Eukaryotic gene expression requires the export of mRNA from the nucleus to the cytoplasm. The DEAD box protein Dbp5p is an essential export factor conserved from yeast to man. A fraction of Dbp5p forms a complex with nucleoporins of the cytoplasmic filaments of the nuclear pore complex. Gfd1p was identified originally as a multicopy suppressor of the rat8-2 ts allele of DBP5. Here we reported that Dbp5p and Gfd1p interact with Zds1p, a protein previously identified as a multicopy suppressor in several yeast genetic screens. By using the two-hybrid system, we showed that Zds1p interacts in vivo with both Gfd1p and Dbp5p. In vitro binding experiments revealed that Gfd1p and Dbp5p bind directly to the C-terminal part of Zds1p. In addition, ZDS1 interacted genetically with mutant alleles of genes encoding key factors in mRNA export, including DBP5 and MEX67. Furthermore, deletion of ZDS1 or both of ZDS1 and the closely related ZDS2 exacerbated the poly(A)+ export defects shown by dbp5-2 and mex67-5 mutants. We proposed that Zds1p associates with the complex formed by Dbp5p, Gfd1p, and nucleoporins at the cytosolic fibrils of the nuclear pore complex and is required for optimal mRNA export.

In eukaryotic cells, protein synthesis requires the synthesis, processing, and export of mRNA. mRNA export occurs through nuclear pore complexes (NPCs),

very large macromolecular assemblies (60 MDa in yeast and about 120 MDa in higher organisms) that are the only channels for transport of molecules and macromolecules between the nucleus and the cytoplasm. NPCs are composed of multiple copies of ~30 proteins called nucleoporins that form a symmetrical, 8-fold assembly of spoke-like structures sandwiched between nuclear and cytoplasmic rings, named the NPC core, and filamentous structures that extend into both the nuclear and the cytoplasmic compartments (1, 2).

In Saccharomyces cerevisiae, nucleoporins required for mRNA export are found in two subcomplexes of the NPC (3–5). mRNA export is either blocked or dramatically reduced by mutations affecting most of the nucleoporins in these complexes (5–8). The strongest mRNA export defects are found in mutants affecting the nucleoporins Nup159p and Nup82p, which are components of the cytoplasmic filaments (5, 9). Interactions occur between the C-terminal coiled-coil regions of Nup82p and Nup159p, and Nup159p is lost from NPCs at 37 °C in strains where either Nup159p or Nup82p have been truncated to remove part of the coiled-coil region (5, 9). A domain at the N terminus of Nup159p provides an important docking site for the essential mRNA export factor Dbp5p/Rat8p, a member of the DEAD box family of RNA helicases (10, 11).

The yeast genome encodes more than 30 DEAD box proteins (DBPs), and one or more participate in every aspect of RNA metabolism from synthesis to turnover (reviewed in Refs. 12–14). It is generally believed that DEAD box proteins are RNA helicases, but only a limited number of DBPs have been studied biochemically. Several DBPs have been shown to unwind short double-stranded RNA substrates, and this sometimes requires participation of additional proteins that interact with the DBP (15–18). Three DEAD box proteins have been shown to mediate dissociation of stably bound proteins from RNA, but the enzymatic properties of most DEAD box proteins have not been determined (19, 20).

The precise function performed by the DEAD box protein Dbp5p is not known. Dbp5p interacts directly with the cytoplasmic filaments of the NPC and binds directly not only to Nup159p but also to Gle1p. The filaments also contain another nucleoporin, Nup42/Rip1p (21). Two other proteins that interact with the filaments are Sac3p and Gfd1p (11, 21–23). The GFD1 gene was identified as high copy suppressor of rat8-2 and gle1-8 mutations and encodes a nonessential coiled-coil protein that interacts with NPCs. Two-hybrid analyses showed that Gfd1p interacts with Gle1p, Dbp5p, and Rip1p/Nup42p (11, 21). Recently, Gfd1p has been found associated in a complex with Nab2p both in vivo and in vitro. Nab2p is a shuttling mRNP protein that accompanies the mRNA through the NPC (24). Gfd1p was localized to the cytoplasm and to the nuclear rim (11). Together, these data suggest that Nup159p, Nup82p, Nup42p/Rip1p, Gle1p, Sac3p, and Gfd1p are co-localized at the fibrils of the NPC, and all of them may contribute to the Dbp5p-binding site.

