membrane anchor in the IMM. Thus, dysregulation of Ca^{2+} homeostasis might be an important prerequisite step for the activation of mitochondrial calpain and of the AIF-mediated pathway. The fact that numerous *in vitro* and *in vivo* studies have documented antioxidant inhibition of AIF-mediated cell death supports the notion that the intracellular ROS level positively regulates AIF cleavage and release. However, additional studies are needed to clarify the precise interaction between ROS and AIF-mediated cell death mechanisms. AIF plays a key role also in mediating cell death in several pathological conditions, such as ischemic injury, neurodegenerative disorders and certain types of cancers. Therefore, improved knowledge about the molecular mechanisms regulating the release of AIF from mitochondria should be beneficial for designing more specific drugs for future treatment of these diseases.

Acknowledgments

This work was supported by grants from the Swedish Research Council, the Swedish and the Stockholm Cancer Societies, the Swedish Childhood Cancer Foundation, the EC FP-6 (Oncodeath and Chemores) as well as the FP7 (Apo-Sys) programs. We apologize to authors whose primary references could not be cited due to space limitation.

References

[1] P. Li, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri, X. Wang, Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, Cell 91 (1997) 479–489.
[2] G. Kroemer, Mitochondrial implication in apoptosis. Towards an endosymbiont hypothesis of apoptosis evolution, Cell Death Differ. 4 (1997) 443–456.
[3] V. Gogvadze, J.D. Robertson, B. Zhivotovsky, S. Orrenius, Cytochrome *c* release occurs via Ca^{2+}dependent and Ca^{2+}independent mechanisms that are regulated by Bax, J. Biol. Chem. 276 (2001) 19066–19071.
[4] M. Ott, J.D. Robertson, V. Gogvadze, B. Zhivotovsky, S. Orrenius, Cytochrome *c* release from mitochondria proceeds by a two-step process, Proc. Natl. Acad. Sci. USA 99 (2002) 1259–1263.
[5] B. Zhivotovsky, S. Orrenius, O.T. Brustugun, S.O. Doskeland, Injected cytochrome *c* induces apoptosis, Nature 391 (1998) 449–450.
[6] R. Tehranchi, B. Fadeel, A.M. Forsblom, B. Christensson, J. Samuelsson, B. Zhivotovsky, E. Hellstrom-Lindberg, Granulocyte colony-stimulating factor inhibits spontaneous cytochrome *c* release and mitochondria-dependent apoptosis of myelodysplastic syndrome hematopoietic progenitors, Blood 101 (2003) 1080–1086.
[7] B. Joseph, P. Marchetti, P. Formstecher, G. Kroemer, R. Lewensohn, B. Zhivotovsky, Mitochondrial dysfunction is an essential step for killing of non-small cell lung carcinomas resistant to conventional treatment, Oncogene 21 (2002) 65–77.
[8] S. Orrenius, B. Zhivotovsky, P. Nicotera, Regulation of cell death: the calcium-apoptosis link, Nat. Rev. Mol. Cell Biol. 4 (2003) 552–565.
[9] S.A. Susin, H.K. Lorenzo, N. Zamzami, I. Marzo, B.E. Snow, G.M. Brothers, J. Mangion, E. Jacotot, P. Costantini, M. Loeffler, N. Larochette, D.R. Goodlett, R. Aebersold, D.P. Siderovski, J.M. Penninger, G. Kroemer, Molecular characterization of mitochondrial apoptosis-inducing factor, Nature 397 (1999) 441–446.
[10] H. Otera, S. Ohsakaya, Z. Nagaura, N. Ishihara, K. Mihara, Export of mitochondrial AIF in response to proapoptotic stimuli depends on processing at the intermembrane space, Embo J. 24 (2005) 1375–1386.
[11] M.D. Miramar, P. Costantini, L. Ravagnan, L.M. Saraiva, D. Haouzi, G. Brothers, J.M. Penninger, M.L. Peleato, G. Kroemer, S.A. Susin, NADH oxidase activity of mitochondrial apoptosis-inducing factor, J. Biol. Chem. 276 (2001) 16391–16398.
[12] M.J. Mate, M. Ortiz-Lombardia, B. Boitel, A. Haouz, D. Tello, S.A. Susin, J. Penninger, G. Kroemer, P.M. Alzari, The crystal structure of the mouse apoptosis-inducing factor AIF, Nat. Struct. Biol. 9 (2002) 442–446.
[13] H. Ye, C. Cande, N.C. Stephanou, S. Jiang, S. Gurbuxani, N. Larochette, E. Daugas, C. Garrido, G. Kroemer, H. Wu, DNA binding is required for the apoptogenic action of apoptosis inducing factor, Nat. Struct. Biol. 9 (2002) 680–684.
