Ion channels and transporters in cancer. 1. Ion channels and cell proliferation in cancer

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Submitted 18 February 2011; accepted in final form 20 March 2011

Becchetti A. Ion channels and transporters in cancer. 1. Ion channels and cell proliferation in cancer. Am J Physiol Cell Physiol 301: C255–C265, 2011. First published March 23, 2011; doi:10.1152/ajpcell.00047.2011.—Progress through the cell mitotic cycle requires precise timing of the intrinsic molecular steps and tight coordination with the environmental signals that maintain a cell into the proper physiological context. Because of their great functional flexibility, ion channels coordinate the upstream and downstream signals that converge on the cell cycle machinery. Both voltage- and ligand-gated channels have been implicated in the control of different cell cycle checkpoints in normal as well as neoplastic cells. Ion channels mediate the calcium signals that punctuate the mitotic process, the cell volume oscillations typical of cycling cells, and the exocytosis of autocrine or angiogenetic factors. Other functions of ion channels in proliferation are still matter of debate. These may or may not depend on ion transport, as the channel proteins can form macromolecular complexes with growth factor and cell adhesion receptors. Direct conformational coupling with the cytoplasmic regulatory proteins is also possible. Derangement or relaxed control of the above processes can promote neoplasia. Specific types of ion channels have turned out to participate in the different stages of the tumor progression, in which cell heterogeneity is increased by the selection of malignant cell clones expressing the ion channel types that better support unrestrained growth. However, a comprehensive mechanistic picture of the functional relations between ion channels and cell proliferation is yet not available, partly because of the considerable experimental challenges offered by studying these processes in living mammalian cells. No doubt, such studies will constitute one of the most fruitful research fields for the next generation of cell physiologists.

That membrane potential ($V_m$) and ion transport may have a role in the cell cycle has been long suggested, at a time when the existence of ion channels was still only a hypothesis. Differentiated cells were generally found to be hyperpolarized compared with quiescent or cycling cells, particularly the neoplastic (15, 32). Information quickly raised after the advent of the patch-clamp technique, which extended the range of structures amenable to measurement down to subcellular organelles. The first mammalian cell types to be addressed were structures amenable to measurement down to subcellular or-rich of the patch-clamp technique, which extended the range of neoplastic (15, 32). Information quickly raised after the advent of cell proliferation cannot be drawn for many reasons. First, the physiological effect of $V_m$ depends on the specific complement of channels expressed by a certain cell. For example, hyperpolarization increases the driving force for Ca$^{2+}$ (i.e., the difference between $V_m$ and the equilibrium potential for Ca$^{2+}$), thus facilitating Ca$^{2+}$ entry from the extracellular space. However, hyperpolarization also tends to close VG Ca$^{2+}$ channels. The balance of these effects on Ca$^{2+}$ influx depends on which calcium conduction pathways are expressed by a certain cell. These pathways have turned out to be very numerous and include nonselective cation channels, voltage-dependent and -independent Ca$^{2+}$ channels, and several Ca$^{2+}$ transporters (126). Second, merely considering the average $V_m$ measured in a population of cells can be misleading. The activity and expression of a given channel type can oscillate during the mitotic process and thus produce fluctuations in $V_m$ in phase with the cell cycle (4, 33, 36). Third, it is increasingly recognized that ion channels exert nonconducting signaling roles that may be totally unrelated to $V_m$ (65). These as well as other reasons suggest caution in inferring $V_m$ changes simply based on modified expression of ion channels. This applies particularly to the VG K$^+$ channels, whose voltage-dependent features are extremely diversified. Therefore, overexpressing a certain K$^+$ channel does not necessarily lead to significant cell hyperpolarization.

These complications explain some early perplexities about the relation among $V_m$, ion transport, and cell proliferation, such as the observation that blocking K$^+$ channels often inhibits proliferation although cycling cells tend to be depolarized. Recent work has coupled the use of ever more specific channel inhibitors with increasingly potent molecular biological methods. The result of these efforts is a blossoming information on specific ion channels and transporters affecting distinct aspects of proliferation in normal as well as neoplastic cells (6, 17, 71, 112, 145). What was perhaps not expected in the “heroic” age is the bewildering complexity of the regulatory interactions between ion channels and the other elements of the signaling network. Although the fact that ion channels contribute to regulate cell proliferation seems now established, a conclusive picture of the ion channel-dependent control of the division cycle in mammalian cells is yet to be reached.