A late step in mRNA export is remodeling of the mRNP complex to remove mRNA-binding proteins needed for mRNA export so that they can return to the nucleus for another round of export. Association of Dbp5p with the cytoplasmic filaments positions it to mediate this remodeling (10, 11, 21, 25). However, Dbp5p also shuttles between the nucleus and cytoplasm (11), and in Chironomus tentans it has been shown that Dbp5p binds to pre-mRNP co-transcriptionally and accompanies the mRNP to and through the nuclear pores (26). An early role for Dbp5p during transcription was also suggested by its genetic interactions with both Gfd1p and Dbp5p.

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and physical interactions with RNA polymerase II components and general transcription factors (27). Thus, Dpb5p could be loaded onto the mRNP early during pre-mRNA biogenesis and may have a later role at the NPC in remodeling the mRNP. By acting on mRNP while bound to the cytoplasmic fibrils of the NPC, Dpb5p may also be able to use the energy of ATP hydrolysis to mediate translocation of the mRNP through the NPC.

Mex67p is thought to play the role of receptor for mRNA because it shuttles between the nucleus and the cytoplasm, can be cross-linked to poly(A) in vivo, and interacts directly with the FG repeats of several nucleoporins (revised in Ref. 28). The recruitment of Mex67p to cellular mRNA is facilitated by the mRNA export adaptor Yra1p in association with the RNA helicase Sub2p (29, 30). Both Yra1p and Sub2p are recruited to genes in a transcription-dependent manner through a complex called TREX (31).

RNA processing factors are recruited to the elongating mRNA through association with the C-terminal domain of the largest subunit of RNA polymerase II. All pre-mRNA processing steps must be completed accurately before the mRNP can be exported (for a recent review see Ref. 32). In yeast, capping of nascent transcripts at their 5′ ends is performed by three enzymes: the RNA triphosphatase, Cet1p; the RNA guanylyltransferase, Ceg1p; and the 7-methyltransferase, Abd1p. Both Ceg1p and Abd1p bind directly to the phosphorylated C-terminal domain (33). The cap structure is recognized by different proteins in the nucleus and in the cytosol (34, 35).

To explore further the role of the Dpb5p in mRNA export, we performed two-hybrid screens to identify additional partners of Dpb5p and its associated factor Gfd1p. We identified Zds1p as a factor interacting with Dpb5p, Gfd1p, and Rip1p. The S. cerevisiae ZDS1 gene and its paralog ZDS2 (gillions of different screens) have been isolated as multicopy suppressors of a disruptor of the 5′-to-3′-exoribonuclease activity (5′-P1) (29) by replacement of the ZDS1 coding sequence with the KAN marker. Double mutant mex67-5 zds1Δ was obtained from the mex67 shuttle strain (MATa ade2 leu2 ura3 his3 trp1 me67::His3 [pUR3-MEX67]) (29) by replacement of the ZDS1 coding sequence with the KAN marker. Introduction of the mex67-5 allele in a pLEU2-mex67-5 plasmid and SPOA treatment to select for pUR3-MEX67 plasmid survival in the strain MATa ade2 leu2 ura3 his3 trp1 me67::His3 zds1Δ:KAN [pUR3-MEX67], the ZDS2 coding sequence was replaced by the TRP1 marker and transformed with pLEU2-MEX67 and pLEU2-mex67-5 to obtain the strains used in Fig. 4C. ceg1Δ mutants strains were obtained by crossing the rat8-2 strain CSYS50 (MATa leu2Δ ura3-53 trp1Δ63 rat8-2) (11) with strain YS245 (MATa ura3-52, leu2-3,112 his3Δ200 trichlorophenol acetyltransferase) and selecting for the rat8-2 allele and a disruption of the chromosomal CEG1 locus. In this strain the wild type CEG1 gene (in the pRS316-CEG1 plasmid) was replaced by ceg1Δ mutant alleles by transforming with plasmids pRS315-ceg1Δ-1, pRS315-ceg1Δ-3, pRS315-ceg1Δ-63, pRS315-ceg1Δ-237, and pRS315-ceg1Δ-250, followed by SPOA treatment. All the ceg1Δ strains were obtained from S. Buratowski and are described previously (41, 42). Strain rat8-2 zds1Δ was obtained by crossing the rat8-2 strain FY23 (MATa leu2Δ ura3-52 trp1Δ63 rat8-2) with YEpSTM1 containing the complete open reading frame of ZDS1. The same fragment was inserted into pGBDU-C1. YEpTIF1 was generated by PCR amplification of a fragment containing the TIF1 gene (between positions −500 from the AUG to +457 beyond the stop codon) and insertion into the SstI and PstI sites of YEplac181 (38). YEpSTM1 containing the STM1 gene in the plasmid YEplac181, was a generous gift containing of P. Linder (Centre Medical Universitaire, Université de Genève).

