Alkaline Response Genes of *Saccharomyces cerevisiae* and Their Relationship to the RIM101 Pathway

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Environmental pH exerts broad control over growth and differentiation, but the molecular responses to external pH changes are poorly understood. Here we have used open reading frame macroarray hybridization to identify alkaline response genes in *Saccharomyces cerevisiae*. Northern or lacZ fusion assays confirmed the alkaline induction of two ion pump genes (*ENA1* and *VMA4*), several ion limitation genes (*CTR3*, *FRE1*, *PHO11/12*, and *PHO64*), a siderophore-iron transporter gene (*ARN4/ENB1*), two transcription factor genes (*NRG2* and *TIS11*), and two predicted membrane protein genes (*YAR068W/YHR214W* and *YOL134W*). Unlike *ENA1* and *SHC1*, these new alkaline response genes are not induced by high salinity. The known pH-responsive genes in other fungi depend on the conserved PacC/Rim101p transcription factor, but induction of several of these new genes relied upon Rim101p-independent pH signaling mechanisms. Rim101p-dependent genes were also dependent on Rim13p, a protease required for Rim101p processing. The Rim101p-dependent gene *VMA4* is required for growth in alkaline conditions, illustrating how Rim101p may control adaptation. Because Rim101p activates ion pump genes, we tested the role of *RIM101* in ion homeostasis and found that *RIM101* promotes resistance to elevated cation concentrations. Thus, gene expression surveys can reveal new functions for characterized transcription factors in addition to uncovering physiological responses to environmental conditions.

Extracellular pH is a key environmental signal that influences growth, physiology, and differentiation (1–9). Specialized responses to pH changes have been characterized in many organisms, but there is limited information concerning the extent and general features of such a response. Our objective is to use *Saccharomyces cerevisiae* as a model to understand transcriptional responses to a change in external pH.

Yeast cells grow more rapidly in acidic media than in neutral or alkaline media. They neutralize the cytoplasm through activity of Pma1p, the plasma membrane H⁺-ATPase, which hydrolyzes ATP to pump protons out of the cell (10). The proton gradient is used for transport of amino acids, nucleotide bases, phosphate, and many other molecules in symport reactions (10). It also provides an electrochemical gradient that favors uptake of cations (11, 12). Thus, the plasma membrane proton gradient is required for accumulation of solutes from the medium, and *PMA1* is an essential gene (10, 13).

In neutral and alkaline conditions, two other ion pumps are vital for growth: the plasma membrane Na⁺-ATPase (called Ena1p or Pmr2p (14)) and the multi-subunit vacuolar membrane H⁺-ATPase (15). Ena1p hydrolyzes ATP to pump Na⁺ out of the cell, and the Na⁺ gradient permits uptake of other cations. The vacuolar ATPase is required for vacuolar acidification, which cannot occur through endocytosis in alkaline media (16, 17). Null mutants lacking these pumps are hypersensitive to alkaline growth conditions and elevated external cation concentrations but are capable of growth in acidic media.

External pH changes cause a transcriptional response in *S. cerevisiae*. There are three known alkaline-inducible genes: the Na⁺-ATPase structural gene *ENA1* (14, 18), *SHC1*, and *SCY1* (19). *ENA1* and *SHC1* are also induced in high salt medium; results for *SCY1* have not been reported. Thus, it is possible that external pH is sensed by factors that also sense external salt concentration, thereby triggering a common signal transduction cascade and activating a common set of genes. Alternatively, it is possible that a unique regulatory pathway senses and responds to external pH changes.

Transcriptional responses to external pH have been characterized in several other fungi. For example, alkaline pH induces transcription of *IPNA* and other penicillin biosynthetic genes in *Aspergillus nidulans* and *Penicillium chrysogenum* (20, 21), the protease gene *XPR2* in *Yarrowia lipolytica* (22), and cell wall protein genes in *Candida albicans* (23–25). These responses depend upon a conserved signal transduction pathway (20, 22, 26–29) that is best understood from work on the *A. nidulans* transcription factor PacC. PacC is required for responses to alkaline pH. It accumulates in an apparently inactive full-length form under acidic growth conditions and is activated by C-terminal proteolytic cleavage under alkaline growth conditions (30). Several alkaline response regulatory genes, *palA, palB, palC, palP, palH, and palI*, are required for proteolytic cleavage of PacC (30, 31). Their sole known function is to promote PacC cleavage, because loss-of-function *pal* mutations cause similar phenotypes to loss-of-function *pacC* mutations and because expression of C-terminally truncated PacC derivatives suppresses *pal* mutant defects (30, 31).

