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Research Article

At acidic pH, the GPA2–cAMP pathway is necessary to counteract the *ORD1*-mediated repression of the hypoxic *SRP1/TIR1* yeast gene

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Abstract

The hypoxic *SRP1/TIR1* gene encodes a stress-response cell wall mannoprotein and this gene is downregulated at acidic pH. The stress-responsive HOG pathway is necessary to maintain hypoxic *TIR1* expression, but only at acidic pH. However, unlike known HOG pathway-dependent genes, *TIR1* is under positive cAMP control and this effect is mediated by *GPA2* but not by *RAS2*. Genetic analysis showed that *ord1* mutation was epistatic to the *gpa2* mutation, thereby indicating that Gpa2p is needed to counteract the Ord1 factor, which is involved in the repression of hypoxic *TIR1* expression, while the HOG pathway appears to be independent from Ord1 repression. In addition, an increased *ORD1* gene expression was observed in the Δ*gpa2* mutant cells, meaning that *GPA2* maintains a low basal level of *ORD1* transcripts. Thus, cAMP allows partial relief of the *TIR1* repression exerted by Ord1p. However, this is contradicted at acidic pH by the HOG pathway requirement because Hog1p is activated under stress conditions when the cAMP cellular content is low. The opposite effects of the GPA2–cAMP and HOG pathways are likely to explain the diminished hypoxic expression of *TIR1* at acidic pH.

Keywords: stress response; hypoxia; *SRP1*; *TIR1*; HOG pathway; GPA2–cAMP pathway; pH regulation; PKA pathway

Introduction

The study of the *SRP1/TIR1* gene (Marguet *et al.*, 1988) has led to the discovery of a Rox1-independent yeast hypoxic pathway (Bourdineaud *et al.*, 2000). The *TIR1* gene has been shown to be induced by low temperatures and to a greater extent by anaerobiosis and hypoxia (Donzeau *et al.*, 1996). The hypoxic *TIR1* gene expression depends on heme absence but is independent from Rox1-mediated repression (Donzeau *et al.*, 1996), contrarily to other hypoxic genes (Zitomer and Lowry, 1992). This is not specific to *TIR1*, since another Rox1-independent gene, *DAN1*, has been isolated (Sertil *et al.*, 1997). Rather, we found a new hypoxic pathway depending on the antagonistic interaction between the repressor *ORD1* and activator *YAP1* (a transcriptional activator involved in oxidative stress response; Wu and Moye-Rowley, 1994). The Ord1 repressor, an intrastrand crosslink recognition protein (Brown *et al.*, 1993; Lambert *et al.*, 1994), was found to bind to both of the −299/−251 and −218/−156 *TIR1* promoter regions. *YAP1* was shown to counteract the hypoxic Ord1-mediated repression. Levels of *ORD1* gene expression were far higher in the Δ*yap1* mutant cells than in wild-type cells, indicating that *YAP1* is necessary to oppose against *ORD1* expression. This explains why the deletion of both Ord1-binding boxes of the *TIR1* promoter allowed a hypoxic bypass of *YAP1* requirements (Bourdineaud *et al.*, 2000).

The hypoxic expression of *TIR1* has also been shown to be downregulated at acidic pH (Bourdineaud, 2000). The stress-responsive HOG pathway appeared necessary to maintain hypoxic *SRP1* expression, but only at acidic pH (Bourdineaud, 2000). All of the other known genes responsive to the HOG pathway contain a positive

promoter element called the stress response element (STRE). The STRE element activates transcription in response to osmotic stress or acidic pH, and is negatively regulated by high levels of activity of the cAMP-dependent protein kinase (protein kinase A; PKA) (Marchler *et al*., 1993). However, the *TIR1* promoter does not contain STRE elements and, unlike all other known HOG pathway-dependent genes, *TIR1* was under positive cAMP control and was positively modulated by protein kinase A at neutral and acidic pH. Surprisingly, this positive cAMP control was found to be mediated by *GPA2* but not by *RAS2* (Bourdineaud, 2000). In order to understand such peculiar regulation, genetic analyses were undertaken. It is here shown that the *ord1* mutation is epistatic to the *gpa2* mutation, and that *GPA2*, whose expression is toxic under stress conditions, is nevertheless required to maintain a low basal level of *ORD1* transcripts at acidic pH.

