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Transcriptional Control of Genes Involved in Yeast Phospholipid Biosynthesis[§]

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Phospholipid biosynthetic genes encode enzymes responsible for phospholipid biosynthesis. They are coordinately regulated by the availability of phospholipid precursors through the inositol-sensitive upstream activating sequence (UAS_{INO}). However, not all phospholipid genes are UAS_{INO}-containing genes and not all UAS_{INO}-containing genes have the same response to the phospholipid precursors. Therefore, the transcriptional regulation of phospholipid genes in response to the availability of phospholipid precursors is still unclear. Here, 22 out of 47 phospholipid biosynthetic genes were identified as UAS_{INO}-containing genes, including *EKI1*, *EPT1*, *INM1*, *IPK2*, *KCS1*, *PAH1*, and *PIK1* which have never been reported before. We also showed, using qRT-PCR technique, that 12 UAS_{INO}-containing genes are down-regulated by 100 μM inositol in the wild type cells and up-regulated by 100 μM inositol in the *ino2*Δ cells. Therefore, it is possible that these genes are transcriptionally regulated by the UAS_{INO} through the negative response of Ino2p to inositol. One other UAS_{INO}-containing gene might be regulated by the positive response of Ino2p to 100 μM inositol. Surprisingly, we found 9 UAS_{INO}-containing genes are not dependent on the response of Ino2p to 100 μM inositol, indicating that they may be regulated by other pathway. Furthermore, we identified 9 and 3 non-UAS_{INO}-containing genes that are possibly regulated by the negative and positive response of Ino2p to 100 μM inositol, respectively. Therefore, these observations provide insight into the understanding of the co-regulated phospholipid biosynthetic genes expression.

Keywords: phospholipid biosynthesis, gene expression, UAS_{INO}, Ino2p, qRT-PCR

Phospholipid structural genes play an important role in regulating yeast cellular processes. These genes encode enzymes responsible for phospholipid synthesis, which define the structural integrity of cells during mitosis. For example, one of the essential phospholipids, phosphatidylinositol (PI), and its metabolites regulate a diverse set of cellular processes such as glycolipid anchoring of proteins (Shields and Arvan, 1999), signal transduction (Divecha and Irvine, 1995; Ohanian and Ohanian, 2001), mRNA export from the nucleus (Odom *et al*., 2000; Saiardi *et al*., 2000a, 2000b; Shears, 2000), vesicle trafficking (Czech, 2000; Martin, 2001) and also serve as reservoirs of second messengers (Greenberg and Lopes, 1996; Henry and Patton-Vogt, 1998; Carman and Henry, 1999).

In the budding yeast *Saccharomyces cerevisiae*, phospholipid metabolism is coordinately regulated by the response to the availability of phospholipid precursor molecules, inositol and choline, in the growth medium (Kelley *et al*., 1988; Dowd *et al*., 2001; Loewen *et al*., 2004; Boumann *et al*., 2006; Gaspar *et al*., 2006). The addition of inositol to yeast cells starved for inositol induces a rapid and significant change in the pattern and synthesis of membrane phospholipids (Kelley *et al*., 1988; Loewen *et al*., 2004) and inositol-containing sphingolipids (Alvarez-Vasquez, 2005). The synthesis of PI, and the consumption of phosphatidic acid (PA) and cytidinediphosphate-diacylglycerol (CDP-DAG) are increased in the presence of inositol. Recent studies have also demonstrated that the addition of phospholipid precursor molecules to actively growing yeast cultures caused changes in the expression of genes and in the phospholipid synthesis and turnover (Santiago and Mamoun, 2003; Jesch *et al*., 2005; Gaspar *et al*., 2006).

Our current understanding of phospholipid gene expression comes from the study of *INO1* expression. *INO1* encodes a key enzyme required in the inositol biosynthetic pathway (Fig. 1). The *de novo* synthesis of PI, one of the major membrane phospholipids in mitotically active cells, requires the product of *INO1* expression. The regulation of *INO1* requires chromatin remodeling activities (Ford *et al*., 2007, 2008; Esposito *et al*., 2009) and the binding of heterodimeric transcriptional activator, Ino2p/Ino4p, to its promoter (Ambroziak and Henry, 1994; Schwank *et al*., 1995). The binding site for the transcriptional activator is a 10-bp *cis*-acting promoter element, the inositol-sensitive upstream activating sequence (UAS_{INO}) (Lopes *et al*., 1991; Koipally *et al*., 1996). The promoters of some yeast phospholipid structural genes contain variants of a 10-bp UAS_{INO} element. It is believed that the coordinated regulation of those phospholipid genes is influenced by the binding of Ino2p to the UAS_{INO} element and such binding depends on its response to the availability of phospholipid precursors. However, not all of the phospholipid genes have the UAS_{INO} element in their promoters and not all UAS_{INO}-containing genes have the same response to the phospholipid precursors. Therefore, our understanding of how cells regulate the phospholipid gene expression in response to the availability of phospholipid precursor molecules is still unclear.

