The mRNA Export Factor Human Gle1 Interacts with the Nuclear Pore Complex Protein Nup155*

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The protein Gle1 is required for export of mRNAs from the nucleus to the cytoplasm in both lower and higher eukaryotic cells. In human (h) cells, shuttling of hGle1 between the nucleus and cytoplasm is essential for bulk mRNA export. To date, no hGle1-interacting proteins have been reported and the mechanism by which hGle1 interacts with the nuclear pore complex (NPC) and mediates export is unknown. To identify proteins that can interact with hGle1, a genome-wide yeast two-hybrid screen was performed. Three potential hGle1-interacting partners were isolated, including clones encoding the C-terminal region of the NPC protein hNup155. This interaction between hGle1 and full-length hNup155 was confirmed in vitro, and deletion analysis identified the N-terminal 29 residues of hGle1 as the hNup155-binding domain. Experiments in HeLa cells confirmed that the nuclear rim localization of the major hGle1 protein variant (hGle1B) was dependent on the presence of these 29 N-terminal residues. This suggests that this domain of hGle1 is necessary for targeting to the NPC. This work also characterizes the first domain in hNup155, a 177 C-terminal amino acid span that binds to hGle1. The mutual interaction between hGle1 and the symmetrically distributed nuclear pore protein Nup155 suggests a model in which hGle1's association with hNup155 may represent a step in the Gle1-mediated mRNA export pathway. Molecular & Cellular Proteomics 3:145–155, 2004.

The highly selective, bidirectional exchange of macromolecules between the nucleus and cytoplasm is mediated by the coordinated efforts of soluble transport factors and components of the nuclear pore complex (NPC). Our knowledge of the physical composition of the NPC and the distinct factors required for protein import and export has grown significantly over the past decade (for reviews see Refs. 1 and 2). In both yeast and vertebrates, at least 30 NPC proteins (nucleoporins, Nups) are required to form the portal through which transport substrates navigate (3, 4). Directional targeting of proteins to and from the nucleus requires specific motifs encoded in the respective primary amino acid sequences, termed nuclear localization sequences (NLSs) and nuclear export sequences (NESs) (5). Different types of NLS and NES motifs are recognized by alternative transport machinery resulting in saturable, noncompeting nuclear import and export pathways. The karyopherin (Kap) family of conserved soluble factors responsible for transporting NLS- and NES-bearing proteins have been referred to as importins, transportins, and exportins (reviewed in Ref. 6).

All transport processes are thought to use the same portal formed by the NPC (7) (for reviews see Refs. 8–10). The most extensively studied mRNA export factor is the shuttling heterodimer TAP-p15 (Mex67-Mtr2 in budding yeast) capable of binding to RNA, to a number of proteins implicated in mRNA processing (reviewed in Refs. 8 and 11), and to specific NPC proteins (12–14). Other protein mediators of mRNA export have been described including the RNA binding, heterogeneous nuclear ribonucleoparticle (hnRNP) proteins. In particular, a subset of hnRNP proteins contain both NLS and NES motifs and shuttle between the nucleus and cytoplasm (10). The NES-bearing hnRNP proteins may act as facilitators of translocation, and a role in mRNA export for members of the karyopherin β family has begun to emerge (15, 16). Other mRNA export factors include NPC-associated proteins such as Gle2/Rae1 (8, 17), which forms a complex with Nup98 and TAP (13), and the DEAD-box helicase Dpb5 (18–21). Finally, the protein Gle1 is also required for mRNA export in both yeast and human cells (22–26). We recently have shown that in human cells Gle1 nucleocytoplasmic shuttling is required for efficient mRNA export (27). Precisely how hGle1 interacts with the NPC en route to and from the cytoplasm is unknown.