Materials and methods

Media and growth conditions

Rich media (YPD) and synthetic complete (SC) media lacking uracil (SC–URA), tryptophan, leucine or histidine were prepared as described (Sherman *et al*., 1986) and were supplemented to a final concentration of 2% with glucose. Hypoxic cultures were grown in glucose-rich medium supplemented with 12 μg/ml ergosterol and 0.2% Tween 80. Hypoxic conditions (decrease in oxygen partial tension) were obtained by static cultures in 16 cm long glass tubes (1.1 cm internal diameter; Pyrex) completely filled with an aerated culture and stoppered. The cultures were then incubated for 4 h at 28°C. The aerated cultures were previously grown to an absorbance at 600_{nm} of 0.5 before being poured in the glass tubes. Buffering of YPD to given pH values was done by adding 50 mM sodium phosphate to the medium and adjusting the desired pH with concentrated HCl or NaOH. Plasmids were transformed into *S. cerevisiae* by the modified LiAc method (Gietz *et al*., 1992).

Strains used

The *Escherichia coli* strain used in this study was XL1Blue (Stratagene). The strains of *S. cerevisiae* used in this study were as follows: S150-2B (*MAT***a** *leu2-3,112 ura3-52 trp1-289 his3-Δ1*), SEY6210 (*MATα leu2-3,112 ura3-52 his3-Δ200 lys2-801 trp1-Δ901 suc2-Δ9*). The Δ*ord1* strain is VB5-8 (*MAT***a** *leu2 ura3-52 trp1 his3 Δord1::LEU2*) and its wild-type congenic counterpart is VB5-3 (*MAT***a** *leu2 ura3-52 trp1 his3*). VB5-3 and VB5-8 were derived from the fourth back-cross between JM43Δ*ord1* and FYF102 (Bourdineaud *et al*., 2000). The following strains are isogenic to SP1: S13-3A (*MATα leu2 ura3 his3 trp1 ade8 can1 tpk2::HIS3 tpk3::TRP1 bcy1::LEU2*) and S13-58-A1 (*MAT***a** *leu2 ura3 his3 trp1 ade8 can1 tpk1^{w1} tpk2::HIS3 tpk3::TRP1 bcy1::LEU2*) (Johan Thevelein). The *cdc25-5* strain is OL520-1 (*MATα cdc25-5 rca1/pde2 ura3 his3 leu2 trp1*) (Michel Jacquet). The Δ*gpa2* strain is JRY79 (JRY28 Δ*gpa2*). JRY28 is the wild-type strain (*MAT***a** *leu2 his3 trp1 ura3 lys2*) (Johan Thevelein). The *hog1* mutant, JBY10, is Δ*hog1::TRP1* in YPH499. The wild-type strain is YPH499 (*MAT***a** *leu2 ura3 his3 lys2 trp1 ade2*) (Michael Gustin).

Plasmids used

pLGΔ5′SRP1 harbours a *TIR1–lacZ* fusion and has already been described (Marguet *et al*., 1986). pJPG34 results from an upstream deletion with BAL-31 exonuclease from the *Bgl*II site (at position −552 in the original *TIR1–lacZ* fusion plasmid) to −394 (Bourdineaud *et al*., 2000). pJPB29 is a *TIR1–lacZ* fusion containing an internal deletion from −299 to −156 (Bourdineaud *et al*., 2000).

β-galactosidase assay

Cells were grown for 4 h in glucose-containing media, either aerobically or hypoxically to exponential phase, and β-galactosidase enzyme assays were performed as described (Miller, 1972), except that cells were broken with glass beads. The enzyme units were calculated as described by Miller. The average of three experiments is reported.

