Control of the cAMP Pathway by the Cell Cycle Start Function, CDC25, in Saccharomyces cerevisiae

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We investigated the relationship in Saccharomyces cerevisiae between the cell cycle start function, CDC25, and two mutants defining components of the cAMP pathway. The thermolabile adenylate cyclase mutant cyrl-2(ts) is phenotypically similar to the temperature-sensitive mutant cdc25(ts) in that both mutants, when shifted to the restrictive temperature, arrest in GI of the cell cycle and permit the initiation of meiosis and sporulation. The mutant bcyl [a lesion resulting in a low level of regulatory (R) subunit and a high level of active, catalytic (C) subunit of the cAMP-dependent protein kinase] suppresses the temperature-sensitive phenotype of cyrl-2(ts) and confers an asporogenous phenotype. We found that cdc25(ts) complemented cyrl-2(ts), and, unlike cyrl-2(ts), was not suppressible by bcyl, demonstrating that CYRl and CDC25 must encode different functions. Also our results indicate that CDC25 does not encode the R subunit of the cAMP-dependent protein kinase. In addition, although the cdc25(ts) bcyl double mutant was temperature sensitive like cdc25(ts), we found that the cdc25(ts) bcyl homozygous diploid was asporogenous like bcyl/bcyl. The inability of the cdc25(ts) bcyl double mutant to sporulate demonstrated that CDC25 does not encode the C subunit of the cAMP kinase, and indicated that the CDC25 function modulates the cAMP pathway to control meiosis and sporulation. Further, the temperature-sensitive phenotype of the double mutant, and hence the inability of bcyl to suppress cdc25(ts), suggested that a second CDC25 cell cycle function exists which is independent of the cAMP pathway. Taken together, our genetic data indicated that the CDC25 protein has a dual regulatory role: (1) to control the decision between proliferation and differentiation by controlling the cAMP pathway; and (2) to control the cell cycle positively by a mechanism independent of the cAMP pathway. cdc25(ts) and cyrl-2(ts) conferred resistance to ammonium and methylamine. This suggests that CDC25 and the cAMP pathway work in concert as an ammonium signal-response system. We conclude that CDC25 controls meiosis and sporulation in response to nutritional stimuli by modulating the cAMP pathway.

INTRODUCTION

Recent genetic and biochemical studies (Matsumoto et al., 1983) of two mutants of the cAMP pathway, cyrl-2(ts) and bcyl, have demonstrated that cAMP-dependent protein phosphorylation plays a major regulatory role in the control of proliferation and differentiation in the yeast Saccharomyces cerevisiae. The lesion cyrl-2(ts) results in a thermolabile adenylate cyclase activity, and has two developmental consequences. First, at the restrictive temperature, cyrl-2(ts) cells arrest in an unbudded, GI state, identical to the arrest phenotype of several of the temperature-sensitive cdc start mutants described previously (Johnston et al., 1977); secondly, diploids homozygous for cyrl-2(ts) sporulate in nutritional medium when shifted to the restrictive temperature, a feature unique to two of the start mutants, cdc25 and cdc35 (Shilo et al., 1978). The lesion in the bcyl mutant results in a low level of the regulatory (R) and a high level of the active, catalytic (C) subunit of the cAMP-dependent protein kinase (Matsumoto et al., 1983). Hence, in bcyl, the cAMP kinase no longer requires cAMP for activation. This is
demonstrated by the observation that bcy1 suppresses the temperature-sensitive growth phenotype of cyr1-2(ts). In addition, diploids homozygous for bcy1 do not arrest the cell cycle normally when nutritionally deprived, and are unable to sporulate. Two major conclusions have been drawn from these results. First, cAMP-dependent protein phosphorylation is required for cell cycle initiation and vegetative growth; secondly, the cAMP-dependent protein kinase must be inactivated before cell cycle arrest or sporulation can ensue. This understanding of the dual role of the cAMP kinase in the control of proliferation and differentiation in yeast raises a number of new questions. First, how are the various cell cycle mutants related to the cAMP pathway? Possibly, some may code for proteins which modulate the adenylate cyclase complex, others for proteins which are substrates of the cAMP kinase, and still others may define functions outside of the cAMP pathway. Secondly, since nutritional signals ultimately control proliferation and differentiation in yeast, it is of interest to determine if nutritional information is mediated by the cAMP pathway.

