WNKs are unique because the lysine required for phosphoryl transfer lies in the phosphate anchor ribbon (kinase subdomain I) instead of β strand 3 (kinase subdomain II), its position in all other members of the protein kinase superfamily (5). The kinase domains of WNKs are located near their N termini. WNKs contain a conserved autoinhibitory domain, first identified in WNK1, and two predicted coiled-coil domains, which are located C-terminal to the kinase domain (6, 9, 11). The WNK1 autoinhibitory domain (residues 485–555) reduces WNK1 autophosphorylation and substrate phosphorylation (9). Similarly, the WNK4 autoinhibitory domain (residues 444–518) was reported to inhibit WNK1 autophosphorylation, suggesting WNKs may modulate the kinase activity of other family members (11).

In this study, we have continued to characterize the biochemical properties and regulation of WNK1 that may contribute to its physiological activities and abnormal function in disease.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Harvest**—MCF7, MDA-MB-231, HT-29, SW480, COS, and HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 1% L-glutamine at 37 °C under 5% CO₂. Mouse distal convoluted tubule (DCT)³ cells were cultured as described above with 110 mg/mliter sodium pyruvate under 10% CO₂. Mouse distal convoluted tubule (DCT)³ cells were harvested in lysis buffer containing 1% Triton X-100, 50 mM HEPES (pH 7.5), 150 mM NaCl, 1.5 mM MgCl₂, 1 mM EGTA, 10% glycerol, 100 mM NaF, 0.2 mM Na₃VO₄, 50 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM benzamidine hydrochloride, 10 mg/mliter leupeptin, 0.5 mg/mliter pepstatin A, and 1.5 mg/mliter aprotinin. The cells were vortexed for 30 s, incubated on ice for 10 min, and centrifuged for 15 min at 14,000 rpm to remove insoluble material.

**Antibodies and Proteins**—The anti-WNK1 antibody Q256 has been described previously (5). A partial cDNA encoding the kinase domain of rat WNK2 was isolated from a cDNA library at the same time WNK1 was originally isolated (5). Rat GST-WNK1(1–555) and the kinase domain of GST-WNK2 were expressed in *Escherichia coli* strain BL21, using standard purification protocols. The kinase domain of rat WNK1 (residues 194–483) was purified as described previously (14). The kinase domain of rat WNK4 (residues 141–436) was expressed from a recombinant baculovirus in Sf9 cells (14). Ser332 of WNK4 was mutated to Ala by site-directed mutagenesis; the mutant protein was purified as described above. The autoinhibitory fragment of WNK1 (residues 485–614) was cloned into a pHis6-Parallel vector and expressed in Rosetta cells (Novagen) using standard protocols (15).

**Immunoblotting**—Proteins from cell lysates were separated on 7.5% polyacrylamide (29:1) gels in sodium dodecyl sulfate, transferred to nitrocellulose membranes, and blocked in 5% nonfat dry milk (5). The membranes were probed with Q256 (1:1000), followed by secondary antibody (1:5000), and visualized by enhanced chemiluminescence.

**In Vitro Kinase Assays**—Cell lysates were incubated with antibody Q256 for 1 h at 4 °C and incubated with protein A-Sepharose beads for an additional hour at 4 °C to immunoprecipitate endogenous WNK1. The beads were washed three times with 1 mliter NaCl, 20 mliter Tris-HCl (pH 7.4) and once with 10 mliter MgCl₂, 10 mliter HEPES (pH 8.0). The beads were washed three times with 1 mliter NaCl, 20 mliter Tris-HCl (pH 7.4) and once with 10 mliter MgCl₂, 10 mliter HEPES (pH 8.0). The beads were washed three times with 1 mliter NaCl, 20 mliter Tris-HCl (pH 7.4) and once with 10 mliter MgCl₂, 10 mliter HEPES (pH 8.0).
were then incubated with 10 mM HEPEs (pH 8.0), 10 mM MgCl₂, 50 μM ATP (γ-32P]ATP), 1 mM benzamidine hydrochloride, and 1 mM dithiothreitol at 30 °C for 20–30 min. Kinase assays with purified proteins were performed as described above, but with 5 mM MgCl₂, 0.5 mM benzamidine hydrochloride, 0.5 mM dithiothreitol, and 0.3 mg/ml bovine serum albumin to stabilize kinases and substrates. The kinase reactions were resolved on gels, dried, and exposed to x-ray film. Incorporation into WNK and substrate proteins was determined by liquid scintillation counting of bands excised from gels.

