Involvement of Ion Channels in Apoptosis

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Involvement of ion channels in apoptosis is linked to critical aspects of this complex cellular process such as coordination of the Ca\textsuperscript{2+} signal, cell shrinkage and mitochondrial integrity. The body of work described below demonstrates the large variety and critical involvement of ion channels in this cellular process. It highlights the emerging roles discovered for this diverse group of membrane proteins in non excitable tissue physiology and pathophysiology.

All cells of multi-cell organisms contain a genetic program ready to be activated for their death, with the actual execution of this program called apoptosis. Apoptotic pathways include several proteins with powerful enzymatic cell degrading potential (mainly belonging to the Bcl-2 and caspase families, and cytochrome C). These proteins are latent most of the cells’ life (either by compartmentalization or by co-factor inhibition). The latent proteins are activated once a “death” signal has been consolidated by the cells’ existing signaling mechanisms. The program also includes elements of environmental awareness as the degraded cells remaining are packed and disposed of (for review see reference 1).

Apoptosis is a very important factor in an organism’s development, especially at the embryonic stage and in normal renewing tissue homeostasis (for example in epithelial and red or white blood cells). On the other hand the same mechanism is activated in pathophysiological conditions such as neuronal or cardiac ischemia (see for example 3). In addition, apoptosis prevention might be one of the necessary steps for the transformation of normal to cancerous cells. 4

Time course and stages of apoptosis progression includes a number of well-defined cellular and morphological changes. 3 These include cell shrinkage or apoptotic volume decrease (AVD), nuclear condensation, DNA fragmentation and formation of sub-cellular apoptotic bodies, that undergo phagocytosis by neighboring cells. Processing of the apoptotic stimulus, involve Ca\textsuperscript{2+} signals that lead to disruption of mitochondrial membranes. The latter leads to the release of proapoptotic factors, activating the caspase machinery that degrades the cell. 4 In parallel to stimulus processing, the cell shrinks, involving the participation of ion channels in the secretion of salts. 3

Methods of experimental induction of apoptosis usually mimic certain cellular process that serve as an apoptosis induction mechanism. Nevertheless, many factors bypass certain cellular pathways that are upstream of the point where they interfere with cellular function. For example, the build-up of cytoplasmic Ca\textsuperscript{2+} concentration probably plays a key role in the convergence of the initial apoptotic signal. 4 Many apoptotic insults involve different ways of chronic cytoplasmic Ca\textsuperscript{2+} elevation. Some include inhibition of cytoplasmic Ca\textsuperscript{2+} sequestering into ER, like Thapsigargin, 7 while others may perforate the membrane specifically for Ca\textsuperscript{2+} by the use of a ionophore, like A23187. 4 Apoptotic inducers may be as diverse as global kinase inhibition (Staurosporine) 9 or Hydrogen Peroxide (H\textsubscript{2}O\textsubscript{2}), mimicking the reactive oxygen species (ROS) 7 produced during apoptosis. However, in many cell types apoptosis is signaled by external ligands of death receptors (such as TNF-α or Fas ligands), which in many cases were used to induce experimental apoptosis (see table). 10

Ion channels are integrated membrane proteins which are exposed on both sides of the membrane. These proteins allow specific ions to cross membranes down their electrochemical gradient, usually in response to an appropriate stimulus. Ion channels are known to control a number of cellular processes including:

1. Change in a given ion concentration.
2. Changes in membrane potential (due to their charge being translocated across the membrane).
3. Changes in the cell osmotic balance.
4. Mediators of a cell’s response to a wide range of both intra- and extra-cellular chemical and/or physical stimuli. 11

Ion channels that have been implicated in apoptosis as regulators of local and global ion content belong to a wide range of channel families. The channels may differ regarding the ions toward which they are permeable, the stimulus that opens the channel, localization of the channel (i.e. plasma membrane, mitochondria etc.) and their overall role as pro- or anti-apoptotic agent (see table). These channels were reported to contribute to one of the following:

1. Cytosolic Ca\textsuperscript{2+} increase, leading to activation of Ca\textsuperscript{2+} dependent apoptotic machinery.
2. Ion fluxes between mitochondria and cytosol, resulting in either initiation of apoptosis or protection of apoptosis stimulated cells.
3. Permeation to large molecules such cytochrome C, facilitating their translocation from mitochondria to cytosol.
4. K\textsuperscript{+} and Cl\textsuperscript{-} efflux (from the cytosol to the extracellular space), leading to and accompanying water efflux and cell shrinkage, also leading to reduction in cytosolic K\textsuperscript{+} and relief of apoptotic inhibition.

