Prevascular Prolamin A Interacts with Narf, a Novel Nuclear Protein*

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Prelamin A is farnesylated and methylated on the cysteine residue of a carboxyl-terminal CaaX motif. In the nucleus, prelamin A is processed to lamin A by endoproteolytic removal of the final 18 amino acids, including the farnesylated cysteine residue. Using the yeast two-hybrid assay, we isolated a novel human protein, Narf, that binds the carboxyl-terminal tail of prelamin A. Narf has limited homology to iron-only bacterial hydrogenases and eukaryotic proteins of unknown function. Narf is encoded by a 2-kilobase mRNA expressed in all human cell lines and tissues examined. The protein is detected in the nuclear fraction of HeLa cell lysates on Western blots and can be extracted from nuclear envelopes with 0.5 M NaCl. When a FLAG epitope-tagged Narf is expressed in HeLa cells, it is exclusively nuclear and partially co-localizes with the nuclear lamina. The farnesylation status of prelamin A determines its ability to bind to Narf. Inhibition of farnesyltransferase and mutation or deletion of the CaaX motif from the prelamin A tail domain inhibits Narf binding in yeast two-hybrid and in vitro binding assays. The prenyl-dependent binding of Narf to prelamin A is an important first step in understanding the functional significance of the lamin A precursor.

Several proteins have been found to be prenylated and methylated at their carboxyl-terminal ends (1, 2). Prenylation is a post-translational modification directed by one of three motifs. The Rab family of Ras-like GTPases are geranylgeranylated on the cysteine residues of CC or CXC motifs, where C is a cysteine and X is any amino acid. The final cysteine of the CXC motif is also carboxymethylated. Other prenylated proteins have a CaaX motif, where C is a cysteine, a is any aliphatic amino acid, and X is an amino acid that specifies whether a farnesyl (in which case X is a serine, alanine, methionine, or glutamine) or a geranylgeranyl (in which case X is a leucine) group will be covalently attached. Following the attachment of the isoprenoid group, the final -aaX amino acids are cleaved, and the cysteine residue is methylated. Prenylation was initially believed to be important only for membrane attachment, but the 15-carbon farnesyl moiety alone does not stably anchor proteins to membranes (3–5). Another role for prenylation appears to be its importance in protein-protein interactions (6).

The only nuclear proteins known to be prenylated in mammalian cells are prelamin A and B-type lamins. Both are farnesylated and carboxymethylated, but prelamin A is further processed by endoproteolysis to mature lamin A, which lacks the final 18 amino acids, including the modified cysteine residue (7–10). Prenylated prelamin A containing a mutation that prevents its endoproteolytic processing can still be incorporated into the nuclear lamina (11). Unprenylated prelamin A accumulates within the nucleus with some incorporation into the nuclear lamina in cells where farnesyl synthesis has been blocked (9, 12). Once farnesyl synthesis is restored, prelamin A is rapidly farnesylated and cleaved into its mature form. Recently, the prelamin A endoprotease activity was characterized from HeLa cell nuclear extracts (13). For proteolysis to occur, prelamin A must be farnesylated and methylated, and the lack of either modification prevents maturation to lamin A. The specific protein or proteins involved in this endoproteolysis are not yet isolated. Furthermore, the cellular role of the prenylated prelamin A precursor is unknown. As a first step toward understanding its function, we searched for proteins that interacted with prelamin A. We now report the discovery of a novel protein that binds the farnesylated prelamin A carboxyl-terminal domain (preAct).1 We have named this protein the nuclear prelamin A recognition factor, or Narf.

EXPERIMENTAL PROCEDURES

Plasmid Construction—preAct contains amino acids 389–664 of prelamin A, which includes the CaaX motif (14). Act C → S has amino acid 661 changed from a cysteine to a serine residue. ActCAaX contains amino acids 389–660 with an artificial stop codon inserted after the amino acid 660 codon in the corresponding cDNA sequence. Act contains amino acids 389–646, and the carboxyl-terminal tail domain of lamin C (Cct) contains amino acids 389–572, which includes the 6 amino acids distinct from lamin A that arise from alternative mRNA splicing (15). The carboxyl-terminal tail domain of lamin B1 (Bct) contains amino acids 391–586 (16).

