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Ca^{2+} Signaling, Intracellular pH and Cell Volume in Cell Proliferation

R. Schreiber
Institut für Physiologie, Universität Regensburg, Universitätsstr. 31, D-93053, Regensburg, Germany

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Abstract. Mitogens control progression through the cell cycle in non-transformed cells by complex cascades of intracellular messengers, such as Ca^{2+} and protons, and by cell volume changes. Intracellular Ca^{2+} and proton concentrations are critical for linking external stimuli to proliferation, motility, apoptosis and differentiation. This review summarizes the role in cell proliferation of calcium release from intracellular stores and the Ca^{2+} entry through plasma membrane Ca^{2+} channels. In addition, the impact of intracellular pH and cell volume on cell proliferation is discussed.

Key words: Cell proliferation — Tumor growth — Intracellular calcium — Calcium signalling — Calcium entry — TRP-Channels — Intracellular pH — pH homeostasis — Sodium-proton exchanger — Cell volume

Introduction

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), intracellular pH ($[pH]_i$) and cell volume are critical in cellular processes. Changes in $[Ca^{2+}]_i$ are key steps in fertilization, development and differentiation, motility, apoptosis and proliferation [8, 34, 49, 95]. Like Ca^{2+}, also pH plays a central role in the regulation of many aspects of cell physiology, and protons may function as second messengers similarly to Ca^{2+} [1]. Relatively small changes in $[pH]_i$ are parallel to changes in cell volume and are involved in cell metabolism, apoptosis and proliferation [37, 49, 95]. In this review we will focus on the role of Ca^{2+} channels, Ca^{2+} signalling, $[pH]_i$ and cell volume for their role in controlling cell proliferation.

Correspondence to: R. Schreiber; email: rainer.schreiber@vkl.uni-regensburg.de

$[Ca^{2+}]_i$ and Cell Proliferation

Changes of $[Ca^{2+}]_i$ are associated with progression through the cell cycle. The cell cycle consists of four primary phases: G_1, the first gap phase; S phase, in which DNA synthesis occurs; G_2, the second gap phase; and the M phase, or mitosis, in which the chromosomes and cytoplasmic components are divided between two daughter cells. When proliferating cells are deprived of growth factors, they exit from the cell cycle after having reached the G_1 phase and enter a quiescent state termed G_0. In the G_0 phase, they stop growing and depress protein synthesis. The transitions between these cell cycle phases are tightly regulated, and major checkpoints at the G_1/S and G_2/M transition points arm the cell with devices that prevent the G_1 or G_2 cell cycle engines from starting replication or mitosis until all is in order and may even command the cell to self-destruct if a problem, such as damaged chromosomes, cannot be repaired [35, 113]. Extracellular Ca^{2+} is required at distinct points in the cell cycle. When proliferating mouse or human fibroblasts are placed into media containing low Ca^{2+}, they cease cellular division and accumulate in G_1 phase [13, 38, 114]. In BALBc/3T3 fibroblasts, this G_1 arrest was reversible and increasing the extracellular Ca^{2+} concentration to normal level enabled cells to undergo DNA synthesis within hours [13]. Transient changes of $[Ca^{2+}]_i$ occur at the exit from quiescence in early G_1 phase, at the G_1/S transition, during the S phase and at the exit from M phase. At the early G_1 phase and at the G_1/S transition the cells are most sensitive to the depletion of extracellular Ca^{2+} [7, 12, 66, 90]. This requirement for extracellular Ca^{2+} in proliferation is modulated by the degree of cellular transformation. Neoplastic or transformed cells continued to proliferate in Ca^{2+}-deficient media, but how this change in extracellular Ca^{2+} dependence affects intracellular Ca^{2+}-dependent pathways is unkown [11, 112]. In addition to the requirement for extracellular Ca^{2+}, intracellular

Ca^{2+} stores are also required for cell proliferation. Depletion of intracellular inositol 1,4,5-triphosphate ($InsP_3$)- sensitive Ca^{2+} stores with the Ca^{2+}-pump blocker thapsigargin arrests cell growth. After removal of thapsigargin, cells re-enter the S phase [94]. The consequence of Ca^{2+}- store depletion includes inhibition of DNA synthesis, protein synthesis and nuclear transport [31, 94].

