Nuclear export of the yeast mRNA-binding protein Nab2 is linked to a direct interaction with Gfd1 and to Gle1 function

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SUMMARY

Nuclear export of messenger RNA (mRNA) is mediated by interactions between soluble factors and nuclear pore complex (NPC) proteins. In *Saccharomyces cerevisiae*, Nab2 is an essential RNA binding protein that shuttles between the nucleus and cytoplasm. The mechanism for trafficking of Nab2-bound mRNA through the NPC has not been defined. Gle1 is also required for mRNA export and Gle1 interactions with NPC proteins, the RNA helicase Dbp5, and Gfd1 have been reported. Here we report that Nab2, Gfd1, and Gle1 associate in a complex. Using immobilized recombinant Gfd1, Nab2 was isolated from total yeast lysate. A similar biochemical assay with immobilized recombinant Nab2 resulted in co-isolation of Gfd1 and Gle1. A Nab2-Gfd1 complex was also identified by coimmunoprecipitation from yeast lysates. In vitro binding assays with recombinant proteins revealed a direct association between Nab2 and Gfd1, and two-hybrid assays delineated Gfd1 binding to the N-terminal Nab2 domain. This N-terminal Nab2 domain is distinct from its RNA binding domains suggesting Nab2 could bind Gfd1 and RNA simultaneously. As Nab2 export was blocked in a gle1 mutant at the restrictive temperature, we propose a model wherein Gfd1 serves as a bridging factor between Gle1 and Nab2-bound mRNA during export.
INTRODUCTION

Trafficking of molecules between the nucleus and cytoplasm proceeds through portals known as nuclear pore complexes (NPCs). NPCs are embedded in a pore formed by the fusion of the inner and outer membranes of the nuclear envelope. Yeast and vertebrate NPCs are highly conserved in architecture with a characteristic nuclear basket, 8-fold central spoke-ring structure that forms an aqueous channel, and cytoplasmic filaments (1). Proteomic analysis has revealed that NPCs are composed of ~30 distinct nucleoporins (Nups) (2,3). While diffusion accounts for the movement of small molecules, such as ions and metabolites, a facilitated, energy-dependent process mediates the rapid transport of larger molecules, such as proteins and messenger RNA (mRNA) (4). Transport rates for macromolecules are estimated at upwards of ~520 to 1000 molecules per second per NPC (5,6).

Protein import and export pathways are mediated by karyopherins (Kaps), a family of shuttling transport factors (7). Kaps bind their cargo by recognition of a nuclear localization sequence (NLS) for import or a nuclear export sequence (NES) for export (8). Key steps in movement through the NPC include initial docking of the Kap-cargo complex, translocation through the aqueous channel and release of the cargo (9). These are presumably dictated by direct interactions between Kaps and Nups (10,11). The directionality of transport is regulated by the small GTPase Ran (10,12). High nuclear RanGTP levels are maintained by the compartmentalization of the guanine nucleotide exchange factor to the nucleus and the GTPase activating protein to the cytoplasm. Thus, during import, high RanGTP levels facilitate release
of cargo from the Kap in the nucleus. In contrast, during export, cargo binding to the Kap is enhanced in the nucleus and release is triggered by RanGTP hydrolysis in the cytoplasm.

Remarkable progress has also been made in the past decade towards identifying factors that play a role in mRNA export. Current models for mRNA export predict the existence of multiple steps at both the nuclear and cytoplasmic faces of the NPC. Recent studies also suggest that packaging the mRNA for export could start as early as transcription (reviewed in 4, 13). During transcription and pre-mRNA processing, the RNA is bound by heterogeneous nuclear ribonucleoproteins (hnRNPs). Some hnRNPs contain nuclear retention signals and are removed from the mRNA prior to export whereas others shuttle between the nucleus and cytoplasm (14). In the budding yeast *Saccharomyces cerevisiae*, Nab2, Hrp1 and Npl3 are shuttling hnRNPs (15-17). Nab2 is imported into the nucleus by an interaction with Kap104 (18,19). Within the nucleus, Nab2 has roles linked to the Thp1 transcription complex, the regulation of pre-mRNA polyadenylation and mRNA processing, and mRNA export (20-23). The Nab2 export mechanism is dependent on the ubiquitin ligase Tom1 and the arginine methyltransferase Hmt1 (24,25). However, it is unclear how ubiquitination and methylation affect the pathway. A recent report shows that one export step involves Nab2 binding to Mlp1 on the nuclear face of the NPC (26). Movement through the NPC may require further interactions with Nups or NPC-associated factors. Since mRNA is tightly linked to hnRNPs, delineating the molecular pathways for mRNA export will require an understanding of the interplay between these mRNA binding proteins and export factors.
Many studies have focused on dissecting the role of budding yeast Mex67 (Tap/NXF1 in vertebrates) in mRNA export pathway. Mex67 forms a heterodimer with another export factor, Mtr2, binds the hnRNP-like factor Yra1, and interacts with FG (phenylalanine, glycine) repeats in several Nups (27-29). Functional associations have also been established between Mex67 and the TREX complex through Yra1 and the splicing factor Sub2 (30-32). Mex67, Yra1 and Sub2 have all been further linked to the SAGA histone acetylase complex (22). Thus, Mex67 is a key player in connecting transcription and mRNA export. Other non-hnRNP factors with roles in mRNA export are Dbp5, Gle1 and Gle2/Rae1 (33-38). Dbp5 is a DEAD-box RNA helicase which localizes to the cytoplasmic face of the NPC through an interaction with Nup159 (39,40). The unwindase activity could provide directionality and represent a terminal step in mRNA export. Recent work has demonstrated that Dbp5 also has a role in transcription (41). Gle2 is required for efficient mRNA export in yeast and has a docking site on Nup116 (37,42,43). The role of Gle2/Rae1 in vertebrate cells is not clear since MEFs and blastocytes from knockout mice exhibit cell cycle defects, but not mRNA export defects (44). However, a complex of Gle2/Rae1, Nup98 and Mex67/Tap/NXF1 has been detected in vitro (45).

Our objective has been to provide insight into Gle1-mediated mRNA export. In budding yeast, Gle1 localizes to NPCs at steady state and gle1 mutants rapidly accumulate polyA+ RNA in the nucleus (36). GLE1 was initially identified in budding yeast by a synthetic lethal screen with a nup100 mutant (36) and as a multicopy suppressor of a nup159/rat7 mutant (46). Genetic
and physical interactions have also been shown between Gle1 and Nup42/Rip1 (36,47,48).