Below, we will describe the possible roles played by specific ion channels in mediating processes crucial for the apoptotic signal propagation, either as mediators of the insult or as effectors activated by upstream stimuli.

Ca\textsuperscript{2+} permeable channels on plasma membrane

Ca\textsuperscript{2+} homeostasis plays a crucial role in apoptosis. It involves the generation, amplification and coordination of a diversity of Ca\textsuperscript{2+} signals by means of interplay between Ca\textsuperscript{2+} stores. 5 One such Ca\textsuperscript{2+} store is the extracellular fluid, which could be accessed by the opening of Ca\textsuperscript{2+} permeable channels located on the plasma membrane. This role is apoptosis is reported to be taken by either P2X (ionotropic ATP receptor activated
by binding of extracellular ATP resulting in the opening of a non-selective, Ca\(^{2+}\)-permeable cation channel. TRP (Transient Receptor Potential, non-selective cation channels, activated by different stimuli) or by Ca\(_{\text{v1}}\) (voltage-dependent Ca\(^{2+}\) selective channels).

P2X channels are the direct receptor of the apoptotic insult (ATP)\(^{12}\) as well as the P2X channels are the direct receptor of extracellular K\(^{+}\) insult (depolarization of the cell). The initial build-up of cytosolic Ca\(^{2+}\) described above, may serve as a messenger for many cellular agents and process. For the purpose of this review we should consider Ca\(^{2+}\)-activated channels, such as K\(_{\text{ATP}}\) and Cl\(_{\text{Ca}}\) channels on the plasma and/or mitochondria and the IP\(_{3}\) receptor (IP\(_{3R}\)) on the ER membrane.

### Intracellular channels on ER and mitochondrial membrane

Ca\(^{2+}\) signal convergence results in activation of intracellular channels that leads to cytochrome C release from mitochondria.\(^{3}\) This section will focus on a few more Ca\(^{2+}\) channels as well as on channels contributing to mitochondrial integrity.

IP\(_{3R}\) are Ca\(^{2+}\)-activated Ca\(^{2+}\) channels, on the ER membrane, which facilitate Ca\(^{2+}\) flow from ER to the cytoplasm, once the cytoplasmic Ca\(^{2+}\) level is intermediate (i.e. a bell shaped dependency in which the channel is closed both when Ca\(^{2+}\) is in the resting level and during Ca\(^{2+}\) overload) and plays a key role in Ca\(^{2+}\) homeostasis.\(^{28}\) It is also recruited to participate in an inter-organellar Ca\(^{2+}\)-cytochrome C self-amplifying signal, which is critical for downstream apoptotic mechanisms.\(^{6,28}\) Cytochrome C can enhance IP\(_{3R}\) activity as it binds the IP\(_{3R}\) with very high affinity and prevents channel closure during Ca\(^{2+}\) overload.\(^{28}\) During apoptosis, small amounts of mitochondrial cytochrome C translocate to the ER (a process that depends on IP\(_{3R}\) activity) and potentiate IP\(_{3R}\). This leads to a bursting Ca\(^{2+}\) overload, which coordinates cytochrome C release from all the cell's mitochondria, leading to activation of the caspase cascade.\(^{28}\)

The events linking the ER released Ca\(^{2+}\) to cytochrome C release happen in the mitochondria and involve channels on both the outer and inner mitochondrial membranes (OMM and IMM respectively). The sequence of events and the exact roles played by particular channels are not fully resolved. A Ca\(^{2+}\) channel sensitive to Ruthenium Red blockade was recently suggested as the Ca\(^{2+}\) route into mitochondria. However, the exact role of the channel in apoptosis as well as its molecular identification is not yet clear.\(^{30}\) In addition, other channels are involved in the maintenance/disruption of IMM potential and the release of cytochrome C from its “resting” location (between OMM and IMM) into the cytosol.