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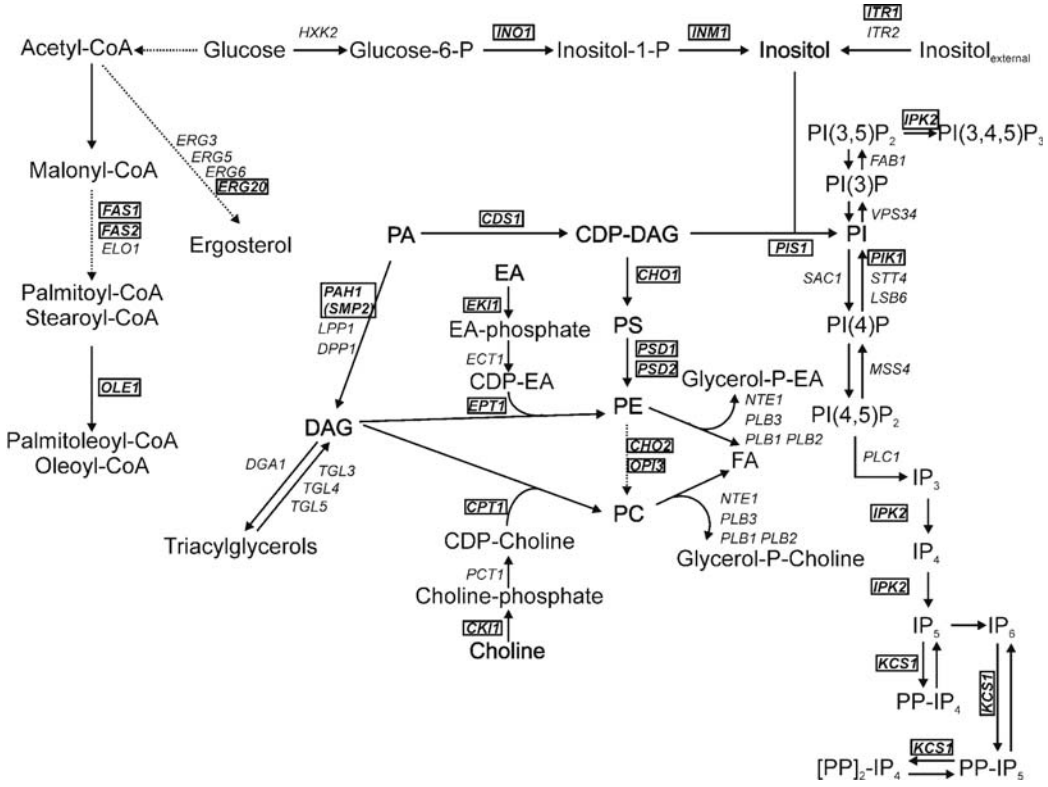

Fig. 1. Phospholipid biosynthetic pathways of *S. cerevisiae*. UAS$_{INO}$-containing structural genes are highlighted in boxes. PA, phosphatidic acid; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; EA, ethanolamine; FA, fatty acid; CDP-DAG, cytidinediphosphate diacylglycerol; DAG, diacylglycerol; IP_3, IP_4, IP_5, IP_6, PP-IP_4, PP-IP_5, [PP]$_2$-IP_4, inositol polyphosphates; PI(3)P, PI(4)P, PI(3,5)P_2, PI(4,5)P_2, PI(3,4,5)P_3, phosphoinositides.

In the present study, sequence analysis was used to search for the UAS$_{INO}$-containing phospholipid genes. We also used quantitative real-time PCR (qRT-PCR) to examine transcriptional regulation of structural genes in the presence or absence of inositol. We have shown that the addition of inositol to logarithmically growing yeast cells results in major and rapid changes of the expression of phospholipid biosynthetic genes. The regulation of most of the phospholipid biosynthetic genes that contain at least one copy of the UAS$_{INO}$ element in their promoter depends on the response of Ino2p to inositol availability. Furthermore, our results identified genes that do not have the UAS$_{INO}$ element might also depend on the response of Ino2p to the inositol availability. Taken together, these observations provide insight into the understanding of the co-regulated genes expression in the phospholipid biosynthetic pathway.

Materials and Methods

Yeast strains and growth conditions

Wild-type (WT) yeast strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and *ino2Δ* strain (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ino2Δ*) were used in this study. WT cells were grown at 30°C in SC (synthetic complete media) containing 2% glucose (w/v) with 10 μM inositol, 100 μM inositol or without inositol. For *ino2Δ* cells, yeast culture was grown at 30°C in SC with 10 μM inositol. When the optical density reached 0.9-1.1, cells were harvested by centrifugation and washed twice with SC without inositol to completely remove inositol. Cells were then resuspended in SC with 10 μM inositol, 100 μM inositol or without inositol, and incubated for 2 h at 30°C.

RNA preparation and first strand cDNA synthesis

The total RNA was prepared as described previously (Ford *et al*., 2007). Briefly, 250 ml cells were harvested and resuspended in 400 μl lysis solution (10 mM Tris HCl; pH 7.5, 10 mM EDTA, 0.5% SDS). Subsequently, equal volume of acid phenol (pH 4.3) was added to the cells suspension. After 1 h incubation at 65°C, the mixture was subject to centrifugation, and the aqueous phase was mixed with an equal volume of acid phenol again. After 5 min of incubation on ice, the aqueous phase was subject to chloroform extraction, ethanol precipitation, and was resuspended in 50 μl DEPC-treated H_2O.

Equal amounts (10 μg) of total RNA were treated with RNase-free DNase (QIAGEN cat.#79254) at 37°C for 1 h, and purified by phenol/chloroform (3:1) extraction and ethanol precipitation. One microgram of pure RNA was used in SYBR GreenER Two-Step qRT-PCR kit (Invitrogen cat#11765-100) for first strand cDNA synthesis and real-time PCR reaction preparation as described in manufacture's manual.

Real-time PCR analysis

Phospholipid biosynthetic genes chosen for this study are shown in Fig. 1. All real-time PCR primers are listed in the supplementary materials. All experiments were repeated twice, and in each experi-

ment, PCR reactions were done in triplicate in a 7,500 sequence detection system (Applied Biosystems, USA). Target DNA sequence quantities were estimated as described previously (Ford *et al*., 2008). Briefly, target DNA sequence quantities were estimated from the threshold amplification cycle number (C_T) using Sequence Detection System software (Applied Biosystems). Each DNA quantity was normalized to the *ACT1* DNA quantity by taking the difference between each gene's C_T and *ACT1*'s C_T value. Furthermore, a ΔC_T value was calculated for each gene by subtracting the C_T value for the sample prepared from SC with 10 μM inositol or 100 μM inositol from the C_T value for the sample prepared from SC without inositol. Each relative RNA fold change was then calculated with the following formula: $2^{(-\Delta C_T)}$.