Besides the interactions with Nups, Gle1 also binds Dbp5 and a novel factor Gfd1 (40, 48).

\textit{GFD1} is a multicopy suppressor of \textit{gle1-8} and \textit{dbp5-2} mutants (40,48). Gfd1 localizes to the cytoplasm and nuclear rim and interacts with Gle1, Dbp5 and Nup42 by two-hybrid analysis (40,48). Precisely how Gfd1 plays a role in Gle1-mediated mRNA export has not been defined.

Finally, the nucleocytoplasmic shuttling of the human (h) homolog hGle1 is required for mRNA export (49), but it is unknown whether Gle1 shuttles in \textit{S. cerevisiae}.

Even though evidence exists for Gle1 having a role in mRNA export in \textit{S. cerevisiae} and vertebrate cells, its connection to the cargo mRNA has not been delineated. To understand the Gle1-mRNA export pathway, we have focused here on the roles of Nab2 and Gfd1. Our studies demonstrate that Nab2 and Gfd1 associate in a complex both in vivo and in vitro. We find that the N-terminal domain of Nab2 interacts with Gfd1. Interestingly, recent work has shown that the N-terminal domain of Nab2 is required for polyA+ RNA export (23), and we observed a Nab2 export block in \textit{gle1-4} mutant cells. Overall, our results support a model wherein the recruitment of Gle1 to a Nab2 complex represents a step in the mRNA export pathway.
EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids- All yeast strains used in this study are listed in Table I. The sequence encoding the green fluorescent protein (GFP) was fused in frame before the stop codon for the chromosomal GFD1 using the gene integration method by Baudin et al. (50). Polymerase chain reaction products were generated with oligonucleotides and a template containing sequences encoding GFP and the Schizosaccharomyces pombe HIS5 (pGFP-HIS5; kindly provided by J. Aitchison). The resulting DNA fragment was transformed into SWY518 using the lithium acetate method and colonies were selected on media lacking histidine. Correct integration was confirmed by immunoblotting with affinity-purified rabbit polyclonal anti-GFP antibodies (kindly provided by M. Linder). The resulting strain was back-crossed twice and the GFP tagged progeny was used in this study. The plasmids used in this study are listed in Table II and were maintained in either BL21 (Nab2-GST (18), pSW1279 and pSW1296) and or DH5α (all others). Expression vector references: pGAD-C1 and pGBD-C1 (51); pGEX-5X (Amersham Pharmacia Biotech); pMAL-cR1 (New England Biolabs).

Yeast Two-hybrid Analysis- Gal4$_{AD}$-Gfd1 and Gal4$_{BD}$-Nab2 constructs were co-transformed into the two-hybrid reporter strain PJ69-4A. Transformants were selected on SC medium lacking leucine and tryptophan and assayed for interaction by growth on SC medium lacking leucine, tryptophan, histidine and adenine. Growth was scored at 30°C. All plasmids
were tested for specificity and ability to self-activate using Gal4\textsubscript{AD}-Snf4 and Gal4\textsubscript{BD}-Snf1.

\textit{Affinity Chromatography}- This assay was performed as described previously (52). For the yeast extracts, wild type (SWY518) or Gle1-TAP-tagged (62) cells were lysed in 20 mM Hepes pH 6.8, 150 mM potassium acetate, 2 mM magnesium acetate, 250 mM sorbitol, 0.1 mg/ml phenylmethylsulfonyl fluoride (PMSF), 1 µg/ml pepstatin, 2 µg/ml aprotinin, and 2 µg/ml leupeptin by passing through a French Press. Following centrifugation at 30,000 x g for 30 min at 4°C, the supernatant was filtered through a 0.45µ filter and desalted in a Sephadex G-25 fine column. The lysate was supplemented with 0.1% Tween-20, 2 mM DTT, and complete protease inhibitor cocktail (Roche). For recombinant protein, \textit{E. coli} cells with pGEX-5X (GST), pSW1296 (GST-Gfd1) or Nab2-pGEX2TK (Nab2-GST) were grown at 37°C in 1L of 2 X YT medium containing 100 µg/ml carbenicillin and 2% glucose to an OD\textsubscript{600} of 1. Protein expression was induced with 0.3 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 3 h at 37°C. Cells were resuspended in 30 ml ice-cold 50 mM Tris pH 8.0, 100 mM NaCl and 2 mM EDTA containing PMSF and complete protease inhibitor cocktail (Boehringer) and lysed in a French Press. Following centrifugation at 30,000 x g for 15 min at 4°C, the supernatant was supplemented with 0.1% Tween-20. Aliquots of yeast and \textit{E.coli} lysates were frozen in liquid nitrogen and stored at 70°C.

For the binding assay, \textit{E.coli} extracts were incubated with Glutathione-Sepharose beads
(Amersham Pharmacia Biotech) for 20 min at 4°C. The beads were washed 3x in binding buffer (20 mM Heps, pH 6.8, 150 mM potassium acetate, 2 mM magnesium acetate, 2 mM DTT, 0.1% Tween-20), 2x with binding buffer containing 0.1 mM ATP and 2x with binding buffer containing 1 M NaCl. The beads were incubated with 1 ml yeast lysate or binding buffer alone for 2 h at 4°C and then washed 6x with binding buffer. Bound proteins were eluted with 100 µl of 1 M NaCl followed by boiling in SDS-sample buffer or eluted directly by boiling in SDS-sample buffer. Proteins eluted with 1 M NaCl were precipitated with trichloroacetic acid/sodium deoxycholate. Blots were probed with monoclonal anti-Nab2 antibodies (kindly provided by J. Aitchison) diluted 1:4000, rabbit polyclonal anti-Gle1 antibodies diluted 1:200 or rabbit anti-Gfd1 peptide antibodies diluted 1:200 (overnight, 4°C). Bound proteins were detected using peroxidase-labeled anti-mouse IgG or anti-rabbit IgG (1h, 23°C) and developed by enhanced chemiluminescence (Amersham Pharmacia Biotech) for anti-Nab2 and anti-Gfd1 or SuperSignal West Femto chemiluminescence (Pierce) for anti-Gle1.

Antibodies- MBP-Gle1 was expressed and purified as described previously (37). The antigen was sent to Cocalico Biologicacls for production of rabbit antiserum WU851. Antiserum to Gle1 was purified by affinity chromatography over a GST-Gle1 Affi-Gel 10 column (Bio-Rad) as described previously (37). Anti-Gfd1 peptide antibodies (Gfd1D/1) were generated by Bethyl Laboratories. Bethyl Laboratories synthesized the peptides Gfd1D9R25 (CDAPDEEPIKKQKP) and Gfd1K85K101 (CKISPVSESLAINPFSQK) for production of
rabbit anti-serum, and purified the antiserum against the Gfd1D9R25 peptide.