RT–PCR analysis of *ORD1* gene expression

The RNA samples were treated with Rnase-free Dnase I to avoid genomic DNA contamination during PCR amplification. Synthesis of cDNA was carried out with the ProSTAR First-Strand RT–PCR kit (Stratagene). Reverse transcriptase reaction used random primers of the kit with 10–15 μg of total RNA. Of the first strand cDNA

synthesis reaction, 4 µl were used as template for PCR amplification. Thirty-five cycles were carried out, each consisting of 30 s of denaturation at 95°C, 30 s of annealing at 48°C, and 30 s of enzymatic primer extension at 72°C. PCR fragments were visualized on a 1% agarose gel. Negative controls included PCR with either water instead of RT mix or 4 µl crude RNA. None of the negative controls resulted in DNA amplification. The actin gene was used as a reference, since *ACT1* is a housekeeping gene. Primer combinations used for RT–PCR were as follows: for *ACT1* the forward primer was 5′-GCT GCT TTG GTT ATT GAT AAC GGT-3′ and the reverse primer 5′-GAT CTT CAT CAA GTA GTC AGT CAA-3′; for *ORD1* the forward primer was 5′-G AAC ACC GGT ATC TCG CCC AAA CAG-3′ and the reverse primer 5′-GC CGT GGC AGG GTT TAA TTG GGA GG-3′. The expected PCR fragment sizes are 561 and 636 bp, respectively, for *ACT1* and *ORD1*.

Results

Although cAMP is an absolute requirement, at acidic pH excess amounts of cAMP appear detrimental to hypoxic TIR1 expression

Although *RAS2* is not involved in the regulation of *TIR1* expression, the guanine nucleotide exchange factor Cdc25, which is known to control the GTP : GDP ratio on the Ras proteins, was nevertheless required for hypoxic *TIR1* expression (Bourdineaud, 2000). Strain OL520-1 carries a *rca1/pde2* mutation that allows uptake of cAMP from the medium, and a temperature-sensitive *cdc25-5* allele. Cells carrying this *cdc25-5* allele showed a rapid drop in the cAMP level upon shift to the restrictive temperature (Camonis *et al.*, 1986). In this strain, the expression of a typical *HOG1*-regulated and STRE-driven gene was increased at restrictive temperature. Indeed, *SOD2–lacZ* expression at 37°C displayed a four-fold increase compared to that observed at 23°C (Flattery-O'Brien *et al.*, 1997). In addition, the expression of 18 new selected genes was found to be increased at restrictive temperature in the same strain (Tadi *et al.*, 1999). However, a very low hypoxic expression of *TIR1* at the restrictive temperature has been described in this strain (Bourdineaud, 2000). At 37°C, supplementation of the growth medium with cAMP (0–10 mM) successfully resulted in a high increase (around 20 times) of the hypoxic expression of *SRP1* at pH 6 (Table 1). This means that null or low levels of cAMP inhibited *TIR1* hypoxic expression. The expression ratio $r_{37°/28°}$ at pH 6 showed a 50-fold increase when the added cAMP concentration was varied from 0 to 10 mM (Table 1), meaning that the Cdc25 factor requirement for *TIR1* expression was efficiently bypassed by supplying cells with cAMP. It is noteworthy that 10 mM added cAMP at pH 6 led to a gene expression which was the same at 28°C and at 37°C.

When hypoxic expression of *TIR1* at 28°C and pH 6 was examined, increasing cAMP concentration induced a drop from 50 to 25 Miller units, meaning that high levels of cAMP were detrimental to hypoxic *TIR1* expression. This negative effect induced by high levels of cAMP was even more pronounced at 28°C and pH 3: for the hypoxic expression of *TIR1* there was a eight-fold decrease when the external cAMP concentration was changed from 0 to 10 mM, and the expression ratio $r_{pH6/pH3}$ at 28°C was increased from 2 to 8 when the added cAMP was varied from 0 to 10 mM (Table 1), showing that at acidic pH, cAMP was detrimental to hypoxic *TIR1* expression. Thus, at

