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Amino Acids Induce Expression of *BAP2*, a Branched-Chain Amino Acid Permease Gene in *Saccharomyces cerevisiae*

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Branched-chain amino acid uptake in *Saccharomyces cerevisiae* is mediated by at least three transport systems: the general amino acid permease Gap1p, the branched-chain amino acid permease Bap2p, and one or more so far unknown permeases. Regulation of the transcription of *BAP2* is mainly subject to the presence of certain amino acids in the medium. The level of transcription is low during growth on a minimal medium with proline as the sole nitrogen source. As assayed with a *lacZ* fusion, the level of transcription is slightly higher (3-fold) on a minimal medium with ammonium ions as a nitrogen source, and transcription is induced about 20-fold by addition of leucine (0.2 mM). As little as 10 μM leucine causes a fivefold induction. Addition of L-leucine to minimal proline medium, on the other hand, has no effect on *BAP2* transcription. The two known permeases for transport of branched-chain amino acids, Gap1p and Bap2p, are thus not active at the same time. The *BAP2* promoter contains one or two putative Gcn4p binding sites and one putative Leu3p binding site. None of the three is needed for induction by leucine. Induction of *BAP2* transcription by leucine is accompanied by an increase in branched-chain amino acid uptake. This elevation is interpreted to be partly the result of an increased level of the Bap2p permease in the plasma membrane, because deletion of *BAP2* slightly decreases the induction of uptake. There is still a leucine-inducible increase in branched-chain amino acid uptake in a Δ*gap1* Δ*bap2* strain, indicating that *BAP2* shares leucine induction with at least one remaining branched-chain amino acid-transporting permease.

A number of permeases in *Saccharomyces cerevisiae* are known to mediate the active transport of amino acids across the plasma membrane. Most of them are more or less specific for one or a few related L-amino acids and exhibit different properties with respect to substrate affinity, specificity, capacity, and regulation (for review, see references 5, 14, and 17).

So far merely one system, the general amino acid permease Gap1p (20), is known to transport all common L-amino acids; it also transports several D-amino acids and related compounds, such as ornithine, citrulline, and toxic amino acid analogs (32).

Gap1p, the high-affinity proline permease Put4p (21), the UEP/DAL5 ureidosuccinate-allantoate permease (28), and the UGA4 GABA permease are subject to the same regulation (14). The synthesis of these permeases is subject to nitrogen-catabolite repression, which operates on the transcription of an array of genes in cells grown in the presence of nitrogen sources such as ammonium ions, asparagine, and glutamine (20, 21, 28).

In addition to the regulation at the transcriptional level, Gap1p is regulated by nitrogen-catabolite inactivation. Gap1p is inactivated, e.g., by addition of ammonium ions to proline-grown cells. This inactivation is dependent on the gene products of *NPI1* and *NPI2* (12), whereas the reactivation process of Gap1p requires the gene product of the *NPR1* gene (13). The *NPR1* gene has been cloned, and the deduced amino acid sequence was found to be homologous to those of protein kinases (30).

Other amino acid transporters, such as the arginine permease Can1p (15), the lysine permease Lyp1p (11), the methionine permease Mtp1p (8), and the histidine permease Hip1p (6), are not affected by the presence of ammonium ions. There are some amino acids, such as alanine and serine, which seem to induce their own uptake (9, 31), while preloading of yeast cells with L-histidine or L-lysine reduces further uptake of these amino acids, probably by feedback inhibition of their specific permeases (6, 24).

As part of our investigations of the uptake of the branched-chain amino acids leucine, isoleucine, and valine in *S. cerevisiae*, we recently reported the identification of a gene, *BAP2*, encoding a branched-chain amino acid permease (10). Deletion of *BAP2* in a Δ*gap1* background reduced leucine and valine uptake about 45% and the isoleucine uptake by about 25%. The uptake of other hydrophobic amino acids, such as L-alanine, L-phenylalanine, and L-methionine, was not affected by the *bap2* deletion (10).

