FEBS Letters 586 (2012) 116–121

journal homepage: www.FEBSLetters.org

The cytosolic domain of human Tom22 modulates human Bax mitochondrial translocation and conformation in yeast

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ARTICLE INFO

Article history:
Received 12 October 2011
Revised 15 November 2011
Accepted 1 December 2011
Available online 17 December 2011

Edited by Vladimir Skulachev

Keywords:
Apoptosis
Bax translocation
TOM
Yeast mitochondria

ABSTRACT

The role of the mitochondrial protein receptor Tom22p in the interaction of pro-apoptotic protein Bax with yeast mitochondria was investigated. Co-immunoprecipitation assays showed that human Bax interacted with different TOM subunits, including Tom22p. Expression of the cytosolic receptor domain of human Tom22 increased Bax mitochondrial localization, but decreased the proportion of active Bax. BN–PAGE showed that the cytosolic domain of Tom22 interfered with the oligomerization of Bax. These data suggest that the interaction with the cytosolic domain of Tom22 helps Bax to acquire a conformation able to interact with the outer mitochondrial membrane.

Structured summary of protein interactions:
BAX and **BAX** physically interact with **TOM22** by anti bait coimmunoprecipitation (View interaction)
BAX physically interacts with **TOM70** by anti bait coimmunoprecipitation (View interaction)
BAX physically interacts with **TOM22**, **TOM70** and **TOM40** by anti bait coimmunoprecipitation (View interaction)
BAX physically interacts with **TOM22** by anti bait coimmunoprecipitation (View interaction)
BAX and **BAX** bind by blue native page (View interaction)
BAX physically interacts with **TOM40** by anti bait coimmunoprecipitation (View interaction)

1. Introduction

Apoptosis, the main form of programmed cell death, is controlled by proteins of the Bcl-2 family [1]. Among them, the pro-apoptotic protein Bax promotes the permeabilization of the mitochondrial outer membrane to apoptogenic factors such as cytochrome c, smac/diablo, or apoptosis inducing factor [2]. The translocation of Bax from the cytosol to mitochondria is a crucial early step of apoptosis, and is associated to conformational changes from a soluble conformation to a membrane-inserted conformation, by exposing more hydrophobic domains of the protein [3,4].

The involvement of outer mitochondrial membrane proteins in the different steps leading to Bax translocation, insertion and activation is still debated [5]. Antibodies directed against Tom22 prevent Bax membrane insertion on isolated mammalian mitochondria and the down-regulation of Tom22 inhibits Bax-dependent apoptosis in human glioblastoma cells [6,7]. These effects are selective for Tom22 since the modulation of Tom20 does not have any effect on Bax translocation [6]. When expressed in yeast, human Bax is less efficiently localized to mitochondria in a strain containing less Tom22p [6] and the consequences on yeast growth are attenuated [8]. These data were challenged by another report [9] that used recombinant N-terminal hexahistidine-tagged Bax added to isolated mitochondria. Since movements of the N-terminal end of Bax are crucial for its conformational changes [10,11], this tagged Bax might not accurately recapitulate the steps underlying Bax translocation. Furthermore, as shown in the original paper describing the production of this recombinant protein [12], it is contaminated by a truncated form of Bax called p18, that promotes the membrane addressing of full-length Bax [13] and does not therefore reflect the behavior of full-length Bax alone. Two other reports that

Abbreviations: ART, apoptotic regulation of targeting; BH, Bcl-2 homology; BN/SDS–PAGE, blue native/sodium dodecyl sulfate polyacrylamide gel electrophoresis; PSC, peptide sensitive channel; TOM, translocase of outer membrane

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doi:10.1016/j.febslet.2011.12.003

challenged the role of Tom22p in Bax effects in yeast were based on the utilization of a mutant carrying a mutation of S184 [14,15], but the deletion of S184 in helix α9 had been shown to change this helix into a constitutive sequence of insertion into the outer mitochondrial membrane [16] and the resulting Bax mutant did not require anymore the presence of Tom22p for mitochondrial translocation [6].

In the present study, we report that, like in mammalian cells, human Bax and yeast Tom22 physically interact. Furthermore, the modulation of this interaction by a competing peptide corresponding to the cytosolic receptor domain of human Tom22, modulated not only the translocation, but also the conformation and the oligomeric status of Bax in the outer mitochondrial membrane.

2. Methods

2.1. Yeast strains and plasmids

The haploid wild-type strain W303-1A (*matα, ade1, his3, leu2, trp1, ura3*) was transformed with the pYES3 plasmid carrying a reprogrammed version of human Bax cDNA (optimized for high expression in yeast) under the control of the GAL1/10 promoter. The human Bax protein produced by this construction is full-length (192 residues) and untagged. Mutations of Proline residues at positions 8, 13, and 168 have been described previously [17]. The pYES2 plasmid carrying a truncated version of human Tom22 (residues 1-82; hTom22(1-82)) under the control of the GAL1/10 promoter has been constructed by amplifying the corresponding fragment from a human cDNA library. Cells were grown in Yeast Nitrogen Base medium supplemented with DL-Lactate as a carbon source to obtain fully differentiated mitochondria. Bax-expression and hTom22(1-82) expression was induced by adding 0.5% galactose to the cultures.

