Heterophilic Interactions of Sodium Channel β1 Subunits with Axonal and Glial Cell Adhesion Molecules*

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Voltage-gated sodium channels localize at high density in axon initial segments and nodes of Ranvier in myelinated axons. Sodium channels consist of a pore-forming α subunit and at least one β subunit. β1 is a member of the immunoglobulin superfamily of cell adhesion molecules and interacts homophilically and heterophilically with contactin and Nf186. In this study, we characterized β1 interactions with contactin and Nf186 in greater detail and investigated interactions of β1 with NrCAM, Nf155, and sodium channel β2 and β3 subunits. Using Fc fusion proteins and immunocytochemical techniques, we show that β1 interacts with the fibronectin-like domains of contactin. β1 also interacts with NrCAM, Nf155, sodium channel β2, and Nf186 but not with sodium channel β3. The interaction of the extracellular domains of β1 and β2 requires the region 169TEEEGKDGDGE181 located in the intracellular domain of β2. Interaction of β1 with Nf186 results in increased Na1.2 cell surface density over α alone, similar to that shown previously for contactin and β2. We propose that β1 is the critical communication link between sodium channels, nodal cell adhesion molecules, and ankyrinG.

The axo-glial complex in myelinated axons is composed of a nodal gap region that contains high density clusters of voltage-gated sodium channels and a juxtaparanodal region that contains voltage-gated potassium channels (1–6). The nodal gap and the juxtaparanode are separated by the paranode, a region containing septate-like junctions composed of cell adhesion molecules (CAMs) that act as diffusion barriers to ion channel movement (2, 6–8). This specific arrangement of voltage-gated sodium and potassium channels results in rapid and efficient saltatory conduction of action potentials (9–12).

Voltage-gated sodium channels are composed of a pore-forming α subunit, from the gene family containing Na1.1 through Na1.9 and at least one β subunit from the gene family containing β1, β1A, β2, β3, and β4 (10, 13–16). Sodium channels are clustered at high density in axon initial segments and nodes of Ranvier of myelinated axons, where they co-localize with members of the Ig superfamily of CAMs such as contactin, NrCAM, and neurofascin 186 (Nf186) (2, 17, 18).

Sodium channel β1 and β2 subunits are also members of the Ig superfamily of CAMs. β1 and β2 colocalize with sodium channel α subunits at nodes of Ranvier, and β1 interacts with contactin and with Nf186 in vitro (9, 17, 19, 20). Nf186, NrCAM, β1, and β2 each interact in vitro with a key cytoskeletal anchoring protein, ankyrinG, that is also localized to nodes of Ranvier (21–23). β1- or β2-mediated homophilic cell-adhesive interactions result in ankyrin recruitment in Drosophila S2 cells, and the interaction of sodium channel α subunits with ankyrinG is greatly enhanced in the presence of β1 subunits in vitro (22, 24). We propose that the sodium channel signaling complex at nodes of Ranvier includes the CAMs contactin, NrCAM, and Nf186 as well as ankyrinG and that sodium channels interact with these proteins via β1 subunits.

In sciatic nerve, Nf186 and NrCAM are early markers of nodal formation that cluster at presumptive nodes prior to sodium channels and ankyrinG (2, 25). In the peripheral nervous system (PNS), Schwann cell contact at the beginning stages of myelin ensheathment is required for sodium channel clustering (26, 27). In optic nerve, developing nodes of Ranvier are defined by clustering of ankyrinG prior to the arrival of CAMs and sodium channels (28, 29), and this process is independent of paranodal axoglial cell adhesion (29). In the central nervous system (CNS), conditioned medium from optic nerve glia induces clustering of sodium channels in retinal ganglion cell axons in vitro (30). In vivo, the presence of oligodendrocytes is necessary for sodium channel clustering and nodal formation in the CNS (30).

Contactin is expressed at paranodes in the PNS, where it interacts with Caspr on the axonal membrane and neurofascin 155 (Nf155) on the Schwann cell membrane to form septate-like junctions that separate the nodal gap from the juxtaparanode (31–33). In the CNS, contactin is expressed in the nodal gap, where it interacts with sodium channels (17, 32), as well as in the paranode. Contactin increases the level of sodium channel expression at the cell surface from 4- to 6-fold in vitro (17), and this interaction is dependent on presence of β1 subunits (9, 17). In β1 (−/−) mice, contactin and sodium channels co-localize at optic nerve nodes of Ranvier but do not associate, confirming that interaction of these two molecules is dependent on the presence of β1 (9). The number of mature nodes of Ranvier is significantly reduced in β1 (−/−) optic nerves, and we have proposed that the loss of sodium channel-contactin interactions at the node may be responsible for this effect (9).