The Cell Cycle Checkpoints

The cell mitotic cycle comprises a sequence of events that must be precisely coordinated, such as DNA replication, chromosome condensation and segregation, duplication and migration of the spindle pole, breakdown of the nuclear envelope, and cytokinesis. Moreover, mitosis must be synchronized with cell growth for cells to maintain their size during proliferation.
Correct progress through the cell cycle is ensured by checkpoint controls that monitor DNA integrity and the completion of each molecular event before allowing transition to the next phase. In eukaryotic cells, the main checkpoints are placed at the G1/S transition, in late S (DNA synthesis) phase, at mitosis (M) entry and at the metaphase to anaphase transition (Fig. 1). Progress through these checkpoints and the synthesis of DNA are under control of cyclin-dependent kinases. These are regulated by the oscillatory expression of three main cyclin classes: G1 and G1/S-cyclins, S-cyclins, and M-cyclins, whose expression increases during the homonymous stages (34, 68, 94). The metaphase to anaphase transition is instead triggered by the anaphase-promoting complex/cyclosome (APC/C; 117). Entry into the cell cycle from a quiescent (Go) phase generally depends on stimulation by mitogens. Exit from mitosis can lead to differentiation, apoptosis, or return to quiescence. All of the above mechanisms can be altered in neoplastic cells.

Ion Channels and The Cell Cycle

Convincing demonstration that ion channels modulate cell proliferation must ultimately rely on results showing that their activity regulates the cell cycle checkpoints. Early evidence suggested that increase in K+ channel expression and activity at the G1/S boundary is often necessary for cells to traverse the cell cycle. However, as discussed earlier, an interpretation of these observations is far from being obvious. The first detailed information about ion channel physiology during the cell cycle stages was obtained in murine zygotes, where high-conductance inward rectifying K+ channels display oscillatory activity (but not expression) during cell cycle, with ensuing depolarization in S and G2 (36). Other classical examples of oscillation in channel activity or expression during cell division are reviewed in Ref. 6. In cases such as these, it is necessary to determine whether the oscillating channel activity regulates the cell cycle proteins or vice versa (as was in fact the case in murine oocytes; 37), or if the two interact with complex feedbacks. Altogether, there is now good evidence that specific channel types can regulate different cell cycle phases. Moreover, ion channels contribute to determine the typical features of cancer cells, such as independence from mitogenic or antimitogenic signals, avoidance of apoptosis, indefinite proliferative potential, and capability of inducing angiogenesis (122). Here, we focus on regulation of the cell cycle proper and omit detailed discussion of the crosstalk between ion channels and mitogenic, angiogenic, and migratory factors. Some of these mechanisms have been extensively reviewed (6, 98, 122, 126) and other will be the object of further reviews in the present series.

As is testified by their central role in sensory systems, ion channels are extremely effective in transducing surface events to the cytosolic protein machineries. They couple the high sensitivity to chemical and electrical signals typical of cooperative allosteric proteins with the capability to produce strong signal amplification because of the considerable energy released by passive ion flow. However, in resting conditions, because of the global symmetry of charge distribution across the lipid bilayer, the average electric field inside the cytoplasm is null (Fig. 2A). Hence, apart from local phenomena that we discuss later, the signal transduction between cell surface and interior cannot be a straightforward electric coupling caused by different steady Vm values in different cellular states. Many possible mechanisms have been proposed with different degrees of experimental support (Fig. 2 and Fig. 3).

Modulation of Ca2+ entry. As indicated earlier, changes in Vm can regulate Ca2+ influx. The classic example is T cell activation, i.e., the response to antigen binding that makes the lymphocyte ready to proliferate and thus produce an effective immune response. T cell proliferation is thought to be triggered by an elevation of the cytosolic free calcium concentration ([Ca2+]i), which activates the Ca2+/PKC-dependent pathways that regulate progress from Go into mitosis (26). In these cells, Ca2+ entry is facilitated by the hyperpolarization produced by K+ channel activation. T cells express VG (Kv 1.3) and Ca2+-dependent (KCa 3.1) K+ channels that are activated sequentially to trigger and sustain cell hyperpolarization (41, 78, 149). A similar pattern is observed in oligodendrocyte...
progenitor cells, in which upregulation of Kv1.3 and Kv1.5 takes place during G1. Blocking Kv1.3 inhibits entry into S both in vitro and in vivo (27).