All cDNAs corresponding to lamin tail domains were amplified by polymerase chain reaction (PCR) using primers containing specific restriction endonuclease sites. The cDNA sequence coding for Act C → S

* This work was supported by National Institutes of Health (NIH) Grant CA66974. The confocal microscopy facility used for this work was established by NIH Grant 1S10-RR10506 and is supported by NIH Grant S-P30-CA13696 as part of the Herbert Irving Cancer Center at Columbia University. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: preAct, prelamin A carboxyl-terminal tail domain; Act C → S, prelamin A tail domain mutant where the CaaX motif cysteine is replaced with a serine residue; ActCAaX, lamin A tail domain mutant where the CaaX motif is deleted; Act, mature lamin A tail domain; Bct, lamin B1 tail domain; Cct, lamin C tail domain; PCR, polymerase chain reaction; GST, glutathione S-transferase; 5′-RACE, 5′-rapid amplification of cDNA ends; PBS, phosphate-buffered saline; ORF, open reading frame; Fe-S cluster, iron-sulfur cluster; CF, cytoplasmic fraction; SW, salt-washed nuclear supernatant fraction; NS, salt-washed nuclear envelope fraction; GDI, Rab GDP-dissociation inhibitor.
was generated using a PCR primer where the cysteine codon TGC was replaced by the serine codon AGC. PCR products were either digested with the desired restriction endonucleases or subcloned into the TA vector pCR2.1 (Invitrogen) and subsequently digested. Amplified cDNAs were purified on agarose gels and ligated into their respective vectors using T4 DNA ligase and subsequently transformed by competent E. coli DH5α. Cells were grown on Luria broth containing 10 μCi/ml, 3000 Ci/mmol). Blotting and washing of membranes were performed using standard methods and dried onto Bio-Design Gel-Wrap using a Bio-Rad model 583 Gel Dryer.

**Preparation of GST Fusion Proteins**—Escherichia coli strain DH5α bacteria were transformed with pGEX-4T-1 or pGEX-Narf and grown overnight. Overnight cultures were diluted 1:10 into fresh Luria broth and grown for 2–4 h at 37 °C. Protein expression was induced by adding isopropyl-β-D-thiogalactoside (Fisher) to a final concentration of 0.5–1.0 mM and growing the bacteria for an additional 6–8 h. Bacterial lysates were prepared by freezing and thawing the cells. Lysis of the cells was accomplished by adding lysozyme or a combination of lysozyme and RNAse into the bacterial lysate. Lysis mixtures were incubated at 37 °C for 30 min and the remaining material was removed by centrifugation at 14,000 × g for 20 min. Nuclear envelopes were washed three times with 1 pellet volume of nuclear extraction buffer containing 0.5 M NaCl, and supernatants were saved and combined. Nuclear envelope pellets were washed twice in 1 pellet volume of nuclear extraction buffer and sonicated briefly with a Dismembrator (Fisher) to suspend the nuclei. Nuclei were resuspended in 1 pellet volume of nuclear extraction buffer and sonicated briefly with a Dismembrator (Fisher) to suspend the nuclei. Stock solutions of anti-NarF antibodies were prepared in PBS-T (PBS, 0.1% Tween 20) and subsequently incubated for 1 h at room temperature or overnight at 4 °C. Antibodies were diluted into fresh blocking solution and incubated with the blots for 1 h at room temperature. Rabbit sera with polyclonal antibodies against lamin A and C or lamin B1 (21) were diluted 1:500. Affinity-purified anti-NarF antibodies were diluted 1:20. Blots were washed for 2 × 10 min with blocking solution and for 10 min with PBS-T (PBS, 0.1% Tween 20) and subsequently incubated for 45–60 min with horseradish peroxidase-conjugated donkey anti-rabbit IgG antibodies (Amersham Pharmacia Biotech) diluted 1:5000 in PBS-T. Unbound antibody was removed by washing once with PBS-T for 15 min and then 4 × 5 min. Bound antibody was detected using ECL Western blotting detecting reagents, and blots were exposed for varying times to Hyperfilm ECL chemiluminescent film (Amersham Pharmacia Biotech).
Liquid β-Galactosidase Assay—S. cerevisiae Y187 cells were used for yeast two-hybrid liquid β-galactosidase assays according to the manufacturer’s protocol (CLONTECH Protocol Handbook PT5204-1). Yeast colonies were co-transformed with pGutB9 constructs containing cDNAs for preAct, Act C → S, ActCΔCαα, Act, Bct, Cct, and the pGADGH-Narf construct described above. Yeast cell homogenates were incubated with the antibodies at room temperature for 1 h. Transfected cells were diluted into solution B (PBS-T, 10% normal goat serum), and cells were washed three times with 2% normal goat serum (Sigma). Primary antibodies were diluted into solution A (PBS-T, 2% normal goat serum (Sigma)) overnight. The reaction was then stopped, and the optical density (λ = 600) was measured on a Beckman DU-6 spectrophotometer. Cultures from six yeast colonies were assayed for each data point, and statistical significance was calculated using a paired two-sample of the means t-test.