Results

Search of UAS$_{INO}$-containing phospholipid genes

It has been shown that transcriptional activation of a UAS$_{INO}$-containing gene, *INO1*, is regulated through the response of Ino2p to the availability of inositol and choline (Lopes and Henry, 1991; Lopes *et al*., 1991; Ambroziak and Henry, 1994; Schwank *et al*., 1995; Koipally *et al*., 1996). The consensus sequence of 5′-WYTTCAYRTG-3′ has been derived as the UAS$_{INO}$ element (Schuller *et al*., 1995; Hoppen *et al*., 2005). Our aim is to understand the coordinated transcriptional control of genes with or without the UAS$_{INO}$ element in the phospholipid biosynthetic pathway. Sequence analysis was performed to search genes that contain a UAS$_{INO}$ element. The DNA sequence of each gene was obtained from the *Saccharomyces* Genome Database (http://yeastgenome.org). Each gene's DNA sequence covered 1 kb upstream of the translation start site and the entire open reading frame. Serial Cloner (Version 1.3r11) was used to search for the consensus sequence 5′-WY TTCAYRTG-3′ (Schuller *et al*., 1995; Hoppen *et al*., 2005). Our results showed that 22 genes out of the 47 genes in the phospholipid pathway contained at least one UAS$_{INO}$ element (Table 1). The locations of the UAS$_{INO}$ elements in these genes fell within the intergenic region, except in *KCS1*, *OPI3*, *PIK1*, and *PSD1*, which fell into their neighboring genes' open reading frame. Most of these UAS$_{INO}$-containing genes had only one copy of the UAS$_{INO}$ element within their promoter region, except for *CHO2*, *FAS1*, *INO1*, *ITR1* and *KCS1*, which had two or more copies of the UAS$_{INO}$ element within their promoter region.

In our sequence analysis, 15 out of those 22 genes that contained at least one UAS$_{INO}$ element have been identified in previous studies (Schuller *et al*., 1995; Santiago and Mamoun, 2003; Jesch *et al*., 2005, 2006). The other 7 phospholipid genes that contained at least one UAS$_{INO}$ element have not been previously reported as UAS$_{INO}$-containing genes. These 7 genes include *EKI1*, *EPT1*, *INM1*, *IPK2*, *KCS1*, *PAH1*, and *PIK1*. *PAH1* catalyzes the dephosphorylation of PA to yield diacylglycerol (DAG) (Fig. 1). *EKI1* and *EPT1* contribute to the cytidinediphosphate (CDP)-choline pathway (Kennedy pathway) for the synthesis of phosphatidylcholine (PC) from DAG. *INM1* is involved in the biosynthesis of inositol. *IPK2*, *KCS1*, and *PIK1* are involved in the PI second messenger system.

Transcriptional profile of phospholipid genes

Next, we used the qRT-PCR strategy to examine the transcription profile of phospholipid genes in response to the presence or absence of the phospholipid precursor inositol. A cDNA probe was prepared from poly(A)$^+$ RNA isolated from WT cells cultivated in SC medium with 10 μM, 100 μM, or without inositol. The transcript levels of 29 phospholipid genes decreased in the presence of 100 μM inositol (Table 2), whereas the transcript levels of 18 phospholipid genes increased in the presence of 100 μM inositol (Table 3). 18 out of 29 of these genes which were repressed by 100 μM inositol contained at least one copy of the UAS$_{INO}$ element. These genes were *CDS1*, *CHO1*, *CHO2*, *CKI1*, *EKI1*, *EPT1*, *ERG20*, *FAS1*, *FAS2*, *INO1*, *IPK2*, *ITR1*, *OLE1*, *OPI3*, *PAH1*, *PIK1*, *PSD1*, and *PSD2*. The other 11 genes, *DGA1*, *ECT1*, *ERG3*, *ERG5*, *ITR2*, *LSB6*, *MSS4*, *NTE1*, *PLC1*, *SAC1*, and *TGL3*, which were repressed by 100 μM inositol do not have any UAS$_{INO}$ elements. There was no significant difference in the increased transcript levels in the absence of inositol between the UAS$_{INO}$-containing genes and non-UAS$_{INO}$-containing genes.

Table 1. Compilation of phospholipid biosynthetic genes containing UAS$_{INO}$ consensus sequence[a] in their promoter regions

Gene	Sequence	Location[b]	Distance from the nearest gene[c]
CDS1	ATGTGAAAA	-160	550
CHO1	CTTTCACAT	-162	505
CHO2	TGTGAAAA	-402	617
	ATGTGAATT	-334	617
	ATGTGAAGA	-265	617
CKI1	TATTCACATG	-196	290
CPT1	ATGTGAAAA	-162	414
EKI1	ATGTGAAAA	-215	889
EPT1	ATTTCACA	-154	495
ERG20	TTTTCACAT	-423	586
FAS1	ATGTGAAAA	-866	1030
	ACTTCACAT	-707	1030
FAS2	TTTTCACAT	-235	505
INM1	TTTTCACG	-445	455
INO1	TCTTCACGT	-357	439
	CATGTGAAAA	-241	439
	ATGTGAAAT	-290	439
	TTCACATG	-179	439
IPK2	TTTTCACAT	-6	478
ITR1	TCTTCACATG	-285	376
	ATGTGAAAA	-219	376
KCS1	TTTCATAT	-463	403
	TTTCATAT	-368	403
OLE1	TGTGAAAT	-868	1007
OPI3	TGTGAAAT	-190	160
PAH1	ATTTCACAT	-593	739
PIK1	TTTCACAT	-684	493
PIS1	CATATGAAGT	-274	348
PSD1	ATGTGAAA	-400	359
PSD2	ATATGAAAA	-244	477

[a] UAS$_{INO}$ consensus sequence: WYTTCAYRTG (Schuller *et al*., 1995; Hoppen *et al*., 2005).
[b] Location: the position of the ICRE sequence relative to the translation start site (+1).
[c] Distance from the nearest gene: the distance between the two neighboring genes' translation start sites.

