Multiple WW Domains, but Not the C2 Domain, Are Required for Inhibition of the Epithelial Na\(^+\) Channel by Human Nedd4*

Received for publication, December 20, 2000, and in revised form, May 18, 2001
Published, JBC Papers in Press, May 18, 2001, DOI 10.1074/jbc.M011487200

Peter M. Snyder‡§, Diane R. Olson‡, Fiona J. McDonald‡, and Daniel B. Bucher‡

From the ‡Department of Internal Medicine and Department of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, Iowa 52242, and ¶Department of Physiology, University of Otago, Dunedin, New Zealand

The epithelial Na\(^+\) channel (ENaC) forms the pathway for Na\(^+\) absorption across the apical membrane of epithelia. The activity of ENaC is controlled by its interaction with Nedd4; mutations that disrupt this interaction increase Na\(^+\) absorption, causing an inherited form of hypertension (Liddle’s syndrome). Nedd4 contains an N-terminal C2 domain, a C-terminal ubiquitin ligase domain, and multiple WW domains. The C2 domain is thought to be involved in the Ca\(^{2+}\)-dependent localization of Nedd4 at the cell surface. However, we found that the C2 domain was not required for human Nedd4 (hNedd4) to inhibit ENaC in both Xenopus oocytes and Fischer rat thyroid epithelia. Rather, hNedd4 lacking the C2 domain inhibited ENaC more potently than wild-type hNedd4. Earlier work indicated that the WW domains bind to PY motifs in the C terminus of ENaC. However, it is not known which WW domains mediate this interaction. Glutathione S-transferase–fusion proteins of WW domains 2–4 each bound to \(\alpha\), \(\beta\), and \(\gamma\)ENaC in vitro. The interactions were abolished by mutation of two residues, WW domain 3 (but not the other WW domains) was both necessary and sufficient for the binding of hNedd4 to \(\alpha\)ENaC. WW domain 3 was also required for the inhibition of ENaC by hNedd4; inhibition was nearly abolished when WW domain 3 was mutated. However, the interaction between ENaC and WW domain 3 alone was not sufficient for inhibition. Moreover, inhibition was decreased by mutation of WW domain 2 or WW domain 4. Thus, WW domains 2–4 each participate in the functional interaction between hNedd4 and ENaC in intact cells.

The epithelial Na\(^+\) channel (ENaC) forms the pathway for Na\(^+\) absorption across epithelia, where it plays a critical role in Na\(^+\) homeostasis (1, 2). Nedd4 decreases Na\(^+\) current by enhancing the rate of ENaC degradation; as a result, there are fewer Na\(^+\) channels at the cell surface (3, 4). This regulation is dependent on the ubiquitin ligase activity of Nedd4, suggesting that Nedd4 might inhibit ENaC via channel ubiquitination (3).

Consistent with this hypothesis, mutation of potential ubiquitination sites in ENaC increased Na\(^+\) current (5).

Nedd4 contains multiple WW domains; three in rat and mouse (6, 7) and four in human and Xenopus (4, 8). Previous studies found that the WW domains bind to PY motifs in the C terminus of \(\alpha\), \(\beta\), and \(\gamma\)ENaC (6, 8, 9), mediating a direct physical interaction between these proteins. ENaC mutations that disrupt this interaction increase renal Na\(^+\) absorption (3, 6, 10, 11), causing an inherited form of hypertension (Liddle’s syndrome) (12). Thus, the interaction between Nedd4 and ENaC is critical for Na\(^+\) homeostasis and blood pressure regulation. An important unresolved question is which WW domain or domains mediate the interaction with ENaC. In vitro, multiple WW domains have the capacity to interact with ENaC PY motifs. For example, all three rat WW domains interacted with ENaC using an in vitro binding assay (6). In contrast, mouse and human WW domain 1 did not interact with ENaC (8, 9). However, it is not known which WW domains interact with ENaC in the intact cell.

