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Apoptosis. Signaling Pathways and Cell Ion and Water Balance

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Abstract—Current data on the alteration of the monovalent ion balance, pH and the membrane potential during apoptosis are summarized and considered with respect to ionic mechanisms of the apoptotic cell shrinkage. A brief survey of the main signaling pathways involved in apoptosis, such as receptor- and mitochondria-mediated pathways of the caspase-dependent and caspase-independent apoptosis is given. The data on the alteration of the distinct ion transporters and channels of the plasma membrane during apoptosis are considered.

Key words: apoptosis, cell ion and water balance, apoptotic cell shrinkage, cell K^+, cell Na^+.

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Apoptosis (or programmed cell death) is a natural process essential for normal tissue development and homeostasis. Unlike necrosis, apoptosis occurs without inflammation because apoptotic cells preserve their content (see review Bar, 1996) and then undergo phagocytosis (see review Savill and Fadok, 2000). The classic apoptotic hallmarks are cell shrinkage, loss of intercellular contacts, blebbing, cytoskeleton disruption, chromatin condensation, nuclear fragmentation, and DNA degradation (see review Häcker, 2000; Bortner, and Cidlowski, 2002). Apoptosis is usually identified by morphological features, internucleosomal DNA cleavage and by determination of the caspase activity. A widely accepted method for the determination of apoptosis in living cells is the Annexin V assay using flow cytometry. Annexin V detects phosphatidylserine exposed on the external side of the plasma membrane during apoptosis. Depending on the cell types, their physiological state and apoptosis-inducing stimuli, the apoptotic features may vary. Some of them may be lacking as occurs in apoptosis of anuclear erythrocytes. Thus, several criteria are needed to identify apoptosis (see review Darzynkiewicz et al., 1998).

The purpose of present review is to consider the data on the changes of ion and water balance in apoptosis with respect to the general apoptotic machinery.

MAJOR SIGNALING PATHWAYS IN APOPTOSIS

The signaling pathways leading to apoptosis are presented in Fig. 1. Apoptosis can be initiated by both exogeneous and endogeneous factors (see reviews Ghobrial et al., 2005; Reed, 2000; Roy and Nicholson, 2000). The essential role in triggering of apoptosis belongs to the cystein proteases or caspases, a family of evolutionarily conserved proteases (see review Earnshaw et al., 1999). Under normal physiological condition caspases are presented as inactive proenzymes; in apoptotic cells they are activated. Caspases are divided into two groups, initiators and effectors (see review Kaufmann and Earnshaw, 2000). Caspases 8, 9, 10, and 12 are initiators and function upstream within apoptotic signaling pathways (see reviews Ho and Hawkins, 2005; Vermeulen et al., 2005). They are capable of activating the downstream effector caspases 3, 6, 7, 14. Caspase 2 has both initiator and effector features (see review Zhivotovsky and Orrenius, 2005). There are multiple targets of effector caspases. Caspase 3 may be responsible, for example, for the cleavage of DNA fragmentation factor DFF-45, gelsolin, PARP, PAK-2 (Janicke et al., 1998). It should be noted that apoptosis is reversible only at the stage of initiation of the caspase cascade.

The mechanisms of caspase activation may be different. The *receptor-mediated pathway* is triggered by members of the death-receptor superfamily including Fas/CD95, TNF, DR-4, DR-5 (see review Kaufmann and Hengartner, 2001). For example, activation of the Fas receptor by a specific ligand or by antibodies leads to Fas binding with the adaptor FADD (Fas-associated protein with death domain). FADD binds to procaspase 8 and activates caspase 8, which activates procaspase 3 (Fig. 1). This type of signal transduction occurs, particularly, in lymphoid cells. In other cells the activation of caspase 8 is not sufficient to activate procaspase 3 (see review Kaufmann and Hengartner, 2001).