2.2. Bax and cytochrome c contents of yeast mitochondria

Mitochondria were isolated from cells grown overnight (14 h). Proteins from isolated mitochondria (50 μg proteins) were precipitated with 0.3 M TCA, washed with acetone, and solubilized in 2% SDS before to be separated on SDS–PAGE, blotted and analyzed with an anti-Bax antibody (N20, Santa-Cruz). Cytochromes content was measured by redox spectrophotometry. Briefly, mitochondria (3 mg proteins) were suspended in the two cuvettes of a double-beam spectrophotometer (Varian Cary 4000). The reference and sample cuvettes were oxidized and reduced with potassium ferricyanide and sodium dithionite, respectively. Difference spectra were acquired between 500 and 650 nm and cytochromes c+c1, cytochrome b and cytochromes a+a3 were quantified from the O.D. differences between 550 *minus* 540, 561 *minus* 575, and 603 *minus* 630 nm, respectively.

2.3. Co-immunoprecipitation assays

Yeast cells were grown in 20 ml cultures and washed in 10 mM tris/maleate buffer (pH 6.8) containing 0.6 M mannitol, 2 mM EGTA, antiproteases (Complete™, Roche) and antiphosphatases (1 mM sodium vanadate, 10 mM sodium fluoride, and PhosStop™, Roche). Cells were resuspended in the same buffer and were broken by vortexing with glass beads for 3 min in a FastPrep (MP Biochemicals). Unbroken cells and nuclei were eliminated by a 10-min 900×*g* centrifugation. Supernatants were added with 1/9 volume of 10× IP buffer (Sigma) and gently shaken at 4 °C for 30 min. Immunoprecipitations were done overnight with 2 μg of mouse monoclonal anti-Bax antibodies (2D2 or 6A7, Sigma). 50 μl Protein G-agarose beads (Sigma) were added and incubated for 4 h. After extensive washing with 5 × 0.5 ml of IP buffer 1× and 2 × 0.5 ml of IP buffer 0.1×, proteins were eluted with 30–50 μl 0.2 M glycine, pH 2.8. Protein samples were solubilized in Laemmli buffer without β-mercaptoethanol.

Western blottings were done with the following antibodies: Bax (rabbit polyclonal N20, Santa-Cruz, 1/5000), yeast Tom22 (custom rabbit polyclonal, Millegen, 1/2000), yeast Tom20, Tom40 and Tom70 (rabbit polyclonal, gifts from Dr Carla Koehler, 1/5000), yeast Porin (mouse monoclonal, Invitrogen, 1/20000), yeast phosphoglycerate kinase (mouse monoclonal, Invitrogen, 1/10000), yeast cytochrome c oxidase subunit II (mouse monoclonal, Invitrogen, 1/10000). Immunoblots were revealed with Western Lightning *plus* (Perkin–Elmer) and quantified with a G-Box imaging system (Syngene).

2.4. Mammalian cells

Cultures of FL5.12 cells, induction of apoptosis and co-immunoprecipitation assays were done as described in [18].

2.5. BN–PAGE analyzes

Mitochondria were suspended at 1 mg/ml in 50 mM imidazole/HCl buffer (pH 7.2) containing 50 mM NaCl and 5 mM ε-aminocaproic acid. Digitonin-solubilization was done for 20 min on ice at detergent/protein ratio of 0.3 (w/w). Non-solubilized material was removed by centrifugation at 21000×*g* for 20 min. The supernatant was supplemented with Coomassie dye solution (5% Serva Blue G in 750 mM ε-aminocaproic acid) at a dye/protein ratio of 0.2 (w/w) and separated on a linear gradient (5–14%) polyacrylamide slab gel. Molecular weight markers were treated in a similar manner before loading on the gel. Second dimension electrophoresis was done on each individual lane from the BN–PAGE. Lanes were excised and layered on the top of a 12.5% SDS–PAGE for the second dimension. Western-Blotting were done with polyclonal anti-Bax (N20, Santa-Cruz, 1/1000).

Electrophysiological studies were done on proteoliposomes containing the outer mitochondrial isolated from a *Δpor1* strain expressing or not C-terminal c-myc tagged human Bax as described in [19].

3. Results

3.1. Mitochondrial human Bax physically interacts with both yeast and mammalian Tom22p

Although the sequence conservation between the products of yeast and human *TOM22* is weak (Fig. 1A), human Tom22 is able to complement the absence of yeast Tom22p suggesting that the interaction between Tom22 and mitochondria-addressed proteins involves a general conformation recognition process [20]. When expressed in yeast, wild-type human Bax (without any tag) largely resides in the cytosol, like in non-apoptotic mammalian cells, and the translocation of Bax to mitochondria is stimulated by introducing point mutations at critical positions that favor the activating conformational change [17]. One of these constitutively mitochondrial and active mutants both in yeast and human cells is BaxP168A [17,21]. This mutation increases the mobility of the C-terminal hydrophobic α9 helix, allowing its movement away from the hydrophobic groove formed by the BH domains.