**Immunoprecipitation** - Cultures of SWY518 (Gfd1) and SWY2535 (Gfd1-GFP) were grown to an OD600 of ~0.5 in rich medium (YPD; 1% yeast extract, 2% peptone, 2% glucose). Whole cell lysates were prepared by glass bead lysis in 20mM Tris pH 6.5, 150mM NaCl, 5mM MgCl₂, 2% Triton X-100 supplemented with complete protease inhibitor cocktail and 1mM PMSF. Immunoprecipitations were conducted as described previously (53). Briefly, 2µl of anti-GFP antibody was mixed with 200 µl of cell extract and 50 µl of Protein A-sepharose beads for 1.5 hours at 4°C. The beads were washed six times with 50 mM Tris pH 6.5, 150 mM NaCl, 0.05% Tween-20 before elution. Blots were probed with anti-Nab2 monoclonal antibodies at a 1:3000 dilution (overnight, 4°C) and peroxidase-labeled anti-mouse IgG (1h, 23°C). The blots were developed by enhanced chemiluminescence.

**Purification of Protein and Soluble Binding Assay** - *E. coli* strains containing pGEX-5X (GST) or pSW1296 (GST-Gfd1) were grown in 1 liter of LB media containing 100µg/ml carbenicillin and 2% glucose at 37°C. When the OD₆₀₀ was ~1, cultures were induced for 3 hours with 0.3mM IPTG and cell pellets frozen at 70°C. Thawed pellets were resuspended in ice-cold lysis buffer (20 mM sodium phosphate pH7.3, 150 mM NaCl, 10 mM EDTA, 0.1 mM DTT) supplemented with complete protease inhibitor cocktail and 0.1 mM PMSF and lysed in a French
Press. The suspension was centrifuged at 9000 x g for 30 min, and Triton X-100 was added to a final concentration of 1%. The supernatant was diluted in lysis buffer containing 1% Triton X-100 and incubated with Glutathione-Sepharose resin for 2 h at 4°C. After washing the resin with 10 ml lysis buffer containing 1% Triton X-100 and 25 ml of lysis buffer the beads were packed into a column. The column was washed with lysis buffer until the OD$_{280}$ of the flow-through was ~0. The protein was eluted in 50 mM Tris pH 9.0, 10 mM glutathione.

MBP and MBP-Nab2 were purified as described above with the following modifications. The lysis buffer was 10 mM sodium phosphate pH 7.2, 30 mM NaCl, 0.25% Tween-20, 10 mM EDTA, 10 mM EGTA and 10 mM β-mercaptoethanol supplemented with PMSF and complete protease inhibitor cocktail. Following lysis, NaCl was added to a final concentration of 0.5 M. The supernatant was incubated with amylose resin (New England Biolabs) overnight at 4°C. After washing with 25 ml column buffer (10 mM sodium phosphate pH 7.2, 0.5 M NaCl, 10 mM β-mercaptoethanol) containing 0.25% Tween-20) the resin was packed in a column and washed with column buffer until the OD$_{280}$ of the flow through was ~0. The bound protein was eluted with column buffer containing 10mM maltose. MBP-Gle2 (kindly provided by L. Strawn) was purified as described previously (29).

Purified proteins were dialyzed into 20 mM Hepes pH 6.8, 150 mM potassium acetate, 2 mM magnesium acetate, 2 mM DTT and 10% glycerol. Binding assays were conducted as described previously with 5 µg of proteins in binding buffer (20 mM Hepes pH6.8, 150 mM
potassium acetate, 2 mM magnesium acetate, 2 mM DTT, 10% glycerol and 0.1% casamino acids). Bound and unbound fractions were separated on SDS-polyacrylamide gels and detected by Coomassie staining.

**Fluorescence Microscopy**— Plasmids expressing Nab2-GFP or Nab2RGG-GFP were transformed into wild type (SWY518), gle1-4 (SWY1191) or gfd1 cells by standard techniques. Stationary cultures grown in SC media lacking uracil (-ura) were diluted and grown overnight at 23°C to early log phase. Half the culture was maintained at 23°C and the other half shifted to 37°C for 1 hour. Cells were then labeled with Hoechst 33258 at a final concentration of 10 µg/mL, washed once in SC-ura and viewed by direct fluorescence microscopy. Images were collected with a 100x objective on an Olympus BX50 microscope using a Photometric CoolSNAP HQ camera (Roper Scientific) and MetaVue software.
RESULTS

Nab2 interacts with Gfd1 in two-hybrid and biochemical affinity assays

In a recent report of a genome-wide two-hybrid interaction analysis, an interaction between Nab2 and Gfd1 was reported (54). To test whether this two-hybrid result was potentially physiologically significant, we further analyzed the specificity of the two-hybrid interaction. In-frame fusions were generated for Gfd1 to the transcriptional activation domain of Gal4 (Gal4_{AD}), and for Nab2 to the DNA binding domain of Gal4 (Gal4_{BD}). These plasmids were co-transformed into reporter strains and the presence of an interaction was assayed by growth on media lacking histidine and adenine. As controls for non-specific interactions, we used fusions of the transcription factors Snf1 to Gal4_{BD} and Snf4 to Gal4_{AD}. As shown in Figure 1A, a two-hybrid interaction was observed specifically between Nab2 and Gfd1, and not between Nab2 and Snf4 or Gfd1 and Snf1.

Next, we used an affinity chromatography assay to determine whether a physical interaction between Nab2 and Gfd1 could be detected in vitro. Gfd1 was expressed in bacteria as a fusion to glutathione-S-transferase (GST) and bound to glutathione sepharose beads. The immobilized GST-Gfd1 was then incubated with buffer alone or total yeast lysate prepared under non-denaturing conditions. Bound proteins were sequentially eluted, first with high salt (1M NaCl) followed by boiling in SDS buffer. Eluted fractions were separated by SDS-PAGE and analyzed by immunoblotting with monoclonal anti-Nab2 antibodies. Nab2 in the yeast
lysate specifically bound to GST-Gfd1 and not GST alone (Fig. 1B, lanes 1 and 3). Additionally, the majority of Nab2 that was bound to GST-Gfd1 was eluted with high salt (Fig. 1B, lanes 1 and 5). The anti-Nab2 antibody did not recognize any proteins in the *E. coli* extract (Fig. 1B, lanes 2, 4, 6 and 8). These results are consistent with Nab2 forming a complex with Gfd1.

