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Possible involvement of *RAS*-encoded proteins in glucose-induced inositolphospholipid turnover in *Saccharomyces cerevisiae*

(cAMP/GTP-binding protein/calcium ion/protein kinase C/cell cycle)

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ABSTRACT **Incubation of yeast *Saccharomyces cerevisiae* at very low (0.02%) glucose levels led to arrest of the cell cycle at the G_0/G_1 phase. Readdition of glucose to these "starved" yeast resulted in cell proliferation. In glucose-starved yeast, glucose stimulated ^{32}P incorporation into phosphatidic acid, phosphatidylinositol, phosphatidylinositol monophosphate, and phosphatidylinositol bisphosphate but not into phosphatidylethanolamine and phosphatidylcholine. Preincubation of yeast with [^{3}H]inositol and subsequent exposure to glucose resulted in rapid formation of [^{3}H]inositol monophosphate and [^{3}H]inositol trisphosphate, presumably derived from phosphatidylinositol and phosphatidylinositol bisphosphate. Under similar conditions, glucose elicited both efflux and influx of Ca^{2+} in yeast. Glucose-induced ^{32}P incorporation into inositolphospholipids and formation of [^{3}H]inositol phosphates were more pronounced in *RAS*-related mutants such as *ras1*, *ras1 ras2 bcy1*, and *RAS2*^{Val19} than in the wild-type strain. These results strongly suggest that glucose stimulates inositolphospholipid turnover, Ca^{2+} mobilization, and subsequent cell proliferation in a manner similar to that of growth factors with mammalian cells, and that *RAS*-encoded proteins are involved in regulation of this glucose-induced inositolphospholipid turnover in yeast.**

Genetic and biochemical studies in the yeast *Saccharomyces cerevisiae* have revealed that cAMP plays an important role as a second messenger in the progression of the cell cycle at the G_0/G_1 phase (1). The intracellular level of cAMP is regulated by adenylate cyclase and GTP-binding proteins in mammalian cells (2). Yeast contains two closely related GTP-binding proteins, encoded by *RAS1* and *RAS2*, that are homologous to mammalian *ras*-encoded proteins (3, 4). Genetic analysis indicates that both *RAS1*- and *RAS2*-encoded proteins are involved in activation of adenylate cyclase (5). RAS2 protein has been shown to activate adenylate cyclase activity in a cell-free system (6, 7). Yeast containing *RAS2*^{Val19} (a *RAS2* allele with a missense mutation, which is analogous to the oncogenic mammalian *ras* gene) has enhanced GTP-independent adenylate cyclase activity and increases intracellular cAMP (5). The *RAS2*^{Val19} strain tends to continue growth without undergoing G_0/G_1 arrest, presumably because of the high intracellular cAMP concentrations (5). Thus, yeast provides a useful model for studying the roles of oncogenes such as the *ras* gene in cell proliferation.

Growth factors, such as platelet-derived growth factor and fibroblast growth factor, activate phospholipase C to induce hydrolysis of membrane inositolphospholipids (8, 9). The initial products of this reaction are diacylglycerol and inositol trisphosphate (InsP_3). Diacylglycerol remains in the membrane and serves as a second messenger for activation of protein kinase C (10, 11). Diacylglycerol is then metabolized to inositolphospholipids by way of phosphatidic acid (PtdOH) and CDP-diacylglycerol. Water-soluble InsP_3 also acts as a second messenger for Ca^{2+} mobilization from intracellular Ca^{2+} stores (12). These two second messengers, as well as cAMP, may play crucial roles in the transition from the G_0/G_1 phase of the cell cycle in mammalian cells (13, 14). Although products of some oncogenes such as *src* and *ras* may exert their effects through modification of these signal pathways (15, 16), the specific modes of their actions have not yet been clarified. In yeast, it is not clear whether molecules similar to growth factors in mammalian cells serve as extracellular signals and stimulate inositolphospholipid turnover, though inositolphospholipids show a very high metabolic turnover (17). The presence of glucose and its derivatives results in a rapid increase in intracellular cAMP in yeast in a manner similar to the effect of various hormones and growth factors on mammalian cells (18). Little is known, however, about the mechanism by which glucose activates adenylate cyclase or about the role glucose plays in cell proliferation apart from its use as a carbon source. In the light of these observations in yeast and mammalian cells, it seemed important to evaluate the effects of glucose and other nutrients on inositolphospholipid turnover in wild-type and *RAS*-related mutant strains of yeast.

