View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Developmental Cell, Vol. 4, 419–429, March, 2003, Copyright ©2003 by Cell Press

The Cytoplasmic Domain of *Xenopus* NF-Protocadherin Interacts with TAF1/Set

Mike A. Heggem and Roger S. Bradley*
Department of Cell Biology and Neuroscience
Montana State University
Bozeman, Montana 59717

Summary

Protocadherins are members of the cadherin superfamily of cell adhesion molecules proposed to play important roles in early development, but whose mechanisms of action are largely unknown. We examined the function of NF-protocadherin (NFPC), a novel cell adhesion molecule essential for the histogenesis of the embryonic ectoderm in *Xenopus*, and demonstrate that the cellular protein TAF1, previously identified as a histone-associated protein, binds the NFPC cytoplasmic domain. NFPC and TAF1 coprecipitate from embryo extracts when ectopically expressed, and TAF1 can rescue the ectodermal disruptions caused by a dominant-negative NFPC construct lacking the extracellular domain. Furthermore, disruptions in either NFPC or TAF1 expression, using NFPC- or TAF1-specific antisense morpholinos, result in essentially identical ectodermal defects. These results indicate a role for TAF1 in the differentiation of the embryonic ectoderm, as a cytosolic cofactor of NFPC.

Introduction

The cadherins comprise a large family of calcium-dependent cell adhesion molecules whose members are involved in important morphogenic events throughout embryogenesis. Cadherin-mediated cell adhesion has been implicated in diverse developmental processes, from cell migration and the sorting of embryonic cells into tissues to the formation of synaptic junctions and the establishment of complex neural circuits (Gumbiner, 1996; Shapiro and Colman, 1999; Takeichi et al., 1997; Tepass et al., 2000). The original members of this family, the classical cadherins, are localized to sites of cell-cell contact at the adherens junction, where they function as dimers, each dimer binding two identical dimers in a neighboring cell (Koch et al., 1999; Pertz et al., 1999). Classical cadherins are transmembrane proteins, with an extracellular domain containing five tandem cadherin-specific repeats and a highly conserved intracellular domain. Several intracellular proteins promote adherens junction formation by binding to conserved regions of the classical cadherin cytoplasmic domain. These include the catenins (α-, β-, and γ-catenin), which govern the interaction of cadherins with the cytoskeleton, the src substrate p120^{ctn}, thought to mediate lateral clustering of cadherins, and IQGAP1, a target of the Rho family of small GTPases (Gumbiner, 2000; Kuroda et al., 1998; Yap et al., 1998).

While classical cadherins remain the best-studied members of the family, several additional proteins have been identified and classified within the cadherin family, many of which exhibit interesting expression patterns that implicate them in important developmental events (Angst et al., 2001). Of particular interest are the protocadherins, which comprise a large subfamily of molecules only distantly related to classical cadherins, to which they share homology solely in their extracellular domain (Frank and Kemler, 2002; Sano et al., 1993; Suzuki, 2000). Rather than five cadherin repeats typical of classical cadherins, the number of extracellular repeats in protocadherins is often six to seven; furthermore, the cytoplasmic domains of protocadherins are not related to classical cadherins and represent novel sequence, and hence little is known concerning interacting cytosolic proteins. Interestingly, protocadherin family members are highly expressed in the nervous system in vertebrates, and are proposed to participate in the subdivision of the brain into functional domains, as well as in the formation of neural circuits (Arndt and Redies, 1998; Hirano et al., 1999). Despite their proposed roles, relatively few studies have addressed the function of protocadherins during development.

We previously isolated a novel protocadherin from *Xenopus*, termed NF-protocadherin (NFPC; Bradley et al., 1998), which exhibits a restricted expression pattern in early embryos, being predominantly localized to the inner or sensorial layer of the ectoderm, as well as to a subset of cells in the neural folds and neural tube. This expression pattern implied a role for NFPC in cell adhesion during embryonic ectodermal differentiation, which was subsequently confirmed by studies designed to misexpress NFPC in early embryos. Ectopic expression of wild-type NFPC, by RNA injection, demonstrated that NFPC can mediate cell adhesion within the embryonic ectoderm in vivo. In contrast, ectopic expression of a dominant-negative form of NFPC, in which the entire extracellular domain is deleted (NFΔE), significantly perturbs ectoderm differentiation, resulting in the formation of large ectodermal blisters pursuant to the dissociation of cells in the inner layer.

While not directly addressed in the previous study, several lines of evidence strongly suggest that cytoplasmic cofactors are essential for NFPC-mediated cell adhesion, and that these factors are distinct from those that bind classical cadherins. First, removing the intracellular domain of NFPC abrogates its ability to promote cell adhesion, demonstrating that the cytoplasmic domain is critical for NFPC function (Bradley et al., 1998). Second, the ability of the intracellular domain of NFPC to act as a dominant-negative, causing dissociation of the ectodermal inner layer, is presumably due to competition with endogenous NFPC for binding intracellular cofactors. Third, the mouse and human homologs of NFPC (termed BH-protocadherin; Yoshida et al., 1998, 1999) exhibit striking amino acid conservation in their cytosolic domain, implying that the function of this domain has been conserved across vertebrates. Finally, as the cytoplasmic domain of NFPC is not homologous to other cadherins and does not contain the consensus

*Correspondence: rbradley@montana.edu

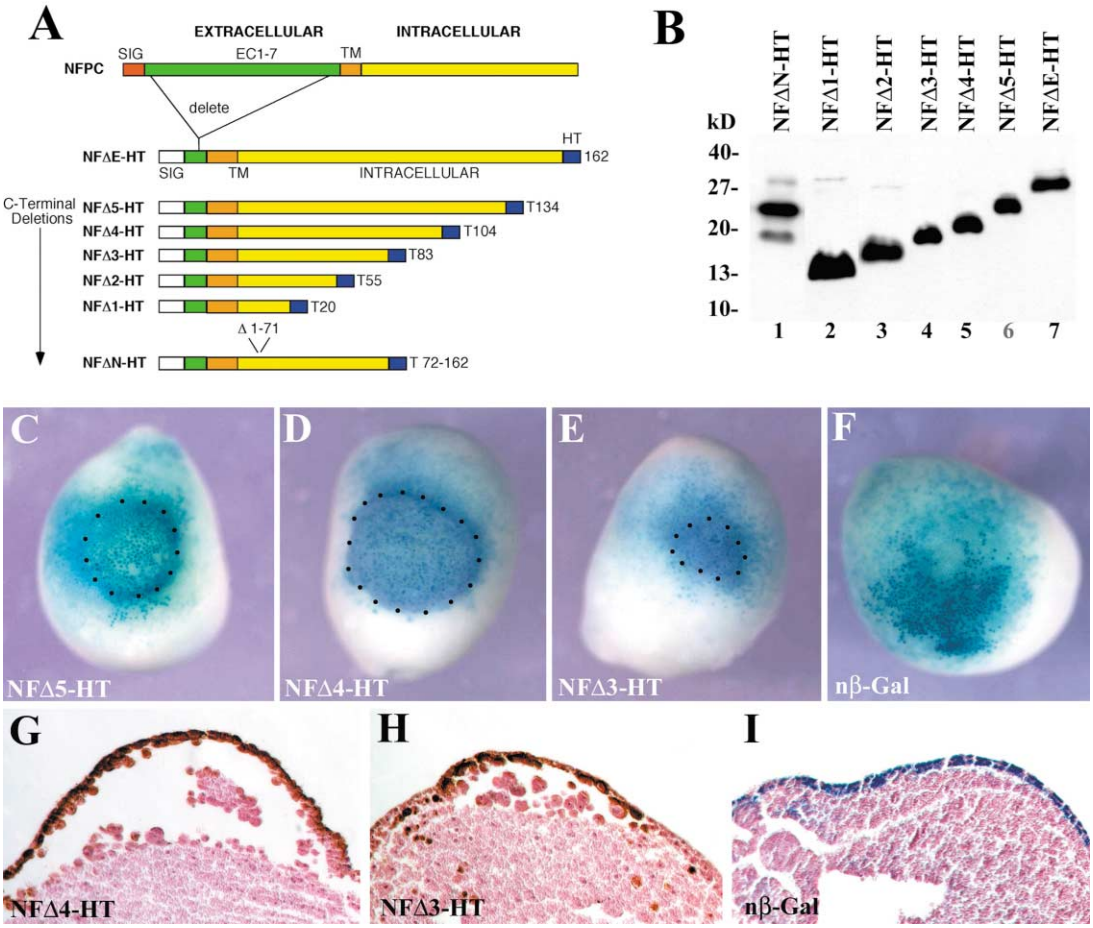

Figure 1. Ectopic Expression of NFPC Dominant-Negative Constructs in the Ectoderm

(A) Schematic diagram of NFPC dominant-negative mutants. In NFΔE, the NFPC extracellular domain is deleted, constructs NFΔ5, NFΔ4, NFΔ3, NFΔ2, and NFΔ1 delete increasing regions of the C terminus of NFΔE, while NFΔN deletes 71 amino acids from the juxtamembrane domain. All constructs are tagged at the C terminus with an RGS-His_6 epitope (HT).