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The abbreviations used are: NPC, nuclear pore complex; Kap, karyopherin; GFP, green fluorescence protein; GST, glutathione S-transferase; MBP, maltose-binding protein; h, human; hnRNP, heterogeneous nuclear ribonucleoprotein; mRNP, messenger RNA-bound hnRNP complex; NES, nuclear export sequence; NLS, nuclear localization sequence; sc, Saccharomyces cerevisiae; GAD, Gal4 DNA binding domain; GAD, Gal4 transactivation domain; Nup, nucleoporin; SMD, synthetic minimal media supplemented with 2% glucose; LTH, leucine, tryptophan, histidine; LTHA, leucine, tryptophan, histidine, adenine; FL, full-length; PBS, phosphate-buffered saline; mAb, monoclonal antibody; r, rat.
To reveal the molecular mechanism by which Gle1 functions in mRNA export, we initiated a series of studies focused on the human (h) homologue (24). At steady state, hGle1 is found both throughout the cytoplasm and nucleoplasm of HeLa cells as well as at the nuclear envelope (24, 27). Recently, two hGle1 protein variants, called hGle1A and hGle1B, were found to be expressed in HeLa cells. However, only hGle1B, the most abundant, shows strong localization to the nuclear rim (27). This suggests a physical association between hGle1B and NPCs, similar to Saccharomyces cerevisiae (sc) Gle1 (3). However, a proteomic-based approach characterizing the composition of vertebrate NPCs (4) together with our recent study on hGle1 nucleocytoplasmic shuttling (27) argue that the association of hGle1 with NPCs is transient. In this study, we report that hGle1 can interact with at least three different proteins, and multiple regions of hGle1 are required to mediate docking at NPCs. The N-terminal region of hGle1 interacts with the nucleoporin hNup155 and is necessary for mediating NPC targeting. Interestingly, previous studies have shown that hNup155 is localized on both the nuclear and cytoplasmic faces of the NPC (28). Taken together, this suggests that the potential interaction of hGle1 with hNup155 on both the cytoplasmic and nuclear sides of the NPC may facilitate mRNA export.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**

Plasmids used in this study are summarized in Table I. pEGFP, pGAD-GH, and pGBT8 expression vectors were purchased from Clontech (Palo Alto, CA); pMal expression vector was purchased from New England Biolabs (Beverly, MA); pGex expression cassettes were purchased from Amersham Biosciences (Piscataway, NJ); pGreen-lantern was purchased from Life Technologies (Grand Island, NY); and pGB was purchased from Stratagene (La Jolla, CA).

**Two-hybrid Screen**

The Matchmaker HeLa cell cDNA library (Clontech) was amplified following manufacturer’s instructions. Gal4 transactivation domain (GAD)-hGle1A (pSW938) was transformed into the PJ69-4A strain (29) and checked for expression and self-activation of the reporter genes. The PJ69-4A/pSW938 cells were grown to an OD600 of 0.6 and checked for expression and self-activation of the reporter genes. The PJ69-4A/pSW938 cells were grown to an OD600 of 0.6 and transformed by the lithium acetate method (30). Cells were plated on synthetic minimal media supplemented with 2% glucose (SMD) and lacking leucine, tryptophan, and histidine (LTH). Plates were incubated at 30 °C for 3–5 days. 6.5 × 10⁵ clones were screened, and colonies able to survive on LTH were replica plated to SMD media lacking LTH and adenine (LTHA). Those growing on LTHA were tested for β-galactosidase expression by scoring blue color with a filter overlay. Twenty-seven positive clones were identified, and all were subsequently tested for nonspecific interactions with a Gal4 DNA binding domain (GAD) fusion to the coding sequence for Lamin C (pLaminC; Stratagene).