Ca^{2+} acts both as a ubiquitous allosteric activator and inhibitor of intracellular enzymes in the cytosol, organelles and nucleus. The best known Ca^{2+}-binding protein is calmodulin. Ca^{2+}/calmodulin regulates numerous intracellular enzymes including phosphodiesterases, adenylyl cyclases, ion channels, protein kinases, and protein phosphatases, i.e., calcium-calmodulin dependent kinase type II (CaM- kinase II) and the CaM-dependent phosphatase calcineurin. The G_1/S transition, the progression from G_2 to M and the metaphase/anaphase transition are specific points of intervention of CaM-kinase II [42, 90]. Activated CaM-kinase promotes phosphorylation of proteins, such as Ca^{2+}/cAMP response element (CRE) binding protein (CREB, a nuclear Ca^{2+}- responsive transcription factor) and Ras (a cytosolic Ca^{2+} responsive transcription factor), that are required for initiating and maintaining the cell cycle [54]. Additionally CaM-kinase and calcineurin-regulating nuclear factors are involved in the DNA division machinery like cyclin-dependent kinases (cdk4) and cyclins (cyclin D1) [42, 90].

Ca^{2+} Release from Intracellular Ca^{2+} Stores

$[Ca^{2+}]_i$ is maintained at nearly 10^{-7} M in resting conditions by active mechanisms, mainly by Ca^{2+} pumps in the endoplasmic reticulum (sacro/endoplasmic reticulum Ca^{2+} ATPase, SERCA) and plasma membrane (plasma membrane Ca^{2+}-ATPase, PMCA). Both Ca^{2+} pumps are extruding Ca^{2+} from cytosol by direct energy consumption. Both extracellular Ca^{2+} and intracellularly stored Ca^{2+} in endoplasmic reticulum (ER) is much higher (5,000–20,000 times) than $[Ca^{2+}]_i$. This enormous concentration gradient allows Ca^{2+} influx and release to occur in a passive way. Ca^{2+} release from intracellular Ca^{2+} stores is triggered by growth factors. Peptide growth factors, such as FGFs, EGF, PDGF bind to receptors with intrinsic tyrosine kinase activity, receptors associated to cytosolic tyrosine kinase or G- protein-coupled receptors. Stimulation of these receptors coupled to phospholipase C (PLC) generates the second messenger inositol 1,4,5-triphosphate ($InsP_3$) and diacylglycerol (DAG) (Fig. 1). $InsP_3$ serves as chemical messenger and induces Ca^{2+} release from intracellular Ca^{2+} stores by activating specific Ca^{2+} channels located in the ER membranes. The $InsP_3$ receptor is a multimeric Ca^{2+} channel that opens after binding to $InsP_3$ and gives rise to a very fast and short increase of $[Ca^{2+}]_i$. The Ca^{2+} spike is limited by $[Ca^{2+}]$ in the stores and by the activity of the calcium pumps SERCA and PMCA [6, 54].

Extracellular Ca^{2+} Influx by Voltage-operated Ca^{2+} Channels

Longer lasting Ca^{2+} signals are generated by activation of extracellular Ca^{2+} entry mediated by Ca^{2+}-permeable cationic channels in the plasma membranes. Different mechanisms of Ca^{2+} entry are realized in excitable cells and non-excitable cells. Depolarization of excitable cells, such as neurons, muscles and secretory cells, activates voltage-operated Ca^{2+} channels (VOCs). Ca^{2+} entry through VOCs modulates the electrical activity of these cells and is largely excluded from nonexcitable cells. In heart and skeletal muscle activation of L-type VOCs by depolarization of the membrane potential causes Ca^{2+} entry and activation of ryanodine receptors. Recruitment of Ca^{2+} sparks produced by clusters of activated ryanodine receptors underlies the Ca^{2+} signal that activates myocyte contraction. The Ca^{2+} rise is rapidly reduced by Ca^{2+} pumps so that $[Ca^{2+}]_i$ remains low [16, 54, 70]. In addition to depolarization, which activates ryanodine receptors, hypertrophic growth of excitable cells is caused by a slow increase of resting $[Ca^{2+}]_i$ by activation of $InsP_3$ receptors, due to long-term agonist increase of $InsP_3$ concentration [53, 54]. The other product of PLC activation, DAG, serves as a signal to translocate and activate the major regulatory kinase, protein kinase C (PKC). In addition, DAG appears to have important PKC-independent effects on Ca^{2+} entry channels [39, 40] (Fig. 1).