*S. cerevisiae* has a PacC-like pathway. Rim101p is a homolog of PacC, and it is activated by C-terminal proteolytic cleavage (32) that depends upon *pal* gene homologs (26, 32–34). However, unlike PacC cleavage, which is completely dependent upon external alkaline pH, Rim101p cleavage occurs under both acidic and alkaline growth conditions (32). Therefore, it is uncertain whether the *S. cerevisiae* *RIM101* pathway also governs pH-responsive gene expression.

Here we have used a gene expression survey to identify pH-responsive *S. cerevisiae* genes. We have used these genes to...
determine the relationship between gene induction by alkaline pH and by salt. In addition, we have asked whether the S. cerevisiae RIM101 pathway is required for alkaline gene expression, and how cleavage of Rim101p impinges upon regulation of alkaline genes. Our results provide insight into both the functional conservation of the PacC/RIM101 pathway as a pH response pathway and upon the nature of external pH as a global transcriptional regulatory signal.

**EXPERIMENTAL PROCEDURES**

**Strains—**Yeast strains (Table I) were derived from strain SK1 (35) through genetic crosses and transformation. The RIM101-HA2, RIM101-531-HA2-TRP1, and rim101Δ12 alleles have been described (32); the rim13Δ::HIS3 and rim13Δ::TRP1 mutations are described below.

**Media, Growth Conditions, and PCR—**Yeast growth media (YPD and SC) were of standard composition (36). For growth in liquid YPD medium at a specified pH, YPD containing 0.1 M HEPES was titrated before autoclaving. Other additions to YPD included 0.4 or 0.8 M NaCl, 1.2 M sorbitol, 50 mM Tris-HCl, pH 8.0, 0.2% SDS, and were incubated at 30° C. Other cultures were grown to saturation in pH 4 YPD or pH 8 YPD and then inoculated at an 0.2 in pH 4 YPD or pH 8 YPD and grown for at least two doublings. β-Galactosidase assays were carried out on permeabilized cells as described (36), except that cells were suspended in 0.15 ml of Z buffer, permeabilized, and assayed with 0.7 ml of 1 mg/ml o-nitrophenyl-β-D-galactoside in Z buffer.

**GeneFilter Analysis—**Yeast ORF1, GeneFilters (Research Genetics, GF1001 and II) were probed with labeled cDNA from strain TLY759 as follows. A late exponential pH 4 YPD culture was used to inoculate 250 ml cultures of pH 4 YPD and pH 8 YPD at an A600 of 0.375. After 2 h, cells were collected by filtration and frozen at −80° C; at this time the pH 4 culture had reached an A600 of 0.905 and pH 3.6, and the pH 8 culture had reached an A600 of 0.625 and pH 7.5. Total RNA was produced as follows. Thawed cell pellets were resuspended in 20 ml AE (50 mM sodium acetate, pH 5.2, 10 mM EDTA) and brought to 1% SDS. Portions of these samples of serial 5-fold dilutions in YPD were spotted on test plates. All cultures and plates were incubated at 30° C.

For β-galactosidase assays, strains were grown to saturation in pH 4 YPD and then inoculated at an A600 < 0.2 in pH 4 YPD or pH 8 YPD and grown for at least two doublings. β-Galactosidase assays were carried out on permeabilized cells as described (36), except that cells were suspended in 0.15 ml of Z buffer, permeabilized, and assayed with 0.7 ml of 1 mg/ml o-nitrophenyl-β-D-galactoside in Z buffer.

