

## Characterization of the pH Signal Transduction Pathway Gene *palA* of *Aspergillus nidulans* and Identification of Possible Homologs

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**We have cloned the *palA* gene of *Aspergillus nidulans*, one of six genes participating in ambient pH signal transduction in a regulatory circuit mediating pH regulation of gene expression. The derived 798-residue PalA protein is 29.4% identical over its entire length to a hypothetical protein from the nematode *Caenorhabditis elegans* and also has possible yeast homologs.**

In the filamentous fungus *Aspergillus nidulans*, as in many other microorganisms, the syntheses of a number of permeases, secreted enzymes, and exported metabolites are appropriately regulated by ambient pH (3). For example, acid phosphatase and the acid-pH-optimum  $\gamma$ -aminobutyrate permease are synthesized preferentially in acidic media, whereas alkaline phosphatase and penicillin are synthesized preferentially in alkaline media (3, 7, 18). pH regulation of gene expression is mediated by the zinc finger transcription factor PacC, whose 73-kDa full-length translation product is proteolyzed to yield a 29-kDa N-terminal fragment able to activate transcription of genes expressed at alkaline pH and prevent transcription of genes expressed at acid pH (15, 17, 19). The products of six genes, *palA*, *-B*, *-C*, *-F*, *-H*, and *-I*, form a signal transduction pathway through which alkaline ambient pH is able to elicit the conversion of the full-length form of PacC to the functional proteolyzed form (1, 3, 5, 15, 19). Loss-of-function mutations in these *pal* genes prevent this proteolytic conversion and mimic the effects of growth at acidic pH (1, 3, 5, 7, 15, 18).

The mechanism of ambient pH signal transduction is an intriguing subject of scientific and biotechnological importance. We have previously shown that the *palB* gene product is likely to be a cysteine protease of the calpain family, albeit not the protease responsible for the final conversion of PacC to its functional form (5). Here, we report the cloning and sequence of *palA*. Amino acid sequence similarity strongly suggests that the *palA* gene product has a homolog in the nematode *Caenorhabditis elegans*. It also has possible homologs in the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*.

The *palA* gene was cloned with linkage group III (containing *palA*) clones from a chromosome-allocated wild-type *A. nidulans* cosmid library (2) and pILJ16 (11) and by identification of *palA*<sup>+</sup> cotransformants by their ability to grow on pH 8 medium (3-5) among *argB*<sup>+</sup> transformants of a strain of genotype *biA1 yA2 wA3 argB2 areA49 palA1*. *palA1*-rescuing activity was found in cosmid W28H12 and further localized to a 5-kb *XbaI-XhoI* fragment (Fig. 1A). To confirm that this fragment contains the *palA* gene itself rather than a gene associated with a fortuitous suppressor activity, strains carrying either of two