The present report describes the initiation of a study of the regulation of *BAP2. BAP2* transcription was studied by means of Northern (RNA) analysis as well as the determination of *BAP2* promoter-mediated *lacZ* expression. Transcription is modestly affected by the nitrogen source in a way opposite that of *GAP1* and other genes under nitrogen-catabolite repression. In addition, we observed induction by several amino acids, particularly leucine.

MATERIALS AND METHODS

Strains and media. The *S. cerevisiae* strains used in this study were M4054 (*MAT***a** *gal2 ura3 gap1-Δ101*) and M4056 (*MAT***a** *gal2 ura3 gap1-Δ101 bap2-Δ1*) (10) (both strains isogenic to S288C [25]), XK25-3A (*MATα leu3-781 ura3-52*) and XK143-2c (*MATα leu3-781 gcn4-101 ura3-52*) (both strains a generous gift from G. B. Kohlhaw), and F212 (*MAT***a** *gcn4-Δ1 ura3-52 ino1 can1*) (a generous gift from A. K. Hopper).

Yeast media were prepared as described previously (10).

Construction of *BAP2-lacZ* translational fusions. A 1.7-kb region, which contains the entire upstream part and the first 54 bases of the open reading frame

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of *BAP2*, was amplified by PCR with the cloned *Pfu* polymerase according to the manufacturer (Stratagene). The following oligonucleotides were used for the amplification: A, 5′-TGACTCCTCTGGATCCCATGCCTC-3′; and B, 5′-ATC GATATCGgATCcGGAGAAGTTTCC-3′. Oligonucleotide B contains two base exchanges (marked in lowercase) compared with the *BAP2* open reading frame in order to generate a *Bam*HI restriction site which permits the in-frame fusion of the *BAP2* open reading frame to that of *lacZ*. The cloned genomic segment described by Grauslund et al. (10) was used as a template for the amplification. The amplified fragment was inserted, after *Bam*HI restriction, into the single *Bam*HI site in front of *lacZ* of the *ARS-CEN* vector pFN8 (26) to create plasmid pTD14. The proper orientation was checked by restriction analysis.

To measure the influence of two putative Gcn4p binding sites and of a putative Leu3p binding site found in the *BAP2* promoter, the binding sites were inactivated by PCR-based site-directed mutagenesis by the method described by Boles and Miosga (2). Plasmid pTD17 is a derivative of pTD14, with only a 683-bp *BAP2* promoter fragment fused to *lacZ* instead of the 1.7-kb promoter fragment in pTD14. To create pTD17, a 1.3-kb *Hin*dIII fragment was removed from pTD14, thereby deleting the most upstream 1,000 bp of the region containing the *BAP2* promoter and 300 bp of pFN8. The 737-bp *Hin*dIII-*Bam*HI *BAP2* promoter fragment of pTD17 was inserted into pUC18 and pUC19 to create pTD18 and pTD19. The following three mutagenic primers were used: G1, 5′-TACT AGGAAGGGCCCTTCAGCGAG-3′, generating an *Apa*I site and changing the putative Gcn4p binding site at −212 (relative to the start codon) from TGACTC to GGGCCC; G2, 5′-GATGAGTTTGAGGGCCCATCATATAAAC-3′, generating an *Apa*I site and changing the possible Gcn4p binding site at −633 from TTACTC to GGGCCC; and L1, 5′-CATATTTTGTGGGATCAGTACTGTCA TCCACATTCC-3′, generating a *Sca*I site and changing the putative Leu3p binding site at −406 from CCGGAACCGG to TCAGTACTGGT. The conditions for the PCRs were as follows: 20 mM Tris-Cl (pH 7.5), 8 mM $MgCl_2$, 40 μg of bovine serum albumin, 200 μM (each) the four deoxynucleoside triphosphates, 1 μM (each) the two primers, 2.5 U of recombinant *Pfu* polymerase (Stratagene), and 20 to 40 ng of plasmid template DNA in a volume of 100 μl. PCR round 1 was performed with pTD18 as the template, M13 universal primer (Promega, catalog no. Q5391), and one of the mutagenic primers G1, G2, or L1 for 1 cycle at 94°C for 4 min, 42°C for 2 min, and 72°C for 2 min, followed by 24 cycles at 94°C for 1 min, 42°C for 2 min, and 72°C for 2 min, and ending with 2 cycles at 94°C for 1 min, 42°C for 2 min, and 72°C for 4 min. PCR round 2 was performed with pTD19 as the template and 40 ng of one of the three 0.3- to 0.6-kb amplified megaprimer fragments from PCR round 1 for 5 cycles at 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min, and then with addition of M13 universal primer and 23 cycles at 94°C for 1 min, 50°C for 2 min, and 72°C 2 min, followed by 2 cycles at 94°C for 1 min, 42°C for 2 min, and 72°C for 4 min. The 0.73-kb fragments were isolated, cut with *Hin*dIII and *Bam*HI, and substituted for the wild-type sequences in pTD17, thereby generating plasmid pTD21 (putative Leu3p site mutated in the *BAP2-lacZ* fusion), pTD23 (putative Gcn4p site at −212 mutated in the *BAP2-lacZ* fusion), and pTD25 (possible Gcn4p site at −633 mutated in the *BAP2-lacZ* fusion). The mutagenesis was checked by restriction analysis with *Sca*I and *Apa*I.