Bcl-2 proteins, an established apoptotic protein family, are suggested to act both as modulators of other channels and as channels themselves.\(^{29,31}\) The latter activity carried out by the Bcl-2 protein BAX, was suggested to generate the transducer of cytoplasmic Ca\(^{2+}\) elevation into cation currents disrupting the IMM potential. Other Bcl-2 proteins have been suggested to form the channel that conducts cytochrome C out of the mitochondria (for further details see\(^{32}\)).

However, it is more accepted that VDAC1 channels on the OMM (which are modulated by Bcl-2 proteins) actually mediate cytochrome C translocation.\(^{33}\) VDAC channels are big pores, with complex behavior that are usually considered as voltage-dependent anion channels. VDAC

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**A schematic sketch illustrating the ion channel types participating in apoptosis.** See text and table for details.
## Summary of Ion Channel Involvement in Apoptosis

<table>
<thead>
<tr>
<th>Channel reference</th>
<th>Channel identification *</th>
<th>Method to induce apoptosis</th>
<th>Apoptotic interactions</th>
<th>Examined in cell type</th>
<th>Channel and/or apoptosis pharmacology **</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ca</strong>(^{2+}) permeable channels on plasma membrane **</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P2X(_1) (^{19})</td>
<td>PCR, Ab</td>
<td>DEX glucocorticoid, SEB super agonist.</td>
<td>Activates SAK. Co-localized with caspase-3.</td>
<td>Thymocytes (but not T-lymphocytes).</td>
<td></td>
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<tr>
<td>P2X(_{7,12,14,20})</td>
<td>+/-, mice, DN, SPh</td>
<td>ATP, Glucose deprivation.</td>
<td></td>
<td>Infected macrophages, mouse macrophages (BAC1), Dendritic cells, DJSC1, Human T-lymphocytes, Human Keratinocytes, Cerebellar granule cells (also P2X6).</td>
<td>BaATP (blocker), Oxidized ATP (opener/inducer).</td>
</tr>
<tr>
<td>Ca(_{1,1}) (L-type) (^{13,22})</td>
<td>UM, NSPh</td>
<td>Serum from diabetic patients, extracellular K(^{+}) elevation.</td>
<td>GH3, insulin secreting and Chromaffin cells.</td>
<td></td>
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<tr>
<td>Ca(_{3}) (T-type) (^{23})</td>
<td>UM, NSPh</td>
<td>Cytokines</td>
<td>Pancreatic cells</td>
<td>Ni(^{2+}) (blocker).</td>
<td></td>
</tr>
<tr>
<td>TRPM2 (^{24,25})</td>
<td>AsOn, DN</td>
<td>H(_2)O(<em>2), TNF(</em>{\alpha})</td>
<td>H(_2)O(_2) activates the channel</td>
<td>Insulinoma: RIN-5F, U937 and TF1 cells.</td>
<td></td>
</tr>
<tr>
<td>TRPM7 (^{27})</td>
<td>Specific RNA silencing</td>
<td>O(_2) glucose deprivation.</td>
<td>ROS activated channel.</td>
<td>Cortical neurons.</td>
<td>Gd(^{3+}) (blocker).</td>
</tr>
<tr>
<td>TRPV1 (^{28})</td>
<td>+/-, mice, SPh</td>
<td>Particulate matter. cAMP and PKA, downstream of channel activation.</td>
<td>Epithelial airways cells, trigeminal ganglion neurons.</td>
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<tr>
<td>Ca(_{2+}) channel on ER membrane **</td>
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<tr>
<td>IP(_{3,1}) (^{18})</td>
<td>Ab</td>
<td>Stauroporine.</td>
<td>cytochrome C potentiates the channel.</td>
<td>HeLa and PC12 cells.</td>
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<tr>
<td>**Channels on mitochondrial membranes **</td>
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<tr>
<td>VDAC1 (^{29})</td>
<td>Ab</td>
<td></td>
<td>Release of cytochrome C, regulated by Bcl-2.</td>
<td>Yeast mitochondria.</td>
<td></td>
</tr>
<tr>
<td>VDAC2 (^{30})</td>
<td>+/- mice</td>
<td>Stauroporine.</td>
<td>Protective, Inhibit BAK.</td>
<td>Mouse liver mitochondria.</td>
<td></td>
</tr>
<tr>
<td>CLIC4 (^{31,36})</td>
<td>HE, AsOn</td>
<td>Etoposide.</td>
<td>Uregulated by p.53</td>
<td>Keratinocytes.</td>
<td></td>
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<tr>