The purpose of the present study was to test the hypothesis that sodium channel β1 subunits participate in heterophilic cell-adhesive interactions with CAMs found in the axo-glial...
complex. We show that β1, expressed in the absence of sodium channel α subunits, interacts with NrCAM, Nf186, contactin, and Nf155. We observed an interaction of the β1 and β2 subunit extracellular domains only in the presence of a region of the β2 intracellular domain, suggesting that cytoskeletal interactions may affect β2-mediated heterophilic cell adhesion. We did not detect β1 interactions with sodium channel β3 subunits in our system. β1-NF186 interactions result in increased sodium channel α subunit cell surface expression in vitro, similar to what we showed previously for contactin and β2. In contrast, although β1 is required for NrCAM interactions with Na1.2, coexpression of β1 and NrCAM did not increase α subunit cell surface expression. These results suggest that β1 subunits interact with multiple CAMs to form the basis of the sodium channel complex at nodes of Ranvier. In addition, the present results showing β1 interaction with Nf155, coupled with our previous observation that β1 (−/−) mice lack septate-like junctions at the periphery of the nodal gap in a subset of CNS and PNS axons (9), support our hypothesis that β1 subunits may also participate in axo-axonic communication at the nodal-paramedian boundary.

MATERIALS AND METHODS

Antibodies and Constructs—Rabbit polyclonal antisera to sodium channel β1 and β2 subunits were described previously (19, 22, 24). A polyclonal anti-β3 antibody was a gift from Dr. William A. Catterall (University of Washington, Seattle, WA) and has been described previously (20). Cy3-conjugated anti-human-Fc antibodies were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). Anti-rabbit horseradish peroxidase antibodies were described previously (24). Alexa Fluor-conjugated secondary antibodies were purchased from Cell Signaling (Beverly, MA), and anti-rabbit horseradish peroxidase was purchased from Image Analysis Laboratory at the University of Michigan. Images were captured using an Olympus DP70 camera and processed using DP Controller version 1.1.65 and DP Manager version 1.1.17 software.

Stable Transfection and Characterization of Cell Lines—CHL cells stably expressing Nav1.2 (17, 42, 43) as well as full-length Nf186, full-length Nav1.2, or sodium channel β2 subunits and the fusion proteins encoding the extracellular domains of contactin and NrCAM were provided by Dr. Claudine Chosanger (University of New Jersey, Piscataway, NJ) and were described previously (35, 36). The Cn and CnIg-Fc constructs were gifts from Dr. Peter Brophy (University of Edinburgh, Edinburgh, Scotland) (34). Nf155-Fc and Cn-Fc cDNA constructs were described previously (17, 42, 43). 1 μg of cDNA encoding NrCAM, Nf155, or Nf186 was transfected into Na1.2, Na1.2/β1, and Na1.2/β2 CHL cells, respectively, at 50% confluence using Fugene 6 according to the manufacturer’s instructions (Roche Applied Science). 24 h later, the cells were replated in the presence of Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum (Invitrogen), 1% penicillin and streptomycin (Invitrogen), and 400 μg/ml G418 (Invitrogen). Clonal colonies were expanded, and expression was verified by Western blot using anti-HA.11 to detect Nf186 (18:1000), anti-Nf155 (1:2500), or anti-NrCAM (1:200) antibodies.

[3H]STX Binding Analysis—Whole cell [3H]STX saturation binding analysis of each cell line was performed using a vacuum filtration method as previously described (42) at a concentration of 5 nM [3H]STX with the addition of 10 μM unlabeled tetrodotoxin (Calbiochem) to assess nonspecific binding. [3H]STX (28 Ci/mmol) was obtained from Amersham Biosciences. Binding data were normalized to protein concentrations using Advanced Protein Assay Reagent (Cytoskeleton, Inc., Denver, CO).