β-Galactosidase assays. Fresh transformants were restreaked on synthetic complete (SC) medium plates without uracil and grown for 1 or 2 days. A slight modification of a standard protocol (1) was used. Four hundred microliters of each of several overnight cultures with various nitrogen sources was used to inoculate 40 ml of the same medium. The cells, incubated at 30°C and grown to an optical density at 600 nm of 0.8 to 1.1, were harvested and washed in cold water (4°C), resuspended in buffer Z (0.06 M Na_2HPO_4, 0.04 M NaH_2PO_4, 0.01 M KCl, 1 mM $MgSO_4$, 0.05 M β-mercaptoethanol) containing 0.01% Triton X-100, and frozen at −80°C overnight. After the permeabilized cells had been thawed, they were washed with buffer Z and resuspended in 150 μl of buffer Z. The assay was performed by addition of less than 20 μl of cell suspension to a prewarmed mixture of 1 ml of buffer Z and 0.2 ml of *o*-nitrophenyl-β-D-galactoside (4 mg/ml) at 28°C. The reaction was stopped by addition of 0.5 ml of 1 M Na_2CO_3. The cells were spun away, and the A_{420} of the supernatant was determined. Units were calculated as described by Miller (23). At least three independent transformants were assayed for each determination.

Preparation of RNA and Northern analysis. Cultures were grown in the indicated media to an optical density at 600 nm of 0.9 to 1.1. Total RNA was isolated by the method described by Brown (3). Forty micrograms of RNA was loaded onto each lane of a formaldehyde agarose gel, electrophoresed, and blotted onto a Hybond-N membrane and hybridized according to the instructions of the supplier (Amersham). *BAP2* mRNA was probed with the 1,230-bp *Eco*RV-*Sac*I fragment, while actin mRNA was probed with a 530-bp *ACT1-Sty*I fragment. The labelled mRNA bands were visualized and counted on a phosphor screen with version 3.3 of the ImageQuant program (Molecular Dynamics, Sunnyvale, Calif.)

Amino acid uptake. An overnight culture was diluted and grown to a cell density corresponding to an optical density at 600 nm of 0.5 to 0.7 (0.065 to 0.090 mg [dry weight]/ml) on the indicated medium at 30°C. The uptake measurements were performed as described previously (10).