(B) Immunoblot analysis of NFPC deletion constructs expressed in embryos. Embryos were injected with RNA encoding the indicated deletion construct, and then harvested at stage 14 and analyzed by Western blot with an anti-His antibody. All deletion constructs were expressed in embryos at expected molecular masses.

(C–H) Ectopic expression of NFΔ5-HT, NFΔ4-HT, and NFΔ3-HT in ectoderm. Embryos were coinjected with RNA encoding either NFΔ5-HT (C), NFΔ4-HT (D), or NFΔ3-HT (E) together with *nLacZ* RNA, fixed at stage 14, and stained for β-galactosidase activity. Compared to control embryos injected with *nLacZ* RNA alone (F), embryos injected with NFΔ5-HT or NFΔ4-HT developed large ectodermal blisters (outline), while embryos injected with NFΔ3-HT exhibit less pronounced ectodermal disruptions. Embryos were injected with NFΔ4-HT (G) or NFΔ3-HT (H), and then immunostained for the His epitope tag and sectioned, revealing the ectodermal blistering.

(I) Control embryo injected with *nLacZ* RNA.

catenin binding site identified in classical cadherins (Stappert and Kemler, 1994), NFPC is unlikely to interact with catenins and may mediate cell adhesion via novel mechanisms. Therefore, to ascertain the mechanism of action of NFPC as a cell adhesion molecule, we sought to identify proteins that interact with the cytoplasmic domain of NFPC. Results presented here demonstrate that the NFPC juxtamembrane domain interacts with a protein, TAF1, previously identified as a histone binding protein involved in mediating transcriptional access to chromatin. In *Xenopus*, TAF1 is normally expressed in the embryonic ectoderm and neural tube, and NFPC coprecipitates with TAF1 when ectopically expressed in embryos. In addition, coinjection of TAF1 and NFΔE can rescue the ectodermal blisters observed upon ectopic expression of NFΔE alone. Furthermore, injection of either antisense TAF1 or NFPC morpholinos results in morphologically identical ectodermal defects due, in part, to a decrease in programmed cell death in the ectoderm. These findings indicate that TAF1 may participate in NFPC-mediated cell adhesion in the embryonic ectoderm, and that both NFPC and TAF1 are required for the proper differentiation of this tissue.

Results

Deletion Analysis of the NFPC Cytoplasmic Domain

Ectopic expression of a mutant NFPC construct lacking the extracellular domain (NFΔE) was previously shown to disrupt the embryonic ectoderm, resulting in the formation of ectodermal blisters (Bradley et al., 1998). This

Table 1. Ectodermal Blisters Produced by NFPC Dominant-Negative Mutants

RNA injected	Number of embryos examined	Number of embryos with blisters	Percentage of embryos with blisters
NFΔ1-HT	83	2	2.4
NFΔ2-HT	94	13	13.8
NFΔ3-HT	117	22	18.8
NFΔ4-HT	178	75	42.1
NFΔ5-HT	131	87	66.4
NFΔN-HT	45	0	0
NFΔE-HT	99	59	59.6
nLacZ	115	0	0

Embryos were injected with RNA encoding deletion constructs, along with *nLacZ* to mark the site of injection, fixed at stage 14, stained in X-gal, and examined for the appearance of ectodermal blisters.

dominant-negative activity of NFΔE allowed a convenient assay to map regions of the NFPC cytoplasmic domain necessary for cell adhesion. Accordingly, several mutant forms of NFΔE were generated, lacking various portions of the intracellular domain, for ectopic expression in *Xenopus* embryos (Figure 1A). Injection of RNA encoding these constructs, tagged with a His epitope (HT) followed by immunoblot analysis of embryo extracts with an epitope-specific antibody, established that all constructs were expressed in embryos at the expected molecular mass (Figure 1B). In addition, whole-mount immunochemical staining of injected embryos revealed that all constructs were appropriately expressed and localized to the plasma membrane (data not shown). These deletion constructs were then assayed for their ability to function as dominant-negatives, similar to NFΔE, perturbing the formation of the embryonic ectoderm. Embryos were injected with RNA encoding the deletion constructs, along with *nLacZ* to mark the site of injection, allowed to develop until neural plate stage (stage 14), and examined for ectodermal blisters. Constructs in which up to 58 amino acids of the C terminus were deleted (NFΔ5-HT and NFΔ4-HT) still function as strong dominant-negatives upon expression in *Xenopus* embryos, resulting in the formation of ectodermal blisters (Figure 1; Table 1). In contrast, deletion of either 79 or 107 amino acids from the C terminus (NFΔ3-HT and NFΔ2-HT) produced increasingly weaker perturbations of the embryonic ectoderm, while deletion of the C-terminal 142 amino acids (NFΔ1-HT) resulted in an almost complete loss of the dominant-negative activity. Similarly, a construct that deleted the juxtamembrane 71 amino acids of the cytoplasmic domain (NFΔN-HT) had no effect on the embryonic ectoderm. Thus, the ability of NFΔE to act as a dominant-negative maps to the first 80 amino acids of the cytoplasmic domain of NFPC. This region, therefore, plays an important role in NFPC-mediated adhesion and likely contains sites for interacting cytosolic factors.

Identification of Proteins Associated with the NFPC Cytoplasmic Domain

This deletion analysis was then used as a starting point for identifying proteins that bind the NFPC cytoplasmic domain. GST-fusion proteins were generated containing the cytoplasmic domains of NFΔ2, NFΔ3, NFΔ5, and NFΔN, and cytosolic extracts from cultured HeLa cells were then incubated with the fusion proteins bound to glutathione-agarose. HeLa cells were chosen because expression of NFPC in HeLa cells, by stable transfection, results in targeting of NFPC to the plasma membrane where it becomes concentrated at sites of cell-cell contact (data not shown), indicating that HeLa cells contain cofactors necessary for NFPC-mediated cell adhesion.

Proteins that bound the fusion constructs were then analyzed by SDS-PAGE followed by silver staining, revealing several proteins that potentially interact with the NFPC cytoplasmic domain (Figure 2A). Initial efforts to isolate interacting proteins have focused on the most prominent of these, the 41 and 39 kDa polypeptides, both of which interact with constructs containing regions necessary for the dominant-negative effect (NFΔ3-GST and NFΔ5-GST) but bind more weakly to NFΔ2-GST, and not with NFΔN-GST or a control frame-shifted construct encoding a nonsense protein that terminates after 12 amino acids (NFfs-GST). Thus, binding of the 41 and 39 kDa polypeptides to the GST-fusion proteins requires the juxtamembrane region of the NFPC cytoplasmic domain, the same region required as a dominant-negative in the ectodermal blister assay.

To isolate and identify the 41 and 39 kDa proteins, the above procedure was scaled up and the two polypeptides were excised and subjected to trypsin digestion. Resultant peptides were sequenced and used to search the NRPD. One protein in the database, human TAF1/Set, matched peptides obtained from both the 41 and 39 kDa proteins. The TAF1 protein was originally identified as a putative oncogene activated by a translocation breakpoint in acute undifferentiated leukemia and was reported to be a nuclear transcription/replication factor (Adachi et al., 1994; Nagata et al., 1995). Consistent with the results from the pull-down experiments, TAF1 exists as two alternatively spliced isoforms α and β, of approximate MW 41 and 39 kDa, respectively, which differ at the N-terminal AUG used for translation (Figure 2B).

To confirm that TAF1 is the identity of the 41 and 39 kDa peptides that bind GST-NFΔ3, we performed an immunoblot analysis of the GST-fusion pull-downs, using an antisera against human TAF1β protein. As shown in Figure 2C, the 39 kDa band was detected in the GST-NFΔ3 lane, but not in the control GST-NFΔN lane. This confirms that TAF1β is the 39 kDa protein that binds to the cytoplasmic domain of NFPC. As this antiserum was raised to an N-terminal peptide specific to the 39 kDa isoform, it does not recognize the 41 kDa TAF1α protein.