Gel Filtration Chromatography—COS or HEK293 cells were harvested in lysis buffer containing 20 mM Tris (pH 7.5), 150 mM KCl, 0.5% Nonidet P-40, 1 mM dithiothreitol, and protease inhibitors. Approximately 5 mg of cleared lysates were then fractionated on Superose 6 (Amersham Biosciences) that had been pre-optimized with the HMW Gel Filtration Calibration Kit (Amersham Biosciences). Each fraction was analyzed for the presence of WNK1 by immunoblotting. Fractions were stained with Coomassie Blue to detect eluted protein standards.

RESULTS

Hypertonic and Hypotonic Stresses Increase WNK1 Autophosphorylation in Epithelial Cell Lines—We previously reported that treatment of HEK293 cells with NaCl increases WNK1 autophosphorylation and that changes in WNK1 autophosphorylation correlate with phosphorylation of the substrates myelin basic protein (5, 9). Given the potential of WNK1 to cause hypertension, we wished to determine whether WNK1 in well-differentiated epithelial cells was also sensitive to NaCl and other osmotic challenges. We found that hypertonic stresses, including glucose, sucrose, mannitol, sorbitol, KCl, NaCl (Fig. 1, A–E), and urea (data not shown), increase WNK1 activity in DCT cells. These cells retain many properties of the distal convoluted tubule from which they were derived (12, 13). In DCT cells, addition of 0.05–0.1 M NaCl to the culture medium produced a 1.5–2.0-fold increase in WNK1 activity, increasing to a maximum activation in the range of 10-fold as the amount of WNK1 present. DCT cells were among those with the smallest amount of full-length WNK1 protein, perhaps consistent with the observation that kidney predominantly expresses a splice form lacking the N terminus of WNK1 to which cancer cell lines MCF7 and MDA-MB-231, was somewhat less sensitive to 0.5 M NaCl than that from DCT cells (Fig. 1F). The extent of activation in these cell lines was not related to the amount of WNK1 present. DCT cells were among those with the smallest amount of full-length WNK1 protein, perhaps consistent with the observation that kidney predominantly expresses a splice form lacking the N terminus of WNK1 to which...
FIG. 2. WNK1 exists as an oligomer. COS or HEK293 cells were fractionated on a Superose 6 column. Protein standards, thyroglobulin, ferritin, and catalase were detected by staining with Coomassie Blue. The fractions were immunoblotted to detect the presence of endogenous and Myc-tagged WNK1.

our antibodies were raised (19). Full-length WNK1 in DCT cells displays the greatest sensitivity to osmotic stress. The next most sensitive cell line was the highly invasive MDA-MB-231 line (20).

In search of other WNK1 activators, mouse DCT cells, breast cancer cell lines, and HeLa cells were treated with numerous agents including epidermal growth factor, insulin, vasopressin, forskolin, parathyroid hormone, dexamethasone, aldosterone, H2O2, fetal bovine serum, and transforming growth factor β. None of these agents caused as much as a 1.5-fold increase in WNK1 activity.

WNK1 Oligomerizes—In characterizing endogenous WNK1, we found that its behavior upon gel filtration suggested that it was in a large complex (Fig. 2). Using the relative position of elution of standard proteins on Superose 6 and assuming the protein is globular, the elution of WNK1 in fraction 12 was consistent with its presence in a complex with a mass of 1000 kDa. The calculated mass of endogenous human WNK1 is ~250 kDa. We also examined the gel filtration profiles of two truncated forms of rat Myc-tagged WNK1 expressed in HEK293 cells. Myc-WNK1-(1–1193), which has a calculated mass of 130 kDa, eluted in fractions 13 and 14, consistent with a mass of ~700 kDa, and Myc-WNK1-(1–1001), which has a calculated mass of 110 kDa, eluted in fraction 14, consistent with a mass of ~600 kDa (Fig. 2). It seems likely that some of the reduction in apparent size of the truncated WNK1 complexes is due to loss of proteins that would normally associate with the C-terminal regions of WNK1 deleted from the expressed fragments. However, if WNK1 is a tetramer, the complexes would be expected to display a reduction in mass roughly four times the mass deleted from the fragment, and this is what was observed. These results are consistent with the possibility that WNK1 is a tetramer.

