Cell Death and Differentiation (2001) 8, 875–877

www.nature.com/cdd

News and Commentary

Conformational change of Bax: a question of life or death

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Bax is a pro-apoptotic member of the Bcl-2 family of proteins. It contains the three Bcl-2 homology domains BH1, BH2 and BH3 that are involved in protein–protein interaction. In healthy cells Bax is 'dormant', located in the cytosol or loosely associated to mitochondria. Upon induction of apoptosis, the protein was shown to translocate to mitochondria, insert into the outer membrane, oligomerize and trigger the release of cytochrome *c*, Smac/Diablo and other proteins from the intermembrane space. These events require various conformational changes of the protein: why? The recent resolution of the 3D structure of full length monomeric Bax by Suzuki *et al*[1] has brought a new insight into the requirement for structural modifications, and the mechanism through which these may occur.

The structure of Bax consists of eight amphipathic helices clustered around helix α5, that constitute the hydrophobic core of the protein. The overall folding of Bax is similar to that of Bcl-x_L[2] and Bid,[3,4] two other members of the Bcl-2 family whose structure had previously been resolved. Like these two proteins, Bax displays a structure reminiscent of the structure of the transmembrane domain of the bacterial toxins, diphteria toxin and colicins, which act as channels for ions and peptides. Whether Bax, Bcl-x_L and Bid form such channels into the mitochondrial membrane is an interesting possibility but this has not yet been demonstrated.

Interestingly, two important domains of Bax, the putative transmembrane domain (helix α9) and the BH3 domain, are masked inside the hydrophobic core of the protein. This is the conformation adopted by Bax in its inactive cytosolic form. Bax is the first protein from the Bcl-2 family for which the structure of the transmembrane domain has been resolved. Bcl-x_L was crystallised without helix α9,[2] and Bid does not display a transmembrane domain. The hydrophobic residues of this amphipathic helix are orientated towards the hydrophobic core of the Bax protein, while residues containing hydroxyl groups are solvent exposed. Such conformation would contribute to the solubility of the protein in the cytosol and prevent its membrane insertion in non-apoptotic cells. In order to insert into the mitochondrial membrane, helix α9 would have to disengage from the hydrophobic pocket. What triggers the exposure of the Bax C-terminus and its translocation to mitochondria? After engagement of the TNF/Fas receptors, the pro-apoptotic BH3-only protein Bid is cleaved by caspase-8, resulting in the translocation to the mitochondria of the truncated form of the protein, tBid.[5] *In vitro*, Bid induces a conformational change in Bax leading to the exposure of the N-terminal residues 1–21. This is followed by insertion of the Bax protein in the outer mitochondrial membrane.[6,7] Bid may interact directly with Bax since a mutant in the BH3 domain of Bid with a reduced affinity for Bax failed to trigger Bax insertion. As a consequence of the conformational change at the N-terminus, the dislocation of helix α9 from the hydrophobic core of the protein may occur. Indeed, mutational analysis suggested an interaction between the C-terminus and the first 20 N-terminal amino acids of Bax.[8] However, determination of the structure revealed that the N- and C-termini are not in close proximity.[1] Since the 12 N-terminal residues do not adopt an ordered conformation, one may suppose that the N-terminus of the recombinant Bax protein used in the NMR studies presented by Suzuki *et al*. is not folded as it normally is in the native protein. The explanation for this may reside in the purification strategy used in these studies: the Bax N-terminus was fused directly to the chitin binding protein that was then cleaved off.[1] Such a construct may have artificially constrained the N-terminus in a conformation that prevents its interaction with the C-terminal domain of Bax. According to Suzuki *et al*., disengagement of helix α9 from the hydrophobic pocket of Bax is a process that requires energy.[1] Therefore, Bid may cooperate with another protein.