TABLE 1. *BAP2* promoter activity

Strain	Nitrogen source	L-Leucine concn (mM)	β-Galactosidase activity (Miller units)
M4054 (Δ*gap1*)	L-Proline	0	0.5
		0.23	0.5
	Ammonium ions	0	1.6
		0.01	8.5
		0.23	28
		2	26
	L-Glutamine	0	2
		0.01	4.7
	Mixed (SC medium)	0.23	37
M4056 (Δ*gap1* Δ*bap2*)	L-Proline	0	1.1
	Ammonium ions	0	1.7
	Mixed (SC medium)	0.23	40

RESULTS AND DISCUSSION

***BAP2* transcription on complete and minimal media.** The effect of different growth media on *BAP2* transcription was investigated by assaying β-galactosidase activity expressed from a *BAP2-lacZ* translational fusion introduced on a centromere-based vector. A strain (M4054) deficient in the general amino acid permease (Δ*gap1*) was chosen in order to possibly simplify the interpretations of the uptake experiments described below. The β-galactosidase activity in M4054 was highest during growth on SC medium (Table 1). Growth on minimal ammonia (MA) medium produced only a little β-galactosidase activity (5% of that measured in cells grown in SC medium); growth on minimal proline (MP) medium, produced even less activity (1.6%). Thus, *BAP2* is certainly not subject to nitrogen-catabolite repression. The same pattern was seen in an isogenic strain (M4056) also deficient in the Bap2p permease (Δ*bap2*); i.e., the absence of this permease has little or no influence on the transcription of the *BAP2-lacZ* fusion (Table 1). Since the main nitrogen source in SC medium and MA medium is the same (38 mM ammonium sulfate), one explanation for the more than 20-fold higher transcription of *BAP2* in SC medium compared with MA medium is the presence of one or several of the amino acids in SC medium.

Leucine is an inducer of *BAP2* transcription. To test the possible effect of individual amino acids, we measured β-galactosidase activity in strain M4054 (Δ*gap1*) after growth on MA medium including only one amino acid at a time. In the first set of measurements, we added each amino acid at the concentration at which it is present in SC medium. Addition of 0.23 mM L-leucine to MA medium lead to 75% of the β-galactosidase activity measured after growth on SC medium. L-Isoleucine and L-valine showed slightly decreased inducing capacities (60 to 70%) (data not shown). The addition to MA medium of other amino acids at SC medium levels also had an inducing effect on *BAP2* expression, although the level of effect was not as high as that of the three branched-chain amino acids (data not shown). Supplementation of MA medium with 3.6 mM L-serine, for example, leads to nearly 50% of the β-galactosidase activity seen after growth on SC medium. However, this concentration of L-serine is nearly 20 times higher than the leucine concentration in SC medium (see Materials and Meth-

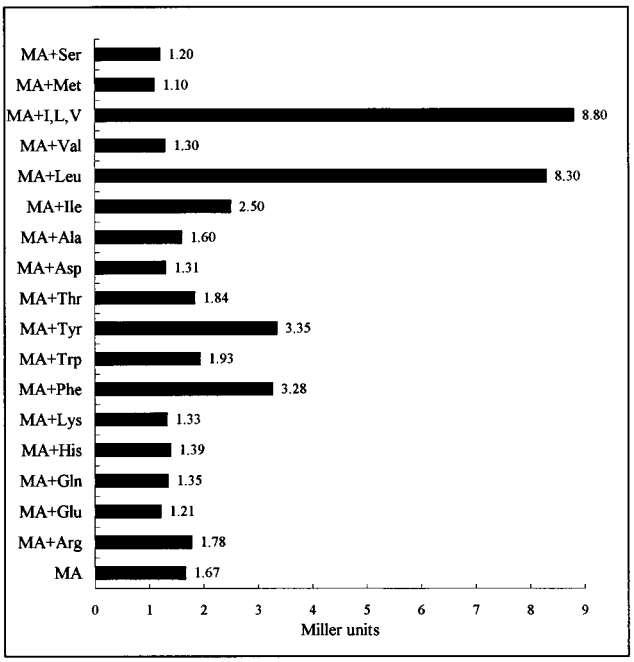

FIG. 1. Potency of various amino acids in induction of the *BAP2* promoter. Strain M4054 (Δ*gap1*), containing a *BAP2-lacZ* fusion in a centromere-based plasmid, was grown on MA medium plus 0.01 mM the indicated amino acid. I, L, V indicates the presence of all three branched-chain amino acids, each at a concentration of 0.01 mM. β-Galactosidase assays were performed as described in Materials and Methods. At least two independent colonies were used for each condition, and the assays were performed in duplicate. The mean β-galactosidase specific activity (Miller units) is shown. The standard deviation was <15%.

