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Minireview

Inositol and Phosphatidylinositol Mediated Glucose Derepression, Gene Expression and Invertase Secretion in Yeasts

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Abstract Glucose repression occurs in many yeast species and some filamentous fungi, and it represses the expression and secretion of many intracellular and extracellular proteins. In recent years, it has been found that many biochemical reactions in yeast cells are mediated by phosphatidylinositol (PI)-type signaling pathway. However, little is known about the relationships between PI-type signaling and glucose repression, gene expression and invertase secretion in yeasts. Many evidences in our previous studies showed that glucose repression, invertase secretion, gene expression and cell growth were mediated by inositol and PI in *Saccharomyces* and *Schizosaccharomyces*. The elucidation of the new regulatory mechanisms of protein secretion, gene expression and glucose repression would be an entirely new aspect of inositol and PI-type signaling regulation in yeasts.

Key words glucose repression; PI-type signaling pathway; invertase secretion; gene expression

Glucose Repression [1,2]

Saccharomyces cerevisiae and other yeasts can grow well on different kinds of carbon sources. However, glucose and fructose are the best carbon sources for their growth. When the medium contains glucose or fructose, the biosynthesis of enzyme catalyzing degradation of other carbon sources will be greatly reduced or stopped. This phenomenon is called glucose repression.

Although much progress has been made in this field, the exact mechanisms of glucose repression in yeasts are still unclear. It is very important to study the exact mechanisms of glucose repression in yeasts, and the elucidation of the mechanisms will have many applications to fermentation industries [3].

Control levels [2]

Glucose repression can be caused at different levels when medium contains glucose, they include: (1) the concentrations of the corresponding mRNAs reduce, which depend on transcription rate of the corresponding genes and the stabilities of mRNAs; (2) the translation rate reduces; (3) the rate of protein degradation increases. The most general reaction of glucose repression involves a parallel decrease at mRNA and protein levels.

Relationship between Mig1 complex and glucose repression [2]

*MIG*1 gene is found to play an important role in glucose repression. Mig1 is a C_2H_2 zinc finger protein that is able to bind the promoters of a variety of genes repressed by glucose. The binding requires a GC box with a consensus sequence (G/C)(C/T)GGGG and also requires an AT-rich region at the 5′ side of the GC box. It has been suggested that the finger 1 of Mig1 recognizes a G(G/A)G triplet and the finger 2 recognizes a (G/C)(C/T)G triplet. The AT-rich region would be required to stabilize the interaction, since it would allow the bending of the DNA and facilitate the following protein-DNA contacts.

For repression to be relieved when the concentration of glucose in the medium decreases, two internal elements of Mig1 protein are also required. One includes two RXXS

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motifs, the potential substrates for some protein kinases. The other important feature of Mig1 is a basic domain at the 3′ side of the zinc fingers, which might be involved in targeting Mig1 to the nucleus.

In addition, two more proteins with zinc fingers similar to those of Mig1 have been identified in *S. cerevisiae*. One of them called Mig2 can bind to the promoter of *SUC*2 and contribute to its repression by glucose.

Gancedo [2] proposed a model for the mode of Mig1's action and its regulation. In the presence of glucose in the medium, Mig1 is located in the nucleus in the yeast cells, where it represses transcription of the genes repressed by glucose. When glucose in the medium is depleted by yeast cells or when yeast cells grow in the non-fermentable carbon sources, the glucose removal causes phosphorylation of Mig1 by catalysis of the Snf1 complex discussed below. In this case, the phosphorylated Mig1 is translocated to cytoplasm and glucose repression on the genes is relieved, leading to transcription of these genes. The whole process is shown in Fig. 1.

