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Review

The pleiotropic effects of heterologous Bax expression in yeast

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Abstract

The finding that the heterologous expression of Bcl-2 proteins in yeast elicits effects that resemble their roles in metazoan apoptosis has contributed to the increasing use of this organism as a model for the study of apoptotic regulation. The pro-apoptotic Bax protein, for example, localizes to the yeast mitochondria, where it acts to promote alterations in mitochondrial physiology and cell death, similar to its ascribed mode of action in higher organisms. These observations lead to the hypothesis that the heterologous Bcl-2 proteins impinge on conserved elements of the apoptotic machinery in yeast. We herein provide a retrospective of the studies aimed at both testing this general hypothesis and investigating the mechanisms of the Bcl-2 proteins using yeast, with a particular emphasis on Bax. We also discuss the evidence for pleiotropic roles of Bax in yeast apoptosis.

Keywords: Bax; Bcl-2 protein; Yeast apoptosis

1. Introduction: The Bcl-2 family members are critical regulators of apoptosis

Apoptosis is a genetically programmed form of cell death that is important for the survival of multicellular organisms by virtue of its functions in eliminating damaged or infected cells [1–4]. Further, loss of normal apoptotic responses can lead to uncontrolled cell growth, contributing to the development of tumours [5]. A number of single cell organisms, including the yeast *Saccharomyces cerevisiae*, have been shown to undergo programmed cell death (PCD) [6,7]. Given that yeast cells have been used extensively to understand the framework of a number of basic biological systems [8], it stands to reason that our understanding of PCD should therefore be greatly enhanced by the current studies of PCD in yeast. Although initially controversial, there is now very little doubt that yeast PCD is similar to the process of intrinsic apoptotic cell death that occurs in mammalian cells [9,10]. This is exemplified by the fact that the yeast genome encodes for a number of orthologous genes that have been shown to be important in mammalian apoptosis. Yeasts have an AIF (Apoptosis Inducing Factor *AIF*), metacaspase (*YCA1*), an IAP (inhibitor of Apoptosis Protein; *BIR1*), OMI/Htr2A (Nuclear Mediator of Apoptosis; *NMA111*), DJ-1 (*HSP31*) as well as a nuclease (*TAT-D*) that is a strong candidate to be involved in DNA degradation during apoptosis [11–15]. As in metazoans, overexpression of the apoptotic proteins can serve to initiate or enhance cell death in yeast, while strains lacking any of these genes show a decreased response to a number of different death stimuli.

Members of the Bcl-2 family of proteins ($n \geq 22$) are important regulators of the metazoan apoptotic process [16]. They are divided into subfamilies based on their apoptotic and anti-apoptotic nature as well as the presence or absence of different Bcl-2 Homology (BH) domains [17,18]. Bcl-2 contains all four BH domains and is a widely studied member of the Bcl-2 anti-apoptotic subfamily. Bax is the best-studied member of the pro-apoptotic Bcl-2 proteins that contain multiple BH domains. In response to pro-apoptotic stimuli, members of the Bax-like protein family act on the mitochondria to induce changes such as the release of cytochrome *c* (cyt *c*) [17–19]. Many additional BH-domain containing proteins have been studied to lesser degrees, including the pro-apoptotic Bad, Bak, and Bim, among others, as well as the anti-apoptotic Bcl-xL, BFL-1 (A1), Mcl-1, and CED-9 [17,20].

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While Bcl-2 proteins have been identified, a great deal of evidence suggests that the complete functional repertoire of Bax and Bcl-2 is not well understood [16]. Although yeast do not have genes encoding Bcl-2 proteins, the heterologous expression of mammalian Bax in yeast nevertheless induces a suppressible lethal phenotype that is associated with characteristics of metazoan apoptosis, including phosphatidylserine externalization, chromatin condensation, DNA breakage, and the formation of apoptotic bodies (Table 1) [21]. Since the initial demonstration that Bax is lethal when expressed in yeast, numerous groups have used yeast as a model system to study the function of Bax [22,23]. Studies have focused on different facets of Bax that range from determining its structure/function, to examining its role in mediating the effects on prions, to determining how it interacts with the mitochondria to using the lethal effects of Bax to screen for novel anti-apoptotic genes. Here we will give a historical perspective of Bax in yeast and we review the establishment of this model system as a powerful platform for the study of the structural/functional/mechanistic properties of Bax and other Bcl-2 family members. We also present the current knowledge on the mechanisms by which Bax promotes deleterious effects in yeast and discuss the use of classical yeast-based approaches, such as functional suppressor screens, to gain functional insights into the evolutionary conserved process of apoptosis.