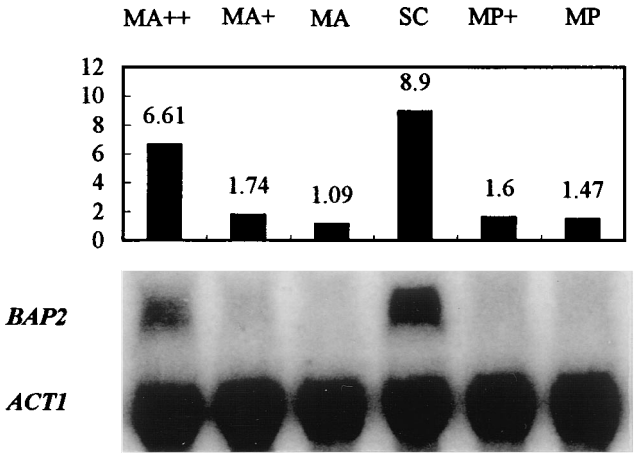

FIG. 2. Northern blot analysis of *BAP2* mRNA steady-state levels in M4054 (Δ*gap1*) cells grown on MA medium, MP medium, or SC medium. The Northern blot was simultaneously probed with a *BAP2* probe and an actin (*ACT1*) probe for standardization. The blot was quantified with the ImageQuant program 3.3 (Molecular Dynamics). The relative values of *BAP2* mRNA after normalization against the actin mRNA are shown as percentages in the upper panel. MA++, cells grown on MA medium with 0.23 mM leucine; MA+, cells grown on MA medium with 0.01 mM leucine; MA, cells grown on MA medium; SC, cells grown on SC medium; MP+, cells grown on MP medium with 0.01 mM leucine; MP, cells grown on MP medium.

ods). In order to compare the potencies of the amino acids, we determined β-galactosidase activity during growth in the presence of each amino acid at 0.01 mM. The results of this experiment are shown in Fig. 1. At this concentration, only leucine showed an induction of *BAP2* promoter-mediated expression as high as fivefold, corresponding to around 22% of that obtained on SC medium. A combination of all three branched-chain amino acids induced a level of expression similar to that leucine induced alone. Thus, L-leucine seems to be an important trigger for the induction of *BAP2* transcription.

Induction of *BAP2* transcription by L-leucine depends on the nitrogen source. It is known that transcription of *GAP1* and *PUT4* depends on the nitrogen source in the growth medium (20, 21). It was therefore obviously necessary to investigate the influence of different nitrogen sources on *BAP2* transcription. We tested three different nitrogen sources in this study: two nitrogen sources to be assimilated easily (ammonia ions and L-glutamine) and one source that, in terms of growth rate, is more difficult to assimilate (L-proline). As shown in Table 1, none of the nitrogen sources tested led to a high level of *BAP2* transcription on its own. However, as already noted, addition of 0.01 mM leucine to MA induced a fivefold increase in *BAP2* transcription. A somewhat lower level of transcriptional induction was seen after the addition of L-leucine to a minimal medium with L-glutamine as the sole nitrogen source. Addition of L-leucine to media with L-proline as a nitrogen source had no effect on *BAP2* transcription. A generalization of these observations might be that nitrogen limitation suppresses induction by leucine, but we have not yet pursued this possibility further.

