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Ste20-Type Kinases: Evolutionarily Conserved Regulators of Ion Transport and Cell Volume

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Ste20 serine/threonine kinases regulate fundamental cellular processes including the cell cycle, apoptosis, and stress responses. Recent studies in *Caenorhabditis elegans* and mammals demonstrate that Ste20 kinases also function in cell volume sensing and Cl^- transport regulation. Yeast Ste20 initiates a shrinkage activated MAPK cascade that regulates organic osmolyte accumulation. Ste20 kinases thus play evolutionarily conserved roles in cellular volume sensing and regulation. They may also function in systemic osmotic homeostasis and to link cell-cycle events with cell volume.

The Founding Member of the Ste20 Kinase Superfamily

Ste (from "sterile") genes were discovered by genetic analysis of mating in the budding yeast *Saccharomyces cerevisiae*. Mating is mediated by the fusion of haploid yeast cells. Haploid cells identify mating partners by pheromones released from cells of the opposite mating type. These pheromones bind to membrane receptors that in turn activate G protein signaling and a downstream kinase cascade (8). Ste20 specifically was identified as a suppressor of mating defects induced by a dominant negative form of a Gβ-subunit required for pheromone signaling (25) and as a dominant activator of the mating response (41). Biochemical characterization demonstrated that *STE20* encodes a serine/threonine kinase (57).

Ste20 is the founding member of a kinase superfamily that is divided into two groups, the p21-activated kinases (PAKs) and the germinal center kinases (GCKs). These two groups are subdivided further into 10 subfamilies: PAK-I and -II and GCK-I through -VIII (5) (FIGURE 1). PAKs are distinguished by the presence of a kinase domain located in the COOH terminus and an NH_2-terminal p21 GTPase-binding domain that mediates binding to small GTPases such as Cdc42 and Rac. GCKs lack GTPase-binding domains, and the kinase domain is located in the NH_2 terminus (FIGURE 1). Ste20-type kinases play essential roles in signaling pathways that regulate fundamental cellular processes, including cell-cycle control, apoptosis, development, cell growth, and cell stress responses (5).

FIGURE 2 shows three well-defined Ste20 signaling pathways that have been identified in yeast by genetic and molecular analyses. In all three pathways, activation of Ste20 triggers a MAPK cascade. Yeast Ste20 signaling cascades control cell growth, mating, and associated cell-cycle events and the osmoregulatory response to hypertonic stress.

Proline-Alanine-Rich Ste20-Related Kinase Regulates Cell Volume-Sensitive K-Cl and Na-K-2Cl Cotransporters

Na-K-2Cl (NKCC) and K-Cl (KCC) cotransporters play critical roles in epithelial transport, intracellular and extracellular ion homeostasis in the central nervous system, and systemic salt and water balance (15). KCCs and NKCCs also play essential roles in cell-volume regulation. Swelling results in the activation of regulatory volume decrease (RVD) mechanisms in virtually all cells. In many cell types RVD is mediated by swelling-induced activation of KCCs resulting in the net efflux of KCl and osmotically obliged water. Regulatory volume increase (RVI) following cell shrinkage is widely mediated by shrinkage-induced activation of NKCCs and the uptake of NaCl and KCl (15).

Activation of KCCs and NKCCs is brought about by serine/threonine dephosphorylation and phosphorylation, respectively (17, 24, 44). Compelling evidence suggests that a common "volume-sensitive" kinase mediates cell volume-dependent regulation of both cotransporters (26–28). A working model originally proposed by John Parker (36, 37) postulates that cell shrinkage activates the kinase (or a kinase cascade), which in turn phosphorylates NKCCs and KCCs, leading to their subsequent activation and inactivation, respectively (FIGURE 3). Cell swelling inhibits kinase activity, allowing protein phosphatases to dephosphorylate both cotransporters. Dephosphorylation inhibits NKCC and activates KCC.

The Ste20-type kinase called proline-alanine-rich Ste20-related kinase (PASK; also known as SPAK) was first described in 1998 by Ushiro et al. (53). PASK is a member of the GCK-VI subfamily, which includes the