Yeast Strains and Genetic Methods—Wild type strains used in this work are FY23 (MATa leu2Δ ura3-52 trp1Δ63) and FY66 (MATa leu2Δ ura3-52 his3Δ200) and are described in Ref. 39. Double mutant zds1Δ zds2Δ was obtained by consecutive substitution in the strain FY23 of the ZDS1 and ZDS2 coding sequences genes by TRP1 and KAN markers, respectively, as described previously (40). Double mutant strain rat8-2 zds1Δ was obtained from strain CSYS64 (MATa leu2Δ ura3-53 his3Δ200 rat8-2) by substitution of the ZDS1 coding sequence by the HIS3 marker and selection for cells that formed the 5′-to-3′-exoribonuclease activity (5′-P1). This strain was used to construct the triple rat8-2 zds1Δ zds2Δ by replacement of the ZDS2 coding sequence with the KAN marker. Double mutant mex67-5 zds1Δ was obtained from the mex67 shuffle strain (MATa ade2 leu2 ura3 his3 trp1 me67::His3 [pUR3-MEX67]) (29) by replacement of the ZDS1 coding sequence with the KAN marker. Introduction of the mex67-5 allele in a pLEU2-mex67-5 plasmid and SPOA treatment to select for pUR3-MEX67 plasmid survival in the strain MATa ade2 leu2 ura3 his3 trp1 me67::His3 zds1Δ::KAN [pUR3-MEX67], the ZDS2 coding sequence was replaced by the TRP1 marker and transformed with pLEU2-MEX67 and pLEU2-mex67-5 to obtain the strains used in Fig. 4C. ceg1Δ mutants strains were obtained by crossing the rat8-2 strain CSYS50 (MATa leu2Δ ura3-53 trp1Δ63 rat8-2) (11) with strain YS245 (MATa ura3-52, leu2-3,112 his3Δ200 trichlorophenol acetyltransferase). Plasmids were obtained using the strain FY23 (MATa ade2 leu2 ura3 trp1 his3Δ200 GFD1-Gal4p-BD [pGFD1-BD]) (37). The plasmid for the production of Gfd1p-Gal4p-BD (pGFD1-BD) was obtained by introducing an EcoRI-XbaI fragment containing the complete coding sequence from the GFD1 gene into the EcoRI and Sall sites of pGDBU-C1 (37). The same fragment was inserted into pGAD-C1 to obtain Gfd1p-Gal4p-AD. The plasmids to express the Gfd1p, Rip1p-C66, and Rip1p-PG baits and the GST-Dpb5p, GST-Gfd1p, and GST-Rip1p-C66 fusions were the generous gifts from F. Stutz (University of Geneva) and are described previously (21). Full-length Zds1p and Zds2p fused to the Gal4p-AD were obtained by cloning a PCR fragment containing the entire open reading frame of ZDS1 or ZDS2 into pGAD-C1. YeP-TIF1 was generated by PCR amplification of a fragment containing the TIF1 gene (between positions −500 from the AUG to +457 beyond the stop codon) and insertion into the SstI and PstI sites of YEplac181 (38). YePSTM1 containing the STM1 gene in the plasmid YEplac181, was a generous gift containing of P. Linder (Centre Medical Universitaire, Université de Genève).

Yeast Two-hybrid Screen—The two-hybrid reporter strain PJ69-4A (37) containing pBDP5-BD or pGFD1-BD was transformed with a set of S. cerevisiae genomic libraries cloned into the activation domain vectors pGAD-C1, pGAD-C2, and pGAD-C3 (37). About 106 transformants were plated on synthetic complete medium plates lacking tryptophan, leucine, and histidine and incubated at 30 °C for 3 days. Plasmids conferring the ability to grow on plates lacking histidine were isolated from appropriate yeast strains, amplified in Escherichia coli, and rechecked. The region of the yeast genome carried on plasmid insertions was determined by sequencing.

