Contributions of Bcl-xL to acute and long term changes in bioenergetics during neuronal plasticity

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Abstract

Mitochondria manufacture and release metabolites and manage calcium during neuronal activity and synaptic transmission, but whether long term alterations in mitochondrial function contribute to neuronal plasticity that underlies changes in organism behavior patterns is still poorly understood. Although normal neuronal plasticity may determine learning, in contrast a persistent decline in synaptic strength or neuronal excitability may portend neurite retraction and eventual somatic death. Anti-death proteins such as Bcl-xL provide neuroprotection at the neuronal soma during cell death stimuli, but also appear to enhance neurotransmitter release and synaptic growth and development. It is proposed that Bcl-xL performs these functions through its ability to regulate mitochondrial release of bioenergetic metabolites and calcium, its ability to rapidly alter mitochondrial positioning and morphology as well as its role in interacting with proteins that directly alter synaptic vesicle recycling. Bcl-xL translocates acutely to sub-cellular membranes during neuronal activity to achieve these changes. After stressful stimuli, pro-apoptotic cleaved delta N Bcl-xL (ΔN Bcl-xL)-induced mitochondrial ion channel activity leads to synaptic depression and this is regulated by caspase activation. During physiological states of decreased synaptic stimulation, loss of mitochondrial Bcl-xL and low level caspase activation occur prior to the onset of long term decline in synaptic efficacy. The degree to which Bcl-xL changes mitochondrial membrane permeability may control the direction of change in synaptic strength. The small molecule Bcl-xL inhibitor ABT-737 has been useful in defining the role of Bcl-xL in synaptic processes. Bcl-xL is crucial to the normal health of neurons and synapses and its malfunction may contribute to neurodegenerative disease.

Introduction

Discovery of the death promoting or preventing activities of Bcl-2 family proteins provided several major revelations in the field of cancer biology as it became clear that cell death was under genetic control and therefore simple expression products could be targeted for altering the death rate of cancer cells. More recently however, there has been an increase in understanding of the complexity of the role of Bcl-2 family proteins, particularly in the areas of metabolic control, mitochondrial bioenergetics and cell growth. How these bioenergetic-promoting properties of Bcl-2 family members are linked with their cell death function has become a major topic of interest. Perhaps nowhere is this more questioned than in the nervous system. In this important post-mitotic cell population, the entire relevance of Bcl-2 family proteins is suspect. Why use a genetically encoded system to kill neurons that are not supposed to die during an organism's lifetime? On the other side, what would be the role of anti-cell death proteins in protecting from a death that is not anticipated to occur? These questions were and are still puzzling, but some murky areas are now becoming clear. The brain uses more oxygen and glucose than any other organ in the body by far. During periods of stress, even more substrates are required (Hertz and Dienel, 2002). Therefore it is reasonable to assume that stressful periods in the brain are similar in some ways to the highly proliferative state of cancer tissues and may require profound adjustments in metabolism and bioenergetics. Nevertheless, there may be differences in approach of the two systems. For example, cancer cells perform glycolysis at high rates, even when they appear to have oxygen available (DeBerardinis et al., 2008; Wise and Thompson, 2010), whereas the brain is exquisitely sensitive to a loss of oxidative metabolism. Therefore it is very likely that metabolic controls in these two cell types differ, although overlap in strategies certainly occurs.

Neurons may use different adaptive mechanisms not only because of the absence of cell cycling, but also because of differences in cell morphology. Neurons have elaborate processes extending in some cases meters beyond the soma, and these neurites form connections with other cells and often function independently of the soma. The large spatial separation between the neurite endings and the soma may result in differences in metabolism between the two subcellular structures.

One metabolic strategy that may be specific to neurons is the use of ketone bodies as a fuel source to help prevent neuronal damage during epileptic seizures. This metabolic shift is regulated by Bcl-2 family proteins (Gimenez-Cassina et al., 2012; Lutas and Yellen, 2013). Actions of the ketogenic diet and other fuel source changes are complex, however, and not yet fully understood (Bough and Rho, 2007; Hartman et al., 2010). In addition to switching fuel sources, another way in which Bcl-2 family proteins regulate metabolism that may be highly relevant to neuronal synapses is to enhance the efficiency of metabolism by altering the leak of hydrogen ions through the mitochondrial inner membrane. These changes in inner membrane conductance occur in response to persistent neuronal activity (Alavian et al., 2011; Chen et al., 2011a), sensed by mitochondria as increases in calcium uptake into the matrix over a highly selective inner membrane channel called the calcium uniporter (Kirichok et al., 2004; Baughman et al., 2011; De Stefani et al., 2011). Increased calcium uptake stimulates matrix enzymes that carry out oxidative phosphorylation. Underlying organismal behavioral changes, changes in activity level of neurons are encoded as changes in neuronal firing patterns, changes in neurotransmitter release or structural alterations needed for long term modifications in excitability or synaptic strength. Implicit in the understanding of neural and synaptic plasticity is that a decrease in neuronal activity may also cause long term changes and may set in motion a process that results in a long term decline in synaptic strength (synaptic rundown), a decline in excitability and accompanying alterations in metabolism followed by neuritic or somatic

neurodegeneration (Hickman et al., 2008; Li et al., 2010; Olsen and Sheng, 2012). In this review, we will focus on the functions of Bcl-xL to change bioenergetic efficiency of neurons in response to neuronal activity. Bcl-xL is a complex molecule that may alter neuronal excitability and enhance synaptic efficacy over the long term by supporting the neuron with long term mitochondrial metabolic changes, changes in mitochondrial morphology, changes in mitochondrial positioning and with alterations in vesicle pool dynamics that enhance synaptic function. Bcl-xL may also play an important role in sensing synaptic rundown to protect against eventual neurodegeneration by opposing pro-apoptotic activities. Despite all of these "pro-life" functions, anti-apoptotic or full length (FL) Bcl-xL can also undergo post-translational modifications that lead to its own conversion into a deadly pro-apoptotic cleavage product (Clem et al., 1998). This latter function may be important not only for producing cell death upon severe stress, but also may contribute to long term decline in levels of synaptic transmission.

BCL-2 family protein in the nervous system: role in programmed cell death in neurons

Programmed cell death or apoptosis is the genetic predisposition of cells to die (Adams and Cory, 2007) during nervous system development (Kroemer and Reed, 2000) or later in the life of the organism. Failure of the death program can lead to unchecked growth of tumor cell populations, while early onset of cell death signaling may begin the process of neurodegeneration such as in Alzheimer's or Amyotrophic Lateral Sclerosis (Yuan and Yankner, 2000). In addition, during pathological brain insults such as ischemia, infection, or trauma, some brain cells die immediately, but others die a delayed death long after the insult, by turning on programmed death pathways (Banasiak et al., 2000).

Programmed cell death in vertebrate cells is also termed apoptosis. Cell death may be initiated by signaling at the plasma membrane or by intracellular pathways that lead to changes in mitochondria (Christofferson and Yuan, 2010).The final common pathway for programmed cell death in many systems is mitochondrial outer membrane permeabilization (MOMP) (Green and Kroemer, 2004; Dejean et al., 2005; Adams and Cory, 2007). In some cases, particularly after cytosolic and mitochondrial calcium overload such as occurs during neuronal excitotoxicity, MOMP may be triggered by an acute inner membrane depolarization (Whelan et al., 2012). MOMP leads to the release of several inter-membrane space proteins such as cytochrome c (Green and Kroemer, 2004; Martinez-Caballero et al., 2005). Release of cytochrome c compromises the ability of mitochondria to produce ATP and eventually to maintain the mitochondrial inner membrane potential (Gottlieb et al., 2002) and its release also serves to activate downstream cytosolic enzyme pathways including caspases that destroy cellular proteins, resulting in dissolution of cell contents (Youle and Strasser, 2008). Pro-death Bcl-2 family proteins regulate the onset of MOMP by producing permeabilization of the outer mitochondrial membrane through their own ion channel function and by interacting with other mitochondrial molecules. In their canonical role, the anti-apoptotic Bcl-2 family proteins protect cells against MOMP by interacting with, and preventing the activities of, the pro-apoptotic family members (Adams and Cory, 2007).

Three categories of Bcl-2 proteins contribute to the regulation of cell death. These are the anti-apoptotic members (such as Bcl-xL, Bcl-2, and Mcl-1, represented in this review by Bcl-xL), the pro-apoptotic members such as Bax and Bak (represented in this review by Bax or ΔN Bcl-xL), and a large group of BH-3 only proteins such as BID, BAD, PUMA and NOXA (Galonek and Hardwick, 2006). The anti-apoptotic members of the group are similar in structure and sequence to the pro-apoptotic Bax and Bak, and in addition to the BH1, 2 and 3 domains, contain a BH4 domain that is important for the anti-apoptotic features of the molecules (Tsujimoto and Shimizu, 2000; Sugioka et al., 2003). Protein-protein interactions are also important for pore forming capabilities of Bax and Bcl-xL. (Wolter et al., 1997; Kaufmann et al.,

2003). The pore forming capabilities of Bax require activation by unmasking of domains in its three-dimensional structure that help target Bax to the mitochondrial membrane. Its insertion into the membrane and its oligomerization are enhanced by the binding of BID and other BH3-only molecules directly to Bax (Shamas-Din et al., 2013). Sequestration of the BH3-only molecules to prevent their ability to activate the pore-forming capacity of Bax is carried out by Bcl-xL and other BH4-containing molecules (Kim et al., 2006). With the onset of a pro-death signal BID or other BH3 molecules are released from sequestration; they then help activate Bax to form a pore in the mitochondrial outer membrane (Kim et al., 2009).

