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Bcl-2 regulation of the inositol 1,4,5-trisphosphate receptor and calcium signaling in normal and malignant lymphocytes: Potential new target for cancer treatment

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Abstract

The anti-apoptotic protein Bcl-2 is a versatile regulator of cell survival. Its interactions with its own pro-apoptotic family members are widely recognized for their role in promoting the survival of cancer cells. These interactions are thus being targeted for cancer treatment. Less widely recognized is the interaction of Bcl-2 with the inositol 1,4,5-trisphosphate receptor ($InsP_3R$), an $InsP_3$-gated Ca^{2+} channel located on the endoplasmic reticulum. The nature of this interaction, the mechanism by which it controls Ca^{2+} release from the ER, its role in T-cell development and survival, and the possibility of targeting it as a novel cancer treatment strategy are summarized in this review.

Keywords

Bcl-2; inositol 1,4,5-trisphosphate receptor; apoptosis; cancer; chronic lymphocytic leukemia; lymphocyte

1. Introduction

It has been almost thirty years since Bcl-2 was discovered [1, 2] and found to be a positive regulator of cell survival [3]; twenty years since the first indication that Bcl-2 regulates intracellular Ca^{2+} dynamics [4, 5], and ten years since an interaction of Bcl-2 and its close relative Bcl-xl with the inositol 1,4,5-trisphosphosphate receptor ($InsP_3R$) was discovered [6, 7]. An inhibitor of the Bcl-2-$InsP_3R$ interaction has recently been developed and shown to induce the death of primary human chronic lymphocytic leukemia (CLL) cells [8, 9],

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raising the possibility that targeting this interaction may become a novel treatment strategy for Bcl-2-positive malignancies. This review will discuss our understanding of how the Bcl-2-InsP_3R interaction promotes cell survival, the evidence that Bcl-2-positive cancer cells exploit this mechanism to avoid cell death, and the efforts to therapeutically target the Bcl-2-InsP_3R interaction. Readers are referred to a number of excellent reviews for expanded information about InsP_3Rs and Ca^{2+} signaling [10, 11] and the Bcl-2 protein family [12, 13].

2. Bcl-2 Family Members and Functions

Bcl-2 is a 26 kDa integral membrane protein that resides on the outer mitochondrial membrane and endoplasmic reticulum (ER) membrane. It is anchored on these membranes by a C-terminal hydrophobic tail and is mainly cytoplasmic in location. Bcl-2 excited great interest when it was discovered to promote cell survival by inhibiting apoptosis [3]. One after another, Bcl-2 relatives were identified, elevating Bcl-2 to *pater familias* stature. Proteins in the Bcl-2 family share sequence motifs referred to as Bcl-2 Homology Domains (BH domains), of which four are recognized (Figure 1). From a functional standpoint, members generally fall into two opposing groups: anti-apoptotic proteins and pro-apoptotic proteins. Anti-apoptotic members such as Bcl-2 typically have four BH domains (BH1-4). Pro-apoptotic members fall into two subgroups: those with three BH domains (BH1-3), such as Bax and Bak, and those with only a BH3 domain, the ‘BH3-only proteins’, including for example Bim, Bad, and PUMA. These distinctions are useful from an operational standpoint, but are undergoing significant revision and clarification as an increasing number of proteins are found to have BH3-like domains but not all are Bcl-2 family members [14]. Moreover, only anti-apoptotic members were originally considered to have BH4 domains, but this distinction is eroding as BH4-domain-like structures become recognized in certain pro-apoptotic family members [14].

One of the most remarkable features of Bcl-2 is its lack of an obvious inherent function, such as kinase, phosphatase or enzymatic activity. Bcl-2 and its anti-apoptotic relatives nevertheless exert widespread influence over many cell functions, ultimately influencing cell survival. The main *modus operandi* involves engagement in diverse interactions. These include homomeric and heteromeric oligomerization involving both Bcl-2 family members and non-family proteins. A major activity of Bcl-2 involves interaction with its pro-apoptotic family members, including Bax, Bak and the BH3-only members. By binding pro-apoptotic family members, Bcl-2 prevents these proteins from oligomerizing and forming pores in the outer mitochondrial membrane, thus releasing cytochrome *c* and activating a cascade of caspase activation, ultimately leading to apoptosis. Bcl-2’s site of interaction with pro-apoptotic proteins is located in a hydrophobic cleft composed of BH1-3 domains (Figure 1). This cleft is occupied by small molecule BH3-mimetics such as ABT-737 that displace pro-apoptotic proteins from Bcl-2 and thus trigger apoptosis [15, 16]. For an in-depth explanation of how Bcl-2 interacts with its pro-apoptotic relatives, thereby preserving outer mitochondrial membrane integrity, the reader is referred to publications by Llambi *et al* [17] and Shamas-Din *et al* [13].

Bcl-2 family proteins also regulate cell survival through their localization to the ER [18, 19]. Moreover, Bcl-2 interacts with a number of proteins in addition to Bcl-2 protein family members, indicating that Bcl-2 has functional significance beyond its direct control of pro-apoptotic relatives (Figure 1). These interactions are mediated through the BH4 domain and include binding of the BH4 domain to $InsP_3Rs$, the serine/threonine protein kinase Raf-1 [20] and the serine/threonine protein phosphatase calcineurin (CaN) [21]. Raf-1 phosphorylates and thereby inhibits the pro-apoptotic protein Bad [20]. The interaction of the Bcl-2 BH4 domain with CaN and the $InsP_3R$ regulates intracellular Ca^{2+} dynamics and cell survival, as addressed in this review.

3. Regulation of Cell Survival and Cell Death by $InsP_3R$-mediated Ca^{2+} Elevation

$InsP_3Rs$ are $InsP_3$-gated Ca^{2+} channels located mainly on the ER [10, 11] (Figure 2). Their central function is to release Ca^{2+} ions from the ER lumen, where Ca^{2+} is stored at high concentration. Ca^{2+} release induces highly regulated and systematic elevations of cytoplasmic Ca^{2+} concentration. $InsP_3R$-mediated Ca^{2+} elevation regulates the activity of many fundamental cellular processes including fertilization, cell cycle entry, cell division, metabolism, and transcription [10, 22]. Ca^{2+} information is encoded in the frequency and amplitude of Ca^{2+} oscillations and decoded by Ca^{2+}-sensitive kinases and phosphatases, in turn regulating the activity of target proteins as diverse as transcription factors, endonucleases, proteases and metabolic enzymes [23, 24].

One of the most important functions of $InsP_3R$-mediated Ca^{2+} signaling is the promotion of cell survival by supporting mitochondrial Ca^{2+} uptake and mitochondrial metabolism. The close proximity of ER-localized $InsP_3Rs$ to mitochondria facilitates Ca^{2+} transfer from the ER lumen into mitochondria [25, 26] (Figure 2). This calcium transfer promotes mitochondrial ATP production by catalyzing the conversion of pyruvate to acetyl-CoA and by activating multiple Ca^{2+}-sensitive enzymes in the citric acid cycle [27, 28]. Insufficient ER-mitochondrial Ca^{2+} transfer results in autophagy, a survival mechanism through which cells digest intracellular components in order to produce ATP [29], but which may lead to cell death if prolonged (Figure 3). Conversely, excessive transfer of Ca^{2+} to mitochondria induces Ca^{2+} overload, resulting in loss of mitochondrial membrane potential, cytochrome *c* release and apoptosis [30, 31] (Figure 3). Therefore, although Ca^{2+} elevation plays a critical role in promoting cell survival, it is also a known inducer of cell death [32, 33] and for this reason $InsP_3R$-mediated Ca^{2+} release from the ER must be tightly regulated. Ca^{2+} elevation triggers apoptosis through a number of different pathways in addition to mitochondrial Ca^{2+} overload. These include activation of Ca^{2+}-sensitive proteases and endonucleases, activation of the pro-apoptotic Bcl-2 family member Bad, and inducing expression of pro-apoptotic Bcl-2 family member Bim [33].