At the N terminus, Nedd4 has a C2 domain, first described as a regulatory domain in protein kinase C (13). Homologous sequences were subsequently identified in a number of other proteins, where they function in the binding and modulation of protein function by Ca\(^{2+}\) and phospholipids (14). In Nedd4, the function of the C2 domain is unknown. Previous work found that increased cytosolic Ca\(^{2+}\) induced the translocation of a C2 domain–glutathione S-transferase (GST) fusion protein to the cell surface, possibly by interacting with annexin XIIIb (15, 16). This suggests that the C2 domain might function in the Ca\(^{2+}\)-dependent localization of Nedd4. Consistent with such a model, ENaC is inhibited by increases in cytosolic Ca\(^{2+}\) (2). However, the role of the C2 domain in the Nedd4-mediated inhibition of ENaC is not known.

The goal of this work was to identify the sequences in human Nedd4 (hNedd4) that are necessary for the inhibition of ENaC. First, we tested the requirement for the C2 domain using a hNedd4 construct lacking the C2 domain. Second, we asked which of the four hNedd4 WW domains participate in the inhibition of ENaC. We used two different expression systems with distinct advantages: (a) Xenopus oocytes, and (b) Fischer rat thyroid (FRT) epithelia. Oocytes reconstitute some aspects of the Nedd4-dependent regulation of ENaC (3, 4). Conversely, FRT cells allowed us to study hNedd4-mediated regulation in a polarized epithelium.

EXPERIMENTAL PROCEDURES

DNA Constructs—hNedd4 was cloned by polymerase chain reaction of cDNA reverse transcribed from kidney polyadenylated RNA (Clontech). The 5′ primer was CCATGAGTTGACACATGCGAAGCAGTGGGAGGTG and included a ClaI site for cloning into pMT3. The 3′ primer was CAGGGCTTGTGAAGTTGAGTTAGGCTTTCC and included a KpnI site. A hNedd4 construct lacking the C2 domain (ΔC2) was generated by site-directed mutagenesis (QuickChange; Stratagene).
Expression and Electrophysiology in Xenopus Embryos—

Expression and Electrophysiology in FRT Epithelia—

Immunoprecipitation of hNedd4—

Inhibition of ENaC by hNedd4.

Bio-Dot SP apparatus (Bio-Rad) and probed with α, β, or γENaC as described above. hNedd4 Binding Assay—αENaC-FLAG was expressed in COS-7 cells by electroporation, as described previously (10). Two days after electroporation, the cells were lysed, and protein was solubilized in TBS containing 1% Triton X-100 and protease inhibitors (0.4 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, 20 μg/ml leupeptin, and 10 μg/ml pepstatin A). Insoluble protein was solubilized in 2% SDS, 1 mM EDTA, 1% 2-mercaptoethanol, and 10 mM Tris (pH 7.4) and diluted 1:10 (1 ml, final volume) in TBS/1% Triton X-100. αENaC-FLAG was immunoprecipitated from 100 μl of lysate (0.74 μg/μl total protein) with anti-FLAG M2 monoclonal antibody (1:1,000; Kodak) and protein A beads (Pierce). The immunoprecipitated αENaC-FLAG could be detected either by metabolic labeling of the cells or by immunoblotting with anti-FLAG M2 antibody (data not shown).

hNedd4 (wild-type or mutant) was generated and labeled with [35S]methionine by in vitro transcription and translation. After SDS-PAGE and fluorography, the protein was quantitated using Kodak Image Station and 1D software. The immunoprecipitated αENaC-FLAG (30 μl immobilized on beads) was then incubated for 16 h with wild-type or mutant hNedd4 (equal specific activities, 12.8–20 μl) in a total volume of 800 μl. The beads were washed three times with TBS/1% Triton X-100, separated by SDS-PAGE, and imaged by fluorography.

