Saccharomyces cerevisiae Protein Pci8p and Human Protein eIF3e/Int-6 Interact with the eIF3 Core Complex by Binding to Cognate eIF3b Subunits*  

Mammalian, plant, and Schizosaccharomyces pombe eukaryotic initiation factor-3 (eIF3) contains a protein homologous to the product of int-6 (eIF3e), a frequent integration site of mouse mammary tumor viruses. By contrast, Saccharomyces cerevisiae does not encode a protein closely related to eIF3e/Int-6. Here, we characterize a novel S. cerevisiae protein (Pci8p, Yil071cp) that contains a PCI (proteasome-COP9 signalosome-eIF3) domain conserved in eIF3e/Int-6. We show that both Pci8p and human eIF3e/Int-6 expressed in budding yeast interact with the yeast eIF3 complex in vivo and in vitro by binding to a discrete segment of its eIF3b subunit Prt1p and that human eIF3e/Int-6 interacts with the human eIF3b segment homologous to the Pci8p-binding site of yeast Prt1p. These results refine our understanding of subunit interactions in the eIF3 complex and suggest structural similarity between human eIF3e/Int-6 and yeast Pci8p. However, deletion of PCI8 had no discernible effect on cell growth or translation initiation as judged by polysome analysis, suggesting that Pci8p is not required for the essential function of eIF3 in translation initiation. Motivated by the involvement of Int-6 in transcriptional control, we investigated the effects of deleting PCI8 on the total mRNA expression profile by oligonucleotide microarray analysis and found reduced mRNA levels for a subset of heat shock proteins in the pci8Δ mutant. We discuss possible dual functions of Pci8p and Int-6 in transcriptional and translational control.

Translation initiation in eukaryotes is a complex series of reactions leading to the formation of an 80 S ribosomal complex containing Met-tRNA\textsuperscript{Met} base-paired with the initiation codon in the mRNA. The largest of eukaryotic initiation factors (eIFs),\textsuperscript{1} eIF3 serves as a scaffold for assembly of other eIFs, thereby promoting ribosome binding of a ternary complex consisting of Met-tRNA\textsuperscript{Met}, eIF2, and GTP and of mRNA in association with the cap-binding complex eIF4F (for review, see Refs. 1 and 2).

Mammalian eIF3 contains 11 non-identical subunits (3) and is very similar in subunit composition to plant eIF3, as both contain homologs of subunits eIF3a–i and eIF3k and only one additional subunit specific to each organism (4–6). The fission yeast Schizosaccharomyces pombe encodes orthologs of nine of these conserved subunits (all except for eIF3k) (7, 8), and eIF3a–c, eIF3e–g, and eIF3i have been identified in a putative eIF3 complex purified from this yeast (9). eIF3 from the budding yeast Saccharomyces cerevisiae is atypical in containing only five subunits present in stoichiometric amounts (eIF3a, Tif32p, eIF3c/Prt1p, eIF3d/Nip1p, eIF3g/Tif35p, and eIF3i/Tif34p) (10, 11). Hcr1p, the budding yeast ortholog of human eIF3j, appears to be a non-stoichiometric peripheral component of yeast eIF3 (12). Importantly, the five-subunit complex purified from budding yeast was sufficient to restore Met-tRNA\textsuperscript{Met} binding to 40 S ribosomes in a heat-inactivated extract from a prt1 mutant and thus possesses a key activity ascribed to mammalian eIF3 (11). All five eIF3 subunits are essential in S. cerevisiae (2). For these reasons, it was proposed that the five-subunit complex isolated from budding yeast represents a catalytically active “core” complex of the critical eIF3 subunits (11). This hypothesis is consistent with the finding that, in fission yeast, two of the five core subunits are essential (eIF3g and eIF3i), whereas two of the “non-core” eIF3 subunits are dispensable (eIF3d and eIF3e) (7–9, 13).

Three of the eIF3 subunits in mammals, plants, and S. pombe contain a conserved region (the PCI 26 S proteasome-COP9 signalosome-initiation factor-3) or PINT (proteasome-Int-6-NIP1-TRIP-15) domain that also occurs in five subunits of the 19 S lid subcomplex of the 26 S proteasome and in six subunits of the COP9 signalosome (14, 15). Two of the eIF3 subunits containing the PCI/PINT domain, eIF3a and eIF3c, may be regarded as core subunits (as they are present in the five-subunit budding yeast eIF3), whereas the third, eIF3e, appears to be a peripheral eIF3 subunit. The gene encoding eIF3e in S. pombe, int6\textsuperscript{6}, is not essential, but strains lacking this gene grow more slowly than the wild-type strain and display moderate reductions in the rate of translation initiation (7, 9). Thus, eIF3e/Int6 in S. pombe may play an accessory role in the general function of eIF3 or may be required only for translation of specific mRNAs.

