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The WNK/SPAK and IRBIT/PP1 Pathways in Epithelial Fluid and Electrolyte Transport

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Abstract

Fluid and electrolyte homeostasis is a fundamental physiological function required for survival and is associated with a plethora of diseases when aberrant. Systemic fluid and electrolyte composition is regulated by the kidney, and all secretory epithelia generate biological fluids with defined electrolyte composition by vectorial transport of ions and the obligatory water. A major regulatory pathway that emerged in the last several years is regulation of ion transporters by the WNK/SPAK kinases and IRBIT/PP1 pathways. The IRBIT/PP1 pathway functions to reverse the effects of the WNK/SPAK kinases pathway, as was demonstrated for NBCe1-B and CFTR. Since many transporters involved in fluid and electrolyte homeostasis are affected by PP1 and/or calcineurin, it is possible that WNK/SPAK and IRBIT/PP1 form a common regulatory pathway to tune the activity of fluid and electrolyte transport in response to physiological demands.

Bodily fluid and electrolyte homeostasis is a fundamental physiological function associated with numerous diseases when aberrant. The systemic fluid and electrolyte composition is regulated by the kidney. In addition, all secretory epithelia generate biological fluids with defined electrolyte composition essential for their specific functions. Fluid and electrolyte composition is determined by vectorial ion transport and the associated osmotic water transport through water channels. Many central and peripheral regulatory inputs ensure tight regulation of bodily fluid and electrolyte composition that respond to systemic, tissue, and cellular changes in fluid volume and electrolyte composition (39). A major regulatory pathway that emerged in the last several years is regulation of ion transporters by the WNK/SPAK kinases and IRBIT/PP1 pathways, the subject of this review. Seminal discoveries in this topic include identification of the WNK kinases in a search for MAPK/ERK homologs (54), the finding that mutations in the WNKs are associated with hypertension (52), the association between the WNK and SPAK/OSR1 kinases and their function in a common regulatory pathway (19, 50), and the regulation of the Na⁺-HCO₃⁻ cotransporter NBCe1-B by IRBIT (47). Several aspects of these topics have been covered by extensive recent reviews (11, 34). Here, we will focus on the relationship between the

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WNK/SPAK and IRBIT/PP1 pathways to suggest that their reciprocal effect on fluid and electrolyte transport may form a common pathway that determines the resting and stimulatory secretory states.

The WNK Kinases as Scaffolding Proteins

The With-No lysine (K) Kinases (WNK) received their name due to the lack of the conserved lysine in subdomain II (27). The crystal structure of the kinase domain of WNK1 revealed that the lysine is contributed by a lysine in subdomain I (37). Mammals have four WNK kinases (FIGURE 1A) with several splice variants (34), with wide expression of WNK1 (9, 38) and WNK4 (28, 48) and more restricted and cell-specific expression of WNK2 (48) and WNK3 (48). The WNKs are very large proteins composed of up to 2,382 residues (WNK1). However, very little is known about their domain structure beyond the homologous kinase domain, the autoinhibitory domain (AID), and the multiple coiled-coil domains. WNKs 1, 2, and 4 also have several proline-rich domains (PRD) that in WNK1 play an important role in the regulation of the renal K⁺ channel ROMK1 (24). The WNKs PRD may also interact with SH3 domains to mediate WNK1-dependent endocytosis that is mediated by the endocytic scaffold intersectin (24). Notably, the PRDs of WNK1 and WNK4 also contain the PPxxF binding ligands for the scaffold proteins Homer (4) that may recruit them to GPCR complexes (53). It is most likely that the WNKs have additional domains in the large stretches between the kinase domain and the COOH terminus (>1,800 residues in WNK1) that can function as scaffolds to mediate the many functions of the WNK kinases (27, 34, 50).