Table 1. cAMP effect on hypoxic *TIR1* expression in a *cdc25-5 pde2* strain

	***TIR1–lacZ* expression (Miller units)**					
	28°C		**37°C**			
cAMP (mM)	**pH 6**	**pH 3**	**pH 6**	**pH 3**	$r_{37°/28°}$ **at pH 6**[a]	$r_{pH6/pH3}$ **at 28°C**[b]
0	50±5	25±2	1	<1	0.02	2
4	45±5	15±1	13±1	2±0.5	0.3	3
10	25±2	3±0.5	23±2	2.5±0.5	1	8

[a] $r_{28°/37°}$ is the ratio of β-galactosidase activities measured in this strain at 28°C to those at 37°C under hypoxia.
[b] $r_{pH6/pH3}$ is the ratio of β-galactosidase activities measured in this strain at pH 6 to those at pH 3 under hypoxia.

acidic pH under hypoxia, *TIR1* behaves like the other *HOG1*-dependent genes.

These results are consistent with the findings obtained with a strain displaying a constitutive high level of PKA activity (*TPK1 tpk2 tpk3 bcy1*). In this strain the decreases in *TIR1* hypoxic expression were 22% and 44% at pH 6 and pH 3, respectively, compared to the wild-type strain (Bourdineaud, 2000). In addition, in a strain showing low levels of PKA activity (*tpk1^{w1} tpk2 tpk3 bcy1*), significant decreases in *TIR1* hypoxic expression were observed at pH 6 and at pH 3 compared to the wild-type strain (Bourdineaud, 2000). In summary, PKA activity is required at both neutral and acidic pHs, but high levels of PKA activity appear detrimental to hypoxic *TIR1* expression, especially at acidic pH.

The GPA2–cAMP pathway is toxic under stress conditions

These results suggest that hypoxic *TIR1* expression is governed by two antagonistic pathways. Indeed, at acidic pH (an environmental condition known to trigger the HOG pathway; Schüller *et al.*, 1994), both the *GPA2* and the *HOG1* genes were required for *TIR1* hypoxic expression (Bourdineaud, 2000). This is surprising, since Gpa2p allows adenylate cyclase stimulation and thus glucose-induced cAMP synthesis (Colombo *et al.*, 1998), while the HOG pathway triggers stimulation of genes under negative cAMP control (Schüller *et al.*, 1994). This antagonism between the GPA2–cAMP and the HOG pathways might explain why, despite an absolute requirement for cAMP, excess amounts of cAMP appear detrimental to hypoxic *TIR1* expression at acidic pH. If this were to be true, then a Δ*gpa2* mutation should restore normal growth under the conditions known to activate the HOG pathway. Indeed, a Δ*gpa2* mutation allowed yeast to grow both at acidic pH and at high salt concentrations when the cell envelope was rendered fragile with SDS (Table 2), so Gpa2p action is toxic under stress conditions known to activate the HOG pathway. This was confirmed by the finding that high PKA activity was toxic under the same stress conditions. A *bcy1 tpk1^{w1} tpk2 tpk3* strain could grow at acidic pH or high salt concentrations in the presence of SDS, whereas the *bcy1 TPK1 tpk2 tpk3* strain could not (Table 3). These findings were consistent with those showing that a Δ*gpa2* mutation had a protective effect on yeast against heat-shock (Colombo *et al.*, 1998), and that reduced cAMP levels could also protect yeast against heat-shock and freeze–thawing treatments (Park *et al.*, 1997; Jamieson, 1998).

The Ord1 factor mediates repression of the *TIR1* gene at acidic pH

In order to explain the GPA2–cAMP pathway role concerning hypoxic *TIR1* expression at acidic pH, the question was raised whether an epistatic relationship might exist between *GPA2* and *ORD1*. Indeed, Ord1p has been shown to be a repressor of the *TIR1* gene (a eight-fold increase in normoxic expression was observed in a Δ*ord1* strain as compared to the wild-type strain; Bourdineaud *et al.*, 2000). In addition, internal deletion constructs showed that Ord1 factor controlled *TIR1* expression through binding on both of the −299/−251 and −218/−156 regions (Bourdineaud *et al.*, 2000). Therefore, the first question to solve was whether the Ord1 factor was involved in the diminished hypoxic *TIR1* expression observed at acidic pH. When compared to the wild-type strain, the hypoxic expression of *TIR1* increased two-fold in the Δ*ord1* strain at acidic pH (Table 4, first line). The expression ratio $r_{pH6/pH3}$ was 3.5 in the wild-type strain and 2.1 in the Δ*ord1* strain, indicating

Table 2. *GPA2* is toxic under stress conditions[a]

	pH 6		pH 3		0.5 M NaCl[b]	
0.015% SDS	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
Wild-type	+	+	+	−	+	−
Δ*gpa2*	+	+	+	+	+	+

[a]Media were YPD supplemented with the indicated compounds. +, growth; −, lack of growth.
[b]The medium was pH 6.