Bcl-xL levels rise in the brain during development *(*Krajewska et al., 2002) and it has become clear that Bcl-xL is necessary for the ongoing health of developing neurons and synapses in the brain (Li et al., 2008; Li et al., 2013). In adult brain, only BID and Bcl-xL continue to be highly expressed (Krajewska et al., 2002) as *e*ven Bax is down-regulated. Therefore it is of utmost importance to understand the function of Bcl-xL in the adult nervous system. To protect neurons, Bcl-xL enhances the release of ATP and phosphocreatine from mitochondria (Gottlieb et al., 2002) but prevents the release of cytochrome *c* (Kluck et al., 1997; Cheng et al., 2001). Another surprising feature is the ability of Bcl-xL to be converted into a pro-apoptotic molecule (Clem et al., 1998; Ofengeim et al., 2012). Bcl-xL has important membrane altering properties as well as protein-protein interactions that may account for its myriad roles in the nervous system.

Mitochondrial ion channels

BCL-2 family protein form ion channels in mitochondrial outer membranes

Both pro- and anti-apoptotic Bcl-2 family proteins produce ion channel activity in the absence of other mitochondrial proteins when inserted into artificial lipid membranes (Schlesinger et al., 1997; Schendel et al., 1998). The three dimensional structure of Bcl-xL is comprised of 7 alpha helices (Muchmore et al., 1996; Schendel et al., 1998; Lessene et al., 2013). Two outer layers of amphipathic helices serve to screen the long hydrophobic alpha helices from the aqueous domain. A long proline-rich loop found between the first and second helices is absent in the pro-apoptotic members of the family. The loop may be vulnerable to protease digestion, and contains phosphorylation sites. The BH1, 2, and 3 domains of Bcl-xL fold together to give a hydrophobic region involved in homo- and heterodimerization with proteins that contain a BH3 domain such as Bax or BAD. The structure of Bcl-xL mimics in some important ways that of the diphtheria toxin membrane translocation domain and the pore-forming domains of bacterial colicins that kill cells via the formation of a highly conductive ion channel in the plasma membrane. Two helices of Bax and Bcl-xL are insufficient to form a pore (Schendel et al., 1998) but their ability to homo- and heterodimerize with each other and other mitochondrial proteins may provide for interactions critical for their pore-forming ability (Billen et al., 2008; Lovell et al., 2008).

The Bcl-xL channel in lipid bilayers is a non-selective channel that favors the conductance (Minn et al., 1997) of cations over anions and displays multiple conductances,. Both Bax and Bcl-xL display similar channel activity with multiple conductances, but Bcl-xL has a linear conductance, whereas Bax appears to be more rectified toward positive potentials, is more anion-selective than Bcl-xL, and has larger peak conductances (Schlesinger et al., 1997).

Bcl-xL–regulated mitochondrial ion channel activity is important for release of ATP during synaptic activity

Specific targeting of mitochondria to synapses is required for normal synaptic transmission at high frequencies (David and Barrett, 2003). The regulated targeting of mitochondria to sites of high energy demand suggests that the mechanisms of ATP production and release by mitochondria could very well be regulated during frequent synaptic events (Ivannikov et al., 2013). In addition, calcium uptake and re-release by mitochondria during neurotransmitter release regulates short term plasticity (Friel and Tsien, 1994; Tang and Zucker, 1997; Mochida, 2011; Lee et al., 2012; Wan et al., 2012) and enhances enzymatic activity of several TCA cycle enzymes (Wan et al., 1989) (Fig. 1).

Mitochondrial ion channels participate in the management of cytosolic calcium levels and in the release of ATP and are therefore potentially extremely important for the regulation of synaptic transmission (Blaustein et al., 1978) (Fig. 1). Different types of neuronal synapses contain different numbers of mitochondria with slightly different properties, depending on whether the main function of the mitochondria is to provide energy or buffer calcium. At some synapses, oxidative metabolism by mitochondria is crucial to successful neurotransmission (Nguyen et al., 1997). Moreover, mitochondrial bioenergetics improve acutely in synapses that have undergone preconditioning, providing for enhanced oxidative competence (Nguyen et al., 1997) and suggesting that an interaction may exist between neuronal plasticity and mitochondrial plasticity (Nguyen and Atwood, 1994).

The interaction between neuronal activity, calcium influx into mitochondria and energy production was further clarified recently in a study in Drosophila neuromuscular junction (Chouhan et al., 2012) . Using a complex array of imaging techniques including genetically encoded calcium/pH indicators, it was shown that neuronal activity enhanced calcium uptake by mitochondria and stimulated mitochondrial activity, followed by an enhancement in NAD(P)H levels in mitochondria, and a hyperpolarization of the inner membrane potential; these events were inhibited by pharmacological agents that blocked mitochondrial calcium uptake. Interestingly, the level of cytosolic calcium remained similar in different neurons despite their very different firing rates, suggesting that a certain level of cytosolic calcium is optimum for energy production during activity. This specific cytosolic calcium level is most likely achieved by an ideal combination of calcium buffering inside, and extrusion out of, the nerve ending. An exciting implication of these novel findings is that in different cell types, similar cytosolic calcium levels may produce various mitochondrial responses to adjust to increasing energy demands.

The discovery of the molecular substrate for the calcium uniporter ion channel (MCU) at mitochondria inner membrane (Fig. 1) has generated a lot of interest in mechanisms of how this regulation may be achieved (Kirichok et al., 2004; Baughman et al., 2011; De Stefani et al., 2011). One important recent finding is that the MCU is regulated by a gate-keeper called MICU that prevents mitochondrial calcium uptake when matrix/cytosolic calcium is low (Mallilankaraman et al., 2012a), but allows for it when cytosolic calcium becomes elevated during neuronal activity. This protein and another important protein partner, MCUR1 allow for activity-dependent calcium uptake into energized mitochondria in order to control mitochondrial enzymes and ATP production in response to acute elevations in cytosolic calcium (Mallilankaraman et al., 2012b). Although not completely understood yet, these interesting findings portend that sites of minute regulatory adjustment will determine activity-dependent energy responses of mitochondria.

After stimulation of ATP production, release of ATP from mitochondria into the cytosol is under the modulatory control of Bcl-xL through its interaction with the voltage dependent anion

channel (VDAC) (Vander Heiden et al., 2001) (Fig. 1). VDAC regulates the uptake of ADP and other metabolites as well as the release of ATP from mitochondria during normal cell activities (Rostovtseva and Colombini, 1997; Mannella and Kinnally, 2008; Maldonado and Lemasters, 2012). Bcl-xL is known to regulate the conductance of VDAC in mitochondrial outer membranes to release ATP during cell death stimuli in cancer cell lines. Bcl-xL, in its anti-apoptotic role, enhances VDAC conductance after a cell death stimulus to enhance ATP release. The extra ATP helps the cell overcome stress and improves the probability of survival (Gottlieb et al., 2002).

If Bcl-xL contributes to ATP release in cancer cells, it may also do so during normal nervous system function. The first evidence that mitochondrial ion channel activity could be regulated to enhance neurotransmitter release came from studies of mitochondrial membrane conductance during synaptic transmission in an intact presynaptic terminal, that of the squid stellate ganglion. Through the use of a double-barreled patch pipette (Jonas et al., 1997) recordings were made both at rest and during and after intense synaptic stimulation (Jonas et al., 1999).

In control recordings within the resting presynaptic terminal, the conductance of mitochondrial membranes was found to be low. In contrast, during frequent electrical stimulation of the presynaptic nerve, a large increase in mitochondrial membrane activity occurred (Jonas et al., 1999). The delay in onset of the mitochondrial activity and the persistence of the mitochondrial activity after stimulation implied that mitochondrial channel activity is not simultaneous with plasma membrane channel activity. This suggested that the increase in activity depended on an intracellular second messenger, most likely calcium (Csordas et al., 2012). In keeping with this, mitochondrial activity was abrogated by removing calcium from the bathing medium during stimulation, demonstrating that the evoked mitochondrial membrane channel activity was dependent on calcium influx into the terminal and by extension into mitochondria (Jonas et al., 1999). In addition, the uncoupler FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone), which depolarizes mitochondria and prevents calcium uptake, eliminated the mitochondrial channel activity. Furthermore, the acute changes in mitochondrial membrane activity were found to be important for short term synaptic plasticity, because FCCP application eliminated short term (posttetanic) potentiation of the synapse following high frequency nerve stimulation.