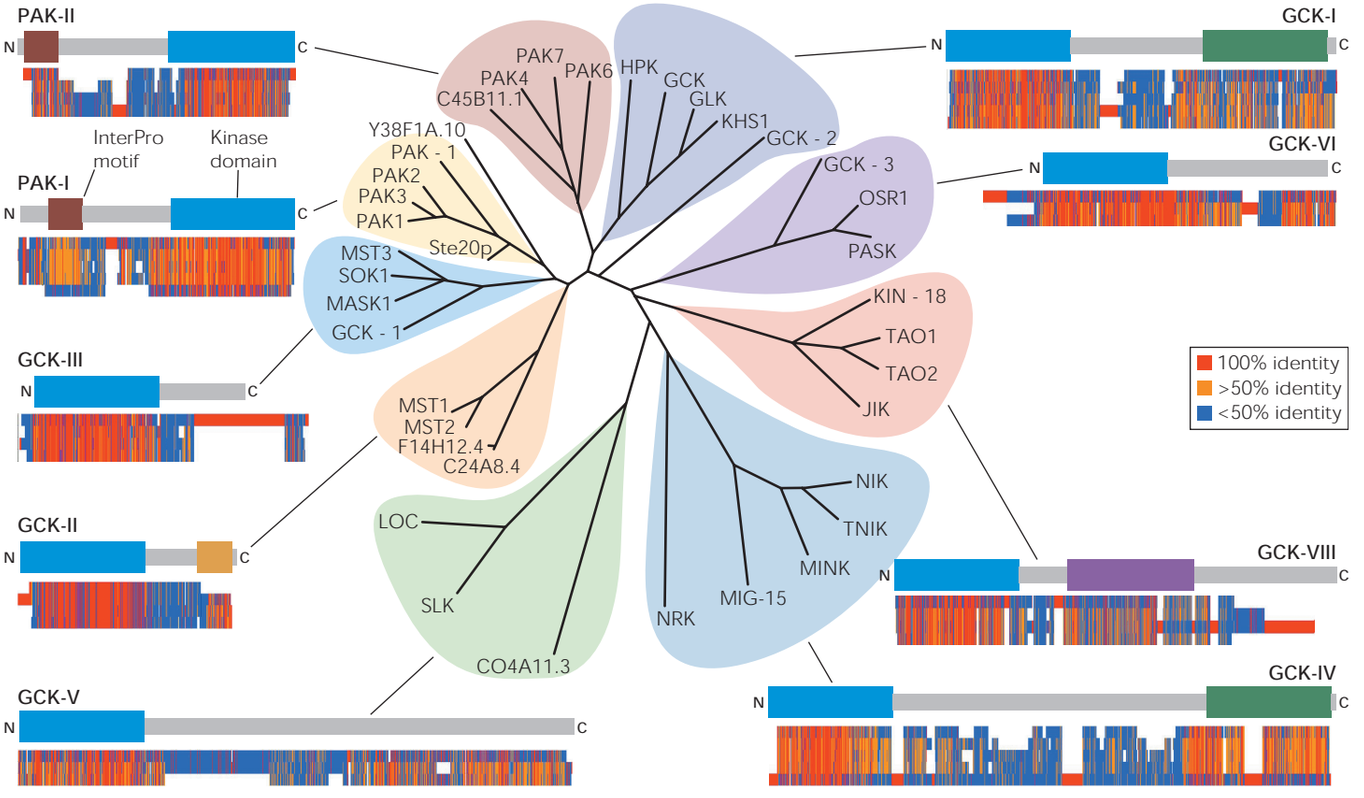

FIGURE 1. **Phylogenetic tree, domain structure, and multiple sequence alignments of the GCK and PAK subfamilies of STE20-type kinases from humans and *C. elegans***

STE20-related kinases were grouped by ClustalW protein alignment, and distance matrices and trees were subsequently calculated using Phylip. p21-activated kinases (PAKs) fall into two structurally similar subfamilies, PAK-I and PAK-II, whereas germinal center kinases (GCKs) fall into eight subfamilies, one of which (GCK-VII) is not represented in *C. elegans*. The human kinases are designated in the phylogram via their common names. Previous symbols, gene aliases, and designated gene names can be found on the HUGO nomenclature website (http://www.gene.ucl.ac.uk/nomenclature/index.html). The HUGO-approved gene names for the kinases in this figure are as follows: MAP4K1 is HPK, MAP4K2 is GCK, MAP4K3 is GLK, MAP4K4 is Nck-interacting kinase (NIK), MAP4K5 is KHS, MAP4K6 is MINK, STK3 is MST2, STK4 is MST1, STK10 is LOC, STK24 is MST3, STK25 is SOK1, STK39 is PASK, OXSR1 is oxidative stress-response protein 1 (OSR1), and the remainder are as designated. The *C. elegans* kinases are shown as cosmid open reading frames for predicted genes or official gene names where designated (*C. elegans* genes are hyphenated, whereas the mammalian genes are not). Y38F1A.10 does not fall into any of the subfamilies, but it is most closely related to the PAKs. For reference, yeast kinase Ste20p, the founding member of this family, is also shown. Representative domain structures are shown for each subfamily, based upon InterPro motifs (http://www.ebi.ac.uk/interpro/). Protein kinase domains (IPR000719) are indicated by blue boxes. Citron-like domains (IPR001180, green box) may be involved in macromolecular interactions, particularly with small GTPases. The SARAH domain (IPR0011524, orange box) facilitates dimerization and is unique to the GCK-II family. PAK domains (IPR000095, brown box) allow the PAK family kinases to bind to members of the p21 and Rho families. Finally, the DUF334 domain (IPR005602, purple box) is of unknown function and is found only in the TAO or GCK-VIII subfamily. Motifs that are functionally conserved generally show up in multiple sequence alignments. In the alignments for each subfamily shown immediately below the domain structures, red indicates 100%, orange >50%, and blue <50% identity. *C. elegans* proteins are the first sequence in each alignment, with the exception of Y38F1A.10, which is last in the PAK-I subfamily alignment. Note that regions unique to a single subfamily member have by default 100% identity in that region and are coded red (for an example, see the *C. elegans* representative of the GCK-III family) but are not conserved among other subfamily members. For a more detailed description of functionally conserved motifs in the STE20p subfamilies, see Dan et al. (5).