Table 2. Genes down-regulated by inositol for WT cells

Gene	Fold change[a]			
	10 μM inositol		100 μM inositol	
	Avg.[b]±SD[c]	*p* value	Avg.±SD	*p* value
CDS1	0.605±0.213	0.20	0.108±0.010	<0.01
CHO1	0.077±0.015	<0.01	0.005±0.002	<0.01
CHO2	0.632±0.626	0.62	0.039±0.023	<0.01
CKI1	0.452±0.038	<0.01	0.486±0.042	<0.01
DGA1	0.237±0.104	0.02	0.081±0.016	<0.01
ECT1	0.195±0.173	0.04	0.492±0.103	0.04
EKI1	0.219±0.053	<0.01	0.144±0.074	<0.01
EPT1	0.631±0.045	0.01	0.104±0.008	<0.01
ERG3	0.316±0.232	0.10	0.082±0.030	<0.01
ERG5	0.752±0.343	0.54	0.055±0.017	<0.01
ERG20	0.00005±0.00003	<0.01	0.137±0.023	<0.01
FAS1	0.267±0.056	<0.01	0.162±0.034	<0.01
FAS2	0.018±0.009	<0.01	0.005±0.001	<0.01
INO1	0.241±0.162	0.04	0.013±0.002	<0.01
IPK2	0.262±0.008	<0.01	0.126±0.004	<0.01
ITR1	0.129±0.101	0.01	0.019±0.011	<0.01
ITR2	0.379±0.164	0.06	0.041±0.010	<0.01
LSB6	0.160±0.059	<0.01	0.426±0.092	0.02
MSS4	0.961±0.443	0.94	0.186±0.029	<0.01
NTE1	0.897±0.228	0.70	0.596±0.033	<0.01
OLE1	0.036±0.015	<0.01	0.002±0.0002	<0.01
OPI3	0.006±0.004	<0.01	0.078±0.001	<0.01
PAH1	0.005±0.0009	<0.01	0.430±0.074	0.02
PIK1	0.434±0.194	0.10	0.046±0.012	<0.01
PLC1	0.587±0.073	0.03	0.052±0.002	<0.01
PSD1	0.0005±0.0001	<0.01	0.002±0.001	<0.01
PSD2	0.337±0.044	<0.01	0.213±0.026	<0.01
SAC1	0.401±0.287	0.17	0.075±0.036	<0.01
TGL3	0.083±0.0003	<0.01	0.276±0.036	<0.01

[a] Fold change: the ratio of a specific gene's relative RNA quantity to *ACT1* re-lative RNA quantity. Each gene's relative RNA quantity has been normalized to its corresponding RNA quantity obtained from no inositol treatment.
[b] Avg.: average fold change
[c] SD: standard deviation

All 6 genes, *CDS1*, *CHO1*, *CHO2*, *OPI3*, *PSD1*, and *PSD2*, involved in the phosphatidylethanolamine (PE) methylation pathway contained at least one copy of the UAS_{INO} element and were down-regulated in the presence of 100 μM inositol (Fig. 1, Table 1, and Table 2). Genes that are involved in the synthesis of PE via DAG and the Kennedy pathway, including *PAH1*, *EKI1*, *ECT1*, and *EPT1*, were also down-regulated in the presence of 100 μM inositol, except for *LPP1* and *DPP1*, which do not have any UAS_{INO} elements. Fatty acid biosynthetic genes *FAS1*, *FAS2*, and *OLE1* contain at least one copy of the UAS_{INO} element and were down-regulated in the presence of 100 μM inositol. *ERG3*, *ERG5*, and *ERG20* are ergosterol biosynthetic genes and were down-regulated in the presence of 100 μM inositol, but only *ERG20* is a UAS_{INO}-containing gene. *PIK1*, *IPK2*, *LSB6*, *MSS4*, *PLC1*, and *SAC1* are in the PI second messenger system and were down-regulated in the presence of 100 μM inositol. From the above genes, only *IPK2* is a UAS_{INO}-containing gene.

Four genes, *CPT1*, *INM1*, *KCS1*, and *PIS1*, that contain at least one copy of the UAS_{INO} element were up-regulated in response to the presence of 100 μM inositol (Table 3). *CPT1* is involved in the process of PC synthesis, *INM1* is involved in PI synthesis, *KCS1* is involved in the PI second messenger system, and *PIS1* is required for PI synthesis from CDP-DAG. The other 14 genes, *DPP1*, *ELO1*, *ERG6*, *FAB1*, *HXK2*, *LPP1*, *PCT1*, *PLB1*, *PLB2*, *PLB3*, *STT4*, *TGL4*, *TGL5*, and *VPS34*, which were up-regulated by 100 μM inositol do not have any UAS_{INO} elements (Table 3). Therefore, our results showed that the transcriptional regulation of most UAS_{INO}-containing genes (18 out of 22) is negatively controlled by the presence of 100 μM inositol.

Role of Ino2p in phospholipid biosynthetic genes expression

It has been demonstrated that only Ino2p is required to bind to the UAS_{INO} element of *INO1* promoter for transcriptional

Table 3. Genes up-regulated by inositol for WT cells

Gene	Fold change[a]			
	10 μM inositol		100 μM inositol	
	Avg.[b]±SD[c]	*p* value	Avg.±SD	*p* value
CPT1	2.518±1.566	0.43	7.787±1.467	0.04
DPP1	0.847±0.061	0.13	1.502±0.192	0.12
ELO1	0.258±0.046	<0.01	1.302±0.045	0.02
ERG6	1.999±0.430	0.09	2.421±0.407	0.07
FAB1	0.870±0.255	0.66	1.306±0.220	0.30
HXK2	0.234±0.066	<0.01	1.916±0.126	0.02
INM1	3.172±1.718	0.33	4.190±0.218	<0.01
KCS1	2.285±0.705	0.21	3.966±0.411	0.02
LPP1	0.072±0.025	<0.01	3.761±0.833	0.08
PCT1	1.323±1.191	0.81	3.34±0	<0.01
PIS1	1.720±0.172	0.05	3.834±0.279	<0.01
PLB1	0.834±0.650	0.82	5.382±1.298	0.08
PLB2	4.218±3.563	0.46	26.35±7.71	0.08
PLB3	0.888±0.378	0.80	6.332±1.893	0.11
STT4	1.416±0.476	0.08	1.973±0.489	0.18
TGL4	0.266±0	<0.01	6.353±1.111	0.04
TGL5	0.769±0.066	0.07	1.994±0.179	0.03
VPS34	11.196±10.812	0.45	21.160±2.265	0.01

Annotation were as described in the footnote of Table 2.

activation (Schwank *et al*., 1995). Furthermore, we have shown transcriptional response of 47 phospholipid biosynthetic genes to the availability of inositol. To further examine the role of Ino2p in the regulation of other phospholipid biosynthetic gene expression, the same qRT-PCR strategy was used to determine the transcription profile of phospholipid genes in the presence or absence of the phospholipid precursor inositol for the yeast *ino2Δ* strain. By comparing the transcription profiles between WT cells and *ino2Δ* cells, we can examine how these genes respond to inositol through Ino2p.