**Expression and Purification of Fusion Proteins in Bacteria**

Glutathione S-transferase (GST)-hGle1 fusion proteins were expressed in Escherichia coli BL21 as described elsewhere (27). Crude lysate was transferred to 0.5 ml of a 50% glutathione bead slurry (Amersham Bioscience) and rotated for 1 h at 4 °C. Washes were performed at 4 °C as follows: 3 × 10 ml of STE (10 mM Tris, pH 8.0, 150 mM NaCl; 1 mM EDTA) plus 1% Triton X-100, 4 × 10 ml of phosphate-buffered saline (PBS), 1 × 10 ml of PBS plus 1.0 mM NaCl for 10 min at room temperature, 2 × 10 ml of PBS. Bound GST-fusion protein was checked for integrity and purity by SDS-PAGE and stored in PBS-containing protease inhibitors (Roche, Indianapolis, IN) at 4 °C for up to a week. Expression of hNup155 fused with maltose binding protein (MBP) or 6XHis was conducted exactly as recommended by the manufacturer (New England Biolabs and Qiagen, Valencia, CA, respectively).

**In Vitro Protein-Protein Interaction**

Ligand Blot Overlay—The ligand blot assay was completed as described elsewhere (31) using His-tagged and MBP-tagged Nup155 constructs. Bound MBP-full-length (FL)-hGle1A or MBP-hGle11,105 was detected by incubation with the affinity-purified anti-hGle1 antibody followed by alkaline phosphatase-conjugated anti-rabbit antibody. Blots were developd via color visualization with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyolphosphate (Promega, Madison, WI).

Affinity Chromatography Assay—[35S]-Labeled FL Nup155 was generated by in vitro transcription/translation using pSW1455 with the TNT® SP6-coupled reticulocyte lysate system (Promega) following the manufacturer’s instructions. In binding experiments, ~2.0 µg of immobilized GST-tagged proteins were washed once with binding buffer (110 mM KOAc, 2 mM Mg(OAc)₂, 20 mM HEPES, pH 7.3, 0.2% Tween-20, 1 mM dithiothreitol, and complete protease inhibitor EDTA-free mixture (Roche) and blocked for 30 min, 4 °C, in 100 µl of binding buffer plus 0.1% bovine serum albumin. [35S]-Labeled Nup155 was subsequently added, and the mixture was incubated at 4 °C for 1 h with gentle continuous mixing. Beads were washed six times with binding buffer and eluted by boiling in SDS sample buffer. Following SDS-PAGE, gels were fixed in Coomassie staining solution, incubated in Amplify™ (Amersham Biosciences) as per manufacturer’s recommendation, dried, and processed for autoradiography.

**Expression and Analysis of GFP-hGle1 Constructs in HeLa Cells**

CCL2 HeLa cells were cultured and transfected as previously described (27). Detection of GFP-derived autofluorescence and staining of NPC proteins using monoclonal antibody (mAb) 414 were carried out exactly as described elsewhere (27). Cells were examined using an Olympus BX50 microscope (Melville, NY). Digital images were acquired with a Photometric CoolSNAP HQ camera (Roper Scientific, Trenton, NJ) using MetaVue 6.0 software (Universal Imaging, Downingtown, PA) and processed in Photoshop 7.0 (Adobe, Mountain View, CA).

**RESULTS**

**A Two-hybrid Screen Identifies an Interaction Between hGle1 and hNup155**

To identify factors required for hGle1 function, we searched for hGle1-interacting proteins in a HeLa cell cDNA library initially using hGle1A fused to GAD in a yeast two-hybrid strategy. From 6 million potential library clones, 27 positives were identified that activated all three reporter genes (see “Experimental Procedures”). The positive clones were grouped into four categories (A–D) based on similar restriction digest patterns (Table II), and further tests for specificity demonststrated a lack of interaction between GAD clones and a GAD-Lamin C fusion (data not shown). The inserts were ana-
lyzed by DNA sequencing, and the predicted coding sequence for the putative interactors was compared with sequences in the GenBank database using the BLAST search program (32).

Four groups of positive clones (A–D, Table II) potentially represented novel hGle1A interactors. The largest group (A) was comprised of clones with fusions to the sequence encoding keratin 10, type 1. In a direct two-hybrid strategy using various domains of hGle1A in fusion with the GAD, we found that the interaction of hGle1 with the GAD-keratin fusion was dependent on the coiled-coil domain in GAD-hGle1A (see Fig. 6 below and data not shown). The remaining clones from groups B, C, and D interacted with hGle1 independent of its coiled-coil domain.

