

Reconstitution of the GTP-dependent adenylate cyclase from products of the yeast *CYR1* and *RAS2* genes in *Escherichia coli*

(cyclic AMP/oncogene/transformation)

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ABSTRACT Plasmids carrying the *CYR1* gene of yeast *Saccharomyces cerevisiae*, which encodes adenylate cyclase, were introduced into the *cya* mutant strain of *Escherichia coli*. The transformants had a GTP-independent adenylate cyclase activity but did not produce cAMP. The *E. coli* transformant carrying the yeast *RAS2* or *RAS2^{val19}* gene had no adenylate cyclase activity. Transformant cells carrying both *CYR1* and *RAS2* produced GTP-dependent adenylate cyclase and cAMP, and those carrying *CYR1* and *RAS2^{val19}* produced GTP-independent adenylate cyclase and a large amount of cAMP. Production of cAMP in the transformant carrying *CYR1* and either *RAS2* or *RAS2^{val19}* was confirmed by staining colonies on maltose-MacConkey plates and by measuring induction of β -galactosidase by isopropyl β -D-thiogalactopyranoside. Mixing a crude extract from the *E. coli* transformant carrying *CYR1* with a crude extract from cells carrying *RAS2* reconstituted the GTP-dependent adenylate cyclase. Reconstitution of the GTP-dependent adenylate cyclase was observed by mixing the plasma membrane fraction of yeast *CYR1 ras1 ras2 bcy1* mutant and a crude extract from the *E. coli* transformant carrying *RAS2* or by mixing a crude extract from the *E. coli* transformant carrying *CYR1* and the membrane fraction of yeast *cyr1 RAS1 RAS2 BCY1* mutant. The data suggest that the yeast GTP-dependent adenylate cyclase consists of catalytic and regulatory subunits encoded by the *CYR1* and *RAS2* genes, respectively.

In yeast *Saccharomyces cerevisiae* cells, cAMP acts as an essential regulator for growth through activation of cAMP-dependent protein kinase (1-4). The regulatory roles of cAMP in yeast have been studied by using cAMP-requiring mutants that are defective in adenylate cyclase or cAMP-dependent protein kinase (1). One of the cAMP-requiring mutants, *cyr1*, carried a lesion in the structural gene for adenylate cyclase (5). The *cyr1* mutation was suppressed by the *bcy1* mutation resulting in a deficiency of the regulatory subunit of cAMP-dependent protein kinase and the production of a high level of cAMP-independent protein kinase (1). The yeast gene coding for adenylate cyclase has been cloned by using complementation of a thermosensitive *tsm0185* mutant, and it has been shown to complement the *cyr1* mutation (6). The adenylate cyclase system of yeast consists of at least two protein components, catalytic and regulatory subunits, and is regulated by guanine nucleotides in the presence of magnesium ions (7) as found in mammalian cells (8). In the absence of the regulatory subunit, the adenylate cyclase activity of the catalytic subunit required manganese ions (7). The regulatory subunit conferred guanine nucleotide

regulation on the catalytic subunit in the presence of magnesium ions (7).

Yeast cells contain two closely related genes, *RAS1* and *RAS2*, that encode proteins that are homologous to mammalian *ras* proteins (9-11). The product of yeast *RAS2* gene has guanine-nucleotide-binding activity (12) and regulates adenylate cyclase activity (13). A yeast strain containing *RAS2^{val19}*, a *RAS2* allele with a missense mutation that is analogous to the oncogenic mammalian *ras* genes (14), has increased levels of an apparently GTP-independent adenylate cyclase activity and of intracellular cAMP (13). Yeast strains deficient in the RAS function exhibit some of the properties of the adenylate cyclase deficient yeasts, and it has been found that the lethality of the *ras1 ras2* genes was suppressed by *bcy1* (13). To investigate the biochemical role of RAS gene products, we have introduced the yeast *CYR1* and *RAS2* genes into *Escherichia coli* and have reconstituted GTP-dependent adenylate cyclase from products of these genes *in vivo* and *in vitro*.

