nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

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Reprinted from Nature, Vol. 372, No. 6506, pp. 567 – 570, 8 December 1994

A yeast gene necessary for bud-site selection encodes a protein similar to insulin-degrading enzymes

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CELLS of the yeast *Saccharomyces cerevisiae* choose bud sites in a non-random spatial pattern that depends on mating type: axial for haploid cells and bipolar for a/α diploid cells[1,2]. We identified a mutant yeast, *axl1*, in which the budding pattern is altered from axial to bipolar. Expression of the *AXL1* gene is repressed in a/α diploid cells. With the ectopic expression of *AXL1*, a/α cells exhibited an axial budding pattern, thus *AXL1* is a key morphological determinant that distinguishes the budding pattern of haploid cells from that of a/α diploid cells[2]. *AXL1* encodes a protein similar in sequence to the human and *Drosophila* insulin-degrading enzymes[3,4] and to the *Escherichia coli ptr* gene product[5]. The axial budding pattern might result from degradation of a target protein by the putative Axl1 protease.

Two classes of genes are necessary for proper bud-site selection[2]: *BUD1-BUD2-BUD5* and *BUD3-BUD4*; the former is necessary for both bipolar and axial budding, the latter for converting a bipolar pattern to axial (Fig. 1*a*). In **a** and α cells budding is proposed to be axial, because all *BUD* genes are active (Fig. 1*b*). In contrast, expression of *BUD3* or *BUD4* may be turned off by the repressor **a**1-α2 in **a**/α cells[2] (Fig. 1*b*), which may lead to bipolar budding in **a**/α cells. The *axl1-1* mutant was identified when screening for mutants with a bipolar budding pattern (Fig. 2*a*, *b*; see legend to Table 1). The position of bud site formation was quantified in cells with a single bud scar and a single bud according to the scheme in Table 1. The *axl1* mutant was mated to *bud3* or *bud4* strains for complementation tests. The *MAT*α genes of the two diploids so formed were disrupted to neglect the effect of repressor **a**1-α2. As they exhibited the axial budding pattern, the *AXL1* gene is distinct from *BUD3* and *BUD4*. *AXL1* was cloned by complementation of the bipolar budding phenotype of an *axl1* strain (see Fig. 3 legend). We also observed that the cloned *AXL1* gene did not complement *bud3* and *bud4* strains. Thus, *axl1*, *bud3* and *bud4* belong to different complementation groups. A disruption allele of *AXL1* (*axl1-Δ1::URA3*) was constructed (Fig. 3*a*). Phenotypes of the *AXL1* null mutation in haploid cells were indistinguishable from those of the *axl1-1* mutant in haploid cells: both budded in a bipolar fashion (Fig. 2*c*, *d*), and the *AXL1* null mutation apparently did not affect the bipolar pattern in **a**/α diploid cells (data not shown). Prior studies showed that mutations in genes required for both axial and bipolar budding (*BUD1*, *BUD2* and *BUD5*) are epistatic to mutations of genes required only for axial budding (*BUD3* and *BUD4*)[2,6,7]. When the epistasis test was done, the *axl1 bud5* strain exhibited the random pattern (Fig. 2*e*, *f*) which means that *bud5* is epistatic to *axl1*. Thus, *AXL1* is indeed a class of genes necessary only for axial budding such as *BUD3* and *BUD4*.