Drosophila gene *fray*, the *Caenorhabditis elegans* gene *gck-3*, and a gene encoding a mammalian protein termed oxidative stress-response protein 1 (OSR1) (FIGURE 1). Ushiro et al. (53) demonstrated that PASK expression is enriched in neurons and transporting epithelia.

Delpire and co-workers identified PASK and OSR1 as proteins that interact with the NH_2 terminals of NKCC and KCC via a PASK-binding motif [(R/K)FX(V/I)] (39). Dowd and Forbush (9) characterized the effect of PASK on NKCC1 activity. NKCC1 is expressed widely and functions in cell-volume regulation, epithelial salt and water secretion, and intracellular ion homeostasis (15, 17). Overexpression of an inactive, dominant negative PASK mutant in human embryonic kidney cells reduces NKCC1 activation in response to cell shrinkage and low cytoplasmic Cl^- levels by ~80% and reduces regulatory phosphorylation of the cotransporter. In contrast, overexpression of wild-type PASK increases NKCC1 activity (9). Similarly, coexpression of NKCC1 with catalytically inactive PASK in *Xenopus* oocytes inhibits shrinkage-induced activation of the cotransporter (14).

Yeast two-hybrid analysis identified several proteins that interact with the regulatory domain of PASK. These proteins include heat shock protein 105, the

MAPK p38, the apoptosis-associated tyrosine kinase (AATYK), a novel tyrosine kinase related to AATYK, and WNK4 [a member of the "with no K" (i.e., lysine) serine/threonine kinase family] (38). All of these proteins contain one or more PASK-binding motifs, and many of them function in cellular stress-response pathways.

WNK4 plays a central role in systemic salt and water balance and has been shown to regulate thiazide-sensitive Na-Cl cotransporter and renal outer medullary K^+ channel (ROMK) activity. Mutations in WNK4 give rise to autosomal-dominant pseudohypoaldosteronism type II (reviewed in Ref. 16). Recent studies by Gagnon et al. (14) suggest that PASK and WNK4 may function together to regulate NKCC1 and KCC2. More recently, Vitari et al. (55) demonstrated that WNK4 as well as WNK1 interact with, phosphorylate, and activate PASK and its homolog OSR1. Activation of PASK and OSR1 by either WNK is required for in vitro phosphorylation of the NH_2 terminus of NKCC1.

The PASK Homolog GCK-3 Regulates a Cell Volume-Sensitive and Cell Cycle-Dependent *C. elegans* ClC Channel

The *C. elegans* oocyte expresses an inwardly rectifying, hyperpolarization-activated Cl^- current that is carried by the "b" splice variant of *clh-3* (6), one of six ClC channel-encoding genes in the worm genome. CLH-3b is regulated by meiotic cell-cycle and cell-volume changes (6, 45, 46). During meiotic cell-cycle arrest, CLH-3b activity is inhibited by serine/threonine phosphorylation of the channel and/or associated regulatory proteins (45, 46). In the inhibited state, CLH-3b activates slowly at hyperpolarized potentials more negative than –60 mV. Upon resumption of the meiotic cell cycle, a process termed meiotic maturation, or during hypotonic cell swelling, CLH-3b is activated by dephosphorylation reactions mediated by the type 1 protein phosphatases GLC-7α and GLC-7β (46) (FIGURE 3). Meiotic maturation- and swelling-induced activation of CLH-3b are accompanied by a striking depolarizing shift in activation potential and an increase in hyperpolarization-induced activation kinetics (6, 45, 46). Dephosphorylation-dependent activation of CLH-3b during meiotic maturation functions to coordinate oocyte ovulation with the meiotic cell cycle (45, 59).

