Protein phosphatase 2A (PP2A) is an essential enzyme which is present in all eukaryotic cells. PP2A has been implicated in regulating various metabolic processes and also in the control of cell cycle progression. In the yeast Saccharomyces cerevisiae, the catalytic subunit of PP2A is encoded by two duplicated genes, PPH21 and PPH22. A third related gene, PPH3, also contributes some PP2A activity. We have used a yeast strain in which a single functional PP2A gene is expressed from a regulated promoter to screen for high copy number suppressors of PP2A depletion. A new gene was cloned, PAM1 (PP2A multicopy suppressor), which in high copy number can bypass the need for a PP2A catalytic subunit. The PAM1 gene encodes a hydrophilic 93-kDa protein that contains two coiled coil motifs and has a highly basic C-terminal tail. High level overexpression of PAM1 inhibits growth and induces a filamentous phenotype.

Many cellular processes are regulated by protein kinases that phosphorylate target proteins on serine and threonine residues (Hunter, 1987). However, eukaryotic cells also contain a number of serine/threonine-specific protein phosphatases that reverse the action of these kinases. Based on chemical properties, these enzymes have been classified into type 1, 2A, 2B, and 2C protein phosphatases (Cohen, 1989). Sequence analysis has shown that the catalytic subunits of the type 1, 2A, and 2B phosphatases are related proteins (Cohen and Cohen, 1989). In particular, protein phosphatase (PP) 1 and 2A (PP2A) are highly similar in sequence.

PP2A is a heterotrimeric enzyme with two regulatory subunits, A and B, and a catalytic C subunit (Cohen, 1989). The catalytic subunit belongs to a protein family that also includes several PP2A-related enzymes such as PPH1/SIT4 (Arndt et al., 1989), PPH3 (Ronne et al., 1991), PX and PPV (Cohen et al., 1990). This protein family is in turn more distantly related to the catalytic subunit of PP1 (Cohen and Cohen, 1989). In the budding yeast Saccharomyces cerevisiae, the A and B subunits of PP2A are encoded by the TPD3 and CDC55 genes (Healy et al., 1991; van Zyl et al., 1992), while the catalytic subunit is encoded by two duplicated genes, PPH21 and PPH22 (Sneddon and Stark, 1990; Ronne et al., 1991). The PP2A-related gene PPH3 can also provide some PP2A activity in the absence of PPH21 and PPH22 (Ronne et al., 1991). It is not known if the Pph3 protein interacts with Tpd3 or Cdc55, nor is it clear that Pph3 has the same in vivo function as PP2A. Yet another PP2A-related enzyme in budding yeast is PPH1/SIT4, which is essential in some yeast strains but dispensable in others (Sutton et al., 1991). A SIT4 homolog in fission yeast, ppe1, seems to be involved in regulating pim1, a homolog of the human RCC1 protein (Matsumoto and Beach, 1993; Shimanauki et al., 1993).

The sequence similarity between PP1 and PP2A is reflected in overlapping substrate specificities. Thus, both enzymes can dephosphorylate a number of proteins involved in different metabolic processes. However, the two enzymes differ in intracellular location. PP1 is largely particle bound, being associated with glycogen particles, actomyosin complexes, and ribosomes (Cohen, 1989). It is therefore thought that PP1 regulates glycogen metabolism, muscle contractility, and protein synthesis, conclusions which in part are supported by biochemical and genetic data (Cohen, 1989). Moreover, PP1 is enriched in the nucleus (Ohkura et al., 1989). In contrast, PP2A occurs as a soluble complex in the cytosol and is excluded from the nucleus (Kinoshita et al., 1993). It is thought to regulate cytosolic processes such as glycolysis, gluconeogenesis, and amino acid catabolism (Cohen, 1989). However, there is evidence that PP2A also contributes to the control of glycogen metabolism (Penf et al., 1991), and a minor role for PP2A in other processes that are regulated by PP1 cannot be excluded (Cohen, 1989).