Relationship between Snf1 complex and glucose repression [4,5]

Snf1 complex encoded by *SNF*1 is a protein kinase. Snf1 has two domains: an amino-terminal catalytic domain and a carboxyl-terminal regulatory domain. At high glucose concentration, the Snf1 regulatory domain binds to the catalytic domain and inhibits the kinase activity; while at low glucose concentration, Snf4, another component of the Snf1 complex, interacts with the regulatory domain, counteracting the inhibitory effect of Snf1. The *SNF*1 is absolutely required for the derepression of the genes repressed by glucose. When the glucose concentration in the medium is very low or there is no glucose in the growth medium, the Snf1 complex is phosphorylated by another unknown kinase in yeast cells. In this case, the Snf1 complex is active and can catalyze the phosphorylation of Mig1 as discussed above. The phosphorylated Mig1 complex fails in binding to the promoters of the genes repressed by glucose and is trans-located to cytoplasm, leading to the active transcription of the genes. Therefore, the Snf1 protein kinase is a central component of the signaling pathway for glucose repression in yeasts. When the glucose concentration in the medium is very high, the Snf1 complex is dephosphorylated and inactive. So Mig1 complex can not be phosphorylated and bind to the promoters of the genes repressed by glucose, see Fig. 1.

So far, it is little known about how the presence of glucose affects the activity of Snf1 and how glucose contributes to repression by the signaling pathway. However, it has commonly been accepted that the signal that really causes glucose repression in yeasts is the intracellular concentration of glucose rather than the glucose flux, and the evidence that cells have a significant concen-

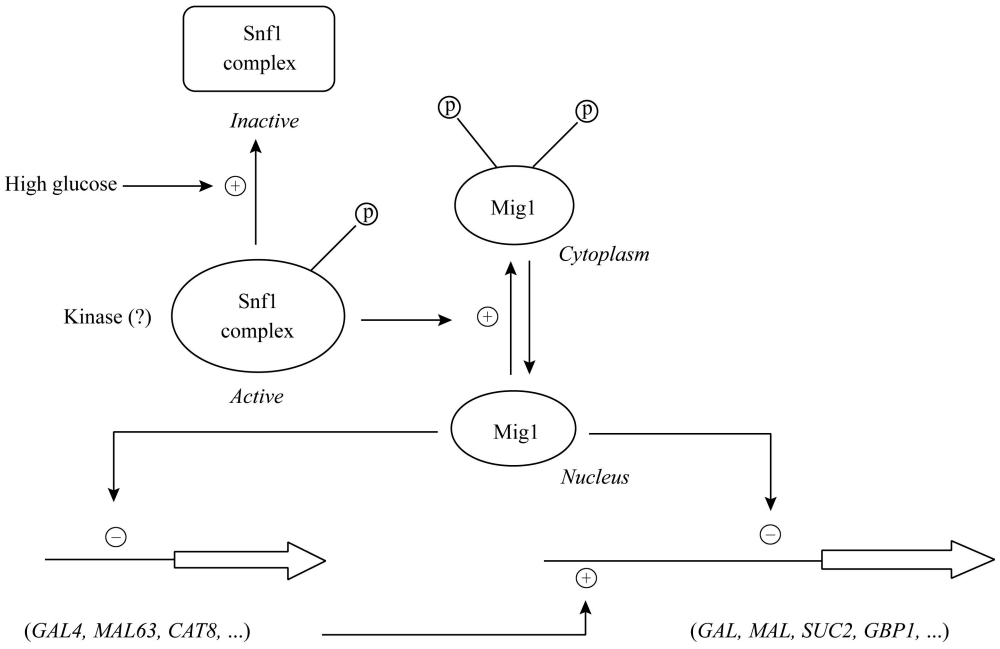

Fig. 1 The mode of the actions of Mig1 and Snf1 in yeast cells

tration of intracellular glucose suggests that glucose itself could be a signaling molecule [4].