RESULTS

Production of Fc Fusion Proteins and Binding Assays—To investigate potential β1-interacting CAMs, we used soluble Fc fusion proteins encoding the extracellular domains of contactin, Nf155, NrCAM, sodium channel β2, or sodium channel β3 (Fig. 1A) (18, 36, 37, 44) as well as full-length Nf186, full-length β2, and the truncation mutant β2STOP (22, 34). Immunoblots of cell supernatants from transiently transfected CHL fibroblasts indicated expression of all of the soluble Fc fusion proteins used (Fig. 1B).

For each binding experiment, CHL cells were transiently transfected with the indicated CAM as described in the figure legends. To investigate binding interactions, cells were incubated in conditioned medium containing a particular Fc fusion protein. 36 h post-transfection and 12 h postincubation, the cells were fixed and stained for the expressed CAM using specific antibodies and for the Fc fusion protein of interest using a Cy3-conjugated anti-human IgG antibody. None of the Fc fusion proteins showed binding to untransfected CHL cells above background (Fig. 1C). CHL cells transfected with β1 only did not bind the Fc protein backbone (Fig. 1C, c). Thus, any interaction that was observed between CHL cells transfected with β1 and incubated with the Fc fusion proteins was interpreted as a specific interaction between β1 and the Fc fusion protein.

Heterophilic Interactions of Sodium Channel β1 Subunits

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We have shown previously that contactin is part of the sodium channel complex at CNS nodes of Ranvier and may be involved in nodal formation during development and remyelination (17). The association of contactin with sodium channels in vitro is dependent on the presence of β1, and this interaction results in increased sodium channel α subunit expression at the cell surface (17). The interaction between β1 and contactin requires the Ig loop domain but not the juxtamembrane, transmembrane, or intracellular domains of β1 (24). We further investigated this interaction by examining the regions of contactin that are important for interaction with β1. β1 interacts with Cn-Fc, a fusion protein containing full-length contactin (Fig. 2A). These results are similar to the interaction previ-
showed that the extracellular domain of NF186 interacts with full-length NF186 and that Caspr may negatively regulate interactions between contactin and NF155 (36). Our next step was to test whether β1 interacts with NF155. We were interested in investigating interactions between β1 and NF155, as β1 (−/−) mice show disrupted paranodal loops. As shown in Fig. 3B, the extracellular domain of β1 interacts with the extracellular domain of NF155 (NF155-Fc), suggesting that β1 and NF155 may interact in a trans manner between adjacent cells. As a positive control, and consistent with previous data, we show that NF155-Fc interacts with contactin (Fig. 3C) (36).

We next investigated whether the nodal protein NrCAM could interact with β1. When β1-transfected cells were incubated with Nr-Fc, we detected only a weak interaction between these two proteins (Fig. 4A). Using the opposite configuration, we detected a strong signal when NrCAM-transfected CHL cells were incubated with β1-Fc (Fig. 4B). Our results suggest that the NrCAM intracellular domain may influence its ability to participate in extracellular cell-adhesive interactions with β1.

β1 Interacts with Sodium Channel β2 but Not β3 Subunits—β1 participates in homophilic (β1-β1) interactions that result in ankyrin recruitment to points of cell-cell contact in Drosophila S2 cells and in mammalian fibroblasts (22, 23). However, heterophilic interactions between β subunits (i.e. β1-β3 or β1-β2) were not tested in those studies. In the next series of experiments, we tested whether β1 interacts heterophilically with either β2 or β3.

As a positive control, CHL cells transfected with full-length β1 were incubated with β1-Fc. We observed β1-Fc binding in
cate examples of NrCAM (A) and incubated with either Nr-Fc or β1-Fc, respectively. Cells were stained with anti-β1EX (green, inset) and anti-NrCAM (B, inset) and Cy3 (red, to detect Fc). A, CHL cells transfected with β1 (green, inset) and incubated with Nr-Fc (red). B, CHL cells transfected with NrCAM (green, inset) and incubated with β1-Fc (red). The arrows indicate examples of NrCAM (B) expressing cells positive for β1-Fc binding. The arrowheads indicate examples of β1-expressing cells negative for Nr-Fc binding (A). Scale bars, 50 μm.

In contrast, we could not detect an interaction between β1- and β3-Fc in parallel experiments (Fig. 5B), suggesting that β1 and β3 do not interact. To perform the reverse experiment, CHL cells were transfected with full-length β3 and then incubated with β1-Fc (Fig. 5C). Again, no interaction between β1 and β3 was observed in this configuration. Ratcliffe et al. (20) showed previously that β3 interacts heterophilically with Nf186. Consequently, as a positive control, CHL cells were transfected with Nf186 and incubated with β3-Fc. Under these conditions, β3-Fc interacted with Nf186 (Fig. 5D). Thus, our β1-Fc and β3-Fc constructs were functional in terms of binding interactions, but they did not interact with each other in our system.