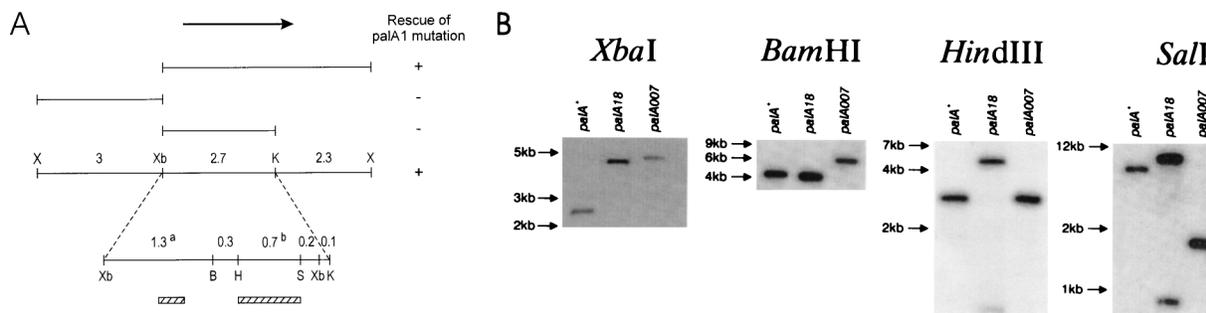


FIG. 1. (A) Rescue of the *palA1* mutation by various restriction fragments, position and orientation of the *palA* transcript, and positions of two *palA*-associated translocation breakpoints. X, *XhoI*; Xb, *XbaI*; B, *BamHI*; H, *HindIII*; S, *SalI*; K, *KpnI*. The arrow at the top denotes the direction and location of the *palA* transcript relative to the 8-kb *XhoI* fragment in the middle. The ability of various restriction fragments, whose sizes are shown in kilobases, to rescue the *palA1* mutation is indicated to the right. The hatched bars at the bottom indicate the positions of fragments used to obtain cDNA clones and as probes for Northern blots. a, contains the *palA007* translocation breakpoint; b, contains the *palA18* translocation breakpoint. (B) Southern blots localizing the *palA18* and *palA007* translocation breakpoints. From left to right are shown *XbaI* digests probed with the 2.6-kb *XbaI* fragment shown in the expanded portion of the Fig. 1A restriction map, *BamHI* digests probed with the 1.3-kb *XbaI-BamHI* fragment shown in the expanded portion of the Fig. 1A restriction map, *HindIII* digests probed with the 2.7-kb *HindIII* fragment extending rightwards from the *HindIII* site shown in the expanded portion of the Fig. 1A restriction map, and *SalI* digests probed with the 0.7-kb *HindIII-SalI* fragment shown in the expanded portion of the Fig. 1A restriction map.

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XbaI

-490 TCTAGACAACCCCGAGCTAGCTATTACCGTACTGACAGTGCAGACTTACCCCTCGATACTCGCAAACCGCGCGGCAA  
 -411 AGTGTGCTTTTAGGCAAATCGAAGGAAGTCGGCGAGGCTCCGCTCGGTGGCAGCATCAAGCATGTATAAGGGTCTGTCGATTATTCTGATCCGCACTG  
 -311 GAGTAAATAACATGATTCTGCTCAGCATCTTGAATATATTAAGATGCGCATCAACGAGCTAGACCGATGATTATCGGTCCCATCGACTGGAGTCCAGGA  
 -211 GTATTGTATTATCTGAACATAGCAATAAATAACGACCTGTTAAATGCACATTGATTATAATTTTTCCGAAGTAGTAAATACAGATAGTGCCTAAGGCA

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-111 CTACCACGTCAATTTGCCCGCTACGTTGCCGTGGGGTCTGCGGGGACAGTTTTTAGCATCCCGTCAACAACAATCGTCCATCCAAGCGCAACACCGC  
 -11 GCATTGCCAAAATGGCCTCGTACGTACGTCCAGTACTTTGCCAAAATATCACCTATTGACAGAAACAGAATATCCTCCGATTCCTTCGCCCGCTCGC  
 1 M A S N I L Q I P F R R S

90 ACACTGTCTCCCTCTCGACCGCTTGACCCAATACATTTCCACCAAATATGACCAGCGGCTGACATGTTGCGAGATGACTTGTCTATTGATCGGTT  
 14 H T V S L S T A L T Q Y I S T K Y D Q R P D M F A D D L L I I D R L

190 ACGAAATGAGGCCATAAACGTGACGGAACACATGTGACGGAATCAGCGGGTGGTTACTTACGCGCGCAACTGAAATGGCTGGGGGAAAGTTTCCA  
 48 R N E A I N V Q E P H V S G I S R L V T Y A A Q L K W L G G K F P

290 GTTGTATGACGTATCAATAATATCATAGATATTCGGGCTGAGTTGACGGTCTAGGTCGGGTTCGAGTTCCCTGGTATCCTGCTTTGGGTTCAACAC  
 81 V D V G V E F P W Y P A F G F N T

390 AAGTCGGCCAGGTACGACGTTTCTCACAGCGAATCTGCTAGGATGTTGGCTAATGTACGACAGTCTCACAGGATAACATCGCTTCGAGCTGGCAAACGT  
 98 S R P V S Q D N I R F E L A N V

490 CATCTTCAACCTCGCGCACTTACTCCCAGCTCGCTTCCGCGTAAACCGCACAAACCGGCTCTCAAGCAAGCATGCACTTTTTCGCCAGGCA  
 114 I F N L A A L Y S Q L A F A V N R T T T D G L K Q A C N Y F C Q A