In summary, calcium uptake by mitochondria inside the synapse triggers mitochondrial ion channel activity that can be recorded with a patch pipette positioned on the mitochondrial outer membrane. Based on the known literature, the findings suggested that a calcium sensitive site within the matrix or the inner face of the outer membrane could trigger the opening of a protein complex that spans the two membranes (Kinnally et al., 1991; Szabo et al., 1992; Petronilli et al., 1999; Halestrap, 2005). Such channel activity could be important for the efflux of calcium as well as ATP and other ions and metabolites from the matrix into the cytosol during short term synaptic potentiation. In addition, calcium efflux from mitochondria during short term synaptic plasticity may also require the mitochondrial sodium/calcium exchanger (NCLX) (Friel and Tsien, 1994; Boyman et al., 2013) (Fig. 1), but whether this exchanger was featured in the recordings is not known. Bcl-xL was found to be necessary for the mitochondrial ion channel activity recorded during synaptic transmission, because a specific inhibitor of Bcl-xL, ABT-737, prevented the activity. ABT-737 is a mimetic of BH3-only proteins such as BAD that bind to Bcl-xL with high affinity within a pocket of the three-dimensional structure that usually binds pro-apoptotic BH-3-containing proteins (Oltersdorf et al., 2005). In cancer cell lines, ABT-737 effectively induces cell death possibly via its ability to displace from full length Bcl-xL the pre-bound pro-apoptotic proteins Bax and Bak (Oltersdorf et al., 2005). In synaptic studies, ABT-737

greatly attenuated mitochondrial ion channel activity recorded during normal synaptic transmission (at times when no cell death was occurring). In addition, marked changes in synaptic response properties were observed after exposure to ABT-737 (Hickman et al., 2008), suggesting that Bcl-xL forms an integral part of the channel complex and that Bcl-xL-regulated mitochondrial activity even in the absence of pro-apoptotic players provides an important modulatory role in synaptic transmission.

Because Bcl-xL contributes to mitochondrial ion channel activity that changes mitochondrial membrane conductance within the presynaptic terminal, Bcl-xL might influence the release of calcium or metabolites into the cytosol that could in turn regulate synaptic responses. In support of this, injection of recombinant Bcl-xL protein into the presynaptic terminal enhanced the rate of rise of postsynaptic responses, resulting in earlier evoked action potentials in the postsynaptic cell compared to control synapses (Jonas et al., 2003). Surprisingly, recombinant Bcl-xL protein potentiated both healthy synapses and those in which the synapse was no longer responding. Injection of Bcl-xL protein into the terminal restored suprathreshold responses in these unhealthy synapses, effectively bringing them “back to life.”

In the synapse as during death stimuli, Bcl-xL might regulate the flux of metabolites across the outer mitochondrial membrane by altering the conductance of VDAC (Vander Heiden et al., 2001; Gottlieb et al., 2002). In keeping with cell line studies, injection of ATP into the synapse enhanced synaptic transmitter release to a similar magnitude as that produced by Bcl-xL injection (Jonas et al., 2003). ATP injection was further found to occlude the effect of Bcl-xL, suggesting that the two agents were acting via the same mechanism. Taken together, these findings suggest a testable model in which the conductance changes of mitochondrial membranes during and after synaptic activity involve a calcium-uptake-dependent increase in ATP release from mitochondria, and raise the intriguing question of whether calcium may also stimulate the manufacture of ATP. These processes clearly fall under the regulation of Bcl-xL and could prove to be as long lasting as the enhancement of synaptic efficacy.

To begin to test these ideas in the mammalian hippocampus, where long term changes in synaptic responsiveness may underlie learning and memory formation, Bcl-xL was over-expressed or depleted in cultured hippocampal neurons (Li et al., 2008). In this system, Bcl-xL overexpression was associated with an increase in mitochondrial targeting to synaptic sites, a large increase in resting cytosolic ATP levels (Alavian et al., 2011), and an enhancement in spontaneous and evoked synaptic responses (Li et al., 2008; Li et al., 2013). Depletion of Bcl-xL produced the opposite findings. Furthermore, Bcl-xL overexpression was correlated with structural alterations in the synapse including enhanced expression of synaptic vesicle numbers, increased synaptic vesicle markers and an increase in postsynaptic markers, consistent with an increase in size and number of synapses. The findings implied that there might be a link between the long lasting metabolic changes in the Bcl-xL overexpressing neurons and permanent alterations of synaptic structure characteristic of synapses with recently strengthened synaptic release properties.

Pro-apoptotic proteolytic cleavage fragment of Bcl-xL causes large conductance mitochondrial ion channel activity correlated with hypoxic synaptic failure

Processes that use a lot of energy such as synaptic vesicle recycling and membrane pumps that maintain ionic homeostasis may put the neuron at risk for metabolic compromise especially if food sources or oxygen supply are low. Therefore, it may follow that severe neuronal stress could result in a long lasting decline in neuronal excitability or in synaptic depression. A decline in neurotransmitter release or recycling can mark a synapse for

elimination, followed subsequently by somatic death if many synapses are sequentially eliminated. Before the onset of decline, a set of changes occurs in mitochondrial membrane activity that negatively affects synaptic function.

Under pro-apoptotic conditions in growth-factor deprived cancer cell lines, Bcl-2 family proteins activate large mitochondrial outer membrane channel activity that participates in release of pro-apoptotic factors from mitochondria (Antonsson et al., 2000; Dejean et al., 2005), either in the absence of any change to the properties of the inner membrane or, as may occur during ischemia (neuronal excitotoxicity), accompanying induction of calcium or oxygen free radical (ROS)-induced depolarization and loss of osmotic regulation (permeability transition) of the inner mitochondrial membrane (Tornero et al., 2011; D'Orsi et al., 2012; Perez-Pinzon et al., 2012). In the synapse, the effects of hypoxia serve as a model to study the role of Bcl-xL in mitochondrial ion channel events activated during severe neuronal injury (Jonas et al., 2004; Jonas et al., 2005) (Fig. 2). The presynaptic terminal is very sensitive to hypoxia, which attenuates synaptic transmission over 10–30 minutes. Patch clamp recordings of mitochondrial membranes at rest during hypoxia reveal large conductance activity not found frequently in controls. The channel activity was found to be larger than that induced by pipette-mediated application of recombinant full length Bcl-xL protein and was instead mimicked by activity of recombinant proteolytically-altered Bcl-xL (ΔN Bcl-xL) that formed a large conductance (Bax-like) channel activity in the outer mitochondrial membrane. In addition, the appearance of the hypoxia-induced channel was prevented by pre-treatment of the synapse with a pan-caspase/calpain inhibitor that prevents the cleavage of Bcl-xL. Appearance of the channel associated with ΔN Bcl-xL during hypoxia most likely arose from specific proteolysis of Bcl-xL and not from general injury, because levels of VDAC were preserved in both caspase/calpian inhibitor-treated and untreated hypoxic synapses, whereas in contrast a decrease in full length Bcl-xL levels during hypoxia was prevented by caspase/calpain inhibition.

Opposite to the response to full length Bcl-xL, when ΔN Bcl-xL protein was injected into the presynaptic terminal during recordings of synaptic transmission, it caused a marked synaptic depression (Jonas et al., 2003; Hickman et al., 2008). The time course of rundown of synaptic responses matched that of hypoxia, suggesting a correlation between the two types of synaptic decline.

More evidence that Bcl-xL protein can produce two different conductance level channel activities came from *in vivo* studies with the Bcl-xL inhibitor ABT-737. When applied to mitochondria within the squid presynaptic terminal just before healthy synaptic transmission, ABT-737 inhibited the channel activity of mitochondrial membranes induced by synaptic stimulation, suggesting that full length Bcl-xL is necessary for this activity (Hickman et al., 2008). Interestingly, however, ABT-737 also was found to inhibit the channel activity of ΔN Bcl-xL (Hickman et al., 2008). During synaptic rundown produced by hypoxia or by direct injection into the synapse of ΔN Bcl-xL, ABT-737 reversed synaptic rundown and enhanced synaptic function, again suggesting that the amplitude of activity at mitochondrial outer membranes may determine the direction of changes in synaptic strength. In addition, ischemic injury in the hippocampus was found to be reversed by ABT-737 in its role to bind to ΔN Bcl-xL. In an *in vivo* model of transient global ischemia, ABT-737 effectively prevented delayed cell death of hippocampal CA1 neurons (Ofengeim et al., 2012). Ischemic death of CA1 neurons was also prevented in a KI mouse containing a form of Bcl-xL resistant to caspase/calpain cleavage (Ofengeim et al., 2012) confirming the specific role of ΔN Bcl-xL in the onset of cell death in hippocampal CA1 neurons during global ischemia.

Synaptic responses decline during long term depression in association with Bcl-xL-regulated Bax-induced mitochondrial channel activity

As we have seen, the enhancement of synaptic responses by full length Bcl-xL, could be related to a difference, not only in size of the conductance of its mitochondrial ion channel compared to that of pro-apoptotic molecules such as Bax or ΔN Bcl-xL, but also to a difference in function of the activity. A key characteristic of the ion channel activity of Bcl-xL is that it can induce ATP exchange across mitochondrial membranes (Vander Heiden et al., 2000; Vander Heiden et al., 2001) while activity of ΔN Bcl-xL or Bax causes release of pro-apoptotic factors such as cytochrome c and in addition causes caspase activation (Fig. 2). The delicate balance between pro- and anti-apoptotic Bcl-2-related functions may thereby regulate mitochondrial metabolism at times of stress and may control the timing of eventual synaptic rundown or death of the soma if neuronal stress overwhelms anti-apoptotic activities (Plas and Thompson, 2002b). Release of factors such as cytochrome c not only activates downstream caspases that inactivate cellular processes, the release also directly compromises mitochondrial function by depriving the mitochondrion of electron transport members; These features may all contribute to the decline in synaptic responses found after full length Bcl-xL cleavage and formation of large conductance outer membrane channel activity.