Table 3. Protein kinase A activity is toxic under stress conditions[a]

	pH 6	pH 3		0.5 M NaCl[b]	
0.015% SDS	**Yes**	**No**	**Yes**	**No**	**Yes**
TPK1 tpk2 tpk3 bcy1	+	+	−	sg	−
tpk1^{w} tpk2 tpk3 bcy1	+	+	+	+	+

[a]Media were YPD supplemented with the compounds indicated. +, growth; −, lack of growth; sg, slowed growth.
[b]The medium was pH 6.

Table 4. Role of Ord1 factor in hypoxic repression of *TIR1* gene at acidic pH

	***TIR1–lacZ* expression (Miller units)**					
	Wild-type		**Δ*ord1***		**$r_{pH6/pH3}$[a]**	
Transforming plasmids	**pH 6**	**pH 3**	**pH 6**	**pH 3**	**Wild-type**	**Δ*ord1***
pLGΔ5′SRP1	390±40	115±15	470±50	225±20	3.5	2.1
pJPB29 (−299/−156)	470±50	205±20	445±45	210±20	2.3	2.1
pJPG34 (5′/−394)	ne[b]	ne	62±5	58±5	ne	1.1

[a]r, ratio of β-galactosidase activities at pH 6 to those at pH 3.
[b]ne, no expression observed.

that the Ord1 factor played a role in the *TIR1* repression at acidic pH. When the wild-type and Δ*ord1* strains were transformed with the plasmid pJPB29, in which the two Ord1-binding boxes were deleted, the hypoxic *TIR1* expression at pH 3 was equivalent for the two strains (Table 4, second line), contrarily to that observed with the plasmid pLGΔ5′SRP1 harbouring the complete promoter. These results obtained with pJPB29 were very similar to those obtained with the complete *TIR1* promoter in a Δ*ord1* strain, confirming the Ord1 role. However, the observed expression ratio $r_{pH6/pH3}$ of 2 in a Δ*ord1* strain suggested that there could be additional repressing control of the *TIR1* promoter. With a plasmid harbouring an upstream deletion (5′/−394), there was equivalent expression at each pH in a Δ*ord1* strain, indicating that the repression observed with pJPG34 is pH-independent and *ORD1*-dependent. Since in a Δ*ord1* strain no pH effect is observed with pJPG34 ($r_{pH6/pH3}=1$), while a pH effect still remains for the complete promoter ($r_{pH6/pH3}=2$), one can infer the likely existence of another repressor acting at acidic pH on sequences located upstream from −394 (Table 4, line 3). However, the diminished expressions observed with pJPG34 means that activating sequences were also deleted.

The *ord1* mutation is epistatic to *gpa2* mutation

When the JRY79 (Δ*gpa2*) strain was transformed with pJPB29, there was a 3.8-fold increase in hypoxic *TIR1* expression at acidic pH compared to the complete promoter (upper part of Table 5; cf. lines 2 and 4). The increase was only 1.3-fold for the same strain at neutral pH. In addition, the expression ratio $r_{pH6/pH3}$ decreased three-fold in the Δ*gpa2* strain when the two Ord1-binding boxes were deleted, strongly suggesting an interplay between *GPA2* and *ORD1*. The Δ*ord1* strain (VB5-8, which is congenic with the wild-type strain VB5-3) was crossed with the Δ*gpa2* strain and the progeny were dissected to yield a Δ*gpa2* Δ*ord1* doubly mutated spore. This double mutant was back-crossed three more times with the VB5-3 wild-type strain, and Δ*gpa2*, Δ*ord1*, Δ*gpa2* Δ*ord1* and wild-type spores

