SRC family kinases in cell volume regulation

David M. Cohen

Division of Nephrology and Hypertension, Department of Medicine, Oregon Health and Science University and the Portland Veterans Affairs Medical Center, Portland, Oregon

Cohen, David M. SRC family kinases in cell volume regulation. Am J Physiol Cell Physiol 288: C483–C493, 2005; doi:10.1152/ajpcell.00452.2004.—SRC family kinases are a group of nine cytoplasmic protein tyrosine kinases essential for many cell functions. Some appear to be ubiquitously expressed, whereas others are highly tissue specific. The ability of members of the SRC family to influence ion transport has been recognized for several years. Mounting evidence suggests a broad role for SRC family kinases in the cell response to both hypertonic and hypotonic stress, and in the ensuing regulatory volume increase or decrease. In addition, members of this tyrosine kinase family participate in the mechanotransduction that accompanies cell membrane deformation. Finally, at least one SRC family member operates in concert with the p38 MAPK to regulate toxicity-dependent gene transcription.

hypotonicity; transient receptor potential; hypertonicity; ion transport; tyrosine kinase

THE ABILITY OF CELLS AND ORGANISMS to withstand environmental osmotic stressors involves early responses, occurring over milliseconds to minutes, and late responses requiring hours to even days to achieve. The rapid component consists of movement of ions and osmotically active organic solutes (organic osmolytes) across the cell membrane. The late phase is characterized by the regulation of de novo synthesis of 1) transport proteins essential for ion and organic osmolyte uptake or efflux; 2) enzymes essential for de novo synthesis of organic osmolytes; and 3) molecular chaperones such as the heat shock proteins that serve to counteract the detrimental effects of elevated intracellular ionic strength.

Although a variety of other cytoplasmic kinases have been implicated in osmotic signal transduction, including protein kinase C (151), myosin light chain kinase (21), and SGK1 (108), the discovery of the parallel mitogen-activated protein kinase (MAPK) modules, and the elucidation of their enormous importance in the cell response to anisotonicity (reviewed in Refs. 15 and 70), has dominated the literature on cell volume regulation. With this review, I call attention to a second essential group of cytoplasmic kinases, the SRC family kinases, with rapidly emerging importance in this field.

RECEPTOR TYROSINE KINASES IN ANISOTONICITY

Tyrosine kinases are broadly divided into receptor protein tyrosine kinases (e.g., EGF receptor and IGF-1 receptor) and nonreceptor protein tyrosine kinases (51). Although not the focus of this review, the role of receptor tyrosine kinases in the cell osmotic response is firmly established. Investigators have noted globally increased protein tyrosine phosphorylation in the context of both hypotonic (20, 119, 138) and hypertonic (113, 133) stress, and have used tyrosine kinase inhibitors to implicate this newly appreciated signaling event in volume regulatory ion transport (37, 118, 138). A variety of environmental stressors, including hypertonic stress and urea stress (29, 62, 116, 159), activate cell surface EGF receptor tyrosine kinase, as well as other peptide growth factor receptors. Initially, stress-dependent tyrosine phosphorylation was attributed to a downregulation of tyrosine-directed phosphatase activity (64), but it soon became clear that other forces were at play. Through a process known as ectodomain shedding, the extracellular EGF receptor-binding domains of membrane-spanning nascent ligands (including EGF and HB-EGF, among others) are cleaved and liberated, whereupon they bind to and activate the EGF receptor in a juxtacrine or paracrine fashion (109). This cleavage in response to stress requires activation of a specific metalloproteinase; the stress-responsive p38 MAPK is also likely required for this process. Parenthetically, this is the same mechanism through which agonists of G protein coupled receptors transactivate the EGF receptor, fueling speculation that a known or orphan G protein coupled receptor might serve as a sensor or effector of osmotic stress.

SRC FAMILY KINASES AND ANISOTONICITY

In contrast to the receptor tyrosine kinases, SRC family kinases are nonreceptor, or cytoplasmic, protein tyrosine kinases. They were initially characterized in the context of cell growth and differentiation; more recently, however, their roles in such diverse processes as cell adhesion and motility, carcinogenesis, immune cell function, and even learning and memory, have been appreciated (135). In addition to SRC itself, there are eight other members of the SRC family including YES, FYN, FGR, BLK, LCK, HCK, LYN, and YRK; together they comprise the largest family of nonreceptor protein tyrosine kinases (51). SRC, FYN, and YES are ubiquitously expressed, whereas the others exhibit more restricted tissue localization (i.e., cells of the central nervous system or immune system; see Ref. 135). As a family, they are most closely related to the ABL, BTK, CSK, and FES kinases. In addition

Address for reprint requests and other correspondence: D. M. Cohen, Division of Nephrology, Mailcode PP262, Oregon Health and Science University and the Portland Veterans Affairs Medical Center, Portland, Oregon 97239 (E-mail: cohenmd@ohsu.edu).
they share similarity with the Eph receptor tyrosine kinases to a much greater degree than the other receptor tyrosine kinase families. A useful dendrogram outlining these relationships is available free of charge at a commercial web site (http://www.cellsignal.com/reference/kinase/tk2.asp).