Beside helix α9, another domain of Bax essential for its pro-apoptotic activity, namely the BH3 domain, is masked in the hydrophobic core of the protein. This domain is required for the formation of Bax/Bax homodimers and Bax/Bcl-2 heterodimers.[9,10] Based on binding affinities studies and on the structure of a complex between Bcl-x_L and a peptide derived from the BH3 domain of Bak,[11] the hydrophobic side chains of the Bak-BH3 peptide (Leu^{59}, Leu^{63}, Ile^{66}, Leu^{70}) were found to be essential for stabilization of the Bcl-x_L-Bak-BH3 peptide complex. In the case of Bax, this domain is located in helix α2 and is orientated such that the side chains of the hydrophobic residues mentioned above face the hydrophobic core of the protein. Therefore, they are not available for interactions and must be exposed following either the rotation of helix α2 or the dislocation of helix α2 or helix α9 from the hydrophobic core. In either case, this would require important rearrangements in the molecule. Bid might be responsible for this conformational change of Bax as, in addition to inducing Bax insertion in the outer mitochondrial membrane, it has been shown to trigger its oligomerization *in vitro*.[7] Since Bax oligomerization occurs via its BH3 domain,[9,10] this domain must be exposed following the interaction with Bid (Figure 1). This may occur subsequently to the dislocation of helix α9 from the hydrophobic core. According to this mechanism, Bid-induced N-terminal conformational change of Bax would trigger the dislocation