In this paper, we demonstrate that glucose acts on yeast to stimulate inositolphospholipid turnover, resulting in formation of inositol phosphates. We also show that the RAS proteins may be involved in the regulation of inositolphospholipid turnover as well as in cAMP formation.

MATERIALS AND METHODS

Media and Chemicals. Liquid culture media used were as follows: YPD, 1% Bacto-yeast extract/2% Bacto-peptone/2% (180 mM) glucose (dextrose); minimal medium, 0.67% yeast nitrogen base without amino acids. Minimal medium contained auxotrophic requirements (50 μg/ml) and various concentrations of glucose as indicated in each experiment. [^{32}P]Orthophosphate, *myo*-[2-^{3}H]inositol (16.2 Ci/mmol; 1 Ci = 37 GBq), *myo*-[2-^{3}H]inositol 1,4,5-trisphosphate (1 Ci/mmol), and $^{45}CaCl_2$ (1.1 Ci/mmol) were from Amersham. Other materials were obtained from commercial sources.

Yeast Strains and Plasmids. The *S. cerevisiae* strain SP1 (*MATa leu2 ura3 trp1 his3 ade8 can1*) was used as the host for yeast plasmids. Starting from SP1, the isogenic mutants KKY1 (*MATa leu2 ura3 trp1 his3 ade8 can1 ras1::URA3*) and KKY2 (*MATa leu2 ura3 trp1 his3 ade8 can1 ras2::URA3*)

Abbreviations: InsP_3, inositol trisphosphate; Mes, 2-(*N*-morpholino)-ethanesulfonic acid; PtdOH, phosphatidic acid; PtdIns, phosphatidylinositol; PtdIns$P$, phosphatidylinositol monophosphate; PtdInsP_2, phosphatidylinositol bisphosphate; PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine; PtdSer, phosphatidylserine; Ins$P$, inositol monophosphate; InsP_2, inositol bisphosphate.

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were constructed by a one-step gene disruption method (19). Strains TTS121 (*MATa leu2 ura3 trp1 his3 ade8 can1 bcy1::URA3*), T27-10D (*MATα leu2 ura3 his3 can1 bcy1 ras1::HIS3 ras2::LEU2*), and TK161-R2V (*MATa leu2 ura3 trp1 his3 ade8 can1 RAS2*^{Val19}) were obtained from M. Wigler and T. Toda (Cold Spring Harbor Laboratory). Strain MC13 (*MATa ade1 ino1-13*) was supplied by S. A. Henry (Albert Einstein College of Medicine of Yeshiva University). Mutant strains KKY1, KKY2, TTS121, and TK161-R2V are theoretically isogenic at all other loci. Plasmids pUC8-RAS1 and pUC8-RAS2 were obtained from F. Tamanoi (University of Chicago).

Glucose Starvation and Assay for ^{32}P Incorporation into Phospholipids. Yeast cells grown to late logarithmic phase in YPD medium were harvested, washed with water, and suspended in minimal medium containing 0.02% (1.8 mM) glucose and 50 μM [^{3}H]inositol (20 μCi/ml). After incubation for 24 hr at 30°C, the cells were harvested, washed with water twice, and resuspended in 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (Mes)/Tris, pH 6.5 (Mes buffer). The cell suspension was preincubated for 60 min at 30°C and then labeled with carrier-free $^{32}P_i$ (60 μCi/ml) for 60 min. The radioactive cells (0.5 ml, 75 μg of protein) were subjected to various stimulants as indicated in each experiment. Reactions were stopped by addition of chloroform/methanol/concentrated HCl (100:200:2, vol/vol) and lipids were extracted by the method of Bligh and Dyer (20). Lipids were separated on thin-layer plates as described (21, 22). The area corresponding to each phospholipid was scraped into vials and the radioactivity was determined.