Genetic data suggest that PP1 and PP2A are involved in regulating cell cycle progression. PP1 is required for completion of mitosis in fungal and insect cells (reviewed by Sneddon and Stark, 1991). PP2A is a negative regulator of the cdc2 kinase in amphibian and mammalian cells (Félix et al., 1990; Yamashita et al., 1990; Lee et al., 1991), and modulation of PP2A activity plays an important role in transformation by the polyoma and SV40 viruses (reviewed by Mumbery and Walter, 1991). Moreover, PP2A inhibits cyclin degradation in amphibian cells (Lorca et al., 1991), and the PP2A-related Sit4 enzyme is required for cyclin accumulation in yeast (Fernandez-Sarabia et al., 1992). In addition to its proposed roles in metabolic regulation and cell cycle control, there is also some evidence that PP2A regulates transcription in mammalian cells (Alberts et al., 1993; Wadzinski et al., 1993) and that it is involved in tissue pattern formation in Drosophila (Uemura et al., 1993).

Partial loss of PP2A activity in fission yeast is reported to cause premature mitosis, while complete loss is lethal (Kinoshita et al., 1990; 1993). Moreover, a disruption of the major PP2A gene in fission yeast, ppa2, partially suppressed a thermosensitive mutation in the cdc25 tyrosine phosphatase (Kinoshita et al., 1993). The latter protein is required to activate the cdc2 kinase at the start of mitosis, and it was therefore proposed that PP2A may inhibit cdc2 through negative control of cdc25 activity (Kinoshita et al., 1993). PP2A is essential also in budding yeast (Sneddon and Strak, 1990; Ronne et al., 1991). However, PP2A-depleted cells do not arrest in a distinct phase.
of the cell cycle. Instead, they acquire an abnormal bud shape, suggesting a role for PP2A in cellular morphogenesis (Ronne et al., 1991). The two regulatory subunits of PP2A, Cdc55 and Tpd3, are not essential, but loss of either protein causes a cold-sensitive cell cycle arrest that seems to involve inhibition of cytokinines (Healy et al., 1991; van Zyl et al., 1992).

We have found that simultaneous loss of PP2H1, PP2H22, and PP2H5 is lethal in yeast, which shows that some PP2A catalytic activity is required for growth under normal conditions (Ronnc et al., 1991). We have now used this lethality to screen for genes which in high copy number can rescue cells that lack PP2A. One such gene, PAM1, is described below. It encodes a 93-kDa hydrophilic protein with two coiled coil motifs and a highly basic C terminus. Overexpression of PAM1 inhibits growth and induces a filamentous phenotype.

EXPERIMENTAL PROCEDURES

Yeast Strains—The yeast strains are listed in Table I. The pam1-51:LEU2 disruption was made by cloning the LEU2 HpaI-SalI fragment between the PAM1 and BglII sites in PAM1, and pam1-51-LEU2 by deleting the 8-kb fragment between the BglII and 5' PstI sites. H109 has a 660-bp EcoRI-Sau3AI fragment carrying the GAL1 promoter inserted between the BglII site and 3' HincII site in the LEU2 5' region. It was made using a LEU2 promoter substitution system that we developed for cloning of heterologous transcription factors in yeast (Ellerstrom et al., 1992). Briefly, a promoter is cloned between the two BglII sites in pHR35 (see below), in place of the LEU2 upstream activating sequence (Martinez-Arias et al., 1984). The plasmid is then cut with Kpn1 to target integration into the LEU2 gene and transformed into strain U457. Integration events (popins) are selected on uracil-less plates. The resulting strain has two tandem copies of the LEU2 promoter which are separated by the URA3 marker. One copy is the wild type chromosomal promoter, and one copy is the mutant promoter from the plasmid. Direct repeat recombination events that lose the URA3 marker and one copy of the promoter (popouts) are then selected on 5-fluoro-orotic acid plates (Boeke et al., 1984). Two types of popouts occur: those that restore the wild type LEU2 gene, and those that leave a mutant promoter on the chromosome. The two events are easily distinguished in U457, which carries the SUP33-a suppressor tRNA gene on the fragment that is deleted in the mutant promoter. Correct popouts can therefore be identified through loss of SUP53-a mediated co-segregational loss of the plasmid and the ability to grow without galactose. Plasmids were rescued from the cells and their ability to suppress PP2A depletion was confirmed by retransformation into H328. In addition to multiple copies of the PP2H genes, we cloned one new gene which we call PAM1 for PP2A Multicopy suppressor. The degree of suppression provided by PAM1 is much weaker than that obtained with PP2H21 or PP2H22, but comparable to that obtained with plasmids containing the PP2H3 gene (Fig. 1A).