In addition to its proposed role in translation as an eIF3 subunit, human eIF3e and the mouse homolog (Int-6) have been implicated in the control of cell growth and tumorigenesis as well as transcriptional regulation (16–18). The int-6 coding sequence was found to be a frequent integration site of mouse sequence.
Pci8p and eIF3e/Int-6 Interact with eIF3 Core Complex

| Primer numbers | Oligonucleotides used in this study | Description | Tag |
|----------------|------------------------------------|-------------|-----|
| 1              | CCCAGCTGATGCGGGATGACCTGTA          | 5' - End of int-6 ORF | MluI |
| 2              | CCCGATTCATGAGAAGCCGCAATCTGAG      | 3' - End of int-6 ORF | BamHI |
| 3              | CCCGATTCATGAGAAGCCGCAATCTGAG      | 5' - End of int-6 ORF | BamHI |
| 4              | CCCGATTCATGAGAAGCCGCAATCTGAG      | MluI         |     |
| 5              | CCCGATTCATGAGAAGCCGCAATCTGAG      | 3' - End of int-6 ORF | BamHI |
| 6              | CCCGATTCATGAGAAGCCGCAATCTGAG      | FLAG         |     |
| 7              | CCCGATTCATGAGAAGCCGCAATCTGAG      | 3' - End of int-6 ORF | BamHI |
| 8              | CCCGATTCATGAGAAGCCGCAATCTGAG      | 5' - End of int-6 ORF | BamHI |
| 9              | CCCGATTCATGAGAAGCCGCAATCTGAG      | MluI         |     |
| 10             | CCCGATTCATGAGAAGCCGCAATCTGAG      | 3' - End of int-6 ORF | BamHI |
| 11             | CCCGATTCATGAGAAGCCGCAATCTGAG      | MluI         |     |
| 12             | CCCGATTCATGAGAAGCCGCAATCTGAG      | 3' - End of int-6 ORF | BamHI |
| 13             | CCCGATTCATGAGAAGCCGCAATCTGAG      | EcoRI        |     |
| 14             | CCCGATTCATGAGAAGCCGCAATCTGAG      | EcoRI        | NdeI |
| 15             | CCCGATTCATGAGAAGCCGCAATCTGAG      | 5' - End of int-6 ORF | EcoRI |
| 16             | CCCGATTCATGAGAAGCCGCAATCTGAG      | MluI         |     |
| 17             | CCCGATTCATGAGAAGCCGCAATCTGAG      | 3' - End of int-6 ORF | EcoRI |
| 18             | CCCGATTCATGAGAAGCCGCAATCTGAG      | MluI         |     |
| 19             | CCCGATTCATGAGAAGCCGCAATCTGAG      | 3' - End of int-6 ORF | EcoRI |

* a Introduced restriction enzyme recognition sites are underlined.

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Mammalian tumor virus (19), and expression of Int-6 is decreased in a consistent portion of human breast and lung carcinomas (20). Human Int-6 interacts with the Tax transactivator of human T cell leukemia virus type 1 (21). Mammalian Int-6 is localized in the promyelocytic leukemia nuclear body (21, 22), whose major constituent, PML protein, is a tumor suppressor and transcriptional regulator (23). In fission yeast, eIF3e/Int-6 was identified as an inducer of multidrug resistance genes dependent on AP-1 transcription factor Pap1p (8), and int6Δ mutants display both caffeine sensitivity (7) and a defect in nuclear partitioning (9, 24). Furthermore, plant eIF3e is also be a distantly related ortholog of the non-core subunit eIF3e (15), which we designate Pci8p. The absence of a third PCI subunit in YIL071c (25) may be the ninth PCI protein in budding yeast. Rpn12p/PSMD8 may also have a PCI domain, although the Conserved Domain Database search does not predict this. If this is the case, then Rpn12p may be the ninth PCI protein in budding yeast.

MATERIALS AND METHODS

Plasmids—The oligodeoxyribonucleotides and plasmids used in this study are listed in Tables I and II, respectively. pAV1427 encodes N-terminally FLAG- and polyhistidine-tagged Gcd6p under the control of the GAL promoter (28) with a unique MluI site located between the coding regions for the N-terminal tags and the first codon of Gcd6p. The DNA segments containing the human int-6 ORF were synthesized from pT7z4 (16) by PCR using primers 1 and 2 and primers 1 and 3 (Table I), of which primer 3 introduces a FLAG epitope coding sequence fused in-frame to the 3' end of the int-6 ORF. After digestion with MluI and BamHI, the fragments were inserted into the 5' -MluI and 3' -BamHI sites of pAV1427 to produce pEMBL-Int-6-5 FL and pEMBL-Int-6-2-FL, respectively. Similarly, the DNA segments encoding yeast Pci8p were synthesized from yeast chromosomal DNA by PCR using primers 4 and 5 and primers 4 and 6, digested with MluI and BamHI, and subcloned into pAV1427 to produce pEMBL-PCI8-5 FL and pEMBL-PCI8-2-FL, respectively. The N-terminal tag coding sequences in pEMBL-Int-6-2-FL and pEMBL-PCI8-2-FL were removed by SacI and MluI digestion, fill-in with Klenow enzyme, and self-ligation, generating pEMBL-Int-6-3 FL and pEMBL-PCI8-3 FL, respectively. Each of these six plasmids was introduced into yeast strain H2557 (see Table III) and examined for expression of the encoded tagged Int-6 or Pci8p protein following induction on galactose-containing medium. As pEMBL-Int-6-5 FL and pEMBL-PCI8-3 FL produced the largest amounts of FLAG-tagged Int-6 and Pci8p, respectively (data not shown), they were selected for further analyses.