The members of group B represented five independent isolates of the same putative interactor with a ~1500-bp insert predicted to code for a 448-aa polypeptide fused to GAD. A GenBank search with this predicted open reading frame identified the mammalian nucleoporin Nup155 (33). Previous studies by others have characterized two budding yeast nucleoporins with similarity to mammalian Nup155, designated scNup170 and scNup157 (34, 35). The C-terminal region of hNup155 is 19% identical to the C-terminal region of scNup170 (Fig. 1). Although we did not isolate a clone corre-
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To determine which region of hGle1 was responsible for the interaction with hNup155, a panel of GBD-hGle1 fusions was generated and tested for interaction with GAD-hNup155C in the two-hybrid assay (Fig. 2A). The N-terminal region of hGle1 (residues 2–372) was found to be sufficient to mediate interaction with GAD-hNup155C. Further deletions of the N-terminal region were tested, and, strikingly, a short span of the first 29 amino acids at the N terminus of hGle1 (hGle12–29) contained all the information necessary to bind hNup155C (Fig. 2, A and B). Because the GBD-hGle1A screen was conducted prior to our knowledge of a second, more abundant hGle1 variant termed hGle1B (27), we subsequently confirmed the interaction between hNup155C and hGle1B using a direct two-hybrid approach (Fig. 2, A and B). Thus, the 29-aa peptide span (also referred to as hNup155-binding domain) is therefore sufficient for the interaction of hGle1A and hGle1B with hNup155 C-terminal domain in a two-hybrid assay.

Structural and functional regions of hNup155 and its homologues have not been reported. As an initial analysis of the hGle1-binding site in the C-terminal region of hNup155 (Fig. 2C; residues 943–1391), the hNup155C region was divided into N-terminal and C-terminal halves at residue 1121 (Fig. 2C; residues 943–1121 and 1121–1391, respectively) with fusions to GAD. Interaction was tested with a FL GAD-hGle1A fusion in the two-hybrid assay. Interestingly, neither half of the hNup155C was capable of interaction with GAD-hGle1A. We next constructed a series of deletion mutants from the N terminus of hNup155C. The initial 10 amino acids of the hNup155C library insert were first removed to yield GAD-hNup155983–1391. Four subsequent GAD-hNup155C fusion proteins were constructed in which the initial 43 (GAD-hNup155987–1391), 78 (GAD-hNup1551021–1391), 111 (GAD-hNup1551054–1391), or 143 (GAD-hNup1551086–1391) amino acid residues of the hNup155C polypeptide were deleted (Fig. 2C). Constructs lacking the first 10 or 43 residues of hNup155C interacted with hGle1; however, the constructs lacking the first 78 or more residues did not interact with hGle1 despite high expression levels of these fusion proteins (data not shown). Therefore, the sequences corresponding to the span between residues 986 and 1020 of hNup155 were important for hGle1 interaction. This region alone was insufficient for interaction as the GAD-hNup155963–1121 fusion protein did not interact with GBD-hGle1A in the two-hybrid assay (Fig. 2C). Thus, the binding site(s) for hGle1 possibly extended over a longer portion of the hNup155C polypeptide. To determine the shortest binding region, deletion mutants were constructed from the C-terminal end of the GAD-hNup155987–1391 polypeptide (in which the initial 43 amino acids were also absent). A minimal hGle1-binding domain of 177 amino acids within hNup155C was identified (Fig. 2C; hNup155987–1159), as delineated in the boxed sequence of Fig. 1.