[14] N. Vahsen, C. Cande, J.J. Briere, P. Benit, N. Joza, N. Larochette, P.G. Mastroberardino, M.O. Pequignot, N. Casares, V. Lazar, O. Feraud, N. Debili, S. Wissing, S. Engelhardt, F. Madeo, M. Piacentini, J.M. Penninger, H. Schagger, P. Rustin, G. Kroemer, AIF deficiency compromises oxidative phosphorylation, Embo J. 23 (2004) 4679–4689.
[15] J.A. Klein, C.M. Longo-Guess, M.P. Rossmann, K.L. Seburn, R.E. Hurd, W.N. Frankel, R.T. Bronson, S.L. Ackerman, The harlequin mouse mutation downregulates apoptosis-inducing factor, Nature 419 (2002) 367–374.
[16] J.A. Pospisilik, C. Knauf, N. Joza, P. Benit, M. Orthofer, P.D. Cani, I. Ebersberger, T. Nakashima, R. Sarao, G. Neely, H. Esterbauer, A. Kozlov, C.R. Kahn, G. Kroemer, P. Rustin, R. Burcelin, J.M. Penninger, Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes, Cell 131 (2007) 476–491.
[17] N. Joza, S.A. Susin, E. Daugas, W.L. Stanford, S.K. Cho, C.Y. Li, T. Sasaki, A.J. Elia, H.Y. Cheng, L. Ravagnan, K.F. Ferri, N. Zamzami, A. Wakeham, R. Hakem, H. Yoshida, Y.Y. Kong, T.W. Mak, J.C. Zuniga-Pflucker, G. Kroemer, J.M. Penninger, Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death, Nature 410 (2001) 549–554.
[18] S.A. Lipton, E. Bossy-Wetzel, Dueling activities of AIF in cell death versus survival: DNA binding and redox activity, Cell 111 (2002) 147–150.
[19] A. Urbano, U. Lakshmanan, P.H. Choo, J.C. Kwan, P.Y. Ng, K. Guo, S. Dhakshinamoorthy, A. Porter, AIF suppresses chemical stress-induced apoptosis and maintains the transformed state of tumor cells, Embo J. 24 (2005) 2815–2826.
[20] N. Apostolova, A.M. Cervera, V.M. Victor, S. Cadenas, A. Sanjuan-Pla, A. Alvarez-Barrientos, J.V. Esplugues, K.J. McCreath, Loss of apoptosis-inducing factor leads to an increase in reactive oxygen species, and an impairment of respiration that can be reversed by antioxidants, Cell Death Differ. 13 (2006) 354–357.
[21] S.J. Chinta, A. Rane, N. Yadava, J.K. Andersen, D.G. Nicholls, B.M. Polster, Reactive oxygen species regulation by AIF- and complex I-depleted brain mitochondria, Free Radic. Biol. Med. 46 (2009) 939–947.
[22] C. Zhu, X. Wang, J. Deinum, Z. Huang, J. Gao, N. Modjtahedi, M.R. Neagu, M. Nilsson, P.S. Eriksson, H. Hagberg, J. Luban, G. Kroemer, K. Blomgren, Cyclophilin A participates in the nuclear translocation of apoptosis-inducing factor in neurons after cerebral hypoxia-ischemia, J. Exp. Med. 204 (2007) 1741–1748.
[23] E.C. Cheung, N. Joza, N.A. Steenaart, K.A. McClellan, M. Neuspiel, S. McNamara, J.G. MacLaurin, P. Rippstein, D.S. Park, G.C. Shore, H.M. McBride, J.M. Penninger, R.S. Slack, Dissociating the dual roles of apoptosis-inducing factor in maintaining mitochondrial structure and apoptosis, Embo J. 25 (2006) 4061–4073.
[24] S.W. Yu, Y. Wang, D.S. Frydenlund, O.P. Ottersen, V.L. Dawson, T.M. Dawson, Outer mitochondrial membrane localization of apoptosis-inducing factor: mechanistic implications for release, ASN Neurol. 1 (2009), doi:10.1042/AN20090046. pii: e00021.
[25] R.T. Uren, G. Dewson, C. Bonzon, T. Lithgow, D.D. Newmeyer, R.M. Kluck, Mitochondrial release of pro-apoptotic proteins: electrostatic interactions can hold cytochrome c but not Smac/DIABLO to mitochondrial membranes, J. Biol. Chem. 280 (2005) 2266–2274.
[26] B.M. Polster, G. Basanez, A. Etxebarria, J.M. Hardwick, D.G. Nicholls, Calpain I induces cleavage and release of apoptosis-inducing factor from isolated mitochondria, J. Biol. Chem. 280 (2005) 6447–6454.