Extracellular Ca^{2+} Influx by Capacitative and Non-Capacitative Calcium Entry

Mitogens activate Ca^{2+} entry by two major pathways. The first mechanism is called capacitative calcium entry (CCE). Depletion of intracellular Ca^{2+} stores activates store-operated Ca^{2+} channels (SOCs) in plasma membranes. The CCE is used in all eukaryotic cells. The second mechanism is called the non-capacitative calcium entry (NCCE). NCCE is carried by store-independent Ca^{2+} channels, like second-messenger-operated channels or receptor-operated Ca^{2+} channels (ROCs) [79] (Fig. 1). These two mechanisms of Ca^{2+} entry may coexist in the same cell and could be activated, dependent on both agonist concentration and channel expression. NCCE is activated by low agonist concentration, while high agonist concentrations induce CCE [66]. NCCE is induced by growth factors, which bind to their surface receptors and increase tyrosine kinase activity by

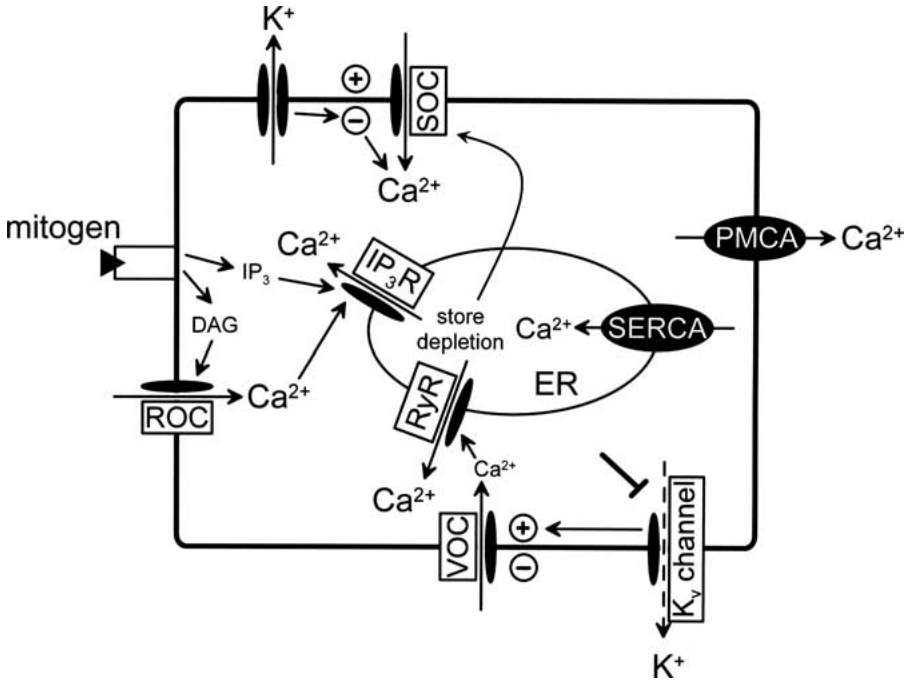

Fig. 1. Proposed mechanisms involved in the regulation of $[Ca^{2+}]_i$ in mammalian cells. Receptor activation by mitogens triggers the formation of diacylglycerol (DAG) and inositol 1,4,5- triphosphate (IP_3). DAG activates receptor-operated channel (ROC)-mediated Ca^{2+} influx. IP_3 activates Ca^{2+} release from endoplasmic reticulum (ER) by activating IP_3 receptors (IP_3R). Subsequent depletion of Ca^{2+} stores in turn activates store-operated channel (SOC)-mediated Ca^{+} influx. In non-excitable cells activation of K^{+} channels causes a hyperpolarization (−) of the plasma membrane and increases driving force for Ca^{2+} entry. In contrast, in excitable cells membrane depolarization (+) by reduced K_v channel activity leads to activation of voltage-operated Ca^{2+} channels (VOC) and Ca^{2+} entry, which is capable of triggering ryanodine receptor (RyR)-mediated Ca^{2+} release, a positive feedback mechanism known as Ca^{2+}-induced Ca^{2+} release. Low $[Ca^{2+}]_i$ under resting conditions is achieved and maintained mainly by active Ca^{2+} transport by Ca^{2+}-Mg^{2+}-ATPases in the ER (SERCA) and plasma membrane (PMCA).