**Northern Blots—**For Northern analysis, 15–20 μg of total RNA per lane was run on denaturing formaldehyde gels, transferred to a nylon membrane and hybridized as described (37). To generate Northern blot probes, genomic DNA was PCR-amplified using the primers designated with “N” followed by the gene name in Table II. The RNA1 and ENO1 PCR fragments were gel purified, labeled with α-32PdCTP using a High prime kit (Roche), and QIAquick PCR purified (Qiagen). All other PCR-amplified fragments were cloned into plasmid pGEM-T Easy (Promega), and plasmid inserts were obtained by NodI digestion, gel purified, and labeled as described above.

**lacZ Fusion Genes—**To generate lacZ fusion genes, 1.5 kilobase pairs of DNA sequence upstream of the desired gene was PCR-amplified from genomic DNA using the primers designated with “L” followed by the gene name in Table II. PCR products were cloned into plasmid pGEM-T Easy (Promega) to yield the plasmids pAED54 (VMA4 5′), pAED50 (YOL154W 5′), pAED51 (ARN4 5′), pAED49 (ENA1 5′), and pAED53 (TSI15 5′). A PsI-EcoRI fragment from plasmid pAED54 was cloned into PsI- and EcoRI-cut plasmid YIp556R (38) to yield plasmid pTL112 (ena4-lacZ), which includes 1.5 kilobase pairs of VMA4 5′ sequences and fuses the VMA4 initiation codon in-frame to lacZ. Also, a PsI-EcoRI fragment from plasmid pAED63 was inserted into PsI- and EcoRI-digested plasmid YIp556R to yield plasmid pTL111 (tis11-lacZ). Similar fusions were created by inserting SpH1-Sphl fragments of plasmids pAED49, 50, and 51 into SplI-cut plasmid YIp556 to yield plasmids pTL107 (ena1-lacZ), pTL105 (yol154W-lacZ), and pTL106 (ura4-lacZ), respectively. For transformation into yeast, each plasmid was cut with Stul to direct integration to the ura3 locus.

**RIM13 Cloning and Analysis—**We identified RIM13 (YMR154C) initially through a S. cerevisiae genomic BLAST search for a PolB homolog (60). Our conclusion that YMR154C is RIM13 is based upon tests of phenotype, complementation, and linkage. For phenotypic tests, a ymr154C::TRP1 insertion-deletion allele (rim13Δ10::TRP1) that removes the entire ORF was introduced into haploid strain AMP1604 by transformation and was moved into meiotic segregants for analysis of diploids. The mutation caused an invasive growth defect in haploids and a sporulation defect in homzygous diploids, as described for rim13Δ (20). Complementation tests: ymr154C::TRP1 strains failed to complement rim13Δ strains for sporulation ability and for expression of a meiotic ime2-2 lacZ reporter gene. In addition, a plasmid carrying YMR154C complemented the Rim101p processing defect of a rim13Δ-1 mutant. For the linkage test, a cross of a ymr154C::TRP1 strain to a rim13Δ strain yielded exclusively 0+/−4− meiotic segregation of invasive growth ability in 24 tetrad, as expected from tight linkage of YMR154C and RIM13. Thus all tests indicate that YMR154C is RIM13.

During the course of our studies, RIM13 was independently described as CPL1 (34).

**TABLE I**

| Strain | Genotype* |
|--------|-----------|
| AMP1604 | RIM101-HA2 MATα ME2-5-lacZ::URA3 |
| AMP1689 | rim13Δ10::TRP1 RIM101-HA2 MATa ME2-5-lacZ::URA3 |
| AMP1800 | RIM101-HA2 MATα arg8 gal80::LEU2 |
| TLY758 | rim101Δ12 MATα TRP1 |
| TLY759 | RIM101 MATα TRP1 |
| TLY814 | Rim13 RIM101-531-HA2 MATa gal80::LEU2 TRP1 |
| TLY816 | rim13Δ::HIS3 RIM101-HA2 MATa gal80::LEU2 TRP1 his3ΔSK |
| TLY818 | Rim13 RIM101-531-HA2-TRP1 MATa gal80::LEU2 |
| TLY820 | rim13Δ::HIS3 RIM101-531-HA2-TRP1 MATa gal80::LEU2 his3ΔSK |
| WXY169 | RIM101-HA2 MATα his3ΔSK gal80::LEU2 |
| WXY170 | RIM101-HA2 MATα his3ΔSK gal80::LEU2 RME1 met4 |

* All strains were of genotype ura3 trpl leu2 lys2 ho::LYS2 rme13Δ::LEU2, except as indicated, and carried additional markers listed in the table.