Isolation of a Novel preAct Binding Protein—We screened a HeLa cell cDNA library using preAct as the bait in a yeast two-hybrid assay. Of approximately 5 × 10^5 colonies screened, five grew on selective medium and showed β-galactosidase activity on a colony filter lift assay. When the “prey” library plasmids from these colonies were isolated and sequenced, three were found to encode the same protein, and the corresponding cDNAs differed slightly from each other only in 5’- and 3’-untranslated regions. The longest cDNA clone was 1560 base pairs and was completely sequenced in both directions. Using a 5'-RACE reaction, we obtained an additional 28 base pairs of upstream sequence, yielding a complete cDNA of 1588 base pairs (Fig. 1). Comparison of this clone with the human EST database failed to find any ESTs with additional upstream sequences, suggesting that we had the full-length cDNA sequence. We named the gene product of this cDNA clone Narf, for nuclear prelamin A recognition factor. The assigned initiation codon appears to be the actual start codon, yielding a 456-amino acid protein with an expected molecular mass of 51,156 Da and a theoretical pI of 6.63. The stop codon occurs at base pair 1369 and is followed approximately 90 base pairs downstream by a polyadenylation signal, which is located 26 base pairs before the polyadenylate tail. When the cDNA is translated into a protein sequence, Kyte-Doolittle hydrophathy analysis showed that Narf is not an integral membrane protein (data not shown).

Narf is Similar to Bacterial Iron Hydrogenases and Eukaryotic Proteins of Unknown Function—The deduced amino acid sequence of Narf was analyzed using a BLAST search. While no known proteins from higher eukaryotes were found to be Narf orthologs, bacterial iron-only hydrogenases and translated open reading frames (ORFs) from eukaryotic genomic DNA sequences showed some identity. The three bacterial hydrogenases with the most overall similarity to Narf are shown in Fig. 2A. Over the entire length of the protein, the D chain from Desulfovibrio fructosovorans NAPD-reducing hydrogenase (HydD in Fig. 2A) is 29% identical and 38% similar. Hydrogenase-1 from Clostridium acetobutylicum (HydA in Fig. 2A) is 30% identical and 38% similar to Narf, while the periplasmic iron hydridease-1 from Clostridium pasteurianum (Phl) in Fig. 2A) is 31% identical and 39% similar. Recently, the latter reaction (22). Iron-only hydrogenases catalyze the formation of H_2 by combining protons and electrons (23). Electrons are imported into the H-cluster active site through four iron-sulfur (Fe-S) clusters. Two of these Fe-S clusters, the FS2 domain (indicated with a^2 in Fig. 2A) and the FS4C domain (indicated with a^c) do not exist in Narf, which is a smaller protein and lacks 136...
amino-terminal acids compared with the hydrogenases. The other Fe-S clusters are the FS4A and FS4B domains (indicated with ∧a or ∧b). Three of the cysteine residues from FS4A and one cysteine from FS4B are conserved in Narf, but they do not make up a functional Fe-S cluster. The only functional domain conserved between Narf and the iron-only hydrogenases are the cysteines forming the H-cluster active site (indicated by arrows).

Other putative proteins with overall similarity to Narf were found by BLAST sequence comparison of Narf with translated genomic DNA sequences from various eukaryotes. None of the ORF translations shown has a known function. The S. cerevisiae protein from the putative ORF YNL240C, which we have named NAR1 on the Stanford Saccharomyces Genome Database (Nar1 in Fig. 2B), is 31% identical and 41% similar to Narf. A translated ORF from chromosome 3 of Schizosaccharomyces pombe (spNarf in Fig. 2B) is 32% identical and 45% similar to Narf, and the translation from a Caenorhabditis elegans ORF on chromosome 3 (ceNarf in Fig. 2B) is 34% identical and 44% similar to Narf. These putative proteins show homology to Narf in regions different from those Narf shares with the iron-only hydrogenases; however, all four of the H-cluster cysteine residues are conserved in all of the proteins. Narf has approximately the same percentages of identical and similar amino acids with the eukaryotic and bacterial proteins, but the eukaryotic proteins are more similar in size.

Characterization of Narf mRNA Expression and Protein Subcellular Localization—The mRNA transcript size and expression pattern of Narf was determined by probing human cancer cell line and human tissue Northern blots with 32P-labeled Narf cDNA (Fig. 3). Only one Narf mRNA transcript of 2 kilobases was detectable on both Northern blots, which is large enough to encode a peptide of the size predicted by the cDNA clone isolated from the yeast two-hybrid assay. Various mRNA expression patterns in several human tissue culture cell lines are shown in Fig. 3A. All cell lines show Narf expression, although mRNA levels vary. The colorectal adenocarcinoma SW480 cell line showed the highest Narf mRNA expression, while lung carcinoma A549 cells and melanoma G361 cells had the lowest levels of expression. As expected, lamin B1 is expressed in all cell types, while lamin A and C are variably expressed. No lamin A/C transcripts are detected in Burkitt’s lymphoma Raji cells, while barely detectable levels are seen in promyelocyte leukemia HL-60 cells and lymphoblastic leukemia MOLT-4 cells. Narf expression is unrelated to lamin A/C expression, since all three of these cell lines show high levels of Narf mRNA. Similarly, high levels of lamin A/C expression in human tissues such as the placenta (Fig. 3B) do not correlate with high Narf expression levels. Narf mRNA is predominantly expressed in skeletal muscle, heart muscle, and brain, although all tissues show some Narf transcripts.