590 GCCGTATCTAGCACCTCCGAACAGACATCGTCCCTGACATGCGCTCCGCCCCGGGAAGACATGGACGAGATGACCTCCGAAGCTTGAAGAGC  
 147 A G I L A H L R T D I V P D M R S A P P E D M D E M T L R S L E E

690 TGCTCTCGACAAGCTCAGGAATGTTTTCGGCAGAAGCCGTGTGGATGGGCTAAAGGATGCATCAATTGCAGACTCGCGGGCAAGTGTGGACTT  
 180 L L L A W Q L E C F W Q K A V M G D G L K D A S I A R L A G Q V S D F

BamHI

790 TTATGGCGATCGCTCGCATCACGCGTCAAGTCAAGTCCGATCAGCCCCGAATGGATCCACCATATGACGGCGAAACAGCATCATTTTGCAGTGCAGCG  
 214 Y G D A C D H A V K S N A I S P E W I H H M T A K Q H H F A A A A

890 CAGTATCGCCAGTCCGTTGGATTGCTGGAGAAGCGCAAGTATGGAGAGGAGTGGCACGGTTACGGGACGCTGTGGCTTGTGTGAATGAAGCGCTCAAAG  
 247 Q Y R Q S L D C L E K R K Y G E E V A R L R D A V A C V N E A L K

990 AGAGCCGGTGGATCAATCGCACGGTGTGGGTGATTGACAGGGGTTAAAGAAATAGAGTAACGGAGGATTGAAGCGCGCCGAGAAGGACAACGATATGAT  
 280 E S R W I N R T V L G D L Q G L K N R V T E D L K R A E K D N D M I

HindIII

1090 TTATCTCAACCCCGTCCCGCCAAGTCCGAGCTCAAGCTTATTGATCGGGCGTGTATGGTTGCGGCTAAGCGCCGTCGCGAGTCAAGCGCAATCTCG  
 314 Y L N P V P P K S E L K L I D R A C M V A A K A P S Q V T D A I S

1190 ATGCTGGGGAAAAAGGCGCTTGGGACAGCCGCTCTTTTGAAGCTGCTCCCGTATGCCGTGACATTCGCGGAGCATTACTCGGACAGGAGAGACC  
 347 M L G E K G P L G Q P L F S K L V P Y A V H I A A S I Y S D R R D

1290 GTCTTGTCAATGAGCGGATTATCGGCGAATGGAGAACATGACGGCAAGTACGCGAGTATGTCACATCCTTTTACATTCTCTATTATGTAACAGGGC  
 380 R L V N E R I I G E L E Q S L D A K L R D

1390 CAGTCTACTATCATCGCTCAATCTCCCGGCTCGTGAAGCCCTTGAAGAGCCCTGGGCTGCGCCATCACTGGTCCGCCATGCCGAAGAAATGCGT  
 400 L L S S L N L P G S L Q A L E K P L G L P P S L V A H A E E M R

1490 CAACAAGACGGCTCAACCGCTCCGCAAGTCCCTTTCGACATCGCCAAAGTCAAATCCAACGACCGCGCGTCTATACCGAAGCGTGAAGCTCCTCG  
 432 Q L R L N R L R K S L L D I A K V K S N D R A V I Y K T A G V E L L C

1590 CCGTGA AAAAGCTGAGGACGAGCTTCCGCGGAAATTTGGCACCGACCGTGGACGCGGAGGCTCTGAAGCGCGCTCCTAACTTACACGAC  
 465 A A E K A E D D A S R R K F G T D R W T R E A S E A A A P K L Y T T

1690 CGCCCGGAAATCGACGGCTACTTCACTCAGCGCAGAGCAGTACACCTGGTGGAGCAAACTGCAGACTCGGAAGCTGTCTTTCGCGTTTTGACC  
 499 A R E I D G Y F T S A Q S S D N L V E Q K L H D S E A V F R V L T

SalI

1790 GGCACGAATCGGACCTTGAAGCTTTTGTCCCAAGTAGTCGACGCGCAACGATACCGCTGAAGTCAAGCGGAGGTGAGTCCGCTCCGACGTGCATCA  
 532 A K I V G R F R D D V K A F V H K R R M E A S Q L E Q

