Nesprin 4 is an outer nuclear membrane protein that can induce kinesin-mediated cell polarization

Kyle J. Roux1,a, Melissa L. Crispa1, Qian Lia, Daein Kimb, Serguei Kozlovb, Colin L. Stewartc, and Brian Burkea,2

1Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL 32610; 2Institute of Medical Biology, Immunos, 8A Biomedical Grove, Republic of Singapore 138648; and 3Center for Advanced Preclinical Research and Mouse Cancer Genetics Program, SAIC-Frederick, Inc., National Cancer Institute, Frederick, MD 21702

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Nucleocytoplasmic coupling is mediated by outer nuclear membrane (ONM) nesprin proteins and inner nuclear membrane Sun proteins. Interactions spanning the perinuclear space create nespin–Sun complexes connecting the cytoskeleton to nuclear components. A search for proteins displaying a conserved C-terminal sequence present in nesprins 1–3 identified nesprin 4 (Nesp4), a new member of this family. Nesp4 is a kinesin-1-binding protein that displays Sun-dependent localization to the ONM. Expression of Nesp4 is associated with dramatic changes in cellular organization involving relocation of the centrosome and Golgi apparatus relative to the nucleus. These effects can be accounted for entirely by Nesp4's kinesin-binding function. The implication is that Nesp4 may contribute to microtubule-dependent nuclear positioning.

The nuclear envelope (NE) forms the interface between the nucleus and cytoplasm acting as a selective barrier that regulates nucleo-cyttoplasmic traffic (1). In addition to this partition function, accumulating evidence reveals an essential role for the NE as a determinant of higher-order nuclear and chromatin organization (2). More surprising are findings that the NE directly impacts cytoskeletal architecture and in this way may help define the mechanical properties of the cell as a whole (3). The molecular bases for these effects are now emerging, uniting aspects of cellular physiology from mechanotransduction to nuclear positioning during differentiation and development (4).

The NE is assembled from several elements, the most prominent being inner nuclear membranes (INMs) and outer nuclear membranes (ONMs) separated by a ~40-nm gap or perinuclear space (PNS). The INM and ONM are joined where they are spanned by nuclear pore complexes (NPCs), the mediators of trafficking across the NE. The ONM also displays connections to the peripheral endoplasmic reticulum (ER). Accordingly, the INM, ONM, and ER represent a single membrane system with the PNS forming an extension of the ER lumen.

The final feature of the NE is the nuclear lamina, a protein meshwork composed primarily of A- and B-type lamins that lines the nuclear face of the INM (2). The lamina is required for NE integrity and provides chromatin-anchoring sites at the nuclear periphery. Remarkably, aberrant A-type lamin expression, which is linked to several human diseases (5), is associated with altered cytoskeletal mechanics (3). The mechanisms underlying this phenomenon represent an intriguing biological problem.

At least 60 NE membrane proteins are known (6). Although most likely reside in the INM, several ONM proteins have been identified (7). These include members of the mammalian nesprin (or syne) family (8, 9), Klarsicht (10) and Msp-300 (11, 12) in Drosophila melanogaster, Anc-1 (13), Zyg-12 (14) and Unc-83 (15, 16) in Caenorhabditis elegans, and Kms2 in the fission yeast Schizosaccharomyces pombe (17). A property that each of these has in common is that they interact with cytoskeletal components. They are also united in possessing a conserved ~50- to 60-residue C-terminal KASH domain (Klarsicht, Anc-1, Syne homology) featuring a single transmembrane segment followed by a short luminal sequence. Localization of ONM KASH proteins depends on tethering by SUN domain proteins of the INM (18). This tethering, involving interactions spanning the PNS, was originally suggested based on findings that localization of Anc-1 depends on an INM protein, Unc-84 (19, 20), a prototype member of the SUN family.