The 2.3-kb DNA segment carrying the PCI8 ORF and its 5' - and 3' -UTRs (0.5 kb each) was amplified from the yeast genome using primers 7 and 9 (Table I), digested with BglII and BamHI, and subcloned into the BamHI site of YCplac111 (29) to generate YCPuPCI8. YCpPCI8-FL, encoding FLAG-tagged Pci8p under the control of its own promoter, was constructed by replacing the 1.0-kb NcoI-BamHI segment of YCPuPCI8 with a 0.5-kb NcoI-BamHI segment of pEMBL-PCI8-3 FL containing the 3' -half of the FLAG-tagged PCI8 ORF. The deletion plasmid pCISA-URA3 was constructed in two steps. First, the 3' -UTR sequence of PCI8 was synthesized by PCR using primers 10 and 9 and digested with BstII and BamHI, which cleave at sites immediately following the PCI8 stop codon and at the end of primer 9, respectively. The resulting 0.5-kb fragment was subcloned into the BamHI site of pNBY51 (30) 3' of the hisG::URA3::hisG cassette. Second, the 5' -UTR of

8 Each of the eight COP9 signalosome subunits is apparently similar to one of the eight proteasome lid subunits (26). Since the signalosome subunit CSN8 contains a PCI domain, the proteasome lid counterpart Rpn12p/PSMD8 may also have a PCI domain, although the Conserved Domain Database search does not predict this. If this is the case, then Rpn12p may be the ninth PCI protein in budding yeast.
**RESULTS**

**Human eIF3e/Int-6 and Yeast Pci8p Bind to Yeast eIF3 in Vivo**—The eIF3e/Int-6 protein (445 amino acids, 52,187 Da) is a component of mammalian eIF3 (16) and contains a conserved domain termed PCI/PINT domain (14, 15). budding yeast homologs have been identified for five of the mammalian eIF3 subunits, but not for Int-6. As the hypothetical *S. cerevisiae* Pci8p protein (444 amino acids, 51,254 Da; GenBank™/EBI Data Bank accession number P40512) contains a PCI/PINT domain (14, 15) and shows no similarity to any other proteins,3 we considered the possibility that Pci8p might be a distantly related, functional ortholog of Int-6. To investigate whether Pci8p and its possible human counterpart (Int-6) could bind to yeast eIF3 in vivo, we constructed plasmids pEMBL-Int-6-5′-FL and pEMBL-PC18-3′-FL for expressing FLAG epitope-tagged versions of these proteins under the control of a galactose-inducible (GAL) promoter. The plasmids were introduced into *S. cerevisiae* strain H2557, and the resulting transformants were grown on galactose-containing medium. Whole cell extracts (WCEs) were prepared from the transformants and subjected to Western analysis using antibodies against the FLAG epitope to characterize the expression levels of FLAG-Int-6 and FLAG-Pci8p. The WCE strain KAY35 encoding FLAG-eIF5 as the sole source of this initiation factor (Table III) was analyzed in parallel for comparison. FLAG-Int-6 migrated at 52 kDa and was expressed at a level approximately one-fourth that of FLAG-eIF5. FLAG-Pci8p also migrated at 52 kDa and was expressed at a level approximately 10-fold higher than that of FLAG-eIF5 (data not shown).

To determine whether FLAG-Pci8p and FLAG-Int-6 can bind to yeast eIF3 in vivo, WCEs from the H2557 transformants just described or from a control transformant bearing an empty vector were immunoprecipitated with anti-FLAG affinity resin, and the immune complexes were subjected to Western analysis with antibodies against the FLAG epitope. Nearly all of Prt1p in the WCE was incorporated into yeast eIF2α subunit Prt1p, and yeast eIF2α subunit Sui2p. The results shown in Fig. 1A indicate that nearly all of FLAG-Int-6 and ~20% of FLAG-Pci8p were immunoprecipitated with anti-FLAG resin (indicated by arrows in the first row, lanes 5 and 8). Interestingly, ~20% of Prt1p (second row, lanes 5 and 8), but little or no eIF2α (third row, lanes 5 and 8), co-immunoprecipitated with both FLAG-Int-6 and FLAG-Pci8p. Under these conditions, nearly all of Prt1p in the WCE was incorporated into yeast eIF3, as indicated by co-immunoprecipitation experiments with antibodies against eIF3a subunit Tif32p (data not shown).

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3 According to the YPD™ Database at www.proteome.com/.