In Situ Poly(A)⁺ RNA Hybridization—Localization of poly(A)⁺ RNA by in situ hybridization with an oligo(dT)₁₀₀ probe coupled to digoxigenin was performed as described previously (43), except in Fig. 5A, where a Cy3-end-labeled oligo(dT)₁₀₀ was used (44). In Vitro Binding Assay—GST fusion proteins were produced in E. coli, purified to glutathione-agarose beads as described previously (21). [³²S]Methionine-labeled proteins were proteins were obtained by coupled T7 transcription-translation in reticulocyte lysates (TNT kit, Promega) as described previously (21). The DNA template for production of Gfd1p was obtained by amplifying yeast genomic DNA with the primers GFD1-5′ (5′-GGG CGA AAT TAA 3′) and both GFD1-3′ (5′-GTG TAA ACT AAT 3′) and included the T7
PhosphorImager. Reduced in E. coli PAGE, and 35S-labeled proteins were detected by using a Fuji FLA-300 GFD1 RNA polymerase promoter and the entire association between the two proteins. We isolated a GST-Dbp5p fusion protein produced in a reticulocyte lysate. Fig. 1 shows that 35S-labeled Gfd1p can be pulled down by GST-Dbp5p bound to glutathione-agarose beads but not by GST alone. This demonstrates that Gfd1p and the fibrils of the NPC (11, 21), we examined the interaction of Zds1p with Rip1p and Glc1p. These two proteins are components of the Nup82p subcomplex, and interactions between Glc1p and Dbp5p and between Rip1p and Gfd1p have been reported (11, 21). Performing a two-hybrid assay in which the interaction is shown by the appearance of β-galactosidase activity, we found that Rip1p but not Glc1p interacts with Zds2p, supporting the idea that Zds1p also interacts with the fibrils of the nuclear pore (Fig. 2C).

Role of Zds1p in mRNA Export

Our data suggest that Zds1p has a positive impact on Dbp5p activity, we found that Rip1p but not Glc1p interacts with Zds2p, supporting the idea that Zds1p also interacts with the fibrils of the nuclear pore (Fig. 2C).

Zds1p Interacts with Dbp5p and Gfd1p in Vitro—To determine whether the two-hybrid interactions between Zds1p and both Dbp5p and Gfd1p are direct, we tested their binding in vitro. The C-terminal part of Zds1p, labeled with 35S]methionine, was synthesized in an in vitro transcription-translation system. The in vitro translated protein was incubated with GST fusions to Gfd1p and Dbp5p produced in E. coli, and purified on glutathione beads. The C-terminal domain of Zds1p, specifically interacted with Gfd1p-GST and Dbp5p-GST but not with Rip1p-GST or GST alone (Fig. 3). None of the GST fusions interacted with in vitro translated luciferase (results not shown; see Ref. 21). These results indicate that Zds1p interacts through its C-terminal domain with both Gfd1p and Dbp5p.

Genetic Interactions between zds1Δ/zds2Δ and Mutants Affecting mRNA Export Factors—Our results demonstrate a direct in vivo interaction between Dbp5p and Zds1p. Dbp5p plays a central role in mRNA export, an essential cellular process. Disruption of either ZDS1 or ZDS2 causes only modest phenotypes, and the double zds1Δ/zds2Δ mutant is viable, although cells have an altered morphology.

To determine whether the function of Dbp5p is affected by the absence of Zds1p, we analyzed the genetic interaction between these genes by constructing a strain lacking ZDS1 (zds1Δ) and containing the temperature-sensitive rat8-2 allele of DBP5. The zds1Δ rat8-2 double mutant showed impaired growth at semi-restrictive temperatures of 32 and 34 °C (data not shown). The impairment was stronger when we also deleted the related ZDS2 gene, suggesting that Zds1p and Zds2p have partially redundant roles with respect to Dbp5p (Fig. 4A). Our data suggest that Zds1p has a positive impact on Dbp5p function.

These findings suggested the possibility that Zds1p and

RNA polymerase promoter and the entire GFD1 coding sequence. The DNA template for production of the C-terminal part of Zds1p was obtained by amplifying yeast genomic DNA with the primers ZDS1-5′ (5′-GGG CGA ATT TAA TAC GAC TCA CTA TAG GGA CAC CAT GGA GCT GTC CAA TAG AGA TAA CGA GAG CAT-3′) and ZDS-INT (5′-GGG CGA ATAT TAA TAC GAC TCA CTA TAG GGA CAC CAT GGA GCT CGA CAAT TGC CAA AAA ACA-3′) and contains the T7 RNA polymerase promoter and ZDS1 sequence starting from codon 535. Attempts to produce full-length Zds1p were unsuccessful.

The in vitro binding assays were performed as described previously (21). Immobilized GST fusion proteins were incubated for 1 h at 4 °C with the product derived from in vitro transcription/translation. The beads were extensively washed, resuspended in 2% Laemmli sample buffer, and analyzed in 10% polyacrylamide gels. Gels were stained with Coomassie Blue, dried, and scanned using a Fuji FLA-300 PhosphorImager. BSA, bovine serum albumin.

RESULTS

Gfd1p Interacts in Vitro Directly with Dbp5p—The two-hybrid interaction between Gfd1p and Dbp5p, as reported previously (11), prompted us to determine whether there is a direct association between the two proteins. We isolated a GST-Dbp5p fusion protein produced in E. coli, and we analyzed its ability to interact with Gfd1p, obtained by in vitro translation in a reticulocyte lysate. Fig. 1 shows that 35S-labeled Gfd1p can be pulled down by GST-Dbp5p bound to glutathione-agarose beads but not by GST alone. This demonstrates that Gfd1p and Dbp5p interact directly.