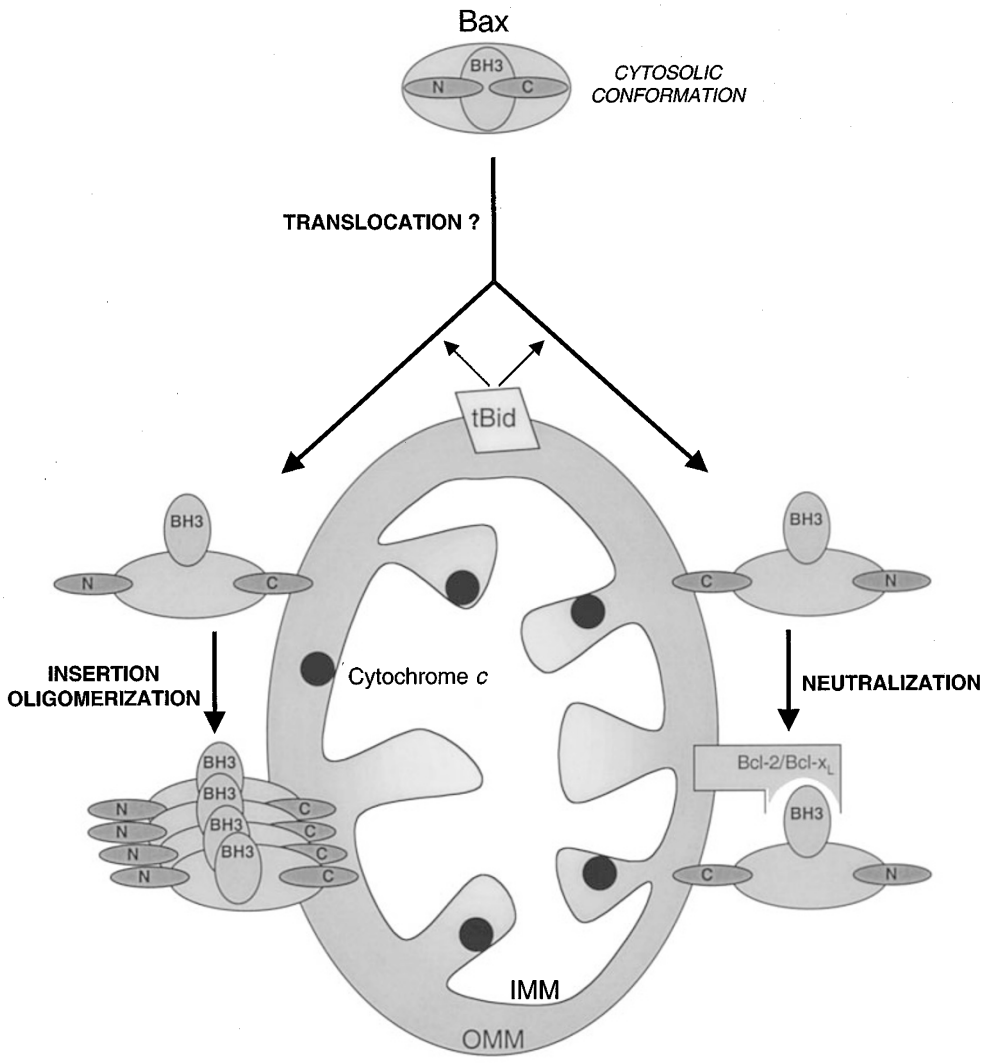

Figure 1 Elucidation of Bax structure revealed that the mitochondrial-targeting domain (C-terminus, helix α9) and the BH3 domain (located in helix α2) are hidden in a hydrophobic pocket.[1] In healthy cells, the protein is maintained in a monomeric 'cytosolic conformation'. Upon induction of apoptosis, Bax translocates to mitochondria by a mechanism that is still undefined. Caspase-8 cleaved Bid (truncated Bid to tBid) induces the exposure of the N- and C-termini of Bax, followed by unmasking of its BH3 domain. Then, Bax inserts via its C-terminus and oligomerizes in the outer mitochondrial membrane (OMM). On the contrary, Bcl-2 or Bcl-x_L prevents Bax insertion and oligomerization of Bax in the OMM, thus neutralizing the protein. Interestingly, Bax may also be neutralized by the adenovirus E1B 19K gene product[21] (not mentioned in the figure). IMM, inner mitochondrial membrane

of helix α9 and exposure of the BH3 domain of the protein. A Bid-independent mechanism for Bax insertion in the mitochondrial membrane was described in Bid$^{-/-}$ cells.[12] Other BH3-only proteins may therefore be able to induce Bax translocation and oligomerization in the mitochondrial outer membrane. *In vitro*, only Bax oligomers are able to trigger the release of cytochrome *c*;[13,14] therefore, Bax oligomerization represents a key step in the release of mitochondrial apoptogenic factors. Interestingly, such oligomers were also detected in mitochondria isolated from cells undergoing apoptosis but not in healthy cells.[15]

In our opinion, at least three important issues remain to be elucidated. The first one concerns Bax translocation to mitochondria (Figure 1). An increase in intracellular pH occurring in the cytosol of cells undergoing apoptosis has been suggested to induce a structural modification of cytosolic Bax followed by its translocation to the mitochondria.[16] However, Suzuki *et al*. showed that the structure of Bax does not vary at pH values ranging from six to eight.[1] One may argue that the structure of recombinant Bax used for these NMR studies is different from the structure of native Bax. Alternatively, the effect of pH on the structure of Bax may be indirect and mediated by a cytosolic factor. This factor would maintain Bax in a cytosolic conformation and prevent its translocation. The second issue concerns the specific targeting to mitochondria. The COOH-terminal domain is essential for Bax translocation to mitochondria.[8,17] However, this domain does not contain a usual mitochondrial targeting signal, and whether or not a mitochondrial receptor of Bax is present in the outer

membrane is still a matter of debate. Mitochondrial Bcl-2 and Bcl-x_L have been shown not to act as membrane receptors for Bax.[17] On the other hand, tBid may be a mitochondrial receptor for Bax. The last issue concerns the mechanism by which Bax triggers the release of cytochrome *c*. Amongst the different mechanisms proposed in the literature,[18] some studies suggest that Bax oligomers form cytochrome *c* channels across the outer mitochondrial membrane.[19] However, the existence of such pores remains to be demonstrated, and the search for the Bax channel still goes on.

In conclusion, elucidation of the structure of monomeric soluble Bax by NMR has allowed a better understanding of the conformational changes required for the translocation of the protein to mitochondria, its insertion and oligomerization. However, much remains to be understood, in particular how Bax triggers the release of apoptogenic factors from mitochondria. Since many data suggest that only Bax oligomers are capable of inducing the permeability of the mitochondria outer membrane,[13,15,20] the next challenge will be to obtain the structure of Bax oligomers.

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