receptor dimerization. Intracellular signalling molecules, e.g., phospholipase C ($PLC\gamma$), phosphatidylinositol-3-kinase (PI-3K), ras activating protein (GAP), ras, mitogen-activated protein kinase (MAPK) and phospholipase A_2 (PLA_2), are activated [65, 91]. In some cells, activation of PLA_2 by bFGF (basic fibroblast growth factor) and other growth factors increases free intracellular arachidonic acid concentration. Arachidonic acid directly contributes to the induction of Ca^{2+} entry from the extra-cellular space and the regulation of cell proliferation [24]. In other cell types, such as exocrine cells from the avian nasal gland, HEK cells, Balb-C 3T3 fibroblast, smooth muscle cells and endothelial cells, arachidonic acid induces Ca^{2+} entry directly, independent of intracellular Ca^{2+}-store depletion and at low concentrations (1–8 μM) [65]. If arachidonic acid is rapidly metabolized, a rise in a large family of eicosanoids occurs [69]. These eicosanoids are well known to be involved in cell proliferation of endothelial cells [111]. On the other hand, arachidonic acid inhibits store-operated Ca^{2+} entry. A concept has been developed, suggesting reciprocal regulation of store-operated and non-store-operated Ca^{2+} entry [62]. It is being discussed, that the production of the intracellular messenger nitric oxide (NO) by nitric oxide synthase (NOS) could modulate cell proliferation in smooth muscle and endothelial cells. NO may act as a mediator of arachidonic acid-induced Ca^{2+} entry [62, 110].

Which Ca^{2+} Channel Is Relevant for Ca^{2+} Entry?

Electrophysiological analysis of channels activated by store-emptying has defined one current, the Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}), which operates mostly in small hematopoietic cells [78, 79]. The activation of this small, highly Ca^{2+}-selective current, appears to require substantial Ca^{2+}-store depletion. In several cell types, receptors can activate a highly Ca^{2+}-selective but distinct current, which is mediated by arachidonic acid [58]. This current, known as arachidonic acid-regulated Ca^{2+}-selective current, can be activated by low agonist concentrations, but is not activated by store-emptying alone [96]. In other cells, store-depletion appears to trigger activation of larger, less selective cation currents [102], which may have significance in modifying membrane potential [28]. The genes encoding mitogen-activated or store-operated Ca^{2+} plasma membrane channels are still elusive. Electrophysiological and fluorimetric measurements suggest that they

form a heterogeneous family, showing different biophysical properties. Many of them are non-selective cationic channels, permeable to calcium, sodium and potassium.

Research on *Drosophila* phototransduction identified a gene encoding a subunit of a Ca^{2+}-permeable channel [36, 64]. Light induces a PLC-dependent activation of an inward cationic current that generates a rapid and a sustained depolarization of the receptor potential [59, 63]. Mutations of the gene cause only a transient receptor potential (TRP). The family of TRPs is a heterogeneous family, with different Ca^{2+} selectivities. The mammalian TRP family can be divided into three subfamilies: First, the TRP-canonical (TRPC) family, comprising seven members, TRPC1-7; second, TRP-vanilloid (TRPV) family, which consists of six members, TRPV1-6; and third, TRP-melastatin (TRPM) family, which contains eight members, TRPM1-8. TRPC channels are directly activated by intracellular messengers, such as DAG (TRP and TRP-like channels [17]) and fatty acids, including arachidonic acid (TRPC3, 4, 6; [39, 97, 118]. TRPC1 channels seem to be involved in bFGF-induced Ca^{2+} entry of endothelial cells [4]. TRPC1 channels are in charge of store-operated Ca^{2+} influx, which is critical for cell proliferation of pulmonary artery smooth muscle cells. In these cells, expression of TRPC1 correlates with the cell proliferation rate. Basal levels of TRPC1 protein expression are significantly higher in proliferating than in growth-arrested cells. Application of antisense oligonucleotides against TRPC1 mRNA reduces cell proliferation [29, 99]. Additionally, in these cells PDFG stimulates proliferation and enhances the expression level of TRP6 mRNA [46, 121]. In contrast, TRPC6 expression is downregulated in an autocrine tumor model [14]. The involvement of other calcium channels is discussed. In Balb/c 3T3 fibroblasts a Ca^{2+}-permeable cation channel with homology to the TRP family has been characterized and named "growth factor channel". This channel is activated by IGF-I, which induces its translocation from the intracellular pools to the plasma membrane [43]. The literature is unequivocal of the involvement of TRPs on store-operated Ca^{2+} entry. The relationship of any particular TRP to SOC is likely to be cell-specific and influenced by such factors as endogenous expression of other TRP members capable of forming heteromultimeric structures, as well as the availability of cellular regulatory mechanisms [104].

