Cpc2/RACK1 Is a Ribosome-associated Protein That Promotes Efficient Translation in Schizosaccharomyces pombe*

Received for publication, April 15, 2003, and in revised form, September 11, 2003
Published, JBC Papers in Press, September 12, 2003, DOI 10.1074/jbc.M303968200

Boris Shor‡§, Jimmy Calaycay¶, Julie Rushbrook¶, and Maureen McLeod‡

From the Morse Institute for Molecular Genetics, Department of Microbiology and Immunology, the ‡Department of Biochemistry, and the ¶Program in Molecular and Cellular Biology, State University of New York Downstate Medical Center, Brooklyn, New York 11203-2098

Cpc2/RACK1 is a highly conserved WD domain protein found in all eucaryotes. Cpc2/RACK1 functions on mammalian signal transduction pathways most notably as an adaptor protein for the βII protein kinase C isozyme. In single cell eucaryotes, Cpc2/RACK1 regulates growth, differentiation, and entry into G₀ stationary phase. The exact biochemical function of Cpc2/RACK1 is unknown. Here, we provide evidence that Cpc2 is associated with the ribosome. Using immunoaffinity purification, we isolated ribosomal proteins in association with Cpc2/RACK1. Polysome and ribosomal subunit analysis using velocity gradient centrifugation of cell lysates demonstrated that Cpc2 co-sediments with the 40 S ribosomal subunit and with polysomes. Conditions known to disrupt ribosome structure alter sedimentation of the ribosome and of Cpc2/RACK1 coordinately. Loss of cpc2 does not dramatically alter the rate of cellular protein synthesis but causes a decrease in the steady state level of numerous proteins, some of which regulate methionine metabolism. Whereas real time PCR analysis demonstrated that transcriptional mechanisms are responsible for down-regulation of some of these proteins, one protein, ribosomal protein L25, is probably regulated at the level of translation.

RACK1 is a highly conserved member of the family of WD-repeat proteins. The distinctive feature of members of this family is the presence of 4–8 repeating units, each consisting of a conserved core flanked by the sequence Trp-Asp (WD) (1). Most WD-repeat proteins include extensions at their amino and carboxyl termini that can be over 600 amino acids long. The first identified member of this family, and the most extensively studied, is the β subunit of G proteins (2). The crystal structure of Gβ revealed that the WD-repeats form a seven-bladed propeller structure, with each blade containing four antiparallel β strands. This structure is believed to describe the architecture of all other WD-repeat-containing domains (3).

RACK1 was initially identified as the founding member of a group of related proteins capable of interaction with protein kinase C isozymes (4, 5). Indirect immunofluorescence studies indicate that RACK1 shuttles β II protein kinase C between different intracellular sites (6). For instance, treatment of cells with phorbol 12-myristate 13-acetate, an activator of protein kinase C, results in coordinate relocalization of both RACK1 and protein kinase C (7). Other studies, however, indicate a more global role for RACK1 in mammalian cells. Notably, RACK1 has been identified as a binding partner for several, seemingly unrelated proteins. Many of these function on signal transduction pathways and include cytokine receptors (8–10), c-Src (11, 12), and the β integrin cytoplasmic domain polypeptide (13, 14). These observations have led to the proposal that RACK1 may function as a scaffold or adaptor protein that recruits other proteins to a signaling complex. Functionally, RACK1 is involved in angiogenesis (15), tumor cell growth (15, 16), neuronal responses to alcohol (17), and apoptosis (18). Despite its involvement in important physiological processes and its high level of conservation, the exact biochemical function of RACK1 remains undefined.

Homologues of RACK1 have been identified in a large number of eucaryotes, including plants and fungi (RACK1 orthologs in various species are known as Cpc2/Asc1/CpcB and Bell; we will use Cpc2 hereafter). Genetic studies in model organisms indicate a pleiotropic function for Cpc2. However, one theme to emerge from these studies is a role for Cpc2 in metabolism and, particularly, in cross-pathway control (18–20).

We isolated Cpc2 as an interaction partner for Ran1/Pat1 kinase (21). Ran1/Pat1 (referred to as Pat1 hereafter) is an inhibitor of sexual development in fission yeast (22, 23). Detailed analysis of cells containing a cpc2 null allele indicates that, like pat1, cpc2 is important for conjugation and sporulation. Additionally, cpc2 is required for G₁ arrest induced by nitrogen limitation. Loss of cpc2 causes cells to exit the mitotic cell cycle and to enter the G₁ stationary phase (21), a phenotype also described for budding yeast CPC2 mutants (24). As indicated above, the function of Cpc2 from several organisms, although not fully understood, indicates a role in the control of amino acid biosynthesis. However, there is no evidence that “cross-pathway control” exists in Schizosaccharomyces pombe (25). Thus, the role for Cpc2 in general amino acid control may reflect its function in a more global metabolic process.

Cpc2 has been identified as a component of several different large protein complexes. Cpc2 co-fractionates with the 80 S ribosome (26). Polysome profiles from Saccharomyces cerevisiae cells containing a CPC2 null allele are aberrant and display an increased number of “half-mer” polyribosomes, indicating a defect in translation initiation synthesis (24). Other evidence for an association between Cpc2 and the ribosome is indirect. For instance, Cpc2 is an abundant protein and is present in approximately equimolar concentration to other ribosomal proteins. However, Cpc2 is not characteristic of other ribosomal proteins. It is acidic, with a pl of 6.04, compared with an average of 10.03 for the other 78 ribosomal proteins (26).
Cpc2 Is a Ribosome-associated Protein

Other physical studies point to an association between Cpc2 and the 19 S proteasome regulatory cap. Since the association is observed only in the absence of ATP (27), these data may or may not be contradictory to those identifying Cpc2 as a component of the ribosome. Recently, immunopurification using tagged Prp4p (a yeast splicing protein) yielded a ribonucleoprotein particle with splicing activity and identified as the yeast prp4p small nuclear ribonucleoprotein. Cpc2 was found physically associated with this complex, indicating its potential to function as a splicing factor (28). In neuronal cell lysates, RACK1 is associated with polysome-bound, poly(A) mRNAs that are being actively translated (29).