Long term synaptic depression (LTD) brought on by low frequency stimulation or by cell signaling is a normal mechanism of synaptic plasticity opposite in some ways to long term potentiation (LTP) brought on by high frequency stimulation (Malenka and Bear, 2004). Despite its role in normal synaptic plasticity, however, long term depression can also serve as a marker for a pre-degenerative synaptic state. In hippocampal CA3 to CA1 synapse, low synaptic activity leads to a long lasting decline in synaptic efficacy, brought about in part by removal of postsynaptic receptors (Malinow and Malenka, 2002; Kessels and Malinow, 2009); this state can be quite stable and may never lead to synaptic demise. It has been described recently that mitochondria are important for a form of LTD associated with normal synaptic plasticity in hippocampal CA1 neurons. In the CA1 dendrite, low frequency activity causes Bcl-xL-sensitive mitochondrially-mediated release of cytochrome c followed by low level activation of caspase 3, which leads to the removal of postsynaptic glutamate receptors from the plasma membrane, resulting in a form of LTD (Li et al., 2010) (Fig. 3). In addition to these findings, however, degenerative changes may also be associated with LTD. In synapses treated with the toxic Abeta protein, LTD was be prevented in Bax -/- mice, implying that Bax actions at mitochondria are necessary for this form of degenerative hippocampal LTD (Olsen and Sheng, 2012). In a model of developmental axonal targeting in spinal neurons, both mitochondrial Bax and caspase 6 activation were found to control axonal loss in response to nerve growth factor withdrawal (Nikolaev et al., 2009). In this scenario, the N-terminus of amyloid precursor protein (APP) bound to death receptor 6 (DR6) to initiate an intracellular cascade resulting in mitochondrial-dependent axonal demise. These studies emphasize the importance of the extracellular environment, animal development and animal behavior in regulation of mitochondria to determine the eventual outcome of neurite and synaptic connectivity.

Mitochondrial dynamics

Mitochondrial movement to presynaptic sites regulates synaptic activity

The transport of mitochondria along axons and dendrites is clearly important for targeting of mitochondria to sites of synaptic activity. Mitochondria use cytoskeletal motors for

moving around the cell; they move in both directions along the axon, as well as remain stationary for prolonged periods of time when they are presumably docked at a site where they are needed (Chang and Reynolds, 2006; Kang et al., 2008; Saxton and Hollenbeck, 2012). Mitochondria in neuronal cultures respond to application of the neurotransmitter glutamate by arresting movement and changing morphology into rounded, short forms, dependent on neuronal activity and elevation of cytosolic calcium or zinc (Rintoul et al., 2003; Malaiyandi et al., 2005; Brustovetsky et al., 2009). Docking of mitochondria at synapses also occurs in response to growth factors such as NGF or in response to intracellular signaling pathways (Chada and Hollenbeck, 2004). The anterograde movement of mitochondria employs microtubules and kinesin motors (Tanaka et al., 1998) and mitochondria link to microtubules via adapter proteins. One such complex involves Milton as the adapter binding to kinesin heavy chain (Glater et al., 2006). Milton was the first mutated mitochondrial targeting protein to be identified in Drosophila in a screen for mutations that affect synaptic transmission in the visual system. The mutant photoreceptors were found to contain abundant somatic mitochondria but completely lacked synaptic mitochondria. Milton was found to regulate mitochondrial movement into the presynaptic nerve ending through its binding to kinesin heavy chain, linking mitochondria to microtubules for transport into synaptic endings. This complex then binds to Miro that directly links to mitochondria (Wang and Schwarz, 2009). In keeping with the idea that complex formation is required for normal synaptic targeting, *dMiro*-mutated flies were found to lack mitochondria in the presynaptic terminals of neuromuscular junctions (NMJ); instead, the mitochondria lined up in regular rows in the soma and failed to be escorted out to the neuritic endings. The end result was a severe defect in synaptic bouton shape and size and an absence of normal microtubule loop formation characteristic of mature synapses. Behaviorally, these flies exhibited defects in locomotion and died prematurely. During high frequency activity at these terminals, there was found to be a slight increase in levels of intracellular calcium compared to controls, consistent with a lack of mitochondrial calcium buffering; this resulted in a more rapid fatigue of neurotransmitter release. Miro has recently been described as containing at least two consensus motifs for calcium binding (Macaskill et al., 2009), thereby providing a mechanism for normal unlinking of mitochondria during syaptic docking at sites of high neuronal activity. Syntaphilin has also been identified as an important anchor for mitochondria at synaptic sites (Kang et al., 2008).

After traveling along microtubules, mitochondria arrive at the synapse, where they either dock by a reinforced interaction between dynein and microtubules (Chen et al., 2009) or bind to an actin-based complex. In certain specialized synapses that release neurotransmitter at extremely high frequencies and fidelities there exists a *mitochondrial adherens complex* made for this purpose. The complex is a collection of filaments that tethers mitochondria very closely to the synapse in a regulated fashion, orienting the matrix cristae perpendicular to the active zone (Rowland et al., 2000). It is likely that such an organization of mitochondria within this specialized synapse enables the mitochondria to carry out timed ATP release and calcium buffering linked precisely to neurotransmitter release.

Mitochondrial morphological changes participate in mitochondrial targeting to synaptic sites

As seen for the *mitochondrial adherens complex*, not only are mitochondrial movement and docking important for regulating synaptic function and neuronal excitability, but changes in mitochondrial morphology also markedly affect these processes. Fusion and fission of mitochondria are processes that occur within many cell types (Bossy-Wetzel et al., 2003; Berman and Hollenbeck, 2013) and dynamically affect mitochondrial shape. Whether

mitochondria exist as an interconnected (fused) network or as individual, discrete organelles most likely depends on the requirements of the individual cell type. The equilibrium between fusion of individual mitochondria and fission of mitochondria into two or several mitochondria is a regulated process involving the replication and segregation of mitochondrial DNA (Shaw and Nunnari, 2002). Proteins that control mitochondrial fission in mammals include the GTPase Drp1 (Smirnova et al., 1998) and Fis1 (Mozdy et al., 2000). Proteins that control fusion include OPA1 (for Optic Atrophy Type 1, a dynamin-related GTPase) (Bossy-Wetzel et al., 2003) and Mitofusin 1 and 2 (Legros et al., 2002; Eura et al., 2003). During programmed cell death, mitochondria fragment under the control of the mitochondrial fission proteins (Karbowski and Youle, 2003; Karbowski et al., 2006; Reddy et al., 2011) and some of the features of cell death can be prevented (Lee et al., 2004) by overexpression of Drp1K38A, a dominant negative mutant of Drp1 that prevents mitochondrial fragmentation (Smirnova et al., 1998).

In neurons, however, it has become increasingly clear that mitochondrial fission plays an integral part in enhancing bioenergetic efficacy of the synapse in a role not previously anticipated. Mitochondrial positioning may be required for energy production during synaptic vesicle mobilization, release, and recycling. ATP-dependent steps in synaptic transmission include refilling single vesicles with neurotransmitter (Takamori et al., 2000), membrane fission during endocytosis (Heidelberger, 2001), and coated pit formation (Smythe et al., 1989; Faundez and Kelly, 2000). Recent evidence suggests that ATP is required for normal functioning of vesicle pools. In *Drosophila*, mobilizing the reserve pool requires ATP. An ATP-sensitive motor, the myosin light chain kinase, which moves vesicles from pool to pool in an energy-dependent manner, is affected by the lack of locally released ATP brought on by the absence of mitochondria at the synapse (Verstreken et al., 2005).

Drp1 may employ mitochondrial fission to create more mitochondria to target mitochondria to nascent synapses during development or during times of synaptic plasticity (Li et al., 2008). In a study of the role of Drp1 in mitochondrial targeting during synaptic plasticity in hippocampal neurons (Li et al., 2004) it was found that 8–9% of mitochondria are found within or close to dendritic spines (the site of contact with the presynaptic cell) particularly during active phases of synaptic development. After repetitive depolarization of the neurons, however, mitochondria change shape from elongated structures to aggregated clusters and 21% redistribute rapidly to dendritic spines, suggesting that acute alterations in mitochondrial morphology could play a role in synaptic plasticity.