**TABLE II**

| Plasmid                                      | Description* |
|----------------------------------------------|--------------|
| pT7p148                                      | int-6 cDNA cloned under T7 promoter (16) |
| pEMBL-yea4                                   | Yeast expression vector, URA3 2μ (GAL) (47) |
| pEMBL-Int-6–5′-FL                           | Yeast expression plasmid for FLAG-tagged Int-6 under P<sub>GAL</sub> |
| pEMBL-PC18–3′-FL                            | Yeast expression plasmid for FLAG-tagged Pci8p under P<sub>GAL</sub> |
| YCplac111                                    | Single-copy LEU2 vector (29) |
| YCpFC18                                      | Single-copy LEU3 PCI8 plasmid |
| YCpFC18-3′-FL                                | Single-copy LEU3-PC18-FLAG plasmid |
| pPC18A-URA3                                  | PC18 disruption plasmid with pci8A::hisG::URA3::hisG |
| pGEX-Int-6                                   | GST-Int-6 fusion plasmid |
| pGEX-PC18                                    | GST-Pci8p fusion plasmid |
| pGEX-p110                                    | GST-human eIF3e fusion plasmid (4) |
| pGEX-p116A                                   | GST-human eIF3a fusion plasmid |
| pT7-PT1                                      | PT1 ORF cloned under T7 promoter (31) |
| pT7-Δ-PT1 series                            | Different parts of PT1 ORF cloned under T7 promoter (12) |

* References are given in parentheses for plasmids constructed previously.
These results suggest that human Int-6 and Pci8p can interact specifically with yeast eIF3 in vivo.

eIF3e/Int-6 and Pci8p Bind Specifically to Purified eIF3 in Vitro—In an effort to confirm these findings in vitro using purified proteins, we constructed GST fusions to Int-6 and Pci8p, purified them from bacteria (see Fig. 1B (upper panel) for Coomassie Blue staining of these proteins), and tested them for interaction with eIF3 purified from yeast. This eIF3 preparation contains polyhistidine-tagged eIF3b/Prt1p, FLAG-tagged eIF3g/Tif35p, and hemagglutinin-tagged eIF3i/Tif34p and was purified by nickel chelation chromatography, followed by anti-FLAG affinity chromatography (33). The purified eIF3 was incubated with the GST fusion proteins or GST alone and was incubated with 10 μg of purified eIF3 (33) in 100 μl of binding buffer (31) for 90 min at 4 °C. The upper panel shows GST alone (~3 μg; lane 2), GST-Pci8p (~0.5 μg of the full-length protein; lane 3), and GST-Int-6 (~1 μg of the full-length protein; lane 4) used for a single binding reaction. Arrowheads indicate the positions of full-length GST and its fusions to eIF3 subunits. The protein complexes were analyzed as described for A. Lane 1, 50% of the input (In) amount of purified eIF3 (5 μg); lanes 2–4, the entire precipitates with GST alone, GST-Pci8p, and GST-Int-6, respectively. HA, hemagglutinin.

Fig. 1. Human eIF3e/Int-6 and S. cerevisiae Pci8p interact with yeast eIF3 in vivo and in vitro. A, co-immunoprecipitation. The transformants of H2557 carrying pEMBL-Int-6-5' FL (FLAG-Int-6), pEMBL-PC18-3' FL (FLAG-Pci8p), or pEMBLyex4 (vector) were inoculated into 50 ml of synthetic complete medium lacking uracil (containing 10% galactose and 2% raffinose) at A600 = 0.05 and grown overnight to A600 = 0.5–2. Cells were collected by centrifugation, suspended in buffer A (40), and broken with glass beads (425–600 μm), by eight pulses of 30 s in a Braun homogenizer at 4 °C with 30 s of cooling between pulses. Homogenized cell extracts were clarified by centrifugation, yielding the supernatants as WCEs, which were used for immunoprecipitation with anti-FLAG affinity resin as described previously (40). The immune complexes were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting using antibodies listed on the left. I, 20% of the input amount of WCEs used for immunoprecipitations; P, the entire precipitated fractions; S, 10% of the supernatant fractions. The asterisk indicates the position of anti-FLAG immunoglobulin. B, GST pull-down experiment. Extracts prepared from isopropyl-β-d-thiogalactopyranoside-induced BL21(DE3) transformants bearing pGEX-4T-1, pGEX-Int-6, or pGEX-PC18 were incubated with 5 μl of glutathione-Sepharose beads (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. After extensive washing, beads attached to GST fusion proteins were incubated with 10 μg of purified eIF3 (33) in 100 μl of binding buffer (31) for 90 min at 4 °C. The upper panel shows GST alone (~3 μg; lane 2), GST-Pci8p (~0.5 μg of the full-length protein; lane 3), and GST-Int-6 (~1 μg of the full-length protein; lane 4) used for a single binding reaction. Arrowheads indicate the positions of full-length GST and its fusions to eIF3 subunits. The protein complexes were analyzed as described for A. Lane 1, 50% of the input (In) amount of purified eIF3 (5 μg); lanes 2–4, the entire precipitates with GST alone, GST-Pci8p, and GST-Int-6, respectively. HA, hemagglutinin.