Anti-Narf antibodies were made in rabbits against a bacterially expressed GST-Narf fusion protein. GST (Fig. 4A, arrowhead) and GST-Narf (Fig. 4A, arrow) were electrophoretically separated on an SDS-polyacrylamide gel and Coomassie Blue-stained. When used to probe a Western blot, preimmune rabbit serum did not detect either GST or GST-Narf (Fig. 4B, left blot), while sera from rabbits immunized against GST-Narf (Fig. 4B, right blot) detected GST (arrowhead), GST-Narf (arrow), and degradation products. To determine whether Narf was expressed in vivo, HeLa cells were fractionated into cytoplasmic and nuclear envelope fractions. The nuclear envelopes were then washed with 0.5 M NaCl to dissociate peripheral membrane proteins. Proteins in the cytoplasmic fraction (CF), salt-washed nuclear envelopes (NS), and salt-wash supernatants (SW) were separated on an SDS-polyacrylamide gel. Proteins from these HeLa cell fractions are shown on a Coomassie Blue-stained gel in Fig. 4C. Anti-Narf antibodies were affinity-purified against the bacterially expressed GST-Narf antigen.
antibodies specifically detected a 52-kDa protein in the SW fraction (Fig. 4D, left blot), which matches the molecular mass predicted by translation of the Narf cDNA. Polyclonal rabbit sera were used to probe for lamin B1 and lamin A/C protein expression. As expected, lamin B1 was exclusively found in the NS fraction (Fig. 4D, middle blot). Lamin B1 and lamin A/C were pre-

**Fig. 2.** Alignment of Narf with bacterial iron-only hydrogenases and eukaryotic proteins. White letters on a black background represent identical amino acids, and black letters on a gray background indicate similar amino acids at the same position in three or more of the aligned sequences. Dots show gaps in the protein alignments that give better overall similarity. A, bacterial iron-only hydrogenases with the most overall homology to Narf from a BLAST sequence comparison. From top to bottom, sequences are as follows: Narf; D. fructosovorans NADP-reducing hydrogenase D chain, GenBank™ accession number D57150 (HydD; 29% identical, 38% similar to Narf); C. acetobutylicum hydrogenase-1, GenBank™ accession number U08760 (HydA; 30% identical, 38% similar to Narf); C. pasteurianum periplasmic iron hydrogenase-1, GenBank™ accession number P29166 (Phf1; 31% identical, 39% similar to Narf); and Narf. Cysteines important for the iron-only hydrogenase active site, or H-cluster, are indicated with arrows. The asterisks mark conserved residues important for the environment around the H-cluster. Other iron-sulfur clusters are marked with carets. B, putative eukaryotic protein sequences found to be similar to Narf using BLAST sequence comparisons with translated genomic DNA. From top to bottom, sequences are as follows: Narf; the translation of *Saccharomyces cerevisiae* putative ORF YNL240C, GenBank™ accession number P23503, which we have named NAR1 on the Stanford *Saccharomyces* Genomic Database (NAR1; 31% identical, 41% similar to Narf); the translation of an *S. pombe* ORF from cosmid C1450 (spNarf; 32% identical, 45% similar to Narf); and the translation of a *C. elegans* ORF from cosmid Y54H5, Contig111 (ceNarf; 34% identical, 44% similar to Narf). Residues conserved between the putative eukaryotic proteins, bacterial iron-only hydrogenases, and Narf are marked with symbols corresponding to those described for A.
dominantly in the NS fraction, but some protein was also found in the SW fraction (Fig. 4D, right blot).

Both the mRNA and protein sizes found for Narf match those expected from its cDNA sequence. Narf does not appear to be a transmembrane protein, since it can be dissociated from the nuclear envelope by salt treatment, which is consistent with its predicted hydrophobicity plot data. These data show that Narf is in the nuclear fraction of cells and is therefore in the appropriate subcellular location for prelamin A association. Narf also appears to be expressed in cells without lamin A. In agreement with the Northern blot data (Fig. 3), Western blot analysis of subcellular fractions from Raji cells, which express Narf but not lamin A/C mRNA, showed that Narf was also found in the SW fraction of Raji cells, while no lamin A/C was detected (data not shown).

**Binding Assays Confirm That Narf Specifically Binds to preAct—**We tested the interactions of Narf with various lamin tail domains using a yeast two-hybrid liquid bait assay (Table I). A comparison of β-galactosidase activities gives an indication of the relative binding strengths of the bait and “prey” fusion proteins. Yeast cells were transformed with the original Narf library clone, pGADGH-Narf, which expresses the GAL4 activation domain fused to Narf. When co-transformed with the empty GAL4 DNA binding vector, pGBT9, yeast cells showed a background level of β-galactosidase activity. Background β-galactosidase activities were also measured when cells were co-transformed with Bet and Cct bait constructs. The only significant β-galactosidase activity was found in cells co-transformed with the original preAct bait construct. Mutation of the CaaX motif cysteine to a serine residue (Act C → S) or deletion of the CaaX motif (ActΔCaaX), decreased β-galactosidase activity to background levels. Similarly, Narf apparently did not associate with the mature Act, which lacks the final carboxyl-terminal 18 amino acids. As S. cerevisiae cells prenylate, carboxymethylate, and endoproteolytically remove -aaX sequences from proteins containing CaaX motifs (2), the fact that Narf does not associate with Act C → S or ActΔCaaX in yeast suggests that Narf requires that preAct be prenylated in order to bind. Narf does not appear to indiscriminately associate with prenylated proteins, since it does not bind Bet, which also has a carboxyl-terminal CaaX motif.