hGle1 and FL hNup155 Interact in Vitro

To confirm the yeast two-hybrid interaction as well as to determine whether hGle1 (A and B variants) interacts with FL hNup155, we conducted affinity chromatography assays (Fig. 3). Bacterially expressed GST-hGle1 fusions were immobilized on beads and incubated with [35S]-labeled FL hNup155 produced in vitro using a rabbit reticulocyte lysate system. To delineate the minimal region of hGle1 required for binding to FL hNup155 in vitro, hGle1 was divided into two halves at amino acid 371, and GST-fusions were generated with each half. In contrast to GST alone and GST-hGle1371–699, significant amounts of [35S]-FL hNup155 were recovered when GST-hGle12–372 was used, confirming that the hNup155-binding domain was located at the N terminus of hGle1 (Fig. 3A). Additional C-terminal deletions of hGle12–372 domain (GST-hGle11,109 and GST-hGle12–29) showed that the first 29 N-terminal amino acids of hGle1 are sufficient for binding to FL hNup155 in vitro. To determine if these 29 residues are necessary for the binding of hGle1A and hGle1B to FL hNup155, we next tested whether bacterially expressed GST-hGle1A329 and GST-hGle1B329 could bind [35S]-labeled FL hNup155 in vitro. As seen in Fig. 3B, the deletion of the first 29 amino acids of hGle1 eliminated hNup155 binding to GST-hGle1A and GST-hGle1B fusions. Together with the yeast two-hybrid experiments, these results indicate that hGle1 can interact with FL hNup155 through the first N-terminal 29 amino acid peptide span and that these residues are necessary and sufficient for the binding of FL hNup155 to both hGle1 protein variants in vitro.

| Category | No. of Isolates | Insert size (kbp) | DNA sequence homology | Ref. |
|----------|----------------|-------------------|-----------------------|------|
| A        | 19             | 1.2–2             | Keratin 10, type 1    | (41) |
| B        | 5              | 1.5               | Human Nup155 (C-terminus) | (28, 33) |
| C        | 2              | 1.2               | elf3-related protein  | (42) |
| D        | 1              | 0.65              | Ubiquitously expressed transcript (UTX) protein | (43) |

**Identification of the Binding Sites Within hGle1 and hNup155**
To further analyze the potential interactions between hGle1 and hNup155, an in vitro assay testing for direct protein-protein interactions was conducted with bacterially expressed proteins (Fig. 4). Two different hNup155 fusions were tested; a His-tagged-hNup155C fusion protein and a maltose binding protein (MBP) tagged-hNup155CN (residues 943–1188). The hNup155 fusion proteins were expressed in E. coli, and total crude lysates were separated by SDS-PAGE. Coomassie staining confirmed expression (Fig. 4; lanes 3, 4, and 5 at arrowheads). Similar gels were transferred to nitrocellulose membranes, and ligand blot assays were completed using cell lysates of bacterially expressed MBP-hGle11–105 (Fig. 4, lane 2) or purified MBP-FL-hGle1A (not shown). Binding of hNup155 fusion proteins were expressed in E. coli, and total crude lysates were separated by SDS-PAGE. Coomassie staining confirmed expression (Fig. 4A; lanes 3, 4, and 5 at arrowheads).
MBP-hGle1-150 was detected using an affinity-purified antibody against hGle1 (Fig. 4B). MBP-hGle1 bound to both of the hNup155C recombinant proteins (Fig. 4B, lanes 2, 3, and 6), but not to MBP alone (Fig. 4B, lane 4). These results strongly suggested that the two-hybrid interaction between hGle1A and hNup155C reflects a direct protein-protein interaction.

The hNup155-binding Domain Is Required for the Localization of hGle1 at NPCs in HeLa Cells