We found yeast Tom22p, Tom40p and Tom70p in BaxP168A-immunoprecipitates (Fig. 1B), suggesting that active Bax was associated to the TOM complex. Additional evidence for the interaction between Bax and yeast Tom22 was obtained by electrophysiology of the PSC, that reflects the activity of TOM: the cation selectivity

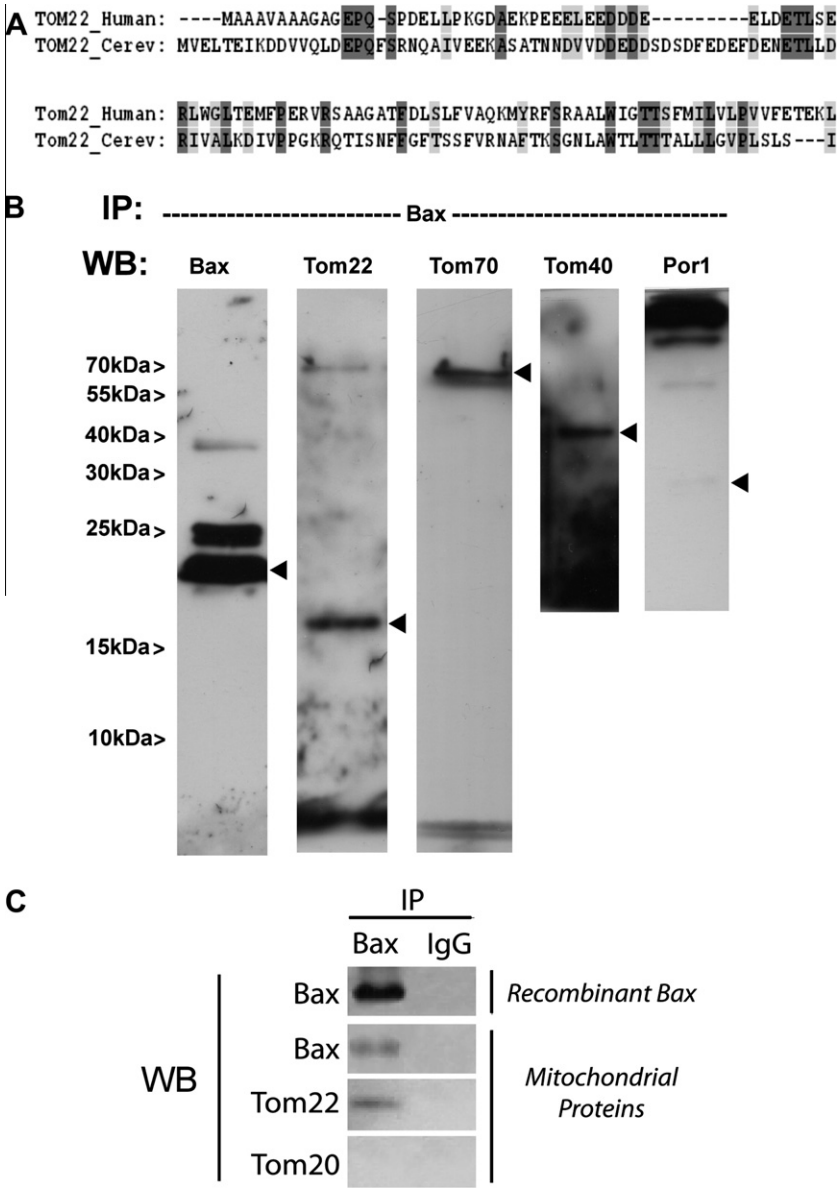

Fig. 1. Interaction between human Bax and TOM components. (A) Sequence alignment of human and yeast Tom22. Dark grey: identical residues; light grey: similar residues. (B) Co-immunoprecipitation of yeast mitochondrial proteins with the constitutively active BaxP168A mutant was done as indicated in the methods section. The intense band at the top of the Por1p lane corresponds to the size of the mouse IgG. A faint band can be detected at 31 kDa, the expected size of Por1p. Bax is detected at 21 kDa with some minor bands above, Tom22 at 18 kDa, and Tom70 and Tom40 at 70 and 40 kDa, respectively. (C) Co-immunoprecipitation of Tom22 but not Tom20 by Bax in apoptotic FL5.12 cells.

(P_K/P_{Cl}) was increased from 4.5 ± 0.9 to 7.5 ± 1.1 (S.D.; n = 17) in the presence of Bax, further supporting the existence of a lingering interaction between Bax and TOM. In parallel, immunoprecipitation of wild-type Bax in pro-lymphocytic FL5.12 cells submitted to apoptosis by IL-3 removal revealed that Bax co-immunoprecipitated with Tom22 but not with Tom20 (Fig. 1C). This result was in accordance with previous reports in glioblastoma cells, where Tom22p but not Tom20p was required for Bax translocation [6].