RESULTS

Inhibition of ENaC by hNedd4—We tested the effect of hNedd4 on ENaC in two different expression systems. In Xenopus oocytes, expression of α, β, and γENaC generated amiloride-sensitive Na+ current (Coexpression of the channel with αNedd4 decreased Na+ current) (Fig. 1A and Ref. 8). We determined whether hNedd4 inhibits ENaC in epithelia, we transiently expressed the channel with or without hNedd4 in FRT epithelial cells. These cells lack endogenous Na+ channels and form a polarized epithelium when grown on permeable filter supports (19, 20). Transfection of FRT epithelia with α, β, and γENaC using cationic lipids generated transepithelial short-
circuit Na\(^+\) current (I\(_{\text{sc}}\)) that was completely blocked by amiloride (19) (Fig. 1B, Control). hNedd4 decreased Na\(^+\) current when coexpressed with ENaC (Fig. 1, B and C, hNedd4). Thus, similar to Xenopus oocytes, hNedd4 inhibited ENaC in epithelial cells.

Requirement for the hNedd4 C2 Domain—The N terminus of hNedd4 contains a C2 domain (Fig. 2A). To test whether the C2 domain is required for the hNedd4-mediated inhibition of ENaC, we generated a hNedd4 lacking the C2 domain (ΔC2). If this domain was required, we predicted that ΔC2 would not inhibit ENaC. We coexpressed ENaC in Xenopus oocytes with increasing amounts of wild-type hNedd4, ΔC2, or an irrelevant protein (SEAP). Wild-type hNedd4, but not SEAP, produced a dose-dependent decrease in Na\(^+\) current (Fig. 3A). Surprisingly, deletion of the C2 domain did not prevent hNedd4 from inhibiting ENaC (Fig. 3A). On the contrary, ΔC2 was a more potent inhibitor of ENaC than wild-type hNedd4. This difference did not result from altered protein expression; immunoprecipitation with a polyclonal antibody against WW domain 2 of ENaC-FLAG (compared with 0–0.8 ng) (mean ± S.E.; n = 6–14). \(p < 0.05\) versus wild-type hNedd4. B, relative amiloride-sensitive I\(_{\text{sc}}\) in FRT epithelia expressing \(\alpha, \beta,\) and γ ENaC (0.07 ± S.E.; n = 12). In some cases, error bars are hidden by data symbols. ∗, \(p < 0.001\) versus wild-type hNedd4. C, immunoprecipitation of ΔC2 or wild-type hNedd4 (or uninjected cells as a negative control (−)) with a polyclonal antibody to WW domain 2 in Xenopus oocytes labeled with \(^{35}\)Smethionine/cysteine. Preimmune serum did not immunoprecipitate hNedd4 (data not shown). In both lanes, faster-migrating bands were also present, either as a result of degradation or initiation of translation at downstream methionines. The intensity of these bands was variable and always less than that of the band corresponding to the full-length protein (see Fig. 6C for examples). D, quantitation of hNedd4 protein (relative to ΔC2) using Kodak Image Station and 1D software (mean ± S.E.; n = 3). The upper band corresponding to full-length protein was quantitated.