In cancer cells, the first studies in transformed fibroblasts had suggested that the dependence of proliferation on extracellular calcium is relaxed. However, subsequent work in cultured cancer cells contradicted that evidence (126). In fact, low threshold (T-type) Ca\(^{2+}\)/H11001-channel activity is required for proliferation of several tumor cell lines (109, 110). These results probably explain why the timing of expression of K\(^{+}\)/H11001 channels during cell cycle in neoplastic cell lines is often similar to the one observed in normal cells. For example, treating breast cancer cells with growth factors leads to Kv10.1 (also known as ether-á-go-go) channel expression during G1. The ensuing hyperpolarization is thought to facilitate calcium influx and thus activate K\(_{\text{Ca}}\) 3.1. This latter maintains the hyperpolarization that sustains the calcium signal throughout the cell cycle, while Kv10.1 is inhibited by CaM activation (105, 106). A similar pattern has been suggested to operate in melanoma cells (102).

Downstream actions of Ca\(^{2+}\). Calcium is thought to regulate the cell cycle by 1) modulating the expression and activity of the transcription factors that control expression of the G1 cyclins and 2) producing direct effects on cyclins, cyclin kinases, and the associated proteins (126). The efficacy of these regulatory actions does not simply depend on [Ca\(^{2+}\)]i elevation but is related to the precise timing of the calcium waves (40). Calcium also exerts direct control of the cell cycle mechanisms by regulating the mitotic spindle and cytokinesis (146).

Particularly, ample evidence on how calcium regulates cell division was obtained in early embryos, which are particularly suitable for cell physiological experimentation because of the large dimension of oocytes and early blastomeres and the rapidity of their cell cycle. In these cells, transient increases of [Ca\(^{2+}\)]i occur at specific cell cycle stages (121, 136), which are mainly controlled by the intracellular stores. In sea urchin oocytes for example, phosphoinositide-dependent Ca\(^{2+}\)-signals control progression through the cell cycle checkpoints at G1/S, G2/M, and mitosis exit (11, 29, 52, 144). The Ca\(^{2+}\)-dependent regulation of the early division cycles has also been thoroughly
studied in frog embryos, where the overall pattern is similar to that observed in sea urchin, although the opacity of *Xenopus* oocytes makes studying \([\text{Ca}^{2+}]\) with optical methods more difficult (56, 92, 101, 124). Similar calcium transients also occur in mouse oocytes, in which however their precise functional role is still debated (7, 43, 62, 72, 82).

The calcium signals observed in oocytes are only partially representative of the general mechanisms, because in cells with higher surface-to-volume ratio the contribution of calcium entry from the extracellular space is considerable. Unfortu-

noted, measuring calcium transients in the small mammalian somatic cells resulted to be much harder than in oocytes, probably because of the highly localized nature of these events (146). Nonetheless, based on different lines of investigation, there is now general agreement that \([\text{Ca}^{2+}]\) also regulates the cell cycle progression in somatic cells, although the evidence is more fragmentary than in oocytes and the timing more difficult to assess.

As to the calcium target, a prominent regulatory importance is generally attributed to the CaM-dependent pathways (31, 66, 123, 137). In sea urchin oocytes, CaM activates around the nucleus before entry into mitosis and on the mitotic spindle poles just before anaphase onset (143). Local calcium signals in the nucleus and around the mitotic spindle have been also detected in *Drosophila* embryos (114). In other species, the evidence is prevalently biochemical. In *Aspergillus nidulans*, Ca-CaM has been found to control the Cdc25-like phosphatases that regulate mitosis entry through stimulation of Cdk1 mitotic kinase (80). Evidence in both fungi and mammalian cell lines suggests that the calcium-dependent regulation of activity and transcription of the cyclin-related proteins are largely mediated by the CaM-dependent kinase II (CaMKII) and calcineurin (e.g., 30, 44, 66, 115). For instance, in fibro-

blasts (67, 96, 142) and rat kidney cells (139), inhibiting CaM or the CaMKs produces arrest in GI. The effect is attributed to inhibition of the cyclin-dependent kinases 4 and 2, to loss of cyclin D1 expression and upregulation of p27.