4. Bcl-2 Regulation of $InsP_3R$-mediated Ca^{2+} Elevation in T-cells

Our work has focused on the role of Bcl-2 in regulating $InsP_3R$-mediated Ca^{2+} signals in T-cells. Bcl-2 is of critical importance in T-cell development and survival. The developing T-cell passes through successive maturational stages within the thymus [34] and Bcl-2 levels

vary considerably throughout these different developmental stages [4, 35, 36] (Figure 4). The earliest precursors from the bone marrow or fetal liver do not express either the TCR or the CD4 and CD8 antigens (*i.e.*, 'double negative stage'), but do express Bcl-2: this provides an element of protection from apoptosis *en route* to the thymus gland. In the cortex of the thymus these immature T-cells express the TCR and both CD4 and CD8 antigens (*i.e.*, 'double positive stage'). Bcl-2 levels are down-regulated at this stage, increasing their sensitivity to Ca^{2+}-induced apoptosis [4]. This facilitates a stringent test of whether or not the T-cells respond to self-antigens, with strong responders undergoing apoptosis ('negative selection') and weak responders avoiding apoptosis ('positive selection') [37, 38]. During negative selection, apoptosis is induced by Ca^{2+}-dependent up-regulation of the pro-apoptotic Bcl-2 family member Bim [39]. Surviving cells advance to the 'single positive stage' (CD4+/CD8−, CD4−/CD8+), where Bcl-2 levels are increased, and enter the circulation to mount immune responses to foreign antigens as mature T cells. Bcl-2 levels are further elevated when a T-cell responds to antigenic stimulation and proliferates, but them decline as the immune response wanes and the T-cell dies, a process referred to as activation-induced cell death.

The role of Bcl-2 in T-cell development was elegantly demonstrated by Bcl-2 knockout and over-expression strategies. The Bcl-2 knockout mouse, developed in the laboratory of Stanley Korsmeyer, demonstrated extensive lymphoid apoptosis [40]. Conversely, enforced expression of Bcl-2 in transgenic mice inhibited negative selection, causing excessive accumulation of thymocytes [41, 42]. Transgenic Bcl-2 inhibits negative selection by a mechanism independent of its ability to antagonize Bax, suggesting a role for Bcl-2-mediated regulation of Ca^{2+} in this process [43, 44].

The $InsP_3R$-mediated Ca^{2+} signals induced by T-cell receptor (TCR) activation are of critical physiological importance in the developing immune system [39, 45, 46]. Positive *versus* negative selection decisions in the thymus may be encoded by distinct Bcl-2-regulated Ca^{2+} signaling patterns [47]. T-cell activation by antigen binding to the TCR triggers a signaling cascade that activates phospholipase C-γ, generating $InsP_3$. $InsP_3$ binds to the $InsP_3R$, inducing channel opening and Ca^{2+} release from the ER, thus stimulating T-cell proliferation [45, 48–50]. Depending on the strength of TCR activation, a variety of Ca^{2+} response patterns are generated, including transient Ca^{2+} elevation, sustained Ca^{2+} elevation, or Ca^{2+} oscillations [51, 52]. TCR activation by physiologically-relevant antigenic peptides in immature thymocytes produce a similar effect: negatively-selecting antigenic peptides induce a strong Ca^{2+} flux, whereas positively-selecting peptides induce a smaller Ca^{2+} flux [53]. Additionally, earlier studies in thymocytes demonstrated that cytoplasmic Ca^{2+} elevation following strong TCR activation by a high concentration of anti-CD3 antibody *in vitro* induces apoptosis, whereas cytoplasmic Ca^{2+} elevation induced by weak TCR activation using lower concentrations of anti-CD3 antibody do not trigger apoptosis [54].

5. Effect of Bcl-2 on Ca^{2+} signaling

We investigated the effect of Bcl-2 on Ca^{2+} signaling patterns in the murine thymocyte line, WEHI7.2. These cells correspond to the double positive stage of thymocyte development

and thus have very low levels of Bcl-2. We find that strong TCR activation by a high concentration of anti-CD3 antibody induces a large transient Ca^{2+} elevation, whereas weak TCR activation by a low concentration of anti-CD3 antibody induces sustained Ca^{2+} oscillations [47]. These Ca^{2+} signaling patterns differ in two ways: high anti-CD3 induces a much more prolonged Ca^{2+} elevation than low anti-CD3 (> 4 min versus < 1 min); and high anti-CD3 triggers a higher peak Ca^{2+} amplitude than low anti-CD3 [47]. High amplitude Ca^{2+} elevation, particularly if continuous and sustained, triggers cell death [32, 33]. Thus, consistent with the earlier findings of other investigators, the critical determinant of whether or not TCR stimulation induces apoptosis appears to lie in both the duration and amplitude of the Ca^{2+} elevation.

We find that Bcl-2 selectively inhibits the pro-apoptotic Ca^{2+} elevation induced by strong TCR activation while enhancing the pro-survival Ca^{2+} oscillations induced by weak TCR activation [47]. The positive effect of Bcl-2 on Ca^{2+} oscillations and its pro-survival effects are consistent with a number of other findings. For example, Ca^{2+} oscillations regulate thymocyte motility during positive selection, thereby modulating interactions with stromal cells [55]. Ca^{2+} oscillations also lead to a sustained activation of CaN [38], which dephosphorylates and thereby activates NFAT, increasing expression of the gene encoding the cytokine interleukin-2 [48, 51, 56].

Moreover, although this review emphasizes Bcl-2-$InsP_3R$ interaction in the context of regulating cell death, the regulatory role of this interaction extends far beyond cell death to include many processes in which Ca^{2+} signaling plays important roles. As an example, elegant experiments by Gillet and coworkers [57] indicate that the zebrafish homolog of Bcl-2, Nrz, interacts with $InsP_3Rs$ and controls cytoskeletal dynamics *via* the regulation of Ca^{2+} trafficking. Cytoskeletal dynamics are important in cancer cell migration and metastasis and Bcl-2, by regulating Ca^{2+}, plays an important role in tumor metastasis and increased tumor vascularity [58].

How Bcl-2 regulates $InsP_3R$-mediated Ca^{2+} release is a major focus in our laboratory. We discovered that Bcl-2 interacts with the $InsP_3R$ [6] and that this interaction involves binding of the BH4 domain of Bcl-2 to a region located within the regulatory and coupling domain of the $InsP_3R$ [59, 60] (Figure 1). We synthesized a 20 amino acid peptide corresponding to the Bcl-2 interaction site on the $InsP_3R$ and found that this peptide, which we refer to as $InsP_3R$-Derived Peptide (IDP), functions as a decoy peptide that binds to Bcl-2 and inhibits Bcl-2-$InsP_3R$ interaction. This peptide, we find, reverses the inhibitory effect of Bcl-2 on $InsP_3R$-mediated Ca^{2+} elevation in T-cells treated with high concentrations of anti-CD3 antibody [59, 60]. This peptide has proven to be a valuable tool in studies of Bcl-2-$InsP_3R$ interaction.