The COOH terminus of CLH-3b interacts with a novel member of the Ste20 kinase superfamily termed

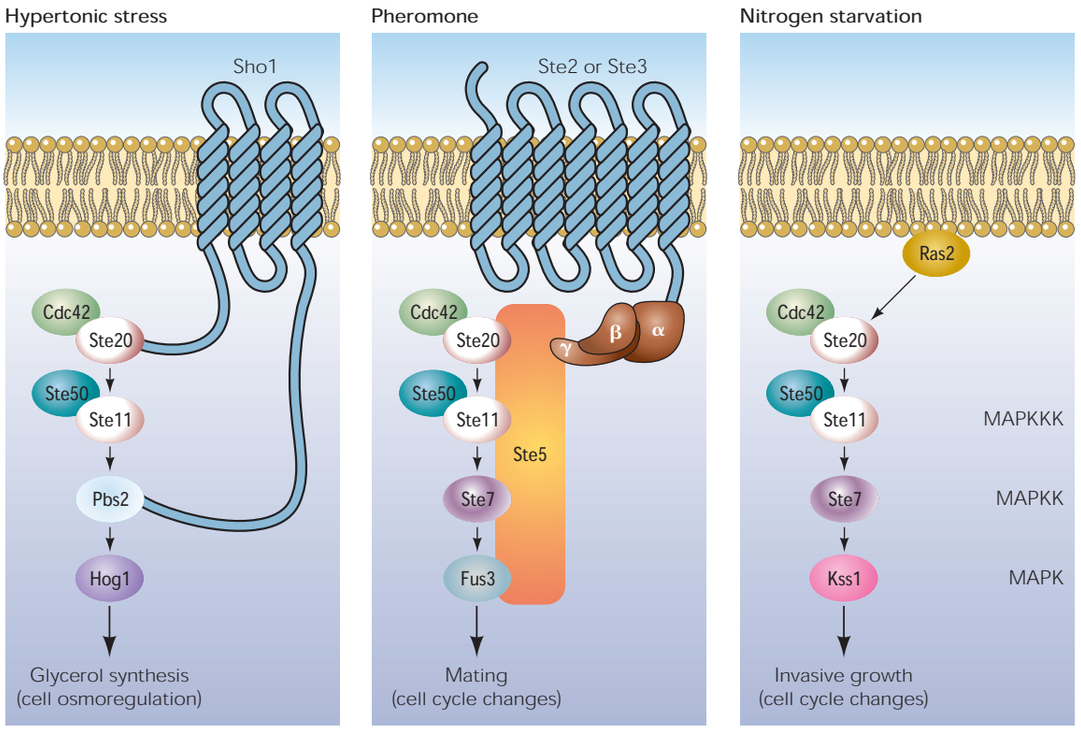

FIGURE 2. **Ste20 signaling pathways in yeast**
Left: hypertonic stress causes cell shrinkage and loss of turgor pressure, leading to activation of a MAPK cascade that induces increased glycerol synthesis. Ste20 initiates the cascade by functioning as a MAPKKKK. *Middle*: diploid yeast cells are formed by fusion or "mating" of a haploid cells with α haploid cells. A haploid cell identifies its mating partner by pheromones released by cells of the opposite mating type. Pheromones trigger a Ste20-regulated MAPK cascade that induces cellular changes required mating, including arrest of the cell cycle in G1 (56). *Right*: diploid a/α yeast cells switch to an invasive filamentous form in response to nitrogen starvation and other signals. The Ste20-initiated MAPK cascade triggers changes in cell-cycle dynamics and induces polarized cell growth (56).

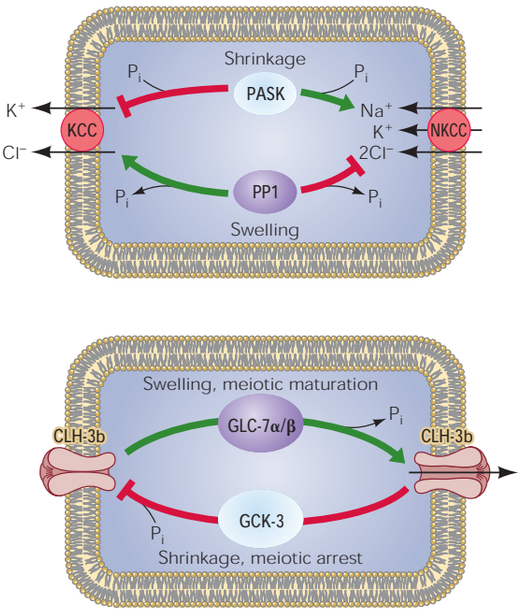

FIGURE 3. **Regulation of cation-Cl⁻ cotransporters and the *C. elegans* ClC anion channel CLH-3b by the Ste20-type kinase homologs PASK and GCK-3**