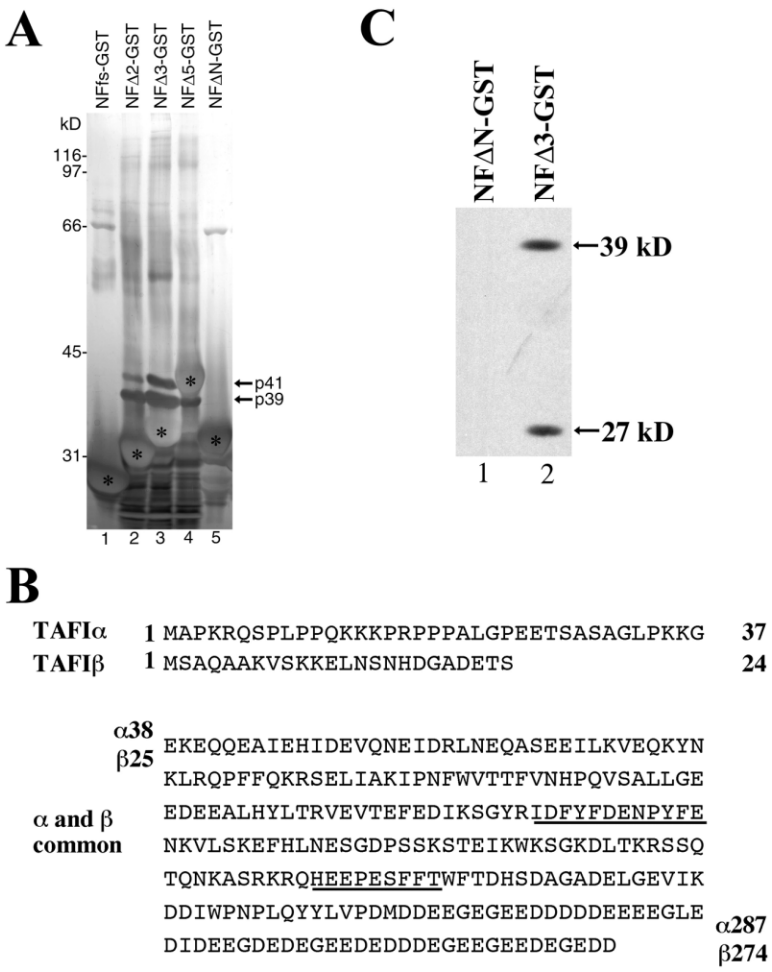

Figure 2. TAF1 Binds the NFPC Juxtamembrane Domain

(A) Proteins that bind the NFPC cytoplasmic domain. HeLa cell extracts were incubated with GST-fusion constructs, and bound proteins were eluted and analyzed by SDS-PAGE and silver stained. The 41 and 39 kDa proteins bind NFΔ3-GST, NFΔ5-GST (partially obscured by the GST-fusion protein band marked by an asterisk), and to a lesser extent NFΔ2-GST, but not NFfs-GST or NFΔN-GST.

(B) The 41 and 39 kDa proteins bound by NFΔ3-GST are identical to human TAF1. The amino acid sequence of human TAF1, including the two alternatively spliced products that differ only at their N terminus, is shown. The two proteolytic fragments identified by microsequencing are underlined.

(C) Immunoblot analysis of interacting proteins. HeLa cell extracts were incubated with NFΔ3-GST or NFΔN-GST, and bound proteins were eluted and analyzed by SDS-PAGE followed by Western blotting with anti-TAF1β antibody. The 39 kDa TAF1β protein binds to NFΔ3-GST, but not the control NFΔN-GST. A 27 kDa peptide is also detected by the antibody, and may represent a proteolytic fragment of TAF1β.

NFPC and TAF1 Interact In Vivo

As TAF1 has previously been described as a nuclear protein, the specificity of the interaction between the NFPC cytoplasmic domain and TAF1 was not immediately obvious and required further testing. Therefore, to confirm that TAF1 can interact with NFPC in vivo, we next examined the subcellular distribution of TAF1β by indirect immunofluorescence. HeLa cells were transfected with a construct encoding a myc epitope-tagged NFPC (NFPC-MT) and stained for expression of the myc epitope and for human TAF1β. Results reveal that while TAF1β is primarily restricted to the nucleus, in approximately 40% of transfected cells, a fraction of TAF1β can be seen at the cell membrane between two adjacent cells expressing NFPC-MT, where it colocalizes with NFPC-MT (Figures 3B and 3C, and data not shown). In contrast, in HeLa cells transfected with a myc-tagged N-cadherin construct (N-Cad-MT), no TAF1β protein is seen at the membrane (Figure 3D). This suggests that TAF1β is not found exclusively in the nucleus in cells in culture, but in the presence of NFPC can localize to the plasma membrane, supporting the idea that TAF1 can interact with the cytoplasmic domain of NFPC.

To determine whether TAF1 can interact with NFPC in vivo in embryos, we next sought to coprecipitate NFPC and TAF1 from *Xenopus* embryos. RNA encoding myc epitope-tagged NFΔE (NFΔE-MT) or NFPC-MT and a His-tagged TAF1β (TAF1β-HT) were coinjected into a single blastomere at the four-cell stage. Embryos were allowed to develop until stage 14, dounced in lysis buffer, and TAF1-HT was precipitated by the addition of Ni-NTA-agarose. Proteins bound to Ni-NTA-agarose were then analyzed by SDS-PAGE followed by immunoblotting with the myc antibody. As shown in Figure 4, while neither NFΔE-MT nor NFPC-MT is precipitated by Ni-NTA-agarose in the absence of TAF1β-HT, in coinjected embryos both NFΔE-MT and NFPC-MT coprecipitate with TAF1β-HT. To confirm the interaction of NFPC and TAF1β, we next asked whether endogenous TAF1β can associate with NFPC. Therefore, embryos were injected with RNA encoding wild-type NFPC, harvested at stage 19, and immunoprecipitated with an anti-TAF1β monoclonal antibody. As shown in Figure 4C, NFPC does coprecipitate with endogenous TAF1β, consistent with TAF1β acting as a cytosolic cofactor of NFPC.

NFΔE-Induced Blisters Are Rescued by TAF1β

While the above results suggest that NFPC and TAF1β can interact in vivo, it was not known whether TAF1β is required for NFPC-mediated cell adhesion. To address this question, we sought to determine whether ectodermal blisters, as caused by the dominant-negative NFΔE, result from the sequestering of endogenous TAF1β by NFΔE. Thus, we asked whether coinjection of *Xenopus*

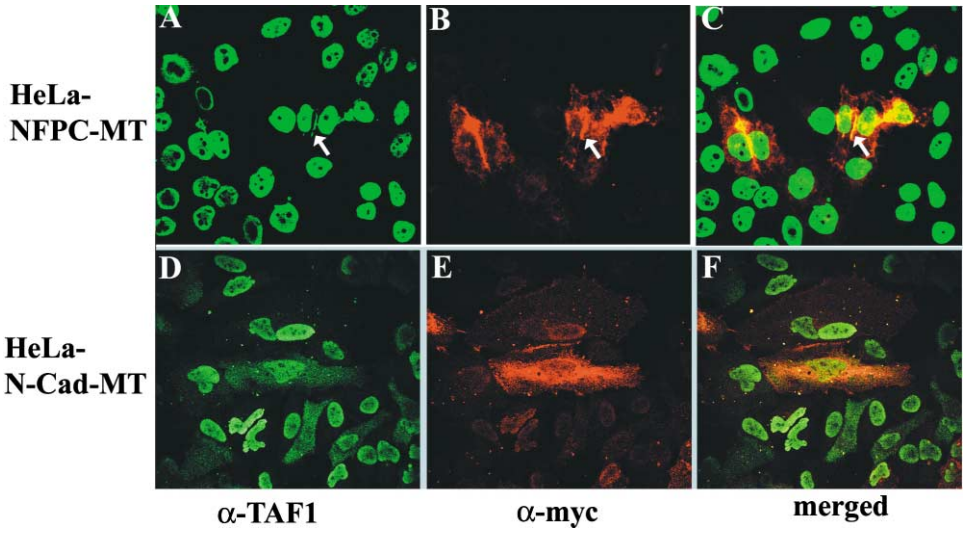

Figure 3. NFPC and TAF1 Colocalize at the Plasma Membrane

HeLa cells were transfected with DNA encoding NFPC-MT or N-Cad-MT, and then processed by immunofluorescence with antibodies to human TAF1β (A and D) and the myc epitope (B and E), and viewed by confocal microscopy. While TAF1β is restricted to the nucleus in cells transfected with N-Cad-MT (D), in cells transfected with NFPC-MT, TAF1β is also found at the membrane where it colocalizes with NFPC (arrows).

