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Yeast mating pheromone α factor inhibits adenylate cyclase

(cyclic AMP/*Saccharomyces cerevisiae*/plasma membranes/mutants/peptides)

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ABSTRACT **The pheromone α factor, secreted by *Saccharomyces cerevisiae* cells of the α mating type, serves to synchronize the opposite mating type (*a* cells) at G1 as a prelude to fusion of the two cell types. We found that, *in vitro*, α factor inhibited the membrane-bound adenylate cyclase of these cells in a dose-dependent manner. Moreover, one class (*ste5*) of *a* cell mutants that grow normally at either 23° or 34°C but that are unable to respond to α factor or to mate at the higher temperature possessed an adenylate cyclase activity that was not inhibited by α factor at 34°C but was fully sensitive to inhibition at 23°C. Furthermore, addition of cyclic AMP to *a* cell culture medium shortened the period of pheromone-induced G1 arrest. We conclude that inhibition of adenylate cyclase activity by α factor may constitute, at least in part, the biochemical mode of action of the pheromone *in vivo*.**

Haploid cells of opposite mating type of *Saccharomyces cerevisiae* can fuse pairwise to form diploid cells. This process is triggered by the action of diffusible oligopeptide pheromones (reviewed in ref. 1). α Factor, the pheromone secreted by α haploids, elicits in *a* haploids the following developmentally specific events, in order of their appearance: an increase in adhesiveness (2); arrest of growth at the G1 stage of the cell cycle with concomitant cessation of nuclear DNA replication (3); and anisotropic cell wall synthesis (4) resulting eventually in the formation of morphologically abnormal cells if mating is precluded by the absence of α cells (5). Exposure of *a* cells to α factor thus synchronizes the culture as unbudded mononucleate cells primed for mating with their α counterparts.

Although much is known about the α factor molecule, including confirmation of its primary structure (6) by solid-phase peptide synthesis (7), little work has been done to elucidate the biochemical mechanism of its action. By analogy with mammalian peptide hormones and neurotransmitters that act via cyclic AMP as an intracellular "second messenger" (8, 9), we decided to investigate the effect of cyclic AMP on the response of *a* cells to α factor *in vivo* and the effect of α factor on yeast adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] *in vitro*. We show that the α pheromone inhibits cyclic AMP production by the enzyme and that this may constitute at least part of its mode of action at the molecular level.

MATERIALS AND METHODS

Chemicals. All biochemicals were of the highest grade commercially available. Radiochemicals were purchased from New England Nuclear. All other chemicals were reagent grade. Purification of α factor and chemical synthesis of the entire molecule and partial segments have been described (7, 10). Commercially prepared synthetic α factor (Peninsula Labs, San Carlos, CA) was also used, after extensive purification (7).

Organisms and Growth Conditions. The standard laboratory yeast strains, X2180-1A (*a*), X2180-1B (α), and P2180A (*a*/α), and conditions for their cultivation have been described (11). The collection of conditionally α-factor-resistant mutants of genotypes *ste2*, *4*, *5*, *7*, *8*, *9*, *11*, and *12* derived from strain 381-G (12) were kindly provided by L. Hartwell.

Preparation of Membranes. Plasma membranes were made by a modification of the procedure of Duran *et al.* (13). Cells were grown in YPD medium (10 ml) to 1–2 × 10^8/ml, harvested by brief centrifugation, washed with 1 M sorbitol/20 mM potassium phosphate, pH 7.0 (solution A), and resuspended in 1 ml of solution A. (All steps, unless otherwise indicated, were conducted at 4°C.) Zymolyase 60,000 (Kirin) [10 mg/ml in 20 mM potassium phosphate (pH 7.0)] was added to a final concentration of 0.4 mg/ml, the mixture was incubated at 30°C for 15 min, and then 4 ml of chilled solution A was added. The spheroplasts were collected by centrifugation at 1000 × g_{av} for 10 min and resuspended gently in 1 ml of 0.8 M sorbitol/10 mM $MgCl_2$/1 mM $CaCl_2$/1 mM $MnCl_2$/0.1 mM Na_2EDTA/50 mM Tris·HCl, pH 7.5 (solution B). An equal volume of concanavalin A (Con A) (0.5 mg/ml in solution B) was added, the mixture was incubated at 30°C for 10 min, and the flocculated spheroplasts were collected by centrifugation at 1000 × g_{av} for 5 min. The spheroplasts were lysed by addition of 5.5 ml of 25 mM 1,4-piperazinediethanesulfonate (Pipes)·HCl, pH 6.2/1.1 mM $MnCl_2$/0.1 mM Na_2EDTA/1 mM phenylmethylsulfonyl fluoride (added from a 0.2 M stock in 95% ethanol) (solution C), followed by shearing in a Potter–Elvehjem homogenizer. The crude plasma membrane fraction (Con A-membranes) was collected by centrifugation at either 20,000 or 105,000 × g_{av} for 30 min and resuspended by gentle homogenization in 1 ml of solution C. As suggested by Duran *et al.* (13), it was assumed that the lectin represented about 20% of the total protein in Con A-membranes.