Plasmids—The URA3 LEU2-2-μm plasmid pH81 and the genomic library made in this vector have been described (Nehlin et al., 1989). The LEU2 promoter substitution vector pH95 was made in three steps. First, a 1920-bp Fad1-EcoRI fragment of YEp13 with the 5' half of the LEU2 gene and adjacent upstream DNA was cloned between the Fad1 and EcoRI sites of pUC18, generating pH92. This plasmid was cleaved with HincII, ligated to BglII linkers, cleaved with BglII, and religated. The resulting plasmid, pH33, has a deletion of 780 bp spanning the LEU2 upstream activating sequence (Martinez-Arias et al., 1984), the sup53 gene, and the yeast 5' to LEU2. In the resulting pH33 has two tandem copies of the BglII linker. The URA3 HindIII fragment was then cloned into the HindIII site of pH93, to generate pH35. Plasmids pHG223, pHG227, and pHG228 were made by cloning different inserts into the BamHI site of pH81 (Nehlin et al., 1989). Thus, pHG223 contains a 3300-bp NdeI fragment carrying the PAM1 gene (Fig. 2), pHG227 a 2700-bp SalI fragment carrying the PP2H3 gene (Ronne et al., 1991), and plasmid pHG228 a 2200-bp XhoI-XbaI fragment carrying the PP2H21 gene (Ronne et al., 1991). Plasmid pHG246 and pHG246 were generated from plasmids pHG223 and PAM1 by ClaI-SalI digestion and religation, thereby removing the LEU2-d marker from these plasmids. Plasmid pHG238, in which PAM1 is expressed from the GAL1-TPK2 hybrid promoter, was made by cloning a 4500-bp NruI-SpeI fragment carrying PAM1 into the BamHI site of pH92 (Ronne et al., 1991).

RESULTS

Cloning of the PAM1 Gene—In an attempt to analyze the cellular function of PP2A, we have screened the yeast genome for genes that in high copy number can suppress the growth defect caused by loss of the PP2A catalytic subunit. For this, we used the previously described yeast strain H328 (Ronne et al., 1991). This strain is disrupted for PP2H21, one of two duplicated genes encoding PP2A, whereas the other gene, PP2H22, is expressed from the galactose-induced GAL1 promoter. H328 is also disrupted for PP2H3, a third more distantly related gene which provides some residual PP2A activity in cells lacking both PP2H21 and PP2H22 (Ronnc et al., 1991). H328 is unable to grow on other carbon sources than galactose, since it is dependent on expression of PP2H22 from the GAL1 promoter to provide the PP2A activity required for survival.

H328 cells were transformed with a yeast genomic library made in the high copy number vector pH81 (Nehlin et al., 1989). Transformants were selected on uracil-less galactose plates and then replicated to glucose and raffinose plates. Colonies that could grow on glucose or raffinose were tested for co-segregational loss of the plasmid and the ability to grow without galactose. Plasmids were rescued from the cells and their ability to suppress PP2A depletion was confirmed by retransformation into H328. In addition to multiple copies of the PP2H genes, we cloned one new gene which we call PAM1 for PP2A Multicopy suppressor. The degree of suppression provided by PAM1 is much weaker than that obtained with PP2H21 or PP2H22, but comparable to that obtained with plasmids containing the PP2H3 gene (Fig. 1A).

Genetic Mapping of the PAM1 Locus—The PAM1 gene was located to chromosome IV in a Southern blot of yeast DNA separated on a contour-clamped homogenous electric field gel (Chu et al., 1986). We proceeded to map the gene by tetrad analysis, using yeast strains in which the PAM1 locus was genetically marked by one-step gene disruptions (see below). We found that the PAM1 gene is on the right arm of chromosome IV, 43 cM from pet14 and 61 centiMorgans from trp4 (Table II). The sequence
containing different plasmids were tested and the ability of glucose. Single colonies were picked from the left has lost both the URA3 marker on the PAM1 plasmid and the ability to grow well on 2% glucose.

**Fig. 1. Suppression of PP2A deficiency by PAM1.** A, H328 cells containing different plasmids were tested for growth on glucose, as previously described (41). B, cosegregational loss of a PAM1 plasmid and the ability of pph21 pph22 pph3 cells to grow on 2% glucose. Triple disrupted cells containing the plasmid were streak purified on 8% glucose. Single colonies were picked to an 8% glucose plate and replicated to different media. The third colony from the left has lost both the URA3 marker on the PAM1 plasmid and the ability to grow well on 2% glucose.