When CHL cells were transfected with full-length β1 and incubated with β2-Fc, we observed no interaction between these two sodium channel subunits (Fig. 6B). As a positive control, we showed that full-length β2 binds β2-Fc (Fig. 6C), consistent with our previous results showing β2-β2 interactions in Drosophila S2 cells (22). Interestingly, performing the reverse experiment, cells transfected with full-length β2 bound β1-Fc (Fig. 6D), suggesting that the β2 intracellular domain may play a role in the interaction of the β2 extracellular domain with the extracellular domain of β1. To test this hypothesis, CHL cells were transfected with β2STOP, a protein lacking the intracellular domain of β2 (22). β2STOP-transfected CHL cells incubated with β1-Fc showed no binding above background (Fig. 6E), suggesting that the intracellular domain of β2 is required for interaction with the β1 extracellular domain. Alternatively, the inability of β2STOP to interact with β1-Fc may be due to a conformational change in the β2 protein due to the lack of an intracellular domain. This possibility is unlikely, however, since β2STOP expression in S2 cells results in cellular aggregation (22), indicating that this construct is able to function as a homophilic CAM (Fig. 6F). Our results suggested that β2-mediated inside-out signaling and/or β2-cytoskeletal interactions may be required for β2-mediated heterophilic cell-adhesive interactions with β1.

To further investigate the role of the β2 intracellular domain in the extracellular interaction of β2 with β1, we created a series of β2 truncation mutants in which increasing numbers of amino acids were deleted from the intracellular domain (Fig. 6A). These truncation mutants are termed β2Δ169-186, β2Δ174-186, β2Δ169-186, and β2Δ164-186, respectively. When β2Δ161-186 was transfected into CHL cells and incubated with β1-Fc, it interacted with β1-Fc to a similar extent as wild type β2 (Fig. 6G). β2Δ169-186, however, showed an impaired ability to interact with β1-Fc, since only a small portion of the transfected cells exhibited positive binding for β1-Fc (Fig. 6, H and I). Further deletion of the β2 intracellular domain, β2Δ169-186, and β2Δ164-186 resulted in the abolishment of β2 binding to β1-Fc despite robust expression of these mutant proteins at the plasma membrane, indicating that the region 169 TEEEGKT-DGEGNA184 in the intracellular domain of β2 is critical for interaction of the β2 extracellular domain with β1.

β1, Contactin, and Nf186, but Not Nf155 or NrCAM, Increase Cell Surface Sodium Channel Expression—β1 increases Na1.2 cell surface expression in transfected cells by ~2-fold over that observed with α alone (17, 42). Coexpression of β2 or β3 with Na1.2 in the absence of β1 resulted in no significant increases in sodium channel cell surface density over α alone, whereas the presence of both contactin and β1 increased Na1.2 at the cell surface by 6-fold (17, 24). We next investigated whether Nf186, Nf155, or NrCAM affected Na1.2 cell surface expression levels. Stable CHL cell lines expressing Na1.2 in combination with β1, Nf186, Nf155, and/or NrCAM were generated by stably transfecting Nf186, Nf155, or NrCAM into the previously established Na1.2 or Na1.2/β1 stable cell lines (17, 24, 42). Two independent stable clones showing robust expression of each respective CAM by Western blot were selected for the binding studies (Fig. 7A). Cell surface sodium channel expres-
sion was measured using whole cell [3H]STX binding as previously described (24). When expressed with Na1.2 in the absence of β1, neither NF186, NF155, nor NrCAM increased Na1.2 cell surface levels over α alone (Fig. 7B). In fact, these CAMs produced statistically significant decreases in [3H]STX binding compared with α alone. We have observed similar results for β2 and contactin (17) but do not as yet have an explanation for these effects. In the presence of β1, neither NF155 nor NrCAM increased cell surface Na1.2 expression over that observed with the Na1.2/β1 cell line (Fig. 7B). In contrast, NF186 did increase Na1.2 density at the cell surface by ~2-fold in the presence of β1 compared with Na1.2/β1. We next investigated whether NrCAM or NF186 might increase the level of sodium channel cell surface expression observed in the presence of β1 and contactin. The cell lines Na1.2/β1/Cn/NF186 and Na1.2/β1/Cn/NrCAM were generated from the previously established Na1.2/β1/Cn line (17). Neither NF186 nor NrCAM increased sodium channel cell surface expression over that observed in the presence of β1 and contactin (Fig. 7B), suggesting that the level of Na1.2 expressed at the cell surface in the presence of β1 and contactin may be the maximal cell surface density of channels attainable in this heterologous system. [3H]STX binding to Na1.2/β1/Cn/NF186 was actually decreased from that observed for Na1.2/β1/Cn; however, we suspect that this could be due to variations in individual cells in the original Na1.2/β1/Cn cell line.