Other anti-apoptotic Bcl-2 family members, including Bcl-xl and Mcl-1, also interact with $InsP_3Rs$ and regulate $InsP_3R$-mediated Ca^{2+} release [50, 61]. Although these anti-apoptotic family members, and Bcl-2, decrease ER luminal Ca^{2+} concentration, this has not been observed in our studies [6, 47, 62]. A recent report also indicates that Bcl-2 may not interact with $InsP_3Rs$ in all circumstances or cell types [63], raising the important question of what actually regulates the Bcl-2-$InsP_3R$ interaction in different types of cells.

Bcl-2 may also regulate ER Ca^{2+} release through other mechanisms besides its interaction with the $InsP_3R$. One proposed mechanism involves Bcl-2 interaction with Sarcoplasmic/Endoplasmic Reticulum-associated Ca^{2+}-ATPases (SERCA). These proteins pump Ca^{2+} ions from the cytoplasm into the ER lumen, maintaining large ER luminal Ca^{2+} stores. This steep Ca^{2+} concentration gradient from ER lumen to cytoplasm facilitates Ca^{2+} efflux from the ER lumen *via* $InsP_3R$ channel opening, leading to cytoplasmic Ca^{2+} elevation. Bcl-2's interaction with SERCA attenuates ER Ca^{2+} filling, indirectly diminishing $InsP_3R$-mediated Ca^{2+} release and Ca^{2+}-mediated apoptosis [64, 65]. Recent findings indicate that HSP70 regulates the Bcl-2-SERCA interaction, maintaining SERCA in an active state that may be essential for apoptosis regulation [66]. Accordingly, an earlier report of the Bcl-2-SERCA interaction finds that Bcl-2 increases the ER Ca^{2+} pool, promoting the high luminal Ca^{2+} concentration required for normal cell function [67].

6. How Bcl-2-$InsP_3R$ Interaction Regulates $InsP_3$-mediated Ca^{2+} Release

The preceding findings illustrate the importance of $InsP_3R$-mediated Ca^{2+} signals in T-cells, and the role of Bcl-2 in regulating these signals. However, it has not been determined how Bcl-2 regulates $InsP_3R$-mediated Ca^{2+} elevation through its interaction with the $InsP_3R$. Oakes *et al* [68] show that Bcl-2 regulates $InsP_3R$ phosphorylation at serine 1755 within the regulatory and coupling domain of the $InsP_3R$ in murine embryonic fibroblasts. Protein kinase A (PKA) phosphorylates serine 1755 and serine 1589 of the $InsP_3R$, increasing $InsP_3$-mediated channel opening and Ca^{2+} release [64, 69]. We previously reported that Bcl-2 decreases $InsP_3R$ phosphorylation, although a specific phosphorylation site was not identified [6]. In recent work, we find that Bcl-2 inhibits $InsP_3R$ phosphorylation at serine 1755, correlating with its inhibition of anti-CD3-induced Ca^{2+} elevation.

The mechanism underlying Bcl-2 regulation of $InsP_3R$ phosphorylation following TCR activation is under investigation in our laboratory. PKA-mediated protein phosphorylation is typically regulated by PP1α [70]. Tang *et al* [71] discovered a direct association between PP1α and $InsP_3R$-1 and established that the association with PP1α reverses PKA-mediated $InsP_3R$-1 phosphorylation. Similarly, others have shown that AKAP9, a multifunctional PKA anchoring protein, docks both PKA and PP1α to $InsP_3R$-1 [72]. Moreover, an $InsP_3$-RPP1α complex has been implicated in Bcl-2-mediated suppression of ER Ca^{2+} release in breast cancer cells [70]. Bcl-2 also binds CaN [21] and increases the association of CaN with $InsP_3Rs$ [72, 73]; this has a neuroprotective effect in primary neuronal cells [73]. Knowledge that Bcl-2 binds CaN, together with evidence that PP1α reverses PKA-mediated $InsP_3R$-1 phosphorylation, stimulated us to hypothesize a role for DARPP-32 (dopamine- and c-AMP-regulated phosphoprotein of 32 kDa) in the regulation of $InsP_3R$-mediated Ca^{2+} elevation by Bcl-2.

DARPP-32 is a PKA-activated and CaN-deactivated PP1α inhibitor studied extensively in the brain [74]. In experiments with medium spiny neurons from DARPP-32 knockout mice, DARPP-32 was shown to regulate dopamine-induced Ca^{2+} oscillations [75]. However, very little is known about the role of DARPP-32 in peripheral tissues, including lymphocytes, although DARPP-32 has been shown to increase the phosphorylation and activity of various ion channels [76]. We recently found that Bcl-2 prevents exaggerated $InsP_3R$-mediated Ca^{2+}

elevation in T-cells by decreasing $InsP_3R$ phosphorylation through a feedback mechanism involving DARPP32 and CaN [77].

Although these recent findings establish a role for Bcl-2 in regulating $InsP_3R$ phosphorylation, other potential mechanisms by which Bcl-2 and/or other anti-apoptotic members of the Bcl-2 family regulate $InsP_3R$-mediated Ca^{2+} signaling should be considered also. For example, one report suggested that the Bcl-2 homologue Bcl-xl, affects Ca^{2+} homeostasis by altering $InsP_3Rs$ levels [78]. More recent evidence indicates that the Bcl-2 protein family member Bok binds to $InsP_3Rs$ and protects them from proteolytic cleavage, although not governing the ability of $InsP_3Rs$ to release Ca^{2+} [63]. Also, the possibility that certain Bcl-2 family members may regulate $InsP_3$ binding affinity should be considered [79].

7. Exploitation of the Bcl-2-$InsP_3R$ Interaction by Cancer Cells and Targeting the Bcl-2-$InsP_3R$ Interaction for Cancer Treatment

Anti-apoptotic Bcl-2 family members such as Bcl-2, Bcl-xl and Mcl-1 play major roles in tumorigenesis by prolonging cancer cell survival (reviewed in 80). Anti-apoptotic Bcl-2 family members also regulate the migration and invasion of colorectal cancer cells [81]. In general, Bcl-2 is prominently expressed in leukemia and lymphoma cells, whereas Mcl-1 is highly expressed in solid tumors in addition to lymphoid malignancies [82]. Moreover, ion channels such as the $InsP_3R$ play extensive roles in regulating cell proliferation and cell death, and are thus emerging as promising targets for cancer treatment [83]. Importantly, cancer cells remodel Bcl-2-regulated intracellular Ca^{2+} fluxes to promote cell proliferation and avoid cell death [58, 84]. This remodeling has important therapeutic implications.