Adenylate Cyclase Assay. Adenylate cyclase activity was measured as the formation of cyclic [^{32}P]AMP from [α-^{32}P]ATP. A sample (25 μl) of Con A-membranes in solution C was added to a mixture containing creatine-*P* (1–2 μmol), creatine kinase (40 μg), cyclic AMP (30 nmol), and Pipes·HCl (pH 6.2) (2.5 μmol). After preincubation for 2 min at the assay temperature, reaction was initiated by addition of [α-^{32}P]ATP (25 nmol, 2–3 × 10^5 cpm/nmol) to give a final volume of 100 μl. (Stock solutions of all phosphorylated compounds were titrated to pH ≈ 7.0 with NaOH.) After a 10-min incubation, reaction was terminated by the addition of 100 μl of 1 mM cyclic AMP/4 mM ATP/2% (wt/vol) $NaDodSO_4$. After addition of 0.8 ml of water and cyclic [G-^{3}H]AMP (20,000 cpm) as an internal standard, cyclic AMP was separated from the reaction mixture by method C of Salomon *et al.* (14) and the radioactivity obtained was measured in aqueous scintillation fluid (Scintiverse, Fisher) with a liquid scintillation counter (Beckman L-3133P) set for double-label detection. Recovery of product was 70–80%. Experimental values were corrected by

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Abbreviations: Con A, concanavalin A; Con A-membranes, yeast plasma membranes prepared with structural reinforcement by Con A; Pipes, 1,4-piperazinediethanesulfonate.

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subtracting blank values (150 cpm or less) obtained from complete reaction mixtures that were quenched immediately after addition of [α-^{32}P]ATP. Duplicate samples always varied by less than 7%. α Factor or synthetic peptides were added from 90% methanol and dried in the assay tubes prior to addition of the other components.

Other Procedures. Determination of protein (11) and monitoring of α-factor-induced G1 arrest (10) were as described. Nucleotides were analyzed on polyethyleneimine-cellulose thin layers with 0.85 M LiCl as solvent or on silica gel thin layers by using chloroform/methanol/water, 30:30:5 (vol/vol). Yeast cyclic AMP phosphodiesterase was assayed according to Londesborough (15, 16).

RESULTS

Adenylate Cyclase Activity in Yeast Plasma Membranes. Adenylate cyclase activity in Con A-membranes was readily measured by the radiochemical assay (Fig. 1). The ^{32}P-labeled product was shown to be cyclic [^{32}P]AMP by cochromatography with authentic cyclic AMP in two different thin-layer systems and by its conversion to 5'-AMP upon treatment with cyclic AMP phosphodiesterase (not shown). After incubation, >97% of the ^{32}P remained as [^{32}P]ATP (as judged by thin-layer chromatography), suggesting that the ATP regeneration system used in the assay was able to cope with the Mg^{2+}-ATPase activity present in yeast plasma membranes (17). Furthermore, the presence of VnO_4^{-3} (100 μM), a potent inhibitor of the ATPase (17), had no effect on adenylate cyclase activity (not shown). Adenylate cyclase activity was Mn^{2+}-dependent and was optimal at pH 6.2, confirming the report of Varimo and Londesborough (18). Enzyme activity was not affected by the presence of F^- (10 mM) or Ca^{2+} (0.1 mM), but was stimulated about 2-fold by the nonhydrolyzable GTP analog, GPP(NH)P (0.1 mM).