Identification of Zds1p as a Component of the Dbp5p-Gfd1p Complex—To follow up on our identification of GFD1 as a high copy suppressor of dbp5-2, we performed yeast two-hybrid screens, using Dbp5p-Gal4-BD and Gfd1p-Gal4-BD as baits and an S. cerevisiae genomic library fused to the GAL4-AD. We used a strain where interaction of the two-hybrid proteins is monitored through activation of a HIS3 reporter gene (37).

Among the proteins identified, we recovered Zds1p in both screens. Co-expression of Dbp5-Gal4-BD or Gfd1-Gal4-BD with Zds1-Gal4-AD allowed growth on media lacking histidine, but neither Dbp5-Gal4-BD, Gfd1-Gal4-BD, nor Zds1-Gal4-AD alone was able to activate the HIS3 reporter gene (Fig. 2A and results not shown). Although ZDS1 and the closely related ZDS2 have been isolated as multicopy suppressors in many genetic screens (see Refs. 36 and 45), we know much less about their physical interactions, and neither was identified in large scale two-hybrid approaches.

All the Zds1-Gal4-AD fusion proteins identified in the two-hybrid screen (seven for Dbp5p and two for Gfd1p) include the C-terminal part of Zds1p (with fusions starting at residues of Zds1p ranging from 530 to 682 (data not shown)), indicating that this is the domain that interacts with Dbp5p and Gfd1p. Most interestingly, this region is highly homologous in ZDS1 and ZDS2 genes, and several observations suggest that the activity of Zds1p may be carried by its C-terminal portion. Thus, truncated alleles of ZDS1, which encodes only amino acids 409–915 or 796–915, partially rescue the phenotypes of the zds1 zds2 double mutant strain (45, 46). Moreover, these truncated proteins are active as negative regulators of Cdc42p (45) or for dosage suppression of temperature-sensitive mutations affecting the yeast capping enzyme Ceg1p (46). Consistent with this, the C-terminal part of Zds2p (amino acids 781–942) was able to interact with Bcy1p in a two-hybrid assay (54).

By using the two-hybrid system, we were also able to detect an interaction between Gfd1p and the full-length Zds1p, although this interaction was weaker than with the truncated forms of Zds1p (Fig. 2B and results not shown). Besides the known interaction with Dbp5p, Fig. 2B also shows that Gfd1p interacts with itself and to a lesser extent with Zds2p. Because of the known physical interactions between Dbp5p, Gfd1p, and the fibrils of the NPC (11, 21), we examined the interaction of Zds1p with Rip1p and Glc1p. These two proteins are components of the Nup82p subcomplex, and interactions between Glc1p and Dbp5p and between Rip1p and Gfd1p have been reported (11, 21). Performing a two-hybrid assay in which the interaction is shown by the appearance of β-galactosidase activity, we found that Rip1p but not Glc1p interacts with Zds1p, supporting the idea that Zds1p also interacts with the fibrils of the nuclear pore (Fig. 2C).
Role of Zds1p in mRNA Export

Fig. 2. Two-hybrid interactions. A, full-length Gfd1p and Dpb5p fused to Gal4p-BD were expressed in the strain PJ69-4A. The resulting strains were transformed with pGAD-C1(vector) or pGAD-encoding the C-terminal part of Zds1p (starting at codon 530) isolated from the two-hybrid genomic library [Zds1p]. Positive interaction was detected by growth on synthetic complete media lacking leucine, tryptophan, and histidine after 6 days at 30 °C. B, yeast cells expressing Gfd1p-Gal4p were transformed with pGAD (vector) or pGAD-based plasmids where the Gal4p-AD was fused to the full-length ZDS1 sequence (Zds1p(f.l.)), the full-length ZDS2 sequence (Zds2p(f.l.)), the C-terminal part of ZDS1 (Zds1p(t)), the entire sequence of GFD1 (Gfd1p), or the entire sequence of DBP5 (Dpb5p). Positive interaction was detected by growth on synthetic complete media lacking leucine, tryptophan, and histidine after 6 days at 30 °C. C, strain EGY40 containing the β-galactosidase reporter plasmid pSH18-34 was transformed with pGAD-encoding the C-terminal part of Zds1p and the indicated baits (fused to the LexA binding domain) as follows: Gld1p, Rip1p-C66, and Rip1p-FG (described in Ref. 21), or plasmid pEG202 (vector). β-Galactosidase activity was determined as described in Ref. 53.