In the *mitochondria-mediated pathway* the inner mitochondrial membrane potential collapses, the electron transport chain protein cytochrome *c* is released

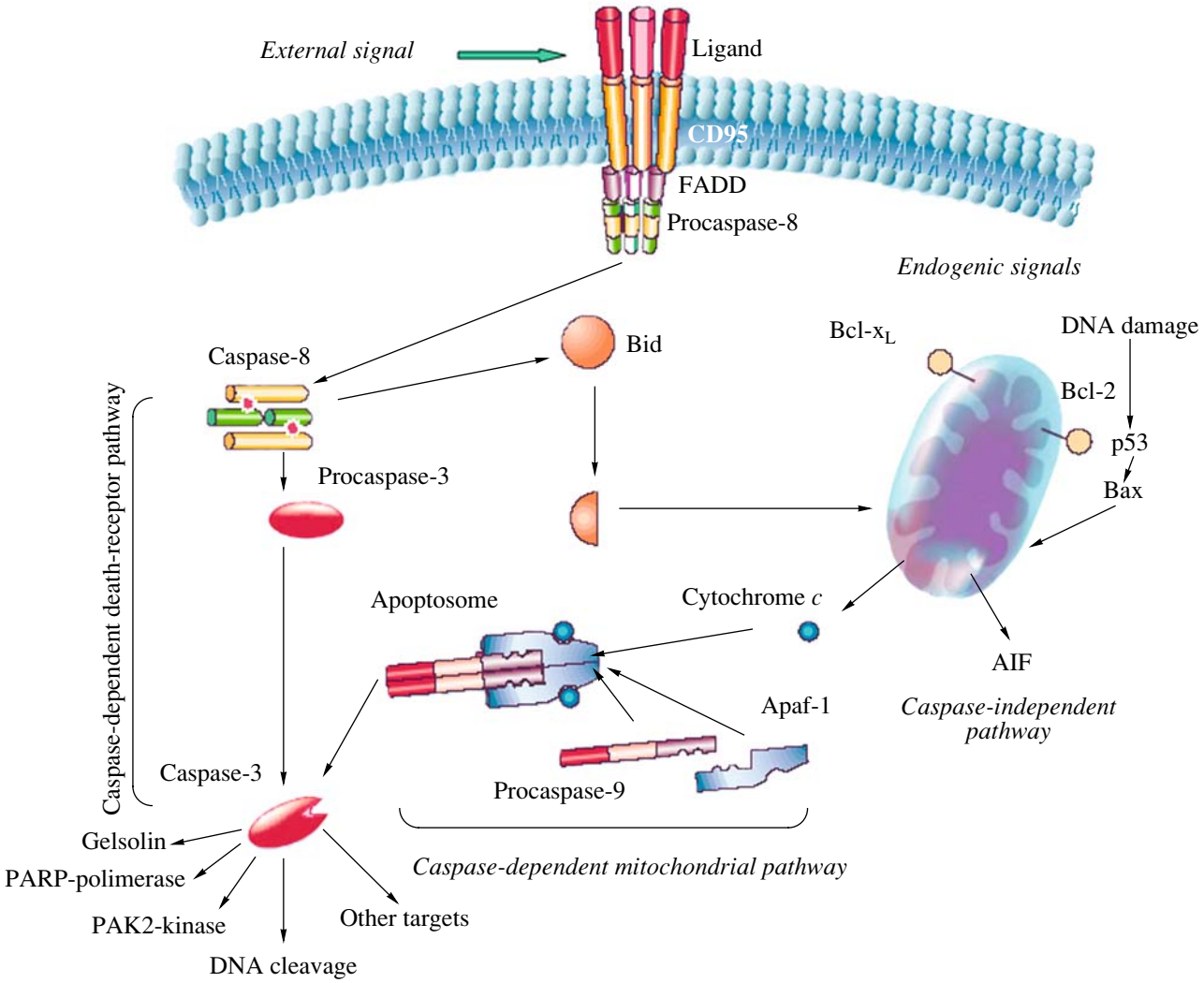

Fig. 1. Signaling pathways in apoptosis (from Hengartner, 2000, modified).
CD95—receptor, one of the death receptor superfamily; FADD—(Fas associated protein with death domain), adaptor; Bid, Bcl-X_1, Bcl-2, Bax—Bcl-2 family members; p53—tumor suppressor protein; Apaf-1—apoptotic protease-activating factor-1; AIF—apoptosis inducing factor; Cytochrome *c*—mitochondrial protein.

from mitochondria and accumulates in the cytoplasm (see review Bernardi et al., 2001) where it binds to the scaffolding protein Apaf-1 (apoptotic protease-activating factor-1). Apaf-1 binds to procaspase 9. These interactions result in the formation of so-called apoptosome where procaspase 9 turns into caspase 9 (see review Green and Reed, 1998). Caspase 9 activates caspase 3. The death-receptor and mitochondrial pathways converge at the level of activation of effector caspases 3 or 7 that are essential, e.g., for the cleavage of DFF-45/ICAD (Tang and Kidd, 1998; Nagata et al., 2003). A proapoptotic Bcl-2 family member, Bid, can also connect the mitochondrial and death-receptor pathways for activation of the caspase cascade (see review Roy and Nicholson, 2000).