<td>K(_{\text{ap}}) (^{37,38})</td>
<td>UM, SPh</td>
<td>Perfused hearts.</td>
<td>Protective</td>
<td>Cerebellar granule neurons, cardiomyocytes.</td>
<td>Diazoxide (opener/protector).</td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{\text{RB}}) (BK) (^{39})</td>
<td>Ab, SPh</td>
<td>Perfused hearts.</td>
<td>Protective</td>
<td>Cardiomyocytes.</td>
<td>Charybdotoxin, Iberiotoxin, PaXilin (blockers), NS-1619 (opener/protector).</td>
</tr>
<tr>
<td>**Cl(^{-}), Na(^{+}), and gap junction channels on plasma membrane **</td>
<td></td>
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<tr>
<td>CLCA2,4 (also CLCA1) (^{40})</td>
<td>HE, PCR</td>
<td>Serum deprivation, Cyclosporin A</td>
<td>Mammary gland cells, HC11, Human hepatoma cell line.</td>
<td></td>
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<tr>
<td>CTR (^{40})</td>
<td>HE of w.t and DN</td>
<td>Cycloheximide, etoposide</td>
<td>Mediates acidification</td>
<td>Mouse mammary C127 cells.</td>
<td></td>
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<tr>
<td>Swell activated CLC (^{45,51})</td>
<td>UM</td>
<td>Perfused heart</td>
<td>Heart.</td>
<td></td>
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<tr>
<td>CLC (^{46})</td>
<td>HE</td>
<td>CD95</td>
<td>Regulated by CD95.</td>
<td>Jurkat T cells.</td>
<td>Gilbenclamide, IAA, DIDS, DPC (blockers)</td>
</tr>
<tr>
<td>Na(_{\text{ap}}) (^{47})</td>
<td>UM, NSPh</td>
<td>Fas ligand</td>
<td>Control cell shrinkage</td>
<td>Jurkat T cells.</td>
<td>STX (blocker).</td>
</tr>
<tr>
<td>Cnx-32, Cnx-43 (^{48})</td>
<td>HE</td>
<td>Many apoptotic stimuli</td>
<td></td>
<td>HeLa cells.</td>
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<tr>
<td>**K(^{+}) channels on plasma membrane **</td>
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<tr>
<td>K(<em>{\text{ap}}) (</em>{1,3}) (^{49,50})</td>
<td>SPh</td>
<td>Fas ligand, FSH.</td>
<td>Caspases increase channel activity.</td>
<td>Jurkat T cells, Porcine granulose cells.</td>
<td>Margatoxin, Charybdotoxin (blockers) SHK (blocked channel but not apoptosis).</td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{2,1}) (^{51})</td>
<td>DN, HE</td>
<td>DTPD, Stauroporine.</td>
<td></td>
<td>Cortical neurons.</td>
<td></td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{11}) (HERG) (^{52})</td>
<td>Ab</td>
<td>H(_2)O(<em>2), TNF(</em>{\alpha})</td>
<td>Conductance promotes apoptosis. Expression recruits TNFR1</td>
<td>HERG expressing and non expressing tumor cells.</td>
<td>Dofetilide (blocker).</td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{1,1}) (BK) (^{53})</td>
<td>SPh</td>
<td>Stauroporine, FCCP, SNAP.</td>
<td></td>
<td>Human and rat smooth muscle cells.</td>
<td>Iberiotoxin (blocker).</td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{3,1}) (SKA) (^{54})</td>
<td>SPh</td>
<td>A33187 (Calcimycin)</td>
<td>T-lymphocytes, thymocytes, erythrocyte.</td>
<td>Charybdotoxin (blocker) Iberiotoxin, Apamin, Agitoxin no effect.</td>
<td></td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{47})</td>
<td>UM, NSPh</td>
<td>H(_2)O(_2).</td>
<td>One-cell mouse embryo (zygote).</td>
<td>Quinin (blocker) Iberiotoxin, Dendrototoxin, Charybdotoxin no effect.</td>
<td></td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{4,1}) (TRAKK) (^{55})</td>
<td>Ab</td>
<td>Arachidonic acid.</td>
<td>Arachidonic acid activates the channel.</td>
<td>Retinal (RGC-5), PC12 cells.</td>
<td></td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{3,1}) (TASK1), K(<em>{\text{ap}}) (</em>{9,1}) (TASK3) (^{56})</td>
<td>DN, HE</td>
<td>Time in culture, Stauroporine.</td>
<td></td>
<td>Cerebellar granule neurons.</td>
<td>Ruthenium red (blocker).</td>
</tr>
<tr>
<td>K(<em>{\text{ap}}) (</em>{1,1}) (ROMK1) (^{57})</td>
<td>HE</td>
<td>Time in culture</td>
<td></td>
<td>Rat hippocampal neurons.</td>
<td></td>
</tr>
</tbody>
</table>