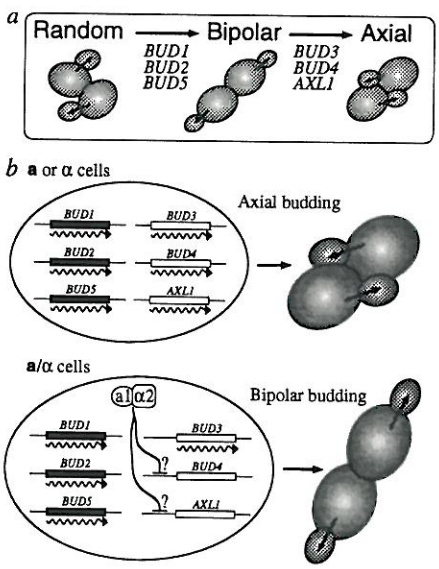


FIG. 1 Models for the morphogenetic pathway for bud-site selection[2]. *a*, Control of budding pattern with *BUD* and *AXL1* genes. The random budding pattern is the basal state. *BUD1*, *BUD2* and *BUD5* are necessary for both bipolar and axial budding. Further addition of *BUD3*, *BUD4* and *AXL1* functions exhibits axial budding. *b*, Two classes of genes control the axial and bipolar budding in **a** and α cells and **a**/α cells. Black bars indicate genes required for bipolar and axial budding, white bars only those for axial budding. Wavy arrows under bars indicate that genes are transcribed. It is proposed that *BUD3* or *BUD4* may be repressed by repressor **a**1-α2 only found in **a**/α cells[2]. However, transcripts of *BUD3* were detected in all cell types (J. Chant, J. Pringle and I. Herskowitz, personal communication).

The expression of *AXL1* may be controlled by the repressor **a**1-α2 in **a**/α diploid cells (Fig. 1*b*). We examined the pattern of *AXL1* expression in **a** and α haploid cells and in **a**/α diploid cells. Figure 2*g* shows that *AXL1* is transcribed in **a** and α haploid cells but not in **a**/α diploid cells. We found that the level of *AXL1* mRNA was higher in **a** cells than in α cells. However, quantification of the budding patterns exhibited no significant difference between **a** and α cells (Table 1). We have no explanation for this occurrence. *AXL1* is the first known gene that is involved in bud-site selection, the expression of which is cell-type-specific.

We determined the nucleotide sequence of a 5.3 kilobase (kb) DNA segment that complements the *axl1* mutation (Fig. 3*a*). This region contains a 3,624 base pair (bp) open reading frame (ORF) that potentially encodes a 1,208 amino-acid polypeptide of 138K (Fig. 3*a*, *b*). The 5′-region of *AXL1* has sequences that resemble **a**1-α2 repression sites (Fig. 3*b*) to which the **a**1-α2 repressor may bind. To examine the effect of ectopic expression of *AXL1*, we prepared a construct (YEUp-AXL1Lp) which can express *AXL1* by the promoter of *LEU2* in all cell types (see legend to Table 1). The ectopic expression of *AXL1* permitted **a**/α cells to exhibit axial budding at high frequency (class 1 = 67%; 37% in diploid cells in which *AXL1* is not expressed) (Table 1). This evidence strongly suggests that *AXL1* is a key morphological determinant for the yeast budding pattern. However, the total promoter activity of *LEU2* on high-copy vector in **a**/α cells was about 10 times higher than that of *AXL1* on low-copy vector in **a** cells (luciferase assay was used as a reporter gene; data not shown). Thus, this result may be due to overexpression rather than only ectopic expression.

The predicted amino-acid sequence of *AXL1* contains regions showing extensive similarity to domains of human and *Droso-*

TABLE 1 Quantitative analysis of bud-site selection by wild-type strains, mutant strains defective in *AXL1, BUD4* and *BUD5* genes, and wild-type diploid strains containing the *AXL1*-expressing plasmid

Strains		Relevant genotype	Class 1	Class 2 (or)	Class 3
DBY747-SB92DL	*MAT***a**	*AXL1*	93	1	6
YAF208	*MAT***a**/*α*	*AXL1/AXL1*	35	7	58
CPY20	*MAT***a**	*axl1-1*	32	8	60
YAF203	*MAT***a**	*axl1-Δ1::URA3*	35	5	60
YAF204	*MAT***a**	*bud5::HIS3*	7	69	24
YAF201	*MAT***a**	*axl1-Δ1::URA3 bud5::HIS3*	8	70	22
YAF209	*MAT***a**	*bud4*	34	7	59
DBY746-SB92DL	*MATα*	*AXL1*	95	1	4
YAF210	*MATα*	*axl1-Δ1::URA3*	34	4	62
YAF208 [YEUp3]	*MAT***a**/*α*	*AXL1/AXL1*	37	3	60
YAF208 [YEUp-AXL1Lp]	*MAT***a**/*α*	*AXL1/AXL1*	67	1	32