Mitochondria are a necessary link in the transduction of apoptotic signals caused by DNA damage, oxidative stress (Aoki et al., 2002) or heat shock (Beere, 2004). The p53 protein is an important transmitter of these signals. The loss or mutation of the *p53* gene results in resistance to apoptosis and tumor development. Under normal conditions p53 is a short-living protein. Cells exposed to ultra-violet light, radiation, chemicals or other factors causing DNA damage have an increased level of p53 (Chen et al., 1996; Bedner et al., 2000). This p53 is capable of activating the expression of the proapoptotic genes and inhibiting expression of the antiapoptotic genes (see review Haupt et al., 2003). Members of Bcl-2 family, which include apoptotic promoters (Bax, Bid, Bik) and apoptotic inhibitors (Bcl-2, Bcl-X_L) are located at the external mitochondrial membrane and compete in the regulation of cytochrome *c* release (see review Hengartner, 2000). Discovery of AIF (apoptosis inducing factor) was an

important step in understanding the apoptosis machinery. This mitochondrial flavoprotein translocates into the nucleus where it binds to DNA and causes caspase-independent chromatin condensation and large-scale DNA fragmentation (see review Cande et al., 2002; Susin et al., 2000). Thus, AIF and cytochrome *c* are important proapoptotic factors.

APOPTOTIC CELL DEHYDRATION

Apoptosis was defined historically as a "shrinkage necrosis" (Kerr, 1971). The concept of apoptotic cell shrinkage originated from microscopic observations of fixed cells and tissues. In current studies of apoptosis living cells are usually used and conclusions about the reduction of cell size are mostly based on measurement of the forward light scattering in flow cytometer (see review Shapiro, 1988). It is assumed that normotonic cell shrinkage during apoptosis is caused by cell dehydration (Okada et al., 2001). This is called "apoptotic volume decrease" (AVD) by analogy with the "regulatory volume decrease" (RVD) that is observed in cells recovering their volume in hypotonic medium. Separation of cells by their buoyant density, i.e., by cell dehydration, is a well-known method for the selection of apoptotic thymocytes and other cells (Thomas and Bell, 1981; Wyllie and Morris, 1982; Cohen et al., 1993; Dumont et al., 2000). The mechanisms of AVD and RVD are thought to be similar (see review Okada et al., 2001). It was reported that apoptosis can be induced by cell dehydration, e.g. by hypertonic shock (Morales et al., 2000; Michea et al., 2002; Rao et al., 2004; Friis et al., 2005). The time of cell shrinkage in apoptosis may vary from tens of minutes to many hours, i.e. within the same range as the time of the apoptosis in toto (Benson et al., 1996; Chang et al., 2000). It was shown that apoptotic shrinkage in HL60, HeLa and U937 cells occurred before phosphatidylserine externalization (McCarthy and Cotter, 1997), cytochrome *c* release, caspase 3 activation and DNA laddering (Maeno et al., 2000).

Cell shrinkage and caspase activation are important features of programmed cell death, but the relationship between these events is not well understood. The treatment of Jurkat cells with the anti-Fas antibody in presence of the pan-caspase inhibitor, z-VAD, abrogates the apoptotic characteristics, including cell shrinkage. When Jurkat cells were treated with either thapsigargin or calcium ionophore A23187 these inhibitors did not prevent cell shrinkage. These findings suggest that cell shrinkage can be an outcome of both caspase-dependent and caspase-independent apoptotic pathways (Bortner and Cidlowski, 1999). It is shown that a reduction of cell volume and chromatin condensation during apoptosis of activated T cells can occur through the AIF activated caspase-independent pathway (Dumont et al., 2000). Shrinkage of Jurkat cells in apoptosis induced through the Fas receptor or by UV radiation was mediated by different initiating caspases, 8 or, respectively, 9 (Vu et al., 2001). All these results indicate that AVD can be observed both in caspase-dependent (i.e., receptor and mitochondrial) and caspase-independent apoptosis. Anti-Fas induced cell shrinkage of Jurkat cells at the stage of the protein kinase C stimulation was blocked by phorbol ester (PMA) and by briostatin-1. Inhibition of protein kinase C with indolocarbazole Gö6976 enhanced the anti-Fas-mediated reduction of cell volume (Gómez-Angelats et al., 2000).