*DN= dominant negative mutant, UM = unidentified molecularly, HE = heterologous expression, Ab = specific antibody, SPh = specific pharmacology, NSPh = non specific pharmacology, AsOn = antisense oligonucleotide, PCR = Polymerase Chain Reaction, w.t = wild type. **blocker = of both channel and apoptosis, opener/inducer = of channel/apoptosis.
channels, however, may play a protective role, as was shown for the VDAC2 isoform that is an inhibitor of Bak oligomerization and therefore it helps keep this proapoptotic protein in chains.\textsuperscript{34} IMM depolarization is an important factor leading to VDAC1 priming to serve as cytochrome C translocator. IMM depolarization is caused by dissipation of H\textsuperscript{+} gradient, probably with the participation of the Cl\textsuperscript{-} intracellular channel, CLIC4.\textsuperscript{35, 36}

The dissipation of H\textsuperscript{+} gradient could be compensated for by activation of K\textsuperscript{+} selective channels on the IMM. These include an ATP compensated (K\textsubscript{ATP}, which is a channel formed by a yet unidentified protein)\textsuperscript{37, 38} and a Ca\textsuperscript{2+} sensitive (K\textsubscript{Ca}, 1.3)\textsuperscript{39} K\textsuperscript{+} channels. Mitochondrial matrix ATP levels drop and Ca\textsuperscript{2+} load activates the two channels, respectively, with these two events accompanying H\textsuperscript{+} gradient dissipation. This protective pathway (involving a not fully resolved mechanism) is suggested to contribute to protective physiological phenomenon and is exploited by specific pharmacological augmentation of K\textsubscript{ATP} to protect hearts during surgery.\textsuperscript{38}

Thus, Ion channels localized on intracellular organelle membranes play a key role in consolidation of the initial apoptotic signal. However, other channels may serve to dissolve such signals and protect cells challenged by an insult.

**Plasma membrane K\textsuperscript{+} efflux channels**

K\textsuperscript{+} homeostasis, (high cytoplasmic compared to the low external concentration) is disrupted during apoptosis. That is, K\textsuperscript{+} ions must leave the cell as an obligatory step in the apoptotic pathway. However, the exact mechanism by which K\textsuperscript{+} depletion promotes apoptosis is not fully resolved. The activity of some apoptotic enzymes was suggested to depend on low K\textsuperscript{+}, perhaps explaining the necessity of K\textsuperscript{+} depletion in apoptosis. K\textsuperscript{+} depletion is strongly linked to cell shrinkage, a hallmark of, but also a possible active contributor to apoptosis.\textsuperscript{1} Therefore, up-regulation of the activity of K\textsuperscript{+} channels probably lie in the basis of increased K\textsuperscript{+} outflow, critically contributing to apoptosis.\textsuperscript{1, 40}

Several reported observations suggest that any K\textsuperscript{+} channel is good for the job of emptying the cell of its K\textsuperscript{+} ions. This implies that following apoptotic stimulation the cell recruits any available K\textsuperscript{+} channels, in the context of the apoptotic stimulus and possible changes in expression profiles. Three arguments support this idea:

1. K\textsuperscript{+} channels belonging to four structurally distinct channel families were reported to be involved in apoptosis (see table).
2. There is correlation between specific channel blockers and prevention of apoptosis in different cell types.\textsuperscript{51, 52}
3. Heterologous expression of several types of K\textsuperscript{+} channels primed the same cell type (for example, cultured hippocampal neurons\textsuperscript{53, 54}) to apoptosis.