Membrane Potential and Ca^{2+} Entry

In excitable cells, i.e., smooth muscle cells, membrane potential controls $[Ca^{+}]_i$ by modulating the activity of sarcolemmal voltage-dependent Ca^{2+} channels [67, 103]. Membrane depolarization opens voltage-gated Ca^{2+} channels, increases Ca^{2+} influx, and raises $[Ca^{2+}]_i$ in smooth muscle cells (Fig. 1) [25, 67, 122]. Membrane depolarization may also promote Ca^{2+} entry via the reverse mode of Na^{+}/Ca^{2+} exchange, which triggers Ca^{2+}-induced Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores and further increases $[Ca^{2+}]_i$ [10, 93]. Persistent membrane depolarization causes sustained increase in $[Ca^{2+}]_i$ and, thus, has a constant stimulatory effect on pulmonary artery smooth muscle cell proliferation [83].

In non- excitable cells, activation of K^{+} channels causes a hyperpolarization of the plasma membrane and increases the driving force for Ca^{2+} entry (Fig. 1). Indeed, membrane depolarization of melanoma cells by voltage clamp decreased and hyperpolarization increased $[Ca^{2+}]_i$, indicating a transmembrane Ca^{2+} flux in accordance with the electrochemical gradient [52, 108]. Moreover, K^{+} -channel blockers inhibit cell cycle progression by membrane depolarization. Blockade of I_K inhibits proliferation of melanoma, T-lymphocytes and human breast carcinoma cells [72]. Among various types of K^{+} channels, the role of EAG K^{+} current in tumorigenesis is the best established to date [5]. The EAG K^{+} channel was first cloned from *Drosophila melanogaster* [109]. Inhibition of EAG expression in tumor cells significantly reduces cell proliferation, whereas promotion of EAG expression stimulates cancer cell growth [77]. In addition, EAG cDNA has been cloned from human breast carcinoma MCF-7 cells and, noticeably, EAG mRNA is not detectable in normal breast [73]. Moreover, the expression of EAG favors tumor progression when transfected cells are injected into immune-depressed mice. The transforming activity of EAG and its ectopic expression in tumor cell lines provides strong evidence for EAG's oncogenic potential [77].

Intracellular pH and Proliferation

The growth of many tumors is associated with changes in the tumor micro-environments leading to hypoxia and decrease of extracellulare pH. Microelectrode measurements demonstrated average extracellular pH values ranging from 6.5–6.9 for tumors and values of 7.0–7.5 for normal cells, with an average difference of 0.5 pH units [33, 105, 115]. Extracellular accumulation of lactate is thought to be a major reason for tumor acidity. Because of regional hypoxia, lactic acid generation is increased in tumors by anaerobic metabolism. In contrast to the acidic environments, the pH_i of tumor cells is generally more alkaline than that of normal cells [98, 105, 115]. It has been shown that alkaline intracellular pH is necessary for various mechanisms involved in cell