**Table II**

| Genetic interval | Parental diphye | Non-parental diphye | Tetratype | x |
|------------------|----------------|---------------------|-----------|---|
| PAM1-aro1        | 40             | 23                  | 136       | 100.7 |
| PAM1-pet14       | 77             | 8                   | 113       | 42.7 |
| PAM1-trp4        | 54             | 14                  | 132       | 60.9 |
| aro1-pet14       | 64             | 3                   | 128       | 38.9 |
| pet14-trp4       | 40             | 22                  | 134       | 91.3 |
| ade1-de8        | 62             | 1                   | 128       | 36.2 |

Analysis (see below) revealed that the 3′ end of PAM1 is located 2 kb 5′ to the CHL4 gene (Kouprina et al., 1993b). This gene, which is also known as CTF17 (Spencer et al., 1990), is involved in mitotic chromosome segregation. It is also known to be located on the right arm of chromosome IV, in the vicinity of the SUP2 gene (Kouprina et al., 1993a). This position is in good agreement with our mapping of the PAM1 locus.

**Sequence of the PAM1 Gene**—The restriction map of the pPAM1 plasmid is shown in Fig. 2, and the nucleotide sequence of the PAM1 gene and its encoded protein in Fig. 3. Deletion mapping revealed that the suppressing activity is mediated by an open reading frame of 830 codons. A plasmid lacking the last 150 codons of PAM1 was still partially active as a suppressor of PP2A depletion. To prove that the activity is mediated by the encoded protein rather than by the plasmid DNA, we also made a frame-shift mutation by filling in the AcrII site in the 5′ part of PAM1. This plasmid was without activity, which confirms that suppression is mediated by the PAM1 protein. There are no sites in the PAM1 promoter that match any of the known consensus sequences recognized by yeast transcription factors (Verdier, 1990). However, some regulatory elements seem to be present, since deletions in the promoter caused a partial loss of the suppressor activity (Fig. 2).

**Structure of the PAM1 Protein**—The PAM1 open reading frame predicts a hydrophilic protein with an apparent molecular mass of 93 kDa. A computer search of the EMBL data bank (Release 34) and the PIR and SWISS protein data bases (Releases 35 and 24) using the FASTA program (Pearson and Lipman, 1988) did not reveal any strong similarity to previously known proteins. However, a number of entries with optimized FASTA scores between 90 and 110 were found, including dystrophin, the Drosophila BicD protein, laminins, myosins, and various intermediate filament proteins. These proteins all contain coiled coil motifs (Cohen and Parry, 1986; Lupas et al., 1991), and the sequence similarities are due to the presence of two such motifs in the PAM1 protein. The two coiled coil motifs are located in the middle of the protein and are separated by 80 amino acid residues. The BicD protein (Wharton and Struhl, 1989), which showed the highest local similarity in a FASTA comparison to PAM1, has a similar pair of coiled coil motifs separated by a spacer region (Fig. 4). BicD is a maternal factor which is involved in establishing anterior-posterior polarity in the Drosophila oocyte. However, the two proteins do not have the same overall organization, since BicD contains several other coiled coil motifs that have no counterparts in PAM1. The second motif in PAM1 has an unusual structure: it contains two staggered leucine heptad repeats and is highly charged (18 of 34 residues). Between the two coiled coil motifs, there is a possible target site for the CAMP-dependent protein kinase (Krebs and Beavo, 1979). The second motif is followed by a polyglutamine tract. Such tracts are found in many proteins and have been proposed to act as flexible hinges that separate different domains (Wootton and Drummond, 1989). A third notable feature in the PAM1 sequence is its highly basic C terminus. Of the 16 C-terminal residues in PAM1, 10 are basic, and there is a stretch of 5 consecutive lysines.