These mechanisms are thought to be also operant in cancer cells, in which however they tend to lose proper control. Accordingly, new therapeutic strategies in oncology are being attempted. A new generation of anti-mitotic drugs has been produced that impede mitotic spindle assembly by specifically targeting CaM (104). Moreover, the human CaMKII inhibitory protein (hCaMKIIβ), which is downregulated in ovarian cancer cells, has been found to decrease the tumorigenicity and growth of human ovarian cancer cells injected in mice by blocking cell cycle and stimulating apoptosis (81).

**Nuclear envelope.** The role of cytosolic calcium transients brings us to consider a complementary but somewhat neglected aspect of intracellular signaling: the function of the nuclear envelope. Studying calcium signals and ion transport in the nucleus presents interpretive as well as technical challenges. The nuclear envelope comprises an outer and an inner nuclear membrane. The former is continuous with the endoplasmic reticulum, the latter often extends into a nucleoplasmic reticulum. These membranes are fused at the nuclear pore com-
plexes. A full electrophysiological distinction of the transport properties of the nuclear pores compared with the nuclear membranes is still lacking, partly because of the difficulties in access and purification of these structures (86). Nonetheless, early evidence with isolated nuclei indicated the existence of electric potentials and ion transport across the nuclear envelope (88). In 1990, patch-clamp studies revealed high-conductance \(\text{K}^{+}\) and \(\text{Cl}^{-}\) channels in extracted nuclear membranes (85, 87), whose relation with the other known channel types is still uncertain (86). Subsequent studies have expanded considerably on the first observations. It is now clear that a full autonomous \(\text{Ca}^{2+}\) handling machinery is available in the nuclear envelope of animal cells. The details and the controversies in the field are reviewed in Refs. 19 and 86. In brief, the \(\text{Ca}^{2+}\) channels typically responsible for calcium release from organelles into the cytosol, i.e., the inositol (1,4,5)-trisphosphate receptor (IP3R), the ryanodine receptor (RyR), and the nicotinic acid-

adenine dinucleotide (NAADP) receptors are all found in the inner nuclear membrane. The outer nuclear membrane, besides IP3Rs, expresses the \(\text{Ca}^{2+}\)-ATPases and the inositol 1,3,4,5-tetraakisphosphate-operated \(\text{Ca}^{2+}\) channels that serve to reload the perinuclear space. The picture is completed by the expression of sodium pumps (48), Na/H exchangers (16), and probably Na/Ca exchangers on the inner nuclear membrane (73). Therefore, mechanisms analogous to those operating in the

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**Fig. 3.** Cell signaling mechanisms that do not depend directly on ion conductance. A: surface charge can modulate the association of second messengers and regulatory proteins with the plasma membrane, because of the effect of local electric fields that rapidly decay a few nanometers away from the membrane surface (89, 103). This mechanism cooperates with the more conventional membrane targeting through interaction between hydrophobic side chains and the lipid bilayer. Surface potentials can contribute to regulate voltage-gated ion channels. B: ion channels can modulate cell signaling by nonconductive mechanisms, such as enzymatic functions of intracellular channel subunits or domains. Channels can also associate with cytoplasmic proteins (e.g., cytoskeletal) and other membrane proteins (e.g., growth factor or adhesion recep-

tors) that in turn regulate the downstream signals.
cytoplasm can regulate the homeostasis of Ca\(^{2+}\) and other ions in the nuclear envelope and nucleoplasmic reticulum. The nucleus seems capable of responding to both nucleoplasmic and cytoplasmic changes in free calcium. In fact, specifically buffering the nuclear Ca\(^{2+}\) levels inhibits cell proliferation (127). Moreover, cytosolic CaM translocates into the nucleus on elevation of [Ca\(^{2+}\)]\(_i\) (91, 141, 148). The relation of nuclear calcium signaling with the cytoplasmic compartments and how the nuclear and cytoplasmic signals interplay in modulating gene expression offer ample matter for future studies.

In principle, ion transport across the nuclear envelope could be also gated by the nuclear pore complex (NPC). The current view is that the NPC contains a wide central channel (with a diameter of \(~10\) nm) that can expand up to about 39 nm (111). The central channel is surrounded by eight smaller peripheral channels with diameters around 8 nm (60, 76). However, the possibility that the NPC is a gated channel and the roles of the accessory peripheral channels are still controversial (19, 23, 86, 88).