MATERIALS AND METHODS

Bacterial and Yeast Strains and Plasmids. *E. coli* strains CA8000 (Hfr *thi*) and CA8306 (Hfr *thi* Δ *cya*) (15) were obtained from S. Harayama. Strain TP2010 (F⁻ Δ *lac recA srl::Tn10 xyl* Δ *cya argH*) (16) was obtained from H. Aiba. Strain DH1 (17) obtained from Y. Komeda was used as host for plasmid construction. *S. cerevisiae* strains AM221-1C (a), AM221-1D (α *cyr1*), and X2180-1B (α *SUC2 mal gal2 CUP1*) were from our stock, and T26-19C (α *leu2 his3 trp1 can1 bcy1 ras1::HIS3 ras2::LEU2*) (13) was obtained from M. Wigler. Plasmid pCEY710 (6) was obtained from P. Masson, and pACYC184 (18) was obtained from K. Tanimoto. Plasmid pGIF5 (19) was from our stock.

Media and Growth Conditions. *E. coli* cells were grown at 37°C in LB medium (20) except for β -galactosidase assay. Ampicillin, chloramphenicol, and tetracycline were added to 50, 30, and 15 μ g/ml, respectively, when necessary. MacConkey indicator plates were prepared from MacConkey agar base (Difco) to which 1% (wt/vol) sugar was added. These plates were used to test for sugar utilization. To assay β -galactosidase activity *E. coli* cells were grown in minimal A medium (20). Yeast cells were grown at 30°C in YPGlu medium [1% (wt/vol) yeast extract, 2% (wt/vol) peptone, and 2% (wt/vol) glucose] and filtration sterilized cAMP was added to 1 mM, when necessary.

Construction of Plasmids. The plasmid pCEY710 was originally cloned by using the ability to complement the *tsm0185* mutant, which is allelic with *cdc35* (6). pHM6 was constructed by inserting the *CYR1* gene from pCEY710 into

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Abbreviations: p[NH]ppG, guanosine 5'-[β , γ -imino]triphosphate; IPTG, isopropyl β -D-thiogalactopyranoside.

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pBR322 (Fig. 1A). pHM10 was constructed by inserting the *CYR1* gene from pHM6 into pACYC184 which is compatible with ColE1-derived replicons and can be propagated concurrently with such replicons in the same bacterial cell (Fig. 1A). To clone the yeast *RAS2* gene, yeast genomic DNA was prepared from X2180-1B cells, and digested with *EcoRI* and *HindIII*. After separation by agarose gel electrophoresis, ≈ 3 -kilobase-long fragments were isolated and ligated to *EcoRI/HindIII*-digested pBR322. The plasmids carrying the *RAS2* gene were screened by comparing the restriction maps with that of the *RAS2* gene (10) and were identified by partial sequencing. The *RAS2*^{val19} allele (in which glycine was substituted to valine at the 19th position of *RAS2* gene product) was obtained from the *RAS2* clone by site-directed mutagenesis (21). The *EcoRI* site was introduced in front of the initiation codon of *RAS2* and *RAS2*^{val19} genes by site-directed mutagenesis, and the *RAS2* and *RAS2*^{val19} genes were inserted into an *E. coli* expression vector carrying the *lacUV5* promoter by substituting the *RAS2* or *RAS2*^{val19} gene for the gene encoding interferon γ of plasmid pGIF5 (19), yielding *placRAS2* and *placRAS2*^{val19}, respectively (Fig. 1B). The *RAS2* and *RAS2*^{val19} genes carrying the *lacUV5* promoter were induced with 1 mM isopropyl β -D-thiogalactopyranoside (IPTG) (Sigma).

Transformation. Transformation of *E. coli* was carried out by the method described by Cohen *et al.* (22).

Assays. Intracellular and extracellular cAMP levels in *E. coli* and yeast cells were determined as described (23). *E. coli* cells were suspended in TEMP buffer (10 mM Tris-HCl, pH 7.4/1 mM EDTA/1 mM 2-mercaptoethanol/0.1 mM phenylmethylsulfonyl fluoride) and were homogenized with an Aminco French pressure cell (J5-598A). The resulting homogenates were centrifuged at $1000 \times g$ for 10 min, and the supernatant fluid was used as a crude extract. Plasma membrane fraction from yeast cells was prepared as described (5), except that Mn^{2+} was omitted from all buffers. Reconstitution of adenylate cyclase was performed as described elsewhere (13). Adenylate cyclase activity was measured at pH 6.2 and at pH 8.5 in the presence of either 5 mM Mn^{2+} or 5 mM Mg^{2+} with or without 0.1 mM guanosine 5'-[β , γ -imino]triphosphate (p[NH]ppG) (Sigma) (5). β -Galactosidase activity was measured by using toluene-treated *E. coli* (20). Protein was measured by the method described by Lowry *et al.* (24) using bovine serum albumin as the standard.