Given the likelihood based on gel filtration that WNK1 oligomerizes, we used two-hybrid analysis to identify regions of WNK1 that associate. The WNK1 constructs tested are shown in Fig. 3B. WNK1-(1–1001) can interact with the C-terminal regions of WNK1 deleted from the expressed fragments (Fig. 3B). WNK1-(217–660) did not interact with itself. WNK1-(1–222) interacted most strongly with WNK1-(217–660). WNK1-(1–222) interacted with residues 481–660, suggesting that the strongest interaction is with the region following the kinase domain, which includes the autoinhibitory domain and a coiled-coil domain. Coding mutations in WNK4 associated with hypertension are in the coiled-coil domain (1). We mutated the comparable residues in WNK1 in the fragment WNK1-(217–660) and found that WNK1-(1–222) also bound to all of these mutants (Fig. 3B). Thus, these mutations, when placed in WNK1, do not cause a detectable change in interactions among WNK1 domains. The catalytic domain of WNK1, residues 217–494, does not include the regions that interact by yeast two-hybrid (residues 1–222 and 481–660), and a comparable fragment behaved as a monomer on gel filtration (data not shown). Co-immunoprecipitation experiments showed that endogenous WNK1 and the truncated protein WNK1-(1–1001) can interact (data not shown), supporting the yeast two-hybrid findings that WNK1 self-associates.

Tests of Interaction between WNK1 and WNK4—WNK4 has been shown to regulate the surface expression of several ion transporters (2–4). Two groups demonstrated that co-expression of WNK4 with the sodium chloride cotransporter reduced its surface expression (3, 21). Interestingly, addition of WNK1 relieved WNK4-mediated inhibition of this ion transporter (3). Therefore, we assessed whether WNK4 also interacts with WNK1 using pairwise yeast two-hybrid tests. Many of the WNK1 baits in Fig. 3A were tested with WNK4-(431–584), WNK4-(431–748), and WNK4-(584–444) (Fig. 3B). Several were also tested with an N-terminal fragment of WNK4 (residues 13–171) (data not shown). Using this approach, no interaction was found between any fragments of WNK1 and WNK4. In addition, co-immunoprecipitation experiments using overexpressed WNK1 and WNK4 in HEK293 cells failed to show that they bind (data not shown). Thus, we obtained no evidence to support the idea that WNK1 and WNK4 interact stably in cells.

Analysis of WNK1 Phosphorylation by Phosphopeptide Mapping—To assess the complexity of autophosphorylation of the WNK1 kinase domain, the protein was subjected to tryptic phosphopeptide mapping (Fig. 4B). We previously identified two sites of autophosphorylation in the activation loop of WNK1: Ser382, a site required for activity, and Ser378, which is dispensable (9). WNK1 Ser382 is partially phosphorylated as the protein is isolated (9). Phosphorylation of these two sites (Ser382 and Ser378) alone is expected to generate up to nine tryptic phosphopeptides (Fig. 4A). The map revealed more than a dozen phosphopeptides (Fig. 4B). We also mapped phosphopeptides in WNK1 S378A following autophosphorylation (Fig. 4C). We observed at least seven tryptic phosphopeptides; phosphorylation of Ser382 alone should account for up to three phosphopeptides. The major peptide missing from this map relative to that from the wild-type protein (labeled 7/8 in Fig. 4B) most likely contains phospho-Ser378. Major phosphopeptides clearly present in both maps (labeled 1 and 4) most likely contain phospho-Ser382, based on differences in these two maps and predicted mobilities based on charge and hydrophobicity (18). These results are most consistent with the presence of at least one additional WNK1 autophosphorylation site within its kinase domain.

For comparison, we examined the tryptic phosphopeptide map of WNK4-(141–436) (Fig. 4D). The map contains a single major phosphopeptide and at least three minor phosphopeptides. The activation loop sequences of WNK4 and WNK1 are identical (Fig. 4A). Therefore, the major WNK4 phosphopeptide, migrating in approximately the same position as WNK1 peptides 1 and 4 (phospho-Ser382), most likely contains phospho-Ser382.