Assay for Generation of [^{3}H]Inositol Phosphates. Yeast cells incubated with [^{3}H]inositol for 24 hr as described above were harvested, washed with water twice, and then incubated in Mes buffer for 2 hr at 30°C. The radioactive cells (1 ml, 150 μg of protein) were stimulated by various compounds as indicated in each experiment and the reactions were terminated as described above. Extraction of lipids and Dowex-1 column chromatography of water-soluble [^{3}H]inositol phosphates was performed as described by Berridge *et al.* (23).

Assay for $^{45}Ca^{2+}$ Efflux and Influx. Glucose-starved yeast cells were prepared as described above except that minimal medium did not contain [^{3}H]inositol. Cells were incubated in Mes buffer for 60 min at 30°C and then labeled with $^{45}Ca^{2+}$ (2 μCi/ml) for 60 min. After incubation, the cells were washed three times with water and resuspended in Mes buffer. The radioactive cells (0.5 ml, 75 μg of protein) stimulated as indicated in each experiment were sedimented by centrifugation at 4°C. The radioactivity determined in the supernatant is a measure of the $^{45}Ca^{2+}$ efflux.

To measure $^{45}Ca^{2+}$ influx, the glucose-starved cells (0.1 ml, 15 μg of protein) incubated in Mes buffer for 2 hr at 30°C were stimulated by 25 mM glucose in the presence of 10 μM $^{45}Ca^{2+}$ (2.5 μCi/ml) as indicated. The reaction was stopped by dilution with 1 ml of ice-cold Mes buffer containing 5 mM $CaCl_2$, filtered quickly on a membrane filter (0.45 μm pore diameter), and washed with 10 ml of the same buffer. The radioactivity taken up by the cells was determined.

RESULTS

Effect of Glucose on Cell Proliferation. When wild-type yeast (SP1) cells in the logarithmic phase of growth were transferred from YPD medium containing 2% (180 mM) glucose to minimal medium without glucose (glucose depletion), the cells became arrested randomly at various stages of the cell cycle and lost their viability. Absolute depletion of glucose from the medium may immediately arrest the progression of the cell cycle. By contrast, when the cells were transferred to minimal medium containing 0.02% (1.8 mM) glucose (glucose starvation), more than 90% of the cells ended up in the nonreplicating, unbudded state in 24 hr. Cell viability was not lost with this treatment, indicating that the cells underwent a G_0/G_1 arrest. Readdition of 2% glucose to the glucose-starved unbudded cells induced cell proliferation within 4 hr (data not shown).

Effect of Glucose on Inositolphospholipid Turnover. Yeast cells were incubated with [^{3}H]inositol in minimal medium containing 0.02% glucose for 24 hr so that all inositol compounds might have a constant specific activity. The cells thus obtained were labeled by $^{32}P_i$ in Mes buffer without addition of nutrients and stimulated by glucose, and the radioactivity in various phospholipids was determined. To eliminate the possible effect of cell growth on phospholipid metabolism, succeeding experiments were carried out in Mes buffer instead of minimal medium as described in *Materials and Methods*. Glucose stimulated ^{32}P incorporation into PtdOH, phosphatidylinositol (PtdIns), phosphatidylinositol monophosphate (PtdIns*P*), and phosphatidylinositol bisphosphate (PtdInsP_2) in a time-dependent manner (Fig. 1 *A* and *B*). The effect of glucose on ^{32}P incorporation into PtdIns was dose dependent; the half-maximal response was