[27] V.J. Yuste, R.S. Moubarak, C. Delettre, M. Bras, P. Sancho, N. Robert, J. d'Alayer, S.A. Susin, Cysteine protease inhibition prevents mitochondrial apoptosis-inducing factor (AIF) release, Cell Death Differ. 12 (2005) 1445–1448.
[28] R. Badugu, M. Garcia, V. Bondada, A. Joshi, J.W. Geddes, N terminus of calpain 1 is a mitochondrial targeting sequence, J. Biol. Chem. 283 (2008) 3409–3417.
[29] E. Norberg, V. Gogvadze, M. Ott, M. Horn, P. Uhlen, S. Orrenius, B. Zhivotovsky, An increase in intracellular Ca^{2+} is required for the activation of mitochondrial calpain to release AIF during cell death, Cell Death Differ. 15 (2008) 1857–1864.
[30] S. Wang, S. Wang, P. Shan, Z. Song, T. Dai, R. Wang, Z. Chi, Mu-calpain mediates hippocampal neuron death in rats after lithium-pilocarpine-induced status epilepticus, Brain Res. Bull. 76 (2008) 90–96.
[31] G. Cao, J. Xing, X. Xiao, A.K. Liou, Y. Gao, X.M. Yin, R.S. Clark, S.H. Graham, J. Chen, Critical role of calpain I in mitochondrial release of apoptosis-inducing factor in ischemic neuronal injury, J. Neurosci. 27 (2007) 9278–9293.
[32] L. Liu, D. Xing, W.R. Chen, Micro-calpain regulates caspase-dependent and apoptosis inducing factor-mediated caspase-independent apoptotic pathways in cisplatin-induced apoptosis, Int. J. Cancer. 125 (2009) 2757–2766.
[33] D. Sanges, A. Comitato, R. Tammaro, V. Marigo, Apoptosis in retinal degeneration involves cross-talk between apoptosis-inducing factor (AIF) and caspase-12 and is blocked by calpain inhibitors, Proc. Natl. Acad. Sci. USA 103 (2006) 17366–17371.
[34] E. Norberg, V. Gogvadze, H. Vakifahmetoglu, S. Orrenius, B. Zhivotovsky, Oxidative modification sensitizes mitochondrial apoptosis-inducing factor to calpain-mediated processing, Free Radic Biol Med. 48 (2010) 791–797.
[35] B. Turk, V. Turk, Lysosomes as "suicide bags" in cell death: myth or reality?, J Biol. Chem. 284 (2009) 21783–21787.
[36] K.F. Ferri, G. Kroemer, Organelle-specific initiation of cell death pathways, Nat. Cell Biol. 3 (2001) E255–263.

[37] I.Y. Churbanova, I.F. Sevrioukova, Redox-dependent changes in molecular properties of mitochondrial apoptosis-inducing factor, J. Biol. Chem. 283 (2008) 5622–5631.
[38] A. Seiler, M. Schneider, H. Forster, S. Roth, E.K. Wirth, C. Culmsee, N. Plesnila, E. Kremmer, O. Radmark, W. Wurst, G.W. Bornkamm, U. Schweizer, M. Conrad, Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death, Cell Metab 8 (2008) 237–248.
[39] B.I. Lee, P.H. Chan, G.W. Kim, Metalloporphyrin-based superoxide dismutase mimic attenuates the nuclear translocation of apoptosis-inducing factor and the subsequent DNA fragmentation after permanent focal cerebral ischemia in mice, Stroke 36 (2005) 2712–2717.
[40] G.T. Kim, Y.S. Chun, J.W. Park, M.S. Kim, Role of apoptosis-inducing factor in myocardial cell death by ischemia-reperfusion, Biochem. Biophys. Res. Commun. 309 (2003) 619–624.
[41] R. Zhang, I. Humphreys, R.P. Sahu, Y. Shi, S.K. Srivastava, In vitro and in vivo induction of apoptosis by capsaicin in pancreatic cancer cells is mediated through ROS generation and mitochondrial death pathway, Apoptosis 13 (2008) 1465–1478.
[42] M. Zhou, M. Baudry, EUK-207, a superoxide dismutase/catalase mimetic, is neuroprotective against oxygen/glucose deprivation-induced neuronal death in cultured hippocampal slices, Brain Res. 1247 (2009) 28–37.
[43] T. Chen, Y.S. Wong, Selenocystine induces caspase-independent apoptosis in MCF-7 human breast carcinoma cells with involvement of p53 phosphorylation and reactive oxygen species generation, Int. J. Biochem. Cell Biol. 41 (2009) 666–676.
[44] N. Zolotarjova, C. Ho, R.L. Mellgren, A. Askari, W.H. Huang, Different sensitivities of native and oxidized forms of Na^+/K(+)-ATPase to intracellular proteinases, Biochim. Biophys. Acta 1192 (1994) 125–131.