Our work indicates that Bcl-2 promotes cancer cell survival by interacting with $InsP_3Rs$ to prevent pro-apoptotic Ca^{2+} elevation (Figure 1), in addition to its known role in binding and inhibiting pro-apoptotic family members. Small molecules that bind to the hydrophobic cleft formed by the BH1-3 domains of Bcl-2 displace pro-apoptotic proteins from Bcl-2 and thus trigger apoptosis [15, 16]. These molecules, including the Bcl-2 selective and platelet-sparing ABT-199, are already in clinical trials for cancers as diverse as lymphoid malignancies, myeloid malignancies and breast cancer [15, 16, 85]. However, cancer cells become resistant to virtually any single therapeutic approach. Therefore, it is essential to target cancer cells from multiple angles if one hopes to achieve a cure. For this reason, efforts are underway to target the Bcl-2-$InsP_3R$ interaction. As summarized above, we have developed a synthetic peptide corresponding to the $InsP_3R$ binding site for Bcl-2 [59, 60] (Figure 1). This $InsP_3R$-Derived Peptide (IDP) inhibits the Bcl-2-$InsP_3R$ interaction by binding to the BH4 domain of Bcl-2, destabilizing Bcl-2's alpha-helical structure [59, 60, 86]. By inhibiting the Bcl-2-$InsP_3R$ interaction, IDP and its protease resistant analog $IDP_{DD/AA}$ decreases Bcl-2's control over $InsP_3R$-mediated Ca^{2+} elevation.

Bcl-2 elevation is a hallmark of chronic lymphocytic leukemia (CLL), the most common form of leukemia in the Western world. Initially, CLL runs an indolent clinical course, providing a unique opportunity to investigate primary CLL cells before they are subjected to chemotherapy. We find that $IDP_{DD/AA}$-mediated inhibition of Bcl-2-$InsP_3R$ interaction induces marked Ca^{2+} elevation and Ca^{2+}-mediated apoptosis in primary human CLL cells,

with minimal if any effect on the viability of normal human lymphocytes [9]. $IDP_{DD/AA}$ also induces apoptosis in Bcl-2-positive cell lines representing the B-cell malignancies, including diffuse large cell lymphoma [87]. Also, in a preliminary study in CLL cells, $IDP_{DD/AA}$ and the BH3-mimetic ABT-737 displayed synergistic cytotoxicity [8]. If confirmed by additional *in vivo* testing, these findings will underscore the value of simultaneously targeting Bcl-2's interaction with both $InsP_3Rs$ and pro-apoptotic family members for cancer treatment.

The usefulness of targeting the Bcl-2-$InsP_3R$ interaction may be dependent upon a number of factors. One factor is the $InsP_3R$ isoform expressed in different types of malignant cells. There are three $InsP_3R$ isoforms, which vary in both tissue distribution and in sensitivity to Ca^{2+} and $InsP_3$ regulation [88]. A recent study discovered that the sensitivity of lymphoma cells to $IDP_{DD/AA}$-induced apoptosis correlated with $InsP_3R$-2 isoform rather than $InsP_3R$-1 or $InsP_3R$-3 [87], which suggests the $InsP_3R$ isoform expression in the malignancy being treated will need to be considered in order to yield optimal therapeutic success. Another factor is the level of Bcl-2 in different types of cancer and the reliance of various cancers on Bcl-2 for their survival [82]. Bcl-2 is typically expressed in lymphoid malignancies, whereas other anti-apoptotic Bcl-2 family members, such as Mcl-1, predominate in non-lymphoid malignancies. Individual anti-apoptotic family members may also differ in their interaction with $InsP_3Rs$ [89]. Also, recent evidence indicates that the BH4 domain of Bcl-xL binds to a different region on the $InsP_3R$ than Bcl-2 [86]. Therefore, $IDP_{DD/AA}$ is more likely to be effective in killing malignant cells where Bcl-2 levels predominate over Bcl-xL levels.

8. Summary and Future Directions

This review has emphasized the role of the Bcl-2 protein in regulating $InsP_3R$-mediated Ca^{2+} elevation, both that which mediates normal cell function and that which can induce cell death. Moreover, the review has focused primarily on this function of Bcl-2 in the contexts of lymphocyte function and lymphoid malignancy. This focus should not be interpreted as an indication, or even a suggestion, that Bcl-2 is the only family member that regulates $InsP_3R$-mediated Ca^{2+} release, as indeed a number of Bcl-2 family members, including both anti-apoptotic and pro-apoptotic, are known to regulate Ca^{2+} release from the ER. Similarly, the main emphasis on lymphoid malignancies should not suggest that these are the only malignancies in which Bcl-2 family members and Ca^{2+} regulation are important. The reader is referred to an extensive review by Roderick and Cook [84] of the many ways that Ca^{2+} signaling toolkit is remodeled by cancer cells to promote their proliferation and survival. In addition, the role of Ca^{2+} signaling in tumor cell migration and metastasis, mentioned earlier in this review, has been thoughtfully reviewed [58].

In sum, the discovery that Bcl-2 and its family members interact with $InsP_3Rs$ and regulate $InsP_3$-induced Ca^{2+} signals has brought widespread attention to Ca^{2+} signaling and its exploitation by cancer cells. Yet, this area of research deserves more recognition among cancer investigators than it seems to garner. This is likely due to the complexity of Ca^{2+} signaling and the specialized nature of techniques employed to study Ca^{2+} and Ca^{2+} signaling. With this oversight comes a loss of opportunity to target Ca^{2+} signaling pathways for cancer treatment. The presently narrow emphasis of the cancer research field on genomics is sure to wane as the rate of progress plateaus and the interest turns back to

biology and physiology, since it may prove difficult to correct defective genes and a better investment of time and resources to develop therapies based on the output of defective genes. We already witness this trend with the skyrocketing interest in autophagy and metabolism, processes involving $InsP_3Rs$ and Ca^{2+} signaling.

For the immediate future, in depth analysis of the differences between Bcl-2 family members in terms of their sites of interaction with $InsP_3Rs$ and their differential effects on Ca^{2+} signaling is likely to generate new ideas and understanding, and to better elucidate the various roles of Ca^{2+} in cancer. In addition, the next steps need to move beyond the present emphasis on Ca^{2+} in processes such as apoptosis and autophagy, to expand our knowledge of the role of Ca^{2+} in metastasis, which remains the major obstacle in our quest for cancer cure.

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Abbreviations

Bcl-2	B-cell leukemia/lymphoma-2
BH	Bcl-2 homology
CaN	calcineurin
ER	endoplasmic reticulum
IDP	inositol 1,4,5-trisphosphate receptor-derived peptide
InsP3	inositol 1,4,5-trisphosphate
InsP3R	inositol 1,4,5-trisphosphate receptor
SERCA	Sarcoplasmic/Endoplasmic Reticulum-associated Ca^{2+}-ATPase
TCR	T-cell receptor

References

1. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Science. 1984; 226:1097–1099. [PubMed: 6093263]
2. Tsujimoto Y, Croce CM. Proc. Natl. Acad. Sci. USA. 1986; 83:5214–5218. [PubMed: 3523487]
3. Vaux DL, Cory S, Adams J. Nature. 1988; 335:440–442. [PubMed: 3262202]
4. Andjelic S, Jain N, Nikolic-Zugic J. J. Exp. Med. 1993; 178:1745–1751. [PubMed: 8228820]
5. Zornig M, Busch G, Beneke R, Gulbins E, Lang F, Ma A, Korsmeyer S, Moroy T. Oncogene. 1995; 11:2165–2174. [PubMed: 7478538]
6. Chen R, Valencia I, Zhong F, McColl KS, Roderick HL, Bootman MD, Berridge MJ, Conway SJ, Holmes AB, Mignery GA, Velez P, Distelhorst CW. J Cell Biol. 2004; 166:193–203. [PubMed: 15263017]
7. White C, Li C, Yang J, Petrenko NB, Madesh M, Thompson CB, Foskett JK. Nature Cell Biology. 2005; 7:1021–1028.