1890 GCGAAGTAAATCGACTCGAAAGCGGCGAAAGCGCAAGGCTCAGGCCCTCAAGACAAAGCGCGGACGACATCAGTTCTGCACTTGTCCGCGAGGC  
 565 S E V N R L E S R R K R K A Q A V K D K A R A D D I S S A L V R E A

XbaI

1990 AGCACGTTTAGAGCGGAGTCCCAATGCAGGCTATCCAGGCTAGTCAATTCAGGACCTCTTTGAATCAGACTGCGCGATTACGACGTCGATCTAGAT  
 599 A R L E R E F P M Q A I Q A S Q P E D L F E S R L R D Y D V D L D

2090 ATGGTTGCGCAGGAGATGCACGATCAGGATCAGATCGTCCGCGAGTGGGACGCGAATCGTGGCTTACGCGGCCATACGGGTGATGCTTCTACAA  
 632 M V A Q E M H D Q D Q I V A Q V R D A N R A F T R A H T G D A S T

KpnI

2190 AGGAGCGTGAGAAGCGCTCCAGGAGCTGGAGAACGGGTACCTGAAGTATAAGGAGATCATTTCAAAATATCGAAGTCCGACGGAAGTTCTATAATGATCT  
 665 K E R E K A L Q E L E N G Y L K Y K E I I S N I E V G R K F Y N D L

2290 CGCGAAGATCGTGGGCGGTTTCAAGGATGATGTCAGGCGCTTGTGCATAAGAGGCGTATGGAGGCTAGTCACTTGAAGCAGTGCCTTCCCCCACC  
 699 A K I V G R F R D D V K A F V H K R R M E A S Q L E Q

2390 TTTTTTTTTATAACCGTCTAATGCGATATAGGGACATATCCAGCGTCCGCGCAATGGCATCCCTGAATATCTCGCTATACGGCAACCCCGAGCAAA  
 726 D I S S V A A M A S L N I S P I R Q P P Q Q T

2490 GGTAGTTTCAAGCCAGTTTCACTCTGCGCGGCTCAGTTCGACGCGCACATTTTAAATCCAGTCAAACCCCAACCTCAACCTCCGTCGAGGCA  
 749 V V S A P V S V S A A A S V P A T H F N P V K P Q P P S Q A

2590 ATCCCAACAGTCAACAACCAAGGCAAGCCGCAACCTGCGGACCTCTAACAGCGCCCGAGCCAAACGCGAGGTTCCGCAAGTACGCCAGGGAT  
 782 I P P Q S Q P Q G E P Q P C G H L

2690 GTGGTCCCCGAAATGGGAATACGCTTGGGCGGGGGTACGACGGCCAGCAGTCTCAGCAAACGTTGGATCCGTCGAAGGGGATGAAGTTCTCATGA

\*

2790 ACCTTAATACGCTACTAAACATATGCTTTGATGTAACATAAGTGTAGACCGTACATACTTGCATACATACACGCACATATCCTCACACTTGATAAGA

\*

2890 GACACGTATGCGTGCCTATTCAAGGGCCATTGTCTACTACAGACGCAAAAGAAATCATTGCGCATGCCCTTAAATACCGATTATACAAAGTTGTTACATT  
 2990 GTCGTCTCAATAGTAAACCTCCTCATGACAGCAGCGGCTCAGCGGCTGCTCC

FIG. 2. Nucleotide sequence of a 3,536-bp region containing the *palA* gene and its derived translation product. Restriction sites shown in Fig. 1A are indicated. The 5' end of all four cDNA clones is starred, as are the 3' ends of the three polyadenylated cDNA clones. The sequence CAAT is present upstream of the cDNAs at -39 and -189. Sequences playing a role in mRNA 3'-end formation in *S. cerevisiae* (9, 10) are underlined.