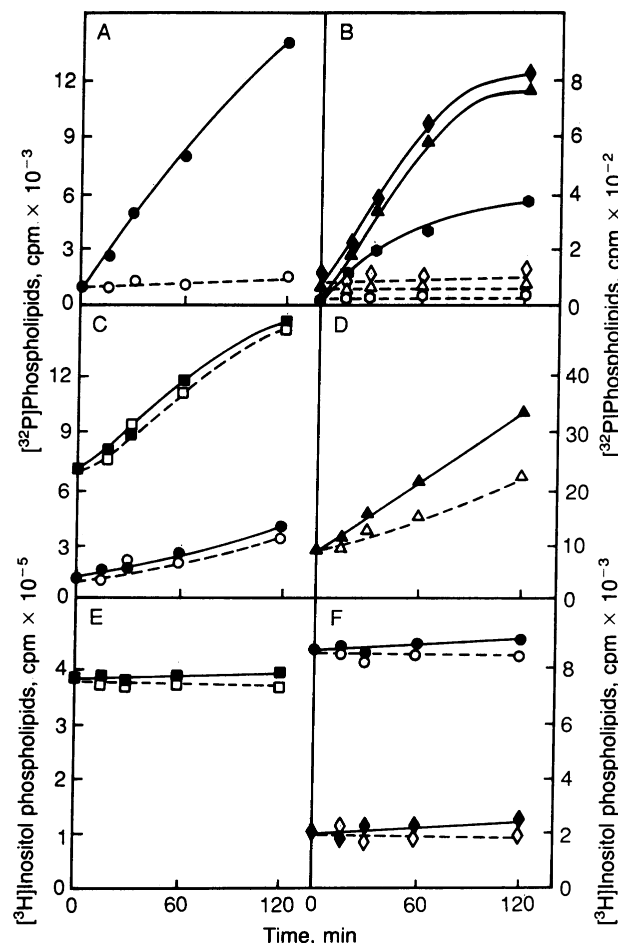

FIG. 1. Time course for glucose-induced changes in ^{32}P and ^{3}H radioactivity in phospholipids. Cells were prelabeled with [^{3}H]inositol for 24 hr in minimal medium. Prelabeled cells were washed, incubated in Mes buffer for 60 min, labeled with $^{32}P_i$ for another 60 min, and stimulated by 25 mM glucose at 30°C for various periods of time as indicated. (*A* and *B*) [^{32}P]Phospholipids. ● and ○, PtdIns; ◆ and ◇, PtdOH; ▲ and △, PtdIns*P*; ● and ○, PtdInsP_2. (*C* and *D*) [^{32}P]Phospholipids. ■ and □, PtdEtn; ● and ○, PtdCho; ▲ and △, PtdSer. (*E* and *F*) [^{3}H]Inositolphospholipids. ■ and □, PtdIns; ● and ○, PtdIns*P*; ◆ and ◇, PtdInsP_2. Closed symbols, stimulated by glucose; open symbols, control. Each experimental value is expressed as cpm per 100 μg of protein. Essentially identical results were obtained in three independent experiments.

obtained at about 10 mM glucose (data not shown). Since there was no difference in ^{32}P incorporation into phosphatidylethanolamine (PtdEtn) and phosphatidylcholine (PtdCho) in the presence and absence of glucose (Fig. 1*C*), the effect of glucose may not simply be due to an increase in the specific activity of intracellular [^{32}P]ATP. Glucose slightly stimulated ^{32}P incorporation into phosphatidylserine (PtdSer) (Fig. 1*D*). Although the exact reason why glucose stimulated ^{32}P incorporation into PtdSer is not known, CDP-diacylglycerol produced from PtdOH may be partly converted to PtdSer (24). In this experiment, inositol, which is the precursor of inositolphospholipids, was not present in Mes buffer. Inositol (50 μM) had no effect on ^{32}P incorporation into inositolphospholipids, PtdOH, PtdEtn, PtdCho, and PtdSer in the presence and absence of glucose. Under the same experimental conditions, the amount of ^{3}H in inositolphospholipids increased only slightly (Fig. 1 *E* and *F*). Since the ^{32}P-to-^{3}H ratio may be equivalent to a specific activity of inositolphospholipids, glucose may stimulate the turnover of phosphates in inositolphospholipids rather than increase the total amount of inositolphospholipids, suggesting that glucose induces the hydrolysis and subsequent resynthesis of inositolphospholipids.