8. Rong Y, Barr P, Yee VC, Distelhorst CW. Biochim Biophys Acta. 2009; 1793:971–978. [PubMed: 19056433]
9. Zhong F, Harr MW, Bultynck G, Monaco G, Parys JB, DeSmedt H, Rong Y-P, Molitoris JK, Lam M, Ryder C, Matsuyama S, Distelhorst CW. Blood. 2011; 117:2924–2934. [PubMed: 21193695]
10. Berridge MJ, Bootman MD, Roderick HL. Nature Rev. Mol. Cell Biol. 2003; 4:517–529. [PubMed: 12838335]
11. Parys JB, De Smedt H. Adv Exp Med Biol. 2012; 740:255–279. [PubMed: 22453946]
12. Youle RJ, Strasser A. Nat Rev Mol Cell Biol. 2008; 9:47–59. [PubMed: 18097445]
13. Shamas-Din A, Kale J, Leber B, Andrews DW. Cold Spring Harb Perspect Biol. 2013; 5:a008714. [PubMed: 23545417]
14. Aouacheria A, Rech de Laval V, Combet C, Hardwick JM. Trends Cell Biol. 2013; 23:103–111. [PubMed: 23199982]
15. Billard C. Mol Cancer Ther. 2013; 12:1691–1700. [PubMed: 23974697]
16. Vandenberg CJ, Cory S. Blood. 2013; 121:2285–2288. [PubMed: 23341542]
17. Llambi F, Moldoveanu T, Tait SW, Bouchier-Hayes L, Temirov J, McCormick LL, Dillon CP, Green DR. Mol Cell. 2011; 44:517–531. [PubMed: 22036586]
18. Annis MG, Yethon JA, Leber B, Andrews DW. Biochim. Biophys. Acta. 2004; 1644:115–123. [PubMed: 14996496]
19. Thomenius MJ, Wang NS, Reineks EZ, Wang Z, Distelhorst CW. J Biol Chem. 2003; 278:6243–6250. [PubMed: 12477729]
20. Wang H-G, Rapp UR, Reed JC. Cell. 1996; 87:629–638. [PubMed: 8929532]
21. Shibasaki F, Kondo E, Akagi T, McKeon F. Nature. 1997; 386:728–731. [PubMed: 9109491]
22. Clapham DE. Cell. 2007; 131:1047–1058. [PubMed: 18083096]
23. Hajnoczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. Cell. 1995; 82:415–424. [PubMed: 7634331]
24. Hajnoczky G, Csordas G, Krishnamurthy R, Szalai G. J Bioenerg Biomembr. 2000; 32:15–25. [PubMed: 11768758]
25. Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T. Science. 1998; 280:1763–1766. [PubMed: 9624056]
26. Drago I, Pizzo P, Pozzan T. EMBO J. 2011; 30:4119–4125. [PubMed: 21934651]
27. Rutter GA, Rizzuto R. Trends Biochem Sci. 2000; 25:215–221. [PubMed: 10782088]
28. Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R. Oncogene. 2008; 27:6407–6418. [PubMed: 18955969]
29. Cardenas C, Miller RA, Simth I, Bui T, Molgo J, Muller M, Vais H, Cheung K-H, Yang J, Parker I, Thompson CB, Birnbaum MJ, Hallows KR, Foskett JK. Cell. 2010; 142:270–283. [PubMed: 20655468]
30. Szalai G, Krishnamurthy R, Hajnoczky G. EMBO J. 1999; 18:6349–6361. [PubMed: 10562547]
31. Hajnoczky G, Csordas G, Das S, Garcia-Perez C, Saotome M, Sinha RS, Yi M. Cell Calcium. 2006; 40:553–560. [PubMed: 17074387]
32. Orrenius S, Zhivotovsky B, Nicotera P. Nature Rev. Mol. Cell Biol. 2003; 4:552–565. [PubMed: 12838338]
33. Joseph SK, Hajnoczky G. Apoptosis. 2007; 12:951–968. [PubMed: 17294082]
34. Takahama Y. Nat Rev Immunol. 2006; 6:127–135. [PubMed: 16491137]
35. Hockenberry DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ. Proc. Natl. Acad. Sci. USA. 1991; 88:6961–6965. [PubMed: 1871110]
36. Linette GP, Grusby MJ, Hedrick SM, Hansen TH, Glimcher LH, Korsmeyer SJ. Immunity. 1994; 1:197–205. [PubMed: 7889408]
37. Hogquist KA. Curr Opinion Immunol. 2001; 13:225–231.
38. Neilson JR, Winslow MM, Hur EM, Crabtree GR. Immunity. 2004; 20:255–266. [PubMed: 15030770]
39. Cante-Barrett K, Gallo EM, Winslow MM, Crabtree GR. J Immunol. 2006; 176:2299–2306. [PubMed: 16455986]

40. Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ. Cell. 1993; 75:229–240. [PubMed: 8402909]
41. Strasser A, Harris AW, Cory S. Cell. 1991; 67:889–899. [PubMed: 1959134]
42. Strasser A, Harris AW, von Boehmer H, Cory S. Proc Natl Acad Sci U S A. 1994; 91:1376–1380. [PubMed: 8108419]
43. St Clair EG, Anderson SJ, Oltvai ZN. J Biol Chem. 1997; 272:29347–29355. [PubMed: 9361016]
44. Williams O, Halligey NTM, Kioussis D, Brady HJM. J Exp Med. 1998; 188:1125–1133. [PubMed: 9743531]
45. Berridge MJ. Critical Reviews in Immunology. 1997; 17:155–178. [PubMed: 9094451]
46. Gallo EM, Cante-Barrett K, Crabtree GR. Nat Immunol. 2006; 7:25–32. [PubMed: 16357855]
47. Zhong F, Davis MC, McColl KS, Distelhorst CW. J Cell Biol. 2006; 172:127–137. [PubMed: 16391001]
48. Lewis RS. Biochem Soc Transactions. 2003; 31:925–929.
49. Fracchia KM, Pai CY, Walsh CM. Front Immunol. 2013; 4:324. [PubMed: 24133495]
50. Erin N, Billingsley ML. Brain Res. 2004; 1014:45–52. [PubMed: 15212990]
51. Randriamampita C, Trautmann A. Biology of the Cell. 2003; 96:69–78. [PubMed: 15093129]
52. Donnadieu E, Bismuth G, Trautmann A. J Biol Chem. 1992; 267:25864–25872. [PubMed: 1464601]
53. Mariathasan S, Bachmann MF, Bouchard D, Ohteki T, Ohashi PS. J Immunol. 1998; 161:6030–6037. [PubMed: 9834085]
54. McConkey DJ, Hartzell P, Amador-Perez JF, Orrenius S, Jondal M. J Immunol. 1989; 143:1801–1806. [PubMed: 2528581]
55. Bhakta NR, Oh DY, Lewis RS. Nat Immunol. 2005; 6:143–151. [PubMed: 15654342]
56. Tomida T, Hirose K, Takizawa A, Shibasaki f, Lino M. Embo J. 2003; 22:3825–3832. [PubMed: 12881417]
57. Popgeorgiev N, Bonneau B, Ferri KF, Prudent J, Thibaut J, Gillet G. Dev Cell. 2011; 20:663–676. [PubMed: 21571223]
58. Prevarskaya N, Skryma R, Shuba Y. Nat Rev Cancer. 2011; 11:609–618. [PubMed: 21779011]
59. Rong Y, Aromolaran AS, Bultynck G, Zhong F, Li X, McColl KS, Herlitze S, Matsuyama S, Roderick HL, Bootman MD, Mignery GA, Parys JB, DeSmedt H, Distelhorst CW. Mol Cell. 2008; 31:255–265. [PubMed: 18657507]
60. Rong Y, Bultynck G, Aromolaran AS, Zhong F, Parys JB, De Smedt H, Mignery GA, Roderick HL, Bootman MD, Distelhorst CW. Proc Natl Acad Sci. 2009; 106:14397–14402. [PubMed: 19706527]
61. Palmer AE, Jin C, Reed JC, Tsien RY. Proc Natl Acad Sci U S A. 2004; 101:17404–17409. [PubMed: 15585581]
62. Hanson CJ, Bootman MD, Distelhorst CW, Wojcikiewicz RJ, Roderick HL. Cell Calcium. 2008; 44:324–338. [PubMed: 18407350]
63. Schulman JJ, Wright FA, Kaufmann T, Wojcikiewicz RJ. J Biol Chem. 2013; 288:25340–25349. [PubMed: 23884412]
64. Volpe P, Alderson-Lang BH. Am J Physiol. 1990; 258:C1086–C1091. [PubMed: 2163198]
65. Dremina ES, Sharov VS, Kumar K, Zaidi A, Michaelis EK, Schoneich C. Biochem J. 2004; 383:361–370. [PubMed: 15245329]
66. Dremina ES, Sharov VS, Schoneich C. Biochem J. 2012; 444:127–139. [PubMed: 22360692]
67. Kuo TH, Kim H-RC, Zhu L, Yu Y, Lin H-M, Tsang W. Oncogene. 1998; 17:1903–1910. [PubMed: 9788433]
68. Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M, Pozzan T, Korsmeyer SJ. Proc Natl Acad Sci U S A. 2005; 102:105–110. [PubMed: 15613488]
69. Wagner LE, Joseph SK, Yule DI. J Physiol. 2008; 586 357-3596.
70. Xu L, Kong D, Zhu L, Zhu W, Andrews DW, Kuo TH. Mol Cell Biochem. 2007; 295:153–165. [PubMed: 16874461]
71. Tang TS, Tu H, Wang Z, Bezprozvanny I. J Neurosci. 2003; 23:403–415. [PubMed: 12533600]