Our results showed that the mRNA levels of 12 phospholipid genes decreased in the presence of 100 μM inositol (Table 4), and the mRNA levels of 35 phospholipid genes increased in the presence of 100 μM inositol in *ino2Δ* strain (Table 5). For the genes repressed by inositol in *ino2Δ* strain, 7 out of 12 genes, *CHO1*, *EKI1*, *INM1*, *ITR1*, *PAH1*, *PSD1*, and *PSD2*, contained at least one copy of UAS_{INO} element (Tables 1 and 4). *CHO1*, *EKI1*, *ITR1*, *PAH1*, *PSD1*, and *PSD2* were also repressed by 100 μM inositol in WT cells (Table 2), indicating that the induction of these genes depends on the presence of 100 μM inositol rather than the presence of Ino2p. These results suggest that these UAS_{INO}-containing genes might be

Table 4. Genes down-regulated by inositol for *ino2*Δ cells

Gene	Fold change[a]			
	10 μM inositol		100 μM inositol	
	Avg.[b]±SD[c]	*p* value	Avg.±SD	*p* value
CHO1	1.542±0.397	0.31	0.412±0.004	<0.01
EKI1	1.392±0.139	0.11	0.116±0.022	<0.01
ERG3	1.546±0.635	0.48	0.399±0.054	<0.01
ERG6	0.0012±0.0002	<0.01	0.016±0.005	<0.01
INM1	0.061±0.057	<0.01	0.014±0.006	<0.01
ITR1	1.042±0.348	0.91	0.060±0.021	<0.01
LPP1	0.111±0.062	<0.01	0.051±0.017	<0.01
PAH1	1.566±1.403	0.73	0.205±0.056	<0.01
PLC1	18.956±16.797	0.40	0.651±0	0.40
PSD1	2.349±1.306	0.41	0.068±0.009	<0.01
PSD2	0.511±0.216	0.20	0.511±0.076	0.02
TGL5	0.115±0.082	<0.01	0.722±0.082	0.08

Annotation were as described in the footnote of Table 2.

Table 5. Genes up-regulated by inositol for *ino2*Δ cells

Gene	Fold change[a]			
	10 μM inositol		100 μM inositol	
	Avg.[b]±SD[c]	*p* value	Avg.±SD	*p* value
CDS1	29.74±27.547	0.41	64.387±62.729	0.42
CHO2	5.205±0	<0.01	2.332±0.785	0.23
CKI1	0.288±0.279	0.13	1.802±1.336	0.61
CPT1	5.225±2.396	0.22	9.623±0.433	<0.01
DGA1	4.350±0.151	<0.01	0.994±0.325	0.99
DPP1	0.560±0.549	0.51	17.065±3.328	0.04
ECT1	0.003±0.001	<0.01	1.634±0.540	0.36
ELO1	0.038±0.030	<0.01	2.207±0.221	0.03
EPT1	8.923±7.873	0.42	34.572±33.077	0.42
ERG5	95.10±1.43	0.41	13.378±12.435	0.42
ERG20	32.486±31.072	0.42	27.787±23.128	0.37
FAB1	23.16±8.840	0.13	15.616±5.205	0.11
FAS1	33.58±33.138	0.43	30.225±27.455	0.40
FAS2	62.000±50.206	0.35	83.393±43.723	0.20
HXK2	4.711±4.044	0.46	81.126±22.843	0.07
INO1	0.436±0.312	0.21	1.476±0.138	0.07
IPK2	3.093±2.804	0.53	6.595±1.461	0.06
ITR2	7.108±1.830	0.08	5.406±2.268	0.19
KCS1	70.838±39.059	0.22	17.393±1.503	<0.01
LSB6	4.603±2.862	0.34	35.576±34.459	0.42
MSS4	33.366±0.694	<0.01	2.603±0	<0.01
NTE1	1.349±0.075	0.04	1.432±0.346	0.34
OLE1	6.621±5.255	0.40	94.113±78.333	0.40
OPI3	70.524±47.260	0.28	359.80±270.54	0.32
PCT1	8.445±6.798	0.39	166.770±57.637	0.10
PIK1	3.510±3.181	0.17	115.14±104.65	0.39
PIS1	0.260±0.189	0.06	34.287±2.727	<0.01
PLB1	2.225±1.693	0.54	6.522±0.113	<0.01
PLB2	4.150±0.878	0.07	97.567±33.122	0.10
PLB3	0.105±0.043	<0.01	7.735±0.722	0.01
SAC1	3.740±2.116	0.32	1.489±0.205	0.14
STT4	21.990±10.909	0.19	4.969±0.310	<0.01
TGL3	8.856±0.993	0.02	7.459±1.542	0.05
TGL4	0.323±0.079	0.01	4.737±1.119	0.08
VPS34	25.872±2.769	0.01	22.7±9.523	0.15

Annotation were as described in the footnote of Table 2.

negatively regulated by 100 μM inositol and the transcriptional control of these genes is irrelevant to the UAS_{INO} element. *INM1* was up-regulated by 100 μM inositol in WT cells but down-regulated by 100 μM inositol in *ino2Δ* cells, suggesting that transcriptional regulation of *INM1* might depend on the binding of Ino2p to its UAS_{INO} element through the positive response of Ino2p to inositol. The other 5 genes repressed by 100 μM inositol in the absence of Ino2p were *ERG3*, *ERG6*, *LPP1*, *PLC1*, and *TGL5*. The expression of *ERG3* and *PLC1* was also repressed by 100 μM inositol in WT cells, indicating that their expression is repressed by inositol and is irrelevant to the Ino2p. On the other hand, *ERG6*, *LPP1*, and *TGL5* which were repressed by 100 μM inositol in *ino2Δ*, cells were induced by 100 μM inositol in WT cells, suggesting that transcriptional regulation of these genes might depend on the indirect positive response of Ino2p to inositol.