WNK1 Phosphorylates Other WNK Family Members—We previously showed that mutation of the major WNK1 activation loop autophosphorylation site Ser382 to Ala drastically reduces both autophosphorylation and protein kinase activity using other substrates (9). Because the kinase domains of WNK family members share high sequence identity, we tested
Peptide mapping suggested that WNK4 autophosphorylates on its activation loop site, Ser\(^{332}\). To confirm this result, Ser\(^{332}\) was mutated to Ala (Fig. 5B). Mutation of Ser\(^{332}\) nearly eliminated WNK4 autophosphorylation, consistent with the requirement for phosphorylation of the activation loop site to yield active WNK1 (9). In addition, mutation of Ser\(^{332}\) resulted in a significant reduction in phosphorylation of WNK4 by WNK1. Thus, we conclude that the major site phosphorylated by WNK1 is the required activation loop site on WNK4. WNK1-(1–555) autophosphorylates at an undetectable rate because it contains the autoinhibitory domain (9). Thus we tested this form of WNK1 as a WNK4 substrate and found that WNK4 was capable of phosphorylating GST-WNK1-(1–555) (Fig. 5C).

The WNK1 Autoinhibitory Domain Inhibits Autophosphorylation of WNK2 and WNK4—A previous report has shown that the WNK4 autoinhibitory domain inhibits WNK1 autophosphorylation, suggesting that one WNK may modulate the activity of distinct WNK family members (11). To determine whether the converse takes place, we asked whether the WNK1 autoinhibitory domain is capable of inhibiting WNK4 autophosphorylation. The purified WNK4 kinase domain was incubated with increasing concentrations of WNK1-(485–614) in vitro (Fig. 6). WNK1-(485–614), but not the autoinhibitory domain of PAK1 (residues 1–231) (data not shown), reduced WNK4 autophosphorylation. In this assay, 1.0 \(\mu g\) of WNK1-(485–614) inhibited 1.0 \(\mu g\) of WNK4 by 44%, and 4.5 \(\mu g\) of WNK1 inhibited 1.0 \(\mu g\) of WNK4 by 65%. In addition, WNK1-(485–614) also inhibited WNK2 autophosphorylation (Fig. 6). In the figure shown, 1.0 \(\mu g\) of WNK4-(485–614) inhibited WNK2 by 10%, and 4.5 \(\mu g\) of WNK1 inhibited WNK2 by 50%.

These data further support the hypothesis that the autoinhibitory domains of WNKs may regulate distinct WNKs, leading to the conclusion that WNKs may interact with one another by affecting the kinase activity of other WNK family members.

**DISCUSSION**

WNKs comprise a distinct branch of the protein kinase family tree, displaying some relationship, although distant, to the Ste20 protein kinases. Ste20 is the prototype mitogen-activated protein kinase kinase kinase kinase (MAP4K), an upstream kinase in a yeast mitogen-activated protein kinase pathway. In addition to its function in controlling blood pressure, WNK1 also has some capacity to serve as a MAP4K in the ERK5 mitogen-activated protein kinase pathway (22), suggesting that it may be regulated by growth factors and stress stimuli that control the ERK5 cascade. Thus, we have screened a large series of potential activators of WNK1 to identify the hormonal and environmental factors that may regulate its protein kinase activity. We confirmed our earlier finding that WNK1 activity is increased substantially by elevated NaCl (5). NaCl and other osmotic challenges activated the kinase in a wide variety of cell lines, including fibroblasts, distal convoluted tubule kidney cells, and colon and breast epithelial cells in different states of transformation. In addition, reduced osmotic pressure caused a small but significant increase in WNK1 activity, indicating that WNK1 senses decreases as well as increases in osmotic strength. In contrast to our initial expectation, we found little or no change in WNK1 activity acutely or over hours in response to many hormones, including several hormones important in the function of epithelial cells and kidney in particular. We also found no significant effect of agents that impact cell proliferation, despite the fact that WNK1 appears to be required for certain growth factor-dependent signaling events (22, 27). This may suggest that the role of WNK1 in growth factor-mediated events is independent of its protein kinase activity. It has been reported that the inhibitory effect of WNK4 on the renal potassium channel, for example, is kinase-
**FIG. 4.** Phosphopeptide analysis of WNK1 and WNK4 autophosphorylation. A, the activation loop sequence of WNK1 and WNK4 and the peptide fragments predicted to be generated after digestion with trypsin. WNK1 Ser^{382} and WNK4 Ser^{332} are marked with an asterisk. B, phosphopeptide map of WNK1-(194–483). Peptide identities are predicted based on mobility as noted in the text. C, WNK1 S378A-(194–483). D, WNK4-(141–436).