Here, we use immunopurification of Cpc2 and polysome profiles to demonstrate that Cpc2 is a ribosome-associated protein. Cpc2 co-sediments with the 40 S subunit and with polysomes in sucrose gradients. Conditions that disrupt ribosomes alter sedimentation of Cpc2. Evidence is presented that Cpc2 has a role in translation, most likely at the subunit-jointing step. Cpc2 does not significantly alter the rate of global protein synthesis but specifically down-regulates the steady state abundance of several cellular proteins. Analysis of a subset of these shows that the decrease of all but one protein, ribosomal protein L25, is due to reduced mRNA levels. Real time PCR analysis of fractionated extracts indicates that the mRNA for ribosomal protein L25 becomes associated with nontranslocating ribosomes in cpc2::ura4 cells. Significantly, rpl25 mRNA is spliced, and both cpc2::ura4 and cpc2− cells process the transcript equally well. Thus, both physical and functional evidence indicate a role for Cpc2 in translation.

EXPERIMENTAL PROCEDURES

Yeast Strains, Plasmids, and Recombinant DNA Techniques—The yeast strains used are SPB27 (h90 ura4-D18 ad6-M210), SPB325 (h90 ura4-D18), SPB325 (h90 ura4-D18 ad6-M210 cpc2::gfp gdfp ura4+), SPB345 (h90 ura4-D18 cpc2::His3 FLAG ura4+), SPB343 (h90 ura4-D18 cpc2::GST ura4+), and SPB344 (h90 ura4-D18 cpc2::GST ura4+ cpc2−). Yeast growth and transformation were as described (30). Antibiotic sensitivities were analyzed by microcapillary liquid chromatography-tandem mass spectrometry using a Finnigan LCQ DECA XP Ion Trap (ThermoFinnigan Corp., San Jose, CA) mass spectrometer. The sequence identities of the proteins were determined by correlating tandem mass spectrometry spectra with the NCBI protein data base using the Sequest algorithm and confirmed by manual evaluation of the spectra.

Sucrose Gradients and Polyribosome Analysis—100 µg of cycloheximide was added to 100 ml of growing cells (except where indicated) prior to centrifugation. Cells were washed and resuspended in TSM1 lysis buffer (10 µl Tris-HCl, pH 7.5, 100 mM NaCl, 30 mM MgCl2, 100 µg/ml cycloheximide) for glass bead breakage. Lysates were clarified by centrifugation at 14,000 rpm for 20 min. 15.5 ml of growing cells (except where indicated) was centrifuged at 14,000 rpm for 20 min. 15 ml of TSM1 buffer (50 mM Tris-HCl, pH 7.0, 50 mM NH4Cl, 12 mM MgCl2). Centrifugation was at 4°C for 3.5 h at 3°C in a Beckman SW41Ti rotor. To disrupt polyribosomes by chelating Mg2+ ions, EDTA was added to a final concentration of 40 µM to a cycloheximide-treated extract. NaCl treatment of extracts was accomplished by the addition of salt to a final concentration of 0.7 M. For RNAse digestion of polyribosomes, extracts were treated with 0.5 mg/ml RNase A for 20 min. Sucrose gradients were collected in 500-µl fractions, and absorbance was measured at 260 nm. To analyze the distribution of Cpc2 on sucrose gradients, fractions were precipitated using 5 µl of 2X deoxycholate and 250 µl of 50% trichloroacetic acid. Pellet extracts were washed with 80% ethanol and resuspended in LDS sample buffer prior to resolution on SDS-PAGE.

For polysome profiles, cells were washed and resuspended in TSM2 buffer (10 µl Tris-HCl, pH 7.4, 100 mM NaCl, 30 mM MgCl2, 50 µg/ml cycloheximide, protease inhibitor mixture (Sigma), 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol) prior to centrifugation. Supernatants were clarified by centrifugation for 20 min at 15,000 rpm. Protein concentrations were calculated using a Bio-Rad assay with bovine serum albumin as the standard.

Immunoblotting—To detect Cpc2 and L7 in either lysates or immune complexes, proteins were resolved on a 4–12% Bis-Tris gradient gel (Invitrogen) and transferred to nitrocellulose membranes (Amersham Biosciences). Membranes were incubated with an anti-FLAG M2 or peroxisome-conjugated anti-MAP-FLAG M2 horseradish peroxidase antibodies (Sigma) were used at a dilution of 1:600, and mouse polyclonal anti-L7 antibodies (GeneTex Inc.) were used at a dilution of 1:500. The secondary antibody was sheep anti-mouse IgG horseradish peroxidase conjugate diluted 1:40,000 (Amersham Biosciences). Blots were developed using the ECL detection kit (Amersham Biosciences). Molecular mass standards were resolved in parallel lanes to determine molecular mass.

SDS–PAGE—For polysome profiles, cell lysates were clarified by centrifugation for 20 min at 15,000 rpm. Protein concentrations were calculated using a Bio-Rad assay with bovine serum albumin as the standard.

Experimental procedures for yeast total RNA isolation were as follows. To obtain yeast RNA, 15.5 ml of growing cells (except where indicated) was centrifuged at 14,000 rpm for 20 min. 15 ml of TSM1 buffer (50 mM Tris-HCl, pH 7.0, 50 mM NH4Cl, 12 mM MgCl2). Centrifugation was at 4°C for 3.5 h at 3°C in a Beckman SW41Ti rotor. To disrupt polyribosomes by chelating Mg2+ ions, EDTA was added to a final concentration of 40 µM to a cycloheximide-treated extract. NaCl treatment of extracts was accomplished by the addition of salt to a final concentration of 0.7 M. For RNAse digestion of polyribosomes, extracts were treated with 0.5 mg/ml RNase A for 20 min. Sucrose gradients were collected in 500-µl fractions, and absorbance was measured at 260 nm. To analyze the distribution of Cpc2 on sucrose gradients, fractions were precipitated using 5 µl of 2X deoxycholate and 250 µl of 50% trichloroacetic acid. Pellet extracts were washed with 80% ethanol and resuspended in LDS sample buffer prior to resolution on SDS-PAGE.

Microscopy—SPB325 cells containing integrated cpc2::gfp were observed using a Nikon Ax100 epifluorescence microscope. Confocal images were captured at 15,000× magnification. To stain nuclei, cells were incubated with 4′,6-diamidino-2-phenylindole as described (31).