CELL K^+ CONTENT AND K^+ CONCENTRATION IN CELL WATER IN APOPTOSIS

It is commonly accepted that reduction of the intracellular K^+ content is essential for AVD (see reviews Bortner and Cidlowski, 2002; Park and Kim, 2002; Yu, 2003a). However, there are few works in which cell ion and water balance in apoptosis was measured in the same experiment. It is necessary to distinguish between changes in cation concentration in the intracellular water and changes in cation content per cell dry mass. It is shown that apoptosis of CEM cells induced by dexamethasone is associated with a decrease in K^+ content by 15% whereas K^+ concentration in cell water is not changed (Benson et al., 1996). Intracellular K^+ concentration was reduced in UV-induced apoptosis of HL60 cells (McCarthy and Cotter, 1997). Using fluorescent probes it was shown that cytosolic K^+ concentration was 110 mM in normal and 50 mM in apoptotic L cells (Barbiero et al., 1995). A decrease in cell K^+ content from 391 to 196 μmol/g dry wt was detected by X-ray elemental microanalysis in UV irradiated U937 cells (Fernández-Segura et al., 1999). Similar results were obtained in human monocytes exposed to oxidized lipoprotein. K^+ content in these cells decreased from 616 to 291 μmol/g dry wt (Skepper et al., 1999). The shift in K^+ content from 416 to 216 μmol/g dry wt was found in apoptosis induced by staurosporine in U937 cells (Arrebola et al., 2005b).

It is believed that a decrease in intracellular K^+ concentration plays a key role in regulation of apoptotic enzymes, e.g. nucleases and caspases (Hughes et al., 1997; Hughes and Cidlowski, 1999). According to our findings (Vereninov et al., 2004a; Yurinskaya et al., 2005a), the dehydration of U937 cells treated with staurosporine was associated with a remarkable reduction of cellular K^+ and a decrease by 7–8% in K^+ concentration in cellular water. It is doubtful that so slight a shift in the K^+ concentration could regulate apoptotic caspases and nucleases. In our opinion, the decrease in K^+ concentration in cell water detected with fluorescent probes should be due to the cell swelling if it is accompanied by a decrease in Na^+ concentrations. However, cell swelling is a feature of necrosis rather than apoptosis (Kerr, 1971). It is necessary to note that if cell shrinkage in apoptosis does not occur, as is observed in etoposide-induced apoptosis of U937 cells, a significant decline in the K^+/Na^+ ratio occurs, but the total K^+ and Na^+ content does not change (Yurinskaya et al.,

2005a). It was shown that the changes in ion composition induced by ionophores (valinomycin, nigericin, amphotericin) caused apoptosis in different cell types (Inai et al., 1997; Oh et al., 1997; Furlong et al., 1998; Marklund et al., 2001).

CELL Na^+ CONTENT AND Na^+ CONCENTRATION IN CELL WATER IN APOPTOSIS

An increase in the intracellular Na^+ concentration from 15 to 30 mM was shown in etoposide treated apoptotic mouse L cells by fluorometry of the Na^+ probe SBFI (Barbiero et al., 1995). Similar results were obtained by the same method in Jurkat cells during apoptosis induced by Fas-ligand (Bortner et al., 2001).

An increase in the Na^+ content from 52 to 197 μmol/g dry wt in apoptotic U937 cells after UV irradiation was shown by X-ray microanalysis (Fernández-Segura et al., 1999). An increase in Na^+ content from 42 to 103 μmol/g dry wt was found by the same method in apoptotic human monocytes exposed to oxidized lipoprotein (Skepper et al., 1999). Na^+ content of U937 cells in apoptosis caused by staurosporine increased according X-ray microanalysis from 63 to 143 μmol/g dry wt, whereas K^+ content reduced from 416 to 216 μmol/g dry wt (Arrebola et al., 2005b).