To examine the hydrolysis of inositolphospholipids, the formation of inositol phosphates after the stimulation by glucose was measured. When cells prelabeled with [^{3}H]inositol were stimulated by glucose, rapid formation of [^{3}H]inositol monophosphate (Ins*P*) and [^{3}H]InsP_3 was observed (Fig. 2*A*). [^{3}H]Inositol bisphosphate (InsP_2) formation could not be detected under the same conditions. In agreement with the previous observation (25), glycerophospho-[^{3}H]inositol formation was observed during the action of glucose. Glycerophospho[^{3}H]inositol may be derived from PtdIns by the action of phospholipases A_1 and A_2 (25). Concentrations of glucose needed for [^{3}H]Ins*P* formation were approximately the same as those needed for ^{32}P incorporation into PtdIns (data not shown). Lithium chloride, which is known to inhibit Ins*P* phosphatase, had no effect on glucose-induced formation of [^{3}H]inositol phosphates. PtdInsP_2 was more efficiently prelabeled by [^{3}H]inositol in the presence of 2% glucose than in the presence of 0.02% glucose. In this case, glucose more dramatically elicited [^{3}H]InsP_3 formation in cells prelabeled with [^{3}H]inositol as shown in Fig. 2*B*. We confirmed that [^{3}H]InsP_3 from yeast cochromatographed with authentic inositol 1,4,5-trisphosphate on Mono Q HPLC (Pharmacia) using the conditions described by Irvine *et al.* (26) (data not shown). Essentially the same results were obtained by using the *ino1* mutant, which is deficient in Ins*P* synthetase activity. Glucose stimulated ^{32}P incorporation into inositolphospholipids and the formation of [^{3}H]inositol phosphates in the *ino1* mutant to an extent similar to that observed in the wild-type strain (data not shown). These results exclude the possibility that glucose is converted to inositol and that glucose therefore stimulates *de novo* synthesis of inositolphospholipids. Together with the observations described above, it is more likely that inositol phosphates were derived from the respective inositolphospholipids by the action of phospholipase C after stimulation by glucose.

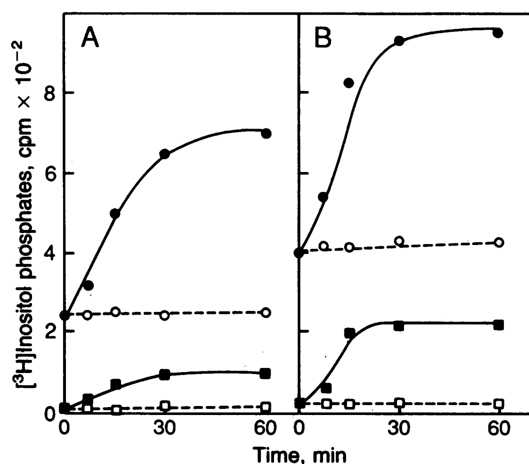

FIG. 2. Time course for glucose-induced generation of [^{3}H]inositol phosphates. Cells were prelabeled with [^{3}H]inositol for 24 hr in minimal medium containing 0.02% glucose (*A*) or 2% glucose (*B*). Prelabeled cells were washed, incubated in Mes buffer for 2 hr, and stimulated by 25 mM glucose at 30°C for various periods of time as indicated. (*A*) Prelabeled in 0.02% glucose. ^{3}H radioactivity in PtdIns, PtdIns*P*, and PtdInsP_2 in the cells at zero time was 403,270, 9390, and 2330 cpm, respectively. (*B*) Prelabeled in 2% glucose. ^{3}H radioactivity in PtdIns, PtdIns*P*, and PtdInsP_2 in the cells at zero time was 413,750, 39,170, and 5310 cpm, respectively. ●, [^{3}H]Ins*P* formation stimulated by glucose; ○, [^{3}H]Ins*P* formation without glucose; ■, [^{3}H]InsP_3 formation stimulated by glucose; □, [^{3}H]InsP_3 formation without glucose. Each experimental value is expressed as cpm per 100 μg of protein. Essentially identical results were obtained in three independent experiments.

Effect of Glucose on Ca^{2+} Mobilization. Since InsP_3 serves as a second messenger for Ca^{2+} mobilization from intracellular Ca^{2+} stores to cytoplasm and subsequently increases Ca^{2+} efflux from cytoplasm, the effect of glucose on Ca^{2+} mobilization in yeast was determined. Glucose stimulated $^{45}Ca^{2+}$ efflux in a time-dependent manner from the cells prelabeled with $^{45}Ca^{2+}$ (Fig. 3*A*). Concentrations of glucose required for $^{45}Ca^{2+}$ efflux are roughly the same as those required for ^{32}P incorporation into PtdIns. Glucose also increased $^{45}Ca^{2+}$ influx into the cells from the medium (Fig. 3*B*). However, the mechanism of stimulation of Ca^{2+} influx by extracellular signals is not known even in mammalian cells.