Northern analysis. To test the unlikely possibility that elements in the *lac* genes are responsible for the regulation seen in Table 1 and Fig. 1, we determined the *BAP2* transcript levels by Northern analysis (Fig. 2). The cells were grown in the indicated media, and the *BAP2* transcripts were detected by hybridization with a *BAP2* gene probe internal to the coding region. An actin probe was used as a loading standard.

As expected, the highest *BAP2* mRNA level was seen after growth on SC medium (Fig. 2) and after growth on MA medium with 0.23 mM leucine (SC medium level). There was only a small amount of *BAP2* mRNA detectable after growth on MA medium with 0.01 mM leucine, and there were even lower levels detectable after growth on MA medium, MP medium, or MP medium with 0.01 mM leucine. The mRNA levels seen in Fig. 2 are thus in line with the β-galactosidase activities measured (Table 1). Because of slight tailing of the *ACT1* band and other sources of background and variations in Northern analysis, we regard Table 1 and Fig. 1 as better reflecting the quantitative pattern of *BAP2* promoter activity than Fig. 2 does.

Induction of *BAP2* transcription with leucine requires neither Leu3p nor Gcn4p. Sequence analysis of the *BAP2* promoter identified putative binding sites for the two transcription factors Leu3p and Gcn4p (10). Gcn4p is the transcriptional activator in a general biosynthetic control system, notably of amino acids (16). Leu3p can act as a transcriptional regulator of several genes in the branched-chain amino acid biosynthetic pathway (7). Leucine has a repressing effect on these genes by affecting the level of α-isopropylmalate, which in turn interacts with Leu3p (29). Leucine and Leu3p also regulate the *GDH1* gene, encoding the $NADP^+$-dependent glutamate dehydrogenase, a key enzyme in nitrogen assimilation (18). In order to look for a possible role of Leu3p and Gcn4p in the induction of *BAP2* transcription, we determined *BAP2-lacZ* expression in *leu3* and *gcn4* mutants. Because of much slower growth of the mutant strains compared with that of the *LEU3* and *GCN4* wild-type strains (which was most pronounced for the *gcn4* mutant) and a lower level of reproducibility of the β-galactosidase activities in these strains (twofold variation), a direct comparison of absolute activities may be of limited value. Nev-

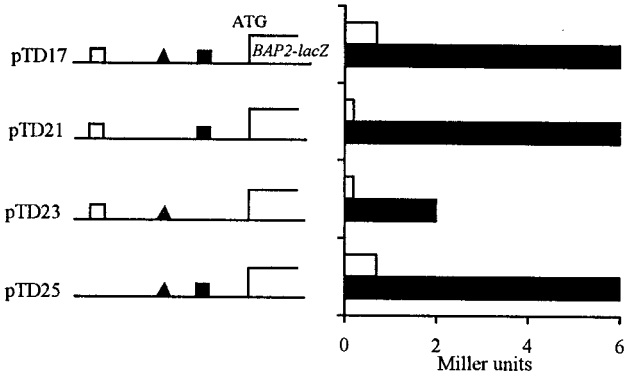

FIG. 3. Transcription levels of the *BAP2-lacZ* fusion assayed as β-galactosidase activity in strain M4054 (Δ*gap1*) as influenced by the inactivation of putative Gcn4p and Leu3p binding sites in the *BAP2* promoter. ■, Putative Gcn4p binding site at −212 (TGACTC); □, possible Gcn4p binding site at −633 (TTACTC); ▲, putative Leu3p binding site at −406 (CCGGAACCGG). Plasmids: pTD17, 683-bp *BAP2* promoter fragment fused to *lacZ*; pTD21, pTD17 with inactivated putative Leu3p binding site; pTD23, pTD17 with inactivated putative Gcn4p binding site; pTD25, pTD17 with inactivated possible Gcn4p binding site. The mean β-galactosidase specific activities (Miller units) are shown. Open bars show activities after growth on MA medium, and filled bars show activities after growth on MA medium plus 0.23 mM leucine. The standard deviations of the β-galactosidase values were <15%.