3.2. The cytosolic part of human Tom22 interferes with Bax conformational changes

Since the full inactivation of *TOM22* gene dramatically decreases yeast growth, it was not possible to use a null strain to investigate further the role of Tom22p on Bax interaction with mitochondria. Instead, we set up a paradigm based upon the competition of Bax binding to Tom22p by a peptide corresponding to the cytosolic part of human Tom22 (82 first residues [22,23]) and lacking the transmembrane domain of the protein. We reasoned that the interaction of Bax with this peptide would prevent Bax interaction with Tom22p and thus impair Bax interaction with mitochondria.

The peptide was co-expressed in yeast with three Bax variants: Bax α, the wild-type protein that is inactive and poorly localized to mitochondria, the double mutant BaxP8G/P13G, that is more active both on mammalian and yeast mitochondria because of the higher flexibility of ART [11,17], and the single mutant BaxP168A that is strongly active both on mammalian and yeast mitochondria because of the higher flexibility of the turn between α8 and α9 helices [17,21].

Unexpectedly, the co-expression of hTom22(1-82) with the three Bax variants increased their mitochondrial localization (Fig. 2A and B). We further determine if Bax was active by measuring the

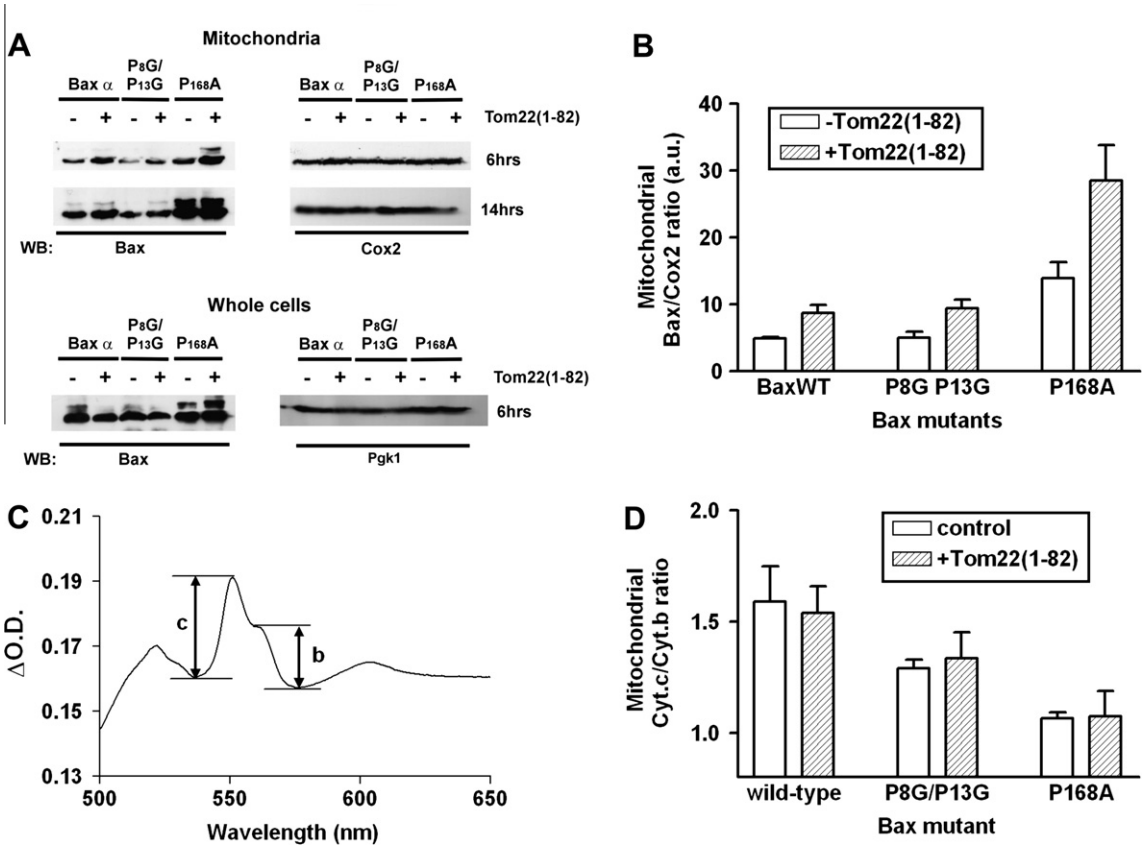

Fig. 2. Co-expression of hTom22(1-82) increases Bax mitochondrial localization. Cells were co-transformed with the pYES3 vector carrying wild-type or mutants of human Bax and the pYES2 vector carrying or not a gene encoding hTom22(1-82). Mitochondria were isolated from cells as indicated in the methods section. (A) Mitochondrial Bax amount was estimated by western-blotting, with subunit II of cytochrome c oxidase (cox2) and Phosphoglycerate kinase (pgk1) serving as controls for mitochondrial and cytosolic proteins, respectively. (B) Quantification of western-blots. Results are average of 3 or 4 independent experiments ± S.E.M. Note that the mitochondrial localization of Bax wild-type may vary from experiment to experiment: in this series of experiments, it is relatively high (as compared to, for instance, data from [17]. (C) The amounts of cytochromes c and b in isolated mitochondria were measured by redox difference spectrophotometry. (D) Quantification of cyt.c/cyt.b ratios. Values are average (±S.E.M.) of 4 independent experiments.