An important role for phosphorylation of Drp1 in neuritogenesis and synapse formation was found recently in cultured hippocampal neurons (Dickey and Strack, 2011). Reversible phosphorylation of Drp1 regulates its GTPase activity. Phosphorylation silences Drp1, preventing mitochondrial fission. In cultured neurons, phosphorylation of Drp1 is correlated with fused mitochondria and neurite outgrowth, but a decrease in synapse formation. On the other hand, increased mitochondrial fragmentation is observed upon dephosphorylation of Drp1. Fragmentation enhances synapse formation, but unlike studies where activation of Drp1 enhances mitochondrial targeting to synapses, in this study activation of Drp1 by dephosphorylation depleted mitochondria from dendrites and depolarized mitochondria, decreasing the length of the dendrites but promoting synapse formation in the short dendrites. Raising intracellular calcium mimics the dephosphorylated state of Drp1, enhancing synaptic growth. The model supports the idea that neuronal activity is necessary for synapse formation, and may be correlated with mitochondrial arrest and dendritic growth arrest as neurites find their targets, connect to each other and mature. These studies demonstrate the complex behavior of Drp1 in regulation of synapse formation and activity but nevertheless emphasize the role of mitochondrial bioenergetics in regulation of these processes. Focusing on the bioenergetic side

of mitochondrial targeting to the synapse, a recent finding in *Drosophila* describes that Drp1 partially rescues a severe bioenergetic defect involving complex I and IV in Pink1 -/- flies, restoring ATP synthesis rates by improving electron transport assembly. This finding implicates Drp1 in preserving inner membrane integrity for the purpose of bolstering synaptic efficacy (Liu et al., 2011).

A Bcl-xL-Drp1 complex regulates mitochondrial positioning at strengthening synapses

Previous studies supported a role for Bcl-2 family proteins including Bcl-xL in altering lipid membrane morphology in cell-free systems (Rostovtseva et al., 2009). Importantly, however, Bcl-xL may also change membrane morphology through its actions on Drp1. Drp1 acts downstream of Bcl-xL (Li et al., 2008; Berman et al., 2009) and upstream of endophilin B1 in regulating mitochondrial membrane remodeling (Karbowski et al., 2004). In hippocampal neurons, Bcl-xL overexpression resulted in increased synaptic numbers and an increase in size of individual synapses, with more synaptic vesicles per synapse, an increase in pre- and postsynaptic markers, and an enhancement of spontaneous synaptic activity. These findings were found to be linked to Drp1-dependent localization of mitochondria to synaptic sites. When Drp1 was disrupted by expression of a dominant negative form of Drp1 that lacks GTPase activity, synapse number and size were disrupted (Li et al., 2008), accompanied by a markedly enhanced mitochondrial length in axons and dendrites and poor targeting to synaptic sites, presumably in part because of the inability to form small mitochondria to localize to diminutive spines and presynaptic boutons. This function was detailed in a study in which Bcl-xL was found to regulate mitochondrial fission and biogenesis, but not, surprisingly, mitochondrial fusion (Berman et al., 2009).

A Bcl-xL/Drp1 complex regulates synaptic vesicle retrieval at squid giant presynapse and at hippocampal synapses

Parallels exist between mitochondrial remodeling and synaptic vesicle remodelling during endocytosis. Similar molecular players overlap in the two systems such as endophilin family members (Farsad et al., 2001). GTPases are necessary for membrane remodeling; they alter membrane curvature and cause the fission of curved membranes from partner membranes. This can result in, for example, fission of mitochondria or, in the case of synaptic neurotransmitter-containing vesicles, in re-uptake of discharged vesicles after they have undergone their fusion with the plasma membrane and release of contents into the synaptic cleft. Drp1 is recruited to mitochondria via the mitochondrial Drp1 anchor Mff but, surprisingly, also to synaptic vesicle membranes by the same protein (Otera et al., 2010; Li et al., 2013).

Recovery of vesicle pools after synaptic depression is regulated by Bcl-xL. Stimulation of the squid giant synapse at 2 Hz produces synaptic depression as synaptic vesicles are rapidly depleted (Swandulla et al., 1991). After this depletion, more reluctantly releasable vesicles are accessed. During continuing stimulation at 2 Hz, vesicles cannot re-populate the initially releasing or “readily releasable” pool, because of continuing stimulation. Extra time is needed for docking of vesicles at the plasma membrane to re-fill the readily releasable pool (Sakaba and Neher, 2001; Sakaba et al., 2005). Therefore the time course of recovery of more reluctantly releasing pools may be more rapid (Sakaba and Neher, 2001); in squid synapse, recovery to the reluctantly-releasing pool is not affected by injection of recombinant Bcl-xL protein but in contrast recovery to the readily releasable pool is regulated by Bcl-xL (Jonas et

al., 2003). Bcl-xL therefore appears to enhance the ability of a subset of docked or readily-releasable neurotransmitter-containing vesicles to become re-available for release after activity.

If endogenous Bcl-xL is necessary for recovery of synaptic vesicle pools, then ABT-737 might affect the rate of recovery from tetanic stimulation. In control squid synapses, recovery of neurotransmitter release after a tetanus generally occurs in less than two minutes. In contrast, in synapses exposed to ABT-737 just before tetanic stimulation, the rate at which the synapse recovers from high frequency firing is decreased (Hickman et al., 2008).

In hippocampus, distinct pools of vesicles also have different probabilities of release (Kidokoro et al., 2004; Virmani et al., 2006; Kavalali, 2007; Denker and Rizzoli, 2010). The readily-releasable pool in hippocampus is defined as the vesicles that are immediately available for release, or “docked” at the active zone (Fig. 4). In these synapses there appear to be approximately 5–10 vesicles that are docked at each active zone, but a single brief stimulus (such as an action potential) may release only one vesicle. The recycling pool makes up 5-20% of vesicles and is defined as the pool of vesicles that continue to be released and re-accumulate during moderate or physiological stimulation. The reserve pool is defined as those vesicles that only release upon extremely intense stimulation and therefore may often remain unused. The reserve pool of vesicles makes up about 80–90% of the vesicles in most terminals but is variable in size.

To determine the role of Bcl-xL in synaptic vesicle recycling of different pools in hippocampal synapses, a number of methods were used including imaging of fluorescent synaptic vesicle constructs and imaging of styryl dyes taken up into synapses upon stimulation (Li et al., 2013). The findings were complex, but indicated that the rate of release of more rapidly-releasing vesicle pools was enhanced by overexpression of Bcl-xL, and, as in squid, Bcl-xL enhanced the rate of recovery to these more readily-releasable pools. It had been found in other models including *Drosophila* neuromuscular junction that reserve pools of vesicles are regulated by mitochondrial positioning and metabolism (Verstreken et al., 2005). The hippocampal study revealed a surprising result for Bcl-xL regulation. Previous studies had suggested that in hippocampus, the readily releasable pool preferentially refills not from reserve vesicles during prolonged activity, but from re-uptake of vesicles for immediate re-use at the plasma membrane during stimulation (Virmani et al., 2006). Although this process is undoubtedly dependent on ATP, analysis suggested that extra ATP production provided by overexpression of Bcl-xL was not necessary for enhanced re-uptake at the plasma membrane during physiological stimulation. Instead, the more rapid re-accumulation of vesicles to the readily-releasable pool in the presence of Bcl-xL depended on the membrane interacting properties of Drp1 (Li et al., 2013). In this new role for Drp1, the Bcl-xL-Drp1 complex enhanced the rate of vesicle uptake by interacting with one of the main endocytosis regulators, clathrin (Fig. 4). In addition to enhancing the rate of endocytosis during nerve stimulation, the complex helped to form normal vesicle shape within the presynaptic terminal (Li et al., 2013). These findings raised a new dilemma. How are the different jobs of Bcl-xL at mitochondria and at synaptic vesicle membranes coordinated to achieve enhanced synaptic efficacy? The answer is so far unknown.

Regulation of the mitochondrial inner membrane proton leak determines neuronal metabolic efficiency

Bcl-xL interacts with the ATP synthase to enhance mitochondrial metabolism in healthy neurons

As described, the targeting of mitochondria to synaptic sites implies that their function in calcium management and ATP production and release is important for the regulation of synaptic transmission. Mitochondria require substrates to carry out oxidative phosphorylation: Oxidation of substrates hyperpolarizes the mitochondrial membrane potential for ATP production. In growing or proliferating cells, growth factors induce cells to increase nutrient uptake from the environment for mitochondrial metabolism (Plas and Thompson, 2002a). Nutrients provide energy sources and building blocks for cell growth (Vander Heiden et al., 2009). During growth factor withdrawal, apoptotic signals become activated leading to a decrease in use of glycolytic or oxidative substrates and eventually to MOMP and/or mitochondrial inner membrane depolarization.

Interestingly, Bcl-xL participates not only in the release of ATP from mitochondria but in the manufacture of ATP. In healthy neurons overexpressing or depleted of Bcl-xL, neuronal metabolic parameters were altered (Alavian et al., 2011; Chen et al., 2011a). The initial findings were quite striking: Overexpression of Bcl-xL in resting neurons led to a large (almost 100%) increase in cytoplasmic ATP levels. Surprisingly, this was accompanied by a decrease in neuronal oxygen uptake, as measured with oxygen-sensitive electrodes positioned over single neurons, and a decrease in aerobic glycolysis, raising the possibility that Bcl-xL overexpression increases mitochondrial efficiency. Interestingly, Bcl-xL was found to markedly increase oxygen uptake during activity compared to controls, in keeping with previous findings of an increase in mitochondrial biomass and larger synapses (Li et al., 2008; Berman et al., 2009; Alavian et al., 2011). Despite this increase in oxygen uptake, however, calculations reveal that the fraction of total oxygen uptake used to make ATP during activity is much higher in Bcl-xL expressing neurons than controls, consistent with an increase in efficiency of mitochondrial metabolism (Alavian et al., 2011; Chen et al., 2011a). Bcl-xL depletion reversed the effects on metabolism, decreasing ATP production and increasing oxygen uptake by the resting cells.