(C and F) Merged fluorescence of (A) and (B), and (D) and (E), respectively.

*TAF1*β RNA rescues the blisters produced by NFΔE. As summarized in Table 2, injection of *NF*Δ*E* RNA alone resulted in 40% of embryos exhibiting ectodermal blisters, whereas coinjection of *NF*Δ*E* and *TAF1*β RNA resulted in only 2% of embryos with blisters. Thus, coinjection of *TAF1*β RNA can reduce the incidence of ectodermal blisters observed with *NF*Δ*E* RNA alone. This implies that TAF1β does act as a cytosolic cofactor of NFPC-mediated cell adhesion, at least within the embryonic ectoderm, and that the dominant-negative effect of NFΔE is due, in part, to the sequestering of endogenous TAF1β protein.

TAF1β Is Required for Proper Ectodermal Development

Coprecipitation and rescue studies described above indicate that TAF1β may be one of the cofactors necessary for NFPC-mediated cell adhesion in the embryonic ectoderm. As the expression pattern of *Xenopus TAF1*β has not been described, we sought to determine whether *TAF1*β is also expressed in the ectoderm, similar to *NFPC*, at the relevant developmental stages at which NFPC is thought to function during ectodermal development. Therefore, *TAF1*β RNA expression was localized in early embryos by whole-mount in situ hybridization. Prior to gastrulation, *TAF1*β is expressed at low levels throughout the ectoderm, which subsequently increases to give strong staining in the ectoderm and neural plate (Figure 5 and data not shown). A control in situ hybridization with a sense *TAF1*β probe showed no detectable staining (data not shown).

The ectoderm of *Xenopus* is unusual in that it consists of two layers, an inner cell layer (sensorial layer) and an outer cell layer (epithelial layer), and our previous research established that *NFPC*, while initially expressed in both layers, becomes restricted to the inner layer by stage 17 (Figure 5A; Bradley et al., 1998). Interestingly, *TAF1*β reveals a similarly restricted expression pattern at stage 17, with *TAF1*β most highly expressed in the inner layer of the epidermis (Figure 5B). To confirm that TAF1 protein is indeed present in ectodermal cells and to examine its subcellular distribution, we next utilized a monoclonal antibody generated against human TAF1α and -β, previously shown to recognize *Xenopus* TAF1 (Matsumoto et al., 1999), to label whole-mount embryos. As shown in Figure 5D, endogenous TAF1 protein is present in the ectodermal cells in both the nucleus and at the cell membrane, consistent with a role for TAF1 as a cofactor of NFPC. Thus both *NFPC* and *TAF1* are normally expressed in the ectoderm, where they may interact at the cytoplasmic face of the cell membrane to regulate the differentiation of this tissue.

To test whether TAF1 is required for proper ectodermal development, we sought to disrupt the expression of TAF1β in the ectoderm by injecting an antisense TAF1β morpholino (TAF1βMO) into embryos. As shown in Figure 5, embryos injected with the TAF1βMO exhibit defects in the normal two-cell layered ectoderm, resulting in an ectodermal bulge, which appears to result from an increase in cells within the ectoderm. In addition, cellular morphology appears altered by the morpholino, as the ectodermal cells lose their cuboidal shape and become more spherical. Significantly, this phenotype is almost identical to that caused by an antisense NFPC morpholino (NFPCMO), whereas embryos injected with a control morpholino (CMO) exhibit no ectodermal disruptions. To verify that TAF1βMO and NFPCMO were effective in preventing expression of their respective proteins, morpholino-injected embryos were dounced in lysis buffer and extracts were analyzed by immunoblot analysis for expression of *Xenopus* TAF1β and NFPC.

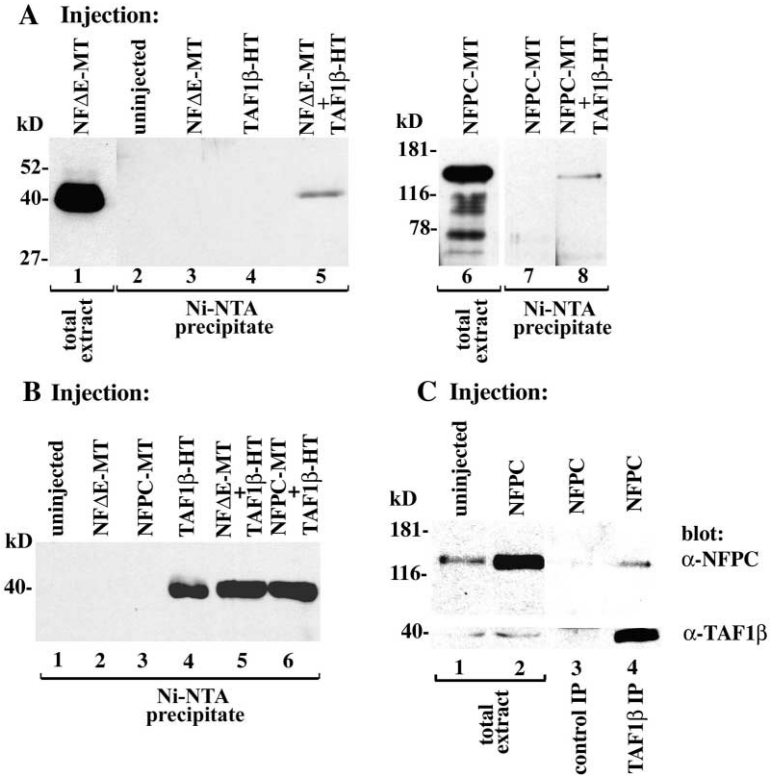

Figure 4. Interaction of TAF1β and NFPC In Vivo

(A) Embryos were injected with RNA encoding either *Xenopus* TAF1β-HT (lane 4), NFΔE-MT (lanes 1 and 3), NFPC-MT (lanes 6 and 7), or coinjected with TAF1β-HT and NFΔE-MT (lane 5) or NFPC-MT (lane 8). Embryos were lysed at stage 14, and incubated with Ni-NTA-agarose to precipitate TAF1β-HT (lanes 2–5, 7, and 8). Bound proteins were then analyzed by immunoblot with an antibody to the myc epitope tag. NFΔE-MT and NFPC-MT are precipitated only in embryos coinjected with TAF1β-HT (lanes 5 and 8).

(B) Precipitated extracts from (A) immunoblotted with the anti-His antibody reveal that TAF1β-HT is precipitated by Ni-NTA.

(C) Embryos were injected with RNA encoding a nonepitope-tagged NFPC, lysed at stage 19, and immunoprecipitated with either a monoclonal antibody to TAF1β to precipitate endogenous TAF1β (lane 4), or a control monoclonal antibody (lane 3). Subsequent immunoblotting with an antibody to TAF1β or *Xenopus* NFPC reveals that NFPC coprecipitates with endogenous TAF1β.

Whereas uninjected and CMO-injected embryos express similar levels of TAF1β and NFPC, embryos injected with TAF1βMO or NFPCMO exhibit a significant decrease in TAF1β and NFPC protein levels, respectively (Figure 5H). Thus both TAF1βMO and NFPCMO are effective in reducing the amount of their corresponding proteins present in embryos, and both cause an apparent excess of cells in the embryonic ectoderm.

To confirm that the two morpholinos result in similar defects, and to investigate the nature of the ectodermal bulges, we next sought to determine whether TAF1βMO and NFPCMO cause an increase in cell proliferation and/or survival, either of which could result in an increase in ectodermal cells. Therefore, to determine whether ectodermal cell mitosis is altered by the morpholinos, embryos were injected with NFPCMO or TAF1βMO, allowed to develop until stage 22, and then injected with BrdU to label cells undergoing DNA synthesis. Subsequent analysis of BrdU-labeled nuclei in the ectoderm revealed no obvious difference between NFPCMO- and TAF1βMO-injected embryos as compared to control embryos (data not shown). We next sought to determine whether cell survival in the ectoderm was altered by NFPCMO or TAF1βMO. Embryos were coinjected with NFPCMO or TAF1βMO, together with *nLacZ* RNA, and then fixed at stage 22, stained for β-galactosidase activity, and subjected to whole-mount TUNEL labeling. Labeled embryos were then serially sectioned and examined for TUNEL-positive cells in the ectoderm. Results reveal that both NFPCMO and TAF1βMO cause a reduction in apoptosis in the ectoderm as compared to CMO-injected embryos (Figures 5I–5K). Quantification of cell death was obtained from sectioned embryos by counting the number of TUNEL-positive nuclei in the injected side of the ectoderm (as determined by X-gal staining). Compared to CMO-injected embryos, embryos injected with either NFPCMO or TAF1βMO show a marked reduction in TUNEL-positive nuclei (Figure 5L). This suggests that the ectodermal defects caused by NFPCMO and TAF1βMO are due, at least in part, to a decrease in cell death, and provides further evidence that NFPC and TAF1β play interrelated roles in the ectoderm.