Fig. 3. The C-terminal domain of Zds1p interacts with Dpb5p and Gfd1p. GST, GST-Dpb5p, GST-Gfd1p, and GST-C66/Rip1p were produced in E. coli and immobilized on glutathione-Sepharose beads. Beads were incubated with an in vitro translated and [35S]methionine-labeled C-terminal fragment of Zds1p. Bound proteins were analyzed by SDS-PAGE, and [35S]-labeled proteins were detected using a Fuji FLA-300 phosphorimaging device (upper panel). The amounts of GST fusions in each reaction were compared by Comassie staining of a gel before autoradiography (lower panel); each GST fusion is indicated by an arrow.

Zds2p might be involved in some aspect of mRNA export. We therefore examined the effect of the absence of ZDS1 and ZDS2 on the growth of a strain carrying a mutant allele of the MEX67 gene. First, we constructed a zds1Δ mex67-5 double mutant, and we analyzed the growth at different temperatures in comparison with growth of the mex67-5 mutant strain. Fig. 4B shows that deletion of zds1Δ abolishes the ability of the mex67-5 mutant to grow at the semi-restrictive temperature of 34 °C. Second, we constructed a strain carrying the zds1Δ zds2Δ mutations, a disruption of MEX67, and carrying a wild type MEX67 gene on a URA3/CEN plasmid. This strain was transformed with a LEU2/CEN plasmid containing either wild type MEX67 or the temperature-sensitive mex67-5 allele. Fig. 4C shows that combining zds1Δ zds2Δ with mex67-5 resulted in synthetic lethality at 30 °C. Therefore, in the absence of both Zds1p and Zds2p, cells producing Mex67-5p as the only form of Mex67p were unable to grow.

Defective Nuclear Export in zds1Δ/zds2Δ Double Mutants—The genetic interactions between ZDS1 and both DBP5 and MEX67 could reflect an involvement of Zds1p and Zds2p in mRNA export. To analyze mRNA distribution in zds1Δ zds2Δ double mutants, we conducted in situ hybridization to localize polyadenylated mRNA. We were able to detect evident accumulation of poly(A)⁺ RNA in nuclei in this strain at low temperature in some cells (Fig. 5A). Although the frequency of nuclear accumulation in the absence of Zds1p and Zds2p is low, we consider it significant, as accumulating cells that showed nuclear accumulation of polyadenylated mRNA were never seen in the isogenic wild type strain.

The requirement of Zds1p and Zds2p for optimal mRNA export was also suggested by exacerbation of the poly(A)⁺ RNA export defects shown by rat8-2 and mex67-5 mutants carrying deletions of ZDS1 or both ZDS1 and ZDS2. Fig. 5B shows that rat8-2 zds1Δ cells and, to a greater extent, rat8-2 zds1Δ zds2Δ cells show substantial nuclear accumulation of poly(A)⁺ RNA, even at room temperature. Moreover, the accumulation of polyadenylated RNA in nuclei in the rat8-2 single mutant at 30 and 34 °C was substantially increased in the rat8-2 zds1Δ and rat8-2 zds1Δ zds2Δ strains. Similarly, the poly(A)⁺ accumulation shown by the mex67-5 zds1Δ double mutant at 34 and 37 °C is stronger than in the mex67-5 single mutant (Fig. 5C). Most interestingly, some cells of the mex67-5 zds1Δ double
was found in a genetic screen for multicopy suppressors of CEG1. To determine whether CEG1 and TIF1 (46) CI, CEG1 encodes the guanylyltransferase mRNA capping enzyme, and TIF1 encodes the translation initiation factor eIF4A. To determine whether CEG1 and TIF1 could be linked to DBP5 function, we constructed strains carrying mutant alleles of both CEG1 and DBP5, and we found that several ceg1 alleles partially suppressed the growth defect of DBP5 (Fig. 6A). However, the interaction can also be seen in vitro. For example, DBP5 and Rip1p interact with the full-length protein is weaker than with the C-terminal half of Zds1p to interact with its binding partners.

Most interestingly, a genetic connection between DBP5 and TIF1 was found in a genetic screen for multicopy suppressors of the synthetic lethality of the double mutant bur6-1 rat8-2. Fig. 6B shows that the double mutant is able to grow at room temperature when TIF1 is overexpressed, but not in a strain transformed with an empty vector. In a similar way, overexpression of STMI, encoding the yeast translation initiation factor eIF4B (47), also suppresses the synthetic lethality of the bur6-1 rat8-2 double mutant (Fig. 6C).