SFKs exhibit a modular architecture dominated by so-called “SRC homology,” or SH domains (Fig. 1). SH1, the catalytic domain, is the site of tyrosine kinase activity. In the inactive state, a key tyrosine in this domain (Y416) blocks the substrate binding site; when autophosphorylated, this residue is displaced and substrate access is unimpeded. SH2 and SH3 are protein-protein interaction domains shared not only with other SFKs but also with many other signaling proteins. The SH2 domain binds phosphotyrosine motifs in either an inter- or intramolecular fashion. An intramolecular interaction of this type figures prominently in SFK regulation. Specifically, SFKs are maintained in an inactive conformation through constitutive phosphorylation of a COOH terminal tyrosine residue (Y527; Fig. 1). This COOH terminal phosphotyrosine binds the upstream SH2 domain, clamping the intervening catalytic SH1 domain in an inaccessible, and hence inactive, state. Unlike the regulatory tyrosine in the catalytic domain, this COOH terminal tyrosine is phosphorylated by another kinase, either COOH terminal SRC kinase (Csk) or Csk homologous kinase (Chk). Removal of the phosphate from this residue by any of several SFK-directed tyrosine phosphatases releases the SH2 domain, and exposes the catalytic domain (see Ref. 158 for review). Of note, the viral oncogene v-Src, lacking this COOH terminal inhibitory tyrosine, is constitutively activated; this mutant protein product entirely accounts for the transforming capacity of the Rous sarcoma virus (106). Interactions mediated via the SH3 domain, in contrast to the SH2 domain, are less dynamic; this motif binds proline-rich sequences harboring the -Pro-X-Pro- motif (where X represents any amino acid).

Activation of SFKs is a complex process; by far the best understood example is SRC itself. As described above, kinases that phosphorylate the COOH terminal tyrosine inactivate SFKs and, conversely, phosphatases that dephosphorylate this residue activate SFKs. Sequence and structural data suggest that this paradigm applies to essentially all SFKs. The extent to which other mechanisms of SRC activation are similarly generalizable, however, remains uncertain. FAK, a nonreceptor tyrosine kinase related to the SFKs, can activate SRC by binding the latter’s SH2 and SH3 domains and disrupting SRC’s inhibitory intramolecular interactions. Activation of receptor tyrosine kinases such as the EGFR and PDGF receptors results in SRC activation. In addition, many studies place SRC downstream of PKC. Subcellular localization of SFKs is influenced by their activation state. Activated SRC, for example, traffics to the cell membrane where amino terminal myristoylation or palmitoylation anchors it to the inner leaflet (135, 158).

The functional relationship between receptor tyrosine kinases and the nonreceptor SFKs is bidirectional and complex (109, 135). SRC, and related family members (see below), physically interact with a variety of cell surface receptor tyrosine kinases (135). Activation of the EGF receptor tyrosine kinase results in SRC activation (109, 135), and SRC itself phosphorylates the EGF receptor on unique tyrosine residues critical for EGF-induced mitogenesis (5, 137). In addition, in the cell response to environmental stress, it appears that, SRC, like the toxin-specific p38 MAPK, is required for the transactivation of the EGF receptor (reviewed in Ref. 109).

Many, and perhaps all, SFKs are influenced by anisotonicity. Hypotonic stress activates LYN (67, 97, 156), LCK (77, 112), and SRC (147). Hyperosmolality activates FYN (59, 112), HCK (68, 113), FGR (68), and YES (112) and exerts a variable effect upon SRC (59, 105). Therefore, although SFKs are activated by both hypo- and hyperosmotic stressors, the profile of individual SFKs activated may be unique to each stimulus. Alternatively, these apparent distinctions may be purely a consequence of the disparate models investigated, and specificity may reside upstream or downstream of SFK activation. There are few, if any, models where bidirectional cell volume regulation has been investigated vis-à-vis SFK activation.

Many osmotically responsive ion transport pathways can be influenced by SFKs, hence these kinases are well poised to regulate cell volume. In many instances, these pathways are influenced by anisotonicity and are influenced by activation or inactivation of SFKs; importantly, however, it has not been firmly established that SFKs mediate the effect of cell swelling or shrinkage on each channel or transporter, so much work remains to be done. The simplest way to conceptualize these events is to consider them in terms of the compensatory response to acute cell volume changes. Acute exposure to a hypertonic environment causes immediate water efflux and resultant cell shrinkage. The subsequent adaptive response is the rapid entry of ions to obligate restoration of cell volume. This process is called regulatory volume increase, or RVI. RVI occurs in response to hypertonic cell shrinkage. In contrast, when cells are acutely exposed to a hypotonic environment, they swell abruptly as water enters. To counteract this process and avert membrane rupture, the cells rapidly dump ions and osmotically active organic solutes thereby tempering the volume increase. This process is regulatory volume decrease, or RVD. The role of SFKs in transport of ions and osmotically active organic solutes (organic osmoles) can be thought of in terms of these two opposing processes, activated independently.

**Fig. 1.** Overview of SRC family kinase (SFK) structure showing numbered SRC homology (SH) domains. Chicken (c)-Src is shown (top); by convention, important residues in SFKs are numbered in accordance with homology to this prototypical kinase. SH1 is the catalytic or kinase domain. An essential regulatory tyrosine in the middle of this domain is shown (Y416; red), phosphorylation of which is required for full activation of the kinase. Phosphorylation of the COOH terminal regulatory tyrosine (Y527; also shown in red) promotes intramolecular interaction with the upstream SH2 domain (red arrow); dephosphorylation of this tyrosine eliminates the interaction and renders the kinase domain accessible and active. The chicken viral (v)-Src proto-oncogene (bottom) lacks the COOH terminal inhibitory Y527 and is therefore constitutively active, accounting for its transforming potential.
to preserve cell volume. The molecular basis for both RV1 and RVD has been extensively studied (see Refs. 71 and 151 for recent reviews); there are universals that apply to virtually all cells and tissues studied, and there are specifics that are highly model or cell-type dependent.