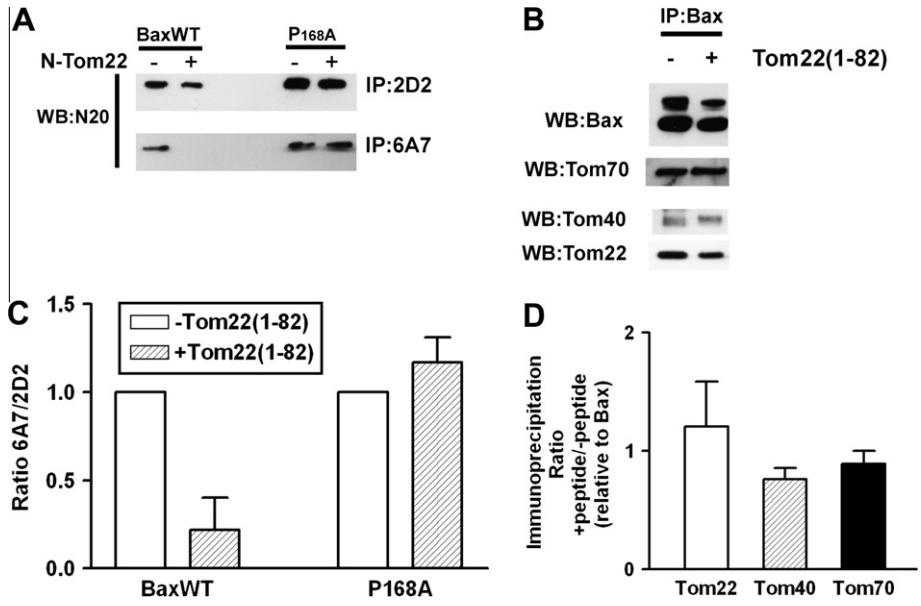

Fig. 3. hTom22(1-82) modulates Bax conformation. (A) Cells were co-expressing wild-type Bax or the mutant P168A and hTom22(1-82). Bax was immunoprecipitated from cell extracts with 2D2 or 6A7 monoclonal antibodies. Western-blots were revealed with the polyclonal N20 antibody. (B) Quantification of western-blots. Ratios of the signal obtained with 6A7 and 2D2 were calculated and the values obtained in the absence of Tom22(1-82) peptide were fixed at 1. Values are averages of three independent experiments (±S.E.M.). (C) Cell extracts from cells co-expressing BaxP168A and Tom22(1-82) were immunoprecipitated with anti-Bax antibody (2D2) and western-blots were revealed with antibodies against TOM subunits. (D) Quantification of (C). Values are average ± S.E.M. of 3 independent experiments.

remaining cytochrome c content of isolated mitochondria (Fig. 2C): cytochrome c release was not stimulated by the presence of hTom22(1-82) (Fig. 2D), showing that the additional amount of mitochondrial Bax was not active. This suggested that, in the presence of hTom22(1-82), inactive wild-type Bax could be addressed to mitochondria. This hypothesis was further tested by comparing immunoprecipitates obtained with two different anti-Bax antibodies: 2D2 that recognizes all Bax conformations, and 6A7 that only recognizes the active conformation [24,25]. Wild-type Bax and Bax-P168A were tested (Fig. 3A). hTom22(1-82) did not change the binding of either 2D2 or 6A7 antibodies to the constitutively active mutant BaxP168A. On the opposite, it decreased the recognition of wild-type Bax by 6A7 (Fig. 3A and B), suggesting that it favored the inactive conformation of Bax. The fact that hTom22(1-82) did not change the interaction between Bax and Tom22p, Tom40p or Tom70p (Fig. 3C and D), further supported the view that the effect of the hTom22(1-82) did not result from a simple competing effect with the TOM complex but had a direct effect on Bax conformation.

3.3. The cytosolic part of human Tom22 interferes with Bax oligomerization

These data suggested that hTom22(1-82) forced Bax to acquire a mitochondria-localized but inactive conformation. Since the activation of Bax depends on its capacity to oligomerize, we investigated the effect of hTom22(1-82) on Bax oligomeric status by BN–PAGE. The mutant BaxP8G/P13G was used for this experiment since it is only partially active and both stimulatory and inhibitory changes could be potentially detected.