In this report, we investigated the relationship of the start mutant cdc25(ts) to two of the mutants of the cAMP pathway, cyr1-2(ts) and bcy1. In addition, we investigated the possibility that diploids homozygous for cyr1-2(ts) and cdc25(ts) are altered in their response to the nutritional signals which control sporulation. Our genetic studies demonstrated that the CDC25 gene product has a dual function: (1) to modulate the cAMP pathway and thereby control proliferation and differentiation; and (2) to control cell cycle initiation by a mechanism which is not mediated by the cAMP pathway. In addition, our data also suggested that the ammonium nutritional signal is mediated by the CDC25 and CYR1 gene products.

METHODS

Yeast strains. All strains used in these experiments are Saccharomyces cerevisiae. Strain 131 (MATa ade2 ura3-52 leu1 can' cyr1/MATa ade2 met8) was the wild-type. Strain X213 was homozygous for the cell-division-cycle (cdc) mutant cdc25(ts), and its origin and construction have been described by Simchen et al. (1972). Strains X213 and 131 have a similar genetic background but are not isogenic. Strain S6537, also homozygous for cdc25(ts), was constructed as follows. Haploid segregants of X213 were outcrossed to another laboratory strain; the resulting heterozygote was sporulated, and temperature-sensitive segregants of opposite mating types, S65 and S37, were mated, yielding S6537. The phenotype of S6537 with respect to growth and sporulation was the same as that of X213. Spontaneous non-temperature-sensitive revertants of S65 and S37 were screened by back crossing to strains wild-type for CDC25, and then checking for the segregation of temperature-sensitive clones. Two revertants, R65 and R37, when independently crossed with wild-type, gave a 4+:0-= segregation of non-temperature-sensitivity, indicating that the reversion event occurred within the CDC25 locus. The two revertants were mated, yielding strain R6537. Strains S6537 and R6537 are by this criterion congeneric. The construction, phenotype and origin of all other strains are described in Table 1.

Media. Presporulation medium (PSP-2) contained 1% (w/v) potassium acetate, 0.67% (w/v) yeast nitrogen base without amino acids (Difco) and 40 μg ml⁻¹ each of adenine, uracil, leucine and histidine, and was buffered with potassium phthalate, pH 5.5. YPA medium contained 10 g yeast extract (Difco), 20 g peptone (Difco) and 20 g potassium acetate per litre of distilled water. YPD plates contained 10 g yeast extract, 20 g peptone, 20 g dextrose and 15 g agar per litre of distilled water. Liquid sporulation medium (SPM) contained 3 g of potassium acetate per litre of distilled water, pH 6.2, adjusted with 12 M-HCl.

Sporulation procedure. Vegetative cultures were grown in liquid PSP-2 or YPA medium at 28 °C. Growth was monitored by direct microscopic counts with a haemocytometer. Cells were harvested at a density of 10⁷ ml⁻¹, washed two times with sterile distilled water and resuspended to the same titre in SPM. Sporulation was assessed microscopically (200 cells or asci were counted) after 48 h incubation. Percentage sporulation efficiency was expressed as 100 times the number of asci divided by the sum of asci and nonsporulating cells. In all cases, four-spored asci were predominant (>80%).