Glucose repression in the utilization of sucrose [5]

Expression of *SUC*2 gene encoding invertase in *S. cerevisiae* is only regulated by glucose repression and not induced by sucrose. In the absence of glucose in the medium, *SUC*2 in the yeast cells is expressed. In the presence of high concentration of glucose, *SUC*2 in the yeast cells is repressed. As discussed above, Mig1 encoded by *MIG*1 binds to the promoter of *SUC*2 and transcription of the *SUC*2 is repressed. It has been shown that the similar phenomenon happens in the cells of *S. pombe* [6]. If *MIG*1 is disrupted by genetic method, the mutants obtained will not synthesize Mig1, leading to derepression of the *SUC*2 gene expression. In the presence of glucose in the medium, the specific rate of sucrose hydrolysis in the *mig*1 mutant will keep stable (10 $mmol \cdot g^{-1} \cdot h^{-1}$), whereas that in its wild type only keeps 1 $mmol \cdot g^{-1} \cdot h^{-1}$.

As mentioned above, besides Mig1, Mig2 can also bind to the promoter of the genes repressed by glucose. Therefore, in some mutants, only when both *MIG*1 and *MIG*2 genes are disrupted, glucose repression on *SUC*2 can be relieved (see Table 1).

Even if *SNF*1 in the yeast cells is disrupted, Mig1 in the mutants still can not be phosphorylated when the mutants obtained are grown in the medium without glucose or with non-fermentable carbon sources, resulting in failure in glucose derepression of the genes repressed by glucose (see Table 1) [5].

PI-type Signaling Pathway in Yeast Cells

The plasma membrane of *S. cerevisiae* cells is mainly composed of phosphatidic acids (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and cardiolipin (CL). Among the major phospholipids, PI is attracting more attention because it has many physiological functions in cell morphology, metabolic regulation and signal transduction. Further elucidation of the physiological function of PI in yeast cells will be very helpful in understanding PI functions in other eukaryotic cells [7].

Addition of inositol in the growth medium can cause significant increase in PI content in yeast cells. For example, Kelly *et al*. [8] reported that in the presence of inositol in the medium, PI content in the plasma membrane of *S. cerevisiae* increased from 10% to 27%. In our previous studies, the same results were obtained [9,10]. This means that added inositol in the medium is a major factor for regulation of PI biosynthesis in yeast cells and many other biochemical reactions in yeasts cells are regulated by inositol [11].

In yeast cells, inositol in PI can be phosphorylated consecutively at 4-position and 5-position in its molecule by kinase, respectively, and converted into phosphatidylinositol 4,5-bisphosphate (PIP_2) via ATP-dependent phosphorylation. When some extracellular stimulants bind to the receptor on plasma membrane of yeast cells, phosphatidylinositide-specific phospholipase C (PLC) will be activated and the activated PLC catalyzes the hydrolysis of PIP_2 in the plasma membrane into inositol 1,4,5-trisphosphate (IP_3) and DAG, the second messenger molecules. Because it is soluble, IP_3 will come to cytoplasm, leading to the release of calcium from the intracellular pool. Many enzymes in the cells will be activated by the increase in the intracellular calcium concentration. DAG produced in the plasma membrane results in the translocation and activation of protein kinase C (PKC). However, the full activation of PKC needs both DAG and PS. After the activation of PKC, a series of changes in pH and membrane will take place [12,13]. The whole process is shown in Fig. 2. Brandao *et al.* [14] found

Table 1 Invertase activities of *mig*1, *mig*1 *mig*2 and *snf*1 mutants

Genotypes	The specific activities of invertase in glucose repressed cells	The specific activities of invertase in the derepressed cells
Wild type	6	586
*mig*1Δ	88	1182
*mig*2Δ	2	765
*mig*1Δ *mig*2Δ	1178	2213
*snf*1Δ	<1	<1
*mig*2Δ *snf*1Δ	<1	<1