In the first dimension (Fig. 4, horizontal direction) BaxP8G/P13G was observed as a range of oligomers between 200 and 350 kDa. In the presence of hTom22(1-82), this profile disappeared and was replaced by a streak below 200 kDa, showing that oligomers formed in the presence of the peptide had a lower size. A second dimension was run by layering the first-dimension lane on top of a SDS–PAGE. These conditions preserve organized protein complexes, such as the oligomers of ATP synthase subunit 9. After this second dimension (Fig. 4, vertical direction), BaxP8G/P13G expressed alone was mainly found at 21 kDa, the size of Bax monomer, with only a minor band at 43 kDa, the size of Bax dimers. In the presence of hTom22(1-82), monomeric Bax could not be detected, and Bax was fully dimeric. This suggests that hTom22(1-82) stimulated the formation of a dimer of Bax that could not further oligomerize.

4. Discussion

Data reported in this paper further support the hypothesis that the mitochondrial translocation of Bax may be modulated by the cytosolic domain of the mitochondrial receptor Tom22. It had been previously reported that purified Bax and mammalian Tom22 physically interacted, and that down-regulating Tom22 impaired apoptosis in human glioblastoma cells [6,7]. A role for Tom22 in Bax effects has also been reported in *Drosophila* [26], where the partial loss of function of Tom22 or Tom70 attenuated the phenotypes linked to heterologous human Bax-expression in flies. Also, the down-regulation of yeast Tom22 decreased the localization of human Bax in yeast mitochondria [6].

In the present work, we show that this functional interaction between human Bax and yeast Tom22p actually resulted from a physical interaction, since we found that Bax co-immunoprecipitated with Tom22p, Tom40p and Tom70p (Fig. 1B). However, the two later might not interact directly with Bax, as already suggested for Tom40p [8], but indirectly through their association with Tom22p. Also, Bax slightly modulates the electrophysiological properties of PSC, the channel associated to yeast TOM. Like previously observed in glioblastoma cells [6], Bax and Tom22 also physically interacted in apoptotic pro-lymphocytic cells (Fig. 1C), showing that this interaction was not limited to one mammalian cell type.

To further investigate the role of this physical interaction between Bax and Tom22p, Bax mutants were co-expressed with the cytosolic receptor domain of human Tom22. We observed an increase of mitochondrial localization of all three Bax variants (Fig. 2A and B) that was not associated to an increase of their activity (Fig. 2D). Furthermore, the peptide decreased the proportion of 6A7-positive wild-type Bax (Fig. 3A and B). This showed that the additional mitochondrial Bax remained under an inactive conformation unable to oligomerize and to form a channel. BN–PAGE analyses indeed supported the hypothesis that the hTom22 receptor peptide forced Bax to acquire a dimeric conformation that could not further oligomerize up to the same size as control Bax (Fig. 4) and may represent a "dead end" in the process.

This effect of hTom22 receptor peptide may provide a new insight on the role of Tom22 in the process leading to Bax translocation and activation. Recent data showed that Bax displays two domains of interactions [27,28]. On this basis, Bax would be able to form stable symmetric dimers or unstable asymmetric dimers, the later being further assembled into high-size oligomers [4].

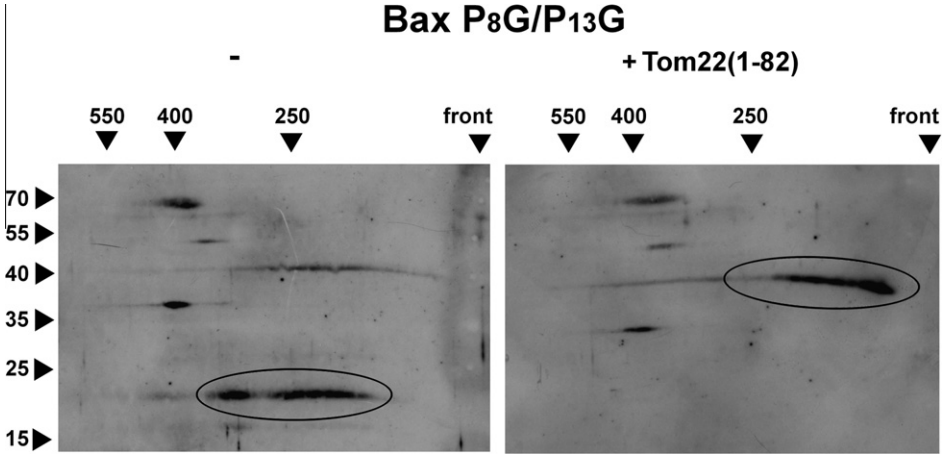

Fig. 4. The peptide corresponding to the cytosolic part of Tom22 favors the dimerization and prevents the oligomerization of Bax. Mitochondria isolated from cells co-expressing the Bax mutant P8G/P13G and Tom22(1-82) were analyzed by two-dimensional BN–PAGE/SDS–PAGE, as described in the experimental procedures section, and revealed with anti-Bax antibody (N20). The experiment shown is representative of 4 independent experiments.