For those genes which were up-regulated by 100 μM inositol in *ino2Δ* cells, 15 out of 35 genes contained at least one copy of the UAS_{INO} element, but only *CDS1*, *CHO2*, *CKI1*, *EPT1*, *ERG20*, *FAS1*, *FAS2*, *INO1*, *IPK2*, *OLE1*, *OPI3*, and *PIK1* were repressed by 100 μM inositol in WT cells (Tables 2 and 5). It has been shown that transcriptional regulation of *INO1* depends on the binding of Ino2p to the UAS_{INO} element through the negative response of Ino2p to inositol (Lopes and Henry, 1991; Lopes *et al*., 1991; Ambroziak and Henry, 1994; Schwank *et al*., 1995; Koipally *et al*., 1996). Therefore,

it is possible that all these genes have the same mechanism of transcriptional regulation as *INO1*. The other three UAS_{INO}-containing genes *CPT1*, *KCS1*, and *PIS1* were induced by 100 μM inositol in both WT and *ino2Δ* cells, indicating that the expression of these three genes is regulated by the availability of inositol and is irrelevant to the presence of Ino2p (Tables 3 and 5). The rest of the other genes up-regulated by 100 μM inositol are non-UAS_{INO}-containing genes. *DGA1*, *ECT1*, *ERG5*, *ITR2*, *LSB6*, *MSS4*, *NTE1*, *SAC1*, and *TGL3* were up-regulated by 100 μM inositol in *ino2Δ* cells, but down-regulated by 100 μM inositol in WT cells, suggesting that transcriptional regulation of these genes probably depends on the negative response of Ino2p to inositol (Tables 2 and 5). The other 11 genes, *DPP1*, *ELO1*, *FAB1*, *HXK2*, *PCT1*, *PLB1*, *PLB2*, *PLB3*, *STT4*, *TGL4*, and *VPS34*, were up-regulated by 100 μM inositol in both WT and *ino2Δ* cells. (Tables 3 and 5). Therefore, the expression of these genes was directly influenced by the availability of the inositol positively.

Discussion

In the yeast *Saccharomyces cerevisiae*, the transcription of structural genes, which encode many phospholipid biosynthetic enzymes, is believed to be coordinately regulated by a *cis*-acting promoter element named UAS_{INO} element (Carman and Henry, 1989; Carman and Henry, 1999; Kagiwada and Zen, 2003; Jesch *et al*., 2006; Jani and Lopes, 2008). The binding of the Ino2p activator to the UAS_{INO} element is in response to the availability of inositol in the environment (Ambroziak and Henry, 1994; Schwank *et al*., 1995). However, some phospholipid biosynthetic genes do not have any UAS_{INO} element in their promoter region. The availability of inositol could also influence their transcriptional activation. Therefore, a complete description of the transcriptional changes of the phospholipid biosynthetic genes can help us to understand the regulation of the coordinated phospholipid signaling pathway.

From sequence analysis, we found that 22 out of 47 phospholipid biosynthetic genes contained at least one or more copies of the UAS_{INO} element in their promoter. Seven of them have never been reported before, including *EKI1*, *EPT1*, *INM1*, *IPK2*, *KCS1*, *PAH1*, and *PIK1*. We have also shown that the addition of inositol to logarithmically growing yeast cells resulted in major and rapid changes of the transcription profile. Based on our findings, we propose that these phospholipid biosynthetic genes can be divided into 2 different categories depended on their regulation or lack of regulation by 100 μM inositol through Ino2p (Table 6). Each category can be further divided into up-regulated by and down-regulated by 100 μM inositol. Twenty-one genes, *CDS1*, *CHO2*, *CKI1*, *DGA1*, *ECT1*, *EPT1*, *ERG5*, *ERG20*, *FAS1*, *FAS2*, *INO1*, *IPK2*, *ITR2*, *LSB6*, *MSS4*, *NTE1*, *OLE1*, *OPI3*, *PIK1*, *SAC1*, and *TGL3*, are regulated by the negative response of Ino2p to inositol either directly or indirectly. Only 4 genes, *ERG6*, *INM1*, *LPP1*, and *TGL5*, are positively regulated by the response of Ino2p to inositol. The other 22 phospholipid biosynthetic genes are directly regulated by 100 μM inositol (Table 6).

The expression of *INO1* has been studied intensively (Ambroziak and Henry, 1994; Kagiwada and Zen, 2003; Ford *et al*., 2007, 2008; Esposito *et al*., 2009). The regulation of *INO1* transcription is dependent on the binding of Ino2p to the UAS_{INO} element, which is in response to inositol availability. Here, we showed that *INO1* expression was up-regulated in the absence of inositol for WT cells (Table 2). The expression of *INO1* was down-regulated in the absence of inositol for *ino2Δ* cells. This result confirms that *INO1* expression depends on the negative response of Ino2p to 100 μM inositol. Eleven other UAS_{INO}-containing genes displayed a similar pattern as *INO1*, including *CDS1*, *CHO2*, *CKI1*, *EPT1*, *ERG20*, *FAS1*, *FAS2*, *IPK2*, *OLE1*, *OPI3*, and *PIK1* (Table 6). Therefore, 12 out of 22 UAS_{INO}-containing structural genes are negatively regulated by inositol through Ino2p. Of these, *CDS1*, *CHO2*, *CKI1*, *ERG20*, *FAS1*, *FAS2*, *OLE1*, and *OPI3* had been previously shown to exhibit a negative response of Ino2p to the inositol (Santiago and Mamoun, 2003; Jesch *et al*., 2005, 2006). The present study is the first report to show that *EPT1*, *IPK2*, and *PIK1* are co-regulated with *INO1* and are likely to be Ino2p targets. One other UAS_{INO}-containing gene, *INM1*, is positively regulated by inositol through Ino2p. The other 9 UAS_{INO} element-containing genes that are not directly regulated by the Ino2p may be regulated by inositol directly or through other signals generated by ongoing phospholipid metabolism (Henry and Patton-Vogt, 1998).