It is hard to correlate the specific K\textsuperscript{+} channel that is activated with a specific apoptotic insult. For example, Staurosporine induced apoptosis that was mediated by K\textsubscript{Ca}, 2.1 channels in neurons,\textsuperscript{5} but in smooth muscle cells it was mediated by K\textsubscript{Ca}, 1.1 channels. The latter mediated also other apoptotic stimuli in these cells.\textsuperscript{5}

Phenotypic differences between cell types might be correlated to the specific K\textsuperscript{+} channel they are using in apoptosis. For example, while expression of the two pore domain channel K\textsubscript{Ca}, 9.1 (TASK-3), is essential for apoptosis in cerebellar granule neurons,\textsuperscript{54} the voltage dependent K\textsubscript{Ca}, 2.1 carries out the same task in cortical neurons.\textsuperscript{2} Most granule neurons are eliminated with cerebellum maturation shortly after birth and die in culture, while cortical neurons can be maintained for long periods in primary cultures. This difference may be attributed to the particular K\textsuperscript{+} channel that the cell uses to mediate apoptosis. It is interesting to note that overexpression of K\textsubscript{Ca}, 9.1 in hippocampal neurons induced apoptosis and arachidonic acid activated K\textsubscript{Ca}, 4.1 (TRAAK) to induce apoptosis in PC12 cells.\textsuperscript{55, 56}

Since K\textsuperscript{+} channels are crucial to apoptosis, specific channel blockers are very indicative tools, especially as these also blocked the death process (see table).\textsuperscript{41, 42} For example, Iberiotoxin, a specific blocker of K\textsubscript{Ca}, 1.1 channels blocks apoptosis in smooth muscle cells,\textsuperscript{4} but failed to influence such a process in T-lymphocytes\textsuperscript{42} and in a mouse embryo cell.\textsuperscript{57} In these two cell types apoptosis was carried out by another Ca\textsuperscript{2+} dependent channel, K\textsubscript{Ca}, 3.1 or by a K\textsubscript{Ca}, 5.1 channel, respectively. Charybdotoxin, a less specific K\textsuperscript{+} channel blocker, was effective in blocking apoptosis in granulose cells as did Margatoxin, pointing to the involvement of K\textsubscript{Ca}, 1.3 channels.\textsuperscript{41} In T-lymphocytes Charybdotoxin also blocked apoptosis, but Iberiotoxin, Apamin and Agitoxin failed to do so, ruling-out K\textsubscript{Ca}, 1.1, K\textsubscript{Ca}, 2 and K\textsubscript{Ca}, 1.3, strongly hinting at the involvement of K\textsubscript{Ca}, 3.1.\textsuperscript{42}

Recently, a mechanism that links K\textsuperscript{+} efflux to the upstream apoptotic stimulus and mitochondrial disruption, was described in neurons.\textsuperscript{58} It involves nitric oxide (NO) increase arising from the stimulus, leading to Zn\textsuperscript{2+} increase and activation of p38 MAPK. The latter activates K\textsuperscript{+} efflux directly, that again was found to be an obligatory step leading to cell death.\textsuperscript{67}

It also should be mentioned that compensatory mechanisms might be activated. For example, K\textsubscript{Ca}, 1.3 was reported to mediate apoptosis but K\textsubscript{Ca}, 1.3 null mice thymocytes exhibited normal apoptosis that was dependent on Ca\textsuperscript{2+} channels.\textsuperscript{47}

Plasma membrane Cl\textsuperscript{-} and Na\textsuperscript{+} channels

Cl\textsuperscript{-} channels must be active during cell volume changes to allow the net salt transport (Na\textsuperscript{+} or KCl) that is accompanied by water movement. In epithelial mammary cells, the expression of two Ca\textsuperscript{2+} activated Cl\textsuperscript{-} channels is changed upon apoptosis induction. CLCA1, which is the dominant isoform under “normal” conditions, is down-regulated while CLCA2 expression is upregulated.\textsuperscript{49} The latter is in agreement with the disrupted expression of this channel in apoptosis resistant tumor cells. CLCA channels were also shown to be a necessary component in apoptosis of heptoma cell line, challenged with Cyclosporin A.\textsuperscript{49} These observations point to the importance of CLCA channels (or their loss) in apoptosis and cancer.