These results suggest that human Int-6 and Pci8p can interact specifically with yeast eIF3 in vivo.
it appears that Int-6 and Pci8p both can interact directly with the same two subunits of yeast eIF3. 4

The Minimal Binding Site in Yeast eIF3b/Prt1p for Int-6 and Pci8p Contains the RNA Recognition Motif (RRM)—We proceeded next to identify the minimal binding site in Prt1p required for its interaction with Pci8p or Int-6. Full-length and various truncated versions of35S-labeled Prt1p (depicted in Fig. 3

A

were synthesized in rabbit reticulocyte lysates and incubated with GST-Pci8p, GST-Int-6, or GST alone. The bound proteins were isolated on glutathione-Sepharose beads and visualized by autoradiography. As reported previously (31), three polypeptides were produced from the construct (pT7-PRT1) that encodes full-length Prt1p (Fig. 3B, first panel, lane 5), but only the largest one corresponding to full-length Prt1p bound specifically to both GST-Pci8p and GST-Int-6 (first panel, lanes 2–4). These results confirm the conclusion reached from Fig. 2 that Pci8p and Int-6 both interact with Prt1p. The 35S-labeled Prt1p polypeptide lacking only the N-terminal 27 residues (construct Δ0) bound to GST-Pci8p and GST-Int-6 above the background level seen for GST alone (second panel); however, none of the polypeptides with more extensive N-terminal truncations bound specifically to either fusion protein (constructs Δ1, Δ2, Δ3, and Δ6 in the third through sixth panels). Thus, the N-terminal boundary of the binding domain lies between residues 28 and 111, within the predicted RRM (35) in Prt1p. Deletion from the C terminus up to residue 261 in Prt1p did not reduce its interaction with GST-Pci8p or GST-Int-6 (eighth panel), whereas deletion to residue 136 abolished the interaction with both fusion proteins (seventh panel). Thus, the C-terminal boundary of the binding domain for Pci8p and Int-6 is between residues 136 and 261 of Prt1p. The minimal binding domain defined by this deletion analysis corresponds to the RRM plus 140 residues flanking it on the C-terminal side. The fact that Pci8p and Int-6 both interacted with the same N-terminal segment of eIF3b/Prt1p supports the idea that these proteins might be functional homologs.

Human eIF3e/Int-6 Binds a Segment of Human eIF3b (p116) Related to the Pci8p-binding Domain in Yeast eIF3b/Prt1p—to test whether human eIF3e/Int-6 also binds to the human hom-
Fig. 3. Identification of the yeast Prt1p (eIF3b) binding site for Pci8p and human Int-6. GST alone (−2 μg), GST-Pci8p (−1 μg), and GST-Int-6 (−1 μg), attached to glutathione-Sepharose beads, were incubated with reticulocyte lysates containing different derivatives of 35S-labeled eIF3b/Prt1p peptides, and the GST fusion complexes were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography as described in the legend to Fig. 2A. The amounts of labeled proteins were quantitated using STORM Model 860 (Molecular Dynamics, Inc.). A, schematic view of different Prt1p derivatives used in this study. The empty box at the top denotes the primary structure of S. cerevisiae Prt1p, with the RRM highlighted by the filled rectangle. Lines below describe the segments of Prt1p with their clone designations and locations in the sequence. The table on the right summarizes the results of binding studies as shown in B. B, GST pull-down experiments with GST-Pci8p and GST-Int-6. Each panel describes the autoradiography of the 35S-labeled Prt1p derivatives depicted in A. Lane 1, 50% input (In) of the Prt1p peptide used in each set of experiments; lane 5, a lighter exposure of lane 1 to show different polypeptides produced from pT7-PRT1 or pT7-Δ0-Prt1p; lanes 2–4, the entire fraction of Prt1p polypeptides bound to GST alone, GST-Pci8p, or GST-Int-6, respectively. Arrowheads indicate the position of each recombinant protein with the expected size.

olog of eIF3b, originally called p116 (4, 32), we attempted to conduct binding studies with a GST fusion to human eIF3b. However, after finding that full-length GST-human eIF3b was not expressed well in bacteria (32), we constructed GST-human eIF3bα containing the portion of human eIF3b corresponding to the minimal binding domain for Int-6 in yeast eIF3b/Prt1p (Fig. 4A). Because it was reported that human eIF3c bound to human eIF3e/Int-6 in yeast two-hybrid assays (22), we also examined a GST fusion to full-length human eIF3c for interaction with Int-6. The two GST fusion proteins or GST alone were purified from bacteria on glutathione-Sepharose beads (Fig. 4B, upper panel) and incubated with the yeast WCE containing FLAG-Int-6 described above for co-immunoprecipitation analysis (Fig. 1A). The bound proteins were visualized by Western analysis using anti-FLAG antibodies. As shown in Fig. 4B (middle panel), FLAG-Int-6 bound to GST-human eIF3bα (lane 3), but not to GST alone (lane 2) or to GST-human eIF3c (lane 4). The unidentified 98-kDa protein present in the WCE that cross-reacts with anti-FLAG antibodies did not bind to any of the GST proteins (Fig. 4B, arrowheads), nor did the yeast eIF3b/Prt1p subunit present in the WCE. This last result suggests that the observed interaction between FLAG-Int-6 and GST-human eIF3bα was not mediated by yeast eIF3. Thus, we conclude that human Int-6 can bind specifically to the RRM-containing region of human eIF3b. The fact that S. cerevisiae Pci8p and human eIF3e/Int-6 interact with the corresponding RRM-containing regions of the cognate eIF3b subunits further supports the idea that Pci8p is the S. cerevisiae ortholog of eIF3e/Int-6.