We next investigated whether the interaction with hNup155 is required for hGle1 localization at NPCs in HeLa cells (Fig. 5). Because overexpression of hNup155 did not have a dominant
Fig. 3. hGle1’s first 29 N-terminal residues are necessary and sufficient for in vitro binding to hNup155. Purified, bacterially expressed GST fusions were immobilized on beads and incubated with [35S]-labeled FL hNup155 (pSW1455) generated in a reticulocyte lysate system. Bound proteins were eluted and analyzed by SDS-PAGE and autoradiography. Input constitutes 50% of in vitro-labeled hNup155 protein. A, top, Coomassie-stained SDS-PAGE of immobilized hGle1A regions fused to GST (asterisks); bottom, autoradiograph of bound FL hNup155. In contrast to GST alone and GST-hGle1A29–659, appreciable amounts of [35S]-hNup155 can bind GST-FL hGle1A (residues 1–659) as well as all hGle1 deletion constructs harboring the first 29 N-terminal amino acids (GST-hGle1A2–372, GST-hGle1A2–105, and GST-hGle1A2–29). B, [35S]-labeled FL hNup155 binds specifically to GST-hGle1A and GST-hGle1B but not to GST alone or to GST-hGle1 protein variants lacking the first 29 N-terminal residues (GST-hGle1A2–99 and GST-hGle1B2–29).

Fig. 4. hGle1 and hNup155C directly interact in vitro. A, Coomassie-stained polyacrylamide gel of bacterially expressed MBP-hGle11–105 (lane 2), MBP (lane 3), MBP-hNup155C (residues 987–1159; lane 4), and His-hNup155C (residues 943–1391; lane 5) from total cell lysates. The arrows note the positions of the respective polypeptides (confirmed by immunoblotting, data not shown). B, Direct interaction of hGle1 and hNup155C by ligand blot assay. A polyacrylamide gel identical to lanes 3, 4, and 5 in A was transferred to nitrocellulose and incubated with lysate from cells expressing MBP-hGle11–105. Affinity-purified antibodies recognizing hGle1 were used to detect the binding of MBP-hGle11–105. The MBP-hGle11–105 fusion does binds to both His-Nup155C (lane 2) and MBP-hNup155C (lane 3) but does not bind MBP alone (lane 4). In lane 6, 1/25th the amount of MBP-hNup155C was tested (as compared with lane 3). In lanes 2, 3, and 6, the lower molecular mass bands represent proteolytic products of the FL fusions (as determined by immunoblotting, data not shown).
negative effect on the intracellular localization of endogenous hGle1 (data not shown), we studied the impact of deleting the first 29 amino acid residues of hGle1 (i.e., the hNup155-binding domain) on the nuclear rim localization of ectopically expressed hGle1. We previously reported that two hGle1 protein variants (hGle1A and hGle1B) are expressed in HeLa cells and that only hGle1B strongly localizes to the nuclear rim (27). After tagging both variants with the green fluorescent protein (GFP) at their N termini, we overexpressed GFP-hGle1A and GFP-hGle1B in HeLa cells. As shown in Fig. 5, A and C, the intracellular distributions of endogenous hGle1 and GFP-hGle1B were similar (27), showing colocalization with the subset of nuclear pore proteins detected by mAb 414 (Fig. 5, B and D) (36). In contrast, GFP-hGle1A, GFP-hGle1B<sub>129</sub>, and GFP-hGle1A<sub>129</sub> (Fig. 5, G, E, and I, respectively) show no detectable nuclear rim localization. These results suggested that in vivo the hNup155-binding domain within hGle1B is required for NPC localization of hGle1B. However, the lack of GFP-hGle1A at the nuclear rim argues that the hNup155-binding domain is not sufficient for NPC targeting of hGle1 in HeLa cells. Interestingly, the ectopic expression of the GFP-tagged C-terminal region of hGle1B harboring the unique 43-aa peptide span (residues 477–698; Fig. 2 and Ref. 27) also does not localize to the nuclear rim (data not shown). This suggests that neither the hNup155-binding domain nor the unique C-terminal domain of hGle1B are alone sufficient for NPC localization of GFP-hGle1B. We propose that the N-terminal hNup155 binding domain and the unique C-terminal 43 amino acids of hGle1B are together required for the nuclear pore targeting of hGle1B. In the absence of either domain (as in the case of hGle1A or GFP-hGle1B<sub>129</sub>), hGle1 fails to localize to the nuclear rim in HeLa cells.