Three mammalian nesprin genes are known [nesprins 1–3 (7)]. For nesprins 1 and 2, the primary transcripts encode a plethora of alternatively-spliced isoforms (9). The largest of these, nesprin 1 Giant (Nesp1G; ~1,000 kDa) and nesprin 2 Giant (Nesp2G; ~800 kDa), reside in the ONM. Smaller isoforms may be found in the INM (21, 22) and elsewhere. The large flexible cytoplasmic domains of Nesp1G and Nesp2G each feature an N-terminal actin binding domain (ABD) followed by multiple spectrin repeats. The third mammalian nesprin (Nesp3) contains an N-terminal binding site for plectin, a cytolinker molecule that may provide a bridge to the intermediate filament system (23).

The nesprins are tethered in the ONM by a pair of Unc-84-related INM proteins, Sun1 and Sun2 (24–27). Because these also interact with nuclear components, including A-type lamins (24, 25, 28), and a histone acetyl transferase [in the case of Sun1 (29)], Sun-nesprin pairs represent links in a molecular chain connecting the cytoskeleton to nuclear structures. We refer to these as LINC complexes (linker of the cytoskeleton and nucleoskeleton). The emerging theme is that all KASH proteins have one or more complementary SUN proteins that function as tethers. In this way, multiple LINC isoforms may exist in and between species.

Are nesprins 1–3 the only mammalian KASH proteins that contribute to the LINC repertoire? We sought to answer this question by searching for proteins containing KASH-like sequences. We report here the characterization of an epithelial-specific KASH protein that represents a fourth branch of the nesprin family and is noteworthy in its ability to bind kinesin-1. Furthermore, its expression is associated with a dramatic separation of the nucleus and centrosome, indicating a possible role in microtubule-dependent nuclear positioning.

Results and Discussion

To identify new nesprin family members we performed a BLASTP search using the human Nes2 KASH domain (KASH2) as probe. This search identified an unknown mouse protein (NP_705850) of 388 amino acid residues and predicted molecular mass of 42 kDa (Fig. L4). Comparison of cDNA


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2To whom correspondence may be addressed. E-mail: kroux@ufl.edu or bburke@ufl.edu.

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However, exclude the possibility that a population of Nesp4 may have the same topology as Nesp1–3 (Fig. S1). We cannot, HA–Nesp4 must be exposed on the ONM. Thus, Nesp4 must versus Triton X-100 (30), revealed that the N terminus of stably expressing HA–Nesp4 (HSG-HAN4), using digitonin nesprin 4 (Nesp4). Differential permeabilization of HSG cells presents an additional member of the mammalian nesprin family, D

Fig. 1. Identification of nesprin 4. (A) Amino acid sequence of mouse protein NP.705805, now designated nesprin 4 (Nesp4). Nesp4 contains a leucine zipper (L Zip; blue), a spectrin repeat (red), and a KASH domain (KASH4; blue highlight). The putative transmembrane sequence (TM) is underlined. (B) Alignment of the KASH domains (KASH1–4) from nesprins 1–3 (Fig. S1) and nesprin 4 (Nesp4) gene (eight exons, 4.2 kb) and protein domain organization. (C) Immunofluorescence microscopy of HSG cells stably expressing HA–Nesp4. The cells were labeled with antibodies against HA and Sun2. DNA is visualized with Hoechst dye. (Bar: 10 μm.)

Localization of Nesp4 to the NE is KASH-dependent. Replacement of the KASH domain with GFP (Nesp4KASH–GFP) leads to a cytoplasmic and nuclear distribution (Fig. 2A). Insertion of a GST module between the Nesp4 and GFP sequences (Nesp4ΔKASH–GST–GFP) results in an exclusive cytoplasmic localization. Presumably its greater bulk limits nuclear entry. Replacement of the luminal portion of the KASH domain with GFP (Nesp4ΔLum–GFP) also results in loss of NE association. However, because the predicted transmembrane domain remains intact, the mutant protein becomes distributed throughout what appears to be the peripheral ER. Conversely, GFP–KASH4, generated by fusion of the Nesp4 KASH domain (KASH4) to the C terminus of GFP, localizes to the NE (Fig. 2A). These data indicate that the KASH domain is both necessary and sufficient for the ONM localization of Nesp4. This view is reinforced by findings that GFP–KASH4 or GFP–Nesp4 will displace HA–Nesp4, endogenous Nesp2G (Fig. S2), and Nesp3 by RNAi leads to loss of NE-associated GFP-KASH4. In the merged images, DNA (blue) was revealed by costaining with Hoechst dye. (Bars: 10 μm.)