**PC18 Is Not Essential, and Its Overexpression or Deletion Does Not Alter the Growth Rate of Wild-type and Mutant eIF3 Strains**—To investigate the physiological function of Pci8p, we investigated whether PC18 is essential for yeast cell growth. Toward this end, wild-type strain H2557 bearing YCpPC18 (LEU2 PC18) was transformed with a pci8Δ::URA3 deletion construct, and deletion of PC18 in the resulting Leu− Ura− transformants was confirmed by PCR analysis (see “Materials and Methods”). By culturing without selection, we found that YCpPC18 was lost from the pci8Δ and PC18 strains with similar frequencies, yielding Leu− Ura+ and Leu− Ura− clones, respectively (data not shown). These results indicate that PC18 is not an essential gene.

The pci8Δ strain lacking YCpPC18 (designated KAY76) and its isogenic wild-type strain H2557 were found to have virtually identical doubling times when grown on rich and minimal media at 18, 25, 30, and 37 °C (data not shown). Overexpression of Pci8p from pEMBL-PC18-3’FL in a transformant of H2557 grown on galactose medium also did not produce a detectable change in cell doubling time (data not shown). Thus, elimination or overexpression of Pci8p did not affect the rate of yeast cell growth under standard culture conditions.

To investigate whether Pci8p is required for wild-type rates of translation initiation, we compared the polysome profiles of pci8Δ strain KAY76 and its isogenic PC18 parent strain H2557 grown to exponential phase on rich medium (YPD) at 30 °C. As shown in Fig. 5, the two strains had very similar size distributions and abundance of polysomes, with the latter quantified by the ratio of polysomes to 80 S monosomes. These findings suggest that the rate of protein synthesis initiation is unaffected by the absence of Pci8p under the culture conditions employed in these experiments.

In an effort to uncover a nonessential stimulatory role for Pci8p in eIF3 function, we introduced the pci8Δ mutation into strains harboring the temperature-sensitive lethal mutation pret1-1, rpg1-1, or nip1-1, altering the yeast eIF3b, eIF3a, and eIF3c subunits, respectively. The resulting double mutants showed growth rates identical to those of the isogenic pret1-1, rpg1-1, and nip1-1 single mutants at the semipermissive temperature for each eIF3 subunit mutation. Additionally, overexpression of wild-type Pci8p from the pEMBL-PC18-3’FL construct during growth on galactose medium did not increase or decrease the growth rates of the eIF3 mutant strains (data not shown). We conclude that Pci8p does not contribute to the
general function of eIF3 in translation initiation under the growth conditions employed in our experiments. Finally, we investigated whether Pci8p might be involved in translational control of GCN4 mRNA, encoding a transcriptional activator of amino acid biosynthetic genes. High level translation of this mRNA is restricted to amino acid starvation conditions and is triggered by limiting the level of the eIF2 ternary complex via phosphorylation of eIF2 by protein kinase Gcn2p (36). Mutants lacking Gcn2p cannot induce GCN4 translation and thus fail to grow on medium containing an inhibitor of histidine biosynthesis, 3-aminotriazole. Mutants in eIF2 or eIF2B subunits that lower ternary complex levels can rescue growth of gcn2Δ cells on 3-aminotriazole medium (2). However, we found that isogenic PCI8 gcn2Δ and pci8Δ gcn2Δ strains (H2557 and KAY76, respectively) both failed to grow on medium containing 5 or 30 mM 3-aminotriazole (data not shown). We also explored the possibility that removal of Pci8p could impair the induction of GCN4 translation in Gcn2p cells by transforming H2557 and KAY76 with a plasmid containing GCN2 and testing the resulting strains for growth on 3-aminotriazole medium. Again, we found that PCI8 GCN2 and pci8Δ GCN2 strains grew indistinguishably on medium containing 30 mM 3-aminotriazole. Thus, removal of Pci8p seems to have no impact on GCN4 translational control, hence the cellular level of ribosome binding of the eIF2 ternary complex.

Gene Expression Profiling in Wild-type and pci8Δ Strains—

FIG. 4. Recombinant human eIF3b segment interacts with FLAG-tagged Int-6. A, shown are the primary structures of eIF3b homologs found in S. cerevisiae and human, drawn schematically with empty boxes. Shaded boxes indicate the minimal binding site for Pci8p and Int-6 found in the S. cerevisiae homolog (Fig. 3) or the homologous segment found in the human eIF3b polypeptide. Filled boxes depict the RRM. The horizontal bar below the human eIF3b schematic indicates its segment (human eIF3bΔ (heIF3bΔ)) fused to GST for the binding studies described for B. Numbers at the ends of each box or bar indicate amino acid positions at the boundary. B, the GST fusion proteins listed at the top were incubated with WCE containing FLAG-Int-6, and the GST fusion protein complexes were analyzed as described in the legend to Fig. 2B. The upper panel shows Coomassie Blue staining of GST (~3 μg; lane 2), GST-human eIF3bΔ (~1 μg of the full-length protein; lane 3), and GST-human eIF3c (~1 μg of the full-length protein; lane 4) employed for the pull-down experiments. The lower panels show immunoblotting of the isolated complexes with the antibodies listed on the left. Arrowheads point to the indicated protein species with the expected sizes. Lane 1, 10% input (In) amount of WCE used for the binding reaction.