Interaction between hNedd4 and ENaC—We took advantage of these mutations to determine the role of each WW domain in the binding of full-length hNedd4 to ENaC. We mutated one or more WW domains and tested the binding of hNedd4 to one of the ENaC subunits (αENaC). αENaC-FLAG was expressed in COS-7 cells, immunoprecipitated, and then incubated with wild-type or mutant hNedd4 (labeled with \(^{35}\)Smethionine). Fig. 5 shows autoradiograms of the in vitro translated hNedd4 probes (bottom panel) and hNedd4 that bound to αENaC-FLAG (top panel). We found that wild-type hNedd4 bound to αENaC-FLAG, but not to immunoprecipitated proteins from COS-7 cells expressing GFP as a negative control (wt-GFP). Deletion of the C2 domain (ΔC2) did not disrupt binding. The WW domain mutations were therefore generated in a construct lacking the C2 domain. Simultaneous mutation of all four WW domains (WW1–4) abolished the binding of hNedd4 to αENaC-FLAG, confirming a role for one or more of the WW domains in the interaction between these proteins. Mutation of WW domain 3 alone was sufficient to abolish binding, but individual mutations of WW domains 1, 2, or 4 were not sufficient to abolish binding. However, these mutations appeared to decrease the binding of hNedd4 to αENaC-FLAG (compared with ΔC2). Thus, WW domain 3, but not WW domains 1, 2, or 4, is required for the interaction between hNedd4 and αENaC.
We did not detect significant binding between hNedd4 and translation. hNedd4 that bound to constructs also lacked the C2 domain. Constructs contained mutations (Val/Ile to Trp; His to Gly) in the indicated WW domains (WW3 alone was intact). Surprisingly, the mutant hNedd4 bound to αENaC-FLAG when WW domain 3 alone was intact (WW domains 1, 2, and 4 were mutated). Together, the data indicate that WW3 is required for hNedd4-mediated inhibition of ENaC in FRT epithelia (Fig. 6, A and B). In contrast, mutation of WW3 nearly abolished inhibition; expression of the WW3 mutant in epithelia or oocytes produced a minimal decrease in Na⁺ current (Fig. 6B). Mutation of WW2 or WW4 had an intermediate effect. In epithelia, both mutants inhibited ENaC, but they did so to a lesser extent than did ΔC2 containing wild-type WW domains (Fig. 6B). Although mutation of WW2 also decreased inhibition in Xenopus oocytes, the WW4 mutant inhibited ENaC to an extent similar to that of ΔC2 (Fig. 6B). The WW domain mutations did not alter the expression of hNedd4 proteins in Xenopus oocytes (Fig. 6, C and D). Thus, WW3 is required for hNedd4-mediated inhibition of ENaC. WW2 and WW4 are not required, but they are also involved in ENaC inhibition. The data are consistent with our observation that WW3 was required for the interaction between hNedd4 and ENaC (Fig. 5) and also suggest a functional role for WW domains 2 and 4.

Our binding studies suggested that WW domain 3 was sufficient alone to bind to ENaC (Figs. 4 and 5). To test whether this interaction would inhibit ENaC, we expressed the channel with hNedd4 containing mutations in WW domains 1, 2, and 4 (WW3 alone was intact). Surprisingly, the mutant hNedd4 decreased Na⁺ current only 20% in FRT epithelia, much less than did ΔC2 hNedd4 (Fig. 7A). This result could not be explained by decreased hNedd4 protein; similar amounts of ΔC2 and mutant hNedd4 protein were expressed (Fig. 7B). Current was also minimally inhibited when only WW domain 2 was intact and was not inhibited at all when WW domain 4 alone was intact (Fig. 7A). Thus, the interaction between a single WW domain and ENaC produced only minimal inhibition of ENaC.

**DISCUSSION**

WW domains in hNedd4 interact with PY motifs in ENaC (6, 8, 9). We found that simultaneous mutation of all four WW domains abolished the inhibition of ENaC by hNedd4. In previous work, inhibition was also abolished by mutation or deletion of ENaC PY motifs (3, 4). Thus, a physical interaction between one or more WW domains and the PY motifs of ENaC is required for hNedd4 to inhibit the channel. Although three of the four hNedd4 GST-WW domain fusion proteins interacted with the PY motifs of ENaC in vitro, several findings suggest...
that the WW domain 3 interaction is critically important. First, GST-WW3 bound more ENaC probe than did GST-WW2 or GST-WW4. This suggests the possibility that WW domain 3 has a higher relative affinity for ENaC than the other WW domains, although we cannot exclude other potential explanations (e.g., differences in conformation or aggregation of the fusion proteins). Second, mutation of WW domain 3, but not of the other WW domains, abolished the binding of hNedd4 to an ENaC subunit. Third, WW domain 3 alone was sufficient to mediate the binding of full-length hNedd4 to ENaC. Because GST-WW3 bound well to all three ENaC subunits, it seems likely that WW domain 3 is also important in the binding of hNedd4 to β and γENaC. Finally, the inhibition of ENaC by hNedd4 was nearly abolished by mutation of WW domain 3. Thus, WW domain 3 was critical not only for the binding of hNedd4 to ENaC in vitro but also for its binding and inhibition of the heteromultimeric channel in the intact cell.