Localization of GFP–KASH4 or GFP–Nesp4 to the ONM is SUN-dependent and can be abolished by expression of SS–HASun1LKDEL (Fig. 2B), a SUN protein dominant negative mutant (24). Similarly, depletion of Sun1/2 by RNA interference results in failure to retain GFP–KASH4 at the NE (Fig. 2C). Our conclusion is that Nesp4 is tethered in the ONM by SUN–KASH interactions and that it defines additional mammalian LINC complex isoforms.

Fig. 2. SUN- and KASH-dependent localization of Nesp4. (A) HeLa cells transiently transfected with either GFP-tagged full-length Nesp4 (GFP–Nesp4) or a variety of GFP-tagged deletion mutants (Nesp4ΔKASH–GFP, Nesp4ΔKASH–GST–GFP, and Nesp4ΔLum–GFP). Deletion of the KASH domain or the luminal portion of the KASH domain prevents localization to the NE. Conversely, GFP fused to the KASH domain (GFP–KASH4) localizes to the NE as revealed by colabeling with an antibody against the NPC protein Nup153. (B) Transfection of SS–HA–Sun1L–KDEL into HeLa cells stably expressing GFP–KASH4 displaces GFP–KASH4 from the NE. (C) Depletion of Sun1 and Sun2 by RNAi leads to loss of NE-associated GFP-KASH4. In the merged images, DNA (blue) was revealed by costaining with Hoechst dye. (Bars: 10 μm.)
However, endogenous Nesp4 was not detected in any common cell line. We therefore surveyed multiple mouse tissue cryosections by immunofluorescence microscopy. Surprisingly, most tissues displayed no evidence of Nesp4 expression. However, Nesp4 was present in the NEs of salivary gland, exocrine pancreas, bulbourethral gland, and mammary tissue (Fig. 3C). The occasional kidney cell was also Nesp4-positive. These data imply that Nesp4 is restricted mainly to secretory epithelia. Northern blot analysis of pregnant mouse mammary RNA revealed a 1.4- to 1.7-kb transcript, consistent in size with Nesp4 cDNA sequences (Fig. S3). Quantitative RT-PCR using multiple primers gave no evidence for alternative splicing (data not shown).

Based on these findings, we examined Nesp4 expression in HC11 mouse mammary cells (31). Upon hormonal induction, HC11 cells differentiate to yield a morphologically heterogeneous population of mammary cells (31). Upon hormonal induction, HC11 cells differentiate to yield a morphologically heterogeneous population secreting milk proteins. Undifferentiated HC11 cultures contain few Nesp4-positive cells (Fig. 3D). However, during the 1- to 2-week differentiation process Nesp4-positive cells accumulate until they represent 40–50% of the population. In all of these cells, Nesp4 is localized exclusively to the NE (Fig. 3D). Additional observations of HC11 cells reveal many that lack Nesp4 but still acquire an alternative splicing pattern (data not shown).

We used anti-GFP immunoprecipitation (IP) of Nesp4ΔKASH–GFP (Fig. 2) expressed in HEK293 cells to identify Nesp4-interacting proteins. In pilot IPs using 35S-labeled cells, in addition to Nesp4ΔKASH–GFP itself, a faint band of 120 kDa was consistently observed (Fig. 4A). This band was absent from IPs containing GFP alone. Scale-up of the IPs and analysis by mass spectrometry revealed that the 120-kDa band contained Kif5B, the heavy-chain subunit of kinesin-1 [also known as conventional kinesin or KIF5 (32)]. Parallel yeast two-hybrid analysis (33), using a membrane proximal region of Nesp4 (residues 129–339) as bait, was performed by Myriad Genetics. This analysis revealed kinesin light chains (KLCs) 1, 2, 3, and 4 as candidate Nesp4 binding partners. Together, these data imply that Nesp4 is a kinesin-binding protein. To verify that we immunoprecipitated Nesp4ΔKASH–GFP, Nesp4AKASH–GST–GFP, and GFP alone from HeLa cells. IPs were analyzed by Western blot using anti-Kif5B. Fig. 4B shows that Kif5B is found in a complex with the Nesp4 cytoplasmic domain. In all likelihood, given the two-hybrid data, formation of this complex is mediated by the KLCs. This notion is supported by the finding that a Nesp4 deletion mutant [Nesp4-GFP(207–307)] encompassing 200 residues contained within the two-hybrid bait construct, will also coimmunoprecipitate with Kinesin-1 (Fig. 4C). A mutant lacking this region [GFP-Nesp4(3–206)] shows no such interaction.