Epithelial cells normally secrete Cl\textsuperscript{-} alongside cations and a mutated Cl\textsuperscript{-} Channel (CFTR) is the basis of a severe epithelial disease, cystic fibrosis (CF). CFTR channels were shown to support apoptosis in mouse mammary cells, where the natural mutant channel (causing the disease), failed to support such a process.\textsuperscript{50}

One role of the CFTR channel is to create an acidic environment, which allows the DNA of dying cells to be fragmented into small pieces. When the CFTR protein cannot fulfill this task, there is an accumulation of mucus with large DNA fragments. This may be the result of a process in which CF cells are not dying “properly” (epithelial tissue must renew constantly), due to a mutation in a channel that is needed for apoptosis. A similar mechanism was suggested in Jurkat T cells, where unidentified Cl\textsuperscript{-} channels also mediate apoptosis and their block prevents cell acidification.\textsuperscript{51}

Another, as yet unidentified, Cl\textsuperscript{-} channel that is activated upon cell swelling plays a role in cardiac cell apoptosis during heart surgery and/or transplantation. However, there are contradicting reports regarding its role(s) as proapoptotic or protective channel. While administration of the Cl\textsuperscript{-} channel blockers NPPB and IAA-94 seemed to enhance apoptosis in rabbit hearts,\textsuperscript{52} NPPB and DIDS protected from apoptosis in rat.\textsuperscript{53}

Na\textsuperscript{+}\textsuperscript{34} and connexin (gap junction)\textsuperscript{35} channels were shown to control cell shrinkage and/or apoptosis in tumor cell lines, but the exact role played in apoptosis by these channels has not been extensively studied. However, Na\textsuperscript{+} inflow is necessary for Jurkat T cells induced shrinkage and conducting Na\textsuperscript{+} channels are required for apoptosis as Saxitoxin (a Na\textsuperscript{+} non specific blocker), also blocked apoptosis.\textsuperscript{54}

The obligatory involvement of channels permeable to different ions in apoptosis suggests...
that the disruption of ion homeostasis is a necessary component of apoptosis propagation.

The Apoptosis-Cancer paradox of ion channels

One of the main motivations for trying to understand the role of ion channels in apoptosis is derived from a possible link to the role of the channels in cancer, where apoptosis is notoriously harder to achieve. As imbalance between apoptosis and proliferation may account for cancerous phenotypes, one may expect that K+ channels, which must be upregulated in order for a cell to die, will be downregulated if the cell is to live forever.

In fact, this is not the case, and a few examples exist of certain K+ or Ca2+ channels that are upregulated in both cellular conditions. These include Kᵥ9.1, Kᵥ1.3, Kᵥ11.1 and P2X7.43,10,5 However, Kᵥ11.1 (HERG) channels were shown to play a dual role: in apoptosis as an active K+ channel and in proliferation as a membrane anchoring protein recruiting growth receptors to the membrane.64 The duality of the Kᵥ11.1 effect was investigated with the use of a natural mutant. It lacks the ability to conduct ions and does not support apoptosis, but retains wild-type abilities to express in the membrane and bind the growth receptor. Specific antibodies used to co-immunoprecipitate Kᵥ11.1 and TNF1R also showed this ability. This study is particularly interesting as it was conducted in different tumor cell lines, some which express the channel and tend to die upon apoptotic insult, while others do not express the channel and are resistant to the apoptotic insult.65

Taken together, the observations summarized here highlight the importance of ion channels in cellular mechanisms that control cellular integrity and fate. These roles played by ion channels in killing a cell are relevant in many clinical situations ranging from protection of the perfused heart during surgery, to exchanging proliferation for apoptosis as a possible tool in the fight against cancer.

References:
Staurosporine induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 2 μM Staurosporine or vehicle were added for 3 or 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.

A23187 (Ca²⁺ Ionophore)

(Antibiotic A23187, Calciomycin, Calcium Ionophore A23187)

Product #: A-600

A23187 induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 2 μM A23187 or vehicle were added for 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.

Okadaic Acid

(Ammomium Salt)

Product #: O-800

Okadaic Acid induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 1 μM Okadaic Acid or vehicle were added for 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.

Puromycin

Product #: P-540

Puromycin induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 2 μM Puromycin or vehicle were added for 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.

Staurosporine

Product #: S-350

Staurosporine induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 2 μM Staurosporine or vehicle were added for 3 or 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.

Thapsigargin

Product #: T-650

Thapsigargin induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 1 μM Thapsigargin or vehicle were added for 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.