Discussion

Protocadherins comprise a large family of developmentally regulated molecules thought to be involved in

Table 2. Rescue of NFΔE-Induced Blisters by *Xenopus* TAF1β

RNA injected	Number of embryos examined	Number of embryos with blisters	Percentage of embryos with blisters
NFΔE-HT	168	72	43
NFΔE-HT + *TAF1β*	175	4	2
TAF1β	149	0	0

Embryos were injected with RNA encoding NFΔE or TAF1β, or coinjected with *NFΔE* RNA and three times the amount of *TAF1β* RNA. Embryos were then scored for the appearance of ectodermal blisters.

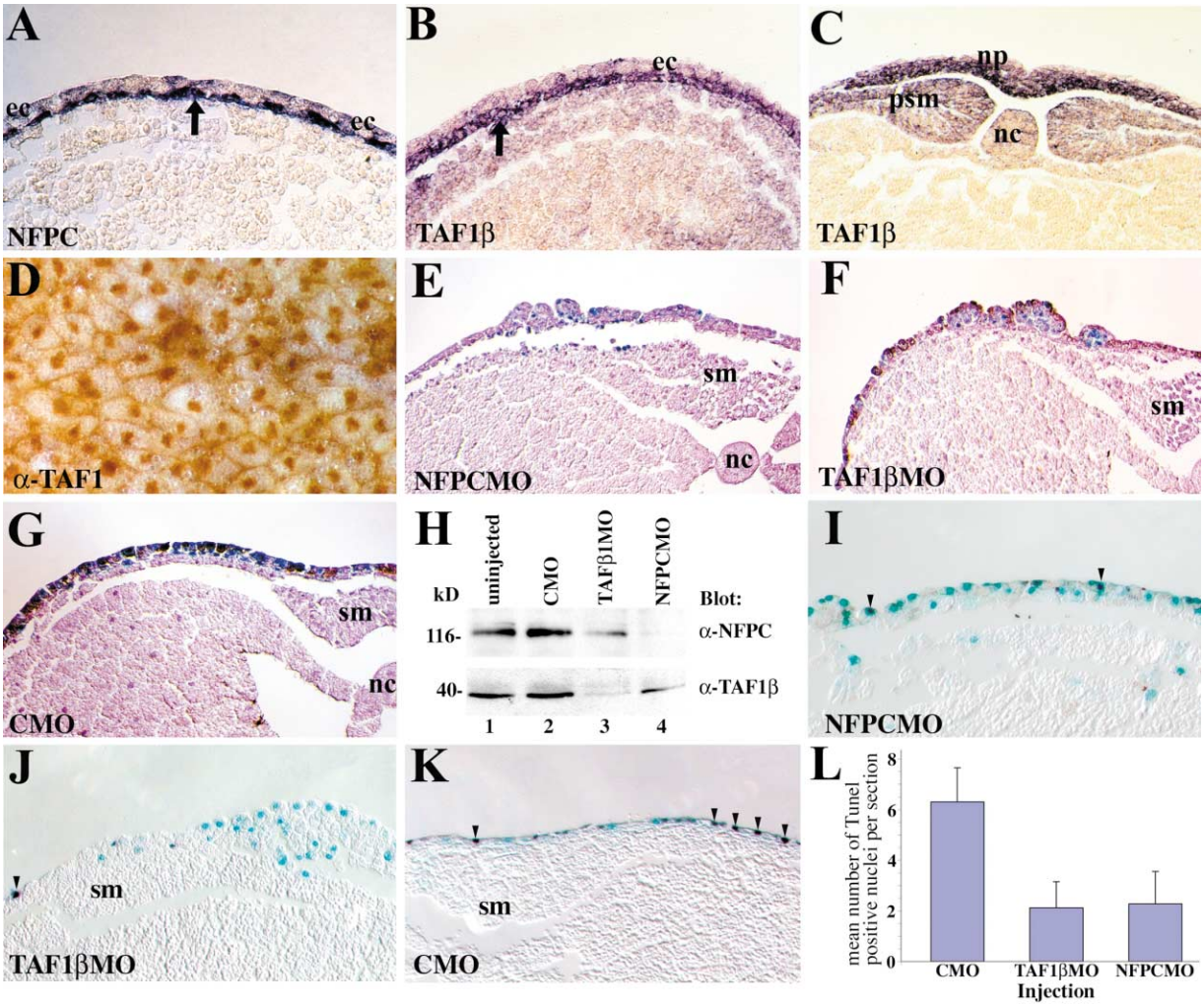

Figure 5. *Xenopus TAF1*β Is Expressed in the Embryonic Ectoderm and Is Required for Proper Ectodermal Differentiation

(A–C) Stage 17 embryos stained by in situ hybridization for NFPC (A) or *TAF1*β (B and C) and viewed in cross-section. *TAF1*β is expressed in the neural plate (np), ventral ectoderm (ec), and presomitic mesoderm (psm). Within the ventral ectoderm, *NFPC* and *TAF1*β show similar expression patterns, with both highly expressed in the inner layer (arrows).

(D) Stage 17 embryo immunostained with a TAF1 monoclonal antibody and viewed in whole-mount. TAF1 is expressed in both the nucleus and at the cell membrane.

(E–H) Antisense NFPC and TAF1β morpholinos disrupt ectodermal differentiation. Embryos were coinjected with NFPCMO (E), TAF1βMO (F), or CMO (G), together with *nLacZ* RNA, fixed at stage 22, stained for β-galactosidase activity, and sectioned. Compared to CMO-injected embryos, embryos injected with NFPCMO or TAF1βMO exhibit similar ectodermal defects.

(H) Immunoblot analysis of morpholino-injected embryos. Compared to uninjected (lane 1) or CMO-injected (lane 2) embryos, injection of TAF1βMO (lane 3) or NFPCMO (lane 4) significantly reduces the amount of TAF1β and NFPC protein present in the embryos, respectively.

(I–L) TUNEL assay for apoptotic nuclei in the ectoderm. Embryos were injected as in (E)–(G), stained for β-galactosidase activity, and then subjected to whole-mount TUNEL assay, sectioned, and analyzed for apoptotic nuclei (dark blue nuclei, arrowheads). NFPCMO-injected (I) and TAF1βMO-injected (J) embryos exhibit fewer apoptotic nuclei in the ectoderm, as compared to CMO-injected embryos (K).

(L) Results shown are the mean number of TUNEL-positive nuclei per section, averaged over 8–12 embryos per morpholino injection.

The following abbreviations were used: nc, notochord; sm, somite.

tissue morphogenesis. That at least some protocadherins function as cell adhesion molecules has been inferred from the sequence homology of their extracellular domains with that of classical cadherins. However, despite the large number of protocadherins recently discovered, information on their roles in development has been notably scarce. In *Xenopus*, NFPC is required for the proper formation of the embryonic ectoderm, and two other protocadherins, Paraxial (PAPC) and Axial protocadherin, also function in cell adhesion, playing important roles in the subdivision of the mesoderm into paraxial and axial domains, and in somite formation (Kuroda et al., 2002; Kim et al., 1998, 2000). However, little is known concerning the mechanism of action of these, or other, protocadherins. Evidence presented here demonstrates that NFPC mediates cell adhesion via novel mechanisms, utilizing the intracellular cofactor TAF1β, and suggests that TAF1β plays a distinct role in the development of the ectoderm in *Xenopus*. While both human TAF1α and -β are capable of interacting with NFPC, as suggested by the GST pull-down experiments, we have only been able to isolate and test *Xenopus*

TAF1β for its ability to bind NFPC. Whether a *Xenopus* TAF1α isoform is expressed in early embryos remains to be determined, though Matsumoto et al. (1999) were also unable to identify a TAF1α isoform from *Xenopus* egg extracts.