**Exocytosis of paracrine agents.** A less direct manner in which ion channels can regulate cell proliferation is by modulating the exocytosis of autocrine or paracrine mediators. Such mechanisms seem to be often implicated in both stem cell and cancer cell proliferation. A nice example is given by the recent observation that downregulation of KCNQ1 channels in non-neuronal and cancer cell proliferation. A nice example is given by the recent observation that downregulation of KCNQ1 channels in embryonic stem cells has been recently found to be inhibited by paracrine neurotransmitters (74). Recently, neuronal ionotropic receptors such as the nicotinic ACh receptors (nAChRs) and the GABA\(_A\) receptors were observed to be also widely expressed outside the nervous system and in cancer cells (42). Most studies have focused on nAChRs, because smoke is a well-known risk factor for cancer. In the central and peripheral nervous system, several \(\alpha\) and \(\beta\)-neuronal nAChR subunits can associate in various stoichiometries that determine specific functional features. Common cerebral forms are the heteropentamer \(\alpha4\beta2\) and the homopentamer (\(\alpha7\)\)_S. In peripheral nervous system and non-neuronal tissues other subunits are widely expressed, such as \(\alpha3\) and \(\beta4\) (42, 129). The nAChRs are permeable to cations, including Ca\(^{2+}\). The permeability to Ca\(^{2+}\) is particularly high in (\(\alpha7\)\)_S receptors, in which it is close to that of NMDA glutamate receptors (45).

Besides ACh, nAChRs are activated by tobacco-derivatives such as nicotine and several carcinogenic nitrosamines structurally analogous to ACh or nicotine (131). In many cancer cell lines, prolonged exposure to nAChR agonists stimulates cell proliferation, angiogenesis, migration, and thus tumorigenicity (24, 35, 42, 53, 107, 108, 129). The steady-state current flowing through nAChRs is proportional to the probability that the channel is active times the probability that it is not desensitized, which depend on both nAChR type and agonist concentration. Steady nicotinic currents cause sustained cell depolarization and Ca\(^{2+}\) influx that are thought to modulate the release of autocrine messengers that regulate the above processes. For example, in pulmonary neuroendocrine cells and neuroendocrine-derived cancer cells such as the small cell lung cancer cells, a steady activation of \(\alpha7\)-containing nAChRs stimulates cell proliferation by increasing the release of serotonin and bombesin. The cellular effect of these molecules is mediated by the PKC/RAF1/MAPK pathway (25, 64, 129). Besides controlling exocytosis, calcium influx through nAChRs probably also produces the usual direct modulation of the intracellular signaling cascades.

However, what is known about the physiology of nAChRs suggests that the cellular responses to chronic concentrations of tobacco derivatives are probably much more complex than suggested by the above discussion. Both normal and neoplastic cells often express multiple nAChR subunits and tonic levels of agonists regulate the surface expression of nAChRs in a subunit-dependent manner (51). In particular, chronic exposure to agonists induces deep desensitization (also named inactivation) of several heteromeric receptors but not of \(\alpha7\)-containing receptors (69). Therefore, whereas the \(\alpha7\)-containing receptors may be assumed to operate as indicated above, at least in first approximation, the heteromeric nAChRs likely contribute to neoplasia in a different way. It has been proposed that the prolonged inactivation of these receptors could promote the neoplastic progression by decreasing GABA release. Such a working hypothesis is based on different lines of evidence. First, GABA is a tumor suppressor in colon carcinoma (63) and lung adenocarcinoma (130). In the latter, it blocks both cAMP-dependent DNA synthesis and cell migration in vitro. This effect of GABA may be analogous to the normal physiological role it exerts on cell cycle during development. The proliferation of embryonic stem cells and peripheral neural crest stem cells has been recently found to be inhibited by paracrine stimulation of GABA\(_A\) receptors (GABA\(_A\)Rs), whose activation leads to cell hyperpolarization (1). It is interesting to notice that this effect on embryonic cell proliferation seems to be opposite to the typical depolarizing role of GABA during the development of the nervous system (12), which depends on a balance of chloride transporters different from the one observed in the adult brain (125). Finally, the regulatory link between cholinergic and GABAergic transmission is suggested by the fact that GABA release is often regulated by nAChR activation in the adult and developing nervous system. In fact, the switch between the excitatory and inhibitory roles of GABA that occurs during brain development is regulated by the spontaneous nAChR activity (77). Moreover, in adult neocortical circuits, steady-state nAChR activation is known to control GABA release (e.g., 2).