RESULTS

Expression of the *CYR1*, *RAS2*, and *RAS2*^{val19} Genes in *E. coli*. The wild-type strain of *E. coli* (CA8000) produced large amounts of intracellular and extracellular cAMP (Table 1). Adenylate cyclase of *E. coli* was completely inactive at pH 6.2, because the pH optimum of this enzyme is 8.5 in the

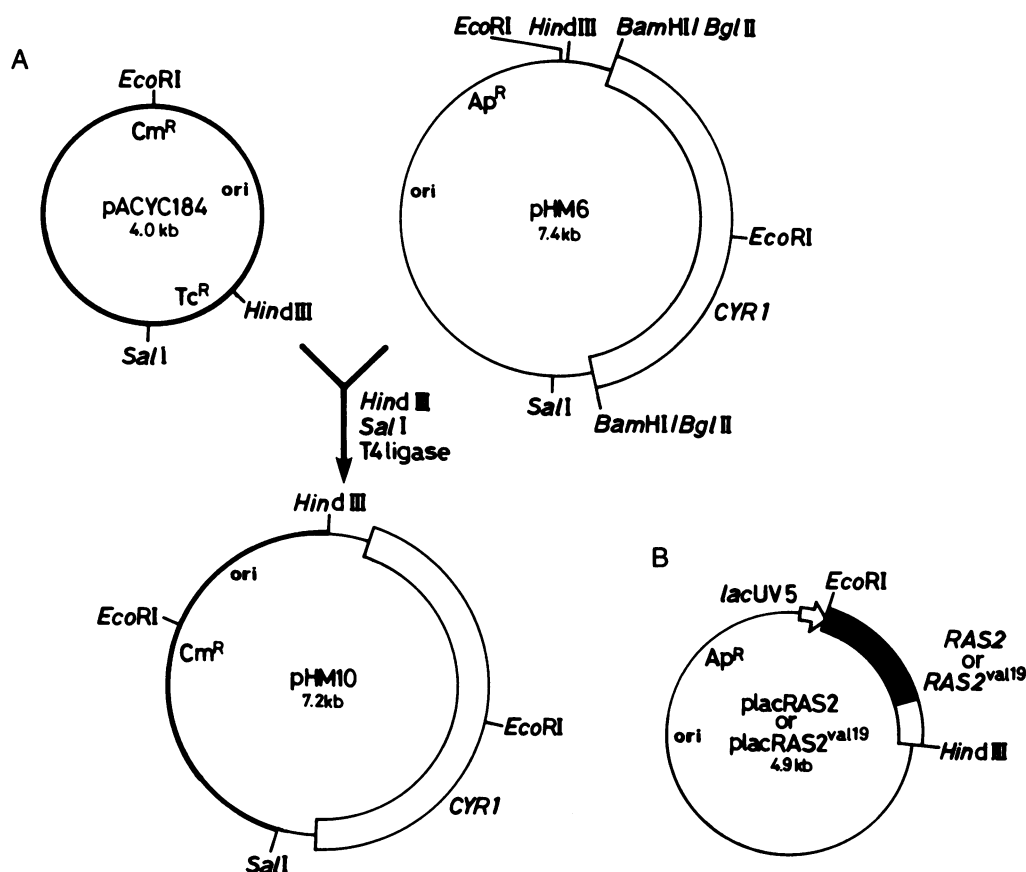


FIG. 1. Construction of plasmids used. (A) Construction of pHM6 and pHM10. pHM6 containing the ColE1 origin of replication was constructed by inserting a 3-kilobase *Bgl* II fragment of pCEY710 containing the *CYR1* gene into the *Bam*HI site of pBR322. Plasmids pHM6 and pACYC184 were digested with *Hind*III and *Sal*I. The digested DNAs were mixed, ligated with T4 DNA ligase, and used to transform *E. coli* to chloramphenicol resistance (Cm^R). Among transformants obtained, clones sensitive both to ampicillin (Ap) and tetracycline (Tc) were selected. The plasmid DNAs were prepared from these clones and characterized by restriction endonuclease digestion. The resulting plasmid, pHM10, carried the replication origin derived from pACYC184 and was compatible with ColE1-derived plasmids such as *placRAS2*. (B) Construction of *placRAS2* and *placRAS2*^{val19}. The *RAS2* and *RAS2*^{val19} genes were inserted into *E. coli* expression vector carrying the *lacUV5* promoter by substituting *RAS2* or *RAS2*^{val19} for the gene encoding interferon γ of pGIF5. pBR322 sequences are indicated by thin lines, and pACYC184 sequences are indicated by thick lines. Yeast DNA is indicated by boxes. A filled-in box shows a coding sequence from the *RAS2* locus. The precise location of the *CYR1* locus was not determined. The *lacUV5* promoter region is indicated by an open arrow.