**Fig. 3.** Northern blot analysis of Narf, lamins A and C, lamin B1, and actin mRNA expression in human cancer cell lines and tissues. From top to bottom, estimated mRNA sizes are as follows: Narf, 2 kilobases; lamin A, 2.9 kilobases; lamin C, 2 kilobases; lamin B1, 3 kilobases; and actin, 2 kilobases with an additional 1.8-kilobase isoform seen in skeletal and heart muscle tissue samples. Actin is included as a control showing mRNA loading for each lane. A, from left to right, human cancer cell lines represented on the Northern blot are as follows: promyelocytic leukemia HL-60, HeLa cell S3, chronic myelogenous leukemia K-562, lymphoblastic leukemia MOLT-4, Burkitt’s lymphoma Raji, colorectal adenocarcinoma SW480, lung carcinoma A549, and melanoma G361 cells. B, from left to right, human tissue samples on the Northern blot are as follows: heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas.

**Fig. 4.** Western blot analysis of bacterially expressed GST or GST-Narf and of HeLa cell cytoplasmic and nuclear fractions. GST or GST-Narf were expressed in bacteria, purified, and electrophoresed on 10% SDS-polyacrylamide gels. A, Coomassie Blue staining of GST (26 kDa, arrowhead) and GST-Narf (86 kDa, arrow). B, Western blots were probed with preimmune rabbit serum (left) or anti-Narf serum (right). Preimmune serum did not detect any bacterial proteins, while GST (arrowhead), GST-Narf (arrow), and GST-Narf degradation products were recognized by anti-Narf antibodies. HeLa cells were fractionated as described under “Experimental Procedures.” Approximately 10–20 μg of each fraction was electrophoresed on 10% SDS-polyacrylamide gels. From left to right, cellular fractions are CF, NS, and SW, C, Coomassie Blue staining of each fraction. D, Western blots probed with affinity-purified anti-Narf rabbit serum (left), anti-lamin B1 rabbit serum (middle), or anti-lamin A/C serum (right). Anti-Narf antibodies detected a 52-kDa protein in the SW fraction that matches the molecular mass predicted by the deduced amino acid sequence. Narf is not cytoplasmic and can be extracted from nuclear envelopes into the SW fraction, indicating that it is not a transmembrane protein. Anti-lamin B1 antibodies detected a single 66-kDa protein in the NS fraction, and antibodies anti-lamin A/C detected lamin A (72 kDa) and lamin C (65 kDa) in the NS, although some lamins A and C were also found in the SW fraction. The migrations of molecular mass standards in kDa are indicated at the left of selected panels.

To confirm the results obtained in the two-hybrid assays, we performed GST binding assays. Lamin tail domains cDNAs were transcribed into mRNA using the Promega RibomAX T7 kit, and the mRNA was in vitro translated in the presence of [35S]methionine using Promega nuclease-treated rabbit reticulocyte lysates. The [35S]-labeled proteins were then incubated with GST or a GST-Narf fusion protein bound to glutathione-Sepharose 4B beads. Beads were washed, and the proteins eluted with SDS-polyacrylamide gel electrophoresis sample buffer were electrophoresed on SDS-polyacrylamide gels and analyzed by autoradiography. preAct, Act C → S, ActΔCaaX, Act, Cct, and Bet were efficiently translated, and roughly equal amounts of each protein were used in the binding assay (Fig. 5A). While a barely detectable amount of preAct bound non-specifically to GST (Fig. 5B, lane 1), much more was retained by
TABLE I

Lamin tail domain interactions with Narf in a yeast two-hybrid liquid β-galactosidase assay

| Bait construct | PreAct | β-Galactosidase units |
|----------------|--------|-----------------------|
| pGBT9 alone    |        | 0.071 ± 0.002         |
| pGBT9-preAct   | Yes    | 0.242 ± 0.015         |
| pGBT9-Act C → S| No     | 0.075 ± 0.006         |
| pGBT9-ActCAaaX | No     | 0.072 ± 0.003         |
| pGBT9-Act     | No     | 0.077 ± 0.002         |
| pGBT9-ActC    | Yes    | 0.076 ± 0.003         |
| pGBT9-Cct     | No     | 0.071 ± 0.002         |

a Statistically significant difference from pGBT9 alone, p < 0.001.