Disruption of the *ACE2* gene[10,11] causes a defect in cell separation. Although *ace2* strains with axial budding produce hemispherical and lustrous colonies with smooth circular outlines, *ace2* mutants with bipolar budding produce rugged and lusterless colonies with notched outlines. An *axl1-1* mutant (CPY20) with bipolar budding was isolated from an *ace2* strain. CPY20 contained a mutation (not *sir* mutation[12]), *axl1-1*, so named because the wild-type allele is necessary for the axial-budding pattern. Each cell with a single bud and a single bud scar was assigned to one of three classes[13]. Cells of three classes are schematically shown. The open and hatched ovals indicate mother cells and buds, respectively; bud scars are depicted as small rings. The caps covering an area around each end of mother cells are shaded. Cells of class 1 would have a single bud and a single scar both in one cap at one end. Cells of class 2 would have one bud or scar in the cap at one end, and the other scar or bud not in the caps. Cells of class 3 would have one bud or scar in the cap at one end, and the other scar or bud in the cap at the other. Class 1 is characteristic of the axial pattern (also found in bipolar pattern); class 2 is characteristic of a random pattern; class 3 is characteristic of a bipolar pattern. At least 300 cells were scored for each strain. Numbers indicate the percentage of cells in each class. Strains used in this analysis were: DBY747-SB92DL, *ace2::LEU2* in DBY747; DBY746-SB92DL, *ace2::LEU2* in DBY746; YAF208, a cross between DBY747-SB92DL and DBY746-SB92DL; CPY20, *axl1-1* in DBY747-SB92DL; YAF203, *axl1-Δ1::URA3* in DBY747-SB92DL; YAF204, *bud5::HIS3* in DBY747-SB92DL; YAF201, *axl1-Δ1::URA3 bud5::HIS3* in DBY747-SB92DL; YAF209, *MAT***a** *bud4* in DBY747-SB92DL and YAF210, *axl1-Δ1::URA3* in DBY746-SB92DL. Square brackets denote the containing plasmid: YEUp3 vector (pUC13 + *URA3* + 2 μm ori DNA); YEUp-AXL1Lp which has a fusion of the *LEU2* promoter and *AXL1* ORF in YEUp3.

phila insulin-degrading enzymes (IDEs)[3,4] which may play a role in the cellular processing of insulin, and *E. coli ptr* gene product (Ptr)[5] that could hydrolyse the B-chain of insulin as well as human IDE[8,9] (Fig. 3*c*). The close similarity of four proteins among distantly related species such as *E. coli*, *S. cerevisiae*, *Drosophila* and human, suggests a common ancestor. It is thus possible that Axl1 functions as a protease, in a manner analogous to IDE. The identity values (%) for each pair-wise alignment are given in Fig. 3*d*. The sequences of the human IDE and *E. coli* Ptr can be aligned, except for one large gap in their amino-terminal regions (Fig. 3*e*). In contrast, the nucleotide sequence of *AXL1* predicts many additional amino-acid residues that cannot be aligned (Fig. 3*e*). The rather low similarity and the many insertions of the additional amino-acid residues suggest that the Axl1 may function as a protease, but that it might have diverged from others. Axl1 does not include a signal peptide, as found in *E. coli* Ptr.