The most prominent and best understood role of the WNKs is the regulation of Na⁺, K⁺, Cl⁻, HCO₃⁻, and Ca²⁺ transporters in epithelia (27, 34, 50) and the brain (12) that is associated with hypertension. The WNKs regulate ion transporters either by determining their surface expression and/or their activity. The regulation can be quite complex and specific to the WNK isoform and the transporter. The details of these regulatory forms are discussed in Ref. 34, and here only few examples will be given. For example, WNK1 and WNK4 reduce the level of the NaCl cotransporter NCC (7, 23, 55) and of ROMK1 (8, 24, 51) in the plasma membrane. However, WNK1 affects surface expression of NCC by suppressing the inhibition exerted by WNK4 (7, 23, 55). Furthermore, WNK4 reduces surface expression of NCC by inhibiting the trafficking of NCC to the plasma membrane in a mechanism that involves Sortilin and results in accumulation of NCC in the lysosomes (62). On the other hand, the WNKs reduce surface ROMK1 by increasing its intersectin-dependent endocytosis (8, 24). WNK1 can also indirectly regulate NCC by phosphorylating SPAK and OSR1 (50), which in turn phosphorylates and activates NCC without affecting its surface expression (34). Interestingly, inhibition of ROMK1 by the WNKs (8, 24) and of NCC by WNK4 (46, 57) is independent of their kinase function, indicating that, in this case, the WNKs function as scaffolds. In the case of ROMK1, the inhibition is mediated by the first 119 residues of WNK1 and requires the PRDs PXXP in WNK1¹⁻¹¹⁹ for the inhibition (8, 24, 51).

Another group of transporters regulated by the WNKs are the Cl⁻ and/or HCO₃⁻ transporters NBCe1-B (58), Slc26a3, Slc26a6 (41), Slc26a9 (16), and CFTR (56, 58). The WNKs inhibit surface expression of NBCe1-B, Slc26a9 (16, 58), and CFTR (56, 58) and activity of all transporters. The kinase function of the WNKs is not required for these effects, and the WNKs act as scaffolds for SPAK (see below).

The SPAK/OSR1 Kinases

In many instances, the WNKs do not act directly on the transporters but rather phosphorylate the sterile 20 family stress kinases SPAK/OSR1 (11, 12). The domain structure of SPAK

and OSR1 are depicted in FIGURE 1B. SPAK and OSR1 are related kinases with conserved kinase domain, a serine-motif (Smotif), and a conserved COOH terminal (CCT) domain (44, 50). Both SPAK and OSR1 interact with the Ca^{2+} binding protein Cab39, which requires the hydrophobic-acidic-hydrophobic motif (WEW in SPAK and OSR1) (36). Cab39 markedly activates the kinases and consequently NKCC1 (17, 21). The role of activation of the SPAK/OSR1 by Cab39 in regulation of other transporters is not known at present. SPAK has, in addition, an alanine- and proline-rich NH_2 -terminal extension. The kinase domain of SPAK and OSR1 are 89% identical, and in many cellular and biochemical assays their function appears redundant (11, 12, 44). However, although knockout of SPAK resulted in enhanced nociceptive threshold, locomotor phenotype, anxiety (22), Gitelman-like syndrome (61), knockout of OSR1, or knock-in of kinase-dead OSR1 are embryonic lethal (30). Indeed, although widely expressed, SPAK and OSR1 do show cell- and tissue-specific expression pattern (12, 34, 44).

The WNKs function as scaffolds and activators of SPAK/OSR1. The SPAK and OSR1 CCT interacts with the [R/K]Fx[V/I] motif that is present at least in one copy in all WNKs (FIGURE 1A). Interaction of SPAK/OSR1 with the [R/K]Fx[V/I] motif was found when studying the regulation of NKCC1 by SPAK (42). Interestingly, this motif is also present in several transporters, including NKCC1 (12, 44), Slc26a3, Slc26a6, and CFTR (based on sequence analysis), although in the latter it has not been verified experimentally as yet, raising the possibility that SPAK/OSR1 directly interacts with these transporters to regulate their function. WNK1 and WNK4 phosphorylate the conserved T185 and S325 of OSR1 and T233 and S373 of SPAK, and phosphorylation of both is required for activation of SPAK/OSR1 (12, 44). As with the WNKs, the effect of the SPAK/OSR1 is specific for a given transporter. For example, the SPAK/OSR1 activates NKCC1 without affecting surface expression, and the kinase function of both the WNKs and SPAK/OSR1 is required (12, 44). Similarly, SPAK/OSR1 activates NCC intrinsic activity (23, 40). On the other hand, SPAK/OSR1 inhibits the activity of NBCe1-B and CFTR (58).