1 The abbreviations used are: ORF, open reading frame; PCR, polymerase chain reaction.
The rim13Δto::TRP1 mutation was constructed as follows. Primers palB-ATG and palB-TAA were used to amplify a TRP1 cassette, and the PCR product was transformed into haploid strain AMP1604. Homologous integration was verified by PCR with flanking primers palB-620 and palB+2526.

The rim13Δ::HIS3 mutation was constructed as follows. Primers Pada-RIM13F and Pada-RIM13R were used to amplify the HIS3-MX6 cassette (Schizosaccharomyces pombe his55) from plasmid pFA6aHis3MX6 (39). Primers pRIM13–5′-F and pRIM13–3′-R were used to amplify 500 base pairs of FRE1, and PHO12 (a duplicated gene pair), metal transport genes including phosphate metabolism genes, which were induced at higher levels at pH 8 than at pH 4 (Fig. 1). These genes were expressed at 10–100-fold higher levels at pH 8 than at pH 4 (Fig. 1). These results indicate that expression of several genes increases substantially in response to a shift from acidic to alkaline pH. We refer to these genes below as alkaline response genes.

To determine whether alkaline pH and high salt induce the same genes, we examined expression of alkaline response genes after treatment of cells with 0.8 M NaCl (Fig. 1). Levels of a control transcript from ENO1/ENO2 (a duplicated gene pair) were unaffected by external pH. Two additional transcripts were not reliably detected on Northern blots but gave alkaline-dependent GeneFilter signals: VMA4, a vacuolar ATPase subunit gene, and YOL154W, a putative cell surface protein gene. To verify pH-dependent expression of these genes and of TIS11, a transcription factor gene, we created strains carrying the respective fusion genes and assayed β-galactosidase activity after growth at pH 4 and pH 8 (Table IV, RIM101 strain). Control ena1-lacZ and arn4-lacZ fusions were expressed at 100-fold higher levels at pH 8 than at pH 4, as expected from Northern analysis, and the lacZ fusion vector with no insert was not expressed at either pH. The vma4-lacZ, yol154W-lacZ, and tis11-lacZ fusions were expressed at 100-fold higher levels at pH 8 than at pH 4. These results indicate that expression of several genes increases substantially in response to a shift from acidic to alkaline pH. We refer to these genes below as alkaline response genes.
to alkaline pH and high salt are distinct.

Requirement for Rim101p in Alkaline Response Gene Expression—To determine whether Rim101p is required for alkaline response gene expression in *S. cerevisiae*, we compared alkaline response transcript levels in *RIM101* and *rim101Δ* strains (Fig. 2). The strains were grown at pH 4 and then shifted to pH 4 or pH 8 for 2 h prior to RNA preparation. Under these conditions, growth of the two strains was comparable. Northern analysis revealed that there are three classes of genes. One class was not induced at pH 8 in the *rim101Δ* strain; this class included *ARN4* and *YAR068W/YHR214W*. The second class was partially induced at pH 8 in the *rim101Δ* strain; this class included *ENA1* and *NRG2*. The third class was induced at pH 8 in the *rim101Δ* strain to levels as great or greater than in the *RIM101* strain; this class included *PHO11*/*PHO12*, *PHO84*, *FRE1*, and *CTR3*. Control transcripts *UBC4* and *ENO1/2* were unaffected by the *rim101Δ* mutation. These results indicate that Rim101p is required for expression of some, but not all, alkaline response genes.