If Nesp4 binds kinesin via the light chains it should recruit KLCs to the NE. We therefore introduced a fluorescent protein-tagged version of KLC1 (mCit–HA–KLC1 (34)) into HeLa cells. mCit–HA–KLC1 alone adopts a generalized cytoplasmic distribution (Fig. 4E). However, coexpression with HA–Nesp4, but not HA–Nesp3 (or Nesp1α; data not shown), causes accumulation of mCit–HA–KLC1 at the NE (Fig. 4F and G). Similar results were obtained with a KLC mutant [Fig. 4H; HA–KLC(TPR6)] containing the presumptive cargo-binding tetra-tripeptide repeat (TPR) domain but lacking the heptad repeat region that interacts with the kinesin heavy chain (35). In contrast, KLC lacking the TPR domain [Fig. 4l; HA–KLC(1–176)] is unaffected by Nesp4 expression. Evidently Nesp4 interacts specifically with the KLC TPR domain. Additional evidence for this interaction between Nesp4 and KLC-1 is provided by the finding that Nesp4ΔKASH–GFP will coimmunoprecipitate with mCit–HA–KLC1 when both recombinant proteins are coexpressed in HeLa cells (Fig. 4D). Based on these findings we would predict that if Nesp4 does indeed function as a binding partner for kinesin-1, then endogenous kinesin should be detectable at the NE of differentiated HC11 cells. Immunofluorescence microscopy of such cells using antibodies against both Nesp4 and Kif5b suggests that this is indeed the case (Fig. S5).

What significance can be attached to the Nesp4–kinesin-1 association? We speculate that Nesp4 could contribute to microtubule-dependent nuclear positioning in secretory epithelial cells. A feature of such cells is that they contain lateral bundles of microtubules that extend from the apical surface with their plus ends oriented toward the basal membrane (36). Nesp4-mediated recruitment to the NE of kinesin-1, a plus-end-directed motor protein, would be predicted to promote nuclear migration toward the base of the cell (Fig. 4J). At present we have no epithelial cell system in which to examine this proposed function for Nesp4. Although differentiated HC11 cells do express Nesp4, their heterogeneity makes them unsuitable for any study of epithelial morphogenesis (Fig. S4). Nonetheless, we can use nonpolarized cells to determine how Nesp4 might affect the interaction of the nucleus with the cytoskeleton and whether it might induce changes in cytoarchitecture. When expressed in HSG cells, both HA–Nesp4 and GFP–Nesp4 usually display a uniform distribution about the nuclear surface. However, in a minority of cells HA–Nesp4 and GFP–Nesp4, but not GFP–KASH4, accumulate at a pole of the nucleus that is invariably distal to the centrosome. The effect is most prevalent in HeLa cells (~50% of cells are affected; Fig. 5A–C and Fig. S6) where expression of Nesp4 leads to the polarization of other NE components, including lamin and NPCs (Fig. 5B and C). This effect may be prevented by treatment of the cells with nocodazole (data not shown) or...
overexpression of mCit–HA–KLC1 (Fig. 5A). The latter should behave in a dominant negative fashion by saturating both kinesin heavy chains and Nesp4. Because microtubules are anchored at the centrosome by their minus ends, the polarization of Nesp4 may be explained by its kinesin-1-mediated anterograde movement across the nuclear surface. In other words, Nesp4 behaves as a kinesin cargo, providing a functional binding site for kinesin-1 at the NE.