The changes in cellular water balance in apoptosis are determined by changes in the sum of cell Na^+ and K^+ contents. Water balance should not be shifted if a decrease in K^+ content is counterbalanced by an increase in Na^+ content. When it is not counterbalanced, then cell shrinkage occurs. This was demonstrated in the comparative study of apoptosis induced by dexamethasone, etoposide and staurosporine in rat thymocytes and human leukemic U937 cells. A decrease in K^+ content of thymocytes induced to apoptosis by dexamethasone was found to be 0.49 mmol/g protein, whereas the increase in intracellular Na^+ content was 0.25 mmol/g protein and shrinkage occurred (Yurinskaya et al., 2005b). Apoptosis of U937 cells treated with staurosporine was accompanied by a decrease in intracellular K^+ content from 1.1 to 0.78 mmol/g protein and by an increase in intracellular Na^+ content from 0.3 to 0.34 mmol/g protein. Cell shrinkage occurred in this case too. In contrast, a decrease in intracellular K^+ content during etoposide-induced apoptosis of U937 cells was counterbalanced by an increase in intracellular Na^+ content, and no cell dehydration was found (Yurinskaya et al., 2005a).

APOPTOTIC CHANGES IN CELLULAR Cl AND P CONTENT

A decrease in the intracellular cation content should be accompanied by a decrease in the anion content. It was shown by X-ray microanalysis that the Cl content of U937 cells during staurosporine-induced apoptosis declined from 143 to 103 mmol/kg dry wt, whereas the P content did not change (Arrebola et al., 2005b). During UV-induced apoptosis of these cells Cl content decreased from 156 to115 μmol/g dry wt whereas P content increased from 299 to 314 μmol/g dry wt (Fernández-Segura et al., 1999). In human monocytes exposed to oxidized lipoprotein Cl content decreased from 152 to 58 μmol/g dry wt and P content reduced from 672 to 465 μmol/g dry wt. (Skepper et al., 1999). The content of so-called anions "not penetrating through the cell membrane," which can be estimated by the difference between the (K^+ + Na^+) content and the content of "penetrating" anions, Cl^-, $H_2PO_4^-$ in apoptosis did not change (Fernández-Segura et al., 1999) or slightly reduced (Skepper et al., 1999; Arrebola et al., 2005b).

INTRACELLULAR pH IN APOPTOSIS

Cytoplasmic acidification by about 0.3–0.4 pH_i units was found in apoptosis by many researchers. Initially it was shown in HL60 cells (Barry and Eastman, 1992). Much of the data on the changes of intracellular pH in apoptosis are reviewed by Lagadic-Gossmann with colleagues (Lagadic-Gossmann et al., 2004). It was reported that acidification of Jurkat cells during CD95-induced apoptosis is caused by inhibition of the Na^+/H^+ exchanger (Lang et al., 2000). Similar results were obtained with Molt4, CEM, and K562 cells (Rich et al., 2000).

What is the role of cytosolic acidification in the apoptotic cascade? The acidification has been observed in mitochondrial, death receptor-mediated, and caspase-independent apoptosis. It was reported that pH_i changes in apoptosis induced via the death receptor occurred after caspase activation whereas acidification in apoptosis induced through the mitochondrial pathway preceded the caspase activation (Furlong et al., 1998; Matsuyama and Reed, 2000). The pH-sensitive endonuclease is believed to play an important role in DNA fragmentation (Barry and Eastman, 1993). On the other hand, it was reported that intracellular acidification in CEM cells did not trigger the effector phase of dexamethasone- and etoposide-induced apoptosis. In these cells caspase 3 had maximum activity nearly to physiological pH_i (Benson et al., 1999). Early cytoplasm alkalinization before caspase activation and DNA fragmentation followed sometimes by acidification was reported (Belaud-Rotureau et al., 2000). It should be mentioned that there is a view that the change in intracellular pH_i is a secondary effect which has no significance in the regulation of apoptosis (see review Shrode et al., 1997).