*Nab2 exists in a complex with Gfd1 in vivo*

The identification of a physical in vitro interaction between recombinant GST-Gfd1 and endogenous Nab2 led us to ask whether a complex between the two endogenous factors could be isolated from yeast cell lysates. To approach this question, we generated a yeast strain with the sequence encoding GFP chromosomally integrated in frame before the stop codon for *GFD1*. The lysates from Gfd1-GFP expressing cells showed that a polypeptide of ~50kDa was recognized by immunoblotting with polyclonal anti-GFP antibodies (data not shown). Next, we conducted co-immunoprecipitation studies with yeast lysates from wild-type cells and Gfd1-GFP expressing cells. After incubation of lysates with anti-GFP antibodies and Protein A sepharose beads, the input and bound proteins were analyzed by SDS-PAGE and immunoblotting with anti-Nab2 antibodies. The blots revealed the presence of a single ~70kDa protein only in the bound fraction from Gfd1-GFP lysates and not from wild type lysates (Fig. 2, lane 3). Our previous studies have shown that the anti-GFP antibodies are specific only for the GFP-tagged proteins (29). Taken together, these results strongly suggest that Gfd1 and Nab2 associate in a complex in vivo.
Recombinant Nab2 and Gfd1 interact directly

The interactions observed thus far did not indicate whether the binding between Gfd1 and Nab2 was direct or required the presence of other adaptor proteins. To analyze whether there was a direct and stable in vitro interaction, we performed soluble binding assays with purified recombinant proteins. Gfd1 was purified from bacteria as a fusion to GST, and Nab2 as a fusion to maltose binding protein (MBP). GST or GST-Gfd1 immobilized on glutathione sepharose beads was incubated with MBP-Nab2. Unbound and bound fractions were separated by SDS-PAGE and detected by Coomassie staining. As seen in Figure 3A (lane 2) the ~50kDa polypeptide corresponded to full-length GST-Gfd1 whereas purified MBP-Nab2 migrated as a major ~120kDa protein (Fig. 3A, lane 3). Analysis of the unbound and bound fractions showed that MBP-Nab2 was found in the bound fraction together with GST-Gfd1 (Fig. 3B, lane 4). In contrast, MBP-Nab2 remained in the unbound fraction with immobilized GST alone (Fig. 3B, lane 1). To determine whether GST-Gfd1 bound MBP-Nab2 specifically, we tested the binding of MBP-Gle2. As shown in Figure 3B (lanes 5 and 6), GST-Gfd1 did not bind MBP-Gle2. Therefore, the interaction between Gfd1 and Nab2 is direct and specific.

The N-terminal domain of Nab2 binds Gfd1

Earlier studies have suggested four potential functional domains in Nab2. The N-terminal domain is required for export of both Nab2 and polyA+ RNA, the glutamine rich
domain is of unknown function, and the RGG and zinc-finger regions are the RNA binding domains (15,23). The RGG domain is also required for binding to the nuclear import factor, Kap104 (18). To identify the domain in Nab2 required for an interaction with Gfd1, the two-hybrid assay was used. A series of GBD-Nab2 deletion constructs were tested for a positive interaction with GAD-Gfd1. As summarized in Figure 4, deletion of the RGG domain had no effect on the interaction with Gfd1. In contrast, deletion of the N-terminal and glutamine rich regions abolished the interaction. Consistent with this result, a construct expressing the N-terminal and glutamine rich domains was sufficient to observe a positive two-hybrid interaction. Further two hybrid analysis revealed that the N-terminal domain of Nab2 was sufficient for Gfd1 binding. Interestingly, this indicated a role for a domain distinct from the RNA binding domains in Nab2, and we concluded that the N-terminal domain is necessary and sufficient for Gfd1 binding.

_Gle1 is required for Nab2 export and associates with Nab2-Gfd1 complexes_

Given that overexpression of _GFD1_ rescues the growth of _glel_ and _dbp5_ mutants (40,48), and Gfd1 interacted with the N-terminal domain of Nab2 (Fig. 4), we speculated that Gfd1 was a good candidate for facilitating Nab2 export. To directly test this possibility, we used an assay Corbett and coworkers developed to study Nab2 import and export (23). Plasmids expressing Nab2-GFP and Nab2RGG-GFP were transformed into wild type and _gfd1_ mutant strains. At
steady state in wild-type cells, Nab2-GFP localizes to the nucleus whereas Nab2RGG-GFP localizes throughout the cell (Fig. 5A and 6A). Therefore, the localization of Nab2RGG-GFP is a useful tool to study Nab2 export. Wild type and gfd1 cells were grown at 23°C and shifted to 37°C for 1 hour or 3 hours (data not shown). To visualize the nuclei, cells were labeled with Hoechst dye after the temperature shift. In gfd1 cells, the localization of Nab2-GFP and Nab2RGG-GFP was not perturbed at 23°C or after incubation at 37°C. Thus, Gfd1 was not required for efficient Nab2 export correlating with the finding that gfd1 cells do not demonstrate a detectable defect in viability or polyA+ RNA export (40,48).

Since Gfd1 physically associates with Gle1 (48), we speculated that Gfd1 could facilitate an interaction between Nab2 and Gle1. To determine whether Gle1 was also present in the Nab2-Gfd1 complex, we performed an affinity chromatography assay. Immobilized recombinant Nab2-GST or GST alone was incubated with yeast lysate from cells expressing Gle1-TAP-tagged. As shown in Figure 7, Gle1-TAP-tagged and Gfd1 were co-isolated in the bound fraction with Nab2-GST (lane 2), but not with GST alone (lane 3). Interestingly, a greater proportion of the total Gfd1 was isolated compared to the relative fraction of bound Gle1-TAP-tagged. To test for a functional connection between Nab2 and Gle1, we used the Nab2 localization assay described above (Fig. 5 and 6). In a gle1-4 mutant, Nab2-GFP localized to the nucleus at both 23°C and after a shift to the non-permissive temperature, 37°C, indicating that import was not affected (Fig. 5G). Strikingly, Nab2RGG-GFP, which was
localized throughout \textit{gle1-4} mutant cells at 23\textdegree C, showed a marked nuclear accumulation at 37\textdegree C (Fig. 6G and P). These results suggested that Gle1 was physically connected to Nab2 complexes and required specifically for Nab2 export but not Nab2 import.
DISCUSSION