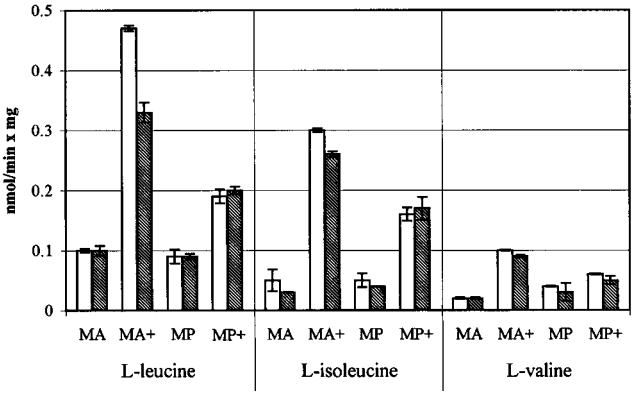

FIG. 4. Uptake of ^{14}C-labelled L-leucine, L-isoleucine, and L-valine as influenced by deletion of *BAP2* and/or the addition of 0.01 mM leucine to the growth medium. Uptake was measured in strains M4054 (Δ*gap1* [open bars]) and M4056 (Δ*gap1* Δ*bap2* [shaded bars]) after growth on MA medium (MA), MA medium with 0.01 mM leucine (MA+), MP medium (MP), and MP medium with 0.01 mM leucine (MP+). All media were supplemented with uracil to satisfy the uracil requirement of both strains.

ertheless, it was clear that a 15- to 20-fold induction of *BAP2* transcription by leucine took place in a Δ*leu3* strain, suggesting that Leu3p is not involved in the induction of *BAP2* transcription by the addition of leucine to MA medium (data not shown). In the Δ*gcn4* strain, the overall level of transcription was lowered, but an 8- to 10-fold transcriptional induction by leucine was still evident (data not shown).

In order to not rely solely on enzyme measurements on extracts from poorly growing strains, the putative Leu3p and Gcn4p binding sites of the *BAP2* promoter were destroyed in the *BAP2-lacZ* fusion by PCR-based site-directed mutagenesis. The mutated versions of the *BAP2-lacZ* fusion were introduced into strain M4054 (Δ*gap1*), and β-galactosidase activities were determined (Fig. 3). The results are in line with those obtained with the *leu3* and *gcn4* mutants. After the inactivation of the Leu3p binding motif (Fig. 3 [pTD21]), induction of β-galactosidase activity by addition of 0.23 mM leucine was as strong as that with the wild-type promoter. On the other hand, the basal-level transcription (defined by growth on MA medium) dropped to 30% of that seen in the wild-type promoter. As a result, the induction ratio rose to 30. However, the main conclusion derived from this result as well as the result obtained with the *leu3* mutant is that the transcriptional induction of *BAP2* by leucine does not require Leu3p.

Mutation of the putative Gcn4p binding site at −212, which fully fits the *GCN4* consensus sequence (TGACTC), lowered the overall level of transcription after growth on MA medium as well as after growth on MA medium with 0.23 mM leucine (Fig. 3 [pTD23]). Nevertheless, the induction ratio remained unchanged (i.e., the induction by leucine still took place). We conclude therefore that Gcn4p is not required for the induction of *BAP2* by leucine, but that Gcn4p stimulates transcription of *BAP2*. A contribution of Gcn4p to basal-level transcription is known for several genes (16). Mutation of the possible Gcn4p binding site TTACTC at −633, which only approximates the consensus, on either MA medium or on MA medium with 0.23 mM leucine had no influence on *BAP2* transcription (Fig. 3 [pTD25]). We therefore consider this site unimportant, at least under the conditions we tested.

Although a putative site for each factor affected basal-level *BAP2* transcription, as seen for several other genes (16, 29), none of the sites was required for the induction by leucine. We therefore assume the existence of an additional regulatory factor that mediates the leucine signal to the *BAP2* promoter, thereby inducing the expression of Bap2p and leucine uptake.