Previous studies showed that 4 UAS_{INO}-containing genes, *CHO1*, *ITR1*, *PSD1*, and *PSD2*, displayed a similar negative response to 100 μM inositol as *INO1* (Jesch *et al*., 2005, 2006). This is in accord with our findings for WT cells (Table 2).

Table 6. Categories of inositol-regulated phospholipid biosynthetic genes

Regulation through inositol directly				Regulation through the response of Ino2p to inositol			
Up-regulation		Down-regulation		Up-regulation		Down-regulation	
UAS_{INO}	Non-UAS_{INO}	UAS_{INO}	Non-UAS_{INO}	UAS_{INO}	Non-UAS_{INO}	UAS_{INO}	Non-UAS_{INO}
CPT1	*DPP1*	*CHO1*	*ERG3*	*INM1*	*ERG6*	*CDS1*	*DGA1*
KCS1	*ELO1*	*EKI1*	*PLC1*		*LPP1*	*CHO2*	*ECT1*
PIS1	*FAB1*	*ITR1*			*TGL5*	*CKI1*	*ERG5*
	HXK2	*PAH1*				*EPT1*	*ITR2*
	PCT1	*PSD1*				*ERG20*	*LSB6*
	PLB1	*PSD2*				*FAS1*	*MSS4*
	PLB2					*FAS2*	*NTE1*
	PLB3					*INO1*	*SAC1*
	STT4					*IPK2*	*TGL3*
	TGL4					*OLE1*	
	VPS34					*OPI3*	
						PIK1	

In the present study, we further examined the response of these genes to 100 μM inositol in *ino2Δ* cells and found they did not show similar patterns as *INO1*, indicating that their response to inositol was not related to the UAS_{INO} element. It is possible that these four genes along with *EKI1*, *ERG3*, *PAH1*, and *PLC1* are negatively regulated by inositol directly and not through the response of Ino2p to inositol (Tables 2, 4, and 6). Furthermore, we found that 3 other UAS_{INO}-containing genes, *CPT1*, *KCS1* and *PIS1*, are positively regulated by 100 μM inositol directly (Table 6). Therefore, our results demonstrated the evidence that the expression of UAS_{INO}-containing genes is not completely regulated by Ino2p.

Our results showed that genes involved in the PE methylation pathway and genes involved in the PE synthesis via DAG were up-regulated in the absence of inositol. These observations suggest that PC synthesis is increased in the absence of inositol. Since PC synthesis is increased, PI synthesis is expected to drop (Fig. 1). Indeed, we observed that *PIS1* was down-regulated in the absence of inositol, indicating the decrease of PI synthesis (Table 3). This 3.8-fold repression of *PIS1* is in accord with previous findings (Jani and Lopes, 2008). Previous studies have also demonstrated that the rate of PI synthesis dramatically increased and PC synthesis decreased when inositol was added to the growth medium of WT cells (Loewen *et al*., 2004; Gaspar *et al*., 2006). Therefore, our findings provide a detailed explanation for such physiological reactions.

In this report, we identified some structural genes that do not have the UAS_{INO} element and might be either positively or negatively regulated by the response of Ino2p to 100 μM inositol (Table 6). It is possible that Ino2p regulates these genes via indirect effects which could represent ongoing phospholipid metabolism. We have also shown that the positive or negative regulation of most of the UAS_{INO}-containing genes depends on the response of Ino2p to inositol availability. Taken together, these observations suggest that Ino2p is mediating either repression or activation of these genes in response to inositol through either direct or indirect effect. Further biochemical analysis, including chromatin immunoprecipitation coupled with real-time PCR analysis and/or promoter site-direct mutagenesis, can provide insight into the coordinated regulation of phospholipid gene expression.

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References

Alvarez-Vasquez, F., K.J. Sims, L.A. Cowart, Y. Okamoto, E.O. Voit, and Y.A. Hannun. 2005. Simulation and validation of modelled sphingolipid metabolism in *Saccharomyces cerevisiae*. *Nature* 433, 425-430.

Ambroziak, J. and S.A. Henry. 1994. *INO2* and *INO4* gene products, positive regulators of phospholipid biosynthesis in *Saccharomyces cerevisiae*, form a complex that binds to the *INO1* promoter. *J. Biol. Chem*. 269, 15344-15349.

Boumann, H.A., J. Gubbens, M.C. Koorengevel, C.S. Oh, C.E. Martin, A.J. Heck, J. Patton-Vogt, S.A. Henry, B. de Kruijff, and A.I. de Kroon. 2006. Depletion of phosphatidylcholine in yeast induces shortening and increased saturation of the lipid acyl chains: evidence for regulation of intrinsic membrane curvature in a eukaryote. *Mol. Biol. Cell* 17, 1006-1017.

Carman, G.M. and S.A. Henry. 1989. Phospholipid biosynthesis in yeast. *Annu. Rev. Biochem*. 58, 635-669.

Carman, G.M. and S.A. Henry. 1999. Phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae* and interrelationship with other metabolic processes. *Prog. Lipid Res*. 38, 361-399.

Czech, M.P. 2000. PIP2 and PIP3: complex roles at the cell surface. *Cell* 100, 603-606.

Divecha, N. and R.F. Irvine. 1995. Phospholipid signaling. *Cell* 80, 269-278.

Dowd, S.R., M.E. Bier, and J.L. Patton-Vogt. 2001. Turnover of phosphatidylcholine in *Saccharomyces cerevisiae*. The role of the CDP-choline pathway. *J. Biol. Chem*. 276, 3756-3763.