Table 5. Interaction between *GPA2* and *ORD1* for hypoxic *TIR1* gene expression

			***TIR1–lacZ* expression[a]**		
Strains	**Relevant genotypes**	**Plasmids**	**pH 6**	**pH 3**	**$r_{pH6/pH3}$[b]**
JRY28	Wild-type	pLGΔ5′SRP1	350±40	100±9	3.5
JRY79	Δ*gpa2*	pLGΔ5′SRP1	230±25	25±5	9.2
JRY28	Wild-type	pJPB29 (−299/−156)	375±40	165±20	2.3
JRY79	Δ*gpa2*	pJPB29 (−299/−156)	300±30	95±10	3.2
VB16-7a	*GPA2 ORD1*	pLGΔ5′SRP1	260±30	90±10	2.9
VB16-1a	Δ*gpa2 ORD1*	pLGΔ5′SRP1	190±20	35±5	5.4
VB16-1b	*GPA2* Δ*ord1*	pLGΔ5′SRP1	315±30	140±20	2.2
VB16-7c	Δ*gpa2* Δ*ord1*	pLGΔ5′SRP1	310±30	160±20	1.9

[a]β-galactosidase activities are given in Miller units.
[b]$r_{pH6/pH3}$ is the ratio of β-galactosidase activities at pH 6 to those at pH 3 under hypoxia.

were selected. Spores were first assayed for blue staining on XGal-containing SC plates, and Δ*gpa2* colonies appeared as pale blue while the Δ*ord1*, Δ*gpa2* Δ*ord1* and wild-type spores gave deep blue colonies. Three random representative mutant and wild-type spores were then selected and assayed for β-galactosidase activity. As the data did not differ significantly (within a 10% limit) between each of the three spores, whatever the screened category, the results are shown for only one strain of each category (lower part of Table 5). At acidic pH, a 55% increase in hypoxic *TIR1* expression was observed in a Δ*ord1* strain as compared to a wild-type strain (Table 5; cf. lines 5 and 7), thus confirming the validity of the data in Table 4. At pH 3, a four-fold increase in hypoxic *TIR1* expression was observed in a Δ*ord1* strain as compared to a Δ*gpa2* strain (Table 5; cf. lines 6 and 7), and the β-galactosidase activities were similar in a Δ*ord1* strain and a Δ*gpa2* Δ*ord1* strain (Table 5; cf. lines 7 and 8). This means that the presence of the *GPA2* gene is not compulsory for *TIR1* gene expression at acidic pH, provided that the *ord1* locus is silent. In addition, the expression ratio $r_{pH6/pH3}$, which was more than 5 in a Δ*gpa2* strain, was decreased to around 2 in the Δ*ord1* and Δ*gpa2* Δ*ord1* strains. Altogether, these results clearly show that *ord1* is epistatic to *gpa2*. Thus, Gpa2p is not directly involved in *TIR1* expression, but is rather required to counteract the Ord1 effect. This was demonstrated by RT–PCR analysis. The *ORD1* transcript level was found increased at acidic pH in Δ*gpa2* mutant cells as compared to the wild-type strain (Figure 1), indicating that *GPA2* is necessary to maintain a low basal level of *ORD1* expression at acidic pH.

An epistatic relationship between *YAP1* and *ORD1* (Bourdineaud *et al.*, 2000) and between *GPA2* and *ORD1* (this work) has been demonstrated. In addition, the HOG pathway has been shown to be necessary for hypoxic *TIR1* expression at acidic pH (Bourdineaud, 2000). It was then legitimate to address the question of whether an epistatic interaction might exist between *HOG1* and *ORD1*. Even so, no definite relationship could be demonstrated between *hog1* and *ord1*. At acidic pH, the observed β-galactosidase activities in Δ*hog1* and Δ*hog1* Δ*ord1* strains were around two-fold below those of a Δ*ord1* strain (data not shown), therefore indicating that at acidic pH the Hog1p requirement for hypoxic *TIR1* expression was independent of Ord1 repression.