With the Con A procedure, detectable adenylate cyclase activity was found only in the plasma membrane fraction. Con A-membranes had about 9-fold higher specific activity for the enzyme than did membranes prepared without Con A. This greater activity appeared to be due to preservation of membrane integrity by the lectin during isolation since, once they had been lysed, preincubation of Con A-treated spheroplasts with 20 mM α-methylmannoside prior to collection of the membrane fraction yielded a preparation with specific activity comparable to that of typical Con A-membranes.

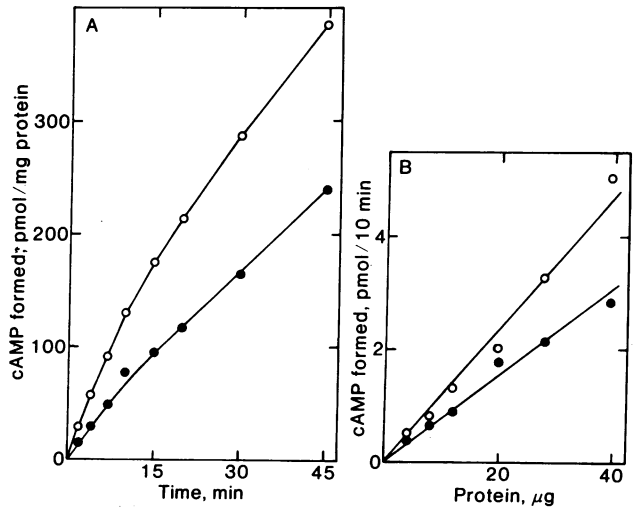

FIG. 1. Time course and dependence on membrane protein concentration of yeast adenylate cyclase activity. Con A-membranes were assayed in the absence (○) and in the presence (●) of 25 μM α factor under standard conditions, except that for the time course a final volume of 1 ml was used and 100-μl samples were removed at the indicated times. cAMP, cyclic AMP.

α Factor Pheromone Inhibits Adenylate Cyclase *In Vitro*. The presence of α factor in the assay inhibited adenylate cyclase activity. This inhibition was manifest as a decrease in the specific activity of the enzyme (Fig. 1). Con A-membranes isolated from X2180-1A (*a*) exposed to α factor for 1 hr before harvesting or made from a respiratory-deficient (*rho*^{o}) derivative of X2180-1A contained adenylate cyclase activity that was just as susceptible to α factor inhibition as the normal preparations. The adenylate cyclase of membranes prepared in the absence of Con A, although of lower specific activity, was equally susceptible to α factor inhibition. Furthermore, enzyme activity in Con A-membranes assayed in the presence of α-methylmannoside or in membranes collected after release of the lectin by preincubation with α-methylmannoside was inhibited similarly by the pheromone. In contrast, solubilization of the adenylate cyclase activity by treatment with 0.1% Triton X-100 completely abolished its susceptibility to α factor inhibition.

The possibility that the observed diminution of cyclic AMP formation was due to enhanced cyclic AMP breakdown in the presence of the pheromone was eliminated by direct assay of cyclic AMP phosphodiesterase. α Factor had no effect on the cyclic AMP phosphodiesterase activity present either in crude extracts or in Con A-membranes, whether assay was conducted under conditions optimal for this enzyme [pH 7.8, 5 mM Mg^{2+} (16)] or under the conditions used for adenylate cyclase. The phosphodiesterase activity present under standard cyclase assay conditions was sufficient to degrade only 0.02% or less of the unlabeled carrier cyclic AMP present.

The inhibition of adenylate cyclase by α factor was dose dependent (Fig. 2*A*). The K_i for α factor was 9 μM (Fig. 2*B*).

α Factor Inhibition of Adenylate Cyclase Is Specific. Synthetic α factor inhibited adenylate cyclase in Con A-membranes (Fig. 2*C*) in a manner identical to that observed with homogeneous natural α factor (Fig. 2*A*), indicating that the inhibition was indeed a consequence of the peptide pheromone itself and not due to a trace contaminant in preparations of natural α factor.