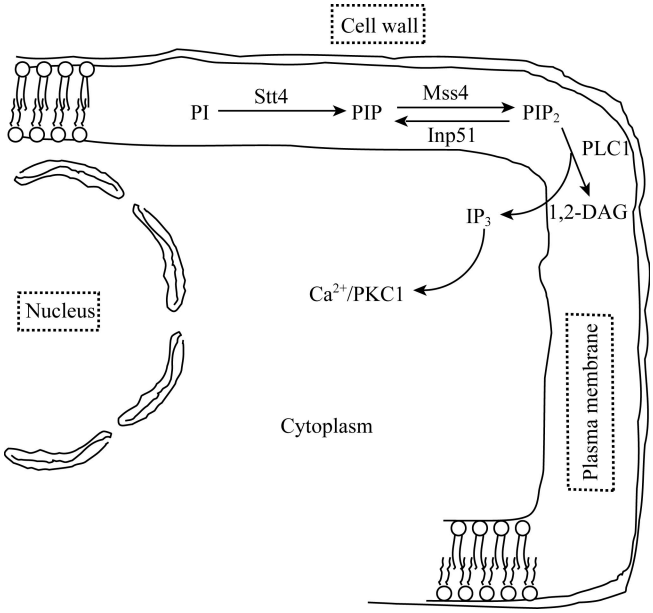

Fig. 2 PI-type signaling in the yeast cells

that the addition of the activators of DAG and PKC to the cell cultures also activates plasma membrane H^+-ATPase and causes an export of H^+ from the cells at the same time. Neomycin can specifically react with PIP_2 to form a complex, and compound 48/80 is a specific inhibitor of PLC. The addition of these two compounds to the cell cultures can completely inhibit the activation of plasma membrane H^+-ATPase and export of H^+ from the cells. These results indicate that PI-type signaling pathway in the yeast cells is involved in the activation of plasma membrane H^+-ATPase and export of H^+ from the cells by added glucose. Coccetti *et al.* [15] found that phospholipase C encoded by *PLC*1 in *S. cerevisiae* is essential for glucose-induced PI turnover and activation of plasma membrane H^+-ATPase as in *plc*1 mutant, glucose-induced PI turnover and activation of plasma membrane H^+-ATPase are completely absent and glucose-induced turnover and activation of plasma membrane H^+-ATPase are restored by reintroducing the *PLC*1 on a multi-copy plasmid into the *plc*1 mutant. They also found that after adding glucose the DAG level increases 1.7-fold in wild type cells, while in the *plc*1 mutant the increase significantly reduces. This indicates that *PLC*1 encoded phospholipase C significantly contributes to DAG production likely through the polyphosphoinositide hydrolysis mentioned above.

Inositol and PI-mediated Invertase Secretion in Yeasts

In recent years, more attention has been paid to protein secretion in the yeast cells and many advances in this field have been made. However, little is known about the relationship between expression of the genes encoding secreted proteins and protein secretion and the changes in phospholipids of plasma membrane in yeast cells. As we know, the whole process during protein secretion in yeast cells always has some connections with the membrane. Indeed, there are many evidences that modification and regulation of many enzymes need some kinds of phospholipids in membrane. David *et al.* [16] reported that there were some relationships between the length of fatty acids in membrane and protein secretion. In recent years, this has been attracting our many interests in relationship between phospholipids, in particular PI and enzyme secretion in yeasts. New elucidation of the regulatory mechanisms of protein secretion would open new sight for inositol and PI-type signaling regulation in yeast.

Invertase is a naturally secreted protein of *Saccharomyces* and *Schizosaccharomyces*, and located in periplasmic space and cell wall of these yeasts after secretion. It has been shown that invertase is an excellent model in the study of protein secretion.

In order to know the relationship between PI content and invertase secretion in *Saccharomyces sp.*, effects of inositol on invertase secretion and phospholipid biosynthesis were examined in our previous studies [17]. We found that invertase secretion in *Saccharomyces sp.* W4 was repressed when the completely synthetic medium contained more than 0.2% (*W*/*V*) glucose while lacked exogenous inositol; however, when the yeast cells were grown in the same medium plus inositol (100 μg/ml), repression of invertase secretion in this yeast strain occurred only at glucose concentration higher than 2.0% (*W*/*V*) or at sucrose concentration above 4.0% (*W*/*V*). More cells grew while less reducing sugar was left with higher glucose or sucrose concentration in the inositol-containing medium. These results demonstrated that inositol supplementation could derepress invertase secretion and cell growth in this yeast strain to some extent. Why does the inositol supplementation derepress invertase secretion and cell growth and what are the biochemical and molecular mechanisms of glucose derepression? In order to answer both two questions, PI contents in the yeast cells that grown in the inositol-containing medium and those grown in the inositol-deficient medium were determined and compared. The results showed that PI contents in the yeast cells grown in the inositol-containing medium were much higher than