72. Tu H, Tang T-s, Wang Z, Bezprozvanny I. J Biol Chem. 2004; 279:19375–19382. [PubMed: 14982933]
73. Erin N, Lehman RAW, Boyer PJ, Billingsley ML. Neuroscience. 2003; 117:557–565. [PubMed: 12617962]
74. Walaas SI, Hemmings HC Jr, Greengard P, Nairn AC. Front Neuroanat. 2011; 5:1–17. [PubMed: 21373368]
75. Tang TS, Bezprozvanny I. J Biol Chem. 2004; 279:42082–42094. [PubMed: 15292232]
76. Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P. Annu Rev Pharmacol Toxicol. 2004; 44:269–296. [PubMed: 14744247]
77. Chang MJ, Zhong F, Lavik AR, Parys JB, Berridge MJ, Distelhorst CW. Proc Natl Acad Sci U S A. 2014; 111:1186–1191. [PubMed: 24395794]
78. Li C, Fox CJ, Master SR, Bindokas VP, Chodosh LA, Thompson CB. Proc Natl Acad Sci U S A. 2002; 99:9830–9835. [PubMed: 12118121]
79. Bonneau B, Nougarede A, Prudent J, Popgeorgiev N, Peyrieras N, Rimokh R, Gillet G. Sci Signal. 2014; 7:ra14. [PubMed: 24518293]
80. Kelly PN, Strasser A. Cell Death Differ. 2011; 18:1414–1424. [PubMed: 21415859]
81. Koehler BC, Scherr AL, Lorenz S, Urbanik T, Kautz N, Elssner C, Welte S, Bermejo JL, Jager D, Schulze-Bergkamen H. PLoS One. 2013; 8:e76446. [PubMed: 24098503]
82. Placzek WJ, Wei J, Kitada S, Zhai D, Reed JC, Pellecchia M. Cell Death Dis. 2010; 1:e40. [PubMed: 21364647]
83. Leanza L, Biasutto L, Managо A, Gulbins E, Zoratti M, Szabo I. Front Physiol. 2013; 4:227. [PubMed: 24027528]
84. Roderick HL, Cook SJ. Nat Rev Cancer. 2008; 8:361–365. [PubMed: 18432251]
85. Juin P, Geneste O, Gautier F, Depil S, Campone M. Nat Rev Cancer. 2013; 13:455–465. [PubMed: 23783119]
86. Monaco G, Decrock E, Akl H, Ponsaerts R, Vervliet T, Luyten T, De Maeyer M, Missiaen L, Distelhorst CW, De Smedt H, Parys JB, Leybaert L, Bultynck G. Cell Death Differ. 2012; 19:295–309. [PubMed: 21818117]
87. Akl H, Monaco G, La Rovere R, Welkenhuyzen K, Kiviluoto S, Vervliet T, Molgo J, Distelhorst CW, Missiaen L, Mikoshiba K, Parys JB, De Smedt H, Bultynck G. Cell Death Dis. 2013; 4:e632. [PubMed: 23681227]
88. Thrower EC, Hagar RE, Ehrlich BE. TRENDS in Pharmacological Sciences. 2001; 22:580–586. [PubMed: 11698102]
89. Eckenrode EF, Yang J, Velmurugan GV, Foskett JK, White C. J Biol Chem. 2010; 285:13678–13684. [PubMed: 20189983]

Highlights

- Marked elevation of intracellular calcium induces apoptosis.
- Inositol 1,4,5-trisphosphate receptors mediate calcium elevation.
- Bcl-2 binds to inositol 1,4,5-trisphosphate receptors to control calcium elevation.
- Bcl-2 inhibits pro-apoptotic calcium elevation in cancer cells.
- The control of calcium by Bcl-2 is a potential target for cancer therapy.