If the nucleus is engaged with centrosomal microtubules via Nesp4 and kinesin-1, we would predict that this interaction could drive the separation of the nucleus from the centrosome (Fig. 4K). In fact, this is exactly what occurs. In nontransfected HeLa cells or HeLa cells expressing GFP–KASH4, the centrosome is located within 2 μm of the nucleus (Fig. 5A, D, and E and Fig. S6). Expression of either HA-Nesp4 or GFP-Nesp4 increases this distance to ~13 μm (Fig. 5A and D–F and Fig. S4). Indeed, values of 40–50 μm are not unusual. To put these numbers in perspective, loss of emerin or A-type lamins leads to a 3- to 4-μm separation between the centrosome and nucleus (37, 38). Our findings suggest that Nesp4 expression does not simply disrupt centrosome tethering, but actively drives the separation of these structures. Furthermore, it can be repressed by coexpression of mCit–HA–KLC1 (Fig. 5F). The only reasonable conclusion is that this effect of Nesp4 is mediated by recruitment of kinesin-1 to the NE (Fig. 4K). Whether the nucleus moves relative to the centrosome or vice versa is unknown. However, studies on centrosome reorientation in migrating fibroblasts suggests that the centrosome itself might remain relatively immobile (39).

In nonepithelial cells, the Golgi apparatus displays a peri-nuclear localization in association with the centrosome (40). Given its effect on centrosome/nuclear positioning, the prediction is that introduction of Nesp4 would cause similar dislocation of the Golgi apparatus. Again, this is precisely what happens. Cells expressing Nesp4, but not GFP–KASH4, display obvious disengagement of the Golgi apparatus from the nucleus (Fig. 5G). Indeed we have observed Nesp4-expressing cells in which the Golgi apparatus lies adjacent to the plasma membrane at the cell margins. This raises the question of whether it might lead to perturbations in membrane trafficking, particularly with respect to the distribution and targeting of exocytic vesicles and the delivery of membrane and secretory proteins to the plasma membrane. Clearly expression of a single ONM protein can induce dramatic changes in cytoarchitecture, effects that are unprecedented for a NE protein. These observations raise the possibility that Nesp4 might indeed contribute to the establishment of secretory epithelial morphology, by promoting kinesin-dependent apical migration of the centrosome and Golgi apparatus and basal localization of the nucleus. We should be in a better position to explore this possibility as mice rendered deficient in Nesp4 become available.

In conclusion, we have identified Nesp4 as a kinesin-1-binding protein of the NE and have been able to demonstrate clear consequences of this binding in vivo. The identification of Nesp4 now completes the association between various mammalian LINC complex isoforms and all major branches of the cytoskeleton. However, Nesp4 is not the only member of the nesprin family to interact with a kinesin. A Nesp1 isoform binds kinesin-2, mediating the transport of membrane to the midbody of cells undergoing cytokinesis (41). This finding raises the possibility that other nesprins at the NE might also bind kinesins. Consequently, Nesp4-expressing cells may not be unique in recruiting kinesin to the NE.
goat anti-kinase 1 (ab15075; AbCam). Polyclonal rabbit anti-Nesp4 was raised against a GST-Nesp4(1–90) fusion protein by Rockland Immunochemicals. GST-fusion protein purification and affinity purification of antisera was performed as described (24). Secondary antibodies, conjugated with AlexaFluor dyes or peroxidase, were from BioSource International/Invitrogen.

Cell Culture and Transfections.  HeLa, HSG, and HEK293 cells were maintained in 6.0% CO2 at 37 °C in DMEM supplemented with 10% FBS, 10% penicillin/streptomycin, and 2 mM L-glutamine. Transfections were performed by using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. HC11 cells (provided by Kermit Caraway, University of Miami, Miami) were maintained and differentiated as described (31).