**Disruption of the PAM1 Gene**—To investigate the function of
PAM1, we carried out one-step disruptions of the gene (Rothstein, 1983). Two disruptions were tested: pam1-62 which deletes the central part of the protein including the two coiled coil motifs and pam1-61 which deletes the start codon and most of the open reading frame. The resulting yeast strains were viable and showed no obvious defects in vegetative growth, mating, or in the ability to use different carbon sources. Nor was thermotolerance, survival of nitrogen starvation, or the ability to study the expression of the PAM1 gene under most conditions, a conclusion which would be consistent with the absence of known regulatory sequence motifs in the gene which is expressed under most conditions, a conclusion which would be consistent with the absence of known regulatory sequence motifs in the gene which is expressed under most conditions, a conclusion which would be consistent with the absence of known regulatory sequence motifs in the gene which is expressed under most conditions, a conclusion which would be consistent with the absence of known regulatory sequence motifs in the gene which is expressed under most conditions, a conclusion which would be consistent with the absence of known regulatory sequence motifs in the gene which is expressed under most conditions, a conclusion which would be consistent with the absence of known regulatory sequence motifs in the gene which is expressed under most conditions, a conclusion which would be consistent with the absence of known regulatory sequence motifs in the gene which is expressed under most conditions. Moreover, while some differences in the amount of mRNA from pam1-62 strain H405, in which the entire probe has been deleted. We were unable to detect any signal under these conditions (data not shown). This argues against the presence of a second gene closely related to PAM1 but does not rule out the existence of more distant related genes.

Expression of the PAM1 mRNA—One possible reason for the apparent lack of a disruption phenotype would be that PAM1 is expressed only under certain conditions, being without function during normal vegetative growth. We therefore proceeded to study the expression of PAM1 in Northern blots with RNA from cells grown on different carbon sources. We found that PAM1 is expressed at a fairly high level in vegetative cells (Fig. 3). Moreover, while some differences in the amount of mRNA from pam1-62 strain H405, in which the entire probe has been deleted. We were unable to detect any signal under these conditions (data not shown). This argues against the presence of a second gene closely related to PAM1 but does not rule out the existence of more distant related genes. However, this conclusion would be consistent with the absence of known regulatory sequence motifs in the PAM1 promoter.

PAM1 Does Not Activate the GAL1 Promoter—A possible mechanism for suppression would be that PAM1 allows expression of the GAL1 promoter on other carbon sources than galactose. To investigate this possibility, we tested the PAM1 gene in two genetic systems. First, we used yeast strain H172, in which the TP2 gene is expressed from the GAL1 promoter (Nehlin et al., 1987; Ronne et al., 1991). We therefore proceeded to investigate whether a second gene closely related to PAM1 is present in the yeast genome, using low stringency hybridizations with a PAM1 probe. To increase our ability to detect a weak signal, we used DNA from the pam1-62 strain H405, in which the entire probe has been deleted. We were unable to detect any signal under these conditions (data not shown). This argues against the presence of a second gene closely related to PAM1 but does not rule out the existence of more distant related genes.
Yeast PAM1 Gene Suppresses Loss of Protein Phosphatase 2A

519) be detected in this system. To permit us to a 1400-bp AccI probe from the tose, since expression of kills the cell. We found that the two al., plasmids from the sources than galactose (data not shown). This indicates that the galactose for growth in the absence of leucine. Due to the complete absence of background growth, very low levels of expression can be obtained from cells containing plasmids. The control vector had no such effect. Moreover, when pph21 pph22 pph3 cells containing the pAM1 plasmid were streak purified on 8% glucose plates, the ability to grow well on 2% glucose co-segregated with the URA3 marker on the plasmid (Fig. 1B). This confirms that the enhanced growth of these cells on 2% glucose is due to the PAM1 plasmid and not to unlinked suppressor mutations.

High Level Overproduction of PAM1 Inhibits Growth—We proceeded to test the effect of high level expression of the PAM1 gene. This can be achieved by growing the cells in the absence of leucine, since the pH81 vector has a defective LEU2 marker that requires a very high copy number (Erhart and Hollenberg, 1983; Nehlin and Ronne, 1990). We found that the PAM1 gene inhibits growth under these conditions. The growth inhibiting activity was mapped to the PAM1 open reading frame, using the same deletions and frame shifts that we used to map the PP2A suppressor activity (Fig. 2). However, the growth inhibition was more sensitive to some of the deletions. Thus, a deletion of the 150 C-terminal codons abolished growth inhibition, as did a deletion down to base -382 in the promoter. A further deletion down to base -143 partially restored growth inhibition. This suggests that a high level of expression is necessary to achieve growth inhibition. This is consistent with the fact that moderate overexpression of PAM1, which suppresses PP2A depletion, does not inhibit growth.