APOPTOTIC CHANGES IN ELECTRICAL POTENTIAL DIFFERENCE ON THE CELL MEMBRANE

Cell depolarization in apoptosis was reported by a number of authors. It was detected in rat thymocytes

with the use of the potential-sensitive dye $DiOC_6$ (Dallaporta et al., 1999). Depolarization determined by flow cytometry with the potential-sensitive probe $DiBAC_4$ was observed in Fas- and thapsigargin-induced apoptosis in Jurkat cells (Bortner et al., 2001). It preceded cell shrinkage which was estimated by forward light scattering. Cell membrane depolarization was detected by electrical measurements in glucocorticoid-induced apoptosis of rat thymocytes (Mann et al., 2001). Cell shrinkage and plasma membrane depolarization, which are thought to be due to a decrease in pump activity, occurred under the experimental conditions simultaneously. Caspase-dependent depolarization caused by pump inhibition was reported in staurosporine-induced apoptosis of MCF7 cells (Düssmann et al., 2003). Similar results were obtained in U937 cells during Fas- and As_2O_3-induced apoptosis (Nolte et al., 2004). However, the authors assume that mechanisms of plasma membrane depolarization may be different in the same cells in dependence of mechanisms of apoptosis. Franco and colleagues (Franco et al., 2006) reviewed various mechanisms of cell depolarization in apoptosis. Apoptosis may be accompanied also by hyperpolarization of the plasma membrane (Zurgil et al., 2000). This effect was observed during dexamethasone-induced apoptosis in mouse thymocytes. In these cells *hyperpolarization* preceded phosphatidylserine externalization and correlated with the changes in the forward light scattering.

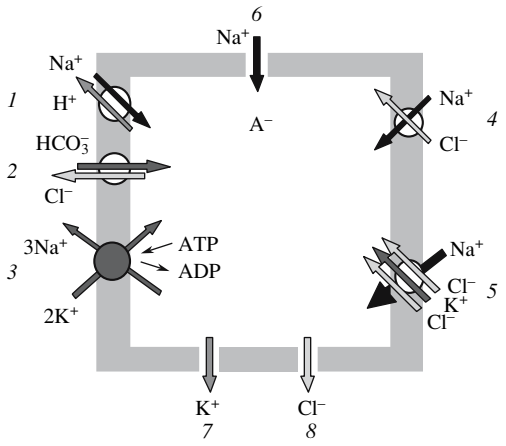

Fig. 2. The major ion pathways across the animal cell plasma membrane (from Hoffmann, 1987, modified).

1—Na^+/H^+antiporter; *2*—Cl^-/HCO_3^--antiporter; *3*—Na^+,K^+-"Pase; *4*—Na^+Cl^--cotransporter; *5*—$Na^+/K^+/2Cl^-$ cotransporter; *6*—Na^+ channels; *7*—K^+ channels; *8*—Cl^- channels, (A^-)—the intracellular anions impermeant the plasma membrane.

Arrows directed up- and downwards show ion fluxes up and down the electrochemical gradient.

MECHANISMS OF INTRACELLULAR ION CONTENT CHANGES IN APOPTOSIS

Generally, two factors generate asymmetric distribution of monovalent ions between cytoplasm and external medium: (1) the presence in the cell of anions which are "impermeant" through the cell membrane, and (2) the transport of ions against the gradient of electrochemical potential on the plasma membrane by ATP hydrolysis or the movement of other ions down their electrochemical gradient (see reviews Vereninov, 1978; Hoffmann, 1987; Macknight, 1987; Sperelakis, 1997; Fig. 2). The intracellular K^+ content depends on the quantity of "impermeant" anions inside the cell, on the pumping of K^+ into the cell and on the rate of dissipation of K^+ electrochemical gradient through the movement of K^+ via channels and other transporters. How are the properties of these ion pathways changed during apoptosis? The available data on this topic are presented below.