However, the interaction between WW domain 3 and ENaC alone was not sufficient for hNedd4-mediated inhibition of ENaC; hNedd4 produced only a small decrease in Na⁺ current when WW domain 3 was the only intact WW domain. This suggests that WW domain 2 and/or WW domain 4 also participate in the functional interaction with ENaC. Consistent with this hypothesis, mutation of WW domain 2 or WW domain 4 decreased ENaC inhibition (although the decrease was less than for the WW domain 3 mutation). Thus, although the interaction with WW domain 3 appears to be most important, WW domains 2, 3, and 4 each participate in the interaction of hNedd4 with ENaC in intact cells. This is also consistent with the previous finding that multiple WW domains were required for a WW domain-GST fusion protein to disrupt the Na⁺

FIG. 6. Effect of WW domain mutations on hNedd4-mediated inhibition of ENaC. A, amiloride-sensitive Iₜₑₐₜ (relative to GFP control) in FRT epithelia expressing α, β, and γENaC (0.07 μg each) with hNedd4 ΔC2 (ΔC2), hNedd4 with mutations in all four WW domains (1–4), or GFP (0.8 μg; mean ± S.E.; n = 20). * p < 0.0001 versus control (GFP). B, relative amiloride-sensitive Iₜₑₐₜ in FRT epithelia and amiloride-sensitive whole-cell Na⁺ current at −60 mV in Xenopus oocytes coexpressing α, β, and γENaC (0.07 μg and 0.2 ng each in FRT and oocytes, respectively) with an irrelevant control protein (GFP in epithelia; SEAP in oocytes), hNedd4 ΔC2, or hNedd4 ΔC2 with mutations in the indicated WW domain(s) (0.8 μg and 0.2 ng in FRT and oocytes, respectively) (mean ± S.E.; n = 16–20). Schematic indicates the wild-type and mutant WW domains. *, p < 0.0001 versus control; **, p < 0.0001 versus control and p < 0.05 versus ΔC2. C, immunoprecipitation of the indicated hNedd4 proteins expressed in Xenopus oocytes (or un.injected cells (−)) with polyclonal antibodies to WW domain 2 or WW domain 1, as indicated. D, quantitation of hNedd4 proteins containing the indicated mutant WW domains (relative to ΔC2) (mean ± S.E.; n = 3–7).

FIG. 7. Single intact WW domains. A, relative amiloride-sensitive Iₜₑₐₜ in FRT epithelia expressing α, β, and γENaC (0.07 μg each) with GFP, hNedd4 ΔC2, or hNedd4 ΔC2 with the indicated mutant WW domains (0.8 μg) (mean ± S.E.; n = 18–19). Schematic indicates the wild-type and mutant WW domains. *, p < 0.0001 versus control; **, p < 0.0001 versus control and p < 0.003 versus ΔC2. B, immunoprecipitation of the indicated hNedd4 proteins expressed in Xenopus oocytes (or un injected cells (−)) with a polyclonal antibody to WW domain 3.
involved in the Ca\textsuperscript{2+}-

An important unresolved question is which of the ENaC PY motifs interact with WW domains 2–4. Mutation or deletion of the PY motif in \(\beta\)ENaC was sufficient to disrupt Nedd4-mediated inhibition of the channel and cause Liddle’s syndrome (3, 12). However, all three PY motifs have the capacity to interact with WW domains 2, 3, and 4 \textit{in vitro} (8), and mutations in any of the three PY motifs increase Na\textsuperscript{+} current (10). Thus, it is possible that the interactions are relatively promiscuous. For example, WW domain 3 might bind to the \(\alpha\)ENaC PY motif in one channel, but it might bind to the PY motif of \(\beta\)ENaC in another channel. Multiple PY motif-WW domain interactions might be required to increase the affinity of the interaction between ENaC and hNedd4. Alternatively, there could be a high degree of specificity in the interaction between WW domains and PY motifs in the intact cell. For example, each WW domain might bind to a different ENaC subunit. Finally, it is possible that WW domains 2–4 bind to PY motifs in different ENaC channels or even different proteins, cross-linking them into a larger complex. Future studies will be required to differentiate between these models. However, each model is consistent with the finding that a single WW domain interaction is not sufficient to inhibit ENaC.