Top: postulated coordinated regulation of swelling-activated K-Cl cotransporters (KCCs) and shrinkage-activated Na-K-2Cl cotransporters (NKCCs). Cell shrinkage is postulated to activate a "volume-sensitive kinase" or a kinase cascade that phosphorylates NKCC and KCC, leading to their subsequent activation and inhibition, respectively. Kinase activity is inhibited by cell swelling allowing protein phosphatases to dephosphorylate both cotransporters. Dephosphorylation inhibits NKCC and activates KCC. PASK likely functions to mediate cell volume-dependent phosphorylation of both cotransporters (9, 14). *Bottom*: cell volume- and meiotic cell cycle-dependent regulation of CLH-3b by GCK-3-mediated protein phosphorylation. Protein dephosphorylation is mediated by the type 1 phosphatases GLC-7α and -7β (46).

GCK-3 (7). Phylogenetic analysis revealed that GCK-3 is a member of the GCK-VI subfamily and is a homolog of mammalian PASK and OSR1 (FIGURE 1). The regulatory role of GCK-3 was assessed by heterologously expressing CLH-3b with or without the kinase in human embryonic kidney cells. As shown in FIGURE 4A, expression of CLH-3b alone produces robust, inwardly rectifying Cl^- currents that activate rapidly at potentials more negative than –20 mV (FIGURE 4B) (7). The voltage sensitivity and kinetic properties are similar to those of native CLH-3b observed after channel activation by oocyte meiotic maturation or swelling (6, 45, 46). Coexpression of GCK-3 with CLH-3b decreases current amplitude, induces an approximately –40 mV shift in channel activation potential, and slows channel activation kinetics (FIGURE 4, A AND B) (7). The changes in CLH-3b functional properties require GCK-3 kinase activity and kinase binding to the channel COOH terminus via an amino acid motif (7) that conforms to the consensus sequence required for PASK binding to KCCs and NKCCs (39).

The reduced current amplitude and voltage sensitivity of CLH-3b when it is expressed with GCK-3 is similar to that observed in vivo prior to meiotic maturation- or swelling-induced channel activation (6, 45, 46). These results indicate that GCK-3 functions to inhibit CLH-3b and that the channel is largely constitutively active in the absence of kinase function. Importantly, cell swelling relieves GCK-3-mediated inhibition of CLH-3b, demonstrating that the kinase functions as part of a signaling pathway involved in cell volume-dependent channel regulation (6).

Single-oocyte RT-PCR analysis revealed that *gck-3* is expressed in the *C. elegans* oocyte (FIGURE 4C). In addition, a transgenic worm strain expressing green fluorescent protein (GFP) under the control of the *gck-3* promoter showed that the kinase is also expressed in the *C. elegans* excretory cell (FIGURE 4C) (7). Knockdown of *gck-3* expression by RNA interference induces constitutive activation of CLH-3b in nonmaturing and nonswollen worm oocytes (FIGURE 4D) (7). Thus GCK-3 functions normally in vivo to inhibit CLH-3b activity in meiotic cell cycle-arrested and nonswollen oocytes (FIGURE 3).

Are GCK-3 and PASK Orthologs?

Orthologs are genes in different species that evolved from a common ancestral gene and perform similar physiological functions. The phylogenetic tree shown in FIGURE 1 indicates that GCK-3 and PASK share a common ancestor. We suggest that the proteins also carry out conserved functions in *C. elegans* and mammals, organisms separated by hundreds of millions of years of evolution (2, 54). If GCK-3 and PASK are indeed orthologs, then the physiological processes that they regulate must be fundamental and highly conserved. What are these processes?

Cell-volume sensing

All cells face constant challenges to their volume either through changes in intracellular solute content or extracellular osmolality. The ability to detect cell size or volume is an ancient and fundamental property of all cells. Indeed, one of the first homeostatic mechanisms that likely arose during cellular evolution was the ability to regulate cell volume and prevent osmotic lysis in the face of swelling.

Ste20 plays a central and well-described role in "cell-volume sensing" in yeast. Yeast respond to cell shrinkage and loss of turgor pressure by accumulating the organic osmolyte glycerol. Glycerol accumulation is mediated by increased glycerol synthesis and reductions in glycerol efflux (reviewed in Refs. 20 and 23).

Glycerol synthesis is regulated in part by Ste20 signaling. FIGURE 2 summarizes the current understanding of the Ste20 osmotic stress signaling cascade. Hohmann and co-workers have reviewed the details of this model in depth (e.g., Refs. 20 and 23). Sho1 is a membrane protein that is localized to sites of polarized cell growth. In response to hypertonic stress, the MAPKK Pbs2 rapidly translocates from the cytoplasm to these regions. Membrane recruitment of Pbs2 is dependent on the interaction of a proline-rich domain in the kinase with the cytoplasmic Src homology 3 domain of Sho1.