Bcl-xL closes an inner membrane proton leak across the ATP synthase.

Oxidative phosphorylation is the main source of formation of ATP in neurons and requires coupling of electron transport (H+ pumping out of the mitochondrial matrix) to ADP phosphorylation (movement of H+ ions through the F_1F_O ATP synthase (Complex-V)). This proton motive force can be disrupted by several uncoupling mechanisms and molecules, including uncoupling proteins 1-5 (Andrews et al., 2005). It is therefore possible that the increase in efficiency of ATP production in Bcl-xL overexpressing cells correlates with a decreased proton leak (decreased uncoupling) during ATP synthase enzyme activity (Fig. 5). By patch clamping submitochondrial vesicles (SMVs) enriched in ATP synthase, and by measuring H+ ion movement using the pH sensitive dye ACMA, it was demonstrated that a leak of H+ ions was prevented during enzymatic activity by the addition of ATP or ADP to the medium, but was re-opened by pharmacological or genetic inhibition of Bcl-xL. Using multiple biochemical and imaging approaches, it was found that the site of interaction of Bcl-xL within the F_1F_O ATP synthase was at the beta subunit in the enzymatic portion or F_1 (Alavian et al., 2011; Chen et al., 2011b), although the leak channel must, by its membrane permeabilizing nature, localize to the membrane portion of the synthase, or F_O.

Bcl-xL acutely alters metabolism during neuronal activity

In squid and hippocampus Bcl-xL acutely regulates synaptic activity. To determine if long term changes in metabolic efficiency regulated by Bcl-xL accompany electrical changes at the neuronal plasma membrane, cultures were stimulated briefly during measurements of ATP levels and the localization of Bcl-xL was within subcellular compartments was determined. Stimulation led to acute translocation of Bcl-xL into mitochondria dependent on the calcium sensor calmodulin (Li et al., 2013). Stimulation also led to acute increases in ATP levels in the neurons, and this was inhibited by the specific Bcl-xL inhibitor ABT-737 (Alavian et al., 2011). Taken together, the findings suggest that metabolic changes in the cell that follow neuronal activity are dependent on Bcl-xL movement into mitochondria during the state of neuronal activity.

Conclusions

Bcl-2 family proteins have well-described roles in the initiation of, and protection from, programmed cell death. In another less well understood and more recently reported function, some members of the family, particularly Bcl-xL, may control mitochondrial positioning, morphology and bioenergetics in order to enhance synaptic strength during development and at times of plasticity in the adult nervous system. These Bcl-xL-regulated mitochondrial signals may also lead to synapse formation or elimination. The striking and myriad functions of Bcl-xL in alterations in synaptic efficacy may serve as a link between growth factor signals at the plasma membrane and mitochondrial events inside axons and dendrites. These processes may in turn signal for synaptic strengthening and synapse stabilization or long term depression followed by neurite degeneration. Although occurring very far from the soma in the distal neurites, these events over the long term may eventually lead to neuroprotection or neurodegeneration of the neuronal cell body.

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Figure Legends

Figure 1. Mitochondrial ion channel components of the Bcl-xL-regulated synapse. Calcium enters the synapse in healthy neurons undergoing stimulation and diffuses to mitochondria; mitochondria take up calcium via the mitochondrial calcium uniporter (MCU) channel. Calcium is buffered in the matrix and stimulates TCA cycle activity resulting in an increase in ATP production. ATP is released across the outer mitochondrial membrane via an interaction between Bcl-xL and VDAC. When free calcium becomes elevated in the matrix after frequent stimulation, it binds to and activates a calcium sensitive inner membrane channel, possibly the mitochondrial permeability transition pore (mPTP), as well as the mitochondrial sodium calcium exchanger (NCLX), to re-release calcium into the cytosol. This is followed by rapid pumping of calcium out of the synapse by the plasma membrane calcium ATPases (PMCA). Under physiological conditions, cytosolic residual calcium promotes short term synaptic plasticity.

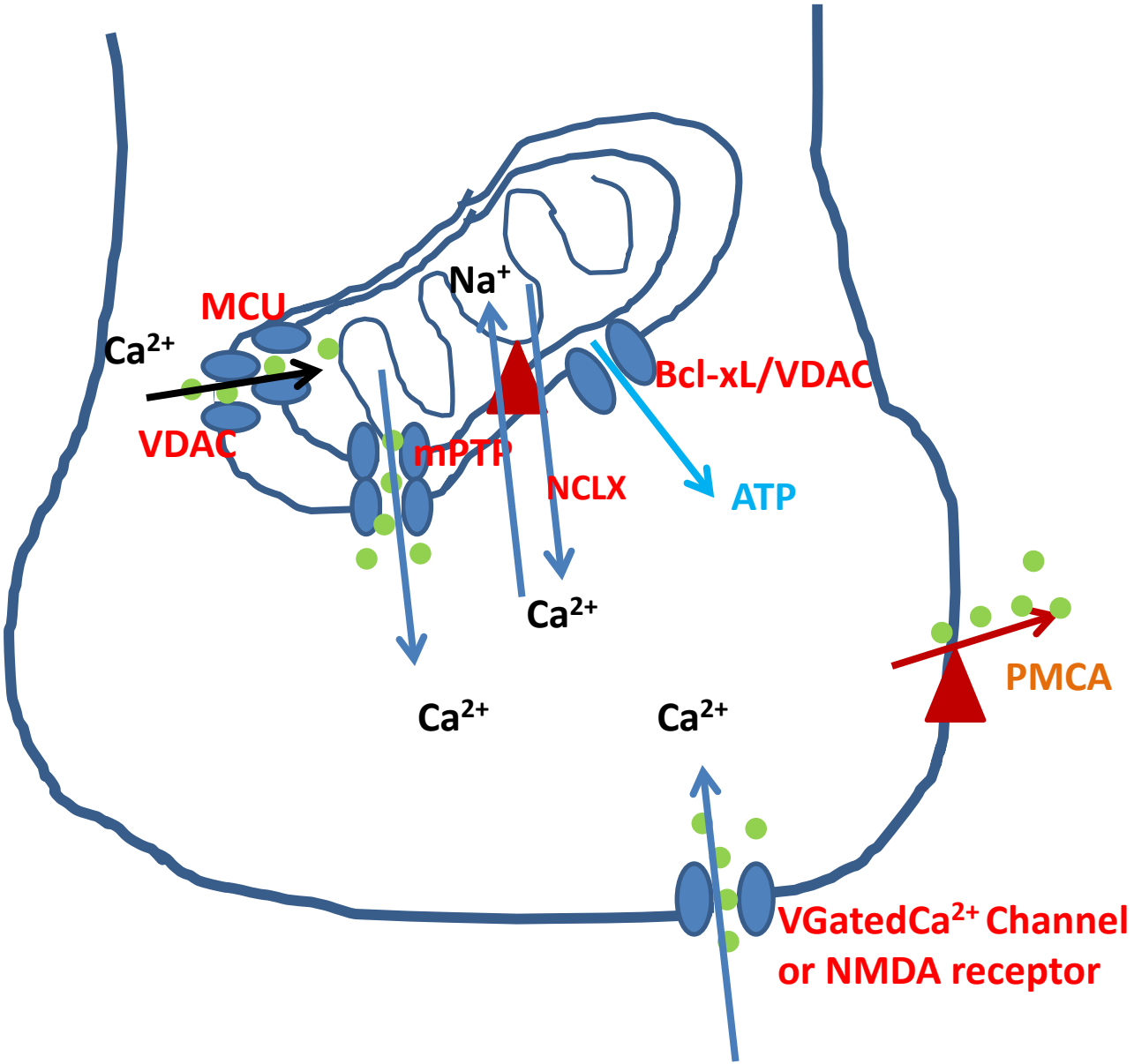
Figure 2. Bcl-xL is cleaved to pro-apoptotic ΔN Bcl-xL during hypoxia. During hypoxia/excitotoxicity, calcium overwhelms the synapse cytosol and mitochondrial matrix. Bcl-xL gets cleaved to ΔN Bcl-xL, which forms large conductance outer mitochondrial membrane channels leading to MOMP. Calcium dysregulation by mitochondria may result in inner membrane depolarization, lack of ATP production and death.