Generation of Stable Cell Lines.  HSG cells stably expressing HA–Nesp4 and HeLa cells stably expressing GFP–KASH4 were generated by G418 (600 μg/mL) selection and subcloning. HEK293 cells stably expressing GFP or Nesp4AKASH–GFP were generated by retroviral transduction. Briefly, the appropriate pLNCX2 plasmid DNA was transiently transfected into the amphotropic retroviral packaging cell line Phoenix A (obtained from Garry Nolan, Stanford University, Stanford, CA). Infective supernatant was recovered 48 h after transfection. Retroviral transduction of HEK293 cells was performed in the presence of 4 μg/mL polybrene. Infected cells were selected with G418.

Immunofluorescence Microscopy.  Cells grown on glass coverslips were fixed in 3% formaldehyde (prepared in PBS from paraformaldehyde, PFA/PBS) and immunolabeled as appropriate (24). To quantify separation of the centrosome/Golgi apparatus from the nucleus the distance from the centrosome to the nearest point on the nuclear periphery was measured by using iPLab Spectrum software (BD Biosciences) calibrated with a stage micrometer.

Tissue Imaging.  Tissues removed from mice after CO2 asphyxiation were frozen in liquid nitrogen-cooled 2-methyl butane, and sections (10 μm) were cut by cryostat and mounted on glass slides. Sections were fixed with 3% PFA/PBS for 10 min, permeabilized with 0.2% Triton X-100, and processed as described (45). Images were collected by using a Leica TCS SPS confocal microscope system running Leica Application Suite 1.8.2 software.

ImmunobLOTS and IPs.  For immunobLOTS, proteins were fractionated by SDS/PAGE and analyzed by Western blot (46). For radiolabeled IPs, 3.5-cm plates of HEK293 cells transiently expressing Nesp were labeled with 50 μCi of 35S-translabel (MP Biomedicals) for 16 h before lysis in 0.5 mL of buffer [150 mM NaCl, 50 mM Tris (pH 7.4), 2.5 mM MgCl2, 0.5% Triton X-100, 1 mM DTT, 10 μg/mL each of chymostatin, leupeptin, antipain, and pepstatin, and 1 mM PMSF]. Lysates were passed through a 21-gauge needle (10×) and centrifuged 16,000 × g for 10 min at 4 °C. The supernatants were rotated for 4 h at 4 °C with protein A Sepharose beads (Sigma) coupled to rabbit anti-GFP. Samples were analyzed by SDS/PAGE and fluorography (46). For the larger-scale IPs, 10-cm plates of HEK293 cells were lysed in 1 mL of buffer and processed as described above. After SDS/PAGE, radiolabeled samples were processed as described (46). A gel with nonradiolabeled samples was silver-stained, and bands unique to the NespAKASH–GFP IP were excised and submitted to the University of Florida’s Interdisciplinary Center for Biotechnology Research proteomics laboratory for tryptic digest before mass spectrometry analysis (QTRAP 4000; Applied Biosystems). Tandem mass spectrophotometric data were searched by using Mascot version 2.2 (Matrix Science) and compiled by using Scaffold 1.7 (Proteome Software).

Northern Blot Analysis.  Twenty micrograms of total RNA samples isolated from mouse mammary tissue were analyzed by Northern blot using a32P-labeled probe targeting the 3′-untranslated region of nesp (residues 222–289). Reverse transcription and PCR were carried out as described (39). Products were fractionated on 6% polyacrylamide-6.0% formaldehyde gels. Gels were stained with silver nitrate and dried and hybridized with 32P-labeled probe. Membranes were exposed to PhosphorImager screens, and bands were quantified using ImageQuant software (Molecular Dynamics).

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Both our findings and those from other laboratories make it increasingly evident that NE represents a nexus of cytoskeletal interactions (7). The implication, of course, is that perturbations in the structure and composition of the NE may have far-reaching effects on cell and tissue organization.

Materials and Methods
Plasmids. A mouse nesp4 cDNA (clone ID 5036575) was obtained from Invitrogen. HA–Nesp4 was inserted into pcDNA 3.1(−). GFP–Nesp4 and GFP–KASH4 were inserted into pEGFP-C1 (Clontech), Nesp4AKASH–GFP, Nesp4AKASH—GST–GFP, and Nesp4AKASH–GFP were inserted into pEGFP-N1 (Clontech), and GFP and Nesp4AKASH–GFP were inserted into pLNCX2 (Clontech), all by standard PCR cloning. GFP–KASH1 and GFP–KASH2 were a gift from Arnoud Sonnenberg (Netherlands Cancer Institute, Amsterdam).