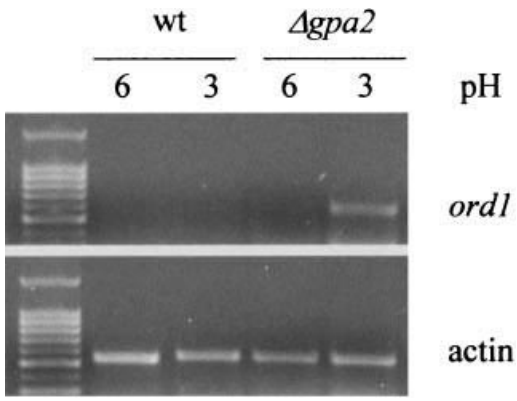

Figure 1. Analysis of *ORD1* expression in a Δ*gpa2* mutated strain. The indicated wild-type and mutated strains were grown for 4 h under hypoxia at 28°C at the indicated pH, and total RNA were extracted. RT–PCR experiments were then carried out with either the *ACT1*- or *ORD1*-specific primers

Discussion

The stress responsive *SRP1/TIR1* gene was shown to be downregulated at acidic pH. Nevertheless, although diminished, *TIR1* expression at acidic pH is sufficient even under normoxia to allow yeast survival (Bourdineaud, 2000). The Ord1 factor is involved in repression of *TIR1* and Gpa2p is shown here to counteract this inhibitory activity. This explains why Gpa2p is required at neutral and acidic pHs and why cAMP allows relief of *TIR1* repression exerted by Ord1p. Thus, Gpa2p requirement could be more critical under conditions associated with a decreased cAMP cellular level, such as a low pH medium—a hypothesis verified here. Furthermore, hypoxic *TIR1* expression at acidic pH is maintained not only by the GPA2–cAMP pathway but also by the HOG pathway (Bourdineaud, 2000). The HOG pathway stimulates the STRE-containing genes, which are under negative cAMP control. However, the *TIR1* promoter does not contain STRE elements. One can speculate that the Hog1p influence on *TIR1* expression at acidic pH is indirect and mediated by a STRE-driven gene yet not identified. Therefore, the *TIR1* gene expression is under the control exerted by two contradictory pathways when the yeast undergoes a low pH: the stress lowers the cAMP content (Jamieson, 1998), which in turn appears detrimental to hypoxic *TIR1* expression (a low cAMP content favours Ord1-mediated repression), and Gpa2p

diminishes HOG pathway efficiency by maintaining sufficient levels of cAMP. This is exemplified by the toxicity of the GPA2–cAMP pathway under stress conditions (Tables 2 and 3). Moreover, a constitutive expression of STRE-driven genes in a Δ*gpa2* strain has already been demonstrated (Colombo *et al*., 1998), and constitutive expression of *GPA2* conferred heat shock sensitivity (Xue *et al*., 1998), demonstrating that Gpa2p action is inhibitory; PKA activity has also been shown to antagonize induction of the general stress response (Smith *et al*., 1998). A regulatory model incorporates the present knowledge on the GPA2–PKA and HOG pathways and tries to explain the decrease in hypoxic *TIR1* expression at acidic pH (Figure 2).