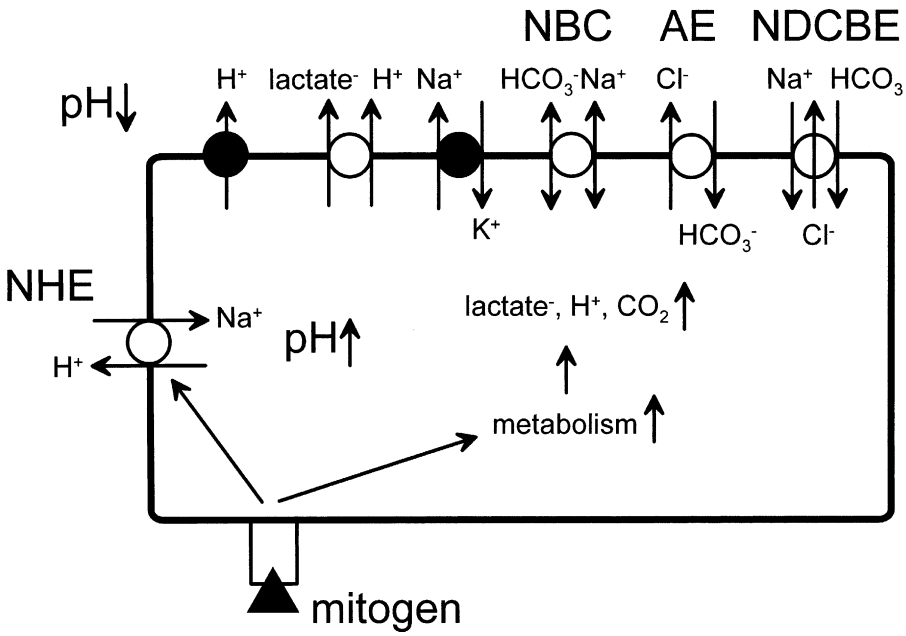

Fig. 2. Summary of the main acid/base transporters of mammalian cells. Receptor activation by mitogens induces activation of the Na^+/H^+ exchanger (NHE) leading to alkalinization of the cytosol. In tumors glycolysis and the production of lactic acid ($lactate^-$) and H^+ is up-regulated. The transporter H^+-ATPase, NHE, $lactate^-$-H^+ symporters, Na^+/HCO_3^- cotransporter (NBC), Cl^-/HCO_3^- exchanger (AE) and Na^+ dependent $C1^-/HCO_3^-$ exchanger (NDCBE) are involved to prevent apoptosis by cellular acidosis. Extracellular accumulation of lactate causes tumor acidity [46, 95]. Black cycles indicate active ATP-dependent transport.

proliferation. Several metabolic enzymes like phosphofructokinase, the rate-limiting step of glyolysis, or the protein, RNA and DNA synthesis have a pH optimum at alkaline pH [23, 55]. Thus it appears to be a general rule that higher pH_i correlate with increased metabolic activity and cell proliferation.

Cells have multiple parallel mechanisms for pH regulation. Such a redundancy of regulatory mechanisms underlines the importance of pH homeostasis for cellular function. The main acid/base transporters of mammalian cells are vacuolar H^+-ATPases, Na^+/H^+ exchangers (NHE), $lactate^-$-H^+ symporters and HCO_3^- transporters [34, 95] (Fig. 2). Because of the reduced fixed buffer capacity of the CO_2/HCO_3^- system, it is assumed that the elevation of pH_i in proliferating cells is due to activation of NHE. The acidic extracellular environment reduces the external HCO_3^- concentrations and therefore diminishes the HCO_3^--dependent H^+ transport [56]. In humans there are nine NHE isoforms, which are members of a gene family called SLC9A [107, 123]. The NHE isoforms differ in tissue distribution and have multiple cellular functions including pH homeostasis, cell volume regulation and transcellular solute transport. The ubiquitous isoform NHE1 has been implicated in carcinogenesis. Treatment of cells with mitogens activates NHE1 by phosphorylation. Additionally, NHE1 is a calmodulin-binding protein and Ca^{2+}/calmodulin appears to regulate the activity of NHE1 [57, 87, 106]. Various other molecules are involved in signal transduction regulating NHE1 activity, such as Ras-mediated ERK, G protein-coupled receptors, PKC, RhoA, and integrin receptors [9, 45, 51, 100, 101]. In contrast, expression of NHE isoforms into NHE antiporter-deficient Chinese hamster ovary cells shows that NHE2 and NHE3 are able to support proliferation of these cells under acid pH conditions [44]. These results are indicating that the ubiquitous "housekeeping" NHE1 isoform is not essential for regulation of pH_i and cell growth and could be replaced by NHE2 or NHE3. But there are compelling evidences, that NHE1 plays an important role in cell proliferation. A recent study of NIH3T3 cells transfected with the E7 oncogene of human papillomavirus type 16 demonstrates that activation of the NHE1 and resulting cellular alkalinization is a key mechanism in oncogenic transformation and is necessary for the development and maintenance of the transformed phenotype [85].