Measurement of Protein Synthesis—For [35S]methionine/cysteine labeling, 4.5 ml of growing cells were mixed with 50 µCi of [35S]-protein labeling mixture (1175 Ci/mmol; PerkinElmer Life Sciences), and incubated for 15 min. 0.5% methylcellulose-concentrated medium was added and mixed with 0.5% of 25% trichloroacetic acid. Samples were heated at 95°C for 20 min and then cooled on ice. Precipitated proteins were filtered through GFC filters (Whatman) and washed twice with 5% trichloroacetic acid and then with 95% ethanol. Dried filters were assayed for [35S] radioactivity by liquid scintillation counting. Protein synthesis rates are expressed as cpm/mg of protein.

Yeast Total RNA Isolation—2 × 10^6 cells were collected by centrifugation and resuspended in 400 µl of TSM2 lysis buffer (10 m Tris-HCl, pH 7.4, 100 mM NaCl, 30 mM MgCl2, protease inhibitor mixture (Sigma), 80 units/ml SUPERasein, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol) prior to centrifugation.
RESULTS

Identification and Immunoprecipitation of Epitope-tagged Cpc2—To immunoprecipitate Cpc2 and its associated proteins, we introduced His$_6$ sequences as well as a FLAG epitope in-frame with the 3′ end of the cpc2 coding region. The construction was integrated to obtain a fusion gene (cpc2::xpc2HF) expressed under control of the endogenous cpc2 regulatory region. The transformants that were examined in a Western blot using anti-FLAG monoclonal antibody and anti-His$_6$ antibody. The immune complexes were resolved on SDS-PAGE and visualized by silver staining (not shown) or staining with Coomassie Blue (Fig. 1C). We observed individual polypeptides present in immune complexes from Cpc2HF cells but absent from those of the parental control cells. Only lysates prepared from cells expressing the fusion protein contained a protein band with the expected molecular weight of Cpc2HF. Since the immunoprecipitation method reproducibly yielded specific polypeptides, the His$_6$ epitope tag was not used for further purification. Notably, most polypeptides associated with Cpc2HF were smaller than 45 kDa. To rule out trivial explanations for the presence of a large number of low molecular weight polypeptides, the eight largest polypeptides (numbered in Fig. 1C) were purified for microsequencing. Ten polypeptides were identified (Table 1). Eight of the ten are known components of either the large or small ribosomal subunits. The other two correspond to Cpc2 and to glyceraldehyde 3-phosphate dehydrogenase. Experiments using a Cpc2-glutathione S-transferase fusion protein suggested that glyceraldehyde 3-phosphate dehydrogenase co-purifies nonspecifically with Cpc2-HF, since it is not present when Cpc2-glutathione S-transferase-associated proteins are examined following purification on glutathione beads (not shown). Cpc2 was identified by mass spectrometry as a component of bands 3 and 4. The gel migration pattern may indicate the presence of different mod-

Fig. 1. Detection of Cpc2H6F and identification of associated proteins. A, Western blot of whole cell extract prepared from control cells (SPB275) or from four SPB345 transformants potentially producing the Cpc2H6F fusion protein (F1 through F4). Equal amounts of protein from each strain (50 μg) were resolved by a 4–12% Bis-Tris gradient gel and subjected to analysis using anti-FLAG M2 monoclonal antibody. Immunodetection was performed using horseradish peroxidase-conjugated anti-mouse secondary antibody and developed using chemiluminescence. B, Western blot of proteins immunoprecipitated from cells containing Cpc2H6F or parental control cells. Whole cell lysate from cells containing Cpc2H6F or Cpc2 is shown (Input). Soluble protein (5 μg) was subjected to immunoprecipitation using anti-FLAG M2 monoclonal antibodies. A portion of the precipitated material (IP) or the supernatant (Sup) was resolved by a 4–12% gradient gel prior to processing for immunoblotting as described above. C, purification and identification of Cpc2H6F-associated proteins. A Coomassie Blue-stained SDS-polycrylamide gel (4–12%) containing a portion of the eluted proteins from anti-FLAG M2 immunopurification of the Cpc2H6F complex was compared with eluted proteins from an identical purification from an untagged strain. Numbers indicate the sequenced protein bands (see Table I).

1 The abbreviations used are: RT, reverse transcriptase; EGFP, enhanced green fluorescent protein; uORF, upstream open reading frame.
I and $60$ subunits (32) (Fig. 2B). Under these conditions, $B$ polyribosomes, and a large increase in the number of free $40$ subunits were dissociated into $40$ and $60$ S subunits, although they migrated more slowly than intact $40$ and $60$ S subunits in sucrose gradients. This suggests that high salt treatment of cell extracts removes some ribosomal proteins. Both Cpc2HF and Rpl7 migrated at the top of the gradient (Fig. 2B). Next, we treated cell extracts with RNase to degrade the mRNA linking translating ribosomes together and collapse polyribosomes into a single $80$ S peak (32). Ribosomal protein Rpl7 and Cpc2 both migrated in sucrose gradients at a position coincident with the $80$ S particles (Fig. 2B). Taken together, these data provide strong evidence that Cpc2 is directly associated with the ribosome.

Cpc2 Is a Cytoplasmic Protein—In some respects, Cpc2 does not fit the classic profile for a ribosomal protein. For instance, Cpc2 is acidic (pI = 6.04), unlike the majority of ribosomal proteins (26). In addition, ribosomal proteins are conserved between the bacterial and eukaryotic kingdoms. Cpc2 is not found in bacteria. We sought independent criteria with which to support or refute the hypothesis that Cpc2 is a ribosome-associated protein. Cpc2 was fused to green fluorescent protein. The fusion allele (cpc2-eGFP) was integrated into the chromosome of $h^{90}$ cells to examine localization of the protein in cells at various stages of the yeast life cycle. $h^{90}$ cells interconvert alleles at the mating-type locus forming a colony of “plus” and “minus” cells that conjugate and undergo meiosis when nutrients become limiting. The subcellular distribution of Cpc2-eGFP was examined by immunofluorescence microscopy (Fig. 3). Cpc2-eGFP was concentrated throughout the cytoplasm and excluded from the nucleus. To confirm the cytoplasmic localization of Cpc2-eGFP, the nucleus was visualized using 4',6-diamidino-2-phenylindole (data not shown). Cytoplasmic localization of Cpc2-eGFP and excluded from the nucleus. To confirm the cytoplasmic localization of Cpc2-eGFP, the nucleus was visualized using 4',6-diamidino-2-phenylindole (data not shown). Cytoplasmic fluorescence of Cpc2-eGFP appeared in conjugating cells, yogotes, and spores. The signal did not vary in any observable way during the cell cycle or when cells were starved of nutrients. Laser-scanning confocal microscopy confirmed the predominantly cytoplasmic localization of Cpc2-eGFP. This localization is consistent with the steady-state cytoplasmic location of ribosomes actively engaged in translation (33).