Effect of Other Nutrients. Several hexoses, including glucose, are effective for cell growth of yeast. We then examined the effects of various hexoses and other nutrients on inositolphospholipid turnover and Ca^{2+} mobilization. Glucose, fructose, and mannose induced ^{32}P incorporation into PtdIns and [^{3}H]Ins*P* formation in roughly parallel fashion (Table 1). However, nonmetabolizable derivatives of glucose such as 2-deoxyglucose and 6-deoxyglucose did not induce

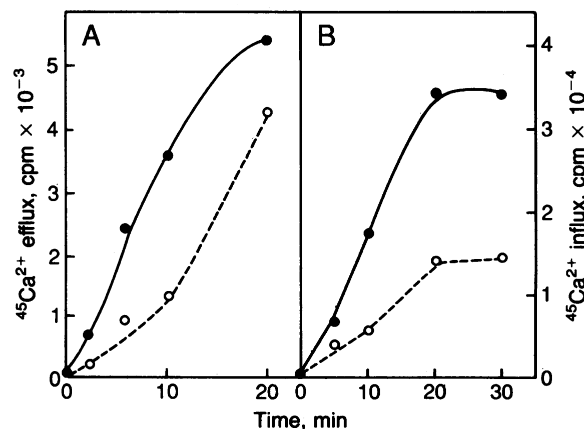

FIG. 3. Time course of glucose effects on $^{45}Ca^{2+}$ efflux and influx. (*A*) $^{45}Ca^{2+}$ efflux. The cells prelabeled with $^{45}Ca^{2+}$ were stimulated by 25 mM glucose at 30°C for various periods of time as indicated. The radioactivity in the cells at zero time was 53,530 cpm. ●, Stimulated by glucose; ○, control. (*B*) $^{45}Ca^{2+}$ influx. The cells were stimulated by 25 mM glucose in the presence of 10 μM $^{45}Ca^{2+}$ for various periods of time as indicated. ●, Stimulated by glucose; ○, control. Each experimental value is expressed as cpm per 100 μg of protein. Essentially identical results were obtained in three independent experiments.

Table 1. Effects of various hexoses on incorporation of ^{32}P into PtdIns, formation of [^3H]Ins*P*, and efflux of $^{45}Ca^{2+}$

Addition	^{32}P incorporation into PtdIns, cpm	[^3H]Ins*P* formation, cpm	$^{45}Ca^{2+}$ efflux, cpm
None	1530	240	1490
Glucose	9340	480	3180
Fructose	6620	450	2930
Mannose	7080	430	2820
2-Deoxyglucose	1820	280	1250
6-Deoxyglucose	1760	260	1160

Cells were prelabeled with $^{32}P_i$/[^3H]inositol, [^3H]inositol, or $^{45}Ca^{2+}$, and then stimulated by 25 mM hexose at 30°C for 60, 30, and 10 min, respectively, for the determination of ^{32}P incorporation into PtdIns, [^3H]Ins*P* formation, and $^{45}Ca^{2+}$ efflux; assays were under conditions similar to those described in the legends to Figs. 1, 2*A*, and 3*A*, respectively. Each experimental value is expressed as cpm per 100 μg of protein. Essentially identical results were obtained in three independent experiments.

these reactions. Nitrogen, sulfate, and various amino acids had no effect on this activity, even in cells that were preincubated without these nutrients. Mating factor α did not induce ^{32}P incorporation into PtdIns and had no effect on glucose-induced ^{32}P incorporation into PtdIns. Hexoses that induced inositolphospholipid turnover elicited $^{45}Ca^{2+}$ efflux, suggesting that Ca^{2+} efflux is coupled to inositolphospholipid turnover during the action of the hexoses.