Table 1. cAMP levels and adenylate cyclase activity of *E. coli* strains and transformants carrying the *CYR1* and *RAS2* genes

Bacterial strain and transformant	cAMP level		Adenylate cyclase activity, units/mg of protein				
	Extracellular, μM	Intracellular, pmol/mg of protein	Mn^{2+} , pH 6.2	Mn^{2+} , pH 6.2, p[NH]ppG		Mg^{2+} , pH 8.5	
				Mg^{2+} , pH 6.2	Mg^{2+} , pH 6.2, p[NH]ppG	Mg^{2+} , pH 8.5	
CA8000	1.20	22.0	0.00	0.00	0.00	0.00	21.2
CA8306	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA8306/pHM6	0.00	0.00	2.26	2.10	0.18	0.16	0.00
CA8306/pHM10	0.00	0.00	2.21	2.24	0.20	0.19	0.00
CA8306/placRAS2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA8306/placRAS2 ^{val19}	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA8306/pHM10,placRAS2	0.11	1.65	4.67	4.50	0.40	1.12	0.00
CA8306/pHM10,placRAS2 ^{val19}	0.58	5.61	4.28	4.17	0.72	1.08	0.00

Adenylate cyclase activity was measured in the presence of either 5 mM Mn^{2+} or 5 mM Mg^{2+} , with or without 0.1 mM p[NH]ppG, at pH 6.2 or 8.5. Essentially identical results were obtained in three independent experiments.

presence of 5 mM Mg^{2+} (25, 26), while the yeast enzyme is active at pH 6.2 but not at pH 8.5. The Δcya mutant of *E. coli* (CA8306) has a deletion in the structural gene for adenylate cyclase, produces no cAMP, and shows no adenylate cyclase activity (Table 1). For the production of yeast adenylate cyclase in *E. coli*, we constructed plasmids designated pHM6 and pHM10. pHM6 is a derivative of pBR322 containing the yeast *CYR1* gene, and pHM10 is a derivative of pACYC184 into which the *CYR1* gene from pHM6 has been recloned (Fig. 1A). Plasmids pHM6 and pHM10 were introduced into Δcya mutant cells. Adenylate cyclase activity of crude extracts of transformants carrying either pHM6 or pHM10 (CA8306/pHM6 or CA8306/pHM10) was detectable in the presence of 5 mM Mn^{2+} at pH 6.2, but was significantly lower in the presence of 5 mM Mg^{2+} at pH 6.2, and no stimulation of adenylate cyclase activity was found in the presence of 5 mM Mg^{2+} and the nonhydrolyzable GTP analog, p[NH]ppG. These transformants produced no detectable amount of cAMP. The results indicate that the *CYR1* gene of yeast can be expressed in *E. coli* cells, but the product of this gene cannot synthesize cAMP in *E. coli* cells.

Plasmids placRAS2 and placRAS2^{val19} were constructed by inserting the *RAS2* and *RAS2*^{val19} genes into an *E. coli* expression vector (Fig. 1B). Transformants carrying either placRAS2 or placRAS2^{val19} (CA8306/placRAS2 or CA8306/placRAS2^{val19}) produced no detectable amounts of cAMP and showed no adenylate cyclase activity at pH 6.2 or at pH 8.5 (Table 1). The *RAS2* and *RAS2*^{val19} on the expression vector could be induced by the presence of 1 mM IPTG in *E. coli*, and the products of these genes had the molecular weight of approximately 39,000 (data not shown), as described by Tamanoi *et al.* (12).

Simultaneous Expression of the *CYR1* and *RAS2* Genes in *E. coli*. Two plasmids, pHM10 and placRAS2, were introduced sequentially by transformation into the Δcya strain of *E. coli*. Because pHM10 and placRAS2 confer resistance to chloramphenicol and ampicillin, respectively, we selected transformants resistant to both antibiotics. It was confirmed by agarose gel electrophoresis that these transformants carried two plasmids simultaneously.