PAM1 Overproducing Cells Acquire a Filamentous Phenotype—We next wanted to examine the terminal phenotype of cells that cease to grow due to PAM1 overproduction. A problem with LEU2 selection is that most cells will arrest due to leucine starvation when they are transferred to leucine-less media, since they have too few copies of the plasmid. These cells outnumber those that have enough plasmids to support growth, but instead cease to grow due to overexpression of the insert. To avoid this problem, we first cloned PAM1 into the LEU2-d vector pJN92 (Ronne et al., 1991), where the insert is expressed from the galactose-induced GAL1 promoter. Cells containing this plasmid were then grown in leucine-less glycercol-lactate media, to select a high plasmid copy number in all cells (Ronne et al., 1991). Finally, expression of PAM1 was induced by adding 2% galactose to the culture, and samples of the cells were removed for microscopy at regular intervals.

Interestingly, we found that PAM1 overproduction induces a filamentous phenotype which resembles the pseudohyphae that are formed during nitrogen starvation (Gimeno et al., 1992). Thus, branched filaments are formed, which contain a mixture of elongated and spheroidal cells. This phenotype ap-

al., 1992). This strain is conditional lethal for growth on galactose, since expression of TPK2 from the GAL1 promoter rapidly kills the cell. We found that the two PAM1 plasmids pPAM1 and pHGZ23 failed to prevent growth of H172 on other carbon sources than galactose (data not shown). This indicates that the GAL1 promoter is not improperly turned on under these conditions.

In a second and more sensitive test, we used yeast strain H109 (Table 1). This strain has the chromosomal LEU2 gene expressed from the GAL1 promoter, and is therefore dependent on galactose for growth in the absence of leucine. Due to the complete absence of background growth, very low levels of expression can be detected in this system. To permit us to assay LEU2 expression, we used two PAM1 plasmids, pHGZ45 and pHGZ46, that were obtained from pHGZ23 and pPAM1 by removing the LEU2-d marker on these plasmids. We found that these PAM1 plasmids failed to permit any growth of H109 in the absence of galactose and leucine (data not shown). This shows that the GAL1 promoter is still tightly regulated in the presence of the PAM1 plasmids. It should be noted that this assay would select for cells with a high plasmid copy number, if this was necessary to derepress the GAL1 promoter. However, prolonged incubation of the plates failed to generate prototrophic papillae, which would have appeared if such selection took place.

PAM1 Suppresses Complete Loss of PP2A—To verify that the PAM1 plasmid is a bona fide suppressor of PP2A depletion, we proceeded to test it in a strain that is disrupted for all three PPH genes, rather than having one of them expressed from the GAL1 promoter. Such triple disrupted strains that are completely deficient for PP2A activity can be constructed by genetic crosses, but they fail to grow under normal conditions. To solve this prob-

FIG. 4. Alignment of coiled coil motifs in PAM1 (residues 342-519) BicD (residues 313-485). Identical amino acid residues are enclosed within boxes.

FIG. 5. Northern blot with mRNA from cells grown in rich media containing different carbon sources. The filter was hybridized to a 1400-bp AccI probe from the PAM1 gene.
We have cloned a gene, PAM1, which in high copy number can suppress the loss of PP2A. There are several possible mechanisms of suppression that have to be considered. A protein phosphatase could suppress the PP2A deficiency directly through its enzyme activity. An example of this is suppression by the PPH3 gene (Fig. 1). Paml shows no sequence similarity to known protein phosphatases, so it is unlikely that Paml is a phosphatase. However, it is possible that Paml could activate a PP2A-related phosphatase, such as Sit4 or Pph3, which in turn suppresses the PP2A deficiency. This could involve control of gene expression or regulation at the protein level of enzyme activity, specificity, or intracellular targeting. Paml shows no similarity to the PP2A regulatory subunits or to other proteins implicated in phosphatase function (Luke et al., 1991; Sutton et al., 1991; Wilson et al., 1991). The C terminus of Paml contains a stretch of polylysine (Fig. 3). Polylysine stimulates PP2A activity against some substrates more than 200-fold (Pelech and Cohen, 1985), and it has been proposed that such basic peptides could mimic a physiological activator of PP2A (8). However, the fact that a Paml protein lacking its 150 C-terminal residues is still partially active as a suppressor (Fig. 2) argues against a crucial role for the polylysine stretch. The fact that Paml functions in a apph3 strain shows that suppression does not involve activation of Pph3. The other PP2A-related enzyme in yeast, Sit4, is essential in the W303-1A background (Sutton et al., 1991), and we were therefore unable to test its role in suppression.