**DISCUSSION**

In this study, we provide the first evidence of a physical interaction between the shuttling mRNA export factor hGle1 and a vertebrate Nup. Using a genome-wide yeast two-hybrid strategy in combination with in vitro binding assays, we have identified three hGle1 protein-protein interactions including a novel interaction with nucleoporin hNup155. This interaction requires the first 29 amino acid residues of hGle1 designated the hNup155-binding domain. In HeLa cells, we demonstrated that in the absence of the hNup155-binding domain hGle1B no longer localizes at the nuclear rim. This suggests that this domain is necessary for NPC localization of hGle1B. Finally, we have identified a possible functional domain within hNup155, a stretch of 177 amino acid residues in the C-terminal half that interacts with hGle1. These experiments lead us to conclude that hGle1 can interact with hNup155 in HeLa cells and that this interaction is necessary for the localization of hGle1B at NPCs. Together, these results contribute to our understanding of the NPC components that may be required for hGle1 to mediate its role in the export of poly(A)<sup>+</sup> RNA from the nucleus.

We originally identified the homologue of hGle1 in budding yeast (scGle1) in a synthetic lethal screen with a *nup100Δ* strain.
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**Fig. 6. Schematic representation of structural and functional features of hGle1 protein variants.** Amino acids 1–29 (open box) delineate hNup155-binding domain at NPCs. Amino acids 105–360 form hGle1’s coiled-coil domain (24), which may be implicated in protein-protein interactions (39). Amino acids 444–483 form hGle1’s nucleocytoplasmic shuttling domain required for hGle1-mediated mRNA export (gray box; Ref. 27). The unique 43-aa C-terminal domain of hGle1B is required for nuclear rim targeting (hatched box). Although not depicted here, hGle1A differs from hGle1B by its last four unique amino acids (27).

The presence of rNup155 on both the nucleoplasmic and cytoplasmic faces of the NPC (28, 38) positions hGle1-binding sites in both compartments. Together with the fact that hNup155 is a relatively abundant nucleoporin (4), hGle1 could potentially interact with hNup155 on both sides of the NPC. This agrees with our analysis of hGle1’s dynamics as a shuttling mRNA export factor (27). Previous analysis of transport factors-Nup interactions has for the most part revealed binding sites in Nup domains that contain phenylalanine glycine (FG) peptide repeats. Our characterization of the interaction between a transport factor (hGle1) and a non-FG Nup (hNup155) is part of a new paradigm. In budding yeast, binding of scGle1 and scDbp5 to non-FG domains in FG-Nups is well documented (20, 21, 22, 25, 37), and recent studies have found a high-affinity Kap95-binding site in the non-FG domain of Nup1 (39). Further studies on hGle1’s affinity for cytoplasmic or nucleoplasmic hNup155-binding sites, as well as domain accessibility within hNup155, will prove instrumental in characterizing the functional interaction between hGle1 and hNup155. Because no reports of domain analysis on Nup155 and no vertebrate Nup155-containing complexes have been published, this work represents the first reported protein-protein interactions for both hGle1 and hNup155.

Although we do not yet know if hGle1A is implicated in mRNA export, work by us and others has shown that both scGle1 and hGle1B act at a critical step in mRNA export from the nucleus (22–27). However, whether the mechanism by which Gle1 mediates mRNA export is conserved between human and budding yeast is still unknown. Although we have documented that hGle1B shuttles (27), it is not known if scGle1 shuttles. Nevertheless, evidence suggests that scGle1 and hGle1 may operate by a similar mechanism. Indeed, expression of chimeric yeast-human Gle1 fusion proteins complement an S. cerevisiae gle1 mutant phenotype (24). Our future studies will aim at deciphering the functional differences between both hGle1 protein variants and isolating additional hGle1 interacting proteins in an attempt at resolving the mechanism(s) underlying hGle1B shuttling and docking at NPCs. As we have identified at least three functional domains within hGle1 (Fig. 6), the subsequent isolation and character-
ization of factors interacting with each domain of hGle1 will set the stage for understanding the molecular link(s) between hGle1 dynamics and the export of mRNA-containing protein complexes from the nucleus.

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