Pbs2 functions as a scaffold that recruits Ste20 and Cdc42, the MAPKKK Ste11, and the Ste11-regulatory protein Ste50. Once this complex is formed, Ste20 activates Ste11 by phosphorylation, which in turn phosphorylates and activates Pbs2. Pbs2 then phosphorylates the MAPK Hog1, which translocates to the cell nucleus and triggers transcription of genes required for glycerol synthesis. Hyperosmotic stress thus activates a Ste20-initiated kinase cascade that triggers a cellular osmoregulatory response. Interestingly, the mammalian Ste20 homologs Pak1 and Pak2 (FIGURE 1) are also activated by hyperosmotic stress and likely function to control kinase cascades (4, 30, 42). Whether they also function to control cellular osmoregulatory responses is not known.

It is clear from studies on mammalian cation-Cl^- cotransporters (9, 14) and the *C. elegans* ClC channel CLH-3b (7) that GCK-3 and PASK function in sensing cell-volume changes. However, it remains unclear how the kinases detect volume perturbations. Do GCK-3 and PASK detect cell-volume changes directly, or do they function downstream of a "volume sensor"? Studies of Ste20 in yeast may provide clues for answering this question.

The trigger for translocation of Pbs2 to Sho1 and initiation of the Ste20 osmoregulatory kinase cascade may