A portion of the α factor molecule that is biologically inactive *in vivo* (1, 10), the COOH-terminal hexapeptide (HN-Pro-Gly-Gln-Pro-Met-Tyr-COOH), did not inhibit adenylate cyclase activity in Con A-membranes. In fact, the presence of this peptide at high concentrations (100-fold molar excess) appeared to reverse inhibition by the intact pheromone somewhat (Table

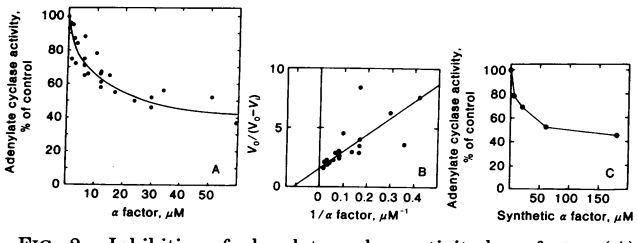

FIG. 2. Inhibition of adenylate cyclase activity by α factor. (*A*) Adenylate cyclase was assayed in the presence of different amounts of α factor. These data are a compilation of five separate experiments using three different preparations of natural α factor. (*B*) Data of panel *A* replotted. The line was determined by linear regression analysis by the least-squares method and yielded an x intercept indicating a K_i of 9 μM. For clarity, four points representing the lowest concentrations of α factor tested have not been included. (*C*) Adenylate cyclase was assayed in the presence of different amounts of synthetic α factor. Replot of the data, as in panel *B*, yielded a K_i of 8 μM.

1). Conversely, an internal segment of the pheromone, H_2N-Leu-Lys-Pro-COOH, which at high concentrations (2–5 mM) is able to transiently arrest the growth of *a* cells *in vivo* (ref. 1; Ciejek, E. M., Dissertation, University of California, Berkeley), did show some slight inhibitory action on the adenylate cyclase *in vitro*, which appeared additive with the effects of the whole pheromone (Table 1).

Heterologous peptides, some of which are hormones that alter the activity of adenylate cyclase in the membranes of other organisms, had little or no effect on the yeast enzyme (Table 2). The only exceptions were slight inhibitions by luliberin (luteinizing hormone-releasing factor) (pyro-Gln-*His-Trp*-Ser-Tyr-Gly-*Leu-Arg-Pro-Gly*-$CONH_2$) and α-melanotropin (α-melanocyte-stimulating hormone) (Ac-Ser-Tyr-Ser-Met-Glu-*His-Phe*-Arg-Trp-*Gly-Lys-Pro-Val*-$CONH_2$). However, these two peptides possess a certain degree of primary sequence homology to α factor (H_2N-Trp-*His-Trp*-Leu-Gln-*Leu-Lys-Pro-Gly*-Gln-Pro-Met-Tyr-COOH), which may account for their effects.

Although only *a* cells respond to the presence of α factor *in vivo*, inhibition by α factor of adenylate cyclase *in vitro* was observed in Con A-membranes from α haploids and *a*/α diploids as well (Table 3); however, this may be due to disruption of normal cell-wall structure during the preparation of spheroplasts (see *Discussion*).

Pheromone Action *In Vivo* Is Mediated by Its Effect on Intracellular Cyclic AMP Level. Addition of cyclic AMP to the culture medium partially antagonized the action of α factor on *a* cells *in vivo*. As shown in Fig. 3, the presence of cyclic AMP during exposure of *a* cells to α pheromone consistently shortened the duration of their arrest in G1, as determined by the percentage of unbudded cells in the population, regardless of minor experimental variations. Cyclic AMP itself did not seem to affect vegetative growth in any way. The fact that providing cyclic AMP exogenously speeded recovery of *a* cells from pheromone-induced G1 arrest suggested that the presence of α factor lowered the intracellular cyclic AMP concentration.

Pheromone-Resistant *a* Cell Mutants Possess an Adenylate Cyclase Activity Insensitive to α Factor Inhibition *In Vitro*. If the influence of α factor on the cyclic AMP level in *a* cells were in fact mediated through an inhibition of adenylate cyclase, then among *a* cell mutants resistant to pheromone action (12, 19) there should be a class that possesses an adenylate cyclase activity resistant to α factor inhibition. Recently, Hartwell (12) selected *a* cell mutants that grow normally at either 23° or 34°C, but that are resistant to α factor-induced growth stasis at the higher temperature. The mutations carried by these α factor-resistant strains fall into at least eight complementation groups. It was found that all of these mutants failed to mate at 34°C, but were mating proficient at 23°C (12).

Table 1. Effect of synthetic fragments of α factor on adenylate cyclase activity

Fragment added	Concentration, mM	Relative activity, % of control	
		−α Factor	+α Factor*
None	—	100	60
COOH-terminal hexapeptide	0.06	105	57
	0.3	95	56
	1.0	126	73
	3.0	106	84
Leu-Lys-Pro	1	111	63
	5	87	49
	10	85	44

* Present at 34 μM.