A group of transporters regulated by the WNKs and the SPAK/OSR1 are the Cl^- and/or HCO_3^- transporters NBCe1-B (58), Slc26a3, Slc26a6 (41), Slc26a9 (16), CFTR (56, 58), and possibly the CLC2 Cl^- channel, since the the CLC2 *C. Elegans* ortholog CLH3 is regulated by the kinases (10). The WNKs inhibit surface expression of NBCe1-B, Slc26a9 (16, 58), and CFTR (56, 58) and the activity of all transporters. This is illustrated in FIGURE 2, and the functional consequences are in FIGURE 3. Expression in HeLa or HEK cells shows that WNK1, WNK4, and their kinase-dead mutants, and SPAK inhibit NBCe1-B and CFTR activity (Ref. 58; FIGURE 2A AND 2B). The WNKs (58) and their kinase-dead mutants markedly reduced surface expression of NBCe1-B (FIGURE 2B) and CFTR (FIGURE 2C). Interestingly, WNK1¹⁻¹¹⁹ recapitulates all the effects of WNK1 (Ref. 58; FIGURE 2A), and the effect of the WNKs were inhibited by kinase-dead SPAK. These findings indicate that the WNKs function upstream of SPAK to act as scaffolds for SPAK. Moreover, although the kinase function of the WNKs is not required for their activity, the kinase function of SPAK is required, and SPAK phosphorelates NBCe1-B and CFTR (58). The pancreatic duct secretes a large volume of a HCO_3^- -rich fluid and expresses WNK1, WNK3, WNK4, and SPAK (31) and thus was used to follow the role of the WNKs and SPAK in fluid and HCO_3^- secretion. Most notably, FIGURE 3 shows that knockout of the WNKs, including WNK1, and of SPAK in the native pancreatic duct markedly enhanced stimulated fluid secretion. This indicates that the WNK/SPAK pathway functions as inhibitor of epithelial fluid and HCO_3^- secretion, most likely by inhibition of HCO_3^- influx by the basolateral NBCe1-B and HCO_3^- secretion by the luminal SLC26 transporters and CFTR (see Ref. 58 for details) to set the basal nonsecretory state of the epithelia.

IRBIT (IP₃Rs Binding Protein Released With IP₃)

Facilitation of ductal secretion by knockdown of the WNKs and SPAK (FIGURE 3A) suggests that they exert tonic inhibition of fluid and HCO₃⁻ secretion. Stimulation of secretions requires reversal of the inhibitory state. IRBIT/PP1 turned to the proteins that reverse the inhibitory effect of the WNKs/SPAK pathway and in addition to directly activate the transporters mediating epithelial fluid and HCO₃⁻ secretion.

IRBIT was discovered multiple times in different contexts, but it was only after the discovery that IRBIT binds to IP₃Rs and dissociates from the IP₃Rs by IP₃ (2) that we began to understand its role in epithelial transport. The various functions of IRBIT have been discussed recently (59), and here we limit the discussion to its role in ion transport. IRBIT comes in two forms. The short and long IRBIT are ubiquitous, with the long IRBIT being identical with the short IRBIT but with an NH₂-terminal extension (3). The IRBIT domains identified so far are shown in FIGURE 1C and include a PP1 binding motif, a PEST domain, a coiled-coil domain, and a PDZ ligand at the end of the COOH terminus (15, 59). The PEST domain has multiple phosphorylation sites (1) and is required for all IRBIT functions described so far (15, 59).