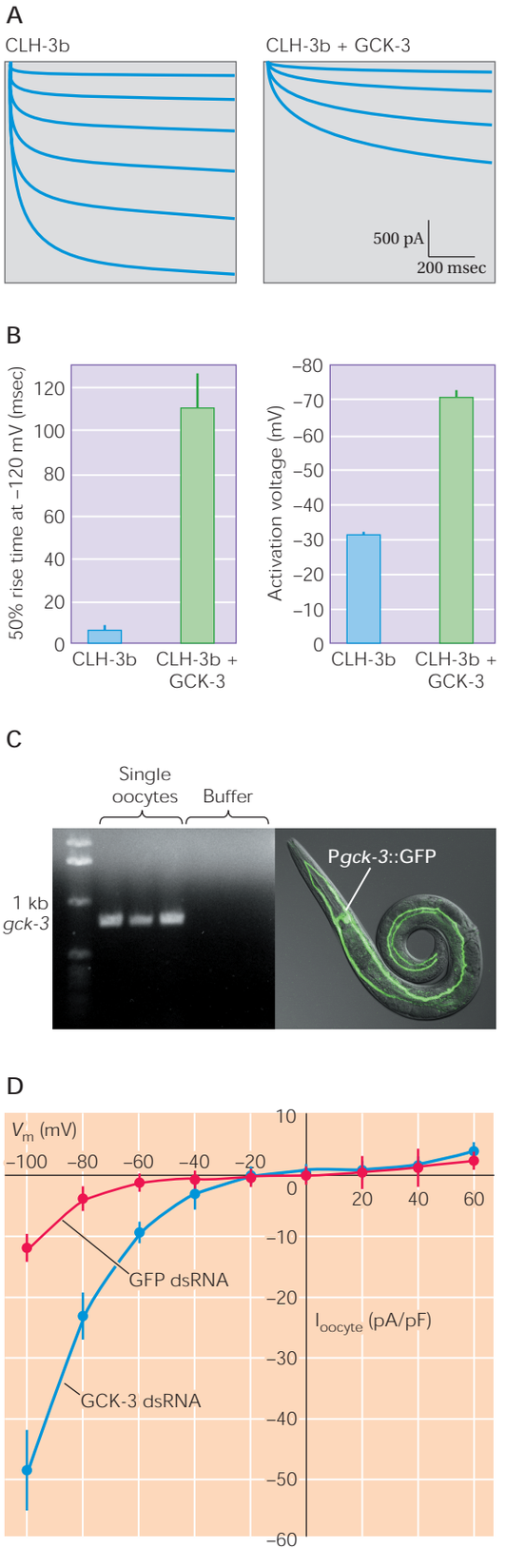

FIGURE 4. **Regulation of CLH-3b activity by GCK-3**

A: Whole-cell currents in human embryonic kidney cells expressing CLH-3b alone or CLH-3b and GCK-3. Each family of current traces shown is the mean of eight cells. Currents were evoked by stepping membrane voltage for 1 s between –140 mV and +60 mV in 20-mV increments from a holding potential of 0 mV. *B*: Kinetics of hyperpolarization-induced current activation (expressed as the time required to reach 50% maximal current activation at –120 mV) and activation voltage of CLH-3b expressed in human embryonic kidney cells alone or coexpressed with GCK-3. GCK-3 decreases channel voltage sensitivity and slows activation kinetics. *C*: *gck-3* and *clh-3* are coexpressed in *C. elegans* oocytes and the excretory cell. *Left*: detection of GCK-3 transcripts in single worm oocytes by RT-PCR. Expected product sizes of amplified GCK-3 cDNA and genomic DNA are 0.8 kb and 3.5 kb, respectively. Transcripts were not detected in samples of oocyte wash buffer. *Right*: combined confocal differential interference contrast and fluorescence micrographs showing expression of the *gck-3* transcriptional reporter P*gck-3*::GFP in the H-shaped excretory cell. *D*: GCK-3 knockdown by RNA interference constitutively activates CLH-3b in meiotically arrested *C. elegans* oocytes. Control worms were microinjected with GFP double-stranded RNA.

be hypertonic stress-induced unmasking of the Sho1 Src homology 3 domain (20). Interestingly, PASK translocates from the cytoplasm to the cytoskeleton in response to hypertonic stress (52). As has been postulated for Sho1, cell shrinkage could induce conformational changes in cytoskeletal and/or other proteins that activate PASK and GCK-3 and/or increase their interaction with regulatory targets. Activation/interaction in turn would trigger a phosphorylation cascade that activates NKCCs and inhibits KCCs (9, 14) and CLH-3b (7). Cell swelling would be expected to have the reverse effect on kinase activity/interactions, thereby allowing net dephosphorylation of regulatory targets by protein phosphatases (e.g., Refs. 21 and 46).

Epithelial transport and systemic salt and water homeostasis

In addition to sensing volume perturbations and maintaining cellular osmotic homeostasis, complex multicellular animals must also regulate systemic salt and water balance and transport fluid and solutes across specialized epithelia into and out of body compartments. As noted earlier, PASK expression is enriched in transporting epithelia (53) and NKCCs and KCCs play essential roles in epithelial ion transport and whole-animal salt and water regulation (15). PASK expression is high in the distal nephron and collecting duct principal cells of the kidney (53). It is thus likely that PASK regulates epithelial transport processes, including those in the kidney that function to control blood volume and ion concentrations. Recent studies demonstrating the likely functional interaction of PASK with WNK kinases (14, 55), which are known to play central roles in regulating renal salt transport (16), underscore this idea.

In *C. elegans*, GCK-3 is expressed not only in the worm oocyte but also in the worm excretory cell (FIGURE 4C) (7). The excretory cell functions as the worm "kidney" and is responsible for whole-animal fluid excretion (32, 33). Disruption of GCK-3 activity by RNA interference causes severe defects in whole-animal osmoregulatory functions (Choe K, Yin X, and Strange K, unpublished observations). Interestingly, a single WNK kinase homolog is present in the *C. elegans* genome and appears to be expressed in the excretory cell (WormBase; http://www.wormbase.org), suggesting the possibility of GCK-3/WNK functional interactions analogous to PASK/WNK interactions observed in mammals (14, 55).

The regulation of both Cl^- channels (i.e., CLH-3b) and cation-Cl^- cotransporters by closely related Ste20-type kinases is intriguing and has important physiological implications for controlling epithelial transport processes and mediating cross-talk (19) between apical and basolateral cell membranes. For example, fluid secretion across secretory epithelia such as the salivary gland, intestine, and lung is mediated by activation of basolateral NKCC1 and apical Cl^- channels such as CFTR (17). Coordinated activation of the cotransporter and inhibition of basolateral Cl^- channels or "leaks" by a common mechanism would increase net secretory Cl^- and water transport. In an analogous fashion, coordinated regulation of Cl^- channels and cotransporters is also important for efficient cell-volume control. Shrinkage-induced activation of NKCC1 and concomitant inhibition of Cl^- leaks by a common kinase would increase the rate of RVI by increasing the rate of net NaCl and osmotically obliged water uptake.

Linking cell cycle and cell volume

In many organisms and cell types, cell-cycle progression is linked tightly to changes in cell volume or size (31, 40, 43, 48). Furthermore, volume-sensitive anion channels and cation-Cl^- cotransporters have been implicated in the regulation of cell-cycle events, cell growth and proliferation, and programmed cell death (10, 34, 35, 44). For example, growth arrest of cervical cancer cells in G0/G1 is accompanied by a 60–70% reduction in volume-regulated anion channel (VRAC) activity. VRAC activity recovers upon reentry into the cell cycle, and inhibition of VRAC causes proliferating cells to arrest in G0/G1 (50). Activation of VRAC is required for the cell shrinkage that accompanies and is an essential component of apoptosis (51). Transfection of cervical cancer cells with a dominant negative KCC1 mutant abolishes swelling-induced KCC activity and significantly decreases cell growth (49). As noted above, oocyte swelling and resumption of the meiotic cell cycle both activate CLH-3b (45).