Methyamine uptake. Cells were pregrown, harvested, washed and resuspended as described above. Samples were taken just prior to methyamine addition, and the protein was determined by the Lowry method. After addition of [³¹C]methyamine (New England Nuclear), final concentration 0.5 mM (2 μCi μmol⁻¹, 74 kBq μmol⁻¹), samples (0-2 ml) were removed at various times and collected by vacuum filtration on a 0.45 μm HAWP Millipore filter. Filters were washed three times with 5 ml of ice cold SPM containing an equivalent concentration of unlabelled methyamine (Aldrich). The filters were placed directly into scintillation vials containing 10 ml 3a70b cocktail (Research Products International Corp., Elk Grove Village, Ill., USA) and counted in a Beckman LS-233 series liquid scintillation counter.
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Table 1. Yeast strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR15-S22H</td>
<td>MATa/CDC25/</td>
<td>Haploid segregant obtained in our laboratory</td>
</tr>
<tr>
<td>131</td>
<td>MATa/CYRI-2(ts) cyrl-2(ts) bcyI</td>
<td>Our stock culture; Simchen et al. (1972)</td>
</tr>
<tr>
<td>S6537</td>
<td>MATa/cdc25(ts)/cdc25(ts)</td>
<td>This work</td>
</tr>
<tr>
<td>R6537</td>
<td>MATa/CDC25/</td>
<td>This work</td>
</tr>
<tr>
<td>CR53a</td>
<td>MATa/CDC25/</td>
<td>This work</td>
</tr>
<tr>
<td>CR48</td>
<td>MATa/cdc25(ts)/cdc25(ts)</td>
<td>Cross between segregant X288-9C and CR15-S22H</td>
</tr>
<tr>
<td>CR41</td>
<td>MATa/cdc25(ts)/+ +</td>
<td>Cross between segregant CR41-9a and CR15-S22H</td>
</tr>
<tr>
<td>X282</td>
<td>MATa/cdc25(ts)/bccyl</td>
<td>Cross between segregant X288-9C and CR15-S22H</td>
</tr>
<tr>
<td>X286</td>
<td>MATa/cyrI-2(ts)cyrI-2(ts)</td>
<td>Cross between segregant X288-9C and CR15-S22H</td>
</tr>
<tr>
<td>X287</td>
<td>MATa/cyrI-2(ts)+ +</td>
<td>Cross between segregant X288-9C and CR15-S22H</td>
</tr>
<tr>
<td>X213</td>
<td>MATa/cdc25(ts)/cdc25(ts)</td>
<td>Cross between segregant X288-9C and CR15-S22H</td>
</tr>
<tr>
<td>131</td>
<td>MATa/cdc25(ts)/+ +</td>
<td>Cross between segregant X288-9C and CR15-S22H</td>
</tr>
</tbody>
</table>

* Addresses: K. Matsumoto, Tottori University, Tottori-Shi, Tottori 680, Japan; G. Simchen, The Hebrew University, Jerusalem, Israel.

Table 2. Temperature sensitivity and sporulation phenotype of various diploid strains carrying cdc25(ts), cyrl-2(ts) and bcyI

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>34 °C</th>
<th>36 °C</th>
<th>26 °C</th>
<th>33.5 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR53a</td>
<td>cyrl-2(ts) + + cdc25(ts)</td>
<td>+</td>
<td>+</td>
<td>70</td>
<td>54</td>
</tr>
<tr>
<td>CR41</td>
<td>cdc25(ts) + + bcyI</td>
<td>+</td>
<td>+</td>
<td>66</td>
<td>48</td>
</tr>
<tr>
<td>CR48</td>
<td>cdc25(ts) bcyI/cdc25(ts) bcyI</td>
<td>–</td>
<td>–</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>X282</td>
<td>bcyI/bcyI</td>
<td>–</td>
<td>–</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>X286</td>
<td>cyrl-2(ts)cyrI-2(ts)</td>
<td>+</td>
<td>–</td>
<td>81</td>
<td>79</td>
</tr>
<tr>
<td>X287</td>
<td>+ + cyrl-2(ts)</td>
<td>+</td>
<td>+</td>
<td>81</td>
<td>32</td>
</tr>
<tr>
<td>X213</td>
<td>cdc25(ts)/cdc25(ts)</td>
<td>–</td>
<td>–</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>131</td>
<td>+ +</td>
<td>–</td>
<td>–</td>
<td>87</td>
<td>49</td>
</tr>
</tbody>
</table>

* Cells of each strain were grown on YPD plates at 26 °C for 24 h, then replica-plated to YPD plates and incubated at 26 °C, 34 °C or 36 °C. Growth was assessed after 48 h.

† Cells of each strain, grown in YPA medium at 26 °C, were shifted to liquid SPM. Sporulation was assessed after incubation at 26 °C or 33.5 °C for 48 h (strains CR48 and X282 were reassessed after 4 d and no increase in sporulation efficiency was observed).

RESULTS
cdc25(ts) is not allelic to cyrl-2(ts) or bcyI

The phenotypic similarities between cdc25(ts) and cyrl-2(ts) prompted us to investigate if the two genes are functionally related. We tested two simple hypotheses: (1) that CDC25 encodes a component of the cAMP pathway, possibly adenylate cyclase (which catalyses the formation of cAMP from ATP), or the C or R subunit of the cAMP-dependent protein kinase (which is activated by cAMP and results in the phosphorylation of specific target proteins); or (2) that CDC25 functions independently of the cAMP pathway. To evaluate these possibilities, a series of crosses were made between cdc25(ts) and cyrl-2(ts), and cdc25(ts) and bcyI. Their phenotypes with respect to temperature sensitivity and sporulation were determined (Table 2), and led us to the following conclusions.