2. The establishment of yeast as a tool for functional studies on Bax

2.1. Bax expression in yeast induces a programmed cell death with apoptotic features

The initial observation that Bax expression confers a lethal phenotype in yeast was made during the course of yeast two-hybrid studies aimed at examining Bax/Bcl-2 interactions [24]. Expression of Bax fused to either DNA-binding or *trans*-activation domains in yeast cells, under the control of a strong promoter, resulted in marked loss of clonogenic capacity that was confirmed to be due to cell death. Further, the effect was specifically suppressed by co-expression of anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1, indicating that both pro- and anti-apoptotic Bcl-2 family members retained certain aspects of their function when heterologously expressed in yeast [24]. Subsequent studies confirmed a lethal phenotype upon expression of Bax, unfused and lacking a signal peptide, under the control of the *GAL1/GAL10* promoter [25]. These observations lead to the hypothesis that the mammalian Bcl-2 proteins act on elements of a conserved endogenous yeast machinery to mediate effects on viability [26]. Importantly, Bax-mediated cell death in yeast, as in mammalian cells, involves a regulated insertion into mitochondrial membranes and mitochondrial dysfunction leading to the release of cyt *c* and apoptosis, supporting the hypothesis that Bax exerts effects in yeast that are comparable to those in its native setting [23].

A seminal study in the field of yeast apoptosis involved the identification of typical markers of mammalian apoptosis in cells mutated for CDC48 [27]. These findings strongly suggested that the basic framework of apoptosis is conserved in lower eukaryotes. The first description of apoptotic phenotypes in yeast induced by members of Bcl-2 family involved the expression of pro-apoptotic human Bak in *Schizosaccharomyces pombe* [28]. The specific phenotypes reported included nuclear envelope breakdown and chromatin condensation and fragmentation, which are characteristics of metazoan apoptosis. The deleterious effects of Bak were dependent on the BH3 domain and suppressed by co-expression of Bcl-xL and Bcl-2, but not by a mutant form of Bcl-2 that does not prevent apoptosis in mammalian cells [29]. Shortly thereafter, similar

Table 1
Bcl-2 and Bcl-2-related proteins and their reported effects in yeast

	Phenotypes observed on isolated yeast mitochondria or upon heterologous expression in yeast.	References
Pro-apoptotic Bcl-2 proteins		
Bax	Loss of mitochondrial membrane potential, and cytochrome *c* release. Induction of oxidative stress, growth-inhibition, and cell death. Elicits cell death upon expression in *Schizosaccharomyces pombe*.	[20,28,27,33,37,106]
Bad	Sensitizes cells to the effects of Bax, yet was also reported to enhance the pro-survival activity of Bcl-xL, Bcl-2 and A1.	[44,167]
Bak	Loss of mitochondrial membrane potential. Induction of cell death upon expression in *Schizosaccharomyces pombe.*	[26,27,37]
Bcl-xS	Enhances the pro-survival activity of Bcl-xL, Bcl-2, and A1.	[37,167]
Bim	Loss of mitochondrial membrane potential, cytochrome *c* release and cell death. Enhances the death-inducing activity of Bax.	[122,166]
BimS	Sensitizes cells to the lethal effects of Bax.	[166]
BNip3	No effect observed.	[44]
BNip3L	No effect observed.	[44]
Noxa	No effect observed.	[44]
Puma	Sensitizes cells to the effects of Bax.	[44]
tBid	Disrupts mitochondrial state-3 respiration and ATP production. Sensitizes cells to Bax-induced cytochrome *c* release and cell death.	[43,44]
Anti-apoptotic Bcl-2 proteins		
A1	Protects from Bax- and Bak-induced cytotoxicity.	[37]
Bcl-2	Promotes cell survival. Increases resistance to oxidative stresses.	[33,37,51]
Bcl-xL	Prevents Bax-induced growth-inhibition and cell death. Increases resistance to oxidative stresses.	[27,37, 106]
BFL-1	Protects from Bax-induced cell death.	[168]
CED-9	Promotes resistance to oxidative stresses.	[51]
Mcl-1	Protects from Bax- and Bak-induced cytotoxicity.	[37,165]