GST-Narf (Fig. 5B, lane 7). Act C → S and ActCAaaX also bound to GST-Narf (Fig. 5B, lanes 8 and 9), but their binding was much weaker than the association of preAct with GST-Narf. The other tail domains showed very weak associations with GST-Narf (Fig. 5B, lanes 10–12), which were not above the background level of preAct and GST association.

preAct Farnesylation Greatly Enhances Narf Binding—Ras and full-length prelamin A have been shown to be correctly farnesylated using nuclease-treated rabbit reticulocyte lysate in vitro translation reactions (9, 24, 25). We have confirmed that preAct is also prenylated in vitro. Mevalonolactone rapidly converts to mevalonate, a farnesyl precursor, when placed in an aqueous environment. When [3H]mevalonolactone was added to in vitro translation reaction mixtures, preAct was 3H-labeled (Fig. 6A, lanes 1 and 4), while Act C → S and ActCAaaX remained unlabeled (Fig. 6A, lanes 2 and 3). This prenylated 3H-labeled preAct behaved like the [35S]methionine-labeled protein and specifically bound to GST-Narf with no detectable association with GST in a binding assay (data not shown).

To confirm that preAct is farnesylated, increasing amounts of the farnesyltransferase-specific inhibitor FPT-II (26) were added to preAct in vitro translation reactions that contained [3H]mevalonolactone. Increasing FPT-II concentrations led to a decrease in the [3H]mevalonolactone labeling of preAct (Fig. 6A, lanes 4–7), with no detectable labeling seen when 150 μM FPT-II was added (Fig. 6A, lane 7). The same concentrations of FPT-II were used in preAct translation reactions containing [35S]methionine. Twenty percent of the translation reaction used for each binding assay was electrophoresed on an SDS-polyacrylamide gel (Fig. 6B). Deletion of the CaaX motif or increasing amounts of FPT-II led to a decrease in GST-Narf binding (Fig. 6C, lanes 9–11). At 150 μM FPT-II, preAct was not detectably prenylated (Fig. 6A, lane 7), and the comparable [35S]methionine-labeled sample was significantly impaired in its ability to bind GST-Narf (Fig. 6C, lane 11). This decrease in binding was not due to FPT-II interfering with protein-protein interactions, since 150 μM FPT-II added to a preAct sample after translation but prior to the binding reaction did not affect preAct binding to GST-Narf (Fig. 6C, lane 12).

To compare the amounts of preAct that specifically bound to GST-Narf upon FPT-II treatment, the autoradiographs shown in Fig. 6, B and C, were scanned, and the bands were analyzed by densitometry. The net ratios of tail domains attached to GST-Narf were calculated by subtracting the background levels related to GST as described under “Experimental Procedures.” The ratios for each sample are shown graphically in Fig. 6D. Ratios from three independent binding assays showed the same decrease in preAct association with GST-Narf with increasing concentrations of FPT-II. Nonprenylated Act C → S (data not shown) and ActCAaaX clearly bind to GST-Narf much more weakly than prenylated preAct. Similarly, in vitro translation and [35S]methionine-labeled Narf does not bind to bacterially expressed, and therefore unprenylated, GST-preAct (data not shown).

When Narf Is Overexpressed in HeLa Cells, It Is Nuclear and Partially Co-localizes with the Nuclear Lamina—Endogenous Narf was shown to be nuclear on Western blots (see Fig. 4). To examine the co-localization of Narf and lamin A within the nucleus, HeLa cells were transiently transfected with a FLAG epitope-tagged Narf under the control of the SV40 early promoter. Transfected cells were grown for a total of 72 h, fixed, and labeled with anti-lamin A/C (Fig. 7, A and D, shown in green) and anti-FLAG (Fig. 7, B and E, shown in red) antibodies. FLAG-tagged Narf was always exclusively nuclear, although fluorescence patterns varied between individual cells. In some cells, exogenous Narf formed large aggregates at the nuclear periphery (Fig. 7B) and co-localized with lamin A and C at the nuclear lamina (yellow in Fig. 7C). Other cells showed a more diffuse intranuclear staining (Fig. 7E), which also partially overlapped with the nuclear lamina (Fig. 7F). These fluorescence patterns probably reflect different levels of exogenous Narf expression.

We also attempted to determine the localization of endogenous Narf in HeLa cells by immunofluorescence microscopy using the same affinity-purified anti-Narf antibodies we used for Western blot detection. Unfortunately, Narf protein levels in individual HeLa cells are too low, or our antibodies are of too low avidity, to detect endogenous Narf using immunofluorescence microscopy (data not shown).