Figure 3. Mitochondrial Bcl-xL participates in determining the strength of synaptic transmission. When synapses undergo frequent, non-pathological activity, calcium buffered by mitochondria stimulates ATP production. Bcl-xL translocates to mitochondria, enhancing ATP production and possibly the ability of mitochondria to buffer calcium during neuronal stimulation. In the opposite condition, when stimulation of the synapse is sub-normal, little calcium enters the cytosol or mitochondria, Bcl-xL levels may decrease in the mitochondria while Bax increases. Low level

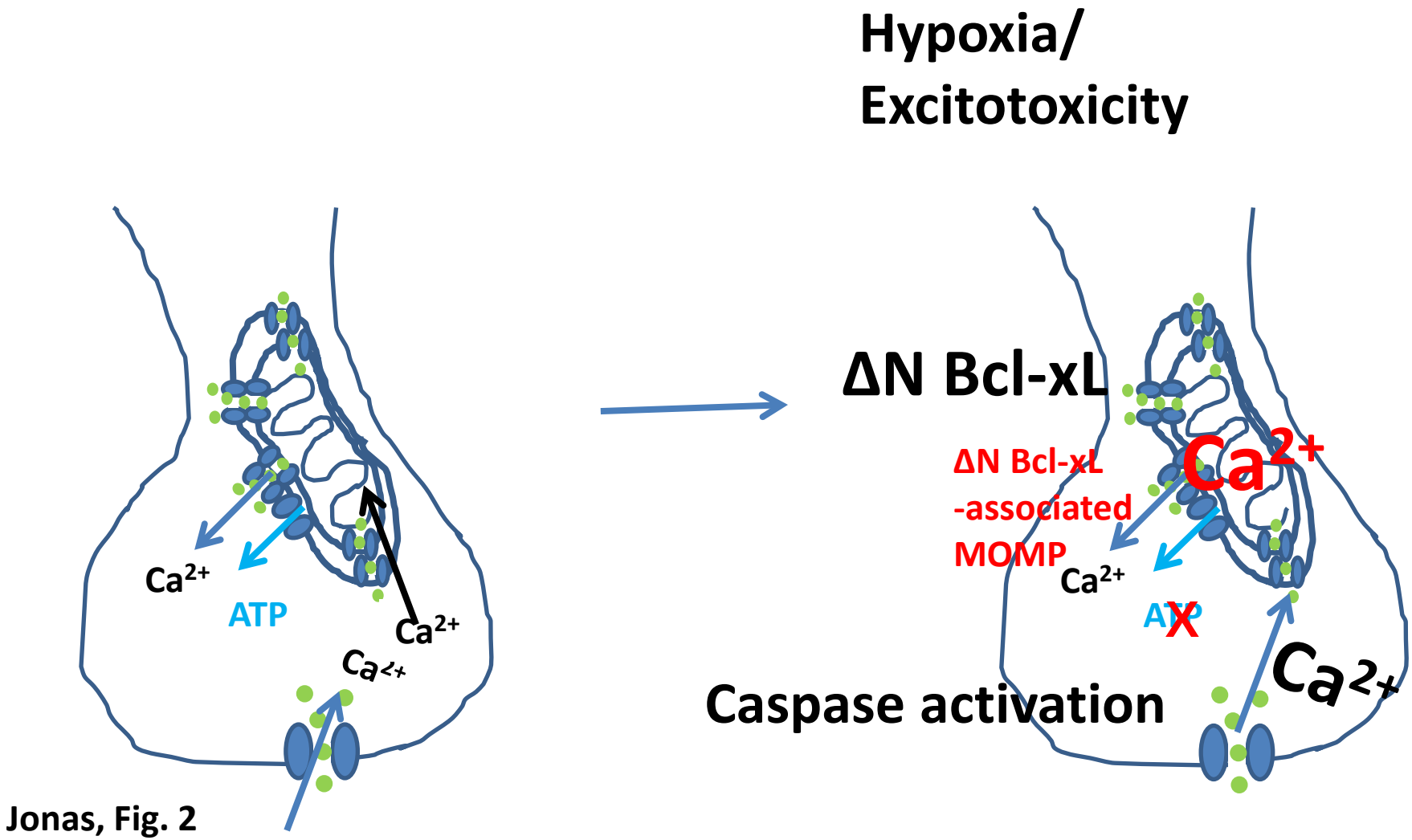
caspase activation occurs. Many of these events at the synapse participate in the onset of long term depression of synaptic responses.

Figure 4. Bcl-xL/Drp1 complex regulates the readily releasable pool at hippocampal and squid synapses. Mitochondria have been shown previously to be important for regulation of reserve pool mobilization. Although Bcl-xL and Drp1, through their actions to regulate mitochondrial positioning, undoubtedly also regulate reserve pool kinetics, new findings indicate that Bcl-xL/Drp1 also interact directly with clathrin at endocytic vesicle membranes to enhance recycling of vesicles after neurotransmitter release. The pool affected by Bcl-xL/Drp1 includes vesicles that are docked and release-ready for fusion. Bcl-xL directly enhances the rate of release from, and recycling to, this readily releasable pool in a calcium/calmodulin dependent manner.

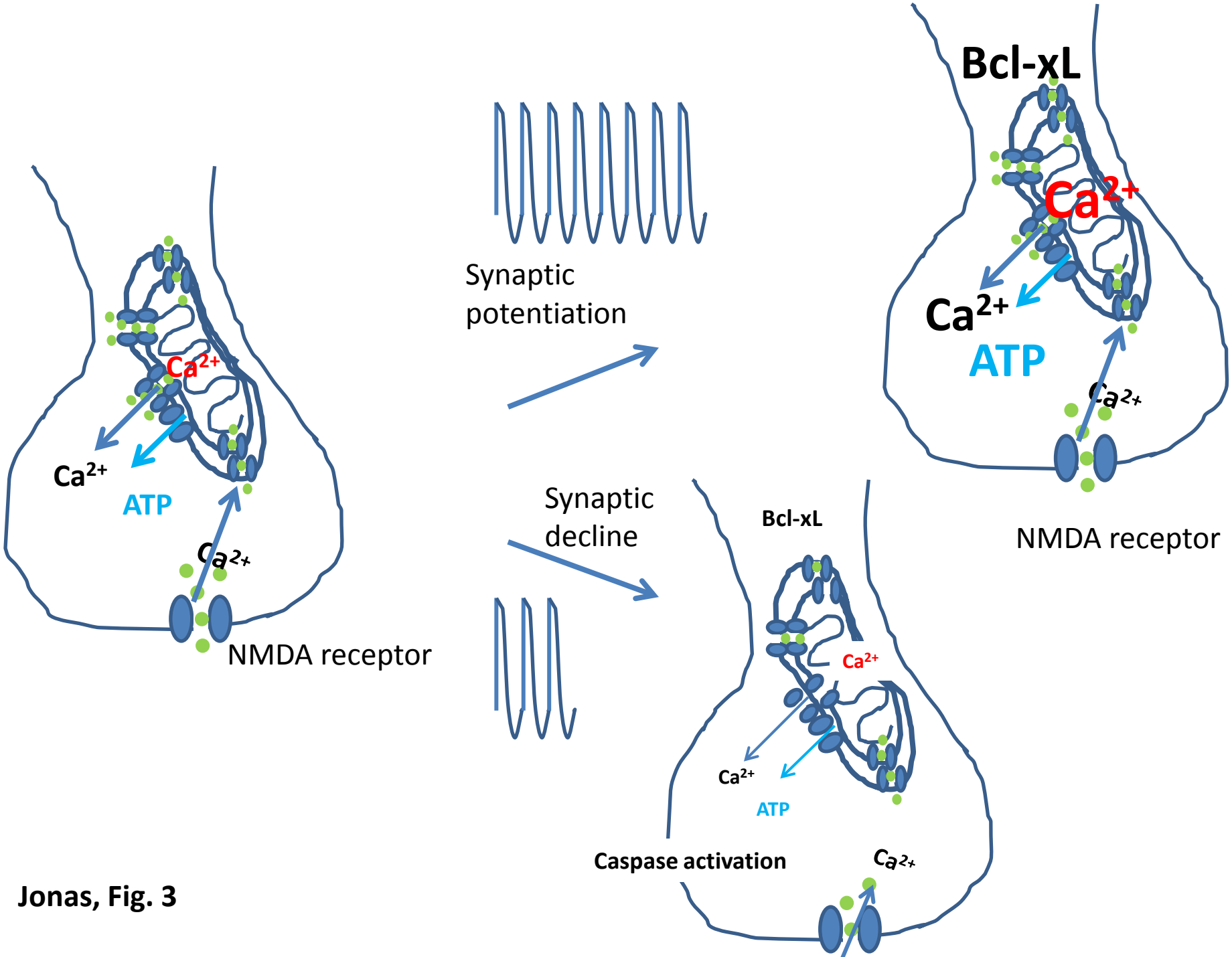
Figure 5. Bcl-xL enhances the efficiency of ATP production at the synapse. Shown is the mitochondrial inner membrane electron transport chain, which pumps out H+ ions to create a gradient across the inner membrane. The energy of the gradient is used to form ATP as H+ ions flow down their concentration gradient across the F_1F_O ATP synthase (complex V). If H+ ions flow through a leak channel, the energy of the gradient will not be used to make ATP and therefore the efficiency ATP synthesis will decrease. Bcl-xL closes the inner membrane leak, enhancing the efficiency of ATP production.