those grown in the inositol-deficient medium. For example, PI content of the yeast cells grown in the inositol-containing medium increased from 16.4% to 23.92% after 24 hours of fermentation, while PI content of those grown in the inositol-deficient medium decreased from 17.54% to 7.18% after the same period cultivation [9,10]. Therefore, we think that the derepression of invertase secretion in this yeast strain is related to PI content in the cells [7,17]. Recently, we found that much more mRNA encoding secreted invertase was detected by RT-PCR in the glucose-derepressed cells grown in the synthetic medium plus inositol than that in the glucose-repressed cells grown in the synthetic medium lacking inositol, These results demonstrated that inositol and PI were involved in invertase gene expression and invertase secretion in yeasts.

In recent years, it has been found that *Schizosaccharomyces pombe* is also an ideal eukaryotic microorganism for biochemical and genetic studies [16,18,19]. Some strains of this yeast are naturally inositol requiring since their genomes do not contain *INO*1 gene encoding inositol-phosphate synthase so that they fail to grow without inositol; while *Saccharomyces* cells can synthesize inositol from glucose-6-phosphate via inositol-phosphate synthase even without inositol in the medium [11,20]. Therefore, we think this fission yeast that is a strict inositol auxotroph would present a useful comparison to *Saccharomyces* in studying the role of inositol and PI in gene expression and enzyme secretion. Because of its failure in inositol biosynthesis, it is very easy to control *S. pombe* cell growth and PI content by adding different concentrations of inositol to the synthetic medium. So far, little is known about invertase secretion and phospholipid biosynthesis in this yeast [21]. The main purpose of our study was to know how inositol affected invertase secretion, *INV*$^+$ gene (encoding invertase) expression, cell growth and phospholipids biosynthesis in *S. pombe*. We found that the specific activity of invertase was at its maximum when the inositol concentration reached 800 μg/100 ml in the synthetic medium containing 2.0% (*W*/*V*) sucrose. However, cell growth steadily increased as inositol concentration increased from 0 to 1000 μg/100 ml. When the inositol concentration was 100 μg/100 ml in the synthetic medium, repression of invertase secretion occurred at sucrose concentration higher than 0.5% (*W*/*V*). However, when *S. pombe* was cultured in the synthetic medium containing 800 μg/100 ml inositol, repression of invertase secretion occurred only at sucrose concentrations above 2.0% (*W*/*V*). Less residual reducing sugar was left in the 800 μg/100 ml inositol-containing medium than that was in the 100 μg/100 ml inositol-containing medium as sucrose concentration increased. More mRNA encoding secreted invertase was detected in cells grown in the 800 μg/100 ml inositol-containing medium (2.0% sucrose) than in those grown in the 100 μg/100 ml inositol-containing medium. These results demonstrated that higher concentra-tion of inositol in the synthetic medium could derepress invertase secretion and *INV*$^+$gene expression in *S. pombe*. PI content (13.8%) and ratio of PI versus PS (1.27) of the yeast cells grown in the synthetic medium with 800 μg/100 ml of inositol were higher than those (6.2% and 0.44, respectively) of the yeast cells grown in the same medium with 100 μg/100 ml of inositol. This meant that PI might be involved in derepression of invertase secretion [22,23].