To delineate the mechanism underlying mRNA transport through the NPC, it is essential to understand the interactions between shuttling hnRNPs and mRNA export factors. In this study, we report that the shuttling hnRNP Nab2 forms a complex with Gfd1. A role for Gfd1 in mRNA export has been previously implicated due to its interactions with Nup42 and the mRNA export factors Gle1 and Dbp5 (40,48). We have assembled a series of biochemical data documenting direct Nab2-Gfd1 binding. This includes yeast two hybrid assays, isolation of endogenous Nab2 using recombinant GST-Gfd1, Nab2 isolation by coimmunoprecipitation with Gfd1-GFP from yeast cell lysates, and soluble binding assays using purified recombinant Nab2 and Gfd1. Since previous in vivo and in vitro evidence has documented an interaction between Gfd1 and Gle1 (40, 48), Gfd1 could link Nab2 export to Gle1 function. Consistent with this conclusion, Nab2ΔRGG export is blocked at the restrictive temperature in a gle1-4 mutant. Moreover, endogenous Gfd1 and Gle1-TAP-tagged are co-isolated using recombinant Nab2-GST. These results highlight a connection between Nab2 and Gle1-mediated mRNA export.

To date, Kap104 and Mlp1 are the only reported protein binding partners for Nab2 (18,26). Incorporating our findings with this work, a model for Nab2 shuttling is presented in Figure 8. Import is mediated by direct binding of Kap104 to the RGG domain of Nab2, and Nab2 accumulates in the cytoplasm of kap104-16 mutant cells (18,19). Since Nab2RGG enters the nucleus, it is possible that other factors may also mediate efficient import (23). However, a role for Kap104 in Nab2RGG import has not been formally excluded. The FG Nups on both
faces of the NPC likely represent the main docking sites for Kap104-mediated import. Nab2 import is perturbed when FG domains in specific Nups on both NPC faces are deleted (55). In the nucleus, the dissociation of Nab2 from Kap104 requires the dual interaction of Kap104 with RanGTP and Nab2 with RNA (18).

For the export of Nab2, one of the first steps at the nuclear NPC face is predicted to be an interaction with Mlp1 (26). However, the Nab2 domain required for Mpl1 interaction has not yet been defined. Our results indicate that a step in the Nab2 export pathway also requires Gle1 function. Based on the lack of a two-hybrid interaction between Nab2 and Gle1 (data not shown), we propose that Gfd1 serves as a bridging factor between Nab2 and Gle1. We found that the Gfd1 binding region in the N-terminal Nab2 domain is distinct from the Nab2 RNA binding domains. Thus, Nab2 may bind mRNA and Gfd1 simultaneously at some point during the export pathway. As shown in Figure 8, reported binding partners to Gle1 are Nup42, Nup159, Dbp5 and Gfd1 (40,48). The DEAD-box RNA helicase Dbp5 also interacts with Nup159 and Gfd1, and Nup42 interacts with Gfd1 (40). Since Nup42 and Nup159 localize to the cytoplasmic NPC face (2), the Gle1-Gfd1-Nab2 complex may form at a terminal step in export. However, a Gle1-ProtA fusion protein has been localized to both faces of the NPC (2), and hGle1 is known to shuttle between the nucleus and cytoplasm (49). Therefore, the Nab2-Gfd1 complex could interact with Gle1 at both NPC faces. Once in the cytoplasm, Nab2 will be released from the mRNA upon binding to Kap104, and then imported into the nucleus for further rounds of transport.
Throughout the mRNA export pathway and as shown in part for Nab2 shuttling in Figure 8, a number of individual protein-protein interactions have been documented (reviewed in 4, 13). However, both the formation of an export-competent hnRNP and the NPC translocation mechanism are likely based on combinatorial and/or overlapping protein-protein interaction networks. For example, we predict additional factors may bind to the N-terminal domain of Nab2 and compensate for the absence of Gfd1. The growth and mRNA export defects observed in nab2N mutant cells are not present in gfd1 mutant cells (40,48), and we found that Nab2 export is not impaired in gfd1 mutant cells. We speculate that Gfd1 and the other functionally redundant proteins serve as bridging factors between Nab2 and Gle1, other mRNA export factors, or Nups. We have also previously shown that inositol hexakisphosphate (IP6) production is required for efficient Gle1-mediated mRNA export (56). Any of these steps along the Gle1 pathway may be modulated by IP6.

It is uncertain whether yeast and human Gle1 utilize similar mechanistic pathways to mediate mRNA export. The ability of yeast-human Gle1 chimera proteins to complement gle1 mutant cells suggests that the pathways may be functionally conserved (56). Four putative functional domains have been identified in hGle1 (49, 57, 58). Both the N and C-terminal domains are required for NPC localization (49, 58). In addition, there is a coiled-coil domain and a region required for nucleocytoplasmic shuttling (49). Thus far, the only binding partner identified for hGle1 is the NPC-localized hNup155 (58). If the yeast and human pathways are
analogous, adaptor proteins similar to Gfd1 could facilitate interactions between hGle1 and shuttling hnRNPs in vertebrate cells.

It has been the long-standing view in the field that Mex67/Tap/NXF1 serves as the transporter for exporting hnRNPs (13). This is based on Mex67/Tap/NXF1 nucleocytoplasmic shuttling and essential interactions with both the RNA-bound Yra1 and nucleoporins. The studies in this paper, combined with previous work, have now linked Gle1 both with an RNA-bound protein (Nab2) and nucleoporins (36, 40, 46, 47, 48, 58), and shown Gle1 shuttling or having access to both NPC faces (2, 49). Thus, Gle1 is also positioned to also play an active role in the translocation mechanism. However, Gle1 and Mex67 clearly function differently in the mRNA export pathway. In cells overexpressing the Nup116 GLFG region, Mex67-GFP accumulates in the nucleus while Gle1-GFP remains predominantly localized at NPCs (29). Moreover, they interact with distinct domains in Nup42; Mex67 binding the N-terminal FG repeat region of Nup42 and Gle1 exclusively the C-terminal non-FG domain (28, 48). We have also not observed any synthetic lethal genetic interactions between gle1 and mex67 mutants (S. Johnson and S. R.W., personal communication). Moreover, in S. pombe MEX67 is not essential for viability as it is in S. cerevisiae (59). This could reflect non-overlapping export pathways for distinct hnRNPs being either Gle1 or Mex67-mediated. Alternatively, we hypothesize that they act at distinct steps in a common pathway. Mex67 may load early onto the RNA during transcription and pre-mRNA processing (22), while Gle1 may intersect later at the stage of NPC translocation. To resolve such sequential steps, future studies will need to be aimed at
pinpointing the spatial and temporal determinants of Gle1 and Mex67 interactions with shuttling
hnRNP proteins and the NPC.
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REFERENCES