TAF1β Is a Cofactor of NFPC

GST pull-down experiments, as well as the coprecipitation studies, indicate that TAF1β can bind the cytoplasmic domain of NFPC. The region of the NFPC cytosolic domain that interacts with TAF1β lies within the membrane proximal 80 amino acids, as this region was found both to interact with TAF1β in the GST pull-down experiments and to be necessary for the disruption of the embryonic ectoderm. This suggests that the dominant-negative effect observed upon ectopic expression of NFΔE is due, in part, to competition with endogenous NFPC for the binding of intracellular TAF1β, which is confirmed by the ability of ectopic TAF1β to rescue the NFΔE-induced ectodermal blisters. Thus, TAF1β can function as a cytosolic cofactor of NFPC within the embryonic ectoderm, and is necessary for proper NFPC function. Interestingly, a construct in which all but the membrane proximal 20 amino acids are deleted (NFΔ1) could slightly disrupt the ectoderm when expressed in embryos, even though it was not capable of binding TAF1β (data not shown). While this region may not be directly involved in binding TAF1β, it may still perturb the binding of TAF1β to endogenous NFPC.

TAF1/Set was originally identified as part of a fusion protein activated in acute undifferentiated leukemia, and was shown to be identical to the template activating factor 1 (TAF1), a host-derived protein required for adenoviral replication, where it is thought that TAF1 interacts with viral basic core proteins to induce structural changes in the adenoviral genome (Adachi et al., 1994; Nagata et al., 1995; von Lindern et al., 1992). TAF1 has since been identified as a component of the INHAT (inhibitor of acetyltransferases) complex, which binds to histones and blocks their acetylation (Seo et al., 2001). In addition, TAF1 interacts with the neuronal Cdk5 kinase activation protein $p35^{nck5a}$, and the activity of Cdk5/$p35^{nck5a}$ is enhanced upon binding TAF1, leading to the hypothesis that TAF1 may function to modulate protein phosphorylation by Cdk5/$p35^{nck5a}$ (Qu et al., 2001). Thus, while the cellular role of TAF1 is not clearly defined, the many functions ascribed to it, including the ability of TAF1 to interact with NFPC, raises questions as to the intracellular localization of TAF1. Immunolocalization studies reveal that most TAF1 protein in a variety of cell types is found in the nucleus (Adachi et al., 1994), suggesting that, at least in cells in culture, little TAF1 protein is found at the plasma membrane. Indeed, immunolocalization of TAF1β protein in HeLa cells transfected with *Xenopus NFPC* shows that the majority of TAF1β is nuclear; however, there is a subset of TAF1β found at the cell membrane, where it colocalizes with NFPC. Similarly, in the *Xenopus* embryonic ectoderm, TAF1 protein can be found in both the nucleus and at the cell membrane, suggesting that the localization of TAF1β at the plasma membrane may depend on the presence of functional NFPC.

Role of TAF1 in Embryonic Ectoderm

In situ hybridization results demonstrate that *Xenopus TAF1β* is expressed in the inner layer of the embryonic ectoderm, where it colocalizes with *NFPC*, and injection of either TAF1βMO or NFPCMO results in virtually identical ectodermal defects, indicating that the two proteins play interconnected roles in the differentiation of the inner layer. In light of the previously reported functions of TAF1 in the nucleus and its ability to bind NFPC as reported here, the question arises as to the exact role of TAF1β in the ectoderm. Is TAF1β normally present at the plasma membrane of the ectodermal inner cells, where it functions as a required cofactor of NFPC, or is TAF1β present only in the nucleus under normal circumstances? In the case of the former, the ectodermal blisters caused by expression of NFΔE are likely the result of NFΔE competing with endogenous NFPC for the binding of TAF1β, thereby disrupting NFPC-mediated cell adhesion. In the latter case, the NFΔE-induced ectodermal blisters might be the result of NFΔE binding TAF1β and effectively removing TAF1β from the nucleus, thereby altering the differentiation of the inner layer cells. We favor the former explanation for several reasons: first, we have previously shown that the ectodermal blisters are specific to NFΔE, in particular, ectopic expression of full-length NFPC does not affect the inner layer cells (Bradley et al., 1998). If blisters were caused solely by the removal of TAF1β from the nucleus, one would expect that ectopic expression of either NFPC or NFΔE would result in the failure of the inner layer cells to properly differentiate. Second, the NFΔE-induced defects can be rescued by ectopic expression of NFPC (Bradley et al., 1998), indicating that it is competition for TAF1β binding the cytoplasmic domain of NFPC and the resultant loss in NFPC-mediated cell adhesion, which results in ectodermal blisters. Finally, our immunolocalization results demonstrate that a subset of endogenous TAF1 protein is normally present at the plasma membrane in ectodermal cells, where it could participate in NFPC-mediated cell adhesion. In this scenario, one function of TAF1β may be to recruit Cdk5/$p35^{nck5a}$ to the cell membrane, where it could phosphorylate NFPC or associated proteins, thereby modulating NFPC-mediated adhesion. In fact, Cdk5/$p35^{nck5a}$ does localize to the membrane in neurons, where it can associate with N-cadherin/β-catenin complexes (Kwon et al., 2000). In addition, Cdk5/$p35^{nck5a}$ can interact with Rac, a member of the Rho family of GTPases in neuronal growth cones (Nikolic et al., 1998). Thus, Cdk5/$p35^{nck5a}$ has been proposed to function in modulating cell adhesion and migration in neurons, by altering N-cadherin-mediated cell adhesion and/or cytoskeletal components. Whether Cdk5/$p35^{nck5a}$ functions in NFPC/TAF1β-mediated cell adhesion in the *Xenopus* ectoderm remains to be tested.

Given the roles of TAF1β in the nucleus and as a cofactor of NFPC, we cannot rule out that *Xenopus* TAF1β may represent a link between NFPC-mediated adhesion and changes in histone acetylation and transcriptional activation. A dual role for TAF1 is reminiscent of β-catenin in classical cadherin-mediated cell adhesion, as β-catenin not only binds to classical cadherins at the adherens junction but also can enter the nucleus in response to activation of the Wnt signaling pathway (reviewed in Miller et al., 1999; Sharpe et al., 2001).

β-catenin can exist in at least three pools within the cell, one bound to classical cadherins at the cell membrane, one bound to TCF transcription factors within the nucleus, and a cytosolic pool that can respond to changes in Wnt signaling or cellular adhesion. However, whether changes in cadherin-mediated cell adhesion can alter the relative abundance of β-catenin in the nucleus is not clear. Similarly, whether TAF1β can shift from a membrane to nuclear localization in response to changes in cell adhesion has yet to be determined. A detailed mapping of the TAF1β protein domains necessary for INHAT activity versus binding to NFPC may help elucidate its dual functions. For example, the ability of TAF1both to promote adenovirus replication and participate in INHAT activity has been shown to require the acidic C-terminal domain. Furthermore, a mutant TAF1β lacking the C terminus no longer localizes to the nucleus, but is found predominantly in the cytoplasm (Matsumoto et al., 1999; Nagata et al., 1998; Seo et al., 2001). Whether this region is also required for binding to NFPC is not known.

A requirement for TAF1β and NFPC in the embryonic ectoderm is underscored by experiments to deplete the two proteins during development. Injection of either the TAF1βMO or NFPCMO causes similar defects: an ectodermal bulge due to both an apparent increase in the number of cells in the ectoderm as well as a change in cell morphology. The increase in ectodermal cells is at least partially due to a decrease in programmed cell death, a process that may be important for the histogenesis of the epidermis. Generally, disruptions in cadherin-mediated cell adhesion are associated with an increase in apoptosis in certain cell lines (Chen et al., 2002; Herniston and Gordon, 1995; Kantak and Kramer, 1998). Why NFPCMO and TAF1βMO result in reduced apoptosis is currently under investigation, though one possibility is that by reducing NFPC-mediated cell adhesion, the ectodermal cells remain in an undifferentiated state, thereby preventing normal cell death associated with epidermal differentiation from occurring. While our evidence suggests that TAF1 functions in NFPC-mediated cell adhesion, at this point we cannot rule out the possibility that NFPC and TAF1 have an independent role in cell survival in the ectoderm. It is interesting to note that the defects caused by either morpholino differ from the ectodermal blisters caused by NFΔE (Bradley et al., 1998). While ectopic expression of NFΔE results in a loss of inner layer cells, NFPCMO and TAF1βMO alter ectodermal cell survival, resulting in an apparent increase in ectodermal cells. This difference may be due to the fact that the morpholinos and the dominant-negative construct disrupt NFPC function by different mechanisms: the morpholinos inhibit RNA translation, while the dominant-negative NFΔE construct blocks the interaction of TAF1β, and possibly other cytosolic proteins, with endogenous NFPC. It is possible that the dominant-negative is more effective at blocking NFPC function, particularly because immunoblot analysis of embryos injected with NFPCMO and TAF1βMO demonstrate low levels of corresponding protein remaining in embryos. Finally, it is also possible that NFΔE can block cell adhesion mediated by related, as yet unidentified, protocadherins present in the embryonic ectoderm, resulting in a more severe defect than that caused by disrupting NFPC alone.