Thus, in *S. pombe*, just like in *Saccharomyces sp.*, the results described above clearly demonstrated that invertase secretion and cell growth could be derepressed to some extent when the yeast cells were grown at higher concentration of inositol. This derepression might be related to higher content of PI in the yeast cells and less glucose in the medium. According to the review articles [3,22] on yeast carbon catabolite repression, the main effect of glucose on *SUC*2 expression takes place at the transcriptional level by binding of the regulatory proteins to the promoter region of *SUC*2 gene (Fig. 2). Therefore, we think the PI content increase may finally cause phosphorylation of Mig1 by PI-type signaling pathway and its translocation to cytoplasm in *S. pombe* in which the similar glucose repression occurs [6]. Then, the activators activate transcription of *INV*$^+$ gene and cause increase in mRNA encoding secreted invertase even in the presence of high concentration of sucrose. Therefore, glucose derepression in *S. pombe* occurred at sucrose concentrations below 2.0% (*W*/*V*) when the fission yeast cells were grown in the synthetic medium plus 800 μg/100 ml of inositol due to high content of PI in the cells. When the yeast cells were grown in the synthetic medium plus 100 μg/100 ml of inositol, the decrease in PI content may cause dephosphorylation of Mig1 and the Mig1 in the nucleus repressed the transcription of *INV*$^+$ gene, so that the decrease in mRNA encoding secreted invertase happened in the presence of high concentration of sucrose. Therefore, specific invertase activity was decreased continuously as sucrose concentration was increased from 0.5% to 5.0% (*W*/*V*) because of lower amount of PI in the cells.

Recently, it has been found that PI 4-phosphate limitation is a major contributing factor to the secretory defect in sec14 cells and PI 4-phosphate plays an important role in the Golgi-to-plasma membrane stage of

invertase secretion [24]. However, they did not mention how the biosynthesis of invertase was enhanced when PI 4-phosphate in Golgi-derived transport vesicles of *Saccharomyces* cells was elevated. In the case of the present study, it is still completely unknown how the PI content increase finally leads to the phosphorylation of Mig1 by the PI-type signaling pathway and derepression of *INV*$^+$ gene expression in *S. pombe*.

As mentioned above, wild type of *S. pombe* is a natural inositol auxotroph [25]. When *S. pombe* was transformed with the two plasmids, pADH-INO and pSPIN-22 that contain a *Pichia pastoris INO*1 gene encoding inositol-phosphate synthase [22,25], the two types of transformants obtained were named as *Sch.P*944 and *Sch.P*1025, respectively. It was interesting that *Sch.P*944 could synthesize a larger amount of inositol than *Sch.P*1205. We found that the invertase secretion in *Sch.P*944 was repressed when the completely synthetic medium lacking exogenous inositol contains more than 1.0% (*W*/*V*) glucose, while specific invertase activity in *Sch.P*1205 was continuously decreased as glucose concentration in inositol-deficient medium was increased from 0.2% (*W*/*V*) to 5.0% (*W*/*V*). This meant that the invertase secretion in *Sch.P*1205 was repressed by the higher concentration of glucose. Our studies also showed that the PI and PS content in *Sch.P*944 were 15.34% and 7.78%, respectively, while those in *Sch.P*1205 were 10.04% and 10.30%, respectively. The results again showed that PI-type signaling pathway might be involved in derepression of invertase secretion [23].

Perspectives

As discussed above, it is still completely unknown how the PI content increase finally leads to the phosphorylation of Mig1 and Snf1 complex by the PI-type signaling pathway and derepression of *INV*$^+$ gene expression in *S. pombe* at higher glucose concentration.

As shown in Table 1, in the *mig*1 or *mig*1*mig*2 mutant cells, glucose has no effect on the corresponding mRNA stabilization and transcription rate. In the *snf*1 mutant cells, even when there is no glucose in the medium or the yeast cells are grown in non-fermentable carbon sources, glucose derepression can not occur. These two types of yeast mutants are very important to elucidate the exact molecular mechanisms of glucose repression and glucose derepression by PI-type signaling pathway.

It has been shown that in *plc*1 mutant, IP_3 and DAG production likely through the polyphosphoinositide hydrolysis of PIP_2 is blocked so that the PI-type signaling pathway can not work. Therefore, the phosphorylation of Mig1 and Snf1 complex by the PI-type signaling pathway and derepression of gene expression in *plc*1 mutant cells at higher concentration of glucose do not occur even more PI is synthesized.

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