These findings reveal a web of genetic and physical interactions connecting mRNA export factors (Dbp5p and Mex67p), nucleoporins (Nup159p, Nup82p, Nup42p/Rip1p, and Gln1p), proteins that associate with NPCs (Sac3p and Gfd1p), and translation initiation factors (eIF4A and eIF4B), suggesting that the fibrils of the NPC where Dbp5p binds might have a role in coupling of mRNA export with translation initiation and that Zds1p/Zds2p might facilitate this. In support of this idea, we found that the zds1Δ/zds2Δ mutant is more sensitive than the isogenic wild type strain to low levels of the translational inhibitor cycloheximide (Fig. 7).

### DISCUSSION

The DEAD box protein Dbp5p is essential for mRNA export, both in yeast and humans (10, 25). A fraction of Dbp5p is localized to the cytoplasmic fibrils of the nuclear pore complex, and it has been suggested that this localization would allow Dbp5p to participate in the remodeling of the mRNP complex as the mRNP emerges from the channel of the NPC. However, Dbp5p also shuttles between the nucleus and cytoplasm (11). To avoid futile disassembly of mRNP complexes in the nucleus, it is likely that a mechanism exists to prevent Dbp5p from removing mRNP proteins from the mRNPs when in the nucleus. Because small DBPs like Dbp5p often require co-factors, one way to regulate its activities spatially would be to permit it to associate with co-factors required for this activity only at specific locations such as the fibrils of the NPC.

To better understand the role of Dbp5p in mRNA export, we conducted two-hybrid screens using as baits Dbp5p and a protein identified originally in a search for high copy suppressors of rat8-2, Gfd1p (11). Clones encoding Zds1-GAD fusions were identified multiple times in both two-hybrid screens.

ZDS1 and its paralogue ZDS2 have been identified in a wide variety of genetics screens as multicopy suppressors. This implies that they are involved directly or indirectly in a large number of seemingly unrelated intracellular processes (36). The reason for the broad spectrum of suppression when ZDS1 and ZDS2 are overexpressed is unknown, but it is not likely that this reflects direct participation of Zds1p in a large number of protein complexes, because only an interaction with Zds2p but not Zds1p was found in a large scale two-hybrid screen (48). The large scale analysis of multiprotein complexes by tandem affinity purification and mass spectrometry only reveals a very small number of partners for Zds1p (49). Specific studies have shown two-hybrid interactions of Zds1p and Zds2p with Sir2p, Sir3p, Sir4p and the yeast telomere-binding protein Rap1p (50) with Bcy1p (51) and the proteins required for cell polarity (51).

Both Dbp5p and Gfd1p interact with the C-terminal portion of Zds1p because this is the only region of the protein present in all of the two-hybrid clones isolated. Furthermore, we showed that the C-terminal portion of Zds1p interacts with Dbp5p and Gfd1p in vitro. However, the interaction can also be observed with the full-length Zds1p. The fact that the interaction with the full-length protein is weaker than with the C-terminal half of Zds1p could reflect Zds1p interacting with multiple partners, some with the N-terminal half and others with the C-terminal half. If a partner that normally bound the N-terminal half of Zds1p is excluded from the nucleus where two-hybrid interactions take place, the N-terminal part of Zds1p might fold so as to interfere with the ability of the C-terminal part of Zds1p to interact with its binding partners. On the other hand, it is also possible that the binding of partners to the N-terminal part influences (and in this case reduces) the interactions of Zds1p with Gfd1p and Dbp5p.

Although we were not able to localize Zds1p at NPCs, this is the most likely place for interactions of Dbp5p, Gfd1p, and Rip1p with Zds1p to occur because Dbp5p, Gfd1p, and Rip1p are known to interact and to be located at the cytoplasmic fibrils of NPCs. Zds1p might associate only transiently with NPCs and could contribute to creating an optimal binding site for Dbp5p.

In situ poly(A)+ RNA hybridization shows that Zds1p and

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2 F. Estruch and C. N. Cole, manuscript in preparation.