In addition to the ectoderm inner cell layer, in situ hybridization analysis demonstrated that *Xenopus TAF1β* is present throughout the early neural plate and neural tube. *NFPC*, in comparison, is primarily restricted to a subset of motor neurons in the ventral neural tube (Bradley et al., 1998). What then is the role of TAF1 in the cells that do not express *NFPC*? It is possible that in these cells TAF1 functions independently of its role in cell adhesion, as a component of INHAT; however, it is also plausible that TAF1β may be a component of cell adhesion complexes mediated by other protocadherins. Given the extensive size of the protocadherin family, it is likely that other protocadherins are expressed in restricted regions of the neural tube, some of which may interact with TAF1β. Further studies to identify protocadherins expressed in the early nervous system, as well as an analysis of their mechanisms of action, will prove useful to understanding how protocadherins contribute to vertebrate neural development.

Experimental Procedures

Constructs and Antibodies

NFPC, NFΔE, and N-cadherin constructs (Bradley et al., 1998) were subcloned into CS2+MT (Turner and Weintraub, 1994) to give NFPC-MT, NFΔE-MT, and N-Cad-MT. NFΔE deletion constructs were generated by PCR, tagged with a C-terminal RGS-His_6 epitope (HT), and subcloned into CS2+. The *Xenopus* TAF1β coding region was obtained by PCR from a *Xenopus* stage 17 cDNA library (Kintner and Melton, 1987), tagged with a C-terminal HT, and subcloned into CS2+ (TAF1β-HT). NFPC antisera was generated by cloning the cytoplasmic domain of *Xenopus* NFPC into the pQE vector (Qiagen), with the resultant fusion protein purified on a Ni-NTA-agarose (Qiagen) column and used to immunize rabbits.

RNA Synthesis and Injection

Synthesis and injection of RNA was performed as described (Bradley et al., 1998). For coprecipitation experiments, two-cell stage embryos were injected with 10 pg RNA encoding NFPC-MT, NFΔE-MT, and/or TAF1-HT, and then harvested at stage 14 and processed as described. For rescue experiments, 10 pg *NFΔE-HT* RNA was injected alone or coinjected with three times the amount of *Xenopus TAF1β* RNA into a single blastomere at the 16-cell stage, and then analyzed at stage 14 for NFΔE-induced blisters.

Immunoblot Analysis and Coprecipitation Experiments

Injected embryos at stage 14 were dounced in lysis buffer A (10 mM HEPES [pH 7.4], 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1% Triton X-100, 1 mM PMSF, 1 μM aprotinin, 5 μM leupeptin, 1.5 μM pepstatin, 0.5 mM iodoacetamide, 15 μM antipain, 3 mM benzamidine) and spun at 10,000 × g. For Western analysis, the above supernatant was analyzed by SDS-PAGE and immunoblotted using an anti-HT antibody (Qiagen) and ECL reagents (Amersham). For coprecipitation studies, the supernatant fraction was incubated with 10 μl of a 1:1 slurry of Ni-NTA-agarose (Qiagen) in lysis buffer B (10 mM HEPES [pH 7.4], 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 20 mM imidazole, 1% Triton X-100) for 30 min at 4°C. Ni-NTA-agarose was then pelleted by centrifugation, washed five times in buffer B, and stripped in Laemmli buffer. Bound proteins were analyzed by immunoblot using the anti-myc monoclonal 9E10 (DSHB) or the anti-HT antibody. Alternatively, *NFPC* RNA-injected embryos were lysed and immunoprecipitated with an anti-TAF1β monoclonal antibody (Matsumoto et al., 1999) followed by immunoblot analysis using the TAF1β monoclonal antibody or the rabbit NFPC antisera.

GST Pull-Downs and Microsequencing

Cytoplasmic deletion constructs of NFΔE were subcloned into pGEX4T-3, expressed in BL21 bacteria, and the resultant GST-

fusion proteins purified on glutathione-agarose (Stratagene). Cytosolic extracts were prepared from 8 L of HeLa cells grown in spinning culture and lysis of the cell pellet by douncing in 50 ml hypotonic lysis buffer (10 mM HEPES [pH 7.9], 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 2 mM DTT, 0.1 mM PMSF, 1 μM aprotinin, 5 μM leupeptin, 1.5 μM pepstatin, 3 mM benzamidine). Nuclei and insoluble material were removed by centrifugation at 5000 rpm, and Triton X-100 was added to the supernatant to a final concentration of 1%, followed by centrifugation. A 100 μl aliquot of this second supernatant fraction was precleared with 25 μl of glutathione-agarose for 1 hr, and then incubated with 25 μl of one of the deletion constructs bound to glutathione-agarose for 30 min at 4°C. The agarose was then pelleted by centrifugation, washed in lysis buffer A, and the bound proteins were removed in Laemmli buffer and analyzed by SDS-PAGE followed by silver staining, or by immunoblot using an anti-human TAF1β rabbit polyclonal (Adachi et al., 1994).

For microsequencing, the above protocol was modified as follows: 500 μl HeLa cell extract was precleared with 50 μl glutathione-agarose, followed by a second preclearing in GST-PGEX bound to glutathione-agarose. The supernatant was incubated with 100 μl GST-NFΔ3 as above, and bound proteins were subjected to SDS-PAGE, transferred to PVDF membrane (MSI), and stained with amido black. HPLC-purified tryptic fragments of the 39 and 41 kDa bands were microsequenced using an ABI 470 protein sequencer (Fischer et al., 1991).

In Situ Hybridization and Immunohistochemistry

In situ hybridizations were performed using a probe corresponding to the coding region of *Xenopus* TAF1β, or an NFPC probe as described (Bradley et al., 1998). Immunohistochemistry of whole-mount embryos was performed as described (Bradley et al., 1998), using a TAF1α/β monoclonal antibody (Matsumoto et al., 1999). For confocal analysis, HeLa cells were grown on glass coverslips and transfected with NFPC-MT or N-Cad-MT using Effectene reagent (Qiagen), fixed after 24 hr, and permeabilized with PBS containing 0.1% Triton X-100. Cells were then incubated in the 9E10 myc antibody and anti-human TAF1β antisera, followed by Cy3- and FITC-conjugated secondary antibodies, and viewed on a Leica TSC/SP laser scanning confocal microscope.

Morpholino Experiments

Antisense morpholinos (GeneTools) comprised the sequences 5′-TCTGTGTCCCCTCAGTCCTCATCAT-3′ (NFPCMO) and 5′-CATGT TGTGTGTGAGGAGAGGTGGT-3′ (TAF1βMO), as well as a standard control morpholino (CMO). For BrdU and TUNEL analysis, 1–5 μM of morpholino was coinjected with *nLacZ* RNA into a single eight-cell blastomere, fixed at stage 22, and processed for β-galactosidase activity; for immunoblot analysis, morpholinos were injected into both cells of a two-cell embryo and harvested at stage 17. To examine mitosis, embryos were injected with BrdU (Hardcastle and Papalopulu, 2000) 2 hr prior to fixing, and then immunostained using an anti-BrdU antibody (Boehringer Mannheim). TUNEL assays were performed on whole-mount morpholino-injected embryos according to protocol (Hensey and Gautier, 1998). Embryos were serially sectioned and in every third section the number of TUNEL-positive nuclei per injected side was counted and an average was obtained for 8–12 embryos for each morpholino.

Acknowledgments

We thank T. Copeland and K. Nagata for reagents, C. Kintner and F. Lefcort for advice, and K. Jones, W. Fischer, and B. Christiansen for technical assistance. This study was supported by grants from NSF (0077235) and NCRR (P20-RR15583).

Received: July 1, 2002
Revised: January 21, 2003

References

Adachi, Y., Pavlakis, G.N., and Copeland, T.D. (1994). Identification and characterization of SET, a nuclear phosphoprotein encoded by the translocation break point in acute undifferentiated leukemia. J. Biol. Chem. *269*, 2258–2262.

Angst, B.D., Marcozzi, C., and Magee, A.I. (2001). The cadherin superfamily: diversity in form and function. J. Cell Sci. *114*, 629–641.

Arndt, K., and Redies, C. (1998). Development of cadherin-defined parasagittal subdivisions in the embryonic chicken cerebellum. J. Comp. Neurol. *401*, 367–381.