**ROLE FOR SFK IN RVD**

For RVD (Fig. 2), the most phylogenetically ubiquitous acute cell volume regulatory ion fluxes are achieved through parallel activation of K⁺ and Cl⁻ channels (71). The molecular identity of cell volume regulatory K⁺ efflux pathways in most contexts has remained elusive, although a role for the following channels seems secure: 1) large conductance (maxi-K⁺) channels (BKCa); 2) intermediate conductance channels activated by relatively high concentrations of cytosolic calcium; 3) small conductance channels activated by relatively low concentrations of calcium; and 4) voltage-gated channels of the Shaker (K₁) family (151). The potentially volume-responsive BKCa is activated by SFKs (82, 83, 149). These channels are also activated by relatively high (i.e., micromolar) concentrations of calcium, a level achieved in response to hypotonic cell swelling in many models. A variety of voltage-dependent potassium channels are regulated by SFKs, including K₁.3 (6, 13, 23–26, 39), K₁.4 (103), K₁.5 (49, 87, 102, 103, 126), K₂ (126), and the K₇ (KCNQ) family (31). Of these, thus far only K₁.3 and K₁.5 have an established role in RVD. K₁.3 participates in perhaps the best studied example of SFK regulation of K⁺ efflux in RVD: the Jurkat T-lymphocyte model. In this model, the SFK LCK appears to be required for inhibition rather than activation of the channel (18). This effect is paradoxical because LCK is generally activated by cell swelling and an increase rather than a decrease in K⁺ channel activity would seem to be more desirable in response to unanticipated volume change in this direction.

Volume-regulated anion channels are also subject to regulation by SFKs and are pivotal in RVD. They conduct the acute chloride efflux that accompanies K⁺ efflux in RVD. Although they have been well characterized in many model systems, their molecular identity is in dispute. The ubiquitously expressed channel (127) has variously been dubbed the volume-sensitive outwardly rectifying Cl⁻ channel (104), volume-regulated anion channel (101), or volume-sensitive organic osmolyte-anion channel (53) and mediates the currents known as swelling-induced Cl⁻ currents or volume-regulated Cl⁻ current (see Ref. 151 for review). Similar to the case with K⁺ efflux above, the clearest understanding of SFK function influencing anion efflux in RVD is in human T cells. A swelling-activated Cl⁻ channel was described in this context, activation of which requires the SFK, LCK. Inhibition or genetic absence of the kinase interferes with the swelling-dependent activation of the channel, and application of purified LCK protein activates the channel (77, 132). In calf pulmonary endothelial cells, swelling-induced chloride currents were blocked by a SRC mutant that was specifically targeted to lipid rafts and caveolae (143). This effect was independent of SRC kinase activity but required the SH2 and SH3 protein-protein interaction domains. Transfection of these cells with wild-type SRC, however, failed to influence the chloride current (143). In addition, in a brain model, the volume-regulated anion channel was sensitive to the SFK inhibitor, PP2 (41).

Electroneutral K⁺-Cl⁻ symport activity is another well-conserved mechanism for effecting RVD (71). Of the four known isofoms (KCC1–4), KCC1, KCC3, and KCC4 are activated by hypotonicity (35, 50, 75, 90, 95, 111, 129), whereas KCC2 is insensitive to cell swelling (107). KCC1 is ubiquitously expressed and KCC2 is confined to the brain (see Ref. 151 for review). It has been suggested that KCC isoforms are activated through Ser/Thr-directed dephosphorylation (reviewed in Ref. 74), although a role for tyrosine phosphorylation has been incompletely explored. For example, in hippocampal neurons, KCC2 is activated by SRC and blocked by tyrosine kinase inhibitors (61). In erythrocytes, K⁺-Cl⁻ symport activity was substantially higher in cells isolated from mice null for the SFKs, FGR, and HCK (14).

Finally, RVD may be accomplished through the rapid efflux of organic solutes, such as amino acids, sugars, methylamines, and polyols. The volume-regulated anion channel contributes to this activity, hence one group’s reference to it as the volume-sensitive organic osmolyte-anion channel (53). Unquestionably, other pathways exist for osmolyte efflux and are