**FIG. 5.** WNK1 and WNK4 phosphorylate each other. A and B, WNK1 was immunoprecipitated from DCT cells with Q256 or Q256 preimmune serum, and the kinase domains of wild-type and S332A WNK4 prepared from Sf9 cells were used as substrates in in vitro kinase assays. A and B are both one of three experiments. WNK4 S332A migrates slightly slower than wild-type WNK4 because its His_{6} tag was not cleaved. C, GST-WNK1-(1–555) was used as a substrate with the kinase domains of wild-type and S332A WNK4. This is one of five experiments. D, the WNK2 kinase domain was used as a WNK1 substrate. This is one of three experiments.
Regulation of WNK1

Immunoblotting WNK1 in many cell lines and tissues indicates that it is expressed ubiquitously (5, 24). In contrast to various analyses of mRNA, protein blots and immunohistochemistry show that WNK1 is relatively abundant in extra-renal epithelia and tissues such as brain (5, 24, 25). These findings suggest two important points. First, significant regulation of WNK1 expression occurs post-transcriptionally; thus, transcript level will be an unreliable predictor of WNK1 abundance. Second, WNK1 is likely to have important functions in many tissues in addition to kidney; these other functions may be the cause of lethality during embryonic development in animals lacking the WNK1 gene (26).

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REFERENCES

1. Wilson, F. H., Dize-Nicodemus, S., Choate, K. A., Ishikawa, K., Nelson-Wilson, C., Desitter, I., Gunel, M., Milford, D. V., Lipkin, G. W., Achard, J. M., Feely, M. P., Dusso, B., Berland, Y., Unwin, R. J., Mayan, H., Simon, D. B., Farfel, Z., Jeunemaître, X., and Lifton, R. P. (2001) Science 293, 1107–1112

2. Yamauchi, K., Roi, T., Kobayashi, K., Sobara, E., Suzaki, T., Itoh, T., Suzuki, A., Hayama, A., Sasaki, S., and Uchida, S. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 4690–4694

3. Yang, C. L., Angell, J., Mitchell, R., and Ellison, D. H. (2003) J. Clin. Investig. 111, 1039–1045

4. Kahle, K. T., Gimenez, I., Hassan, H., Wilson, F. H., Wong, R. D., Forbush, B., Aronson, P. S., and Lifton, R. P. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 2064–2069

5. Xu, B., English, J. M., Wilsbacher, J. L., Stippec, S., Goldsmith, E. J., and Cobb, M. H. (2000) J. Biol. Chem. 275, 16795–16801

6. Verissimo, F., and Jordan, P. (2001) Oncogene 20, 5562–5569

7. Erlich, P. M., Cui, J., Chazaro, I., Farrer, L. A., Baldwin, C. T., Gavras, H., and DelStefano, A. L. (2003) Hypertension 41, 1191–1195

8. Kokubo, Y., Kumaide, K., Inanote, N., Tanaka, C., Banno, M., Takuchii, S., Kawano, Y., Tomoike, H., and Miyata, T. (2004) J. Hum. Genet. 49, 507–515

9. Xu, B., Min, X., Stippec, S., Lee, B. H., Goldsmith, E. J., and Cobb, M. H. (2002) J. Biol. Chem. 277, 48456–48462

10. Lee, B. H., Min, X., Heise, C. J., Xu, B., Chen, S., Shu, H., Luby-Phelps, K., Goldsmith, E. J., and Cobb, M. H. (2004) Mol. Cell 15, 741–751

11. Wang, Z., Yang, C. L., and Ellison, D. H. (2004) Biochem. Biophys. Res. Commun. 317, 939–944

12. Friedman, P. A., and Gesek, F. A. (1992) EMBO J. 11, 3003–1311

13. Kahle, K. T., Wilson, F. H., Nelson-Williams, C., and Lifton, R. P. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1110–1114

14. Wang, Z. Y., Yang, C. L., and Ellison, D. H. (2004) J. Biol. Chem. 279, 22473–22477

15. Hoflaur, P. L., and Ho, Y. (2004) J. Biol. Chem. 279, 22473–22477

16. Howell, B. W., Gertler, F. B., and Cooper, J. A. (1997) EMBO J. 16, 121–132

17. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) Methods Enzymol. 201, 110–149