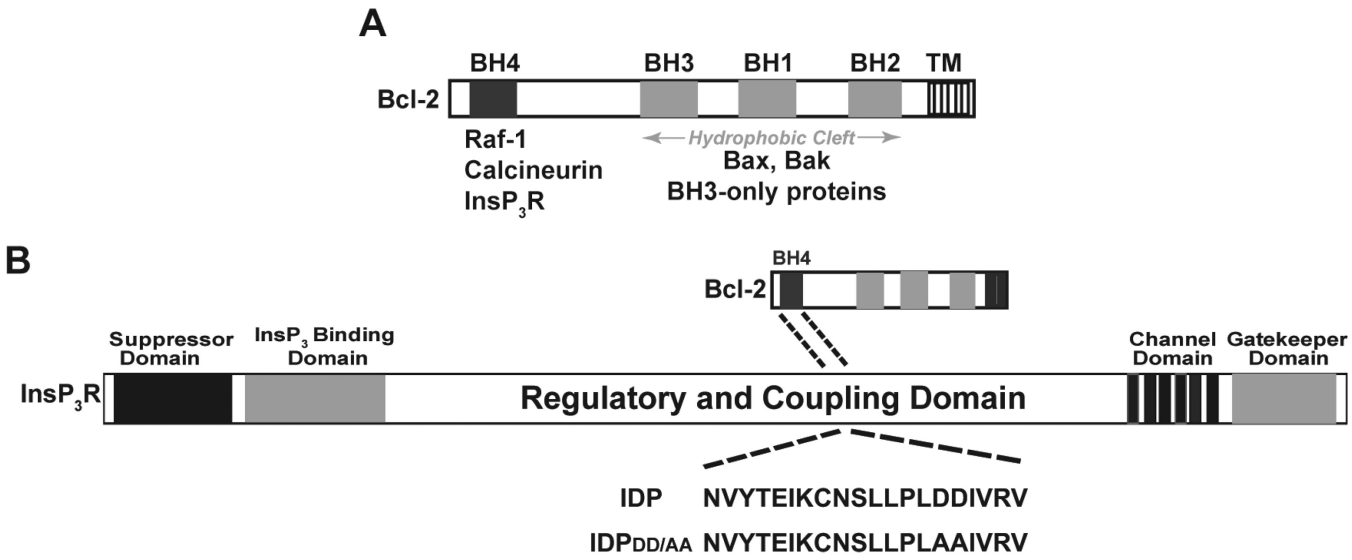

Figure 1. Bcl-2-InsP3R interaction and its peptide inhibitor

(A) The location of Bcl-2 homology (BH) domains is shown, together with proteins known to interact with the BH4 domain and with a hydrophobic cleft composed of BH 1-3 domains. (B) The diagram illustrates the interaction of the BH4 domain of Bcl-2 with a region within the regulatory and coupling domain of the $InsP_3R$. IDP is a synthetic peptide corresponding to a 20 amino acid sequence within the Bcl-2 binding site on the $InsP_3R$. $IDP_{DD/AA}$ is a modification of IDP that eliminates a predicted cleavage site and increases the peptide's activity when introduced into cells by fusion with the cell penetrating peptide of HIV TAT.

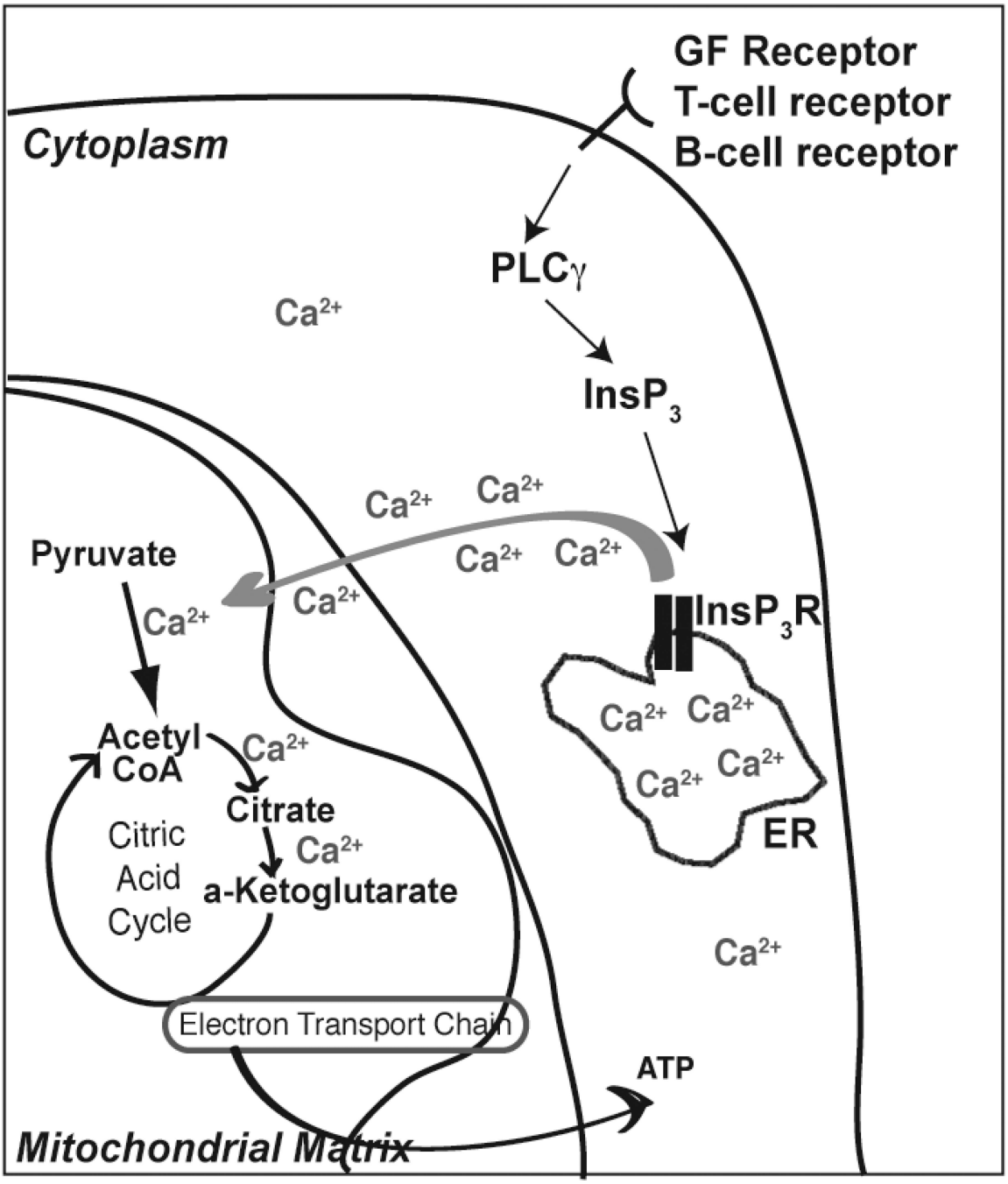

Figure 2. ER-mitochondria Ca^{2+} transfer

$InsP_3R$-mediated transfer of Ca^{2+} from the ER lumen into the mitochondrial matrix is of vital importance since Ca^{2+} activates multiple steps in the citric acid cycle, promoting ATP production.