This study shows that *AXL1* is a haploid-specific gene and that ectopic expression of *AXL1* allowed **a**/*α* cells to exhibit an axial budding pattern. Thus, Axl1 is likely to be a key morphological determinant that converts the bipolar budding to axial. Although the structures and roles of the Bud3 and Bud4 are unknown, they may contribute to the establishment of axial

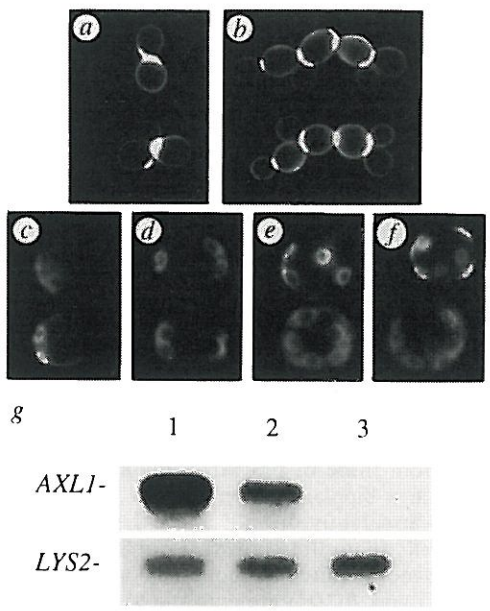
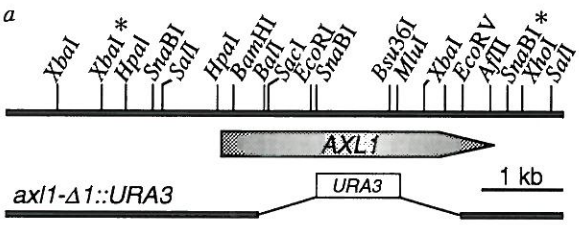


FIG. 2 Expression of *AXL1*. *a–f*, Phenotypes of strains defective for *AXL1* (*a*, *b*) and epistatis relationship of *bud5* and *axl1* null mutations (*c–f*). Cells were treated with Calcofluor[14] to stain bud scars. Fluorescence micrographs of strain DBY747-SB92DL (*ace2*) (*a*); CPY20 (*ace2 axl1-1*) (*b*); DBY747 (wild-type) (*c*); YAF203 (*axl1-Δ1::URA3*) (*d*); YAF204 (*bud5::HIS3*) (e); and YAF201 (*axl1-Δ1::URA3 bud5::HIS3*) (*f*). These strains are isogenic except at the *ACE2*, *AXL1* and *BUD5* loci. *g*, Northern analysis of the *AXL1* gene. RNAs from the following strains were electrophoresed, blotted and probed with *AXL1* DNA (*MluI–EcoRV* fragment) and *LYS2* DNA (*XhoI–EcoRV* fragment) as a control for cell-type-nonspecific gene: lane 1, DBY747 (*MAT***a**); lane 2, DBY746 (*MATα*); and lane 3, YAF106 (*MAT***a**/*α*). DBY746 (*MATα his3Δ ura3-52 leu2-3, 112 trp1-289*) and DBY747 (*MAT***a** *his3Δ ura3-52 leu2-3, 112 trp1-289*) were wild-type strains obtained from the Yeast Genetic Stock Center, California. YAF106 is the result of a cross between DBY746 and DBY747. Total RNA was isolated from wild-type strains. Poly(A)$^+$RNA (8 μg) of each cell type was resolved by electrophoresis on a 1.2% agarose gel. Hybridization was as described elsewhere[15]. Genotypes of other strains used for this study are shown in Table 1 legend.