We assayed expression of *lacZ* fusion genes in *RIM101* and *rim101Δ* strains to extend this analysis (Table IV). At pH 8, the *ena1-lacZ* fusion was expressed at slightly lower levels in the *rim101Δ* strain than in the *RIM101* strain, and the *arn4-lacZ* fusion was expressed at 30-fold lower levels in the *rim101Δ* strain than in the *RIM101* strain. These results were consistent with Northern analysis. We found that *vma4-lacZ* and *yol54W-lacZ* were expressed at 10–100-fold lower levels at pH 8 in the *rim101Δ* strain, compared with the *RIM101* strain. Expression of *tis11-lacZ* was similar in *rim101Δ* and *RIM101* strains. Taken together, Northern analysis and *lacZ* fusion assays indicate that the set of alkaline response genes that are largely dependent upon Rim101p includes *ARN4*, *VMA4*, *YAR068W/YHR214W*, and *YOL54W*.

Role of Rim101p Processing in Alkaline Signal Transduction—Rim101p, like PacC, is activated by proteolytic removal of a C-terminal segment. We used a *rim13Δ* mutant to deter-
mine whether C-terminal proteolysis is required for alkaline response gene expression. The rim13Δ null mutation, like rim13-1 (32), blocks Rim101p processing, as determined with epitope-tagged Rim101-HA2p expressed from the RIM101 genomic locus: a haploid RIM13 RIM101–531-HA2 strain yielded mainly the 531 strain grew poorly in the presence of AMP1689 (RIM13 RIM101–531-HA2), and AMP1800 (RIM13 RIM101–HA2, lane 2) to yield meiotic progeny of two tetrads (lanes 3–6 and 7–10, respectively). Epitope-tagged Rim101-HA2p was visualized on an anti-HA immunoblot of extracts ofYPD-grown cells. Migration of full-length Rim101-HA2p at 98 kDa and of processed Rim101-HA2p at 90 kDa (32) was confirmed on this gel with control RIM13 RIM101–HA2 and rim13-1 RIM101–HA2 extracts.

FIG. 3. Dependence of Rim101p processing on RIM13. Strain AMPl689 (rim13Δ:TRP1 RIM101-HA2, lane 1) was crossed to strain AMPl800 (RIM13 RIM101–HA2, lane 2) to yield meiotic progeny of two tetrads (lanes 3–6 and 7–10, respectively). Epitope-tagged Rim101-HA2p was visualized on an anti-HA immunoblot of extracts ofYPD-grown cells. Migration of full-length Rim101-HA2p at 98 kDa and of processed Rim101-HA2p at 90 kDa (32) was confirmed on this gel with control RIM13 RIM101–HA2 and rim13-1 RIM101–HA2 extracts.

**TABLE V**

| Gene fusion | pH of growth medium | RIM13 RIM101 strain | rim13Δ rim13Δ strain | RIM12 RIM101–531 strain | rim13Δ rim13Δ strain |
|-------------|---------------------|---------------------|----------------------|------------------------|----------------------|
| ena1-lacZ   | 4                   | 0.27                | 0.10                 | 2.7                    | 1.7                  |
|             | 8                   | 21.7                | 25.9                 | 102.6                  | 75.8                 |
| arr4-lacZ   | 4                   | 0.02                | 0.03                 | 0.04                   | 0.04                 |
|             | 8                   | 2.6                 | 0.1                  | 12                     | 6.0                  |
| vma4-lacZ   | 4                   | 0.01                | 0.01                 | 0.02                   | 0.02                 |
|             | 8                   | 5.9                 | 0.3                  | 10.7                   | 5.5                  |
| yol154W-lacZ| 4                   | 0.2                 | 0.1                  | 4.7                    | 2.6                  |
|             | 8                   | 6.4                 | 0.3                  | 12.6                   | 12.4                 |

* Each lacZ fusion gene was integrated at the URA3 locus of strains TLY814 (RIM13 RIM101–HA2), TLY816 (rim13Δ: HIS3 RIM101–HA2), TLY818 (RIM13 RIM101–531–HA2), and TLY820 (rim13Δ: HIS3 RIM101–531–HA2). Strains were grown to saturation inYPD pH 4 and then grown for at least two doublings inYPD pH 4 orYPD pH 8, as indicated. β-Galactosidase activities (Miller units) are the mean of three or four independent transformants, and standard deviations were within 30% of each mean.