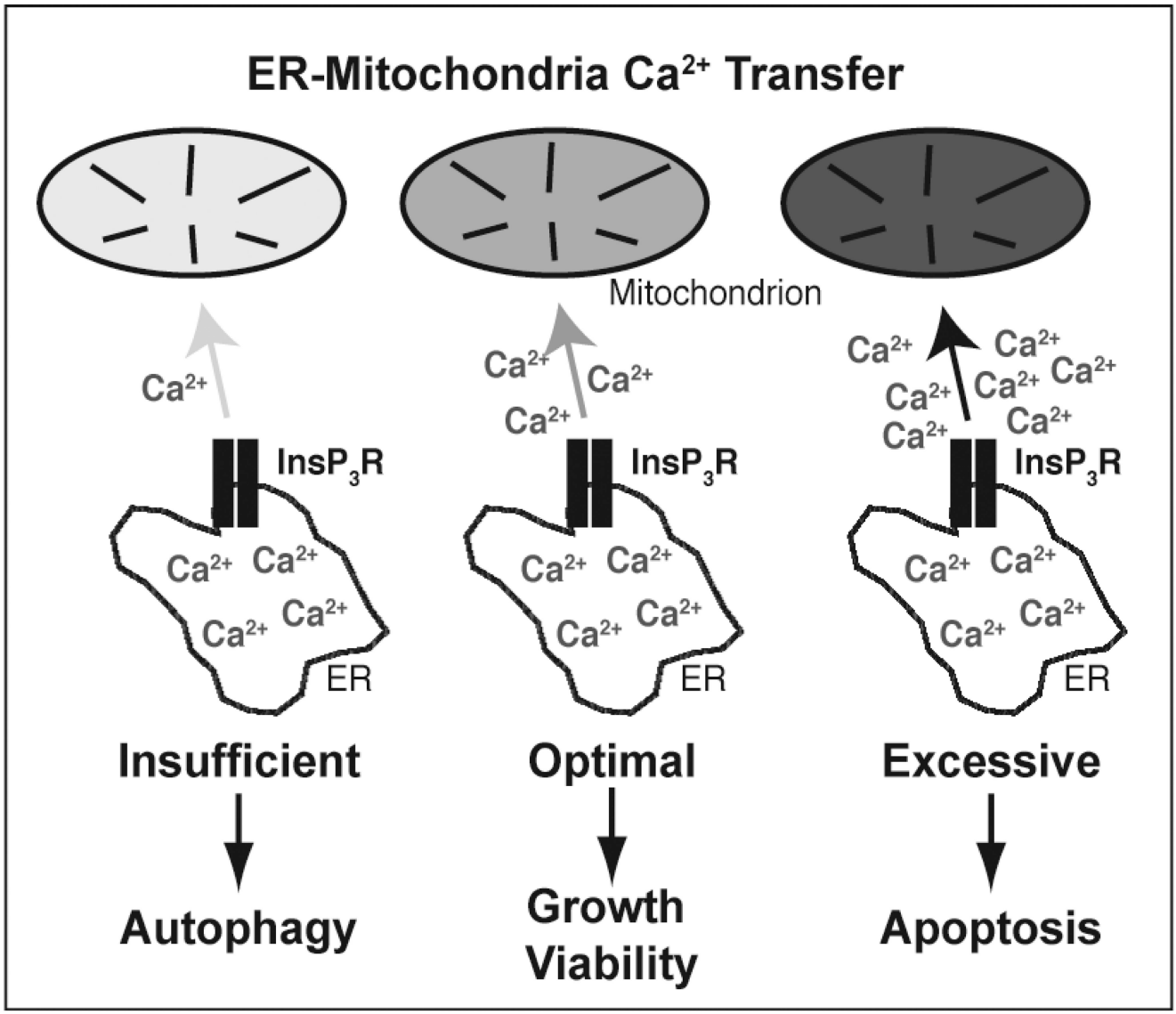

Figure 3. Control over $InsP_3R$-mediated Ca^{2+} release and its importance

The consequences of insufficient or excessive Ca^{2+} transfer from ER to mitochondria illustrate why cells have developed a number of mechanisms, including interaction with Bcl-2, to control $InsP_3R$ channel opening and Ca^{2+} release.

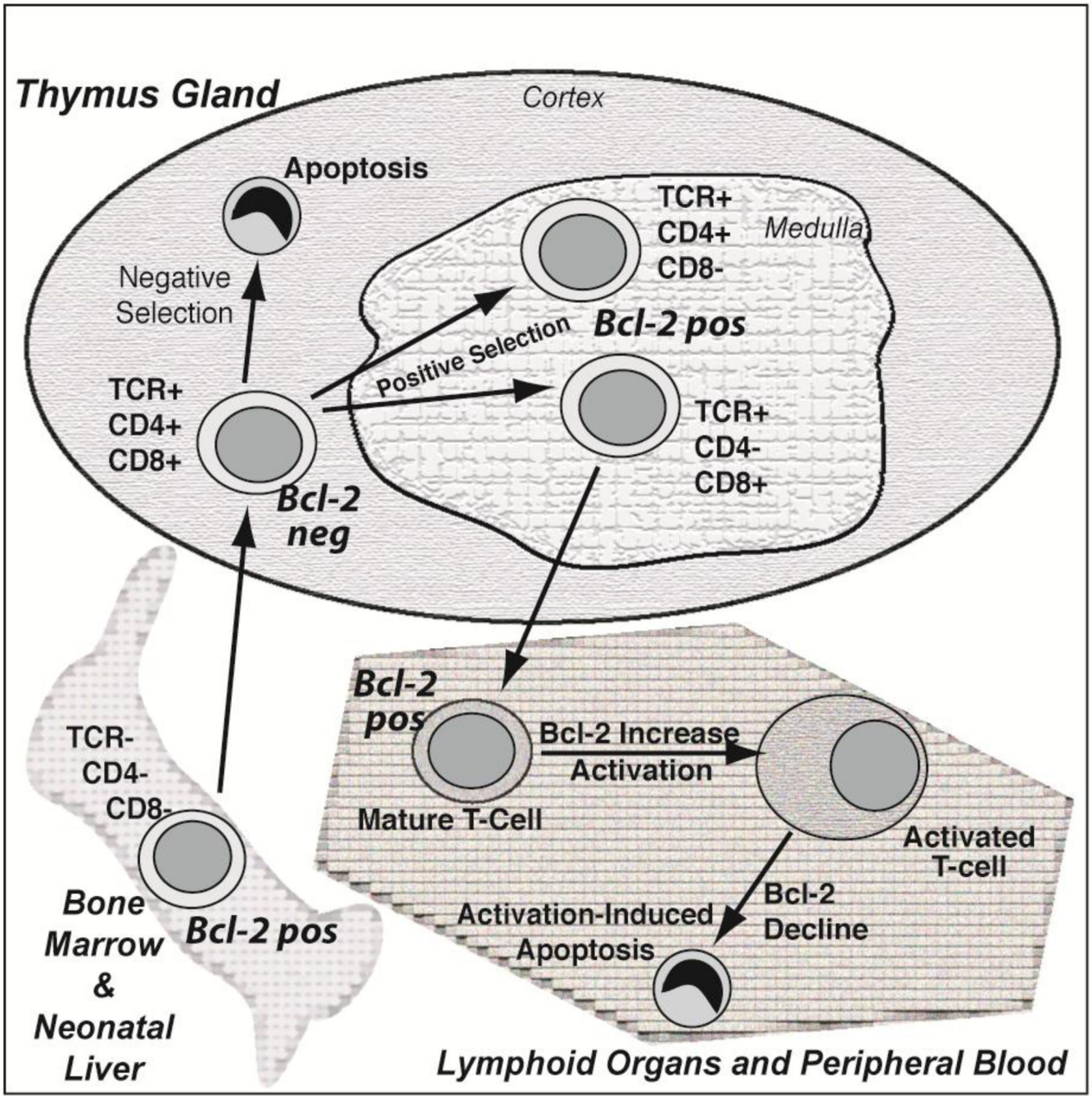

Figure 4. Dynamic fluctuations in Bcl-2 levels throughout T-cell development and adult life

The complexity of the immune system is dependent, in part, upon variation in Bcl-2 expression. Bcl-2 levels are sufficient to repress apoptosis during the earliest stages of lymphocyte precursor development in the fetal liver and bone marrow, and during the journey from these distant organs to the thymus gland. Bcl-2 levels then decline upon entry into the thymic cortex, allowing for apoptotic death of most young thymocytes through negative selection, a process that deletes thymocytes that strongly react with self-antigens. Thymocytes that only weakly react with self-antigens are allowed survive in a process of

positive selection and enter the thymic medulla where Bcl-2 levels become elevated in preparation for survival of mature lymphocytes in peripheral organs including blood and lymph nodes. Bcl-2 levels further increase when a mature lymphocyte is activated to proliferate in response to antigenic stimulation, but then decline later as the immune response declines, an apoptotic process referred to as activation-induced cell death.