---

**Fig. 2.** Signaling events initiated by hypotonicity and the attendant regulatory volume decrease (RVD) that require the participation of SFKs. SRC (here representing either SRC itself or a related SFK family member) is likely indirectly activated by hypotonicity, probably through an integrin-dependent mechanism; integrins are responsive to membrane tension. SRC may also be activated by PKC and via activation of receptor tyrosine kinases, such as the EGF receptor. SRC and related SFKs directly or indirectly enhance phosphorylation of a variety of ion transport proteins including large-conductance (maxi-K⁺) channels (BKCa), volume-sensitive organic osmolyte anion channel (VSOAC), and isoforms of KCC and voltage-gated K⁺ channel (Kᵥ). Calcium entry in response to hypotonic cell swelling requires SRC-dependent activation of TRPV4 in some tissues; other calcium entry pathways (not shown) and pathways of calcium release from intracellular stores (unlabeled) are operative in the setting of RVD but their dependence on SFK activation has not been reported. Although best studied in the context of hypertonic stress (see Fig. 3), limited data support a role for EGFR transactivation in the response to cell swelling. The peptide mitogen and EGF receptor ligand, HB-EGF, is tethered to the cell membrane. HB-EGF activates Ras and phospholipase C (PLC). ERK activation by Ras requires sequential activation of components of an entire MAPK module. PKC may also be activated by relatively high concentrations of calcium; and small conductance channels activated by relatively low concentrations of calcium; and voltage-gated channels of the Shaker (Kᵥ) family (151). The potentially volume-responsive BKCa is activated by SFKs (82, 83, 149). These channels are also activated by relatively high (i.e., micromolar) concentrations of calcium, a level achieved in response to hypotonic cell swelling in many models. A variety of voltage-dependent potassium channels are regulated by SFKs, including Kᵥ.3 (6, 13, 23–26, 39), Kᵥ.4 (103), Kᵥ.5 (49, 87, 102, 103, 126), K₂ (126), and the K₇ (KCNQ) family (31). Of these, thus far only Kᵥ.3 and Kᵥ.5 have an established role in RVD. Kᵥ.3 participates in perhaps the best studied example of SFK regulation of K⁺ efflux in RVD: the Jurkat T-lymphocyte model. In this model, the SFK LCK appears to be required for inhibition rather than activation of the channel (18). This effect is paradoxical because LCK is generally activated by cell swelling and an increase rather than a decrease in K⁺ channel activity would seem to be more desirable in response to unanticipated volume change in this direction.

Volume-regulated anion channels are also subject to regulation by SFKs and are pivotal in RVD. They conduct the acute chloride efflux that accompanies K⁺ efflux in RVD. Although they have been well characterized in many model systems, their molecular identity is in dispute. The ubiquitously expressed channel (127) has variously been dubbed the volume-sensitive outwardly rectifying Cl⁻ channel (104), volume-regulated anion channel (101), or volume-sensitive organic osmolyte-anion channel (53) and mediates the currents known as swelling-induced Cl⁻ currents or volume-regulated Cl⁻ current (see Ref. 151 for review). Similar to the case with K⁺ efflux above, the clearest understanding of SFK function influencing anion efflux in RVD is in human T cells. A swelling-activated Cl⁻ channel was described in this context, activation of which requires the SFK, LCK. Inhibition or genetic absence of the kinase interferes with the swelling-dependent activation of the channel, and application of purified LCK protein activates the channel (77, 132). In calf pulmonary endothelial cells, swelling-induced chloride currents were blocked by a SRC mutant that was specifically targeted to lipid rafts and caveolae (143). This effect was independent of SRC kinase activity but required the SH2 and SH3 protein-protein interaction domains. Transfection of these cells with wild-type SRC, however, failed to influence the chloride current (143). In addition, in a brain model, the volume-regulated anion channel was sensitive to the SFK inhibitor, PP2 (41).

Electroneutral K⁺-Cl⁻ symport activity is another well-conserved mechanism for effecting RVD (71). Of the four known isofoms (KCC1–4), KCC1, KCC3, and KCC4 are activated by hypotonicity (35, 50, 75, 90, 95, 111, 129), whereas KCC2 is insensitive to cell swelling (107). KCC1 is ubiquitously expressed and KCC2 is confined to the brain (see Ref. 151 for review). It has been suggested that KCC isoforms are activated through Ser/Thr-directed dephosphorylation (reviewed in Ref. 74), although a role for tyrosine phosphorylation has been incompletely explored. For example, in hippocampal neurons, KCC2 is activated by SRC and blocked by tyrosine kinase inhibitors (61). In erythrocytes, K⁺-Cl⁻ symport activity was substantially higher in cells isolated from mice null for the SFKs, FGR, and HCK (14).

Finally, RVD may be accomplished through the rapid efflux of organic solutes, such as amino acids, sugars, methylamines, and polyols. The volume-regulated anion channel contributes to this activity, hence one group’s reference to it as the volume-sensitive organic osmolyte-anion channel (53). Unquestionably, other pathways exist for osmolyte efflux and are...
also potentially influenced by SFKs. In erythrocytes of the spiny dogfish, for example, swelling-activated efflux of the organic osmolyte triethylamine was blocked by nonspecific inhibition of tyrosine kinases. The SFK LYN and the related cytoplasmic kinase SYK were implicated because increased activation of these kinases was observed in response to several stimuli known to cause cell swelling in this model (67), although evidence for direct involvement is needed.

**ROLE FOR SFK IN RVI**

In contrast to RVD, substantially less is known about the role of SFKs in the molecular mechanisms of RVI after hypertonic stress (Fig. 3). In vitro, RVI is generally a less robust phenomenon than RVD. The best-characterized means through which hypertonically stressed cells accumulate ions include activation of Na⁺/H⁺ antiporters and Cl⁻/bicarbonate exchangers, and through Na⁺, K⁺, 2Cl⁻, or Na⁺-Cl⁻ cotransport (71, 151).