The function of WW domain 1 is not known. It does not bind to ENaC subunits \textit{in vitro} (8), and mutation of WW1 did not prevent hNedd4 from inhibiting ENaC, suggesting that it does not bind to ENaC in the intact cell. Rather, the WW domain 1 mutant inhibited ENaC to a greater extent than did wild-type hNedd4. Perhaps the binding of WW domain 1 to one or more other proteins modulates the localization or function of hNedd4. Identification of such proteins may provide new candidate genes for the pathogenesis of hypertension.

The interaction between WW domain 3 and ENaC was most important for the inhibition of the channel. Interestingly, this domain is not present in rat or mouse Nedd4 (which contain only three WW domains); based on sequence similarity, the three WW domains of rat and mouse Nedd4 correspond to WW domains 1, 2 and 4 in hNedd4 (8). This suggests that the pattern of interactions between the WW domains and PY motifs and the affinity of the interaction between WW domain 3 and ENaC may differ between species. Thus, it may be difficult to extrapolate PY motif-WW domain binding data between different species, and it may therefore be particularly important to study human Nedd4 to understand the role of this protein in human blood pressure control and hypertension.

Previous work suggested that the Nedd4 C2 domain may be involved in the Ca\textsuperscript{2+}-dependent translocation of Nedd4 to the cell surface through a mechanism potentially involving an interaction between the C2 domain and annexin XIIIb (15, 16). This might be important for the binding of Nedd4 to ENaC at the cell surface. Such a mechanism could be involved in the regulation of ENaC by cytosolic Ca\textsuperscript{2+}. Surprisingly, we found that the C2 domain was not required for the hNedd4-mediated inhibition of ENaC. Instead, a mutant hNedd4 lacking the C2 domain inhibited ENaC better than did wild-type hNedd4. Perhaps under basal conditions, the C2 domain blocks the interaction and/or inhibition of ENaC by hNedd4. Increased cytosolic Ca\textsuperscript{2+} might relieve this block, resulting in channel inhibition by hNedd4. Deletion of the C2 domain might allow hNedd4 to inhibit the channel in the absence of an increase in Ca\textsuperscript{2+}. Such a model is consistent with the observation that increased cytosolic Ca\textsuperscript{2+} inhibits Na\textsuperscript{+} absorption via ENaC. Alternatively, Nedd4 might interact with ENaC at an intracellular location, rather than at the cell surface.

Inhibition of ENaC by hNedd4 plays a critical role in Na\textsuperscript{+} homeostasis and blood pressure control. Mutations in ENaC that abolish the interaction between these proteins cause Liddle’s syndrome, a genetic form of hypertension. We found that mutations in WW domains 2, 3, and 4 disrupted the ability of hNedd4 to inhibit ENaC. Mutations in the ubiquitin ligase domain also disrupted inhibition (3). Thus, it seems possible that loss of function mutations in hNedd4 could increase renal Na\textsuperscript{+} absorption, identifying hNedd4 as a candidate gene for hypertension. An understanding of the molecular requirements for the binding and inhibition of ENaC by hNedd4 will facilitate the search for sequence variations that alter this regulation and may provide new insights into the basic mechanisms of blood pressure control and the pathogenesis of hypertension.

Acknowledgments—We thank Michael Welsh, John Stokes, Christopher Benson, and our other laboratory colleagues for helpful discussions and Brittany Thomas, Tien Vinh, Sarah Hestekin, Potene Garnatos, Elizabeth Wood, and Alex Kipp for technical assistance. We acknowledge the University of Iowa DNA Core Facility for assistance with oligonucleotide synthesis and DNA sequencing.