18. Xu, Q., Modrek, B., and Lee, C. (2002) Nucleic Acids Res. 30, 3754–3766

19. Deimling, M., Haibe-Kains, B., Hennuy, B., Lallemand, F., Gonze, I., Cardoso, F., Piccart, M., DeStefano, A. L. (2003) Science. 300, 701–707

20. Wilson, F. H., Kahle, K. T., Sahab, E., Lalliots, M. D., Ranson, A. K., Hoover, R. S., Hebert, S. C., Gamba, G., and Lifton, R. P. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 680–684

21. Xu, B., Stippec, S., Lenzert, L., Heise, B. H., Zhang, W., Lee, Y. K., and Cobb, M. H. (2004) J. Biol. Chem. 279, 7826–7831

22. Kahle, K. T., Wilson, F. H., Leng, Q., Lalliots, M. D., O’Connell, A. D., Dong, K., Lacroix, M., Haibe-Kains, B., Hennuy, B., Laes, J. F., Lallemand, F., Gonze, I., DeStefano, A. L., Wilson, F. H., Wong, R. D., Forbush, B., Aronson, P. S., and Lifton, R. P. (2003) Nat. Genet. 35, 372–376

23. Xu, B., Min, X., Stippec, S., Lenzert, L., Heise, C. J., Lifton, R. P. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 663–668

24. Zambrowicz, B. P., Apolinário, M., Ramirez, S., Rodan, A. L., Jager, E., Piggott, J., Ghanem, H., Hsu, J., Xuan, Z., Wang, W., Ling, J., Hsu, Y., Wang, Y., Li, Y., Lu, Y., Yang, X., Shi, Z. Z., Sparks, M. J., Van Slipstenhorst, J., Vogel, P., Walker, W., Xu, X., Zhang, C., and Sands, A. T. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 14109–14114

25. Xu, B., Stippec, S., Chu, P. Y., Lazrak, A., Li, X. J., Lee, B. H., English, J. M., Ortega, B., Huang, C.-L., and Cobb, M. H. (2005) Proc. Natl. Acad. Sci. U. S. A., in press

FIG. 6. The WNK1 autoinhibitory domain inhibits the protein kinase activity of WNK2 and WNK4. A, the WNK1 autoinhibitory domain plus a C-terminal coiled-coil domain (residues 485–614) was incubated with the kinase domains of WNK4 (residues 141–436) and WNK2 in vitro kinase assays. Each is one of three experiments. B, graphical representation of data from the experiment shown in A.

independent (23). These results suggest that WNK family members act by both kinase-dependent and kinase-independent mechanisms.

Our evidence points to the likelihood that WNK1 exists as a tetramer. In contrast, the isolated kinase domain is an active monomer; it apparently does not require oligomerization to increase protein kinase activity. At ~250 kDa, the WNK1 monomer has numerous sites of possible protein-protein interaction with no other identifiable enzymatic activities. Its potential, even as a monomer, to bind to other proteins and organize complexes is great. Perhaps an important function of oligomerization, in addition to further increasing the scaffolding capability, is to help suppress protein kinase activity. Two-hybrid analysis results suggest that a region just N-terminal to the WNK1 kinase domain interacts within or adjacent to the WNK1 autoinhibitory domain, consistent with the idea that the autoinhibitory domain may be tethered near the kinase domain, in addition to its linkage through primary sequence. The isolated autoinhibitory domain of WNK1 will inhibit the activity of the catalytic domain, suggesting the possibility that the autoinhibitory domain of one WNK1 molecule may also inhibit the kinase activity of another WNK1 molecule within the tetramer. Thus, oligomerization may confer tighter regulation on WNK1 enzymatic activity.

A few studies have suggested functional interactions between WNK1 and WNK4. For example, WNK1 was found to inhibit the effect of WNK4 on the sodium chloride cotransporter (3). Although the idea that WNK1 may form hetero-oligomers with other WNK family members is appealing, we were unable to detect a stable interaction between WNK1 and WNK4 using several different methods. However, we were able to show, as suggested previously (11), that the autoinhibitory domains can work on other members of the family. Significantly, we did find that the kinases phosphorylated one another. We suggest that the capacity of WNK1 to phosphorylate WNK4 and vice versa may imply a regulatory hierarchy. WNK1 and WNK4 may form their own cascade.