Table 2. Effect of various peptides and proteins on yeast adenylate cyclase

Addition	Amount, μg*	M_r	Relative activity, % of control
None	—	—	100
α Factor	4	1,684	45
Bovine serum albumin	10	68,000	99
Insulin	8	6,000	115
Glucagon	8	3,500	102
Bacitracin	8	1,400	114
α-Melanotropin	4	1,700	78
Luliberin	4	1,200	81
Oxytocin	4	1,000	98

* Added to standard 100-μl reaction mixtures.

We examined the adenylate cyclase activity in Con A-membranes prepared from the parent strain and from at least one representative of all eight mutant classes. We found that seven of the eight types of mutants had essentially normal levels of adenylate cyclase activity and were sensitive to α factor inhibition when assayed at either 23° or 34°C (Table 4). In contrast, four independently isolated strains (42e, 64c, 66a, and 206c) of one complementation group (*ste5*), when grown up at either 23° or 34°C, yielded Con A-membranes in which the adenylate cyclase activity was insensitive to α factor inhibition when assayed at the higher temperature but inhibited by the pheromone at the lower temperature (Table 4). Representative data are shown for one of the four *ste5* mutants, 42e (Fig. 4). Enzyme activity in Con A-membranes prepared from a mixture of wild-type and mutant cells showed roughly the expected intermediate level of inhibition by α factor at 34°C.

DISCUSSION

We have found that α factor inhibits the membrane-bound adenylate cyclase of yeast cells *in vitro* (Figs. 1, 2, and 4). Micromolar concentrations of the pheromone were required for this effect. In contrast, under the conditions standardly used for bioassay of the pheromone (at 2×10^6 *a* cells per ml), α factor is able to elicit its characteristic responses at concentrations around 10 nM (1, 10). On the other hand, the concentration of α factor required for inducing biological responses *in vivo* depends markedly on the *a* cell concentration (20, 21). Because the plasma membranes we prepared were a relatively crude fraction and were assayed at a concentration equivalent to 5×10^8 cells per ml in order to reliably detect adenylate cyclase activity, the level of α factor required for *in vitro* inhibition of the enzyme appears to be in the physiological range. At the highest concentrations of pheromone tested, the maximum inhibition of adenylate cyclase observed was 60% (Fig. 2). Perhaps a certain percentage of the enzyme molecules are inaccessible to α factor (due to membrane eversion) or perhaps some of the enzyme molecules become desensitized or uncoupled from regulatory components during membrane isolation, which may explain the lack of complete inhibition. On the other hand, even well-documented hormone-sensitive adenylate cyclase systems in mammalian cells do not show particularly dramatic changes in enzyme activity in response to inhibitory effectors (22). The inhibition of adenylate cyclase by α factor occurred without a detectable lag and resulted in an apparently permanent reduction in reaction rate (Fig. 1).

With the Con A procedure, we have demonstrated that adenylate cyclase is exclusively a plasma membrane enzyme in yeast cells. Similarly, Con A has been used quite recently to prepare, from cultured mammalian cells, plasma membranes

Table 3. α Factor inhibition of adenylate cyclase from haploid and diploid cells

Strain	Mating type	Adenylate cyclase activity, pmol/min per mg	Relative activity, % of control	
			−α Factor	+α Factor*
X2180-1A	a	30.8	100	42
X2180-1B	α	31.9	100	46
P2180A	a/α	28.4	100	49

* Present at 30 μM.

highly enriched for adenylate cyclase that remains coupled to β-adrenergic receptors (23). Membranes prepared from a yeast strain lacking mitochondrial DNA had adenylate cyclase of the same specific activity and susceptibility to α factor inhibition as that from normal cells, confirming that the activity measured was not complicated by a mitochondrial function.