FIG. 5. Effect of PCI8 deletion on polysome profiles. Yeast strains H2557 (PCI8) and KAY76 (pci8Δ) growing exponentially on YPD medium at 30 °C were treated with cycloheximide for 5 min prior to harvesting the cells. WCEs were prepared and resolved by velocity sedimentation on 15–40% sucrose gradients as described previously (41). Fractions were collected while scanning continuously at A254. The x axis indicates A254/0. The positions of different ribosomal species are indicated. P/M, ratio of A254 in the combined 2–4-mer fractions to that in the 80 S peak.
Mammalian Int-6 has been implicated in transcriptional regulation in addition to its association with eIF3. To investigate the possibility that Pci8p is involved in transcriptional control in yeast, we compared the total mRNA expression profiles of KAY76 (pci8Δ::URA3) and its isogenic wild-type strain H2557U. We constructed this strain by integrating wild-type PCI8::URA3 into the loci of H2557 since KAY76 contains URA3 inserted at the pci8Δ locus. We then prepared polyadenylated mRNA from these two strains grown on YPD medium at 30 °C and processed the samples for two-chip oligonucleotide microarray analysis. Since this system allows estimates of the mRNA expression levels of the genes encoding different eIFs, PCI8, and RRI1 in wild-type yeast cells (H2557U), the results from all four experiments could be compared directly with one another, yielding a mean of four different comparisons. Table V represents a summary of genes that showed an average difference in expression of >2-fold. Only genes demonstrating at least a 1.5-fold change between the mutant and wild type in all four comparisons were included.

Interestingly, the levels of mRNAs encoding five heat shock proteins or their relatives (Hsp30p, Ddr2p, Yro2p, Sse2p, and Pir3p) and Ygr138cp, a putative transmembrane protein of the drug/H+ antiporter 12 family, were 2-fold or more lower in the deletion mutant versus the wild-type strain (Table V). Expression of HSP30, DDR2, YRO2, and SSE2 has been shown to be induced by heat (37, 38). Pir3p is a cell wall membrane protein that is 80% identical to Hsp150p/Pir2p, but its expression is not induced by heat (39). Functional genomic analyses from several laboratories indicate that these genes belong to different groups of genes regulated by a variety of cellular stresses, including heat, osmolarity, and cell-damaging agents. Thus, the genes listed in Table V might compose part of a novel stress-responsive regulon whose expression depends on Pci8p under our experimental conditions. This activity of Pci8p may be akin to the eIF3-independent role of fission yeast int-6 in transcription of multidrug resistance genes (8).

### DISCUSSION

In this report, we showed that one of the eight predicted PCI domain proteins encoded in S. cerevisiae, Pci8p, can interact in vivo and in vitro with the eIF3 complex (Fig. 1). We identified the binding partners of Pci8p as the eIF3b/Prt1p and eIF3a/Ti32p subunits (Fig. 2B) and localized a Pci8p-binding site in the N-terminal part of eIF3b/Prt1p that contains an RRM (Fig. 3). Interestingly, the human eIF3e/Int-6 protein also bound to the yeast eIF3 complex (Fig. 1) and the isolated eIF3b/Prt1p subunit (Fig. 2A) via the N-terminal RRM domain (Fig. 3). Furthermore, human Int-6 interacted with the corresponding RRM-containing segment of the human eIF3b protein (p116) (Fig. 4). These results support the notion that yeast Pci8p is a divergent ortholog of human Int-6. They further suggest that the binding site for human Int-6 in the cognate eIF3b subunit is conserved in yeast eIF3b/Prt1p and that Pci8p can interact with this domain in a manner similar to the interaction of Int-6 with eIF3b/p116, as shown schematically in Fig. 6.

**Interactions between eIF3e/Int-6 and Other eIF3 Subunits—** Results from our binding assays provide new insights into the subunit interactions in mammalian eIF3. The interaction we detected between human eIF3e/Int-6 and eIF3b/p116 (Fig. 4) may be instrumental in tethering Int-6 to the human eIF3 core complex (Fig. 6B). However, there are likely other contributing interactions, as human Int-6 interacts with eIF3e/p110 in the yeast two-hybrid assay (22). Although FLAG-Int-6 did not bind to GST-human eIF3c in our binding assays (Fig. 4), there is evidence that plant eIF3e directly interacts with the cognate plant eIF3c subunit (27). On the other hand, Yen and Chang (24) reported that fission yeast eIF3e/Int6 interacts with eIF3d/Moe1 in yeast two-hybrid and far-Western assays, that this interaction is conserved with the cognate human homologs, and that it may depend on the PCI domain in human Int-6. Perhaps the PCI domain of eIF3e/Int-6 can interact simultaneously with eIF3d and eIF3c to bridge the interaction between eIF3d and the eIF3 core complex (Fig. 6B). Consistent with this model, disruption of moe1Δ reduces the level of Int6 and vice versa (24). The absence of one of the eIF3 non-core subunits e and d may lead to loss of the other from the eIF3 complex,
Pci8p and eIF3e/Int-6 Interact with eIF3 Core Complex