Adenylate cyclase activity in crude extracts prepared from the transformant carrying pHM10 and placRAS2 (CA8306/pHM10,placRAS2) was detected in the presence of 5 mM Mn^{2+} at pH 6.2. The stimulation of adenylate cyclase activity was not observed in the presence of 5 mM Mn^{2+} and p[NH]ppG but was observed in the presence of 5 mM Mg^{2+} and p[NH]ppG at pH 6.2 (Table 1). These transformant cells produced significant amounts of intracellular and extracellular cAMP (Table 1). The transformant carrying pHM10 and placRAS2^{val19} (CA8306/pHM10,placRAS2^{val19}) produced large amounts of intracellular and extracellular cAMP and showed relatively high adenylate cyclase activity in the

presence of Mg^{2+} at pH 6.2 without p[NH]ppG (Table 1). The results indicate that the yeast *CYR1* and *RAS2* genes can be expressed in *E. coli* cells and that the products of these genes can reconstitute GTP-dependent adenylate cyclase that can catalyze synthesis of cAMP in *E. coli*.

To confirm that cAMP is synthesized in transformant cells carrying pHM10 and either placRAS2 or placRAS2^{val19}, plasmids were introduced into TP2010 (*recA* Δcya Δlac) cells. Transformant cells were grown on maltose-MacConkey agar. As shown in Fig. 2, transformants carrying pHM10 and either placRAS2 or placRAS2^{val19} formed deep red colonies, but all other transformants carrying pHM10, placRAS2, or placRAS2^{val19} formed white colonies. Similar results were obtained when these transformants were grown on arabinose-MacConkey agar. These results indicate that cAMP is synthesized in transformants carrying pHM10 and either placRAS2 or placRAS2^{val19}.

To confirm further the cAMP accumulation in transform-

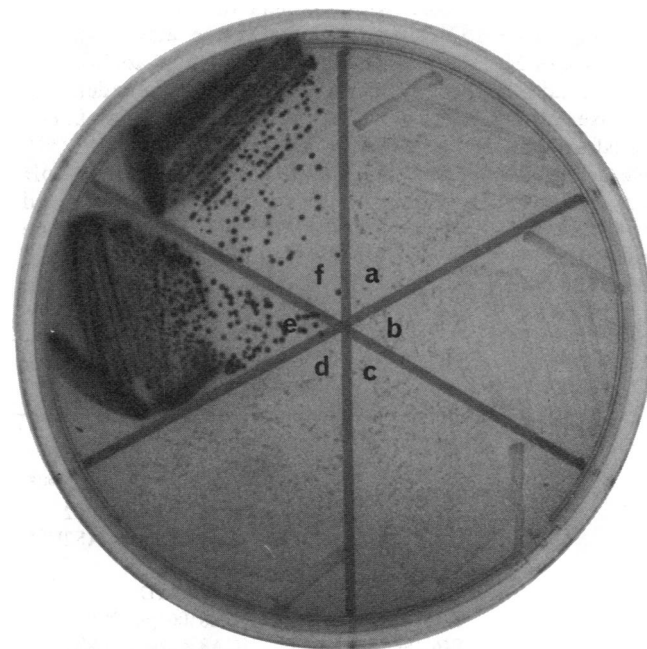


FIG. 2. Sugar utilization of transformants carrying the *CYR1* and *RAS2* genes grown on maltose-MacConkey agar. The transformant cells were streaked on a maltose-MacConkey indicator plate supplemented with ampicillin, chloramphenicol, and IPTG (1 mM) and incubated at 37°C: a, TP2010/pACYC184,pBR322; b, TP2010/pACYC184,placRAS2; c, TP2010/pACYC184,placRAS2^{val19}; d, TP2010/pHM10,pBR322; e, TP2010/pHM10,placRAS2; and f, TP2010/pHM10,placRAS2^{val19}.