1. Fahrenkrog, B., and Aebi, U. (2003) *Nat. Rev. Mol. Cell. Biol.* **4**, 757-766

2. Rout, M. P., Aitchison, J. D., Suprapto, A., Hjertaas, K., Zhao, Y., and Chait, B. T. (2000) *J. Cell Biol.* **148**, 635-651

3. Cronshaw, J. M., Krutchinsky, A. N., Zhang, W., Chait, B. T., and Matunis, M. J. (2002) *J. Cell Biol.* **158**, 915-927

4. Lei, E. P., and Silver, P. A. (2002) *Dev. Cell* **2**, 261-272

5. Ribbeck, K., and Gorlich, D. (2001) *EMBO J.* **20**, 1320-1330

6. Smith, A. E., Slepchenko, B. M., Schaff, J. C., Loew, L. M., and Macara, I. G. (2002) *Science* **295**, 488-491

7. Weis, K. (2003) *Cell* **112**, 441-451

8. Mattaj, I. W., and Englmeier, L. (1998) *Annu. Rev. Biochem.* **67**, 265-306

9. Suntharalingam, M., and Wente, S. R. (2003) *Dev. Cell* **4**, 775-789

10. Gorlich, D., and Kutay, U. (1999) *Annu. Rev. Cell. Dev. Biol.* **15**, 607-660

11. Bednenko, J., Cingolani, G., and Gerace, L. (2003) *Traffic* **4**, 127-135

12. Nakielny, S., and Dreyfuss, G. (1998) *Curr. Biol.* **8**, 89-95

13. Reed, R., and Hurt, E. (2002) *Cell* **108**, 523-531

14. Mili, S., Shu, H. J., Zhao, Y., and Pinol-Roma, S. (2001) *Mol. Cell. Biol.* **21**, 7307-7319

15. Anderson, J. T., Wilson, S. M., Datar, K. V., and Swanson, M. S. (1993) *Mol. Cell. Biol.*
16. Kessler, M. M., Henry, M. F., Shen, E., Zhao, J., Gross, S., Silver, P. A., and Moore, C. L. (1997) *Genes Dev.* **11**, 2545-2556

17. Flach, J., Bossie, M., Vogel, J., Corbett, A., Jinks, T., Willins, D. A., and Silver, P. A. (1994) *Mol. Cell. Biol.* **14**, 8399-8407

18. Lee, D. C., and Aitchison, J. D. (1999) *J. Biol. Chem.* **274**, 29031-29037

19. Aitchison, J. D., Blobel, G., and Rout, M. P. (1996) *Science* **274**, 624-627

20. Hector, R. E., Nykamp, K. R., Dheur, S., Anderson, J. T., Non, P. J., Urbinati, C. R., Wilson, S. M., Minvielle-Sebastia, L., and Swanson, M. S. (2002) *EMBO J.* **21**, 1800-1810

21. Gallardo, M., Luna, R., Erdjument-Bromage, H., Tempst, P., and Aguilera, A. (2003) *J. Biol. Chem.* **278**, 24225-24232

22. Rodriguez-Navarro, S., Fischer, T., Luo, M. J., Antunez, O., Brettschneider, S., Lechner, J., Perez-Ortin, J. E., Reed, R., and Hurt, E. (2004) *Cell* **116**, 75-86

23. Marfatia, K. A., Crafton, E. B., Green, D. M., and Corbett, A. H. (2003) *J. Biol. Chem.* **278**, 6731-6740

24. Duncan, K., Umen, J. G., and Guthrie, C. (2000) *Curr. Biol.* **10**, 687-696

25. Green, D. M., Marfatia, K. A., Crafton, E. B., Zhang, X., Cheng, X., and Corbett, A. H. (2002) *J. Biol. Chem.* **277**, 7752-7760

26. Green, D. M., Johnson, C. P., Hagan, H., and Corbett, A. H. (2003) *Proc. Natl. Acad. Sci.*
27. Santos-Rosa, H., Moreno, H., Simos, G., Segref, A., Fahrenkrog, B., Pante, N., and Hurt, E. (1998) *Mol. Cell. Biol.* **18**, 6826-6838
28. Strasser, K., Bassler, J., and Hurt, E. (2000) *J. Cell Biol.* **150**, 695-706
29. Strawn, L. A., Shen, T., and Wente, S. R. (2001) *J. Biol. Chem.* **276**, 6445-6452
30. Strasser, K., and Hurt, E. (2001) *Nature* **413**, 648-652
31. Strasser, K., Masuda, S., Mason, P., Pfannstiel, J., Oppizzi, M., Rodriguez-Navarro, S., Rondon, A. G., Aguilera, A., Struhl, K., Reed, R., and Hurt, E. (2002) *Nature* **417**, 304-308
32. Zenklusen, D., Vinciguerra, P., Wyss, J. C., and Stutz, F. (2002) *Mol. Cell. Biol.* **22**, 8241-8253
33. Tseng, S. S., Weaver, P. L., Liu, Y., Hitomi, M., Tartakoff, A. M., and Chang, T. H. (1998) *EMBO J.* **17**, 2651-2662
34. Zhao, J., Jin, S. B., Bjorkroth, B., Wieslander, L., and Daneholt, B. (2002) *EMBO J.* **21**, 1177-1187
35. Snay-Hodge, C. A., Colot, H. V., Goldstein, A. L., and Cole, C. N. (1998) *EMBO J.* **17**, 2663-2676
36. Murphy, R., and Wente, S. R. (1996) *Nature* **383**, 357-360
37. Murphy, R., Watkins, J. L., and Wente, S. R. (1996) *Mol. Biol. Cell* **7**, 1921-1937
38. Brown, J. A., Bharathi, A., Ghosh, A., Whalen, W., Fitzgerald, E., and Dhar, R. (1995) *J.
Biol. Chem. 270, 7411-7419

39. Schmitt, C., von Kobbe, C., Bachi, A., Pante, N., Rodrigues, J. P., Boscheron, C., Rigaut, G., Wilm, M., Seraphin, B., Carmo-Fonseca, M., and Izaurralde, E. (1999) EMBO J. 18, 4332-4347