We propose that β1 plays a key role in the interaction of sodium channels with CAMs at nodes of Ranvier. Our previous results showed that contactin and sodium channels do not interact in the absence of β1 in vitro or in vivo (9, 17). Ratcliffe et al. showed that the interaction of NF186 with the sodium channel complex is dependent on the presence of the β1 subunit in transfected cells (20). We were next interested in determining whether β1 is required for the interaction of NrCAM with Na1.2. Lysates from the Na1.2/NrCAM and Na1.2/β1/NrCAM cell lines used in the binding studies were immunoprecipitated with either nonimmune IgG or the anti-Na1.2 antibody, Sp112. Immunoblotting with anti-NrCAM antibodies
1. The error bars indicate means ± S.E. for Nav1.2 and the control IgG were negative for NrCAM staining despite similar NrCAM expression levels in the two cell lines (Fig. 7C, lanes 4 and 5). This result indicates that, similar to contactin and Nf186, β1 is required for the interaction of NrCAM with the sodium channel α subunit.

**DISCUSSION**

Sodium channel β1 subunits are multifunctional proteins that are involved in channel modulation as well as cell adhesion (17, 22–24, 42). β1 subunits interact homophilically in vitro, resulting in cellular aggregation and ankyrin recruitment to points of cell-cell contact, as well as heterophilically in vitro with contactin and Nf186 (17, 20, 22, 24). The present data identify the CAMs Nf155, NrCAM, and sodium channel β2 subunits as additional heterophilic binding partners of β1 in vitro. In contrast, sodium channel β3 subunits do not appear to interact with β1. Nf155 and NrCAM are important for formation and maintenance of both CNS and PNS nodes of Ranvier (2, 3, 6). β2 is critical for sodium channel cell surface expression in vitro and in vivo (19, 46, 47). We show that contactin, Nf186 and NrCAM increase channel cell surface expression in the presence but not in the absence of β1, similar to that shown previously for contactin and β2 (17, 24). Thus, we propose that β1 functions as the critical communication link between sodium channels, ankyrinG, and other CAMs at the node of Ranvier and may participate in paranodal septate-like junction formation via interaction with Nf155.

We have previously described the interaction between sodium channel β1 subunits and the CAM contactin (17, 24). This interaction depends on the presence of the β1 Ig loop domain and results in a 4–6-fold increase in Nav1.2 expression at the cell surface in vitro (24). In the present study, we show that the fibronectin-like repeats of contactin, but not the Ig loop domains, are essential for β1 interactions. In β1(−/−) mice, the association of sodium channels with contactin is disrupted, confirming the interpretation of our in vitro results that β1 subunits are required for formation of the sodium channel-contactin complex (9). We have proposed that the loss of sodium channel-contactin interactions in vivo results in destabilization of sodium channel cell surface expression at nodes and the subsequent decrease in the number of mature CNS nodes of Ranvier observed in β1(−/−) mice (9). Our present results suggest that the “horseshoe” configuration of contactin (48–50) is not required for its interaction with β1, leaving the Ig domains of contactin free to interact with other CAMs while simultaneously interacting with β1. This proposed arrangement would thus increase the number and diversity of CAMs that can be involved in the sodium channel complex.