The interaction between Bax and Tom22 would help the translocation step of Bax towards the outer mitochondrial membrane, but would not be sufficient to promote the full activation of the protein. Hence, the hTom22 receptor peptide may "force" the translocation step even though Bax is not under the adequate conformation to oligomerize, leading to the formation of a membrane-localized but inactive dimer.

These observations made in the yeast model system that allows to reproduce the translocation and activation steps of Bax, will now be evaluated in mammalian cell lines to evaluate the effects on apoptosis. Like in yeast, it is difficult to down-regulate mammalian Tom22 without compromising cell survival. The expression of hTom22(1-82) will be a valuable tool to investigate further the role of Tom22 in apoptosis. Another point of interest is the respective role of TOM receptors Tom20, Tom22 and Tom70. It has been suggested that Tom20 could serve as a receptor for Bcl-2 [29] but down regulation of Tom20 did not alter Bax-dependent apoptosis in glioblastoma cells [6]: this suggests that Tom20 and Tom22 might have distinct functions in regulating the mitochondrial localization of Bcl-2 family members. However, data suggest that both receptors are involved in the same mitochondrial import pathway [30]. This may indicate that distinct domains of both Tom20 and Tom22 are involved in their import function and in their capacity to modulate Bcl-2/Bax translocation. The expression of shorter domains of the cytosolic part of Tom22 might help to clarify this point.

Acknowledgements

This work was supported by grants from the CNRS, the Université of Bordeaux-Segalen, the Conseil Régional d'Aquitaine, and the Association pour la Recherche contre le Cancer (to S.M.). T.T.R. was recipient of a Ph.D. fellowship from the Ministère de la Recherche et de la Technologie. H.A. was recipient of a Ph.D. fellowship from the Fondation pour la Recherche Médicale. The authors thank Dr Carla Koehler (UCLA) for the kind gift of anti-TOM subunits antibodies and Dr Kathleen W. Kinnally (NYU) for helpful comments.