As its name indicates, IRBIT interacts with all three IP₃ receptor isoforms to regulate their function (2, 13, 14). IRBIT specifically interacts with the IP₃ binding domain in the NH₂ terminus of the IP₃Rs (1). In fact, IRBIT competes with IP₃ for binding to the same site on IP₃Rs and right shift the dose response for IP₃ binding, channel opening, and Ca²⁺ release from ER in response to IP₃ (1, 13, 14). These effects required phosphorylation of at least four serines in the PEST domain with phosphorylation of S68 being required for phosphorylation of the other sites (13). The kinase(s) that phosphorylate IRBIT are not known at present, although IRBIT has consensus sites for PKD, AMPK, CamK-II-IV, and CK1 (15). The PDZ ligand is required for interaction of IRBIT with the IP₃Rs (14) and formation of complexes with and activation of the HCO₃⁻ transporters (58). Importantly, in the resting state, when cellular IP₃ levels are low, most IRBIT is bound to the IP₃Rs to reduce their spontaneous activity. Thus, in effect, the IP₃Rs function to buffer the level of IRBIT available for binding to other target proteins, where IRBIT is released from IP₃Rs only when the cells are stimulated to generate IP₃. Indeed, in pancreatic ducts, IP₃Rs are clustered at the apical pole, the site where most IRBIT is located (60).

IRBIT Activates Cl⁻ and HCO₃⁻ Transporters

The role of IRBIT in epithelial fluid and HCO₃⁻ secretion was revealed with the discovery that IRBIT interacts with and activates the ubiquitous Na⁺-HCO₃⁻ transporter NBCe1-B (47). NBCe1-B is a member of the electrogenic NBCe1 subfamily that is part of the superfamily of Na⁺-coupled HCO₃⁻ transporters (NCBT) (6, 43). Subsequently, IRBIT was shown to activate NHE3 (25), CFTR (60), members of the SLC26 transporters family (Park S and Muallem S, unpublished observations), and other members of the NCBT superfamily (6) that have an NH₂-terminal autoinhibitory domain homologous to that of NBCe1-B (33, 47). In addition, *residues 591–696* in the COOH terminus of NHE3 was proposed as the IRBIT binding site (25). Further analysis narrows the potential NHE3 IRBIT binding site to *residues 606–651*, which show good homology with NBCe1-B(37–62) (JHH, unpublished observation), further pointing to this site as the IRBIT binding site. Notably, the autoinhibitory domain of the NCBT that binds IRBIT is not present in CFTR or the SLC26 transporters. This indicates that there are multiple IRBIT interacting domains and perhaps several mechanisms for activation of the transporters by IRBIT. Indeed, binding the IRBIT PEST domain is essential for its interaction with NBCe1-B but not with CFTR, although it is required for activation of both transporters (60).

How might IRBIT activate the transporters? One mechanism can be by removal of autoinhibition. Information for such a mechanism is available only for NBCe1-B, and a similar mechanism may apply to the other NCBT with an autoinhibitory domain, like NBCe1-C, NBCn1, and NDCBE. However, note that the NH₂ terminus of NBCn1 and NDCBE is only partially conserved with that of NBCe1-B. Deletion analysis showed reduced inhibition of NBCe1-B by stepwise truncation of the NH₂ terminus, with complete removal of autoinhibition by deletion of the first 85 residues on NBCe1-B, defining the autoinhibitory domain as NBCe1-B(1–85) (32). The deletions also prevented activation of NBCe1-B by IRBIT with deletion of the first 16 residues was sufficient to eliminate activation of NBCe1-B by IRBIT (32). However, this study did not determine the effect of the deletions on IRBIT interaction with NBCe1-B, and in vitro studies revealed that NBCe1-B(1–16) is not the IRBIT interacting domain (47). In vitro binding assays showed strong binding of IRBIT to NBCe1-B(1–62) and no binding to NBCe1-B(1–36). However, IRBIT binding was not observed with NBCe1-B(37–85), yet the possibility of IRBIT binding to NBCe1-B(37–62) or other sequences in this region was not examined (47). Hence, the most likely IRBIT binding domain is within NBCe1-B(37–62). Binding of IRBIT to this domain removes the autoinhibition to activate NBCe1-B.