Fig. 6. Subunit structures and interactions of eIF3 found in S. cerevisiae (A) and human (B). Ovals indicate polypeptides composing eIF1, eIF5, and eIF3. The circles labeled eIF2 denote the trimeric eIF2 complex, and the plugs attached to them indicate Met-tRNA\textsubscript{Met}. Yeast eIFs implicated in correct AUG selection by genetic approaches (42) and their human homologs are pale blue. The rounded rectangle indicates the 40 S subunit. Double-headed arrows indicate direct interactions between different eIFs and 40 S ribosome. The eIF3 subunits were named after genes encoding them (A) or their sizes in kDa (B), and letters in parentheses indicate the unified eIF3 subunit nomenclature proposed by Burks et al. (6). Primary structures of some eIF3 subunits are drawn with N- and C-terminal ends, evolutionarily conserved domains (ovals), and less conserved charged domains (thick lines). The five eIF3 core subunits found in S. cerevisiae are pale orange, whereas peripheral, non-essential subunits are white. Proteins containing the PCI/PINT domains are shown in red. Brackets refer to N (N-term)- and C (C-term)-terminal subcomplexes of eIF3. For A, direct contacts indicate direct interactions (12, 31). Thick arrows indicate interaction of Pci8p, the possible eIF3e ortholog, found in this study. For B, direct contacts between the five core subunits are deduced from the model in A, except for the eIF3a-eIF3b (RRM) interaction in human (32). Human eIF3 subunits f, h, k, and l are not shown, since no information is available for their interactions.

TABLE V
Summary of genes showing a significant change in mRNA expression level between the pci8Δ strain (KAY76) and its isogenic wild-type strain (H2557U)

| ORF    | Gene       | Function                                           | Accession no. | Mean -fold decrease* |
|--------|------------|----------------------------------------------------|---------------|----------------------|
| YCR021C| HSP30      | Heat shock protein involved in cellular pH homeostasis | S0000615      | 25.9                 |
| YGR135C| DDR2       | Hypothetical transmembrane protein of the drug/H\textsuperscript{+} antiporter 12 family | S0003370      | 12.8                 |
| YOL052C| YOL052C    | Heat shock protein (DNA damage-responsive)         | S0005413      | 6.1                  |
| YBR054W| YRO2       | Heat shock protein similar to Hsp70p               | S0002556      | 5.9                  |
| YKL163W| PIR3       | Cell membrane protein similar to Hsp150p/Pir2p     | S001646       | 3.7                  |
| YBR168C| SSE2       | Heat shock protein of the Hsp70p family            | S0000373      | 2.4                  |

* Mean of four comparisons.
tivity by binding to eIF3e/Int-6 (43). In contrast, the eIF3e/Int6 protein of fission yeast has been implicated more directly in eIF3 function (see Introduction). Thus, it was conceivable that Pci8p also plays a role in translation by binding to the same site in the eIF3 core as human Int-6 does. At odds with this prediction, binding of Pci8p to yeast eIF3 has little or no effect on the general function of this complex in translation initiation under the growth conditions of our experiments (Fig. 5 and other data not shown). It is still possible that binding of Pci8p to eIF3 could alter the translation of specific mRNAs (as suspected for S. pombe Int6), or the protein may have to be induced or undergo a post-translational modification that is restricted to a specific stress condition or developmental state in order to influence eIF3 function. Alternatively, Pci8p may have lost a role in translation and become specialized as a transcriptional regulator, as described below.

Is Pci8p Involved in Transcriptional Control?—Growing evidence has suggested that eIF3e/Int-6 has dual regulatory functions in the nucleus and cytoplasm (see Introduction). Accordingly, we investigated the effect of deleting PCI8 on genome-wide expression of mRNAs using microarray technology. The results showed that pciΔ mutants exhibit reduced expression of mRNAs for several heat-shock- or stress-inducible proteins (Table V). It remains to be determined whether Pci8p functions directly or indirectly to enhance the expression of these mRNAs, although a recent report of two-hybrid interaction between Pci8p and Tfb1p (the transcription factor IIH subunit of RNA polymerase II holoenzyme) may favor a direct role in transcription (44). The possible role of Pci8p in stress-inducible transcription is reminiscent of a recent finding that, in plants, the occurrence of PR500, a free form of the proteasome, induces the expression of mRNAs for several heat shock- or stress-inducible proteins (45). The fact that budding yeast Pci8p can bind to eIF3 and also influence the expression of heat shock mRNAs may indicate that it too performs dual functions in the nucleus and cytoplasm.

It was recently reported that the COP9 signalosome promotes cleavage of a ubiquitin-like protein, NEDD8, from proteins conjugated with it (46). This activity appears to be conserved from budding yeast to humans, even though S. cerevisiae encodes only one COP9 signalosome subunit ortholog termed Rri1p, a Mov34 family protein previously called D0888 (10) or YDL216c (14, 15). In S. cerevisiae, deletion of RRI1 is not lethal, but allows accumulation of a NEDD8-conjugated protein (46). Since the COP9 signalosome is a hetero-octamer containing six PCI proteins and two Mov34 family proteins (26), Pci8p may be directly involved in this COP9 signalosome activity and activate transcription by regulating NEDD8 conjugation of one or more transcription factors. According to our microarray data, RRI1 expression is very low and comparable to that of PC18 (Table IV). The similar expression levels and dispensability of PC18 and RRI1 are at least consistent with this interesting model.

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