MCU
Ca2+
VDAC
Na+
mPTP
NCLX
Bcl-xL/VDAC
ATP
Ca2+
Ca2+
Ca2+
PMCA
VGatedCa2+ Channel
or NMDA receptor
Calcium

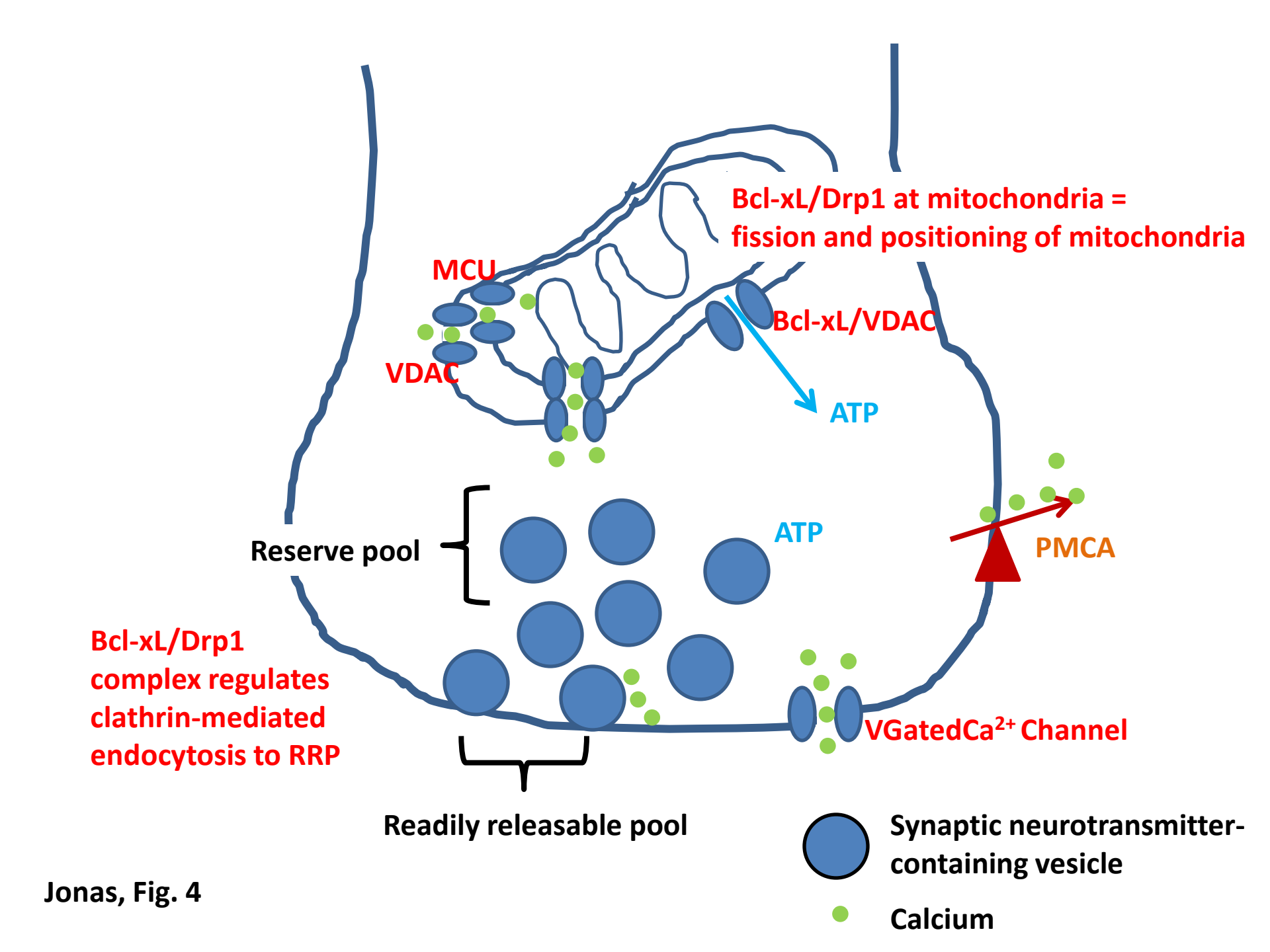
Jonas, Fig. 1


Hypoxia/
Excitotoxicity
ΔN Bcl-xL
ΔN Bcl-xL
-associated
MOMP
Ca2+
Ca2+
ATP
X
Ca2+
Ca2+
Ca2+
Caspase activation

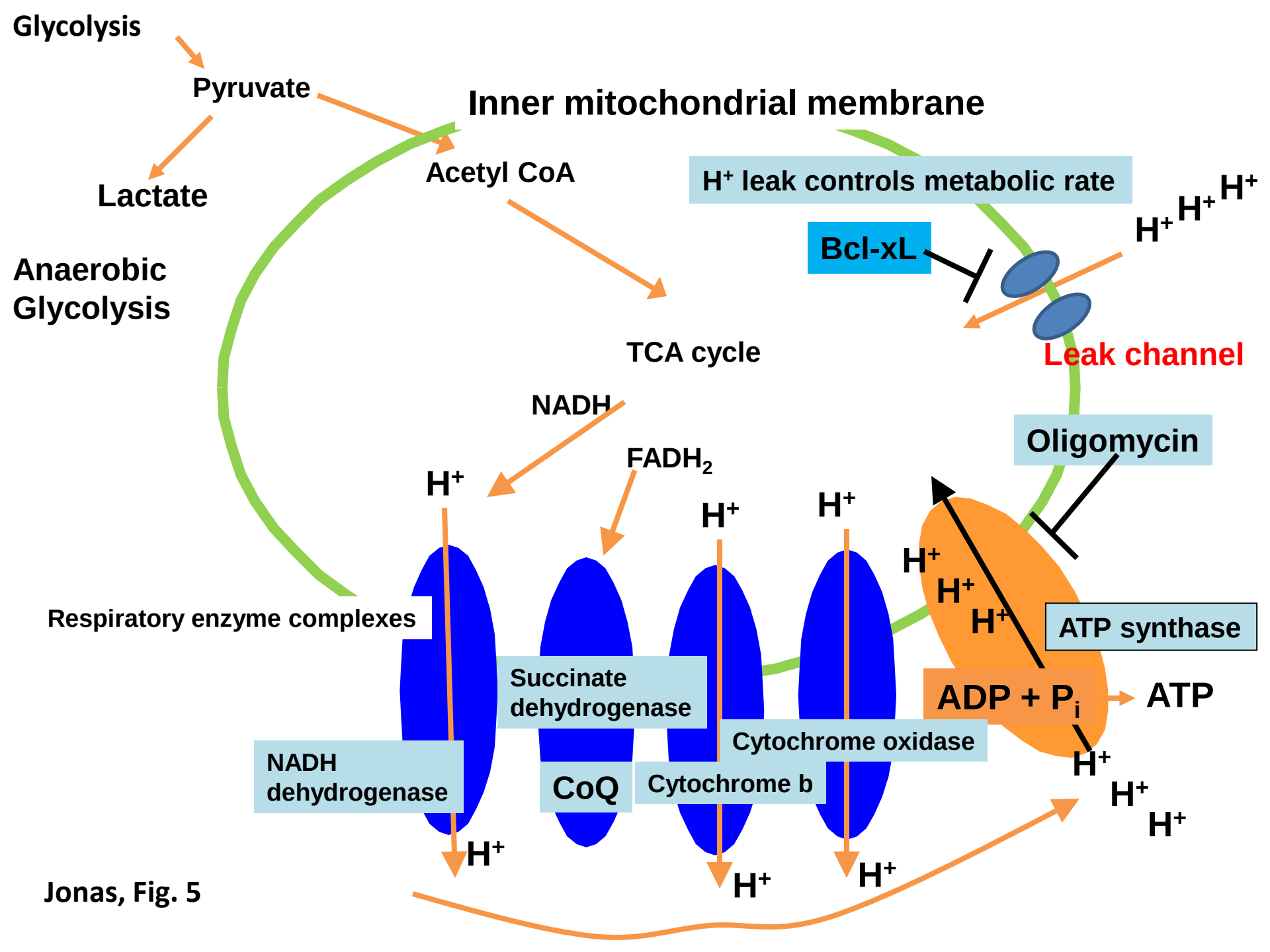
Jonas, Fig. 2


Bcl-xL
Ca2+
Ca2+
ATP
Ca2+
NMDA receptor
Synaptic potentiation
Synaptic decline
Ca2+
Ca2+
ATP
Ca2+
NMDA receptor
Bcl-xL
Ca2+
Ca2+
ATP
Caspase activation
Ca2+

Jonas, Fig. 3


Bcl-xL/Drp1 at mitochondria =
fission and positioning of mitochondria
MCU
VDAC
Bcl-xL/VDAC
ATP
ATP
Reserve pool
PMCA
Bcl-xL/Drp1
complex regulates
clathrin-mediated
endocytosis to RRP
VGatedCa2+ Channel
Readily releasable pool
Synaptic neurotransmitter-
containing vesicle
Calcium

Jonas, Fig. 4


Glycolysis
Pyruvate
Lactate
Anaerobic Glycolysis
Inner mitochondrial membrane
Acetyl CoA
TCA cycle
H+ leak controls metabolic rate
Bcl-xL
Leak channel
Oligomycin
NADH
FADH2
Respiratory enzyme complexes
Succinate dehydrogenase
NADH dehydrogenase
CoQ
Cytochrome b
Cytochrome oxidase
ATP synthase
ADP + Pi
ATP
Jonas, Fig. 5