Bradley, R.S., Espeseth, A., and Kintner, C. (1998). NF-protocadherin, a novel member of the cadherin superfamily, is required for *Xenopus* ectodermal differentiation. Curr. Biol. *8*, 325–334.

Chen, M.W., Vacherot, F., de la Taille, A., Gil-Diez-de-Medina, S., Shen, R., Friedman, R.A., Burchardt, M., Chopin, D.K., and Buttyan, R. (2002). The emergence of protocadherin-PC expression during the acquisition of apoptosis-resistance by prostate cancer cells. Oncogene *21*, 7861–7871.

Fischer, W.H., Karr, D., Jackson, B., Park, M., and Vale, W. (1991). Microsequence analysis of proteins purified by gel electrophoresis. In Methods in Neurosciences, M.P. Conn, ed. (San Diego: Academic Press), pp. 69–84.

Frank, M., and Kemler, R. (2002). Protocadherins. Curr. Opin. Cell Biol. *14*, 557–562.

Gumbiner, B.M. (1996). Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell *84*, 345–357.

Gumbiner, B.M. (2000). Regulation of cadherin adhesive activity. J. Cell Biol. *148*, 399–403.

Hardcastle, Z., and Papalopulu, N. (2000). Distinct effects of XBF-1 in regulating the cell cycle inhibitor $p27^{XIC1}$ and imparting a neural fate. Development *127*, 1303–1314.

Hensey, G., and Gautier, J. (1998). Programmed cell death during *Xenopus* development: a spatio-temporal analysis. Dev. Biol. *203*, 36–48.

Hermiston, M.L., and Gordon, J.I. (1995). In vivo analysis of cadherin function in the mouse intestinal epithelium: essential roles of adhesion, maintenance of differentiation, and regulation of programmed cell death. J. Cell Biol. *129*, 489–506.

Hirano, S., Ono, T., Yan, Z., Wang, X., Sonta, S., and Suzuki, S.T. (1999). Protocadherin 2C: a new member of the protocadherin 2 subfamily expressed in a redundant manner with OL-protocadherin in the developing brain. Biochem. Biophys. Res. Commun. *260*, 641–645.

Kantak, S.S., and Kramer, R.H. (1998). E-cadherin regulates anchorage-independent growth and survival in oral squamous cell carcinoma cells. J. Biol. Chem. *273*, 16953–16961.

Kim, S.H., Yamamoto, A., Bouwmeester, T., Agius, E., and Robertis, E.M. (1998). The role of paraxial protocadherin in selective adhesion and cell movements of the mesoderm during *Xenopus* gastrulation. Development *125*, 4681–4690.

Kim, S.H., Jen, W.C., DeRobertis, E.M., and Kintner, C. (2000). The protocadherin PAPC establishes segmental boundaries during somitogenesis in *Xenopus* embryos. Curr. Biol. *10*, 821–830.

Kintner, C.R., and Melton, D.A. (1987). Expression of *Xenopus* N-CAM RNA in ectoderm is an early response to neural induction. Development *99*, 311–325.

Koch, A.W., Bozic, D., Pertz, O., and Engel, J. (1999). Homophilic adhesion by cadherins. Curr. Opin. Struct. Biol. *9*, 275–281.

Kuroda, S., Fukata, M., Nakagawa, M., Fujii, K., Nakamura, T., Ookubo, T., Izawa, I., Nagase, T., Nomura, N., Tani, H., et al. (1998). Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin-mediated cell-cell adhesion. Science *281*, 832–835.

Kuroda, H., Inui, M., Sugimoto, K., Hayata, T., and Asashima, M. (2002). Axial protocadherin is a mediator of prenotochord cell sorting in *Xenopus*. Dev. Biol. *244*, 267–277.

Kwon, Y.T., Gupta, A., Zhou, Y., Nikolic, M., and Tsai, L. (2000). Regulation of N-cadherin-mediated adhesion by the p35-Cdk5 kinase. Curr. Biol. *10*, 363–372.

Matsumoto, K., Nagata, K., Miyaji-Yamaguchi, M., Kikuchi, A., and Tsujimoto, M. (1999). Sperm chromatin decondensation by template

activating factor I through direct interaction with basic proteins. Mol. Cell. Biol. *19*, 6940–6952.

Miller, J.R., Hocking, A.M., Brown, J.D., and Moon, R.T. (1999). Mechanisms and function of signal transduction by the Wnt/β-catenin and Wnt/Ca2+ pathways. Oncogene *18*, 7860–7872.

Nagata, K., Kawase, H., Handa, H., Yano, K., Yamasaki, M., Ishimi, Y., Okuda, A., Kikuchi, A., and Matsumoto, K. (1995). Replication factor encoded by a putative oncogene, set, associated with myeloid leukemogenesis. Proc. Natl. Acad. Sci. USA *92*, 4279–4283.

Nagata, K., Saito, S., Okuwaki, M., Kawase, H., Furuya, A., Kusano, A., Hanai, N., Okuda, A., and Kikuchi, A. (1998). Cellular localization and expression of template-activating factor I in different cell types. Exp. Cell Res. *240*, 274–281.

Nikolic, M., Chou, M.M., Lu, W., Mayer, B.J., and Li-Huei, T. (1998). The p35/Cdk5 kinase is a neuron-specific Rac effector that inhibits Pak1 activity. Nature *395*, 194–198.

Pertz, O., Bozic, D., Koch, A.W., Fauser, C., Brancaccio, A., and Engel, J. (1999). A new crystal structure, Ca2+ dependence and mutational analysis reveal molecular details of E-cadherin homoassociation. EMBO J. *18*, 1738–1747.

Qu, D., Qing, L., Lim, H., Cheung, N.S., Li, R., Wang, J.H., and Qi, R.Z. (2001). Protein SET binds neuronal Cdk5 activator $p35^{nck5a}$ and modulates Cdk5/$p35^{nck5a}$ activity. J. Biol. Chem. *277*, 7324–7332.

Sano, K., Tanihara, H., Heimark, R.L., Obata, S., Davidson, M., St. John, T., Taketani, S., and Suzuki, S. (1993). Protocadherins: a large family of cadherin-related molecules in central nervous system. EMBO J. *12*, 2249–2256.

Seo, S., McNamara, P., Heo, S., Turner, A., Lane, W.S., and Chakravarti, D. (2001). Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncogene. Cell *104*, 119–130.

Shapiro, L., and Colman, D.R. (1999). The diversity of cadherins and implications for a synaptic adhesive code in the CNS. Neuron *23*, 427–430.

Sharpe, C., Lawrence, N., and Martinez Arias, A. (2001). Wnt signaling: a theme with nuclear variations. Bioessays *23*, 311–318.

Stappert, J., and Kemler, R. (1994). A short core region of E-cadherin is essential for catenin binding and is highly phosphorylated. Cell Adhes. Commun. *2*, 319–327.

Suzuki, S.T. (2000). Recent progress in protocadherin research. Exp. Cell Res. *261*, 13–18.

Takeichi, M., Uemura, T., Iwai, Y., Uchida, N., Inoue, T., Tanaka, T., and Suzuki, S.C. (1997). Cadherins in brain patterning and neural network formation. Cold Spring Harb. Symp. Quant. Biol. *62*, 505–510.

Tepass, U., Truong, K., Godt, D., Ikura, M., and Peifer, M. (2000). Cadherins in embryonic and neural morphogenesis. Nat. Rev. Mol. Cell Biol. *1*, 91–100.

Turner, D.L., and Weintraub, H. (1994). Expression of acheate-scute homolog 3 in *Xenopus* embryos converts ectodermal cells to a neural fate. Genes Dev. *8*, 1434–1447.

von Lindern, M., van Baal, S., Wiegant, J., Raap, A., Hagemeijer, A., and Grosveld, G. (1992). can, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3′ half to different genes: characterization of the set gene. Mol. Cell. Biol. *12*, 3346–3355.

Yap, A., Niessen, C., and Gumbiner, B. (1998). The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening, and interaction with p120ctn. J. Cell Biol. *141*, 779–789.

Yoshida, K., Yoshitomo-Nakagawa, K., Seki, N., Sasaki, M., and Sugano, S. (1998). Cloning, expression analysis, and chromosomal localization of BH-protocadherin (PCDH7), a novel member of the cadherin superfamily. Genomics *49*, 458–461.

Yoshida, K., Hida, M., Watanabe, M., Yamaguchi, R., Tateyama, S., and Sugano, S. (1999). cDNA cloning and chromosomal mapping of mouse BH-protocadherin. DNA Seq. *10*, 43–47.