A decrease in the Na^+,K^+-ATPase pump activity during apoptosis was reported in many papers (see review Yu, 2003b). Degradation of the pump α and β subunits during dexamethasone-induced apoptosis was shown in thymocytes by immunoblotting (Mann et al., 2001). Degradation of catalytic and regulatory subunits of the Na^+,K^+-ATPase, a decrease in the ouabain-sensitive Rb^+ uptake, and proapoptotic action of ouabain (an inhibitor of the Na^+,K^+-ATPase) were found in Jurkat cells during Fas-induced apoptosis (Bortner et al., 2001). Degradation of the pump regulatory subunit was observed in staurosporine-induced apoptosis in MCF7 cells (Düssmann et al., 2003). Ouabain-sensitive Rb^+ uptake by P31 cells reduced during apoptosis was caused by amphotericin B (Marklund et al., 2001). It was shown by X-ray microanalysis of U937 cells that Rb^+ influx via the pump decreased in staurosporine-induced apoptosis (Arrebola et al., 2005a, 2005b). A decrease in the number of ouabain-binding sites and in their affinity to ouabain was found in Jurkat cells during CD95-induced apoptosis. The authors concluded that the Na^+,K^+-ATPase conformation was altered (Nobel et al., 2000). It was found that the cellular ATP content was reduced in apoptosis (Komatsu et al., 2000; Yang et al., 2002; Wang et al., 2003). However, there are reports that ATP content may increase in apoptosis (Zamaraeva et al., 2005).

Data on the effect of ouabain on apoptosis are controversial. It was shown that overexpression of Bcl-2 in transfected PW cells was accompanied by an increase both in resistance to apoptosis and in activity of the Na^+,K^+-ATPase pump. Resistance to apoptosis was lost in transfected cells treated with ouabain (Gilbert and Knox, 1997). Ouabain increased CD-95-mediated cell shrinkage (Nobel et al., 2000). It was concluded that inhibition of the Na^+,K^+-ATPase potentiated apoptotic cell shrinkage. Ouabain aggravated Fas-induced apoptosis in activated lymphocytes (Esteves et al., 2005). The opposite result was obtained in HL60 cells in UV-induced apoptosis. Inhibition of the Na^+,K^+-ATPase by ouabain decreased the number of apoptotic cells with

reduced volume and the number of apoptotic bodies (McCarty and Cotter, 1997). The contradictions probably can be explained by different mechanisms of apoptotic induction: via the death receptor pathway in one case and via the mitochondrial pathway in the other. Orlov with collaborators (Orlov et al., 1999) found that ouabain suppressed caspase activity irrespective of inducers.

K^+ channels. A least 14 species of K^+ channels are considered to be involved in apoptosis (see reviews Yu, 2003a; Remillard and Yuan, 2004; Burg et al., 2006). Apoptosis can be prevented by blocking K^+ channels. Thus, apoptotic cell shrinkage of eosinophils was inhibited by 4-aminopyridine, a well-known blocker of K^+ channels (Beauvais et al., 1995). Quinine and Ba^{++} blocked shrinkage of HeLa and U937 cells associated with apoptosis induced by staurosporine or TNF (Maeno et al., 2000). AVD of mice lymphocytes was prevented by blocking of calcium-sensitive K^+ channels with quinine, clotrimazole or charybdotoxin (Elliot and Higgins, 2003).

It was shown that the activity of K^+ channels was changed in apoptosis (Lang et al., 2006). An enhanced integral conductivity of the channels K_V and maxi-K was observed during apoptosis in vascular muscle cells (Ekhterae et al., 2001; Krick et al., 2001). An increase in activity of channels K_V 1.3 was observed in Jurkat cells in Fas-induced apoptosis (Storey et. al., 2003). In contrast, a reduction of the integral conductivity of the channels K_V 1.3 was observed in Fas- and ceramide-induced apoptosis in Jurkat cells (Szabò et al., 1996; Gulbins et al., 1997). A mathematical modeling of the cell's ion and water balance in apoptosis lead Vereninov and coauthors to the conclusion that apoptotic cell shrinkage could be caused by opening of K^+ channels only in particular cells and under limited conditions (Vereninov et al., 2004b).

There are findings that neuron voltage-sensitive *Na^+ channels* are activated in apoptosis caused by oxygen deprivation (Banasiak et al., 2004). Inhibition of Na^+ influx with a Na^+ channel blocker, saxitoxin, prevented apoptosis induced through Fas-receptor in Jurkat cells (Bortner and Cidlowski, 2003). The role of the Cl^- channels in apoptotic cell shrinkage has attracted great attention as they are known to be cell volume regulators under anisosmotic conditions (Maeno et al., 2000). Cl^- efflux through ORCC channels was observed in Jurkat cells in Fas-induced apoptosis (Szabò et al., 1998). Activation of Cl^-- channels VSOR was found in apoptosis of HeLa cells (Shimizu et al., 2004) and mouse cardiomyocytes (Wang et al., 2005; Okada et al., 2006).