Esposito, M., P. Konarzewska, O. Odeyale, and C.H. Shen. 2009. Genewide histone acetylation at the yeast *INO1* requires the transcriptional activator Ino2p. *Biochem. Biophys. Res. Commun.* 391, 1285-1290.

Ford, J., O. Odeyale, A. Eskandar, N. Kouba, and C.H. Shen. 2007. A SWI/SNF- and INO80-dependent nucleosome movement at the *INO1* promoter. *Biochem. Biophys. Res. Commun.* 361, 974-979.

Ford, J., O. Odeyale, and C.H. Shen. 2008. Activator-dependent recruitment of SWI/SNF and INO80 during *INO1* activation. *Biochem. Biophys. Res. Commun.* 373, 602-606.

Gaspar, M.L., M.A. Aregullin, S.A. Jesch, and S.A. Henry. 2006. Inositol induces a profound alteration in the pattern and rate of synthesis and turnover of membrane lipids in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 281, 22773-22785.

Greenberg, M.L. and J.M. Lopes. 1996. Genetic regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*. *Microbiol. Rev*. 60, 1-20.

Henry, S.A. and J.L. Patton-Vogt. 1998. Genetic regulation of phospholipid metabolism: yeast as a model eukaryote. *Prog. Nucleic Acid Res. Mol. Biol.* 61, 133-179.

Hoppen, J., A. Repenning, A. Albrecht, S. Geburtig, and H.J. Schuller. 2005. Comparative analysis of promoter regions containing binding sites of the heterodimeric transcription factor Ino2/Ino4 involved in yeast phospholipid biosynthesis. *Yeast* 22, 601-613.

Jani, N.M. and J.M. Lopes. 2008. Transcription regulation of the *Saccharomyces cerevisiae* PIS1 gene by inositol and the pleiotropic regulator, Ume6p. *Mol. Microbiol*. 70, 1529-1539.

Jesch, S.A., P. Liu, X. Zhao, M.T. Wells, and S.A. Henry. 2006. Multiple endoplasmic reticulum-to-nucleus signaling pathways coordinate phospholipid metabolism with gene expression by distinct mechanisms. *J. Biol. Chem.* 281, 24070-24083.

Jesch, S.A., X. Zhao, M.T. Wells, and S.A. Henry. 2005. Genome-wide analysis reveals inositol, not choline, as the major effector of Ino2p-Ino4p and unfolded protein responsetarget gene expression in yeast. *J. Biol. Chem*. 280, 9106-9118.

Kagiwada, S. and R. Zen. 2003. Role of the yeast VAP homolog, Scs2p, in *INO1* expression and phospholipid metabolism. *J. Biochem.* 133, 515-522.

Kelley, M.J., A.M. Bailis, S.A. Henry, and G.M. Carman. 1988. Regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae* by inositol. Inositol is an inhibitor of phosphatidylserine synthase activity. *J. Biol. Chem.* 263, 18078-18085.

Koipally, J., B.P. Ashburner, N. Bachhawat, T. Gill, G. Hung, S.A. Henry, and J.M. Lopes. 1996. Functional characterization of the repeated UAS_{INO} element in the promoters of the *INO1* and *CHO2* genes of yeast. *Yeast* 12, 653-665.

Loewen, C.J., M.L. Gaspar, S.A. Jesch, C. Delon, N.T. Ktistakis, S.A. Henry, and T.P. Levine. 2004. Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. *Science* 304, 1644-1647.

Lopes, J.M. and S.A. Henry. 1991. Interaction of *trans* and *cis* regulatory elements in the *INO1* promoter of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 19, 3987-3994.

Lopes, J.M., J.P. Hirsch, P.A. Chorgo, K.L. Schulze, and S.A. Henry. 1991. Analysis of sequences in the *INO1* promoter that are involved in its regulation by phospholipid precursors. *Nucleic Acids Res.* 19, 1687-1693.

Martin, T.F. 2001. PI (4,5)P(2) regulation of surface membrane traffic. *Curr. Opin. Cell Biol.* 13, 493-499.

Odom, A.R., A. Stahlberg, S.R. Wente, and J.D. York. 2000. A role for nuclear inositol 1,4,5-trisphosphate kinase in transcriptional control. *Science* 287, 2026-2029.

Ohanian, J. and V. Ohanian. 2001. Sphingolipids in mammalian cell signaling. *Cell. Mol. Life Sci.* 58, 2053-2068.

Saiardi, A., J.J. Caffrey, S.H. Snyder, and S.B. Shears. 2000a. Inositol polyphosphate multikinase (ArgRIII) determines nuclear mRNA export in *Saccharomyces cerevisiae*. *FEBS Lett*. 468, 28-32.

Saiardi, A., J.J. Caffrey, S.H. Snyder, and S.B. Shears. 2000b. The inositol hexakisphosphate kinase family: Catalytic flexibility and function in yeast vacuole biogenesis. *J. Biol. Chem.* 275, 24686-24692.

Santiago, T.C. and C.B. Mamoun. 2003. Genome expression analysis in yeast reveals novel transcriptional regulation by inositol and choline and new regulatory functions for Opi1p, Ino2p, and Ino4p. *J. Biol. Chem.* 278, 38723-38730.

Schuller, H.J., K. Richter, B. Hoffmann, R. Ebbert, and E. Schweizer. 1995. DNA binding site of the yeast heteromeric Ino2p/Ino4p basic helix-loop-helix transcription factor: structural requirements as defined by saturation mutagenesis. *FEBS Lett.* 370, 149-152.

Schwank, S., R. Ebbert, K. Rautenstrauss, E. Schweizer, and H.J. Schuller. 1995. Yeast transcriptional activator *INO2* interacts as an Ino2p/Ino4p basic helix-loop-helix heteromeric complex with the inositol/choline-responsive element necessary for expression of phospholipid biosynthetic genes in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 23, 230-237.

Shears, S.B. 2000. Transcriptional regulation: a new dominion for inositol phosphate signaling? *Bioessays* 22, 786-789.

Shields, D. and P. Arvan. 1999. Disease models provide insights into post-golgi protein trafficking, localization and processing. *Curr. Opin. Cell Biol*. 11, 489-494.