40. Hodge, C. A., Colot, H. V., Stafford, P., and Cole, C. N. (1999) EMBO J. 18, 5778-5788

41. Estruch, F., and Cole, C. N. (2003) Mol. Biol. Cell 14, 1664-1676

42. Ho, A. K., Raczniaik, G. A., Ives, E. B., and Wente, S. R. (1998) Mol. Biol. Cell 9, 355-373

43. Bailer, S. M., Siniossoglou, S., Podtelejnikov, A., Hellwig, A., Mann, M., and Hurt, E. (1998) EMBO J. 17, 1107-1119

44. Babu, J. R., Jeganathan, K. B., Baker, D. J., Wu, X., Kang-Decker, N., and van Deursen, J. M. (2003) J. Cell Biol. 160, 341-353

45. Blevins, M. B., Smith, A. M., Phillips, E. M., and Powers, M. A. (2003) J. Biol. Chem.

46. Del Priore, V., Snay, C. A., Bahr, A., and Cole, C. N. (1996) Mol. Biol. Cell 7, 1601-1621

47. Stutz, F., Kantor, J., Zhang, D., McCarthy, T., Neville, M., and Rosbash, M. (1997) Genes Dev. 11, 2857-2868

48. Strahm, Y., Fahrenkrog, B., Zenklusen, D., Rychner, E., Kantor, J., Rosbach, M., and Stutz, F. (1999) EMBO J. 18, 5761-5777

49. Kendirgi, F., Barry, D. M., Griffis, E. R., Powers, M. A., and Wente, S. R. (2003) J. Cell
50. Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F., and Cullin, C. (1993) *Nucleic Acids Res.* **21**, 3329-3330

51. James, P., Halladay, J., and Craig, E. A. (1996) *Genetics* **144**, 1425-1436

52. Allen, N. P., Huang, L., Burlingame, A., and Rexach, M. (2001) *J. Biol. Chem.* **276**, 29268-29274

53. Ho, A. K., Shen, T. X., Ryan, K. J., Kiseleva, E., Levy, M. A., Allen, T. D., and Wente, S. R. (2000) *Mol. Cell. Biol.* **20**, 5736-5748

54. Uetz, P., Giot, L., Cagney, G., Mansfield, T. A., Judson, R. S., Knight, J. R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S., and Rothberg, J. M. (2000) *Nature* **403**, 623-627

55. Strawn, L. A., Shen, T., Shulga, N., Goldfarb, D., and Wente, S. R. (2004) *Nat. Cell Biol.* **6**, 197-206.

56. York, J. D., Odom, A. R., Murphy, R., Ives, E. B., and Wente, S. R. (1999) *Science* **285**, 96-100

57. Watkins, J. L., Murphy, R., Emtage, J. L., and Wente, S. R. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 6779-6784

58. Rayala, H. J., Kendirgi, F., Barry, D. M., Majerus, P. A., and Wente, S. R. (2004) *Mol. Cell. Proteomics* **3**, 145-155
59. Yoon, J.H., Love, D. C., Guhathakurta A., Hanover J. A., and Dhar R. (2000) *Mol. Cell Biol.*

   **20**, 8767-8782

60. Bucci, M., and Wente, S. R. (1997) *J. Cell Biol.* **136**, 1185-1199

61. Yang, X., Hubbard, E. J., and Carlson, M. (1992) *Science* **257**, 680-682

62. Ghaemmaghami, S., Huh, W., Bower, K., Howson, R.W., Belle, A., Dephoure, N.,

   O’Shea, E. K. Weissman, J. S. (2003) *Nature* **425**, 737-741
ABBREVIATIONS

DTT, dithiothreitol; Gal4<sub>AD</sub>, Gal4 transcriptional activation domain, Gal4<sub>BD</sub>, Gal4 transcriptional binding domain; GFP, green fluorescent protein; GST, glutathione S-transferase; hnRNP, heterogeneous nuclear ribonucleoprotein; Kap, karyopherin; MBP, maltose binding protein; NES, nuclear export sequence; NLS, nuclear localization sequence; NPC, nuclear pore complex; Nup, nucleoporin; PMSF, phenylmethylsulfonyl fluoride; SC, synthetic complete; TAP-tagged, tandem affinity purification tagged.
FIGURE LEGENDS

Table I: Yeast strains used in this study.

Table II: Plasmids used in this study.

Figure 1: Nab2 and Gfd1 form a complex.

(A) Two hybrid interaction between Nab2 and Gfd1. The indicated proteins were fused to Gal4BD or Gal4AD and expressed in the strain PJ69-4A. Positive interactions are indicated by growth on synthetic complete media lacking leucine, tryptophan, histidine and adenine after 4 days at 30°C.

(B) GST-Gfd1 or GST immobilized on glutathione-sepharose beads was incubated with wild-type yeast extract or buffer alone. Bound proteins were first eluted with high salt (1 M NaCl) and then with SDS-sample buffer, followed SDS-PAGE analysis and immunoblotting with anti-Nab2 antibodies. Proteins were detected using anti-mouse IgG coupled to horseradish peroxidase. Samples loaded represent 1/20th of the total input compared to the total eluted fraction.

Figure 2: Nab2 coimmunoprecipitates with Gfd1-GFP from yeast lysates.

Extracts from wild-type or Gfd1-GFP cells were immunoprecipitated with anti-GFP antibodies.
Bound proteins were separated by SDS-PAGE and immunoblotted with anti-Nab2 antibodies. Proteins were detected using anti-mouse IgG coupled to horseradish peroxidase. Samples loaded represent 1/10th of the total input compared to the total bound fraction.

**Figure 3: Nab2 and Gfd1 interact directly in vitro.**

Purified GST or GST-Gfd1 immobilized on glutathione-sepharose beads was incubated with purified MBP-Nab2 (*) or MBP-Gle2 (**). Input proteins (A) and the unbound and bound fractions (B) were resolved by SDS-PAGE and detected by coomassie staining. The purified GST-Gfd1 contained full length GST-Gfd1(←) and two additional proteolytic bands.

**Figure 4: The N-terminal domain of Nab2 interacts with Gfd1.**

(A) Domain structure of Nab2. Q-rich: Glutamine (Q); RGG: Arginine (R), glycine (G); CCCH motif: cysteine (C), histidine (H) (15,23).

(B) A plasmid expressing Gal4<sub>AD</sub>-Gfd1 was co-transformed with the indicated Gal4<sub>BD</sub>-Nab2 deletion plasmids into the PJ69-4A two-hybrid reporter strain. Positive interactions (+) are indicated by growth at 30ÚC on synthetic complete media lacking leucine, tryptophan, histidine and adenine.
Figure 5: Nab2 import is not affected in gfd1 or gle1 mutants at 37°C.