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Z29561  MATFGFLSAPLKSTNEVDLVLKPLTSYIDNVYNTSDNNRSDVAEAVQE.LNKLRSKACQPLDKHQSAID...VLTRYDQLVAIENKIIISATQNPVVF 95
PalA    MAS.NILQIPFRRSHTVLSLTALTQYISTKY...DQRPFDMFADLLI.IDRLRNEA.INVQEPHVSIGS...RLVTYAAQLKWLGGKFPVDVG...VEF 87
Bro1p   MKP.YLFDLKLKDKTEKLDWKKGLSSYLKKSYSG.SSQWRTFYDEKATSELDHLRNNNA.NGELAP..SSLSBQNLKYYSFLEHLYFRLGSKGSRK...MDF 92

Z29561  KWKDAFDKGSFLFSSRASL..SLSDGSFERAAVLFNIGLSMSQIGAAQPFHTDDEIKVSAKLFQQSAGVFAARLRDVLGMVQQEPTDMLPDTLAALSALM 193
PalA    PWYPAFG...FNTRSFPV..SQDNIREFELANVIFNLAALYSQLAFVNRRTTDDGLKQACNYFCQAAGILAHLRDIDVPMRSAPPEDMDEMTLRSLEELL 181
Bro1p   TWYDAEY...SSAQKGLKYTQHTLAFBKSTLFLNIAVIFTQIA...RENINEDYKNSIANLTKAFSCFEYLSFNFL...NSPSVDLQSENTRELANIC 181

Z29561  TAQAQEAIYIKGHKDKMKA...TSMVKISAQVAEFYSEA...QKMMSKDIVRGLWDDKWSAIVSGKNLAYQALAQYHQHSEVCGEARQIGEQLSRLAESLKL 288
PalA    LAQAQECFWQKAVMDGLKD...ASIALRAGQVSDFYGDACDHAVKSNAI...SPEWIHMTAKQHFAAAQYRQSLDCLEKRYGEEVARLRDAVAC 273
Bro1p   HAAEQELFVLKLLNDQISSKQYYLISKLSRATCNLFQKCHDFEMKEIDDDVAIYGEKPKWTTVTKLHFYKLSAYYHGLHLEENRVGEAIFLDFSMQQ 281

Z29561  FDTAQK...YLPRDITGIWDIYPS.VSKAHAAAKKDNDFIYHEKVSDFRTLPLPKAVL...AKPTPMQTPMTPSFRDMFAVLVPPVQVHNAMQS 375
PalA    VNEALKESRWINRTVLGDLQGLKNRVTEDLKRABKDNNDMIYLNPPPKSELKIDRACHVAAKAPSQVTDIAISMLGKGPLGQPLFSKLVYAVHIAASI 373
Bro1p   LISSLPFKTWL..VEFIDFDGFKETLEKKQKELIKDNDFIYHESVPAVVQVDSIK..ALDAIKSPTEWELBEPYMQDVANKYDLSYRGIIPLDVYEKESI 377

Z29561  YDARKAELVNMETV.RMREATQLMNGVLASLNLPAALDDVTTSTETLPESLK...LKSARKLQN...GGSSEIMRFLSELPTLYQRNEDILTETSRI 464
PalA    YSDRRDRLVNERIIGELNMTDKLRDLLSSLNPLGSLQALEKPLGLPPSLV...AHAEBMRQQ...DGLNRLRKSLLDIAKVSNDRAVYTEGVEL 463
Bro1p   YSEKATLLRKQ.VEETETANLEYSSFIEFTNLRLLSLEKQFSDGNIFSNNTDQGLMRDQIQTWCKFIQTNEFRDIEEQMNKIVFKRQKI...LEI 472

Z29561  LNEEKESDDTMRKQLGT.KWTRMSSEQLTGLVTEIGYRGIILHTASNADKMKV.EKFESHROGIEELLSKNESELRSIPG...QTAHATGETDTRVQL 558
PalA    LAEKAEDDASRRKFGTDRWTREASEAAAPKLYTTAREIDGYFTSAQSSDNLVE.QKLDHSEAVFRVLTGTNRDLEAFVPS..SRRATIPPEVEREVSR 560
Bro1p   LSALPNDQKENVTKL...KSSLVAASNSDEKLFACVK...PHIVEINLLNDNGKIWKKFDEFNRNTPP.QPSLLDIDDTKNDKILELKLQV 556