In an attempt to gain additional information on the interaction between IRBIT and the autoinhibitory domain, we modeled the structure of the entire human NBCe1-B (NCBI ref. NP_001091954) NH₂-terminus 400 amino acids using the ROBETTA online full-chain protein structure prediction server (29), which generated the model shown in FIGURE 4. The green domain is predicted with high homology confidence (ROBETTA/Ginzu confidence score of 57.000) and is homologous to the NH₂ terminus of the crystal structure of human erythrocyte band-3 cytoplasmic domain (cdb3). A potential fold of *residues 1–81* of NBCe1-B was predicted by ROBETTA utilizing de novo modeling source (confidence score of 2.094993). The first 62 residues that include the IRBIT binding region are shown in orange, and *residues 63–100* are shown in blue. The turn-loop- α -helix on the left includes *residues 37–62*. If the structure of NBCe1-B(1–81) resembles the actual structure, it is likely that the structure in FIGURE 4 represents the open state of NBCe1-B when bound to IRBIT, and the autoinhibitory domain is not inhibiting NBCe1-B. The turn-loop- α -helix contains a stretch of conserved positive charges that may interact with the PEST domain that is required for interaction with and activation of NBCe1-B (47, 59, 60). One or the two helices in blue may function as a lever to swing the autoinhibitory domain toward the cdb3 homologous domain (green) to form the close autoinhibited state. It will be interesting to test several predictions of this model. As mentioned above, the IRBIT binding domain is not present in CFTR and SLC26 transporters, and thus they must be regulated by a different mechanism or by equivalent IRBIT binding domains.

IRBIT Antagonizes the Effects of the WNK/SPAK Pathway

Another mechanism by which IRBIT activates the HCO₃[−] transport is antagonizing the effect of the WNK/SPAK pathway to restore surface expression of the internalized transporters. IRBIT has a PP1 binding site, and PP1 can dephosphorylate the Ser/Thr residues phosphorylated by the WNKs/SPAK/OSR1. Measurement of NBCe1-B (FIGURE 2A) and CFTR activity (FIGURE 2C) shows that IRBIT prevented the inhibition by both the WNK and the SPAK kinases and further activated the transporters. Analysis of surface expression showed that IRBIT reversed internalization of the transporters, restoring their normal surface expression (FIGURE 2B AND D). It is of note that when activating the transporters or antagonizing the effect of the WNK/SPAK kinases IRBIT does not increase surface expression beyond the expression observed in the absence of the WNK/SPAK and IRBIT. This indicates that IRBIT has two effects on the transporters: antagonizing the internalizing effect of the WNK/SPAK kinases and activating the transporters. The central

role of IRBIT in regulating the HCO_3^- transporters in vivo is shown in FIGURE 3B in which knockdown of IRBIT almost eliminated stimulated fluid secretion by the pancreatic duct. Moreover, the reduced secretion in the absence of IRBIT could be partially recovered by knockdown of SPAK (58), further emphasizing the interplay between the IRBIT/PP1 and the WNK/SPAK pathways.

In principal, IRBIT can reverse the effect of the WNK/SPAK kinases either by inhibition of endocytosis or by stimulation of exocytosis, both of which were shown to affect the WNK/SPAK-regulated transporters. For example, the WNKs regulate the surface expression of ROMK1 by activation of the intersectin-dependent endocytic pathway (24, 51). This effect is mediated by proline-rich domains in WNK1¹⁻¹¹⁹ that acts as a scaffold to recruit the endocytic complex to ROMK1 (24, 51). On the other hand, WNK4 acting through SPAK inhibited trafficking and exocytosis of NCC that was independent of dynamin (7, 23) and was reversed by dominant-negative sortilin that targets material to the lysosomes (62). At present, it is not known whether IRBIT reverses the effect of the WNK/SPAK pathway by inhibition of endocytosis or stimulation of exocytosis. However, since WNK1¹⁻¹¹⁹ mediates the reduced surface expression of NBCe1-B and CFTR as was found with ROMK1, it is possible that IRBIT inhibits endocytosis to reverse the effect of the WNK/SPAK pathway.