FIG. 3 Restriction map, nucleotide and predicted amino-acid sequences of *AXL1*; similarity of Axl1 with human and *Drosophila* IDEs and *E. coli* Ptr; pair-wise sequence comparison and schematic representation of these proteins. *a*, Restriction map of *AXL1*. The nucleotide sequence from the *Xba*I to the *Sna*BI sites marked by asterisks was determined (DDBJ accession number D17787). The position of ORF and structure of the fragment used to disrupt *AXL1* are noted. *b*, Nucleotide sequence of 5′ upstream region of *AXL1* with the N-terminal amino-acid sequence of Axl1. Possible **a**1-α2 repression sites (5′-ATGTNNNNNNNTACATCA-3′)[16–18] and two *Hpa*I sites are underlined. *c*, Alignment of the amino-acid sequences of human and *Drosophila* IDEs[3,4], yeast Axl1 and *E. coli* Ptr[5]. White and shaded letters indicate identical and conserved residues, respectively. Dashes indicate gaps to produce optimal alignment. *d*, Pairwise sequence comparison. Alignments and calculations were done using the FASTA and ODEN programs[19]. *e*, Schematic representation of *E. coli* Ptr, human IDE and yeast Axl1. Regions that have strong similarities (shaded boxes) and no similarity (black boxes) are noted.

METHODS. To clone the *AXL1* gene, the *axl1-1* strain was transformed using a yeast genomic library[15]. A plasmid (pSH1CU) that complements the *axl1* defect was isolated. A 1.4 kb *Eco*RI–*Xba*I fragment of the insert was subcloned into integrative vector YIp5[20] that contains a *URA3* marker. This construct was used to direct integration by homologous recombination into CPY20 (*axl1-1*). One of the transformants (*axl1*$^-$ URA$^+$) was crossed with a wild-type strain defective in *URA3*. In all 21 tetrads, the *URA3* marker segregated with the bipolar pattern, confirming genetic linkage between the mutant locus and the cloned DNA. For gene disruption, the *Eco*RV–*Ball* fragment was replaced with the *URA3* marker. This was used to replace the wild-type *AXL1*[21] gene. Disruption was confirmed by Southern analysis.