Recent evidence has shown a role for NrCAM and Nf186 in delineating presumptive PNS nodes of Ranvier prior to sodium channel and ankyrinG clustering (2, 18). NrCAM (−/−) mice exhibit a delay in nodal development as well as a delay in the localization of ankyrinG to PNS nodal areas (2). In CNS myelinated axons, ankyrinG has been shown to define nodal regions prior to CAM and sodium channel clustering (28, 29). AnkyrinG (−/−) mice exhibit disrupted localization of sodium channels, neurofascin, and NrCAM at initial segments of unmyelinated cerebellar granule cell axons and myelinated Purkinje neurons (28, 51). Thus, coordinated temporal and spatial adhesive events between NrCAM, Nf186, sodium channels, and ankyrinG are critical to sodium channel clustering and may exhibit different mechanisms in the PNS compared with the CNS. In the present study, we show that sodium channel β1 subunits interact with the nodal, ankyrin-binding CAMs NrCAM, Nf186, and β2, in addition to contactin. Furthermore, we show that, like contactin and Nf186, the interaction of NrCAM with the sodium channel complex is dependent on β1. The interac-

**FIG. 7.** Coexpression of β1 and Nf186 increases while coexpression of β1 with Nf155 or NrCAM has no effect on Na<sub>1.2</sub> cell surface density. A, Western blot analysis of Na<sub>1.2</sub> or Na<sub>1.2β1</sub> or Na<sub>1.2β1/Cn</sub> cell lines that were transfected with Nf155, Nf186, or NrCAM as indicated. Immunoblots were probed with anti-NF155 (1:2000), anti-HA.11 (to detect Nf155, 1:1000), and anti-NrCAM (1:2000), respectively. B, [3H]STX binding to intact CHL cells expressing either Na<sub>1.2</sub> alone or Na<sub>1.2β1</sub> with a combination of contactin, Nf155, Nf186, NrCAM, and/or β1. The error bars indicate means ± S.E. for Na<sub>1.2</sub> (n = 6), Na<sub>1.2/Nf155</sub> (n = 3), Na<sub>1.2/Nf186</sub> (n = 3), Na<sub>1.2/NrCAM</sub> (n = 3), Na<sub>1.2β1/Nf155</sub> (n = 3), Na<sub>1.2β1/Nf186</sub> (n = 6), Na<sub>1.2β1/NrCAM</sub> (n = 3), Na<sub>1.2β1/Cn</sub> (n = 5), Na<sub>1.2β1/Cn/Nf186</sub> (n = 3), and Nav1.2β1/Cn/NrCAM (n = 3) cells. For all cell lines, binding was performed on at least two different stable clones. 10 μM tetrodotoxin was used to determine nonspecific binding as previously described (8). [3H]STX binding was normalized to protein using Advanced protein assay reagent (Cytoskeleton, Inc., Denver, CO). * statistically significant compared with Na<sub>1.2</sub> (p < 0.05). ** statistically significant compared with Na<sub>1.2β1</sub> (p < 0.05). *** statistically significant compared with Na<sub>1.2β1/Cn</sub> (p < 0.05). C, immunoprecipitation ([P] of Na<sub>1.2</sub> with NrCAM from stably expressing Na<sub>1.2β1</sub>NrCAM or Na<sub>1.2β1</sub>/NrCAM CHL cell lines. Equal amounts of cell lysates were immunoprecipitated with 5 μl of either nonimmune IgG or Sp111II for Na<sub>1.2</sub>. Immunoblots were probed with anti-NrCAM antibodies (1:200). Cell lysates indicate equal expression of NrCAM in both cell lines (lanes 4 and 5). showed that Na<sub>1.2</sub> and NrCAM associate in the Na<sub>1.2β1</sub>/NrCAM cell line but not in the absence of β1 (Fig. 7C, lane 3). The control IgG and Na<sub>1.2</sub>/NrCAM lanes (Fig. 7C, lanes 1 and 2) were negative for NrCAM staining despite similar NrCAM expression levels in the two cell lines (Fig. 7C, lanes 4 and 5). This result indicates that, similar to contactin and Nf186, β1 is required for the interaction of NrCAM with the sodium channel α subunit.
tion of β1 with contactin, with NF186, or with β2 (17) results in significant increases in Na_1,2 cell surface expression in vitro. We showed previously that Na_1,2 channels interact weakly with ankyrinG in CHL cells, and coexpression of β1 greatly enhances this interaction (24). In the present study, interaction of β1 with the ankyrin-binding CAMs NrCAM, NF186, and β2 could result in increased interaction of sodium channels with the endogenous ankyrinG protein in CHL cells (23), stabilizing sodium channels at the cell surface. We propose that β1 may act to stabilize sodium channels at the nodal plasma membrane in vivo through multiple extracellular interactions with ankyrin-binding CAMs as well as through its own intracellular ankyrin-binding domain. Furthermore, we propose that the reduction in action potential conduction velocity, reduction in the integral of the optic nerve compound action potential, loss of sodium channel cell surface expression, and reduction in the number of mature nodes of Ranvier observed in β1 (−/−) and/or β2 (−/−) mice (9, 19) result from the loss of these critical interactions between nodal CAMs and sodium channels.