DISCUSSION

Narf Is a Novel Nuclear Protein—Prelamin A is farnesylated and carboxymethylated on the cysteine residue of a carboxy-terminal CaaX motif (7–10). This post-translationally modified cysteine residue is removed from prelamin A when it is endoproteolytically processed into mature lamin A, which lacks the final 18 amino acids of prelamin A. This processing event appears to occur within the nucleus (9, 12, 27). The prelamin A endoprotease activity has been examined in HeLa cell nuclear extracts, and endoproteolysis requires both farnesylation and carboxymethylation of the prelamin A CaaX motif cysteine residue (13). The significance of the prenylated prelamin A precursor is unclear, since the inhibition of prelamin A processing or cellular prenylation reactions does not prevent prelamin A from being incorporated into the lamina (9–12). We report here a novel nuclear protein, Narf, which binds to the prenylated prelamin A carboxy-terminal tail domain. Narf does not associate with the mature lamin A tail domain and only weakly interacts with preAct mutants where the CaaX motif cysteine has been replaced with a serine residue or where the CaaX motif has been deleted. The inhibition of farnesyltransferase leads to a dose-dependent reduction in preAct prenylation and a concurrent decrease in Narf association. Unlike the prelamin A endoprotease activity, Narf does not appear to require cysteine carboxymethylation in order to bind preAct. No significant sequence identity is seen when Narf is BLAST-aligned with other proteases, -aaX endoproteases, carboxymethyltransferases, or farnesyltransferases from yeast or mammals. Narf may be a component of a prelamin A endoprotease complex, and if so, it is interesting to note that Narf mRNA and protein are expressed in cells that do not have any lamin A.

Narf is similar to bacterial iron-only hydrogenases and putative eukaryotic proteins, but sequence analysis of Narf does not show any protein motifs that might indicate function. Bacterial iron-only hydrogenases take electrons generated from pyruvate oxidation and combine them with protons to make...
hydrogen gas (23). The only domain conserved between Narf and the bacterial hydrogenases is the H-cluster active site, a structurally unique site that coordinates six Fe-S clusters between four cysteine residues (22). None of the other Fe-S clusters, involved in electron transfer, are conserved between the iron-only hydrogenases and Narf. Similarly, the cysteine residue implicated in proton donation, cysteine 299 in C. pasteurianum hydrogenase-1, corresponds to alanine 171 in Narf. There is no other uncoordinated cysteine residue near the conserved H-cluster that might fill this proton donation role. We have yet to determine whether Narf contains Fe-S clusters, but without the components required to import electrons or supply protons, it is unlikely that Narf is a functional hydrogenase.

None of the putative eukaryotic proteins found to be similar to Narf have been purified or characterized. All of these proteins appear to share the cysteine residues found in the H-cluster active site of the bacterial iron-only hydrogenases. Four other cysteine residues are also conserved between the eukaryotic and bacterial proteins, although they are part of different Fe-S clusters in the bacterial enzymes. While the overall percentages of amino acids similar and identical to Narf are approximately the same for the eukaryotic and bacterial proteins, the eukaryotic proteins are more similar in length and appear to share more clusters of amino acids with Narf than the hydrogenases. It remains to be determined whether Narf or the eukaryotic proteins contain Fe-S clusters or share any Fe-S clusters in the bacterial enzymes. While the overall sequence and the dissociation of Narf from nuclear envelopes by its association with lamin A, since Narf specifically recognizes preAct, and not just a prenylated cysteine residue, came from in vitro binding assays. Although mutation or removal of the CaaX motif from preAct prevented detectable binding in yeast, the high concentrations of proteins in the in vitro binding reactions were able to detect the weak associations of Narf with Act C → S and ActΔCaaX. Both yeast two-hybrid and in vitro assays, however, clearly demonstrated that prenylated preAct was greatly enhanced in its ability to bind Narf.

That the preAct was specifically farnesylated and not geranylgeranylated is suggested by data showing that rabbit reticulocyte lysate contains a farnesyl group and not a geranylgeranyl group to p21CaaX in vitro (25). Our data showed that FPT-II, an inhibitor of farnesyltransferase, decreased the incorporation of any [3H]mevalonolactone derivatives, which would include farnesyl and geranylgeranyl, in a dose-dependent manner. This inhibition of preAct prenylation also led to a dose-dependent decrease in the ability of Narf to bind preAct. Although FPT-II treatment never brought Narf and preAct association down to the levels seen for Narf and ActΔCaaX, this result may be explained by the fact that [3H]mevalonolactone labeling of preAct upon 150 μM FPT-II treatment, there may be residual prenylated proteins that are too weak to be seen on x-ray film but are still available to bind to Narf. This small amount of protein may have been enough to be detectable only when labeled by [35S]methionine. The high concentration of FPT-II required to decrease farnesylation (FPT-II has an in vivo IC50 of 75 nm) (26) is also not surprising, since in vitro translation reactions do not exactly duplicate in vivo protein concentrations. Rabbit reticulocyte lysates appear to have higher concentrations of farnesyltransferase than that found in cells, and therefore more inhibitor is required to prevent prenylation. A further confirmation that Narf preferen-

![Fig. 5. Binding of lamin tail domains to GST-Narf.](image-url)
Prenylated Prelamin A Binding Protein