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Fig. 4. Genetic interactions between rat8-2 and mex67-5 with zds1Δ or zds1Δ zds2Δ. A and B, the indicated strains (see “Experimental Procedures”) were spotted on YPD plates and incubated at different temperatures for 4 days. C, triple mutant mex67-5 zds1Δ zds2Δ containing the wild type MEX67 gene in a CEN/URA3 plasmid was transformed with pMEX67/CEN/LEU2 (right) or pmex67-5/CEN/LEU2 (left) and streaked on 5′FOA (upper) and YPD plates (lower). Synthetic lethality was analyzed by incubation at 30 °C for 4 days.
Zds2p are not absolutely required for mRNA export. However, at low temperature, when growth of the zds1 zds2 double mutant is compromised (46), a fraction of the cells shows a strong poly(A)\(^{+}\) RNA nuclear accumulation that never occurs in the wild type strain. This result could be indicative of a requirement for Zds1\(p\)/Zds2\(p\) in mRNA export only under specific circumstances, such as a particular cell cycle stage or in cell aging. However, the results presented in this work suggest a general functional role for Zds1\(p\) in mRNA export. We have found that the mRNA export defects of dbp5\(\) and mex67\(\) mutant cells were exacerbated when ZDS1 or both ZDS1 and ZDS2 were disrupted. Moreover, the Hurt laboratory has evidence for a genetic connection between SUS1 and ZDS1, which links Zds1\(p\) to another conserved factor involved in mRNA export. Most interestingly, deletion of SUS1 has been found to be synthetically lethal with mutations in DBP5 (52). The requirement of Zds1\(p\)/Zds2\(p\) for mRNA export seems to be absolute when the activity of the export receptor Mex67\(p\) is compromised, as shown by the lethality of the triple zds1 zds2 mex67-5 mutant. Because physical interactions suggest that Zds1p associates with the complex formed by Dbp5p, Gfd1p, and nucleoporins at the cytosolic fibrils of the NPC, it is tempting to speculate that Zds1p could work by facilitating the interaction of this complex with the proteins bound to the mRNA during its translocation through the NPC. This role could be functionally redundant with other proteins. In this way, it has been suggested recently that Gfd1p, like Zds1p nonessential for export, would act by bridging mRNA export factors and nucleoporins (24).

The proposed role for Dhp5p at the pore in remodeling the protein interaction at the mRNP, together with the overlapping genetic interactions of ZDS1 and DBP5 with both CEG1 and TIF1, suggests a function for the complex in coupling the last steps of mRNA export with translation initiation. To initiate

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3 S. Rodriguez-Navarro and E. Hurt, unpublished results.

**FIG. 5.** Exacerbation of the dbp5\(^{+}\) and mex67\(^{+}\) export defect by deletion of ZDS1. A, double mutant zds1Δ zds2Δ cells were incubated for 4 h at 16 °C in YPD, and poly(A)\(^{+}\) RNA was visualized by fluorescence in situ hybridization analysis using a Cy3-end-labeled oligo(dT)\(_{50}\) probe. B and C, the indicated strains were incubated for 1 h at the indicated temperatures. In all cases, cells were fixed, and in situ hybridization was performed using a digoxigenin-conjugated oligo(dT)\(_{50}\) probe, followed by incubation with a fluorescein isothiocyanate-conjugated anti-digoxigenin antibody. RT, room temperature; WT, wild type; DAPI, 4,6-diamidino-2-phenylindole.

**FIG. 6.** Genetic interactions between dbp5\(^{+}\) and genes suppressed by overexpression of ZDS1. A, suppression of rat8-2 growth defect by ceg1\(^{+}\) mutations. Double mutant rat8-2 ceg1\(^{+}\) cells containing the wild type CEG1 gene or the indicated ceg1\(^{+}\) mutant alleles were spotted on YPD plates and incubated at different temperatures for 4 days. B, suppression of the bur6-1 rat8-2 synthetic lethality by overexpression of TIF1. Double mutant bur6-1 rat8-2 containing the wild type DBP5 gene in a CEN/URA3 plasmid was transformed with the multicopy vector YEplac181 empty (YEplac181) or this vector containing the TIF1 gene (YEpTIF1). Transformants were streaked in 5 FOA plates and incubated for 5 days at room temperature. C, suppression of the bur6-1 rat8-2 synthetic lethality by overexpression of STM1. Suppression was analyzed as in B but using a YEplac181-derived plasmid containing the STM1 gene (YEpSTM1).
transcription, the cap-binding protein Cbc20p/Cbc80p is thought to be replaced by eIF4E. Dbp5p might participate in removal of translation, the cap-binding protein Cbc20p/Cbc80p is thought to be replaced by eIF4E. Dbp5p might participate in removal of translation inhibitor cycloheximide and incubated for 3 days at 30 °C. The broad spectrum of mutations suppressed by ZDS1 and Steve Buratowski for the strains and plasmids used in these studies.

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Role of Zds1p in mRNA Export

FIG. 7. Cycloheximide sensitivity of the zds1Δ zds2Δ double mutant. Double mutant Zds1Δ Zds2Δ cells and isogenic wild type cells were spotted on YPD plates containing the indicated amounts of the translation inhibitor cycloheximide and incubated for 3 days at 30 °C. The broad spectrum of mutations suppressed by ZDS1 and Steve Buratowski for the strains and plasmids used in these studies. We thank Cathie Heath for careful proofreading of the paper.

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