Inositolphospholipid Turnover in *RAS*-Related Mutants. To test the hypothesis that RAS proteins modulate inositolphospholipid turnover as well as adenylate cyclase activity, ^{32}P incorporation into inositolphospholipids and formation of [^3H]inositol phosphates in yeast strains carrying mutant *RAS* genes were determined. We used *ras1, ras2, bcy1, ras1 ras2 bcy1*, and *RAS2*^{Val19} strains as *RAS*-related mutants. *ras2* cells have lower levels of intracellular cAMP; disruption of both *RAS1* and *RAS2* genes is lethal because of very low concentration of intracellular cAMP (5). The *bcy1* mutant is defective in the regulatory subunit of cAMP-dependent protein kinase and suppresses the lethality resulting from disruption of both *RAS1* and *RAS2* genes (5, 27). Cells containing *RAS2*^{Val19} have high levels of intracellular cAMP (5). Table 2 shows the effects of glucose on inositolphospholipid turnover in the *RAS*-related mutants. Glucose stimulated ^{32}P incorporation into PtdOH, PtdIns, PtdIns*P*, and PtdInsP_2 in the *RAS*-related mutants. Enhancement of ^{32}P incorporation into PtdIns is in the order *ras1 ras2 bcy1*, *ras1*, and wild-type strains. There was little difference among these strains in ^{32}P labeling of PtdEtn and PtdCho in the presence and absence of glucose. Under the same conditions, glucose only slightly increased ^3H radioactivity in PtdIns in the *RAS*-related mutants.

We next studied the formation of inositol phosphates in the *RAS*-related mutants. Cells were incubated with glucose for 60 min in the presence of [^3H]inositol to measure accumulation of [^3H]inositol phosphates. As with ^{32}P incorporation into PtdIns, glucose induced [^3H]InsP_3 formation in the *RAS*-related mutants; the magnitude of [^3H]InsP_3 formation was of the same order as that of ^{32}P incorporation into PtdIns. The ratio of [^3H]InsP_3 to [^3H]inositolphospholipids, which may reflect the amount of InsP_3, increased after stimulation with glucose. Although we did not exclude the possibility that *RAS* proteins affect inositol transport into cells, this possibility is unlikely because few differences were seen in radioactivity of inositolphospholipids and glycerophosphoinositol in the *RAS*-related mutants as compared to wild-type strains. Under the same conditions, ^{32}P incorporation into PtdIns and [^3H]InsP_3 formation in the *bcy1* strain were roughly the same as those observed in the wild-type strain.

Table 2. ^{32}P incorporation into PtdIns and formation of [^3H]InsP_3 in *RAS*-related mutants

Strain	Addition	^{32}P incorporation into PtdIns, cpm	[^3H]PtdIns, cpm	[^3H]InsP_3 formation, cpm
SP1 (wild-type)	None	1,050	481,960	140
	Glucose	7,650	512,160	360
KKY1 (*ras1*)	None	1,080	481,630	150
	Glucose	16,320	522,120	620
KKY2 (*ras2*)	None	1,010	533,190	150
	Glucose	8,190	566,760	420
T27-10D	None	2,250	557,630	290
(*ras1 ras2 bcy1*)	Glucose	32,670	587,310	1160
TTS121 (*bcy1*)	None	1,110	461,120	130
	Glucose	6,370	481,970	330
TK161-R2V	None	1,370	541,670	250
(*RAS2*^{Val19})	Glucose	25,270	571,720	920

Cells were prelabeled with [^3H]inositol in minimal medium containing 2% glucose as described in the legend to Fig. 2*B*. For determination of ^{32}P incorporation into PtdIns, prelabeled cells were washed, incubated in Mes buffer for 60 min, labeled with 50 μM inositol (20 μCi/ml) and $^{32}P_i$ for another 60 min, and stimulated by 25 mM glucose for 60 min at 30°C as indicated. For determination of [^3H]InsP_3 formation, prelabeled cells were incubated in Mes buffer for 60 min, labeled with 50 μM [^3H]inositol (20 μCi/ml) for another 60 min, and stimulated by 25 mM glucose for 60 min at 30°C as indicated. Other details are described in *Materials and Methods*. Each experimental value is expressed as cpm per 100 μg of protein. Essentially identical results were obtained in three independent experiments.

Therefore, enhancement of inositolphospholipid turnover in the *ras1 ras2 bcy1* strain is not simply due to the *bcy1* mutation. It is noted that glucose induced ^{32}P incorporation into PtdIns and [^3H]InsP_3 formation in the *RAS2*^{Val19} strain to a level similar to that induced in the *ras1 ras2 bcy1* strain. Under similar experimental conditions, glucose stimulated cAMP formation in the wild-type and *RAS2*^{Val19} strains but not in the *ras1 ras2 bcy1* strain (data not shown).