**Fig. 6. Inhibition of preAct farnesylation leads to a decrease in GST-Narf binding.** A, preAct (lane 1), Act C → S (lane 2), and ActΔCaaX (lane 3) were synthesized by *in vitro* translation in the presence of γH)mevalonolactone, and proteins were electrophoresed on a 12.5% SDS-polyacrylamide gel that was treated with a fluorographic reagent, dried, and exposed to x-ray film. PreAct was also synthesized in the presence of γH)mevalonolactone with varying amounts of the farnesyltransferase inhibitor FPT-II, and translation products were electrophoresed on a 10% SDS-polyacrylamide gel that was treated with a fluorographic reagent, dried, and exposed to x-ray film. From left to right, samples are as follows: preAct (lane 4) and preAct synthesized with 1.5 μM (lane 5), 15 μM (lane 6), or 150 μM (lane 7) FPT-II. PreAct was labeled with γH)mevalonolactone (lanes 1 and 4), while Act C → S (lane 2) and ActΔCaaX (lane 3) were not labeled. Increasing amounts of FPT-II decreased the γH)mevalonolactone labeling of preAct (lanes 5–7). B, preAct and ActΔCaaX tail domains were synthesized by *in vitro* translation in the presence of [35S]methionine and varying amounts of FPT-II. Proteins were electrophoresed on 10% SDS-polyacrylamide gels that were dried and exposed to x-ray film. From left to right, samples are as follows: preAct (lane 1); ActΔCaaX (lane 2); preAct with 1.5 μM (lane 3), 15 μM (lane 4), and 150 μM (lane 5) FPT-II added during synthesis; and a control reaction (lane 6) where preAct was synthesized without inhibitor but 150 μM FPT-II was added during the binding reaction to ensure that the inhibitor did not interfere with protein-protein interactions. C, for each sample, 4-fold more reaction mixture than the amount shown in B was incubated with GST (lanes 1–6) or GST-Narf (lanes 7–12) attached to glutathione-Sepharose 4B. Beads were washed with PBS, and bound proteins were eluted into SDS sample buffer and electrophoresed on 10% SDS-polyacrylamide gels as described for B. FPT-II inhibition of preAct farnesylation prevents GST-Narf binding in a dose-dependent manner (lanes 9–11). The control reaction where FPT-II was added during the binding assay did not affect GST-Narf binding (compare lanes 7 and 12). The migrations of molecular mass standards in kDa are indicated at the left of selected panels. D, the autoradiographs shown in B and C were analyzed by densitometry as described under "Experimental Procedures." Ratios of [35S]-labeled proteins specifically bound to GST-Narf confirm that preAct must be prenylated in order to bind GST-Narf. The values shown are in arbitrary units.

Ras-like GTPase involved in intracellular membrane trafficking. The geranylgeranylation of several Rabs is required for their association with the Rab GDP dissociation inhibitor (GDI), a protein important for membrane extraction and recycling of Rab proteins (34–37). Changing the prenyl moieties attached to Rabs by substituting a farnesyl CaaX motif in place of the normal CXC or CC geranylgeranyl motif significantly reduces the association of prenylated Rabs with GDI (36, 38). While the final cysteine residue of the CXC motif is normally carboxymethylated, this modification was not necessary for geranylgeranylated Rab3A to bind to its GDI (34). Residues amino-terminal to the Rab prenylation motifs have also been found to be important for GDI association (36), and a single amino acid point mutation in Rab1B prevents its interaction with GDI, even when Rab1B is geranylgeranylated (39).

Signal transduction molecules are also prenylated, and their protein-protein interactions can be affected when the attached prenyl moieties are changed. Farnesylated yeast Ras2 binds to and activates adenyl cyclase with 100 times higher affinity than unprenylated recombinant Ras2 (40). The coupling of heterotrimeric G-proteins to their receptors also relies upon prenylation. The γ subunit involved in the association of G-proteins with activated rhodopsin must be farnesylated in order for this interaction to occur, and geranylgeranylation could...
Narf is a nuclear protein that interacts with a prenylated partner. Narf has no homology to other known prenyl-dependent binding proteins, including enzymes that recognize and modify prenylated substrates such as the -aa endoprotease and carboxymethyltransferase. Prenylation of preact greatly enhances its association with Narf, while carboxymethylation does not appear to be required. The functional significance of the association of Narf with prenylated prelamin A is still unknown. Since Narf is expressed in cells that do not have any lamin A or C, it may function in the cell independent of its association with preact, and prelamin A may be important in regulating these functions in a cell type- and differentiation-specific manner. Alternatively, Narf may be important in prelamin A processing, or it may anchor prenylated prelamin A in the intranuclear space, allowing prelamin A to organize nuclear proteins into a higher order structure. The isolation of Narf is an important first step in determining the role of prelamin A in the cell.

Acknowledgments—We acknowledge the NCI, National Institutes of Health, for allocation of computing time and staff support at the Frederick Biomedical Supercomputing Center of the Frederick Cancer Research and Development Center. We also thank Theresa Swayne (Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York) for help with confocal microscopy.

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