First, cyrl-2(ts) and cdc25(ts) complemented each other (Table 2, strain CR53a). This was not intragenic complementation, since CYRI and CDC25 are on different chromosomes.
(Matsumoto et al., 1982; M. L. Tripp & R. Piñon, unpublished results). Since CYRI encodes the catalytic subunit of adenylate cyclase (Matsumoto et al., 1984), this complementation test indicated that CDC25 is not the catalytic subunit of adenylate cyclase. However, these data do not eliminate the possibility that CDC25 is a regulatory component of adenylate cyclase.

Secondly, the phenotypes of the cdc25(ts) +/+ bcyl1 heterozygote and the cdc25(ts) bcyl homozygote (Table 2, strain CR41) led us to conclude that CDC25 does not encode the R subunit of the cAMP-protein kinase. Although the primary lesion in bcyl1 is unknown, Matsumoto et al. (1983) have shown that the consequence of the bcyl1 mutation is a low level of the R subunit, and an unregulated C subunit (i.e. cAMP independent). The arguments which led to our conclusion took into account this effect of the bcyl1 lesion, and can be stated as follows. If CDC25 encodes R, then, at the restrictive temperature, Rcdc25(ts) must inactivate (possibly by binding irreversibly) the C subunit of the cAMP-kinase. Hence, cdc25(ts) should be dominant in a heterozygous condition. However, the cdc25(ts) allele is recessive [i.e. cdc25(ts)/+ is not temperature sensitive; data not shown], suggesting that CDC25 does not encode the catalytic subunit of adenylate cyclase (Matsumoto et al., 1982). Moreover, the temperature-sensitive phenotype of the double homozygote is tight, like that of the bcyl, suppression argues that the product of CDC25 is not the C subunit of the cAMP-kinase. That is, in bcyl, the C subunit is unregulated due to a low level of R subunit; hence, if CDC25 did encode the C subunit of the cAMP-kinase, then the cdc25(ts) mutation should result in a temperature-sensitive C subunit. The presence of a wild-type R subunit would be of no consequence in the double mutant, since the C subunit would be inactive at 34°C [the restrictive temperature of cdc25(ts)]. As a consequence, the negative control over sporulation, conferred by bcyl1, would be released. This is clearly not the case, as the level of sporulation in strain CR48 is the same low level as that of X282 (Table 2). Moreover, the fact that bcyl1 suppresses cyri-2(ts) [cyri-2(ts) bcyl1 is not temperature sensitive; Matsumoto et al., 1982] but does not suppress cdc25(ts) [cdc25(ts) bcyl1 is temperature sensitive; Table 2, strain CR48] is consistent with the conclusion that cdc25(ts) and cyri-2(ts) define different functions.

cyri-2(ts) confers altered nutritional sensitivity

Sporulation in S. cerevisiae is a differentiative process which includes meiosis and ascospore formation. Sporulation is triggered in diploids heterozygous at the mating-type locus
Control of the yeast cAMP pathway by CDC25

Table 3. Effect of sporulation inhibitors on the sporulation efficiency of the cyrI-2(ts) heterozygous and homozygous diploids

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Percentage sporulation efficiency†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No inhibitor</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>X287</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>X286</td>
<td>81 ± 3</td>
</tr>
</tbody>
</table>

* For the genotype of each strain, see Table 1.
† Cells of each strain grown in liquid YPA medium at 28 °C were shifted to liquid SPM containing no inhibitor, or containing the indicated concentration of ammonium or methylamine. The pH in all cases was 6.2. Sporulation was assessed (three replicates per treatment) after incubation at 28 °C for 48 h. Sporulation was re-assessed at 4 d, and no significant change was observed. The values are means ± SEM of three replicates.