Antibodies. The following antibodies were used in this study: the monoclonal anti-lamin A/C (XB10) (42), anti-Nup153 (SA1) (43), anti-HA (12CA5; Covance), anti-γ-tubulin (GTU-88, Sigma), anti-golgi (58K-9; Sigma); polyclonal rabbit antibody anti-HA (ab9110; AbCam), anti-Sun2 (44), anti-GFP (ab290; AbCam), and polyclonal goat anti-kinase 1 (ab15075; AbCam). Polyclonal rabbit anti-Nesp4 was raised against a GST-Nesp4(1–90) fusion protein by Rockland Immunochemicals. GST-fusion protein purification and affinity purification of antisera was performed as described (24). Secondary antibodies, conjugated with AlexaFluor dyes or peroxidase, were from BioSource International/Invitrogen.


Fig. S1. Nesp4 is exposed on the ONM. Immunofluorescence microscopy of HSG cells stably expressing HA-Nesp4. The cells were fixed with formaldehyde and permeabilized with either an ice-cold solution of digitonin (0.003%) or a room temperature solution of 0.2% Triton X-100. The cells were then double-labeled with a mAb against HA tag and polyclonal antibodies against either lamins A and C or actin. The anti-HA antibody clearly detects HA–Nesp4 (Nesp4) after digitonin permeabilization, whereas lamins A and C (but not actin) remain inaccessible to antibody. The conclusion is that the N terminus of Nesp4 must be exposed on the cytoplasmic face of the NE, which is consistent with the view that Nesp4 is a type II ONM protein. DNA is revealed by staining with Hoechst dye. (Bar: 10 μm.)
Fig. S2. Competition between KASH domain proteins for tethering at the NE. GFP–Nesp4 or GFP–KASH4 displaces Nesp2G from the NE of HeLa cells. Transient transfection of HSG–HAN4 cells with either GFP–KASH4 or GFP–KASH2 displaces HA–Nesp4 from the NE. DNA is revealed by staining with Hoechst dye. (Bar: 10μm.)
Fig. S3. Nesp4 is expressed in murine mammary tissue. Northern blot analysis of RNA extracted from mammary tissue of nonpregnant or E13 and E15 pregnant mice. The Nesp4 probe labeled a 1.4- to 1.7-kb band that is induced during pregnancy. Transcript size is consistent with mouse cDNA sequences available in GenBank. Each lane contains 20 µg RNA.
Fig. S4. Differentiated HC11 cells lacking Nesp4 (green) display epithelioid morphology, are delineated by the junctional marker ZO1 (red; A), and express whey acidic protein (WAP, red; B). After differentiation, the cell population appears highly heterogeneous. DNA (blue) is revealed by staining with Hoechst dye.
Fig. S5. Differentiated HC11 cells express abundant kinesin-1 heavy chain (Kif5B) as revealed by Western blot analysis. (A) Relative to α-tubulin, the level of Kif5B in HC11 cells is comparable with that seen in HeLa cells. (B) In those HC11 cells expressing Nesp4, Kif5B can be detected at the NE (arrowheads). In contrast, cells lacking Nesp4 display little or no enrichment of Kif5B at the NE. DNA is revealed by staining with Hoechst dye.
Fig. S6. Exogenous Nesp4 induces separation of the nucleus from the centrosome in HeLa cells. Immunofluorescence microscopy of HeLa cells transiently transfected with GFP–Nesp4. An anti-γ-tubulin antibody was used to detect the centrosomes (red). The transient expression of GFP-Nesp4 (green) is associated with dramatic displacement (yellow arrows) of the nuclei (blue) from the centrosomes. In comparison, the centrosomes of nontransfected cells are immediately adjacent to the nuclei. DNA is revealed by staining with Hoechst dye. (Bar: 10 μm.)