Table 2. Induction of β -galactosidase

Bacterial strain and transformant	β -Galactosidase activity, units	
	Without IPTG	With IPTG
CA8000	36	2,669
CA8306	17	201
CA8306/pHM10	11	195
CA8306/placRAS2	43	181
CA8306/placRAS2 ^{val19}	70	170
CA8306/pHM10,placRAS2	105	778
CA8306/pHM10,placRAS2 ^{val19}	89	920

Bacterial cells were grown in minimal A medium supplemented with 0.4% glucose, 1 mM MgSO₄, 10 μ M thiamine and appropriate antibiotics with or without 1 mM IPTG to the logarithmic phase and were assayed for β -galactosidase activity.

ants carrying *CYR1* and either *RAS2* or *RAS2*^{val19}, the ability of IPTG to induce β -galactosidase was examined. Induction of β -galactosidase by IPTG was observed in transformants carrying *CYR1* and either *RAS2* or *RAS2*^{val19} but not in those carrying either of these genes independently (Table 2). The level of β -galactosidase induced by IPTG in these transformants was lower than that observed in the wild-type *E. coli* and corresponds to the difference in the intracellular levels of cAMP produced (Table 2).

Reconstitution of GTP-Dependent Adenylate Cyclase *in Vitro*. Crude extracts of *E. coli* transformants carrying pHM6 (CA8306/pHM6) and plasma membrane fractions from wild-type (AM221-1C) and *CYR1 ras1 ras2 bcyl* (T26-19C) yeast strains were prepared so that they would have comparable levels of adenylate cyclase activity in the presence of Mn²⁺ at pH 6.2 (Table 3). Crude extracts of *E. coli* transformants carrying either placRAS2 or placRAS2^{val19} and membrane fractions of the yeast *cyr1 RAS1 RAS2 BCY1* mutant (AM221-1D) had no adenylate cyclase activity in the presence of Mn²⁺ or Mg²⁺ (Table 3). Crude extract of the transformant carrying pHM6 mixed with extracts of the transformant carrying placRAS2 had adenylate cyclase activity in the presence of Mg²⁺ at pH 6.2 that was stimulated about 2-fold by the addition of p[NH]ppG (Table 3). The adenylate cyclase activity, in the mixture of crude extracts from *E. coli* transformants carrying pHM6 and carrying placRAS2^{val19}, was at the fully induced level in the presence of Mg²⁺ at pH 6.2, and no further stimulation of the activity was observed by the addition of p[NH]ppG (Table 3). Mixing the membrane fractions of yeast *cyr1 RAS1 RAS2 BCY1* mutants with that

of *CYR1 ras1 ras2 bcyl* mutants reconstituted adenylate cyclase that was active in the presence of Mg²⁺ and was stimulated about 4-fold by the addition of p[NH]ppG. Mixing the membrane fractions of yeast *CYR1 ras1 ras2 bcyl* mutants and crude extract from *E. coli* transformants carrying placRAS2 reconstituted adenylate cyclase that was active in the presence of Mg²⁺ and was stimulated about 4-fold by the addition of p[NH]ppG. However, the adenylate cyclase activity of the mixture of the membrane fractions of the *CYR1 ras1 ras2 bcyl* mutants and crude extract from *E. coli* transformants carrying placRAS2^{val19} was at the activated level in the presence of Mg²⁺, and no further stimulation of activity was observed by the addition of p[NH]ppG (Table 3). Mixing crude extract of *E. coli* transformant carrying pHM6 and the membrane fraction of yeast *cyr1 RAS1 RAS2 BCY1* mutant cells reconstituted adenylate cyclase that was stimulated by the addition of p[NH]ppG. These results indicate that the products of *CYR1*, *RAS2*, and *RAS2*^{val19} genes of yeast synthesized in *E. coli* cells are equally functional as those synthesized in yeast cells and that the product of the *CYR1* gene and the *RAS2* gene reconstitute GTP-dependent adenylate cyclase *in vitro*.

DISCUSSION

Plasmids carrying the yeast *CYR1* and *RAS2* genes can direct the synthesis of GTP-dependent adenylate cyclase in *E. coli* cells. The *CYR1* product synthesized in *E. coli* had adenylate cyclase activity in the presence of magnesium ions as well as manganese ions, but it was not GTP-dependent, and it failed to produce cAMP. The *RAS2* product alone exhibited no adenylate cyclase activity, but it was required for the expression of GTP-dependent adenylate cyclase activity of the *CYR1* product. The production of cAMP by the yeast *CYR1* and *RAS2* gene products in *E. coli* was confirmed by using MacConkey-indicator plates and by assaying β -galactosidase activity induced with IPTG. These results suggest that the catalytic activity of the *CYR1* product is regulated by the *RAS2* product, so as to be stimulated by the addition of p[NH]ppG. This conclusion is supported by the report that the *RAS2* gene product is a GTP-binding protein (12). As observed *in vitro*, the GTP-stimulation of the adenylate cyclase activity of the products of the *CYR1* and *RAS2* genes was about 2-fold, but the level of GTP-stimulation was about 4-fold when the yeast plasma membrane fraction was added. This might mean that the yeast plasma membrane fraction contains some unknown factor(s) necessary for reconstitution of the fully active enzyme. In yeast there are two genes,