The sequence of Cpc2-associated polypeptides

| Band | Sequence | Products | Accession no. | Mass kDa |
|------|----------|----------|---------------|---------|
| 1    | SHxKFEQPR | Rpl3-A/B | P40372/P36584 | 43.8    |
| 2    | SHxKFEQPR | Rpl3-A/B | P40372/P36584 | 43.8    |
| 3    | PEQLVLRATL | Cpc2 | Q10281 | 34.8 |
| 4    | PEQLVLRATL | Cpc2 | Q10281 | 34.8 |
| 5    | AIPKVINGF | Rpl3 | O60138 | 27.5 |
| 6    | AIPKVINGF | Rpl5-A/B | P52822/074306 | 33.3 |
| 7    | PFIKAVKSSP | Rpl5-A/B | P52822/074306 | 33.3 |
| 8    | AVGNKRLSK | Rps1-A/B | Q09781/O94438 | 28.5 |
| 9    | MRKNIYRAN | Rps6-A/B | P05792/Q9/COZ7 | 27.5 |

Loss of cpc2 Causes Phenotypes Associated with Defects in Translation—To investigate a potential function for Cpc2 in translation, we examined the sensitivity of cpc2::ura4 cells to paromomycin, cycloheximide, and verrucarin A at $30^\circ$C. Wild type cells grew well on YEAm medium containing 2.5 mg/ml paromomycin, whereas cpc2::ura4 cells failed to form colonies (Fig. 4A). Paromomycin increases codon misreading (34). No sensitivity to either cycloheximide or verrucarin A was observed (not shown), both of which act on the $60$ S subunit to inhibit peptide bond formation. This argues against the possibility that cpc2::ura4 cells are generally more permeable or susceptible to drugs and suggests that a specific defect in...
translation results from the absence of Cpc2. Importantly, plasmids containing cpc2 cDNA or a genomic clone bearing cpc2 complement the paromomycin sensitivity (not shown). Interestingly, growth of cpc2::ura4 cells was moderately inhibited by low temperature (13 °C). Under these conditions, cpc2::ura4 cells accumulated misshapen and multiseptate cells (not shown). Cold-sensitive growth has been reported previously for yeast mutants containing alterations in one of several components of the translation apparatus (35).

Next, we compared protein synthesis rates in wild type and cpc2::ura4 cells. Exponentially growing cells were transferred to medium devoid of glucose or medium to which glucose was added. Incorporation of [35S]methionine/cysteine into protein was measured over a 1-h period. As shown in Fig. 4B, glucose withdrawal completely inhibits [35S]methionine incorporation into newly synthesized proteins in yeast (36). In the presence of
glucose, the rate of protein synthesis rate increased equally well in both wild type and cpc2::ura4 cells immediately following the addition of radioactive label. However, 60 min following the addition of [35S]methionine/cysteine, the rate of protein synthesis in cpc2::ura4 cells was 18% slower than that of wild type cells.

Loss of Cpc2 Leads to a Decrease in 80 S Monosomes and Polyribosomes—To assess the importance of Cpc2 for efficient translation initiation in vivo, we compared the polyribosome profile of extracts prepared from wild type and cpc2::ura4 cells grown in complete yeast extract medium (Fig. 5). Cells and cell extracts were treated with cycloheximide to immobilize translating 80 S ribosomes on mRNA. When initiation is blocked, ribosomes complete elongation of mRNA but reinitiate inefficiently. This results in an accumulation of 80 S ribosomes and a reduction in the polysome/monosome ratio (37). The polysome/monosome ratios were similar for cpc2::ura4 and wild type cells (3.4 versus 3.7). However, cpc2::ura4 cells displayed a 38% increase in 40 plus 60 S subunits and a 21–27% decrease in 80 S monosomes and polysomes compared with wild type cells. These observations indicate that cpc2 is not essential for translation initiation but may have a role in subunit joining or stability. It was reported that loss of Cpc2 causes an accumulation of half-mer ribosomes (24). Half-mers are stalled on mRNAs due either to a subunit-joining defect or to a defect in the assembly of 60 S subunits (38–40). We did not observe the large shoulder on polysome peaks that usually indicates the presence of half-mers.

Loss of cpc2 Leads to a Deficiency in a Subset of Highly Expressed Cellular Proteins—During the course of these studies, we observed that numerous protein bands were consistently reduced in cpc2::ura4 cell extracts compared with extracts prepared from cpc2+ cells (Fig. 6). This and the observation that Cpc2 is not essential for global protein synthesis prompted us to determine whether Cpc2 might regulate the abundance of a subset of cellular proteins. Cell extracts prepared from cpc2+ and cpc2::ura4 cells were fractionated by

Fig. 3. Cpc2 is localized in the cytoplasm and excluded from the nucleus. A montage of cells containing the Cpc2-EGFP fusion protein during various stages of the yeast life cycle. The arrows indicate conjugating cell pairs. Images were captured using fluorescence microscopy (A) or confocal laser imaging (B).

Fig. 4. Loss of cpc2 causes defects associated with protein synthesis. A, wild type cells (SPB173) or cpc2::ura4 cells (SPB182) were diluted as indicated and spotted on YEA medium or YEA containing 2.5 mg/ml paromomycin. B, exponentially growing cells were shifted to EMM medium with or without glucose in the presence of 50 μCi of [35S]methionine. Cells were processed as described under “Experimental Procedures” at the indicated times. Incorporation rates were calculated as cpm/A600. Three independent samples were counted, and S.E. values did not exceed 1.0%.

Fig. 5. Polysome profile analysis of wild type cells and cpc2::ura4 cells. cpc2::ura4 cells (SPB182) and wild type cells (SPB173) were grown at 30°C. Cells were collected and processed for polysome analysis by velocity sedimentation of whole cell extracts on 7–47% (w/v) sucrose gradients. Gradients were fractionated by scanning at 254 nm, and the resulting absorbance profiles are shown, with sedimentation from left to right.
sequences of these were examined. Primer sets corresponding to the se-
"The identification of proteins by liquid chromatography-tandem mass spectrometry following in gel torycic digestion is indicated to the right of each band.