[45] M.A. Smith, M. Rudnicka-Nawrot, P.L. Richey, D. Praprotnik, P. Mulvihill, C.A. Miller, L.M. Sayre, G. Perry, Carbonyl-related posttranslational modification of neurofilament protein in the neurofibrillary pathology of Alzheimer's disease, J. Neurochem. 64 (1995) 2660–2666.
[46] S.M. Smerjac, O.A. Bizzozero, Cytoskeletal protein carbonylation and degradation in experimental autoimmune encephalomyelitis, J. Neurochem. 105 (2008) 763–772.
[47] R. Kowara, Q. Chen, M. Milliken, B. Chakravarthy, Calpain-mediated truncation of dihydropyrimidinase-like 3 protein (DPYSL3) in response to NMDA and H_2O_2 toxicity, J. Neurochem. 95 (2005) 466–474.
[48] P. Gulati, B. Markova, M. Gottlicher, F.D. Bohmer, P.A. Herrlich, UVA inactivates protein tyrosine phosphatases by calpain-mediated degradation, EMBO Rep. 5 (2004) 812–817.
[49] S. Gelinas, C. Chapados, M. Beauregard, I. Gosselin, M.G. Martinoli, Effect of oxidative stress on stability and structure of neurofilament proteins, Biochem. Cell Biol. 78 (2000) 667–674.
[50] J.C. Troncoso, A.C. Costello, J.H. Kim, G.V. Johnson, Metal-catalyzed oxidation of bovine neurofilaments in vitro, Free Radic. Biol. Med. 18 (1995) 891–899.
[51] M.A. Gallego, B. Joseph, T.H. Hemstrom, S. Tamiji, L. Mortier, G. Kroemer, P. Formstecher, B. Zhivotovsky, P. Marchetti, Apoptosis-inducing factor determines the chemoresistance of non-small-cell lung carcinomas, Oncogene 23 (2004) 6282–6291.
[52] H. Wang, S.W. Yu, D.W. Koh, J. Lew, C. Coombs, W. Bowers, H.J. Federoff, G.G. Poirier, T.M. Dawson, V.L. Dawson, Apoptosis-inducing factor substitutes for caspase executioners in NMDA-triggered excitotoxic neuronal death, J. Neurosci. 24 (2004) 10963–10973.
[53] E.C. Cheung, L. Melanson-Drapeau, S.P. Cregan, J.L. Vanderluit, K.L. Ferguson, W.C. McIntosh, D.S. Park, S.A. Bennett, R.S. Slack, Apoptosis-inducing factor is a key factor in neuronal cell death propagated by BAX-dependent and BAX-independent mechanisms, J. Neurosci. 25 (2005) 1324–1334.
[54] T.H. Hemstrom, B. Joseph, G. Schulte, R. Lewensohn, B. Zhivotovsky, PKC 412 sensitizes U1810 non-small cell lung cancer cells to DNA damage, Exp. Cell Res. 305 (2005) 200–213.
[55] A.K. Liou, Z. Zhou, W. Pei, T.M. Lim, X.M. Yin, J. Chen, BimEL up-regulation potentiates AIF translocation and cell death in response to MPTP, Faseb J. 19 (2005) 1350–1352.
[56] C. Zhu, X. Wang, Z. Huang, L. Qiu, F. Xu, N. Vahsen, M. Nilsson, P.S. Eriksson, H. Hagberg, C. Culmsee, N. Plesnila, G. Kroemer, K. Blomgren, Apoptosis-inducing factor is a major contributor to neuronal loss induced by neonatal cerebral hypoxia–ischemia, Cell Death Differ. 14 (2007) 775–784.
[57] T. Hisatomi, T. Nakazawa, K. Noda, L. Almulki, S. Miyahara, S. Nakao, Y. Ito, H. She, R. Kohno, N. Michaud, T. Ishibashi, A. Hafezi-Moghadam, A.D. Badley, G. Kroemer, J.W. Miller, HIV protease inhibitors provide neuroprotection through inhibition of mitochondrial apoptosis in mice, J. Clin. Invest. 118 (2008) 2025–2038.
[58] Y. Murakami, Y. Ikeda, Y. Yonemitsu, M. Onimaru, K. Nakagawa, R. Kohno, M. Miyazaki, T. Hisatomi, M. Nakamura, T. Yabe, M. Hasegawa, T. Ishibashi, K. Sueishi, Inhibition of nuclear translocation of apoptosis-inducing factor is an essential mechanism of the neuroprotective activity of pigment epithelium-derived factor in a rat model of retinal degeneration, Am. J. Pathol. 173 (2008) 1326–1338.