With respect to Na⁺/H⁺ antiport activity, NHE-1, -2, and -4 are activated by hypertonic stress, whereas NHE-3 is inhibited by it (71). NHE-1 is ubiquitously expressed and of the greatest relevance in RVI (151). Although hypertonic activation of NHE-1 was associated with activation of the SFKs, FGR, and HCK (68), inhibition of these and related kinases with PP1 failed to influence the hypertonic stimulation of NHE-1 (59). In contrast, inhibition of NHE-3 in response to CD95 receptor stimulation (a model of apoptotic cell shrinkage) required the SFK, LCK (72), although pharmacological inhibition of SFKs failed to influence inhibition of NHE-3 in another model (59).

Among Cl⁻/bicarbonate exchangers, AE-2 but not AE-1 is an effector of RVI (71). SFKs may play a role in agonist-inducible activation of AE-1 (110), although no data yet support a role in cell volume regulation.

Na⁺-K⁺-2Cl⁻ and Na⁺-Cl⁻ symport also contribute to RVI in a wide variety of tissues (71). Several Ser/Thr kinases including isoforms of PKC (44, 78) and MAPK (63, 78) have been implicated in regulation of NKCC1. However, the role of SFKs has been investigated in only one context. Erythrocyte NKCC, upon upregulation by the Ser/Thr phosphatase inhibitor calyculin, was modestly blocked by pharmacological inhibition of SFKs with PP1 (30).

Na⁺ channels and nonspecific cation channels may also be instrumental in RVI (71, 151). Activity of the epithelial sodium channel, ENaC, when heterologously expressed in NIH3T3 fibroblasts, can be inhibited by the peptide hormone endothelin-1. This effect is completely blocked by pretreatment with the SFK inhibitor PP2 (36). Nonselective cation channels in liver cells are activated by hypertonic stress. In this model system, intracellular dialysis with recombinant SRC leads to current activation even in the absence of osmotic stress (27). In further support of a potential role for SRC in RVI, cells genetically deficient in this kinase exhibit markedly increased susceptibility to apoptosis in response to transient hypertonic stress (86).

With respect to regulation of RVI effector pathways by SFKs, what is perhaps most striking is the relatively few model systems (and within each, the limited number of transport pathways) that have been investigated; clearly there are ample opportunities for investigation, especially in light of recent observations regarding the key role for SFKs in the regulation of gene transcription by hypertonic stress (see below).

**SPECIFICITY OF SFK FUNCTION IN THE CONTEXT OF RVI AND RVD**

It is clear from the preceding data that diverse ion transport pathways are regulated by any of a number of SFKs in the setting of hypotonicity and its associated RVD response, and by hypertonicity and the resultant RVI response. The molecular basis for the functional specificity accompanying the response to either stimulus, however, is poorly understood. For example, although both RVI and RVD increase activation of SFKs, in each case this SFK activation has been implicated in the upregulation of quite dissimilar ion transport events (Figs. 2 and 3). It is probably overly simplistic to consider SFK activation monolithic. Some measure of specificity toward individual SFK target proteins is likely provided by the specific signature of individual SFKs activated by a given volume regulatory stimulus (either cell swelling or shrinkage). As alluded to earlier, some SFKs (e.g., SRC) may be activated by both hypertonicity and hypotonicity (although not necessarily in the same cell type or model system). In such cases, it may be the selective activation of other SFKs that confers specificity to the cell response. In addition, there are numerous other signaling pathways responsive to anisotonicity that directly or
indirectly impinge upon the SFKs and likely influence their function. Some are detailed in Figs. 2 and 3. The net effect is that individual SFKs integrate a large number of afferent signaling events in response to cell swelling or shrinkage, and this integration determines the activation signature of the individual SFKs. It is also likely that additional parallel SFK-independent signals are triggered by anisotonicity and converge upon the individual SFK target proteins (e.g., MAPK activation of NKCC); in such cases, the target transport proteins themselves and not the SFKs serve as the integrators of diverse signaling inputs, translating them into ion flux.

**SFKs IN NONOSMOTIC ION TRANSPORT PATHWAYS**

In addition to RVD and RVI, other ion transport processes may be subject to regulation by SFKs. These molecular events, although not directly related to cell volume regulation and therefore not the focus of this review, may nonetheless provide useful precedents for signaling pathways operative in the setting of anisotonicity. In one well-studied example, acidosis activates the sodium bicarbonate cotransporter. This process appears to require the action of SRC, based on inhibitor data and overexpression of the negative SRC regulator Csk (22, 114, 115, 117).

SFKs also regulate the ion fluxes that accompany neurotransmission, and these models have been scrutinized in extraordinary detail. Neurotransmitter receptors may be classified (19) as either ionotropic, with the receptor itself serving as the ion channel, or metabotropic, where a G protein coupled receptor indirectly activates ion flux via an intervening signal transduction pathway. The NMDA receptor, an example of the former, contributes to fast excitatory neurotransmission in the central nervous system. Simultaneous binding of both glycine and glutamate to the extracellular portion of the receptor opens a mono- and divalent cation channel. Activation of SFKs, and likely SRC itself, enhances NMDA receptor activity (120). The other principal glutamate receptor in CNS, the AMPA receptor, is also under the control of SFKs. This channel physically interacts with the SFK, LYN, in the cerebellum. Ligand engagement of the receptor activates LYN, which in turn participates in downstream signaling events (42). Therefore, ionotropic glutamate receptors may activate SFKs, as occurs with the NMDA receptor, or may in turn be activated by SFKs, as occurs with the AMPA receptor. The ion fluxes engendered by interaction of metabotropic neurotransmitters with their respective ligands are also heavily influenced by kinases of the SRC family (reviewed in Ref. 45).