REFERENCES

1. Benos, D. J., Awadya, M. S., Ismaiel, I. I., and Johnson, J. P. (1995) \textit{J. Membr. Biol}. \textbf{143}, 1–18

2. Garty, H., and Palmner, L. G. (1997) \textit{Physiol. Rev}. \textbf{77}, 359–396

3. Goulet, C. C., Volk, K. A., Adams, C. M., Prince, L. S., Stokes, J. B., and Snyder, P. M. (1998) \textit{J. Biol. Chem}. \textbf{273}, 30012–30017

4. Abriel, H., Loffing, J., Rehliun, J. F., Pratt, J. H., Schild, L., Horischer, J. D., Rotin, D., and Staab, O. (1999) \textit{J. Clin. Invest}. \textbf{103}, 667–673

5. Staab, O., Gautschi, I., Ishikawa, T., Breitschopf, K., Cicchianoer, A., Schild, L., and Rotin, D. (1997) \textit{EMBO J}. \textbf{16}, 6325–6336

6. Staab, O., Dho, S., Henry, P., Correa, J., Ishikawa, T., McGlade, J., and Rotin, D. (1996) \textit{EMBO J}. \textbf{15}, 2371–2380

7. Kumar, S., Harvey, K. F., Kinoshita, M., Copeland, N. G., Noda, M., and Jenkins, N. A. (1997) \textit{Genomics} \textbf{40}, 435–443

8. Farr, T. J., Lodgington-Lawson, S. J., Snyder, P. M., and McDonald, F. J. (2000) \textit{Biochem. J}. \textbf{345}, 503–509

9. Harvey, K. F., Dioudem, A., Komaitonana, P., Jolliffe, C. N., Day, M. L., Paraisivam, G., Cook, D. L., and Kumar, S. (1999) \textit{J. Biol. Chem}. \textbf{274}, 12525–12530

10. Snyder, P. M., Price, M. P., McDonald, F. J., Adams, C. M., Volk, K. A., Zeiher, B. G., Stokes, J. B., and Welsh, M. J. (1995) \textit{Cell} \textbf{83}, 969–978

11. Schild, L., Lu, Y., Gautschi, I., Schneebaerger, E., Lifton, R. P., and Rossier, B. C. (1996) \textit{EMBO J}. \textbf{15}, 2381–2387

12. Lifton, R. P. (1996) \textit{Science} \textbf{272}, 676–680

13. Nishimura, Y. (1988) \textit{Nature} \textbf{334}, 661–665

14. Nalefski, E. A., and Falke, J. J. (1996) \textit{Protein Sci}. \textbf{5}, 2375–2390

15. Plant, P. J., Yeger, H., Staab, O., Howard, P., and Rotin, D. (1997) \textit{J. Biol. Chem}. \textbf{272}, 32329–32336

16. Plant, P. J., Lafton, F., Leact, S., Verkade, P., Simons, K., and Rotin, D. (2000) \textit{J. Cell Biol}. \textbf{149}, 1473–1484

17. McDonald, F. J., Snyder, P. M., McCray, P. B., Jr., and Welsh, M. J. (1994) \textit{Am. J. Physiol}. \textbf{266}, L728–L734

18. McDonald, F. J., Price, M. P., Snyder, P. M., and Welsh, M. J. (1995) \textit{Am. J. Physiol}. \textbf{268}, C1157–C1163

19. Snyder, P. M. (2000) \textit{J. Clin. Invest}. \textbf{105}, 45–53

20. Sheppard, D. N., Carson, M. R., Osteggdaard, L. S., Denning, G. M., and Welsh, M. J. (1994) \textit{Am. J. Physiol}. \textbf{266}, L405–L413

21. Espanel, X., and Sudoh, M. (1999) \textit{J. Biol. Chem}. \textbf{274}, 17284–17289
Multiple WW Domains, but Not the C2 Domain, Are Required for Inhibition of the Epithelial Na+ Channel by Human Nedd4
Peter M. Snyder, Diane R. Olson, Fiona J. McDonald and Daniel B. Bucher

J. Biol. Chem. 2001, 276:28321-28326.
doi: 10.1074/jbc.M011487200 originally published online May 18, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011487200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 21 references, 8 of which can be accessed free at http://www.jbc.org/content/276/30/28321.full.html#ref-list-1