SDS-PAGE. The identity of the abundant proteins absent in cpc2::ura4 cells was determined by mass spectrometry of gel-purified proteins from cpc2" cells. Six bands were analyzed, and these contained proteins present in the S. pombe protein data bases. Three were enzymes required for methionine biosynthesis and utilization: methionine synthase, homocysteine synthase, and S-adenosylmethionine synthase. Methionine, an important metabolite in yeast cells, is required for protein synthesis and methyl-group transfer through S-adenosylmethionine (41). Methionine stimulates meiosis and lowers cAMP levels in S. pombe (42). Since cpc2::ura4 cells either completely lacked or exhibited reduced levels of methionine biosynthetic enzymes, we determined whether exogenously added methionine could rescue the meiotic or growth defects observed minimal defined medium at 30 °C. However, the addition of methionine inhibited cell growth and did not rescue the meiotic defect (not shown). The other three proteins identified by mass spectroscopy were pyruvate kinase, thiazole biosynthetic en-

Cpc2 Deficiency Affects the Ability of rpl25–1 RNA to Associate with Translating Ribosomes—To define the mechanism(s) causing a decrease in the steady state abundance of each of the six proteins in cpc2::ura4 extracts, the mRNAs of several of these were examined. Primer sets corresponding to the se-
sequences of sam1" (chosen as a representative of the methionine biosynthetic pathway), rpl25–1, and thi2 were used to obtain and amplify specific cDNAs from either wild type cells or cpc2::ura4 cells in a quantitative PCR. For rpl25–1, which is spliced, the primers were designed to distinguish between pro-

Fig. 6. Identification of proteins with a lower steady state level in cpc2::ura4 cells. Coomassie Blue staining pattern in SDS-polyacrylamide gels of lysates prepared from wild-type cells (SPB173) or cpc2::ura4 cells (SPB182). Individual protein bands were analyzed by densitometry. Student's t test was used to identify statistically signif-

The decreased levels of both sam1 and thi2 RNAs were observed in the absence of cpc2. In contrast, the abundance of rpl25–1 RNA was similar in both strains (Fig 7A). The decreased amounts of both sam1 and thi2 RNA were sufficient to account for the lowered protein abundance of each in cpc2::ura4 cells. In contrast, the decline in the level of ribosomal protein Rpl25 in cpc2::ura4 cells is not likely to be caused by an inability to accumulate its mRNA transcript.

Next, we examined the distribution of sam1, thi2, and rpl25–1 transcripts on ribosomes prepared from exponentially growing wild type or cpc2::ura4 cells. Sucrose gradients were

Fig. 7. Steady-state transcript levels examined by a kinetic RT-PCR analysis. A, total RNA prepared from the wild type (SPB173) and mutant (SPB182) cells was used as template for real time RT-PCR as described under "Experimental Procedures." The relative mRNA amounts were normalized to the abundance of the actin mRNA. B, cpc2::ura4 cells and their isogenic wild type parent cells were grown at 30 °C. Cells were collected and processed for polysome analysis by velocity sedimentation of whole cell extracts on 7–47% (w/v) sucrose gradients. Gradients were fractionated by scanning at 254 nm. RNA was purified from each fraction and quantitated in an RT-PCR as described under "Experimental Procedures." For each gene, the relative amount of RNA in each fraction was calculated as a percentage of the total RNA for that gene.
fractionated by centrifugation to separate ribosomal subunits, monosomes, and polyribosomes. Gradient fractions were processed to obtain RNA, and primer sets corresponding to \textit{sam1}, \textit{thi2}, and \textit{rpl25-1} were used in a quantitative PCR of the RNA from each fraction. As illustrated in Fig. 7, all three RNAs were primarily associated with higher order polyribosomes in \textit{cpc2} cells. For both \textit{sam1} and \textit{thi2}, a nearly identical pattern of polyribosome association was observed in \textit{cpc2::ura4} cells. By contrast, the distribution of \textit{rpl25-1} mRNA in \textit{cpc2} cells differed from that in \textit{cpc2::ura4} cells. Specifically, loss of \textit{cpc2} caused a shift in the abundance of \textit{rpl25-1} RNA to fractions containing 80 S monosomes and lower molecular weight polyribosomes. These data indicate that the decrease in ribosomal protein L25-A observed in \textit{cpc2::ura4} cells is probably due to a defect in recruitment of its mRNA to polyribosomes.

**DISCUSSION**

\textit{Cpc2 Is Associated with the Ribosome—}Immunopurification of epitope-tagged Cpc2 consistently led to co-precipitation of a number of small molecular weight proteins. To identify these, a subset was excised from a polyvinylidene difluoride SDS-gel electroblot for N-terminal sequencing. This identified proteins of both the 40 and 60 S ribosome subunits. The fission yeast ribosomal proteins share \textasciitilde 65–85\% identity with their \textit{S. cerevisiae} orthologues (43). If the high level of sequence conservation is indicative of functional conservation as well, we might assume that, like \textit{S. cerevisiae}, \textit{S. pombe} contains 32 small subunit ribosomal proteins and 48 large subunit proteins, 21 of which range in molecular mass from 30 to 45 kDa (44). The number of ribosomal proteins detected in our screen is far fewer, suggesting that the polypeptides identified here represent a particular subset of ribosomal proteins that specifically associate with Cpc2. The precise spatial location of these proteins on the eucaryotic ribosome remains undefined. Besides their role in translation, ribosomal proteins may also be involved in other cellular processes, such as DNA damage and transcriptional control (45, 46).

The interaction between Cpc2 and ribosomal proteins could be specific to ribosomal subunits, to translating ribosomes, or to a particular stage of the ribosome’s life cycle, such as ribosome biogenesis. To determine whether Cpc2 co-fractionated with specific ribosomal fractions, we used velocity gradient centrifugation of cell lysates. This analysis showed that Cpc2 co-sedimented with 40 and 80 S ribosomal particles and polyribosomes but not with free 60 S subunits. Moreover, conditions known to disrupt ribosomes altered sedimentation of Cpc2. The observation that Cpc2 remains associated with 80 S ribosomal particles upon RNase A treatment argues that it is not an RNA-binding protein.