Z29561  RQFMSQWNEVTTDRELEKELKNTNCDIANDFLKAMAENQLINEEHISKEKI.AQIFGDL...KRRVQSSLDQETLMNQIQANNTFTGK 646
PalA    RSCISEVNRLESRRRKAQAVKDK..ARADDISSALVREARLEREFPMQAIQASQFEDLFESRLRDYDVLDMVAQEMHQDQIQIVAVRANRAFTRAH 658
Bro1p   KGHAEDLRTLKEERSRNLSERLDE..INNDITKLLIINKGKSDVE...LKDLFEVELEKFEPLSTRIEATYKQSSMIDDIKAKLDEIFHLS 644

Z29561  TGSSTGAERERILKMLAQASDAYVELK...ANLEEGTKFYNDLTPILVRLQKQVDFAFARQTEKEDLMRQLQLSIVSGQAAKAVVDGVNSLVSSY 739
PalA    TGDASTKEREKALQLENGYLKYEKII...SNIYEVGRKFYNDLAKIVGRFRDDVKAFVHKRRMEASQLEQD...ISSVAAMASL... 736
Bro1p   NEFKDKSSGEEKFLEDRKNFFDKLQEAVKSFISFASDLKPGKIEFYDLSFNMSRDLAERVVRVAKQTEDSTANSPAPP...LPPLDSKASV... 724

Z29561  LTGGTNAAQSPANAPRPPPP...RPAAPSVESPIPPRTPQSSMQATPGAPPQYNPYQQQQQPQMQQFQQHPGYQQPMPYQG 819
PalA    ...NISPIRQPP...QQTVVSAVPSV...SAAASVPAPTHFNPKPQPQPPSQAIPPOSQPQGPQPCGH 797
Bro1p   ...VGGPFLLPQKSAAFQSLSRQGLNLGDQFNKLSAGSDL...PQGPPIPPRTY..EASPYAATPTMAAPPVPPKQSQEDMYD. 806

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FIG. 3. Alignment of the derived PalA sequence with those of a hypothetical *C. elegans* protein (Z29561) and Bro1p of *S. cerevisiae*. Vertical lines indicate identities; dots between sequence lines denote similarities (A/G, D/E, F/Y/W, I/L/M/V, K/R, N/Q, and S/T). Alignments of PalA to Z29561 and Bro1p were carried out individually with Bestfit from the Genetics Computer Group package (6) and then combined and edited by eye.

translocation-associated *palA* mutant alleles were subjected to Southern hybridization analysis along with a wild-type strain. The detection of restriction fragment polymorphisms correlated with chromosomal rearrangement mutations establishes physical linkage between a cloned fragment and a mutationally identified gene (8). By using several probes from the region shown in Fig. 1A and an appropriate set of digests, the chromosome III *palA007* translocation breakpoint was localized to the 1.3-kb *XbaI-BamHI* fragment and the chromosome III *palA18* translocation breakpoint was localized to the 0.7-kb *HindIII-SalI* fragment (Fig. 1B). (*palA007* [formerly *npeC007*] is associated with a translocation involving chromosomes II and III [18], whereas *palA18*, whose selection was described previously [1], is associated with a translocation involving chromosomes III and VIII.) The localization of the two *palA*-associated translocation breakpoints within the 5-kb *XbaI-XhoI* fragment in conjunction with the ability of this fragment

to rescue the *palA1* mutation provides unequivocal evidence that it contains the *palA* gene. In Northern blots, the 2.7-kb *XbaI-KpnI* fragment and the two smaller fragments denoted by hatched bars at the bottom of Fig. 1A hybridized to a single mRNA with a size of ~2.8 kb. Levels of this mRNA do not seem to be influenced by the PacC-mediated pH regulatory system: Northern blots with total RNA from an alkalinity-mimicking *pacC11* mutant or an acidity-mimicking *palF15* mutant showed no appreciable differences in relative *palA* mRNA levels when compared to wild-type levels, in marked contrast to PacC-regulated transcripts in an experiment similar to that shown in Fig. 1 of Tilburn et al. (19) (data not shown). We did consistently find lower relative *palA* mRNA levels in wild-type cells grown at pH 4 than in wild-type cells grown at pH 6.5 or 8 (over which range there is no variation) (data not shown), but this might relate to differential mRNA lability under the growth and/or extraction conditions used. The lack

of influence of extreme mutations in genes of the PacC-mediated pH regulatory system and the lack of effect of changing the growth pH for the wild type between pH 6.5 and pH 8 argues very strongly that the PacC-mediated pH regulatory system, of which the *palA* gene product is a component, does not regulate *palA* expression.