References

[1] Cory, S. and Adams, J.M. (2002) The Bcl2 family: regulators of the cellular life-or-death switch. Nat. Rev. Cancer 2, 647–656.
[2] Sharpe, J.C., Arnoult, D. and Youle, R.J. (2004) Control of mitochondrial permeability by Bcl-2 family members. Biochim. Biophys. Acta 1644, 107–113.
[3] Renault, T.T. and Manon, S. (2011) Bax: Addressed to kill. Biochimie 93, 1379–1391.
[4] Westphal, D., Dewson, G., Czabotar, P.E. and Kluck, R.M. (2011) Molecular biology of Bax and Bak activation and action. Biochim. Biophys. Acta 1813, 521–531.
[5] Ott, M., Norberg, E., Zhivotovsky, B. and Orrenius, S. (2009) Mitochondrial targeting of tBid/Bax: a role for the TOM complex? Cell Death Differ. 16, 1075–1082.
[6] Bellot, G., Cartron, P.F., Er, E., Oliver, L., Juin, P., Armstrong, L.C., Bornstein, P., Mihara, K., Manon, S. and Vallette, F.M. (2007) TOM22, a core component of the mitochondria outer membrane protein translocation pore, is a mitochondrial receptor for the proapoptotic protein Bax. Cell Death Differ. 14, 785–794.
[7] Cartron, P.F., Bellot, G., Oliver, L., Grandier-Vazeille, X., Manon, S. and Vallette, F.M. (2008) Bax inserts into the mitochondrial outer membrane by different mechanisms. FEBS Lett. 582, 3045–3051.
[8] Ott, M., Norberg, E., Walter, K.M., Schreiner, P., Kemper, C., Rapaport, D., Zhivotovsky, B. and Orrenius, S. (2007) The mitochondrial TOM complex is required for tBid/Bax-induced cytochrome c release. J. Biol. Chem. 282, 27633–27639.
[9] Sanjuán Szklarz, L.K., Kozjak-Pavlovic, V., Vögtle, F.N., Chacinska, A., Milenkovic, D., Vogel, S., Dürr, M., Westermann, B., Guiard, B., Martinou, J.C., Borner, C., Pfanner, N. and Meisinger, C. (2007) Preprotein transport machineries of yeast mitochondrial outer membrane are not required for Bax-induced release of intermembrane space proteins. J. Mol. Biol. 368, 44–54.
[10] Goping, I.S., Gross, A., Lavoie, J.N., Nguyen, M., Jemmerson, R., Roth, K., Korsmeyer, S.J. and Shore, G.C. (1998) Regulated targeting of BAX to mitochondria. J. Cell Biol. 143, 207–215.
[11] Cartron, P.F., Moreau, C., Oliver, L., Mayat, E., Meflah, K. and Vallette, F.M. (2002) Involvement of the N-terminus of Bax in its intracellular localization and function. FEBS Lett. 512, 95–100.
[12] Montessuit, S., Mazzei, G., Magnenat, E. and Antonsson, B. (1999) Expression and purification of full-length human Bax alpha. Protein Expr. Purif. 15, 202–206.
[13] Cartron, P.F., Oliver, L., Juin, P., Meflah, K. and Vallette, F.M. (2004) The p18 truncated form of Bax behaves like a Bcl-2 homology domain 3-only protein. J. Biol. Chem. 279, 11503–11512.
[14] Valentijn, A.J., Upton, J.P., Bates, N. and Gilmore, A.P. (2008) Bax targeting to mitochondria occurs via both tail anchor-dependent and -independent mechanisms. Cell Death Differ. 15, 1243–1254.
[15] Ross, K., Rudel, T. and Kozjak-Pavlovic, V. (2009) TOM-independent complex formation of Bax and Bak in mammalian mitochondria during TNFalpha-induced apoptosis. Cell Death Differ. 16, 697–707.
[16] Suzuki, M., Youle, R.J. and Tjandra, N. (2000) Structure of Bax: coregulation of dimer formation and intracellular localization. Cell 103, 645–654.
[17] Arokium, H., Camougrand, N., Vallette, F.M. and Manon, S. (2004) Studies of the interaction of substituted mutants of BAX with yeast mitochondria reveal that the C-terminal hydrophobic alpha-helix is a second ART sequence and plays a role in the interaction with anti-apoptotic BCL-xL. J. Biol. Chem. 279, 52566–52573.
[18] Teijido, O. and Dejean, L. (2010) Upregulation of Bcl2 inhibits apoptosis-driven BAX insertion but favors BAX relocalization in mitochondria. FEBS Lett. 584, 3305–3310.
[19] Pavlov, E.V., Priault, M., Pietkiewicz, D., Cheng, E.H., Antonsson, B., Manon, S., Korsmeyer, S.J., Mannella, C.A. and Kinnally, K.W. (2001) A novel, high conductance channel of mitochondria linked to apoptosis in mammalian cells and Bax expression in yeast. J. Cell Biol. 155, 725–731.
[20] Saeki, K., Suzuki, H., Tsuneoka, M., Maeda, M., Iwamoto, R., Hasuwa, H., Shida, S., Takahashi, T., Sakaguchi, M., Endo, T., Miura, Y., Mekada, E. and Mihara, K. (2000) Identification of mammalian TOM22 as a subunit of the preprotein translocase of the mitochondrial outer membrane. J. Biol. Chem. 275, 31996–32002.
[21] Cartron, P.F., Arokium, H., Oliver, L., Meflah, K., Manon, S. and Vallette, F.M. (2005) Distinct domains control the addressing and the insertion of Bax into mitochondria. J. Biol. Chem. 280, 10587–10598.
[22] Komiya, T., Rospert, S., Koehler, C., Looser, R., Schatz, G. and Mihara, K. (1998) Interaction of mitochondrial targeting signals with acidic receptor domains along the protein import pathway: evidence for the 'acid chain' hypothesis. EMBO J. 17, 3886–3898.
[23] Yano, M., Hoogenraad, N., Terada, K. and Mori, M. (2000) Identification and functional analysis of human Tom22 for protein import into mitochondria. Mol. Cell. Biol. 20, 7205–7213.
[24] Wolter, K.G., Hsu, Y.T., Smith, C.L., Nechushtan, A., Xi, X.G. and Youle, R.J. (1997) Movement of Bax from the cytosol to mitochondria during apoptosis. J. Cell Biol. 139, 1281–1292.
[25] Peyerl, F.W., Dai, S., Murphy, G.A., Crawford, F., White, J., Marrack, P. and Kappler, J.W. (2007) Elucidation of some Bax conformational changes through crystallization of an antibody-peptide complex. Cell Death Differ. 14, 447–452.
[26] Colin, J., Garibal, J., Mignotte, B. and Guénal, I. (2009) The mitochondrial TOM complex modulates bax-induced apoptosis in Drosophila. Biochem. Biophys. Res. Commun. 379, 939–943.
[27] Gavathiotis, E., Suzuki, M., Davis, M.L., Pitter, K., Bird, G.H., Katz, S.G., Tu, H.C., Kim, H., Cheng, E.H., Tjandra, N. and Walensky, L.D. (2008) BAX activation is initiated at a novel interaction site. Nature 455, 1076–1081.
[28] Gavathiotis, E., Reyna, D.E., Davis, M.L., Bird, G.H. and Walensky, L.D. (2010) BH3-triggered structural reorganization drives the activation of proapoptotic BAX. Mol Cell. 40, 481–492.
[29] Motz, C., Martin, H., Krimmer, T. and Rassow, J. (2002) Bcl-2 and porin follow different pathways of TOM-dependent insertion into the mitochondrial outer membrane. J. Mol. Biol. 323, 729–738.
[30] Yamano, K., Yatsukawa, Y., Esaki, M., Hobbs, A.E., Jensen, R.E. and Endo, T. (2008) Tom20 and Tom22 share the common signal recognition pathway in mitochondrial protein import. J. Biol. Chem. 283, 3799–3807.