NHE1 is also involved in cell survival and has anti-apoptotic activity. Cellular acidosis and decrease in cell volume are both triggers of apoptosis [49]. Malignant gliomas exhibit an elevated steady-state pH_i, which is shown due to increased activation of NHE1 activity [56]. In the highly invasive 1-LN human prostate tumor cell line the binding of plasminogen type II (Pg2) to depeptidyl peptidase IV (DPPIV) causes an induction of an intracellular Ca^{2+}-signalling cascade accompanied by a rise in pH_i. It is shown that Pg2 activates phospholipase C, which induces Ca^{2+} release from Ca^{2+} stores and additionally regulates pH_i, via an association of

NHE3 linked to DPPIV, both necessary for tumor cell proliferation and invasiveness [30]. In androgen-independent prostate cancer cells, intracellular ATP induces growth arrest by enhancing Ca^{2+} entry, which causes a rapid and sustained decrease of pH_i. This intracellular acidification is linked to mitochondria function and particularly to the F_0F_1-ATPase. In addition, intracellular acidification reduces Ca^{2+} release from ER and cell proliferation. This finding suggests that the existence of cross-talk between pH_i and Ca^{2+} homeostasis can in turn reduce the amount of releasable Ca^{2+} from the ER. In prostate cancer cells, intracellular acidification is one of the major mechanisms leading to growth arrest induced by ATP [41].

Cell Volume and Proliferation

Activation of NHE by mitogenic factors is paralleled by activation of Na^+-K^+-$2Cl^-$ cotransport and of system A for neutral amino acid transport [2, 15, 47, 74, 75, 80]. A consequence of the activation of these transport systems is an increase of cell volume. Swelling co-activates K^+ and Cl^- effluxes, which initiate regulatory volume decrease. Block of such K^+ and Cl^- channels inhibits cell proliferation and decreases [^{3}H]- thymidine incorporation [48, 71, 72, 117]. Two hypotheses have been proposed for the link of K^+ channel activity and DNA synthesis. The first assumes that, by controlling membrane potential, K^+ channel activity influences $[Ca^{2+}]_i$ (*see* above). The second hypothesis proposes that K^+ channel activity would control ion influx-efflux ratio and the cell volume [18, 88]. The cell-volume hypothesis is supported by the findings, that the membrane potential of neuroblastoma cells as well as that of astrocytes is poorly sensitive to K^+ channel blockers [61, 76, 88] and the proliferation of tumor cells is weakly dependent on extracellular Ca^{2+} concentration [20]. In fact, Rouzaire- Dubois et al. reported that inhibition of proliferation of neuroblastoma cells by K^+ channel blockers is associated with cell swelling [19, 89]. It is known that a close connection exists between cell size and division [32, 68, 84]. In general, cells must double their size before they divide so that they maintain a constant average size over generations. In neuroblastoma and glioma cells, the relationship between proliferation rate and cell volume is bell-shaped and proliferation is optimal within a cell-volume window. Beyond an optimal cell volume the proliferation rate decreases [19]. Indeed, osmotic alterations of cell volume modify cell proliferation. Hypertonic shrinkage inhibits [82, 86, 119] and slight osmotic swelling has been shown to accelerate cell proliferation [3]. When exposed to enhanced extracellular ionic strength, the cells may overcome cell shrinkage by cellular accumulation of osmolytes, which then allows them to proliferate normally [50, 119, 120].

How alterations of cell volume interact with the cell cycle control is speculative. It has been shown that cell swelling stimulates protein kinases ERK-1 and ERK-2 [92], proteins probably involved in regulation of cell cycle. Cell growth (increase in cell size) and division (increase in cell number) are controlled by two distinct but interconnected pathways: the target of rapamycin (TOR) pathway and the mitogen-activated protein kinase (MAPK) and cyclin pathway. At the membrane level, mitogens and growth factors stimulate the activity or expression of nutrient transporters, non-selective cations, K^+ and Cl^- channels [21, 26, 116, 117]. The resulting fluxes of nutrients and ions are associated with water fluxes and cell volume changes. It is well known that conformation and activity of macromolecules are dependent on their crowding and on the ratio of hydration versus osmotically active water. Macromolecules in the cell sterically "crowd" each other in their cohabited aqueous space and this steric interaction can be enhanced by the preferential hydration of molecules. Because of these properties, relatively small changes in macromolecule concentration lead to large changes in their activity [22, 27, 60, 81]. Consequently, changes in cell volume should alter the activity of macromolecules by crowding, including proteins in cell growth and division pathways [19].

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