**SFKs IN ANISOTONIC REGULATION OF CALCIUM HOMEOSTASIS**

In contrast to the sodium, potassium, and chloride movement accompanying RVD and RVI, net entry or efflux of calcium is of little direct osmotic consequence in cell volume regulation. Intracellular calcium concentration, however, appears to be an essential component of the signaling events necessary for orchestrating the cell response to anisotonicity (88, 140). In the setting of osmotic cell swelling, intracellular calcium is increased in a wide variety of cell culture models (see Ref. 71 for a review). This signaling event is a prerequisite for subsequent RVD in some (142, 153) but not all (7, 100, 134) cell types. Volume regulatory transport pathways that require an increase in intracellular calcium in at least one model system include those for efflux of K+ (described above) and Cl−, and efflux of the organic osmolytes taurine and sorbitol (reviewed in Ref. 151). In marked contrast to the prominent role in RVD, participation of intracellular calcium in RVI is less clear (71). The mechanism through which intracellular calcium is increased in response to hypotonic cell swelling is highly model dependent. In most cases, calcium entry predominates although calcium release pathways are well described. Examples of the former include stretch-activated calcium channels, voltage-operated L-type calcium channels, Na+/Ca2+ exchange, and the TRP family of nonselective cation channels (see Refs. 54 and 151 for reviews; see below). Swelling-induced intracellular calcium release occurs via either ryanodine- or inositol 1,4,5-trisphosphate-sensitive pathways. In addition, coordinated calcium entry and calcium release have been demonstrated in multiple models. Examples of the latter include calcium-induced calcium release in a variety of epithelia (43, 139, 155), as well as the inverse relationship where calcium entry is dependent on prior calcium release (4, 10, 48, 89, 92, 94, 141).

As alluded to above, mounting evidence points to a role for one or more members of the TRP family of calcium channels in cell volume regulation. In addition, new data underscore a role for SFKs in this process. The TRP channels comprise a family of six-transmembrane domain nonselective cation channels broadly divisible into three classes: 1) TRPC, or classic (short) TRP channels; 2) TRPM, or melastatin (long) TRP channels, so named for canonical member, melastatin; and 3) TRPV channels, named for the vanilloid-responsive founding member, TRPV1. Three additional classes of closely related channels include the mucolipins, polycystins, including polycystin-2, a molecular culprit in polycystic kidney disease, and the sensor of noxious cold, ANKTM1 (11). TRP channels of the TRPC family are widely expressed, and are generally activated by engagement of receptor tyrosine kinases and G protein coupled receptors in a phospholipase-dependent fashion; they are likely instrumental in store-operated calcium entry. TRPM channels serve diverse functions, befitting their large size; some, dubbed “chanzymes,” even harbor catalytic kinase domains. Members of the TRPM family sense taste, anisotonicity, and noxious cold; others mediate calcium and magnesium uptake from the gut and kidney. TRPV channels are generally temperature responsive, although several members sense anisotonicity or serve to reabsorb calcium in the gut and kidney. In contrast to the ubiquitously expressed TRPC channels, expression of TRPV channels is primarily restricted to the nervous system and a few epithelia, such as the kidney and intestine (11).

With respect to TRP channel function and osmoregulation, data are emerging from diverse model systems. In yeast, a TRP channel homolog is activated by hypertonic stress (17). In Caenorhabditis elegans, the TRP channel OSM-9 is essential for appropriate avoidance of osmotic gradients (12); its genetic absence in worms can be complemented by its mammalian homolog, TRPV4 (81).

In mammalian systems, three TRP channels respond to anisotonicity in vitro: TRPM3, TRPV2, and TRPV4. TRPM3 is most abundantly expressed in kidney, although expression was also detected in central nervous system and testis (38, 76). Heterologous expression in human embryonic kidney-293 cells
resulted in constitutive cation influx, and exposure of TRPM3-expressing cells to hypotonic medium (200 mosmol/kgH2O) was associated with an increase in intracellular calcium (38).

TRPV2 is primarily activated by heat and growth factors. Among other tissues, it is expressed in mouse aortic myocytes; these cells exhibit calcium entry in response to hypotonicity (96). Treatment of myocytes with antisense oligodeoxynucleotides against murine TRPV2 abrogated TRPV2 expression and the hypotonicity response. When heterologously expressed in Chinese hamster ovary cells, TRPV2 was activated by both membrane stretch and hypotonic cell swelling (96).

Comparatively little is known about signaling events leading to regulation of TRPM3 and TRPV2 by anisotonicity, whereas more details are available for TRPV4. Although TRPV4 was also identified in other contexts (16, 152), its significance in osmoregulation was solidified when it was cloned as the mammalian homolog of the C. elegans osmosensory protein, OSM-9 (79, 128). When heterologously expressed, TRPV4 is highly sensitive to a decrease in ambient tonicity (79, 128). This fact, coupled with the observation of some groups that TRPV4 is expressed in the blood-brain barrier-deficient brain nuclei responsible for sensing systemic tonicity (79, 80), suggested that TRPV4 might serve as the elusive sensor of plasma osmolality. Whether TRPV4 functions as a sensor or as an effector remains in dispute; knockout mice exhibit a relatively subtle phenotype with modestly elevated plasma [Na+] and an aberrant response to both salt and water stress (80, 93).