Our studies confirm and extend those using \textit{S. cerevisiae} that reported co-fractionation of CPC2 and the ribosome (24, 26, 47). This and other indirect evidence support the assignment of Cpc2 as a ribosomal protein. For instance, in \textit{S. cerevisiae}, CPC2 is present in \textasciitilde 100,000 copies/cell. This value is consistent with the steady state abundance of most other ribosomal proteins (48). Microarray analysis of the abundance of budding yeast transcripts during several different environmental insults shows that regulation of \textit{cpc2} resembles regulation of other ribosome proteins (49). The association between Cpc2 and the ribosome is not likely to be limited to the yeasts. Mammalian RACK1 and yeast Cpc2/ASC1 are structurally and functionally conserved (21).

**The Function of Cpc2 as a Ribosome-associated Protein—**It appears unlikely that Cpc2 contributes to either the stability or biogenesis of 60 or 40 S subunits, because these defects generally cause an imbalance of one ribosomal subunit over the other (for example, see Ref. 39). Polysome profiles revealed that the ratio of free 40 to 60 S subunits in \textit{cpc2::ura4} cells is similar to that of wild-type cells. Ribosome biogenesis occurs primarily, although not exclusively, in the nucleolus (50, 51). We observed that a functional EGFP-tagged Cpc2 polypeptide was excluded from the nucleolus and localized to the cytoplasm. We also detected a decrease in the amount of polysomes and 80 S polyribosomes accompanied by an increase in 40 plus 60 S subunits in \textit{cpc2::ura4} cells. This result is consistent with the finding that loss of budding yeast Asc1 leads to half-mers (24). Together, these data indicate that subunit joining may be inefficient. Alternatively, loss of \textit{cpc2} may alter the balance between 80 S couples and free subunits.

\textit{Cpc2 Has a Role in Translation of Specific RNAs—}Several lines of evidence indicate that Cpc2 plays a role in protein synthesis, although it is not essential for the process. We did not detect substantial changes in the rate of \[^{35}\text{S}\] methionine incorporation in the \textit{cpc2::ura4} cells. In addition, \textit{cpc2} played an essential role in translation initiation, in its absence, cell lysates would probably contain a decreased polyribosome content accompanied by an increase in free 80 S ribosomes. This is observed when translation initiation is inhibited in \textit{S. cerevisiae} cells lacking essential translation initiation factors (52, 53). We observed a decrease, not an increase, in 80 S ribosomes in the complete absence of Cpc2. Consistent with its role in translation, cells lacking Cpc2 exhibited sensitivity to the aminglycide antibiotic paromomycin. Paromomycin is believed to bind the decoding site of the small ribosomal subunit to induce translational misreading.

Cpc2 appears to modulate translation of a specific subset of mRNAs. The abundance of several polypeptides decreases in the absence of Cpc2. We focused on Rpl25-A, Sam1, and Thi2 and found that Cpc2 is necessary for efficient recruitment of the \textit{rpl25-1} mRNA into polyribosomes. Loss of \textit{cpc2} leads to the addition of the steady-state level of Rpl25-A and shifts \textit{rpl25-1} transcript to the monosome fraction. This effect is specific to \textit{rpl25-1} mRNA because the association between both \textit{sam1} and \textit{thi2} mRNA with polysomes is independent of Cpc2. This result cannot be explained by the difference in coding region size of \textit{rpl25-1} mRNA. Polysome densities of one ribosome per 30–40 nucleotides are possible in eucaryotes. Thus, the 1000-bp \textit{rpl25-1} mRNA would accommodate multiple polyribosomes, so that \textit{rpl25-1} transcript can be actively translated in the wild type cells. Since the abundance of \textit{rpl25-1} transcript is not altered in \textit{cpc2::ura4} cells, Cpc2 is probably a positive regulator of Rpl25-A translation.

If Cpc2 has a general and moderate effect on protein synthesis, how does it cause a selective decrease in some metabolic genes? We observed two \textit{cis} elements in the 5’ leader region of \textit{rpl25-1} mRNA, a short uORF and a potential stem-loop structure, that could potentially contribute to translational control of the gene. The ability of a stem-loop structure to block the progress of 40 S subunits along mRNA during “scanning” is believed to be a cause for translation inhibition in both yeast and mammalian cells (54). It has been shown that translation initiation in \textit{S. cerevisiae} is very sensitive to the presence of stem-loop structures in the 5’-untranslated region (55, 56). Therefore, the inhibitory effect of the potential \textit{rpl25-1} stem-loop structure may be synergistic with absence of Cpc2. Not all proteins down-regulated in \textit{cpc2} cells contain a recognizable stem-loop structure in their 5’-untranslated leader region. Interestingly, most belong to the methionine metabolic pathway, indicating that an upstream regulator of this pathway may be sensitive to loss of Cpc2.

Loss of \textit{cpc2} causes pleiotropic phenotypes, including G2 cell cycle delay (21). There are now examples emerging in the literature that demonstrate a link between protein synthesis...
Cpc2 Is a Ribosome-associated Protein

growth. Contrary to expectations, the addition of methionine thionine can rescue the defects of eIF4A-dependent translational regulation. It is intriguing to structures and short uORFs that have been implicated in Ded1 Sam1 and Thi2 stability also contribute to the decrease in their level of their mRNA. Moreover, our studies directly demonstrate that the decrease in Rpl25A cannot be attributed to the splicing of Rpl25-1 RNA. The basis for the decrease in the two other proteins we examined was probably caused by a decrease in the mRNA abundance of each. Thus, the evidence does not indicate that Cpc2 is required for efficient proteosome activity, although we cannot rule out the possibility that changes in Sam1 and Thi2 stability also contribute to the decrease in their abundance in cpc2::ura4 cells.

Using tagged ASC1, the S. cerevisiae homologue of Cpc2, as bait in a two-hybrid experiment to identify associated proteins, 13 proteins were found (57). Three were ribosomal proteins: RpL13-A, RpL27-B, and RpL7-A. ASC1 also interacted with proteins known to function during ribosome biogenesis (Kri1p and Sik1p), RNA processing (Prp43), or DNA metabolism (Rfa1p). Both RpL13A and RpL7A were also identified in our co-purification experiments using tagged Cpc2. RpL27-B has a molecular mass of 15 kDa and would not have been identified in our immunoprecipitation experiment. On the basis of its cytoplasmic localization and effects on the levels of 80 S monosomes, Cpc2 is unlikely to function in ribosome biogenesis.