The peptide specificity of the inhibition of adenylate cyclase supported its physiological significance (Tables 1 and 2). However, our finding that adenylate cyclase from all yeast cell types was inhibited *in vitro* by α factor appeared to argue against the biological relevance of this effect. On the other hand, several observations indicate that α haploids and a/α diploids may differ from a haploids mainly in their ability to exclude α factor from the cell surface. First, in addition to responding to the pheromone, a cells rapidly degrade α factor, whereas α cells and diploids do not (10, 24). In contrast, spheroplasts of α haploids and diploids proteolytically cleave α factor rapidly and in a manner similar to that observed with whole a cells (24). Second, under appropriate conditions, whole a cells bind considerably more ^{3}H-labeled α factor (1) or fluorescein isothiocyanate-labeled α factor (25) than do α cells. Third, a cells secrete an undecapeptide pheromone, a factor, which elicits the same spectrum of effects in α cells, as does the α pheromone in a cells (26). If its mode of action is similar to that which we propose here for α factor, then α haploids should also possess a pheromone-responsive adenylate cyclase. Presumably intact cells of the two different mating types respond only to the appropriate pheromone because they contain some specific

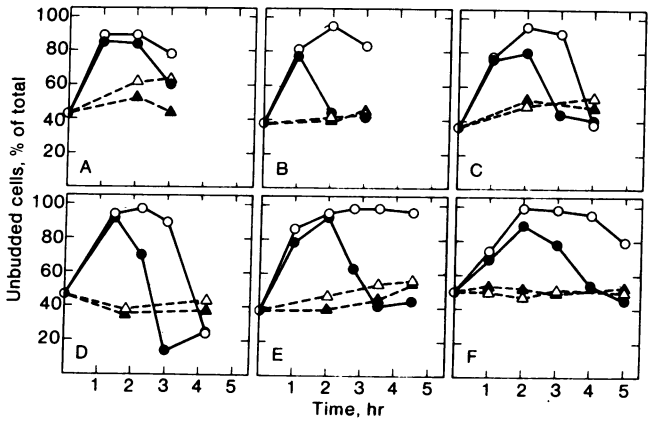

FIG. 3. Effect of cyclic AMP on α factor-induced G1 arrest of a cells. G1 arrest in response to the pheromone was scored as an increase in unbudded cells (7, 10). Conditions were (*A–C*) 30°C, 5 × 10^6 cells per ml, and, when present, 80 ng of α factor per ml or 10 mM cyclic AMP or both; (*D*) 30°C, 5 × 10^6 cells per ml, and, when present, 80 ng of α factor per ml or 1 mM cyclic AMP or both; (*E* and *F*) 23°C, 2 × 10^6 cells per ml, and, when present, 200 ng of α factor per ml or 1 mM cyclic AMP or both. O—O and ●—●, Cultures with α factor; △--△ and ▲--▲, control cultures without pheromone. ● and ▲, Cultures that received cyclic AMP.

Table 4. α Factor inhibition of adenylate cyclase activity from temperature-conditional pheromone-resistant mutants and their parent strain

Strain*	Mutation	Relative activity† 24°C	Relative activity† 34°C
381-G	None (STE$^+$)	0.62	0.66
50b	*ste2-1*	0.64	0.51
63b	*ste4-1*	0.63	0.52
82b	*ste4-3*	ND	0.60
42e	*ste5-1*	0.64	1.12‡
64c	*ste5-2*	0.72	1.10‡
66a	*ste5-3*	0.87	1.06‡
206c	*ste5-5*	0.78	0.97‡
214a	*ste7-4*	0.55	0.53
59a	*ste8-2*	ND	0.69
62c	*ste9-1*	0.65	0.58
236f	*ste9-5*	0.65	0.58
44b	*ste11-2*	0.64	0.65
59c	*ste12-1*	0.63	0.62

* Values shown are for cultures grown overnight at 23°–28°C. (Essentially the same results were obtained for cultures grown overnight at 34°C; however, their adenylate cyclase activity was assayed in the presence and absence of α factor only at the higher temperature.)

† Number given is the ratio of the adenylate cyclase activity measured in the presence of α factor (26 μM) to that measured in the absence of the pheromone. ND, not determined.

‡ α Factor concentration was 35 μM.

transmission system that permits interaction of that pheromone with adenylate cyclase. Hence, removal of the cell wall from α cells and a/α diploids with the hydrolytic enzyme we used to prepare plasma membranes probably allows access of α factor to the adenylate cyclase from which it normally is excluded.