A role for signaling by arachidonic acid metabolites in TRPV4 function has recently been proposed (150), perhaps consistent with earlier data broadly implicating this pathway in various models of RVD (see Ref. 151 for a review). PKC may also function in TRPV4 activation, although not in the context of hypotonic swelling (32).

**TRP CHANNELS ARE SUBJECT TO REGULATION BY SFKs**

TRPV4 undergoes tyrosine phosphorylation in response to hypotonic stress, principally on Tyr253 in the amino terminal cytoplasmic domain (156). This phosphorylation event is sensitive to inhibition of SFKs, either pharmacologically or via transfection of dominant negative-acting SFK. Multiple SFKs interact with TRPV4, as the proteins can be reciprocally coimmunoprecipitated. TRPV4 is a substrate of SFKs in vitro, and mutation of the principal SFK phosphorylation site abolishes channel gating by hypotonicity (156). Although these data strongly suggested that regulation of TRPV4 was mediated through SFKs, this view is not universally accepted. Vriens et al. (148) reported that mutation of Tyr253 failed to influence the tonic response and that inhibitors of SFK failed to affect hypotonicity-inducible calcium transients. However, a second function of TRPV4, that of nociception, was later shown to also require participation of SFKs. In addition to a role in pain associated with application of pressure or an acidic solution to the tail (131), TRPV4 plays a role in pathological pain conditions, such as the neuropathic pain that accompanies membrane stretch and hypotonic cell swelling (96).

In further support of the importance of this mode of regulation, very recent data support a role for SFKs in the regulation of representatives of all three major families of TRP channels (46, 57, 58, 145).

**ANISOTONIC REGULATION OF NONTRANSPORT PROTEINS BY SFKs**

SFKs have also been implicated in phosphorylation of substrates other than transport proteins in response to anisotonicity. In other contexts, SFKs phosphorylate an enormous array of proteins involved in cell adhesion, motility, cytoskeletal organization, cell cycle progression, apoptosis, and differentiation (135). In contrast, only a handful of SFK substrates have been investigated in models of anisotonicity. Osmotic shock results in SRC-dependent tyrosine phosphorylation of the platelet endothelial cell adhesion molecule protein in vascular endothelial cells (105). The insulin receptor substrate, Gab-1, became newly tyrosine phosphorylated after sorbitol treatment in adipocytes; this process was sensitive to pharmacological inhibition of tyrosine kinases in general, and the SFKs specifically (55). In Chinese hamster ovary cells, hypertonic stress caused the tyrosine phosphorylation of a number of proteins including the actin binding protein, cortactin. Tonicity-dependent cortactin phosphorylation could be blocked by the SFK inhibitor PP1 and was reduced in FYN-deficient fibroblasts (59). A role for EGFR in osmotic signal transduction has been established and was discussed above. SRC phosphorylates EGFR directly in other contexts (5, 137). Transactivation of EGFR in response to hypertonic stress requires activation of the SFK, YES (112). Further impacting this signaling axis, hypertonic stress increases expression of a number of EGFR ligands; in the case of HB-EGF, this process is at least partially dependent upon SFK activation (66). In a similar fashion, osmotic stress upregulates expression of COX-2, a key enzyme in arachidonic acid metabolism. Pharmacological inhibition of SFKs reduced this effect in cultured cells derived from the kidney inner medulla (157). In cultured fibroblasts, dominant-negative-acting SRC blocked the ability of hypertonicity and other environmental stressors to upregulate tyrosine phosphorylation of caveolin-1 (146). Of note, several of these signaling events also potentially require activation of MAPK cascade components (66, 157). This type of organization is consistent with the postulated role for SRC in activation of the small G protein, Ras, which resides “upstream” of the Raf/MEK/ERK MAPK module. Ras is activated by hypertonic stress (136) and is potentially instrumental in cell volume regulation (73, 91, 136, 144).

**MECHANOTRANSDUCTION: SFKs, INTEGRINS, AND OSMOTIC STRESS**

A final point regarding the impact of SFKs upon early events in cell volume regulation is the relationship between SRC activation and integrin function. Integrins are the major mediators of cell-matrix adhesion, and are also important in cell-cell interaction. Members of this large family generally exist on the cell surface in an inactive state; engagement with a matrix constituent then activates signaling events reminiscent of those initiated by receptor tyrosine kinases and G protein coupled receptors (52). Ultimately, integrin engagement globally affects cell function, influencing adhesion, motility, proliferation...
and differentiation. As anchoring points, integrins sense membrane stress and provide a nexus for mechanotransduction (1). Classically, this mode of physical stress-sensing ion transport operates independently of any agonist, responding only to membrane deformation. Well-studied examples of integrin-dependent mechanotransduction include hair cell activation in the inner ear and models of shear stress (1, 34). The SFK SRC was one of the earliest identified effectors of the integrins; the constitutively active viral oncogene v-Src confers anchorage-independent growth by subverting integrin function (52). Integrin engagement activates SRC in several native model systems, mirroring the effect of v-Src (135).