Role in Fission Yeast—Methionine is an important metabolite in yeast cells. It is required for protein synthesis and methyl-group transfer through S-adenosylmethionine (41). The addition of methionine stimulates meiosis and lowers cAMP levels in S. pombe cells (42). This, together with the observation that cpc2::ura4 cells have reduced levels of methionine biosynthetic enzymes, led us to test whether exogenously added methionine can rescue the defects of cpc2::ura4 cells in meiosis or growth. Contrary to expectations, the addition of methionine severely inhibited growth of cpc2::ura4 cells and did not rescue its defect in meiosis. In budding yeast, loss of Cpc2 allows expression of amino acid biosynthetic genes in the absence of starvation. These disparate results may be explained by the absence in fission yeast of a cross-pathway control mechanism for expression of amino acid biosynthetic genes (25).

Taken together, our findings suggest that Cpc2 modulates levels of expression of some metabolic enzymes and affects production of the ribosomal protein RpL25-A. Both the physiologic role and the mechanism of such regulation remain unclear. However, it is intriguing that production of methionine/ S-adenosylmethionine and ribosomal proteins in the cells are linked to protein synthesis.

Acknowledgments—We thank Drs. Christopher Helen, Tatyana Pestova, and Donald Mills for helpful discussions. We also thank members of the McLeod laboratory for comments and support. Betty Leung is acknowledged for the preparation of many reagents, plasmids, and yeast strains. Dr. Charles Hoffman is especially appreciated for helpful comments and advice concerning the manuscript.

REFERENCES

1. Neer, E. J., Schmidt, C. J., Nambudripad, R., and Smith, T. F. (1994) Nature 371, 297–300
2. Neer, E. J., Smith, T. F., Neer, E. J., and Smith, T. F. (1996) Cell 84, 175–178
3. Sniegowski, J., Boheim, A., Lambracht, D. G., Hammon, H. E., and Sigler, P. B. (1996) Nature 379, 369–374
4. Ron, D., Chen, C. H., Caldwell, J., Jamiieson, L., Orr, E., and Mosley-Rosen, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 839–842
5. Ron, D., Chen, C. H., Orr, E., and Mosley-Rosen, D. (1998) FASEB J. 12, 35–42
6. Cukai, M., and Mosley-Rosen, D. (1999) Pharmacol. Res. 39, 253–259
7. Ron, D., Jiang, Z., Yao, L., Vagts, A., Diamond, I., and Gordon, A. (1999) J. Biol. Chem. 274, 27039–27046
8. Geisjen, N., Spaurgaren, M., Raaijmakers, J. A., Lammers, J. W., Koenders, L., and Coffer, P. J. (1999) Oncogene 18, 5126–5130
9. Usachova, A., Smith, R., Minshull, R., Bails, G., Steng, S., Crane, E., and Colamontoni, O. R. (2001) J. Biol. Chem. 276, 22948–22953
10. Kiely, P. A., Sant, A., and O’Connor, R. (2002) J. Biol. Chem. 277, 22581–22589
11. Chang, B. Y., Orczy, K. R., Macleod, E. M., and Cartwright, C. A. (1998) Mol. Cell. Biol. 18, 3245–3256
12. Chang, B. Y., Harte, R. A., and Cartwright, C. A. (2002) Oncogene 21, 7639–7649
13. Liliental, J., and Chang, D. D. (1998) J. Biol. Chem. 273, 2379–2383
14. Besson, A., Wilson, T. L., and Yong, V. W. (2002) J. Biol. Chem. 277, 22503–22509
15. Berns, H., Humer, R., Hengerer, B., Kieser, F. N., and Battegay, E. J. (2000) FASEB J. 14, 2549–2558
16. Sang, N., Severino, A., Russo, P., Balsi, A., Giordano, A., Mileo, A. M., Paggi, M. G., and De Luca, A. (2001) J. Biol. Chem. 276, 27026–27033
17. Ron, D., Vagts, A. J., Doehrmann, D. P., Yaka, R., Jiang, Z., Yao, L., Crabbe, J., Griset, J. E., and Diamond, I. (2000) FASEB J. 14, 2303–2314
18. Kruger, D., Koch, J., and Barthelmess, I. B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 211–215
19. Hoffmann, B., Mosch, H. U., Sattlegger, E., Barthel, B., Hänsebusch, A., and Braus, G. H. (1999) Mol. Microbiol. 34, 807–822
20. Hoffmann, B., Wiazek, C., Lagopodi, S. A., and Braus, G. H. (2000) Mol. Microbiol. 37, 28–41
21. Meleod, M., Shor, B., Caparosso, A., Wang, W., Chen, H., and Hu, L. (2000) Mol. Cell. Biol. 20, 4916–4927
22. Nurse, P. (1985) Mol. Gen. Genet. 198, 497–502
23. Ino, Y., and Yamamoto, M. (1985) Mol. Gen. Genet. 198, 416–421
24. Chantrel, Y., Gainsie, M., Lions, C., and Verdiere, J. (1996) Genetics 148, 559–569
25. Erickson, F. L., and Hanning, E. M. (1995) Yeast 11, 157–167
26. Link, A. J., Eng, J., Schieltz, D. M., Carmack, E., Mize, G. J., Morris, D. R., Garvik, B. M., and Yates, J. R., III (1999) Nat. Biotechnol. 17, 676–682
27. Verma, R., Chen, S., Feldman, R., Schieltz, D., Yates, J., Doehrmann, D., and Deshaies, R. J. (2000) Mol. Cell Biol. 21, 3425–3439
28. Strauss, S. W., Bryant, D. E., Hechter, E., Moore, R. E., Young, M. K., Lee, T. D., and Abeliovich, A. (2002) Mol. Cell 9, 31–44
29. Angenstein, F., Evans, A. M., Settlage, R. E., Moran, T. S., Ling, C. S., Kintsova, A. Y., Shabanowitz, J., Hunt, D. F., and Greenough, W. T. (2002) J. Neurosci. 22, 8827–8844
30. Alfa, C., Fantes, P., Hyams, J., McLeod, M., and Warbrick, E. (1993) Experiments with Fission Yeast, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
31. Sambrook, J., and Russell, D. W. (2000) Molecular Cloning: A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
32. Greenwald, B., and Shatzkin, A. J. (1979) Eur. J. Biochem. 94, 41–50
33. Atchison, J. D., and Rout, M. P. (2000) J. Cell Biol. 151, F23–F26
34. Palmer, E., Wilhelm, J. M., and Sherman, F. (1979) J. Mol. Biol. 128, 167–175
35. Jinezuez, A., and Vazquez, J. R. (1999) Biochem. 38, 2311–2316
36. Ashe, M. P., De Long, S. K., and Sachs, A. B. (2000) Mol. Cell Biol. 20, 833–848
37. Naranda, T., Kainuma, M., MacMillan, S. E., and Hershey, J. W. (1991) Mol. Cell. Biol. 17, 145–153
38. Eisinger, D. P., Dick, P. A., and Trumpower, B. L. (1997) Mol. Biol. Cell. 17, 5136–5145
39. Eisinger, D. P., Dick, P. A., Denke, E., and Trumpower, B. L. (1997) Mol. Cell. Biol. 17, 5136–5145
49128