Other pathways. It was reported that spontaneous apoptosis of thymocytes is accompanied by the activation of the *Na^+/H^+-antiporter*, *$Na^+/HCO_3^-/CO_3^{2-}$-cotransporter* and Cl^-/HCO_3^--exchanger (Tsao and Lei, 1996). In contrast, inhibition of the Na^+/H^+-exchanger was observed during apoptosis in Jurkat, Molt4, CEM, and K562 cells (Lang et al., 2000; Rich et al., 2000). Inhibition of Na^+, K^+, $2Cl^-$ cotransporter was found in P31 cells during amphothericin-induced apoptosis (Marklund et al., 2001). It should be noted that alteration of many ion pathways have been observed in apoptosis. However, the role of the particular pathways in the disturbance of the total ion balance in apoptosis is poorly understood.

First attempts to study the time course of the ion balance alteration in apoptosis have been made recently (Arrebola et al., 2005a, 2006). The data obtained by X-ray microanalysis indicate that there are two phases in the changes of intracellular K^+, Na^+, and Cl^- concentration in U937 cells induced to apoptosis by staurosporine. The early phase is characterized by a decrease in intracellular K^+ and Cl^- content concomitant with cell shrinkage which occur before activation of caspase 3. The late phase starts with caspase 3 activation and is accompanied by a decrease in the K^+/Na^+ ratio due to a significant increase in Na^+ content (Arrebola et al., 2005a). Bortner and Cidlowski (Bortner and Cidlowski, 2003) came to conclusion that an early increase in the intracellular Na^+ concentration plays a crucial role in initiating apoptosis.

CONCLUSIONS

The data on the mechanisms of cell shrinkage in apoptosis appear to be scanty and many questions still remain open. Nevertheless, a number of hypotheses on the sequence of events during induction of apoptosis have been suggested (Krick et al., 2001; Platoshyn et al., 2002; see reviews Yu, 2003a; Remillard and Yuan, 2004). According to one of the current schemes (Yu, 2003a) an apoptotic inducer activates K^+ channels; channel opening results in plasma membrane hyperpolarization, then mitochondrial swelling and depolarization mediated by Bcl-2/Bid factors occur. These changes cause a decrease in the ATP level and therefore the Na^+,K^+-ATPase dysfunction, the plasma membrane depolarization and cell shrinkage. Concomitant cytochrome *c* release from mitochondria leads to the formation of apoptosomes and to the DNA degradation.

Analysis of ionic events during apoptosis confirms the concept that apoptosis is a phenomenon that can be realized in diverse ways. For example, etoposide-induced apoptosis of U937 cells is not accompanied by cell shrinkage while typical shrinkage is observed in staurosporine-induced apoptosis. It should be added that the lack of shrinkage during etoposide-induced apoptosis in U937 cells is not a feature of etoposide as an inducer because it causes apoptosis with shrinkage in rat thymocytes (Vereninov et al., 2003; 2004a; Yurinskaya et al., 2005a, 2005b). Changes in the monovalent ion balance in apoptosis are found in all studied cases and should be considered as an obligatory feature of apoptosis. However, the ionic changes occur even when there is no apoptotic cell shrinkage. Evidently,

these changes are significant per se, irrespective to the apoptotic cell dehydration.

There are two apparent discrepancies in the current concepts on the mechanism of apoptotic cell shrinkage. First, a decrease in the sodium pump activity is thought to be responsible for apoptotic decrease in cell K^+ content. However, a deficiency in the pumping rate of Na^+ and K^+ should lead to the cell swelling whereas shrinkage of cells is inherent in apoptosis. Second, the opening of the K^+ channels is believed to play a significant role in the loss of intracellular K^+ during apoptosis. Nevertheless, the opening of these channels should lead to hyperpolarization of apoptotic cells rather than depolarization observed in most experiments. An attempt to overcome these discrepancies has been undertaken recently by Vereninov and coauthors (Vereninov et al., 2007). They studied unidirectional fluxes of monovalent cations in apoptotic U937 cells and came to conclusion that shrinkage is caused by a complex decrease in the sodium pump activity, in Na-Cl symport, and in the integral permeability of Na^+ channels.

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