Alternatively, Paml could inhibit a kinase whose activity is lethal unless balanced by PP2A. An obvious candidate would be the cAMP-dependent kinase, since overexpression of this kinase is lethal in yeast (Nehlin et al., 1989). However, PAML does not suppress the galactose-dependent lethality of the GAL1:TPK2 fusion, nor does it modify the pleiotropic phenotype of bcyI cells, which have a deregulated kinase (data not shown). This suggests that Paml does not inhibit the cAMP-dependent kinase. It is still possible that Paml could act by inhibiting another protein kinase which remains to be identified.

Finally, it is possible that Paml does not affect protein phosphorylation, but instead acts directly on a system that is regulated by PP2A, preventing the lethal effect of PP2A depletion. A problem with testing this hypothesis is that we do not know why PP2A is required for growth. In addition to its proposed roles in metabolic regulation (Cohen, 1989) and gene expression (Alberts, 1993; Wadzinski et al., 1993), PP2A has also been implicated in control of cell shape (Ronne et al., 1991) and gene expression (Alberts, 1993; Wadzinski et al., 1993). If the essential function of PP2A is to inhibit the cdc2 kinase, one would expect a mitotic catastrophe to occur in PP2A-depleted cells (Russell and Nurse, 1987). However, PP2A-depleted budding yeast cells do not arrest in mitosis or in any other specific stage of the cell cycle (Ronne et al., 1991).
Similarly, depletion of PP2A in fission yeast by disruption of ppa2 and exposure to okadaic acid failed to cause a mitotic catastrophe (Kinosita et al., 1993). Instead, binucleate cells accumulated, suggesting a defect in cytokinesis.

Interestingly, we found that the need for PP2A is partially relieved by high amounts of glucose (Fig. 1B). This raises the possibility that the essential function of PP2A could be in the control of carbon metabolism. PP2A regulates several glycolytic and gluconeogenic enzymes (Cohen, 1989), and the PP2A inhibitor okadaic acid rapidly stimulates gluconeogenesis in mammalian cells (Haystead et al., 1989). It is conceivable that loss of PP2A could activate gluconeogenesis also in yeast, which would cause futile cycling of intermediates in cells grown on glucose. Such a condition is expected to be partially relieved by high amounts of glucose. However, it is also possible that the survival of PP2A-depleted cells on high glucose is due to a generally enhanced growth under these conditions.

The aberrant bud shape of PP2A-depleted cells and the elongated shape of PP2A overproducing cells suggests a possible role for PP2A in controlling cell shape (Ronne et al., 1991). In this context, it is interesting that Paml shows some limited similarity to cytoskeletal coiled coil proteins such as myosin and BicD in that it activates the CAMP-dependent kinase (Gimeno et al., 1992), and it is possible that the elongation is the first visible step in pseudohyphal growth (Wharton and Struhl, 1989). The fact that Paml overexpression causes a filamentous phenotype (Fig. 6) could indicate a similar role for Paml in the control of cell polarity in yeast. However, it should be emphasized that Paml differs from classical coiled coil proteins such as myosin and BicD in that it does not necessarily reflect the normal function of Paml.

PP2A is also involved in pseudohyphal growth, which is stimulated by the ras2va119 allele (Wharton and Struhl, 1989). The fact that Paml overexpression in fission yeast by disruption of ppa2 and exposure to okadaic acid failed to cause a mitotic catastrophe (Kinosita et al., 1993). Instead, binucleate cells accumulated, suggesting a defect in cytokinesis. Interestingly, we found that the need for PP2A is partially relieved by high amounts of glucose (Fig. 1B). This raises the possibility that the essential function of PP2A could be in the control of carbon metabolism. PP2A regulates several glycolytic and gluconeogenic enzymes (Cohen, 1989), and the PP2A inhibitor okadaic acid rapidly stimulates gluconeogenesis in mammalian cells (Haystead et al., 1989). It is conceivable that loss of PP2A could activate gluconeogenesis also in yeast, which would cause futile cycling of intermediates in cells grown on glucose. Such a condition is expected to be partially relieved by high amounts of glucose. However, it is also possible that the survival of PP2A-depleted cells on high glucose is due to a generally enhanced growth under these conditions.

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