**FIG. 2.** Role of Rim101p in expression of alkaline response genes. Strains TLY758 (rim101Δ, lanes 1 and 2) and TLY759 (RIM101, lanes 3 and 4) were grown in pH 4 YPD (lanes 1 and 3) and pH 8 YPD (lanes 2 and 4) for preparation ofRNA. Transcript levels were detected by Northern analysis with probes for the genes listed at the left side of each panel. The numbers under each lane are the number of cpm’s in that lane, normalized to the number of cpm’s in the ENO1/2 control lane and expressed as a percentage of the TLY759 pH 8 signal (lane 4).
were spotted on a control YPD plate and on YPD with these additions on media containing the cationic aminoglycoside hygromycin B. Growth was visualized after 2–6 days at 30°C. Metal limitation that arises from disruption of the plasma membrane proton gradients, which normally supply energy for nutrient and ion transport (10). Prior studies indicated that yeast responds by expressing ENA1, a plasma membrane Na\(^+\)-ATPase gene (14, 18). We found here that VMA4 is also induced by alkaline conditions. VMA4 encodes a subunit of the vacuolar H\(^+\)-ATPase, which is required for growth in alkaline media (43, 44). VMA4 is a logical target of regulation, because Vma4p levels are thought to govern vacuolar ATPase assembly (15, 45). Our results argue that cells adapt to alkaline conditions through increases in both vacuolar and plasma membrane ion pump activity. In addition, we found that alkaline induction of VMA4 depends upon RIM101. Thus, alkaline conditions may induce an adaptive increase in vacuolar ATPase activity through RIM101-dependent VMA4 induction.

A second effect of external alkalinity may be nutrient and ion limitation that arises from disruption of the plasma membrane proton gradient. Consistent with this idea, several alkaline response genes are transporters, and others are targets of known solute-specific regulatory pathways: CTR3 and FRE1 are targets of the copper-responsive transcription factor Mac1p (46, 47); YOR154W is a target of zinc-responsive transcription factor Zap1p (48); PHO11/PHO12 and PHO84 are targets of the phosphate-responsive transcription factor Pho4p (49, 50). The fact that Pho11p and Pho12p are acid phosphatases (51) further suggests that their induction by alkaline pH is not a specific response to alkaline conditions. However, alkaline response genes do not correspond entirely to other sets of starvation- or stress-induced genes. ENA1, HXT2, and SHC1 are induced by high salt (19, 40, 41, 52), but other alkaline response genes are not. In addition, alkaline response genes are not uniformly induced by stationary phase, heat shock, sporulation, growth inhibition by 3-aminotriazole or lack of calcineurin (53–56). Thus, the response to alkaline pH may be in part a stress and starvation response, but it has unique attributes that distinguish it from other growth limitation conditions.

Because alkaline conditions inhibit yeast growth, some alkaline response genes may facilitate competition with bacteria. In S. cerevisiae, the alkaline-induced transporter Arn4p imports a bacterial siderophore-iron complex (57), thus capitalizing on a bacterial iron acquisition mechanism. In A. nidulans and P. chrysogenum, penicillin synthesis is stimulated at alkaline pH (20, 21). ARN4 and the penicillin biosynthesis genes depend upon Rim101p or PacC for expression. Therefore, the functions of some Rim101p/PacC-dependent alkaline response genes may only be apparent in competitive growth or survival tests.

Despite limited information on alkaline response genes in other eukaryotes, there is an example of a conserved alkaline response gene. YOL154W is the closest S. cerevisiae homolog of the alkaline inducible C. albicans gene PRA1, which encodes a cell surface protein with similarity to Zn metalloproteases (25). YOL154W is known to be a zinc limitation gene (48), as are its homologs in Aspergillus species (58). However, expression of YOL154W/PRA1 in alkaline media cannot solely be due to impaired zinc uptake, because other zinc limitation genes (48) are not alkaline-induced. We found that expression of S. cerevisiae YOL154W, like C. albicans PRA1 (26), depends upon Rim101p. These observations suggest that the relationships among Rim101p, zinc metabolism, and YOL154W/PRA1 may be conserved in diverse fungi. In S. cerevisiae Rim101p interacts with the zinc response transcription factor Zap1p (59). We suggest that Rim101p-Zap1p interaction may be the basis for conserved pH- and zinc-responsive control of YOL154W/PRA1. We anticipate that many other S. cerevisiae alkaline response genes will have alkaline-regulated homologs in other eukaryotes and that other Rim101p/PacC target genes will be conserved.