Ste20-type kinases play important roles in regulation of the cell cycle, cell growth, cell proliferation, and apoptosis (5, 47) (FIGURE 2). It is reasonable to speculate therefore that members of this kinase family may function to link these events to the activity of volume-sensitive anion channels and cation-Cl^- cotransporters. What regulatory mechanisms could mediate such a link? As noted above, recruitment of Ste20 to a scaffold complex comprised of Sho1 and Pbs2 is important for triggering the hypertonic stress response in yeast (20). Regulated changes in the cellular and subcellular compartmentalization of Ste20-type kinases could thus function to coordinate the activity of membrane proteins with cell-cycle events and other cellular processes. In this regard it is interesting to note that yeast Ste20 has been shown recently to regulate programmed cell death induced by hydrogen peroxide. Hydrogen peroxide triggers the translocation of Ste20 from membrane and cytoplasmic locations into the cell nucleus where it mediates histone H2B phosphorylation, which is required for apoptosis (1). Translocation of Ste20-type kinases away from the plasma membrane would obviously limit their ability to phosphorylate and regulate membrane-associated proteins such as channels and cotransporters.

Regulation of Ste20-type kinases by molecules whose activity correlates with cell volume could also function to link volume-sensitive channels and cotransporters with cell growth and cell-cycle events. For example, cyclins interact with and regulate cyclin-dependent kinases (11, 22). The cytoplasmic levels of cyclins change throughout the cell cycle, and these molecules have been proposed to coordinate cell-cycle events with cell volume (43). During development, the volume of a *C. elegans* oocyte increases ~200-fold prior to induction of meiotic maturation and ovulation (18, 29). Interestingly, the sensitivity of CLH-3b to swelling is inversely related to oocyte size: channel activation requires much greater cell swelling in small, early-stage oocytes compared with larger, later-stage oocytes (45). A simple hypothesis that would explain these observations is that the concentrations of putative GCK-3 regulatory proteins fall as oocytes grow and develop. Growth-dependent reduction of GCK-3 activity below a critical level could lead to net protein dephosphorylation and activation of CLH-3b in maturing oocytes. Inhibition of GCK-3 could also participate in the regulation of meiotic cell-cycle progression as has been proposed for X-PAKs in *Xenopus* oocytes (3, 12, 13). Oocyte swelling may activate CLH-3b by artificially lowering GCK-3 regulatory protein concentrations, thereby inhibiting kinase function.

Conclusions and Future Perspectives

It is clear from the above discussion that GCK-3 and PASK play critical roles in regulating cellular and epithelial ion-transport pathways. An important challenge for the future is to define in detail the specific cellular, tissue, and whole-animal physiological processes in which these kinases function. It will also be important to examine the roles of other members of the Ste20 superfamily in ion-transport regulation. Recent studies have demonstrated that the GCK-IV subfamily kinase Nck-interacting kinase (NIK) (FIGURE 1) binds to, phosphorylates, and activates the Na^+/H^+ exchanger NHE1 (58), suggesting that Ste20-type kinases may play widespread roles in regulating solute and water transport. In addition, Piechotta et al. (39) identified PASK-binding motifs in a variety of membrane ion-transport proteins including Cl^-, Na^+, K^+, and Ca^{2+} channels, neurotransmitter transporters, and ion exchangers.

Elucidation of the molecular mechanisms by which cells sense volume perturbations and activate regulatory responses has been a longstanding and vexing problem in physiology. Given the essential requirement of cellular osmotic homeostasis for all organisms, it is likely that cell volume-sensing mechanisms are highly conserved. The demonstration that Ste20, GCK-3, and PASK function to regulate volume-sensitive metabolic and transport pathways in organisms ranging from yeast to mammals supports this idea. Detailed characterization of the signaling cascades in which GCK-3 and PASK function is crucial for defining how they perform their physiological roles. The genetic and molecular tractability of yeast has proven to be invaluable for defining Ste20 signaling mechanisms (20) (FIGURE 2). Similarly, forward and reverse genetic analyses in *C. elegans* may allow elucidation of GCK-3 signaling pathways. Insights gained from genetic and molecular characterization of GCK-3 signaling in worms will likely provide important insights into PASK signaling in mammals. Ultimately, a combination of genetic, molecular, physiological, and biophysical characterization of GCK-3 and PASK should provide unique insight into how cells sense changes in their size and transduce those changes into appropriate molecular and cellular responses. Detailed knowledge of GCK-3 and PASK signaling will also likely provide new insights into the regulation of epithelial transport processes and systemic osmotic homeostasis and may provide new therapeutic targets for the treatment of water- and ion-balance disorders.

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