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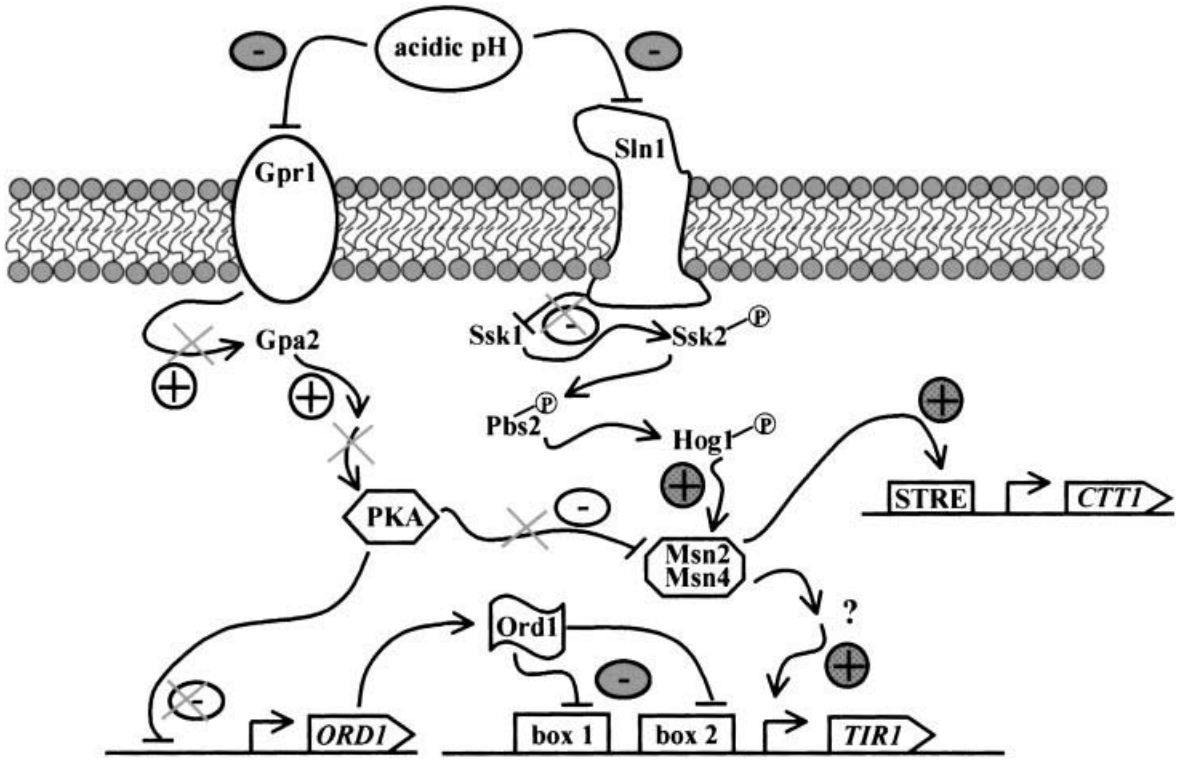

Figure 2. Tentative model to explain the decrease in hypoxic *TIR1* expression at acidic pH. The stress-responsive *CTT1* gene has been included in this model because it is representative of the genes activated by the HOG pathway through the STRE-element (Marchler *et al*., 1993). This stress response via STRE is strongly counteracted by high activity of PKA. This is due to the fact that the nuclear localization of the STRE-binding transcription factors Msn2 and Msn4 (Martinez-Pastor *et al*., 1996) is negatively affected by high PKA activity (Görner *et al*., 1998). The acidic pH is sensed by the HOG pathway (Schüller *et al*., 1994), which transmits signals from the Sln1 plasma membrane sensor (Maeda *et al*., 1994). Sln1 is a protein kinase phosphorylating Ssk1 and thereby inactivating it. However, Sln1 is inactivated by stress, resulting in activation of Ssk1 and subsequently in stimulation of the downstream components of the HOG pathway, including the Msn2 and Msn4 factors. At the same time the level of cAMP decreases following stress application (Márquez and Serrano, 1996), which implies a stress-imposed reduction of PKA activity. Then the PKA inhibition exerted on Msn2 and Msn4 activity is relieved, allowing a strong induction of STRE-driven gene expression. However, the hypoxic *TIR1* expression has to cope with a double bind at acidic pH: the decreased level of PKA activity allows HOG pathway-mediated stimulation of *TIR1* expression but relieves the PKA inhibition on *ORD1* gene expression, thereby facilitating the Ord1 repression of *TIR1* gene. The indicated negative influence of the acidic pH on the Gpa2 protein-coupled receptor Gpr1 (Xue *et al*., 1998) does not mean necessarily that Gpr1 receptor activity is decreased but tries to schematize an overall negative effect of the acidic pH on the Gpa2–cAMP–PKA pathway. The interrogation point means that it is unknown how the HOG pathway stimulates the *TIR1* gene at acidic pH

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