A genomic region with a size of 3,536 bp encompassing the 2.7-kb *XbaI-KpnI* fragment hybridizing to the *palA* message and including the fragments in which the *palA*-associated translocation breakpoints occur was sequenced (Fig. 2). Sequencing of cDNA clones from a  $\lambda$  gt10 library (16) revealed the presence of five introns with sizes of (moving from 5' to 3') 49, 50, 52, 45, and 50 nucleotides, respectively (Fig. 2). Four cDNA clones, all clearly independent as shown by their differing 3' ends (vide infra), all have the same 5' end, which is only 14 bp upstream of the putative initiation codon (Fig. 2). This could mean either that the *palA* mRNA has an exceptionally short leader region or that reverse transcription does not normally proceed beyond this point (perhaps because of stable secondary structure in the mRNA). Three of the four cDNA clones are polyadenylated, each at a different site (Fig. 2). Taking poly(A) tails into account, the length of these polyadenylated cDNAs (longest is 2,641 bp) agrees reasonably well with the 2.8-kb estimate of messenger size, suggesting that their 5' end is either the transcription start point or quite near to it. There are no potential PacC binding sites (GCCARG [19]) in the 490 bp upstream of the initiation codon, consistent with the absence of PacC-mediated transcriptional regulation.

The derived amino acid sequence of *palA* contains 798 residues and has a calculated molecular mass of 89.4 kDa. If the *palA* protein product PalA is located in the nucleus, the very basic sequence RRKRK beginning at residue 573 is a possible nuclear localization signal or part of one. The C terminus of PalA is exceptionally proline rich, with proline accounting for 16 of the last 59 residues. The positions of the *XbaI*, *BamHI*, *HindIII*, and *SalI* sites bordering the *palA007* and *palA18* translocation breakpoints (Fig. 1A) in the Fig. 2 sequence should be particularly noted. The fact that all extant *palA* mutations, including *palA007* with its breakpoint in the promoter, 5' untranslated region, or 5' portion of the coding region and *palA18* with its breakpoint in the central part of the coding region, have the same phenotype argues very strongly that this is the null phenotype, particularly as it is indistinguishable from that of the *palB* disruption (5) allele.

In Bestfit (6) pairwise alignments (Fig. 3), PalA shows 29.4% identity over 850 residues with an 882-residue hypothetical protein from the nematode *C. elegans* (accession number Z29561), 23.1% identity over 769 residues with a 701-residue hypothetical protein from the fission yeast *S. pombe* (accession number Z54354) (not shown), and 20.8% identity over 852 residues with the 844-residue Bro1p protein (14) of the budding yeast *S. cerevisiae*. Although no function has yet been identified for the probable *C. elegans* PalA homolog (or the possible *S. pombe* homolog), Bro1p is likely to be in a pathway that interacts with (possibly sharing downstream targets with) the protein kinase C-mitogen-activated protein kinase pathway in *S. cerevisiae* (14). Like Bro1p (14), PalA contains putative SH3 domain-binding motifs (consensus PXXP [12, 13, 20]) with PVPP beginning at residue 317 and with PVKP and PQPP beginning at residues 770 and 775, respectively. A very recent database entry (accession number Z75183) for a hypothetical protein encoded by chromosome XV of *S. cerevisiae* has 30.2% identity over 732 residues in a Bestfit alignment with PalA.

Although the role of PalA in pH signal transduction remains to be elucidated, the identification of a probable homolog in *C. elegans* (29.4% identity over the entire length of PalA) and

possible homologs in *S. pombe* and *S. cerevisiae* opens the way to a multiorganism approach to the mechanism of pH signaling or at least its evolutionary origins. The *C. elegans* probable homolog is of particular interest because it raises the enchanting possibility of conservation of pH signal transduction between the fungal and animal kingdoms.

**Nucleotide sequence accession number.** The EMBL accession number for the *palA* sequence is Z83333.

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