Although some evidence indicates that tobacco-related nitrosamines can modulate GABA expression in tumor cells (130), drawing conclusions about the interaction of the cholinergic and GABAergic signaling during the neoplastic processes would be premature, as very few functional studies exist about nAChRs and their relation to GABA release in non-neuronal cells. In particular, it will be necessary to understand whether, in cells that express multiple nAChR subunits, different receptor isomers are specifically associated with the secretion of...
specific transmitters, for example, through some form of compartmentalization (Fig. 2C). Alternatively, it is possible that different cancer cell clones express distinct nAChRs as well as different secretion machineries, e.g., GABAergic compared with serotonergic.

Membrane surface potential. Different peptide or lipid side chains exposed to the intra- and extracellular faces of the plasma membrane can modify the local surface electrostatic charge (89). This can produce local alteration in the electric field that, although decaying very rapidly with distance, can have at least two physiological effects.

First, they can modulate membrane targeting of cytoplasmic proteins and second messengers (Fig. 3A), thus summing their effects to those produced by the hydrophobic side chains that typically regulate molecular anchorage to the lipid bilayer (28). The first such evidence concerned the myristoylated alanine-rich PKC substrate (MARCKS; 140), whose proper association with PKC depends on both insertion of the myristoyl chain into the lipid bilayer and the electrostatic interaction of the polybasic domain of MARCKS with acidic phospholipids in the membrane (70). Analogous evidence has next accumulated for other signaling proteins and second messengers (47, 58, 90, 100, 132), suggesting that surface charges may control some aspects of cell signaling implicated in cell proliferation. Importantly, the surface potential can be modulated quickly enough to regulate the membrane association of signaling molecules during the normal course of cell physiological processes such as phagocytosis (150).

Second, surface potential adds to the more conventional electrodiffusion mechanisms in regulating $V_m$ (103). In this way, it can contribute to control VG channels. In fact, the surface charge density can be estimated by studying how the voltage-dependent properties of VG channels vary as a function of the extra- or intracellular ionic strength (59). Application of this and other methods indicates that the surface charge density is different in normal and neoplastic cells, such as different secretion machineries, e.g., GABAergic compared with serotonergic.

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and several small GTPases (10, 21). The evidence that most concerns us here is related to Kv11.1 (also known as ERG), another channel type widely expressed in tumors (13). In neoplastic tissue, Kv11.1 regulates functions as different as cell proliferation, apoptosis, invasion, and angiogenesis. These effects are often mediated by recruitment of Kv11.1 into multiprotein membrane complexes, which generally include integrins and growth factor receptors (118). In primary acute myeloid leukemias, for example, Kv11.1 regulates cell proliferation of normal and leukemic progenitors (120). In these cells, Kv11.1, the β1-integrin subunit and FLT-1 [the vascular endothelial growth factor (VEGF) receptor 1] associate to form a regulatory platform that modulates angiogenesis (by controlling VEGF secretion), cell proliferation (probably through MAPK), and cell migration (probably through the PI3K/Akt pathway; 119). Although the formation of multiprotein complexes in this and other physiological contexts (75) suggests that some form of conformational coupling between these proteins does occur, how this relates to ion transport is unclear.

Ion Channels and Cell Cycle Checkpoints: An Overview

Figure 1 illustrates the role of \([\text{Ca}^{2+}]_i\) and several channel types in different cell cycle stages. Evidence is particularly abundant for the G1/S and G2/M transitions. In general, detailed studies of the relations between ion channels and the mitotic control in intact mammalian cells are still limited, because of the difficulty of applying the methods of cell physiology to cells whose size is often small and whose mitotic cycle is generally very long.

Calcium signals seem to be repeatedly necessary throughout the cell cycle. Recent work has begun to address the modulation of single stages in neoplastic cells, suggesting that specific entry pathways may be active in different phases. For example, TRPC1 channels control cytokinesis in human gliomas (18), whereas the related TRPC6 regulate the G2/M transition in gliomas (39) and oesophageal cancer (134). Further evidence is available for VG calcium channels. Cav1 (L-type) channels tend to be expressed in nonproliferative phases, whereas expression of Cav3 (T-type) currents often increases during the proliferative phases. This is observed in normal as well as cancer cells, although the precise physiological significance is uncertain (110).