PP1 and Calcineurin

Two phosphatases, PP1 and calcineurin (PP3, formally PP2B), have been identified as potential phosphatases that dephosphorylate Ser/Thr by the WNK/SPAK kinases to terminate their effect. Several ion transporters have a PP1 binding motif. This was reported for NKCC1 (18), and a sequence search for PP1 binding motifs (45) revealed the presence of such motifs in NBCe1-B, NHE3, KCC, and ROMK1. In addition, WNK1 and WNK4 may have a PP1 binding site (49). Moreover, the activity of NKCC1 (18, 20), KCC (5), NBCe1-B, and CFTR (58) is affected by PP1. More recently, it was shown that the Ca^{2+} -regulated phosphatase calcineurin regulates the phosphorylation and surface expression of NCC (26, 35). These effects may be mediated by dephosphorylation of WNK3 and WNK4 that displays increased phosphorylation in response to inhibition of calcineurin in vivo (26, 35). These findings suggest a prominent role for PP1, calcineurin, and likely other phosphatases in the regulation of the transporters regulated by the WNK/SPAK pathway.

The studies with NBCe1-B and CFTR suggest that IRBIT mediates the effects of PP1 (58). Thus IRBIT has a PP1 binding site (13) and recruits PP1 to NBCe1-B and CFTR, which is required to reverse the effects of the WNK/SPAK and further activate the transporters (58). Another recent study suggested that PP1 acts mainly on IRBIT to dephosphorylate IRBIT and inhibit activation of NBCe1-B by IRBIT when expressed in *Xenopus* oocytes that lack SPAK/OSR1 (32). The conclusion is based on mutation of the IRBIT PP1 binding motif to reduce or enhance binding of PP1. However, the effect of the mutations on interaction of IRBIT with PP1 and NBCe1-B was not examined. This finding is in contrast to the findings in mammalian cells where inhibition of PP1 by the native PP1 inhibitor I2 or the pharmacological inhibitor Tautomycin inhibited the transporters and acted similar to WNKs and SPAK (58). In addition, mutation of the IRBIT PP1 binding site eliminated interaction of NBCe1-B and CFTR with PP1 (58). Whether the presence of WNKs and SPAK/OSR1 in mammalian cells or other factors are responsible for the different findings is not clear.

Other scaffolds that recruit PP1 to ion transporters are members of the AATYK kinases. These kinases bind PP1 in a kinase-independent manner and interact with and dephosphorylate SPAK, resulting in inhibition of NKCC1 (20). Interestingly, in addition to a PP1 binding site, IRBIT has the potential calcineurin binding motif LxVP that is located within the conserved AHCYL1 domain. Similarly, the AATYK kinases have an LxVP

motif. It will be of interest to determine whether calcineurin regulates several of the transporters that are regulated by the WNK/SPAK kinases and the role of IRBIT and AATYK in this form of regulation. The relationship between the WNK/SPAK and IRBIT/PP1 pathways in secretory epithelia discussed here is summarized in FIGURE 5.

The antagonistic effect of the WNK/SPAK and IRBIT/PP1 pathways raise the question of whether the mechanism in FIGURE 5 is a general mechanism and the two pathways form a regulatory pair to set the activity of ion transporters based on physiological demands. At present, we do not have information on whether IRBIT affects the activity of transporters other than NBCe1-B, CFTR, and the SLC26 transporters that are regulated by the WNK/SPAK kinases, in particular the transporters that are associated with hypertension and K⁺ homeostasis like NKCC1, NCC, KCC, ENaC, and ROMK1. Furthermore, the WNKs and SPAK/OSR1 have diverse effects on these transporters with inhibition or activation of the transporters while affecting or not affecting their trafficking and surface expression. This information is required before the full potential of IRBIT as a regulator of ion transport and homeostasis can be appreciated. It is clear that we are only at the beginning of understanding how the IRBIT/phosphatases and the WNK/SPAK kinases pathways communicate to regulate epithelial fluid and electrolyte transport. Based on the available information, we would predict that IRBIT plays a major regulatory role in epithelial fluid and electrolyte secretion, at least through reversing WNK/SPAK-mediated phosphorylation of Ser/Thr sites that are recognized by PP1 and perhaps calcineurin.