Table 3. Reconstitution of GTP-dependent adenylate cyclase

Source of <i>CYR1</i> product	Source of <i>RAS2</i> or <i>RAS2</i> ^{val19} product	Adenylate cyclase activity, units		
		Mn ²⁺	Mg ²⁺	Mg ²⁺ , p[NH]ppG
CA8306/pHM6	—	6.39	0.09	0.10
—	CA8306/placRAS2	0.00	0.00	0.00
—	CA8306/placRAS2 ^{val19}	0.00	0.00	0.00
CA8306/pHM6	CA8306/placRAS2	6.39	0.23	0.46
CA8306/pHM6	CA8306/placRAS2 ^{val19}	6.90	0.58	0.58
T26-19C	—	6.21	0.04	0.04
—	AM221-1D	0.00	0.00	0.00
T26-19C	CA8306/placRAS2	6.22	0.13	0.45
T26-19C	CA8306/placRAS2 ^{val19}	6.11	1.16	1.04
CA8306/pHM6	AM221-1D	6.40	0.21	0.75
T26-19C	AM221-1D	6.20	0.35	1.47
AM221-1C	AM221-1C	6.50	0.30	1.59

Crude extracts from *E. coli* transformant (CA8306/pHM6, CA8306/placRAS2, and CA8306/placRAS2^{val19}) and membrane fractions from yeast wild-type (AM221-1C), *cyr1* (AM221-1D), and *ras1 ras2 bcyl* (T26-19C) strains were prepared independently and were mixed as indicated. Adenylate cyclase activity was measured in the presence of 5 mM Mn²⁺, or 5 mM Mg²⁺, or 5 mM Mg²⁺ and 0.1 mM p[NH]ppG at pH 6.2.

RAS1 and *RAS2*, which encode proteins of 309 and 322 amino acids, respectively (10). These two proteins are nearly 90% homologous to each other for the first 180 amino acids and then diverge radically (10). Neither *RAS1* nor *RAS2* is by itself an essential gene, but *ras1 ras2* mutant cells do not survive (27, 28). Compared to wild-type strains, intracellular cAMP levels were slightly low in *ras1*, significantly repressed in *ras2*, and undetectable in *ras1 ras2 bcy1* cells (13). Therefore, the *RAS1* and *RAS2* gene products may have similar functions, but the exact role of the *RAS1* protein is yet to be elucidated.

Production of cAMP was not observed in *E. coli* cells containing the *CYR1* gene, even though adenylate cyclase activity was detected in the presence of either manganese ions or magnesium ions, but the production of cAMP was observed only when both the *CYR1* and *RAS2* products were synthesized in *E. coli* cells. It is, therefore, most likely that the complex constituted from the *CYR1* product and the *RAS2* product in *E. coli* cells catalyzes the production of cAMP in the presence of GTP. The adenylate cyclase activity of the *CYR1* product was high in the presence of *RAS2^{val19}* product and there was no further stimulation of adenylate cyclase activity by p[NH]ppG. It has been indicated that the human H-*ras* product p21 has a GTPase activity which is impaired by a mutation at position 12 of p21 molecule (29–31). Since the yeast *RAS1* gene product also has a GTPase activity that is reduced by a missense mutation (32), it is suggested that the yeast *RAS2^{val19}* gene product may have reduced GTPase activity and constitutively activate the *CYR1* gene product.

Although we have not demonstrated that RAS protein interacts directly with adenylate cyclase, one hypothesis supported by our data is that the yeast GTP-dependent adenylate cyclase is made up of catalytic and regulatory subunits encoded by the *CYR1* and *RAS2* genes, respectively. Mammalian cells contain GTP-dependent adenylate cyclase (8), and it was recently found that the human H-*ras* gene could substitute for the yeast *RAS* genes in yeast cells (27). The system described here should allow further comparative studies on the roles of yeast and mammalian RAS genes.

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