Wild-type, gfd1 and gle1-4 mutant cells harboring a Nab2-GFP plasmid were grown at 23°C in synthetic media lacking uracil to log phase. Cells were maintained at 23°C (data not shown) or shifted to 37°C for 1 hour before visualization by direct fluorescence microscopy. Corresponding Hoechst staining (middle) and Nomarski (right) are shown.

Figure 6: Nab2 export is blocked in a gle1 mutant at 37°C.

Wild-type, gfd1 and gle1-4 mutant cells harboring a Nab2RGG-GFP plasmid were grown at 23°C in synthetic media lacking uracil to early log phase. Cells were maintained at 23°C or shifted to 37°C for 1 hour before visualization by direct fluorescence microscopy. Corresponding Hoechst staining (middle) and Nomarski (right) are shown.

Figure 7: Co-isolation of endogenous Gfd1 and Gle1-TAP-tagged with Nab2-GST

Nab2-GST or GST immobilized on glutathione-sepharose beads was incubated with Gle1-TAP-tagged yeast cell extract. Bound proteins were eluted with SDS-sample buffer, and analyzed by SDS-PAGE and immunoblotting with anti-Gle1 or anti-Gfd1 antibodies. Proteins were detected using anti-rabbit IgG coupled to horseradish peroxidase. Samples loaded represent
1/25\textsuperscript{th} of the total input compared to the total bound fraction.

**Figure 8: Model for export of Nab2-bound mRNA.**

Nab2 import is mediated by Kap104 binding to the RGG domain and is also dependent on FG containing Nups (18,19,55). These FG Nups are localized on both faces of the NPC and could represent docking sites for Kap104. In the nucleus, Nab2 binds RNA during transcription and then shuttles to the cytoplasm transporting its cargo mRNA (20,21). One of the first steps in its exit path is predicted to be binding with Mlp1 that localizes to the nuclear face of the NPC (26). The N-terminal domain of Nab2 is required for its export (23), and we report Gfd1 as a binding partner to this domain. Since Gfd1 and Gle1 interact (48) and Gle1 is present in Nab2-Gfd1 complexes, we propose that Nab2 could interact with Gle1 through Gfd1 to facilitate a terminal step in export.
**Table 1:** Yeast Strains used in this study

| Strain            | Genotype                                                                 | Source                  |
|-------------------|---------------------------------------------------------------------------|-------------------------|
| SWY518            | *Mata ade2-1::ADE2 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100*          | (60) This study         |
| SWY2535           | *Matα Gfd1-GFP: HIS5 ade2-1::ADE2 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100* | This study              |
| SWY1191           | *Matα gle1-4 ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100*         | (36) Research Genetics  |
| gfd1              | *Mata gfd1::KanMX ura30 leu20 his31 met150*                               |                         |
| PJ69-4A           | *Mata trp1-901 leu2-3,112 ura3-52 his3-200 gal4 gaL8*                     | (51)                    |
| Gle1-TAP-tagged   | *Mata Gle1-TAP::His3MX ura30 leu20 his31 met150*                          | (62), Open Biosystems   |
**Table II:** Plasmids used in this study

| Plasmid    | Description                                           | Source |
|------------|-------------------------------------------------------|--------|
| pSW431     | Gle2 full length in *BamHI* in pMAL-cR1               | (37)   |
| pSW449     | Gle1 full length in *BamHI* in pMAL-cR1               | (36)   |
| pSW456     | Gle1 full length in *BamHI* in pGEX-3X                |        |
| pSW1251    | Gld1 full length in *EcoRI/SalI* in pGAD-C1           | This study |
| pSW1257    | Nab2 full length in *EcoRI/SalI* in pGBD-C1           | This study |
| pSW1279    | Nab2 full length in *EcoRI/SalI* in pMAL-cR1          | This study |
| pSW1296    | Gld1 full length in *BamHI/SalI* in pGEX-5X           | This study |
| pSW1510    | Nab2 (aa 205-525) in *EcoRI/SalI* in pGBD-C1          | This study |
| pSW1512    | Nab2 (aa 206-272) in *EcoRI/BglII/BamHI/SalI* in pGBD-C1 | This study |
| pSW1521    | Nab2 (aa 1-205) in *EcoRI/SalI* in pGBD-C1            | This study |
| pSW1554    | Nab2 (aa 1-97) in *EcoRI/SalI* in pGBD-C1             | This study |
| Nab2-GST   | Nab2 full length in *BamHI* in pGEX2TK                | (18)   |
| pSE1111    | Snf4 in pGAD                                          | (61)   |
| pSE1112    | Snf1 in pGBD                                          | (61)   |
| pAC719     | Nab2-GFP, 2µM, URA3                                    | (23)   |
| pAC980     | Nab2RGG-GFP, 2µM, URA3                                 | (23)   |

aa: amino acids
FIGURE 2

Strain: Gfd1 Gfd1-GFP Gfd1 Gfd1-GFP

kDa
110
79
62

Input Bound

IP: Anti-GFP
Immunoblot: Anti-Nab2
FIGURE 3

A

|   | GST | GST-Gfd1 | MBP-Nab2 | MBP-Gle2 |
|---|-----|----------|----------|----------|
| 1 |     |          |          |          |
| 2 |     |          |          |          |
| 3 |     |          |          |          |
| 4 |     |          |          |          |

B

Resin: GST  GST-Gfd1  GST-Gfd1
Input: MBP-Nab2*  MBP-Nab2*  MBP-Gle2**

kDa

120
100
80
60
50
40
30
25

1  2  3  4  5  6

U  B  U  B  U  B
FIGURE 4

A

Nab2 domains
N-term Q-rich RGG CCCH motif

- Required for Nab2 & PolyA+ RNA export
- Binds RNA; import factor Kap104
- Binds RNA

B

GAL4\textsubscript{BD} Constructs:

| Construct                  | Length | Growth on LTHA co-transformed with GAL4\textsubscript{AD}-Gfd1 |
|----------------------------|--------|---------------------------------------------------------------|
| Full length                | 525    | +                                                             |
| \Delta RGG                 | 205-273| +                                                             |
| RGG+CCCH motif             | 205    | -                                                             |
| N-term+Q-rich              | 205    | +                                                             |
| N-term                     | 97     | +                                                             |

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FIGURE 6

23°C

Nab2ΔRGG-GFP  Hoechst  Nomarski  Nab2ΔRGG-GFP  Hoechst  Nomarski

wild type
A  B  C  J  K  L

gfd1Δ
D  E  F  M  N  O

gle1-4
G  H  I  P  Q  R

37°C
FIGURE 7

Resin: - Nab2-GST GST

kDa 110

Anti-Gle1

79

Anti-Gfd1

25

- Input - Bound -

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