Cpc2 Is a Ribosome-associated Protein

40. Edskes, H. K., Ohtake, Y., and Wickner, R. B. (1998) J. Biol. Chem. 273, 28912–28920

41. Thomas, D., and Surdin-Kerjan, Y. (1997) Microbiol. Mol. Biol. Rev. 61, 503–522

42. Schweingruber, A. M., Hilti, N., Edenharter, E., and Schweingruber, M. E. (1998) J. Bacteriol. 180, 6338–6341

43. Gross, T., and Kaufer, N. F. (1998) Nucleic Acids Res. 26, 3319–3322

44. Wood, V., Gwilliam, R., Rajandream, M. A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J., Baker, S., Basham, D., Bowman, S., Brooks, K., Brown, D., Brown, S., Chillingworth, T., Churcher, C., Collins, M., Connor, R., Cronin, A., Davis, P., Feltwell, T., Fraser, A., Gentles, S., Goble, A., Hamlin, N., Harris, D., Hidalgo, J., Hodgson, G., Helroyd, S., Hornaby, T., Howarth, S., Huckle, E. J., Hunt, S., Jagels, K., James, K., Jones, L., Jones, M., Leather, S., Mclnair, S., Meehan, P., Meule, S., Mungall, K., Murphy, L., Niblett, D., Odell, C., Oliver, K., O’Neill, S., Pearson, D., Quail, M. A., Rabbinowitsch, E., Rutherford, K., Rutter, S., Saunders, D., Seeger, K., Sharp, S., Skelton, J., Simmonds, M., Squares, R., Stevens, K., Taylor, K., Taylor, R. G., Tivey, A., Walsh, S., Warren, T., Whitehead, S., Woodward, J., Velek, G., Aert, R., Robben, J., Gryn, B., Wentrup, I., Vanstreels, E., Rieger, M., Schafer, M., Muller-Auer, S., Gabel, C., Fuchs, M., Fritze, C., Holzer, E., Moestl, D., Hilbert, H., Borzym, K., Langer, L., Beck, A., Lehrach, H., Reinhardt, R., Pohl, T. M., Eger, P., Zimmermann, W., Wedler, H., Wambutt, R., Portallo, B., Goffeau, A., Cadieu, E., Dreano, S., Gleux, S., Lelaure, V., Motter, S., Malbert, F., Aves, S. J., Xiang, Z., Hunt, C., Moore, K., Hurst, S. M., Lucas, M., Rochet, M., Gaillard, C., Tallada, V. A., Garzon, A., Thode, G., Daga, R. R., Cruzado, L., Jimenez, J., Sanchez, M., Del Rey, F., Benito, J., Dominguez, A., Revuelta, J. L., Moreno, S., Armstrong, J., Forsburg, S. L., Cerrutti, L., Lowe, T., Mccombe, W. B., Paulsen, I., Potashkin, J., Shpakovski, G. V., Ussery, D., Barrett, B. G., and Nurse, P. (2002) Nature 415, 871–880

45. Naora, H. (1999) Immunol. Cell. Biol. 77, 197–205

46. Ruggero, D., and Pandolfi, P. P. (2003) Nat. Rev. Cancer 3, 179–192

47. Garrels, J. I., Mclaughlin, C. S., Warner, J. R., Futcher, B., Latter, G. L., Kehn, R., Schwender, B., Volpe, T., Anderson, D. S., Mesquita-Fuentes, R., and Payne, W. E. (1997) Electrophoresis 18, 1347–1360

48. Warner, J. R. (1999) Trends Biochem. Sci. 24, 437–440

49. Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Storz, G., Botstein, D., and Brown, P. O. (2000) Mol. Biol. Cell 11, 4241–4257

50. Melese, T., and Xia, Z. (1995) Curr. Opin. Cell. Biol. 7, 319–324

51. Scheur, U., and Hock, R. (1999) Curr. Opin. Cell. Biol. 11, 385–390

52. Marten, M. J., Vazquez De Aldana, C. R., Qua, H., Chakraborty, K., and Hinnebusch, A. G. (1997) Mol. Cell. Biol. 17, 4474–4489

53. Maita, T., and Maitra, U. (1997) J. Biol. Chem. 272, 18333–18340

54. McCarthry, J. E. (1998) Microbiol. Mol. Biol. Rev. 62, 1492–1553

55. Bettany, A. J., Moore, P. A., Cafferkey, B., Bell, L. D., Goode, A. R., Carter, B. L., and Brown, A. J. (1989) Yeast 5, 187–198

56. Oliveira, C. C., Van Den Heuvel, J. J., and McCarthry, J. E. (1993) Mol. Microbiol. 9, 521–532

57. Daga, R. R., and Jimenez, J. (1999) J. Cell Sci. 112, 3137–3146

58. Grallert, B., Kearsley, S. E., Lenhard, M., Carlson, C. R., Nurse, P., Boye, E., and Labib, K. (2000) J. Cell Sci. 113, 1447–1458
Cpc2/RACK1 Is a Ribosome-associated Protein That Promotes Efficient Translation in *Schizosaccharomyces pombe*

Boris Shor, Jimmy Calaycay, Julie Rushbrook and Maureen McLeod

*J. Biol. Chem. 2003, 278:49119-49128.*

doi: 10.1074/jbc.M303968200 originally published online September 12, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303968200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 56 references, 26 of which can be accessed free at
http://www.jbc.org/content/278/49/49119.full.html#ref-list-1