Leucine is an inducer of branched-chain amino acid uptake. The leucine-induced transcriptional activation of *BAP2* is expected to create an increased potential for uptake of the branched-chain amino acids. In order to see if such an effect could be detected and quantified, the uptake of the three branched-chain amino acids in strains M4054 (Δ*gap1*) and M4056 (Δ*gap1* Δ*bap2*) was measured after growth on MA medium, MA medium with 0.01 mM leucine, MP medium, and MP medium with 0.01 mM leucine (Fig. 4). Indeed, leucine uptake in strain M4054 (Δ*gap1*) was three to four times higher after growth on MA medium with 0.01 mM leucine than after growth on MA medium alone (Fig. 4, bars 1 and 3). The leucine uptake by one or several unidentified permeases in strain M4056 (Δ*gap1* Δ*bap2*) was also induced by the addition of leucine (Fig. 4, bars 2 and 4), and in this case was induced two- to threefold. This shows that the amount or activity of the additional permeases capable of transporting the branched-chain amino acids (or at least one of them if there are several) is also positively influenced by leucine in the medium. It is conceivable that transcriptional activation is involved, as we also found in this case for *BAP2*. Deletion of *BAP2* caused no change in uptake in cells grown on MA medium or MP medium (Fig. 4, bars 1 and 2 and 5 and 6). This is consistent with the low level of transcription of *BAP2* in cells on these media. On the MP medium, addition of leucine doubled the uptake of leucine and tripled the uptake of isoleucine in both strains (Fig. 4, bars 5 and 7 and 6 and 8 for leucine and bars 13 and 15 and 14 and 16 for isoleucine). This finding, together with the observation that leucine does not induce *BAP2* transcription in cells grown on MP medium, again indicates the existence of at least one extra permease that is in some way positively influenced by leucine. This permease must be active in cells on MP medium, in contrast to the Bap2p permease. It may be noticed that 0.01 mM leucine in the medium induced the transcription of *BAP2* and the uptake of leucine about 5-fold, while the presence of SC amounts of amino acids in the medium induced

the transcription about 23-fold (Table 1), but with no further increase in the uptake of leucine. One reason for this lack of proportionality between *BAP2* mRNA and leucine uptake is probably the regulation of Bap2p activity.

The uptake of isoleucine and valine followed in most respects the pattern described above for leucine uptake. The main difference was the minor contribution of Bap2p to uptake of isoleucine (Fig. 4, bars 9 and 11 and 10 and 12) and valine (Fig. 4, bars 17 and 19 and 18 and 20) after induction with leucine on MA medium. Addition of leucine to MP medium stimulated isoleucine uptake by an even higher factor than it stimulated leucine uptake (Fig. 4, bars 13 and 15 and 14 and 16). Valine uptake was only slightly elevated (Fig. 4, bars 21 and 23 and 22 and 24).

Our results demonstrate that amino acids, especially leucine in micromolar amounts, act as a regulatory signal for the uptake of branched-chain amino acids in *S. cerevisiae*. Leucine induces transcription of the branched-chain amino acid permease gene *BAP2*, and increased uptake of the branched-chain amino acids is observed. A similar role of leucine in regulation of uptake of dipeptides in *S. cerevisiae* has been described by Island et al. (19), and it was recently shown that the addition of leucine induces the transcription of the dipeptide transporter gene *PTR2* (27). It is tempting to suggest that *BAP2* and other amino acid transporters together with the dipeptide transporter are regulated via a common regulatory mechanism. As a prokaryotic analogy, *Escherichia coli* contains the so-called leucine-responsive regulatory protein, Lrp, through which leucine affects the transport of branched-chain amino acids. In addition, Lrp influences the expression of operons involved in amino acid biosynthesis, amino acid degradation, and dipeptide transport (for review, see reference 4). It is remarkable that sensing of leucine plays a particular role in regulation of several genes both in *E. coli* and in *S. cerevisiae.*

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