As is clear from earlier discussion, the cell cycle regulation by K\(^+\) channels is anything but straightforward. Different K\(^+\) channel types can produce distinct effects at different stages, in a cell-specific manner (26, 105). In all probability, such a variety turns on two points. First, distinct K\(^+\) channels serve numerous cellular functions related to proliferation. Besides modulating the calcium signal and interacting with adhesion and growth factor receptors, they participate in controlling the cell volume (135). Moreover, K\(^+\) channels can mediate the cellular response to the environmental redox state and \(P_{O_2}\) through a variety of molecular mechanisms, ranging from direct heme binding (138) to the intervention of specific domains sensitive to the redox state, such as the intracellular per-arn-t-sim (PAS) helix-loop-helix motif (93). Second, the heterogeneity of K\(^+\) channels may further increase in neoplastic tissue, because different malignant cell clones can be selected that express specific channel types that facilitate tumor growth. An example is overexpression of the K\(^+\) channels that regulate the secretion of angiogenetic factors in human gliomas, which may help proliferation of cancer cells in their typical hypoxic environment (84). In some cases, the \(V_m\) of cycling cells is regulated by the ratio of different channel isoforms. For example, in neuroblastoma \(V_m\) can be controlled by Kv11.1 and blocking this channel inhibits mitosis (4). In these cells, \(V_m\) tends to depolarize during the S phase. The effect is caused by oscillation of the expression balance of the full-length Kv11.1a isoform and the N-deleted Kv11.1b (33). The ratio between Kv11.1b and Kv11.1a is higher in S, which leads to cell depolarization because of the deeper steady-state deactivation of Kv11.1b compared with the full-length isoform (79). Once again, the effects on \(V_m\) could be accompanied by modulatory effects specific to the channel isoforms, as suggested by the fact that the truncated Kv11.1b lacks the PAS domain.

Nevertheless, the recent observation that embryonic stem cells stop cycling when hyperpolarized by activation of GABA\(_A\)Rs (thus independently from K\(^+\) channels) gives further support to the old notion that \(V_m\) itself has a role in mitosis, at least in nonneoplastic cells. Differently from the K\(^+\) channel-dependent hyperpolarization, the GABA\(_A\)R-dependent signal was found to specifically target the S phase checkpoint kinases of the PI3K-related family and the histone variant H2AX (1). However, such difference could merely reflect the fact that embryonic stem cells may lack a G1 checkpoint (22). Therefore, in these cells, regulation of proliferation would necessarily occur in the “DNA damage” S/G2 checkpoint, typically involved in controlling the structural integrity of chromatin (8). Much wider evidence is available about the role of chloride channels in controlling the cell volume changes occurring during mitosis (133) and cell migration (135). Such evidence is too abundant to summarize here. In brief, contrary to yeast and other organisms, the presence of a strict cell volume checkpoint in the mammalian cell cycle is still uncertain. However, it is worth mentioning that recent results indicate that cell division in both normal glial progenitors and glioma cells is preceded by a premitotic cytoplasmic condensation linked to chromatin condensation (54). This process depends on chloride efflux through CIC3 channels. These are upregulated during the M phase and colocalize with both the plasma membrane and the mitotic spindles. In these cells, knocking down CIC3 considerable delays mitosis (55).

Conclusions

In summary, the cell cycle phases are often accompanied by oscillations of ion channel expression and activity. The ensuing changes in \(V_m\) can regulate calcium entry or voltage-dependent proteins or be a by-product of the cell volume control. Ion channels also exert a variety of other specific signaling roles related to the mitotic cycle that are only beginning to be unraveled. Their functional flexibility makes them very suitable to coordinate multiple signaling pathways and integrate the intra- and extracellular environment, in cooperation with adhesion and growth factor receptors. Some of these processes are preserved and other are altered in neoplastic cells, whose biophysical heterogeneity likely reflects the progressive selection of malignant cell clones with increasing independence from extrinsic control of proliferation. Studying this mechanistic complexity in living cells proposes considerable exper-
mental challenges. Nonetheless, a wider application of physiological methods to study mammalian cells in real time during the cell cycle certainly constitutes one of the most promising field of cell physiology.

GRANTS

The author’s research has been lately funded by the University of Milano-Bicocca (FAR), the Italian Ministry of University and Scientific Research (PRIN), and the Fondazione Banca del Monte di Lombardia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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