DISCUSSION

It is well known that depletion of growth factors arrests mammalian cells at the G_0/G_1 phase of the cell cycle and that readdition of growth factors stimulates phospholipid turnover followed by DNA synthesis (8, 9, 28). Our results with yeast showed the following: (*i*) Glucose starvation arrests yeast cells at G_0/G_1 phase and readdition of glucose induces cell proliferation. (*ii*) Glucose induces ^{32}P incorporation into inositolphospholipids and PtdOH with the formation of [^3H]inositol phosphates. (*iii*) Glucose elicits $^{45}Ca^{2+}$ efflux and influx. These results strongly suggest that glucose stimulates phospholipase C activation to hydrolyze inositolphospholipids in a manner similar to that of a variety of hormones and growth factors in mammalian cells. Glucose has been shown to stimulate inositolphospholipid turnover in β cells of pancreatic islets (29). Nonmetabolizable derivatives of glucose such as 2-deoxyglucose and 6-deoxyglucose have no effect on inositolphospholipid turnover in either β cells or yeast. In contrast, 2-deoxyglucose and 6-deoxyglucose have been shown to stimulate cAMP formation in yeast cells (18). It is, therefore, conceivable that glucose itself is responsible for adenylate cyclase activation, and glucose metabolites result in phospholipase C activation. However, the nature of receptors that recognize glucose metabolites remains to be established.

In mammalian cells, inositolphospholipid turnover is linked to activation of protein kinase C and Ca^{2+} mobilization (10, 12). Although phorbol 12-myristate 13-acetate, known as a specific activator of protein kinase C (30), induces the

phosphorylation of a yeast protein of about 105 kDa (31), protein kinase C has not been shown to exist in yeast. InsP_3 serves as a second messenger for Ca^{2+} translocation in various eukaryotic cells; however, it is not known whether InsP_3 induces Ca^{2+} mobilization in yeast cells. Identification of protein kinase C and its substrate in yeast and the elucidation of the effect of InsP_3 on Ca^{2+} mobilization are necessary for a better understanding of the role of inositolphospholipid turnover in cell proliferation.

Genetic analysis has shown that cAMP and its dependent protein kinase are essential for the transition from the G_0/G_1 phase of the cell cycle (1). It is not yet established that inositolphospholipid turnover is directly related to the progression of the G_0/G_1 phase of the cell cycle. We have recently obtained a mutant that undergoes a G_0/G_1 arrest under an appropriate condition. Since glucose does not stimulate inositolphospholipid turnover in this mutant under the same condition (unpublished data), this phospholipid turnover may be related to the transition of the G_0/G_1 phase of the cell cycle.

It has been shown that RAS2 protein rather than RAS1 protein plays a major stimulatory role in the regulation of adenylate cyclase (5). Our results suggest that RAS1 protein may play a major inhibitory role in glucose-induced inositolphospholipid turnover, while RAS2 protein is less important in this regard. It should be noted, however, that when a missense mutation was introduced into the *RAS2* gene, the mutant RAS2 protein activated inositolphospholipid turnover. Although we do not know the mechanism by which RAS proteins modulate inositolphospholipid turnover, a conformational change may convert the RAS2 protein to an activating molecule. We speculate that *RAS2*^{Val19} cells tend to continue growth without entering a G_0/G_1 arrest because levels of inositolphospholipid turnover and cAMP are higher than in wild-type cells. Since Ha- and Ki-ras proteins are homologous to yeast RAS proteins (3, 4), it is possible that in mammalian cells ras proteins affect inositolphospholipid turnover as well as cAMP formation, leading to uncontrollable cell growth.

Several lines of evidence indicate that agonist-induced activation of both phospholipase C and adenylate cyclase is regulated by GTP-binding proteins such as G_s, G_i, and G_o proteins in mammalian cells (2, 32, 33). Glucose stimulates inositol phospholipid turnover but it does not stimulate cAMP formation in the absence of RAS proteins as shown in the *ras1 ras2 bcy1* strain. It is conceivable that GTP-binding proteins other than RAS proteins may be responsible for phospholipase C activation in yeast, and that inositolphospholipid turnover is dually regulated by RAS proteins and these putative GTP-binding proteins. Yeast cells offer several advantages over mammalian cells because of the ease of applying a genetic approach. Although much biochemical analysis is necessary, the yeast system described in this paper proved to be a useful model in understanding the mode of action of RAS proteins on inositolphospholipid turnover.

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