There are several classes of alkaline response genes, defined by their relationships to the RIM101 pathway. One class of genes is independent of the RIM101 pathway and includes PHO11/12, PHO84, CTR3, FRE1, and TIS11. In contrast to the previously known alkaline response genes in C. albicans, Y. lypolytica, and Aspergillus species, which are all dependent on Rim101p/PacC, this new class may account for Rim101p-independent biological responses to pH (26). A second class is only weakly dependent on Rim101p and includes ENA1 and NRG2. A third class is dependent on Rim101p but is not expressed in acidic medium in the presence of truncated Rim101–531p and includes ARN4 and VMA4. For this class, Rim101p signaling is necessary but not sufficient to promote alkaline expression. A fourth class is also dependent on Rim101p but is expressed in acidic medium in the presence of Rim101–531p and includes YOL154W. The response of this class parallels the PacC/Rim101p target genes of other fungi in that Rim101p signaling is both necessary and sufficient for expression. This finding provides strong genetic evidence that the S. cerevisiae RIM101 pathway is a pH-sensing pathway. We have yet to determine the molecular basis for control of alkaline response genes by Rim101p, but the presence of a PacC recognition sequence (GCCARG) is not sufficient to predict alkaline inducibility or RIM101 dependence. For example, the UBC4 gene has three GCCARG sites within 500 base pairs upstream of its start, but its expression is neither alkaline-induced nor Rim101p-dependent. We expect that detailed analyses of these different classes of S. cerevisiae alkaline response genes will reveal novel mechanisms of pH-dependent gene regulation.

The fact that ion pump genes are among Rim101p targets led
us to infer that Rim101p has a role in general ion homeostasis. This idea is supported by the sensitivity of rim101 mutants to elevated cation concentrations. Thus we can now ascribe functional importance to the activating Rim101p cleavage that occurs even in acidic media in *S. cerevisiae* (32). In addition, these observations may explain why *C. albicans* rim101/pim1101 strain mutants grow in the liver, but not in the kidney, in infected mice (8): replication in the kidney probably requires greater salt tolerance. Whether Rim101p/PacC pathways in other fungi have similar roles in ion homeostasis remains to be determined. However, the discovery of a new role for *S. cerevisiae* Rim101p illustrates one way that comprehensive gene expression surveys can provide new functional information.

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Alkaline Response Genes of *Saccharomyces cerevisiae* and Their Relationship to the RIM101 Pathway

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Alkaline response genes of *Saccharomyces cerevisiae* and their relationship to the RIM101 pathway.

*Teresa M. Lamb, Wenjie Xu, Aviva Diamond, and Aaron P. Mitchell*

**Pages 1853 and 1854:** We reported in Tables IV and V that a *vma4-lacZ* fusion gene showed alkaline-induced, Rim101p-dependent expression. Subsequent analysis showed that these strains carried an *arn4-lacZ* fusion gene rather than the *vma4-lacZ* fusion gene. Therefore, our conclusion that *VMA4* expression responds to pH and to Rim101p is in error.

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Regulation of *Staphylococcus aureus* pathogenesis via target of RNAIII-activating protein (TRAP).

*Naomi Balaban, Tzipora Goldkorn, Yael Gov, Miriam Hirshberg, Nir Koyfman, Harry R. Matthews, Rachael T. Nhan, Baljit Singh, and Orit Uziel*

**Page 2661:** The left column, line 8 from the bottom, should read: “...universal reverse primer 5′-AACAGCTATGACC-ATG-3′.”

**Page 2662, Fig. 5:** The arrow of Ery is drawn backward. Instead of pointing clockwise, it should point counterclockwise.

These changes do not affect the conclusions of the paper.