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FIGURE 1. The known domains of the WNKs, SPAK/OSR1, and IRBIT

Known domains are marked only when first mentioned. For the WNKs, proline-rich domains (PRD) are in green, the coiled-coil domains (CCD) are in orange, the kinase domains are in red, the autoinhibitory domains (AID) are in purple, and the SPAK/OSR1 binding motifs [R/K]Fx[V/I] are in magenta. For the SPAK/OSR1, the proline- and arginine-rich domain (P/ARD) is in green, the kinase domain is in red, the serine-rich (S) motif is in purple, the WEW-containing domain that binds Cab39 is in pink, and the conserved COOH-terminal domain (CCT) is in brown. For IRBIT, the protein phosphatase 1 (PP1) binding motif is in green, the PEST domain is in red, the CCD is in orange, and the PDZ motif is in turquoise.

FIGURE 2. IRBIT antagonizes inhibition of NBCe1-B and CFTR activity and surface expression by the WNKs and SPAK

A and *C*: NBCe1-B activity as the Na⁺-dependent recovery from an acid load (*A*) and the whole cell CFTR current (*C*). *B* and *D*: the total and surface expression of NBCe1-B (*B*) and CFTR (*D*). The transporters were expressed in HeLa cells alone or with the indicated mutants of WNKs and SPAK and with or without IRBIT. WNK¹¹⁻¹¹⁹ is the NH₂ terminus 119 residues of WNK1 that do not include the kinase domain; the notation KD notes kinase-dead for both the WNKs and SPAK. The examples shown illustrate the reversal by IRBIT of the reduced surface expression and activity of the transporters by the WNKs and SPAK. The results were taken from Ref. 58.

FIGURE 3. WNKs and SPAK inhibit and IRBIT stimulates pancreatic duct fluid secretion
Sealed pancreatic ducts in primary culture were treated with the indicated WNKs or SPAK siRNAs or the PP1 inhibitor tautomycin (*A*) or si-IRBIT (*B*) and were used to measure stimulated fluid secretion. Knockdown of the inhibitors WNKs and SPAK enhanced secretion, whereas inhibition of PP1 that is required to reverse the effect of the WNK/SPAK and knockdown of the activator IRBIT reduced secretion. The results were taken from Refs. 58, 60.

NBCe1-B N terminal domain

FIGURE 4. Model of the NH₂ terminus 400 residues of NBCe1-B

Model prediction and construction is given in the text. *Residues 1–62* that include the IRBIT binding domain are in orange, *residues 62–100* are in blue, and the domain predicted with high homology confidence is in green.

FIGURE 5. Model for regulation of fluid and HCO_3^- secretion by the WNK/SPAK and IRBIT/PP1 pathways

In the resting state, SPAK is scaffolded by WNK, which recruits SPAK to the HCO_3^- transporters. SPAK phosphorylates NBCe1-B, SLC26A6, and CFTR (indicated with red arrows and red -P) to sequester most of them in intracellular organelles (blue arrows), NBCe1-B in organelles next to the basolateral membrane, and SLC26A6 and CFTR likely in the same organelles next to the luminal membrane. In the absence of cytoplasmic IP₃ IRBIT is bound to the IP₃ receptors that in secretory cells are clustered at the apical pole. This results in low secretory activity. When the cells are stimulated with an IP₃-generating agonist, IP₃ binds to the IP₃ receptors to dissociate IRBIT that now recruits PP1 to the transporters to dephosphorylate them. The organelles with the dephosphorylated transporters that are complexed with IRBIT now fuse with the plasma membrane to insert NBCe1-B in the basolateral membrane and SLC26A6 and CFTR in the luminal membrane. IRBIT remains bound to a specific site in each of the transporters in a mode similar to binding of IRBIT to the NH₂ terminus autoinhibitory domain of NBCe1-B. Insertion of the transporters in the plasma membrane and their activation by IRBIT results in stimulation of fluid and HCO_3^- secretion.