Our results show that sodium channel β1 and β2 subunits interact through heterophilic cell-adhesive interactions in addition to the interaction of each of these proteins with the pore-forming α subunit (10, 52). Our observation that a region of the β2 intracellular domain is required for heterophilic cell adhesion between the β2 and β1 extracellular domains raises some interesting possibilities. β2 subunits participate in homophilic cell adhesion, resulting in the recruitment of ankyrin to points of cell-cell contact in Drosophila S2 cells (22). β2STOP, a β2 truncation mutant lacking the intracellular domain, maintains homophilic adhesion yet does not recruit ankyrin (22). In the present study, when full-length β1 was incubated with soluble β2-Fc, no interaction was detected between these two subunits. However, when full-length β2 was incubated with soluble β1-Fc, interactions between the extracellular domains of β1 and β2 were observed. These results suggested that a region in the intracellular domain of β2 must be present for heterophilic interaction with the extracellular domain of β1 to occur. Using a series of β2 truncation mutants, we found that the intracellular 1669−1689pentapeptide region of β2 is critical for extracellular, heterophilic adhesion with β1. We propose that an interaction in the β2 intracellular domain, possibly with ankyrin, stabilizes extracellular, heterophilic cell adhesive events. We showed previously that a single residue in the β1 intracellular domain, Tyr1689, is critical for ankyrin binding and that phosphorylation of this residue negatively regulates ankyrin interactions (22). Whereas we have not yet defined the ankyrin-binding domain of β2, the region that we have identified as critical for β2 heterophilic adhesion is located in a region of the protein similar in position to the ankyrin-binding domain of β1. The β2 intracellular domain does not contain tyrosine residues and is not similar to other ankyrin-binding domains reported in the literature (22). However, it does contain three possible casein kinase II phosphorylation sites according to the PhosphoBase program (available on the World Wide Web at www.cbs.dtu.dk/databases/PhosphoBase/) provided by the Technical University of Denmark. These putative sites include QKL1689DDL, DL1689EEF, and EGK175DGE. The residues Thr1689 and Thr178 are located in the region determined to be required for heterophilic adhesion. It will be interesting in the future to investigate whether this is indeed the location of the ankyrin-binding domain of β2 and, if so, whether it is regulated by phosphorylation.

β1 (−/−) mice exhibit disrupted axo-glial contacts in a subset of axons (9). In these axons, the septate-like junctions at the nodal-paranodal boundaries are everted or pulled away from the axon, leaving all other septate-like junctions, seen as transverse bands by transmission electron microscopy, intact. We proposed that axonal β1 subunits located at the nodal-paranodal border may interact in cis with axonal contactin molecules in the paranode or in trans with glial NF186 molecules at the nodal-paranodal border region. Alternatively, because we have also shown β1 expression in oligodendrocytes (53), it is possible that glial β1 subunits may interact in trans with axonal β1 subunits, in cis with glial NF155, or in trans with axonal contactin molecules. In the present study, we tested the hypothesis that β1 interacts with NF155 and showed that these molecules interact in our assay. Thus, we propose that β1 is associated with sodium channels at the node of Ranvier, where it serves a dual function as a channel modulator and as a CAM. In addition, we propose that β1 may be expressed in the absence of sodium channels in the nodal-paranodal border region of axons or in oligodendrocytes, where it functions as a CAM only. Interaction of β1 with sodium channels at nodes of Ranvier results in modulation of channel function and stabilization of channel cell surface expression through interactions with β2, contactin, NF186, NrCAM, and ankyrinG. Axonal β1 subunits located at the nodal-paranodal border may also interact in cis with paranodal contactin or in trans with glial NF155. β1 subunits expressed in oligodendrocytes may participate in the formation of septate-like junctions at the nodal-paranodal border through cell-adhesive interactions in cis with NF155 and/or in trans with contactin. It will be necessary to perform in vivo experiments using β1 mutant constructs to investigate the importance of the different functional domains of β1 on the maintenance and stabilization of nodes of Ranvier.

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