b

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GAGATTCCTAAAAGCTAAAACAAATCCATTGATAATATAATAGTAGGTTGGTCAATTCAC     176
CGCAGATTCAATGTAAGTATTGATACTCCATAAATGTTAACTTCAGGTTGATATTCGTTA     236
                                   HpaI
CAAAAATCTTAGTTTGCTTTTCATTACTATCTGAACGGAATGCTCAACATTTTTCTTATT     296
CTACAATTGTACAATGTCACGTAAACAGTTTGATTTGTGTATTTACAGTGGAAACATTTT     356
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GACAATTGCTTGAATTTGCTGAACACTTTTCGAGGTACATTCTGATAAAGAGTGCATTAT     476
TAGTTATTTAGAGTATATTAAGACATCTTCTCGAAGATATATAAATTCGTAAAGGAAACA     536
AAAATTTCGGGATAACAAAAAAGTTTCTTCTTTTTACGTATTAAATGGTCTATTCATCGA     596
TCCTATTTCACTATCAATCCTATTATATTATCAACTTTTGCATTTCTGCTTCCACCAAAG     656
TCGACGGCTGTTTCTCATCGTTTATGTAATATCATAACGCATGTATGACATTATATCAGT     716
GGCATGAGCGCTAGTCCATGAAATAGGAGTTTAACTCCAACATACGGAACATTCGCATTA     776
TCGCACACGTAAGACTCGATATATTAAGGAAGGAATTTCCGAGTTGGATTAATAAAGGAA     836
AAATAAAATATAGTGTTGGAAATATAGTTGCCTCAGAATAATATCAATATAATATTAAAA     896
CGTATATAGTTAGATACCCGGTATTATTCGAGTACGCATTAATTCAGCAGTATGACTTTC     956
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GGAATTGACTATCGTCACTGAAGAGTATTCGTATTATGGTATTATGATACATTGTCTAAA    1076
AAAGGATACCATTTTTCTTCAATAGAACAAGAAGAGAATCTAAGGACACCATTTCGAGAA    1136
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GCGGAGTTACTTGATGCGGTATTTTACATGCCTTTTTTTCACTGCAAGAAAAAAATGAAAC    1436
ACTTATTTACACGATTTTCAGGATAGTTTACGCTGGTGAGTATGCAGAACAGTTAACACC    1496
                                                   HpaI
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GCAACTGAATAAGTTTTTTTACTGATGTCCTTGAGAGAAGTAACTAATTATGAAGTCTCG    1616
                          M  S  L  R  E  V  T  N  Y  E  V  S      12
TTTTACATCCCGTTGTCTTACAGCAACAGGACTCATAAAGTTTGTAAACTGCCAAACGGA    1676
F  Y  I  P  L  S  Y  S  N  R  T  H  K  V  C  K  L  P  N  G      32
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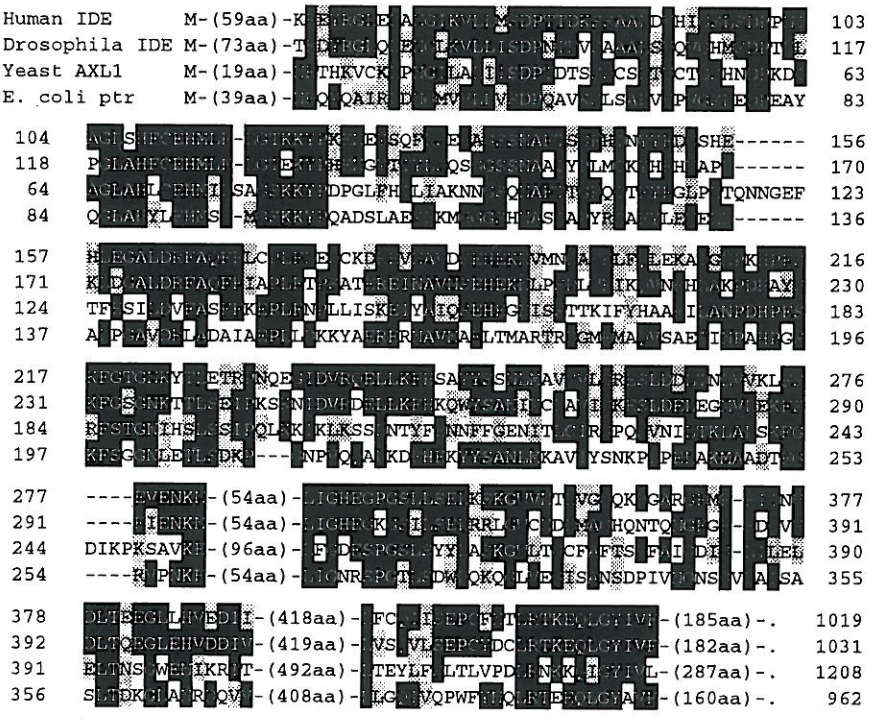
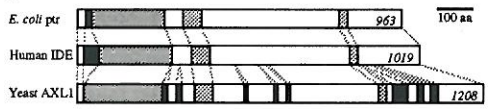
c



d

	Drosophila IDE	*E. coli* ptr	Yeast AXL1 (%)
Human IDE	45	26	19
Drosophila IDE		26	19
E. coli ptr			21

e



budding with Axl1. We have recently identified a mutation (*rax1*) that can convert the budding pattern of *axl1* null strain from bipolar to axial (unpublished data). The putative amino-acid sequence of Rax1 contained several conserved residues of B-chains of the insulin-related hormone superfamily (A.F. *et al.*, unpublished data). Characterization of this gene may provide new insight into the control of bud-site selection. □

Received 16 May; accepted 31 October 1994.

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ACKNOWLEDGEMENTS. We thank I. Herskowitz, J. Chenevert and S. Sanders for helpful discussions and providing *bud* mutants, R. A. Roth for providing human IDE cDNA, H. Hashimoto for providing vectors YCU1, J. Chant, J. Pringle and I. Herskowitz for permission to cite unpublished results, and I. Herskowitz and M. Ohara for helpful comments on the manuscript.