Consistent with their role in mechanotransduction, integrins (and therefore SFKs) participate in osmotic signal transduction and cell volume regulation. Expression of $\beta_1$-integrin is increased by hypertonic stress in a kidney epithelial cell model (124); this molecule also forms a complex with at least two other integral membrane proteins, CD-9 and HB-EGF (99, 125), that are themselves osmotically inducible (3, 66, 123). One of these components, HB-EGF, is subject to ectodomain cleavage and juxtacrine action in response to hypertonic stress as discussed above. A number of anisotonicity-induced transport processes including neurotransmitter release are blocked by a peptide inhibitor of integrin function (60, 85, 130). Hepatocyte cell volume is increased by hypotonicity and by insulin. In both cases, SFKs transmit the activating signal from integrins to downstream elements such as MAPKs (121, 147). Again underscoring the potential relationship between osmotic and mechanical stimuli, physical membrane traction on $\beta_1$-integrins in ventricular myocytes activates a chloride current, which is blocked by pharmacological inhibition of SFKs (8). In addition, with respect to late events in cell volume regulation (see below), integrin clustering has been associated with increased transactivation by the tonicity-responsive transcription factor, TonEBP (56). SFKs also mediate shedding of the adhesion molecule, L-selectin, from hypertoniaically stressed neutrophils (113); this process requires upregulation of a "sheddase" activity analogous to the mechanism of EGF receptor transactivation by hypertonic stress. In aggregate, these data serve to underscore the close relationship between SFK activation and the functioning of integrins (and perhaps other adhesion molecules) in the cell response to anisotonicity.

**SFKs IN HYPERTONIC TRANSCRIPTIONAL REGULATION: TonEBP, p38, AND FYN**

Although the bulk of this review has emphasized the role of SFKs in regulating the early response to anisotonicity, a recent and important study suggests that SFKs might also influence the late adaptive component of cell volume regulation. In the setting of hypertonic stress, after initial rapid ion fluxes have abated and volume has been restored toward normal, cells are left with an increase in intracellular ionic strength. Because this adversely affects enzyme function, among many other variables, organisms have adopted biochemical strategies to preserve cell volume while returning ionic concentration toward normal. Organic osmolytes are synthesized or imported in response to hypertonic stress. Although these compounds can be almost instantly “dumped” when hypertonic stress is abruptly alleviated, their accumulation requires many hours and even days. The delay occurs because maximal accumulation of these solutes requires de novo synthesis of osmolyte transport proteins, including the Na$^+$-Cl$^-$-betaine (GABA transporter; BGT1) and Na$^+$-myo-inositol symports. Synthesis of the enzyme aldose reductase is also required, as it catalyzes an essential step in the intracellular production of the organic osmolyte sorbitol (reviewed in Refs. 9 and 33). Transcription of these four proteins (and many others) is tightly regulated by the tonicity-responsive DNA binding protein TonEBP (OREBP, NFAT5) (40). Hypertonic stress increases expression, nuclear translocation, and likely phosphorylation of TonEBP; all of these mechanisms facilitate TonEBP interaction with its DNA consensus motif, the TonE (ORE) element, present in varying copy number in the upstream 5$'$ flanking sequence of the osmolyte synthesis and transport genes (47, 154). TonEBP is essential for the hypertonicity response; genetic absence of this transcription factor renders cells incapable of upregulating expression of aldose reductase in response to hypertonic stress (84).

Much recent work has appropriately focused on the role of the tonicity-responsive MAPK, p38, in transcription mediated via TonE/TonEBP interaction; in most but not all cases, activation of p38 is required (69, 98, 122). An additional level of complexity, however, is emerging. Ferraris and coworkers (28) noted that the transactivating ability, or transcriptional competence, of a molecular chimera containing the TonEBP transactivation domain could be blocked with inhibitors of tyrosine kinase. Ko and coworkers (65) then established a potential molecular basis for this intriguing observation. Consistent with earlier findings, they noted that TonE-dependent transcription was partially blocked by pharmacological inhibition of p38 or by expression of dominant negative-acting p38 mutant. But they also observed that TonE-dependent transcription could be partially blocked with pharmacological inhibition of SFKs or via expression of dominant negative-acting mutant of the SFK, FYN (65). This group had previously shown that Fyn was activated by cell shrinkage (59). Moreover, in a FYN-deficient fibroblast cell line, inhibition of p38 alone almost completely blocked TonE-dependent transcription in response to hypertonic stress. FYN does not appear to influence TonEBP nuclear translocation (65), but other modalities of regulation have not been explored. These data, although acquired primarily through heterologous expression of an artificial reporter construct in an in vitro model, strongly support a role for SFKs in mediating tonicity-dependent transcription. Although direct evidence is lacking, these authors speculated that the FYN SH3 domain may interact with any number of canonical SH3 binding motifs in TonEBP.

In summary, SFKs integrate a large amount of upstream signaling information in the context of anisotonicity, including membrane tension and receptor activation, and transduce that information to a wide variety of cell volume regulatory effectors, including channels, transporters, and signaling proteins. In addition, SFKs are involved in the long-term adaptive response to anisotonicity by regulating transcription of tonicity-inducible genes. It is likely that SFKs participate in many more molecular aspects of cell volume regulation than has previously been recognized.

**ACKNOWLEDGMENTS**

The author thanks Florian Lang for careful reading of this manuscript.
This work was supported by the National Institutes of Health, the Department of Veterans Affairs, and the American Heart Association.

REFERENCES


SFK IN CELL VOLUME REGULATION

Invited Review