The fact that cyclic AMP added to culture medium shortened the period of α factor-induced G1 arrest (Fig. 3) suggested that the α factor inhibition of adenylate cyclase seen *in vitro* does indeed reflect the actual mode of action of the pheromone *in vivo*. Despite permeability problems and the possibility of degradation by phosphodiesterase, enough cyclic AMP presumably can enter the cells to circumvent pheromone inhibition of adenylate cyclase. In a similar way, supplying cyclic AMP exogenously overcomes catabolite repression of several yeast enzymes (27). In fact, we attempted measurements (by radioimmunoassay) of the cellular content of cyclic AMP, but had considerable difficulty in obtaining quantitatively reproducible values, possibly due to perturbation of cellular metabolism during sampling. Similarly, intracellular cyclic AMP levels are difficult to measure reproducibly even in well-studied mammalian systems, and *in vivo* changes are often rather small (8, 28). Nevertheless, commitment of yeast cells to a new round of cell division has been correlated by others with an increase in intracellular cyclic AMP concentration (29).

That the α pheromone acts *in vivo* by inhibiting adenylate cyclase was supported by our finding that all four strains carrying mutations in the *ste5* gene had an adenylate cyclase activity that was inhibited by α factor at the permissive temperature (23°C), but was completely resistant to the pheromone at the restrictive temperature (34°C), regardless of the temperature at which the cells were grown (Table 4 and Fig. 4). These results also indicate that the function, and not the synthesis, of the *ste5* gene product is temperature sensitive in these mutants. In addition to conferring resistance to α factor-induced growth stasis and *in vitro* insensitivity of adenylate cyclase to α factor inhibition, mutations in *ste5* also render strains mating defective, regardless of whether the cells are a or α

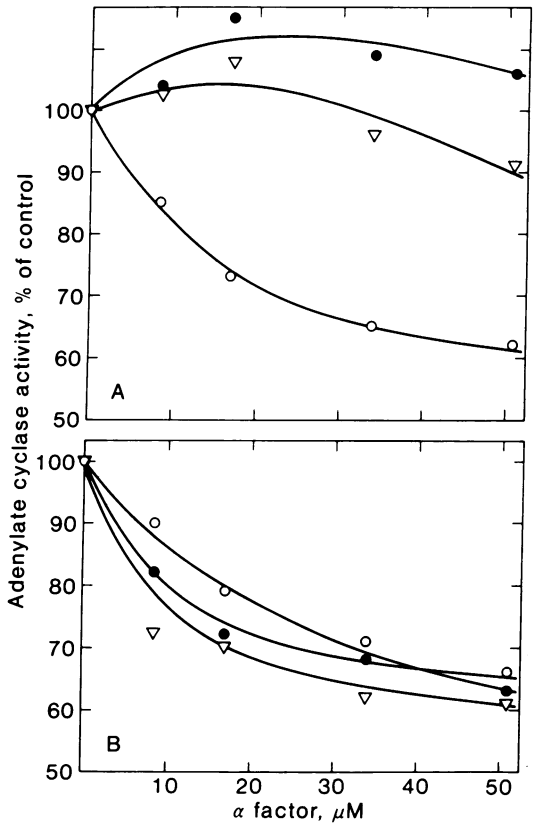

FIG. 4. Effect of temperature on α factor inhibition of adenylate cyclase from a conditionally pheromone-resistant mutant and its parent. Con A-membranes prepared from mutant 42e (●), from its normal parent 381-G (○), and from a mixture of approximately equal numbers of both cell types (▽), grown at 28°C,were assayed for adenylate cyclase activity in the presence of different amounts of α factor either at 34°C (*A*) or at 23°C (*B*).

haploids (12). This lack of mating-type specificity suggests that the *ste5* gene product is present in both cell types and may be a common component of their pheromone-inhibitable adenylate cyclases. It is possible that *ste5* encodes a function analogous to the GTP-binding regulatory protein which has been shown in mammalian systems (30) to modulate the catalytic activity of adenylate cyclase upon interaction with hormone–receptor complex. In any event, the integrity of the plasma membrane is crucial for the α factor inhibition of adenylate cyclase observed *in vitro*, since solubilization of the enzyme with a nonionic detergent completely abolished any response to the pheromone.

Taken together, our results demonstrate that the biochemical mechanism of α factor action involves its inhibition of the adenylate cyclase in the *a* cell plasma membrane. Therefore, yeast may provide a model system for biochemical and genetic dissection of the molecular features of a eukaryotic effector-responsive adenylate cyclase.

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