(MATa/MATα) when ammonium is depleted from the medium. Starvation for any one of several other essential nutritional compounds, including a sulphur, phosphorus, biotin or carbon source, results in cell cycle arrest, but does not normally trigger sporulation (Hartwell, 1974). In non-glucose nutrient media (for example YPA), other sources of nitrogen, such as amino acids, are sufficient to support proliferation; however, when the cells reach high density, sporulation ensues. Hence, amino acids support proliferation, but do not inhibit sporulation. This has been demonstrated in studies in which ammonium, and the non-metabolizable ammonium analogue methylamine, were shown to be potent inhibitors of sporulation, while amino acids (except for glutamine), nucleotides, nitrate and nitrite had no inhibitory effect (Piñón, 1977). These observations suggest that depletion of ammonium is a specific signal which triggers cell cycle arrest and entry into the sporulation pathway. Here, we investigated whether the ammonium signal is mediated by the cAMP pathway. To test this possibility, we examined the effects of ammonium and methylamine on the sporulation of the cyrI-2(ts) homozygous diploid, strain X286. For comparison purposes, the experiments reported in Table 3 were done at 28 °C, the optimal sporulation temperature of the heterozygous control X287. Although 28 °C is a permissive temperature for cyrI-2(ts), the level of adenylate cyclase activity is only 5–10% of that of the wild-type (Matsumoto et al., 1983). Hence, a difference in the level of sporulation between the two strains is viewed as a consequence of their respective cAMP levels. The ammonium and methylamine data (Table 3; these data are representative of eight independent experiments) clearly showed that the cyrI-2(ts) homozygote, X286, was resistant to both ammonium and methylamine. Methylamine at levels of 10 mM showed no sign of toxicity to the vegetative growth of any of these yeast strains (data not shown). Hence, inhibition of sporulation by methylamine was not due to a general toxic effect. We cannot say conclusively whether the reduction in cAMP levels brings about ammonium resistance by causing a direct reduction in cAMP-dependent protein kinase activity, or if the ammonium signal-transduction system in the cyrI-2(ts) homozygous diploid is in some way altered as an indirect consequence of the mutation. However, other adenylate cyclase mutants have been isolated in which exogenous cAMP is required for growth, and upon cAMP depletion, these cyr homozygous diploids sporulate in nutrient medium (Matsumoto et al., 1983). Importantly, bcyI suppresses all of these cyr mutants. We conclude that the most likely possibility is that the resistance of cyrI-2(ts) to ammonium and methylamine was due to a reduced level of cAMP.

cdc25(ts) confers altered nutritional sensitivity

From the data above, we argue that ammonium inhibition of sporulation is mediated through the cAMP pathway. If the CDC25 product modulates the cAMP pathway, then it follows that cdc25(ts) may also show resistance to ammonium inhibition of sporulation. Consistent with this model, the cdc25(ts) homozygous diploids X213 and S6537 were substantially more resistant than the wild-type 131 or the congenic revertant R6537 to ammonium and methylamine (Table 4; these data are representative of over 20 independent experiments).
Fig. 1. Uptake of $[^{14}\text{C}]$methylamine in wild-type strain 131 (○) and cdc25(ts) strain X213 (●) homozygous diploids in SPM at 34 °C. The cells were transferred from PSP-2 (26 °C) to SPM (34 °C) at 10$^7$ cells ml$^{-1}$. $[^{14}\text{C}]$Methylamine (0.5 mM; 2 μCi ml$^{-1}$, 74 kBq ml$^{-1}$) was added at time zero, samples were taken at the indicated times and the c.p.m. of $^{14}\text{C}$ taken-up were determined as described in Methods. Each point represents a single sample. Similar results were obtained in three separate experiments. Total cellular protein was determined just before $[^{14}\text{C}]$methylamine addition.

Table 4. Effect of sporulation inhibitors on the sporulation efficiency of wild-type, revertant, and cdc25(ts) homozygous diploids

<table>
<thead>
<tr>
<th>Strain*</th>
<th>No inhibitor</th>
<th>Ammonium</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>1 mM</td>
</tr>
<tr>
<td>131</td>
<td>55 ± 4</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>R6537</td>
<td>60 ± 4</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>X213</td>
<td>68 ± 4</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>S6537</td>
<td>61 ± 2</td>
<td>63 ± 2</td>
</tr>
</tbody>
</table>

$[^{14}\text{C}]$Methylamine

<table>
<thead>
<tr>
<th></th>
<th>0.5 mM</th>
<th>1 mM</th>
<th>2 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>26 ± 4</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>R6537</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X213</td>
<td>61 ± 8</td>
<td>28 ± 2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>S6537</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*For the genotype of each strain, see Table 1.
†Cells of each strain grown in PSP-2 at 26 °C were shifted to liquid SPM containing no inhibitor, or containing the indicated concentration of ammonium or methylamine. The pH in all cases was 6.2. Sporulation was assessed (three replicates per treatment) after incubation at 33.5 °C for 48 h. Sporulation was re-assessed at 4 d, and no significant change was observed. The values are means ± SEM of three replicates.

cdc25(ts) is not a permeability mutant

One explanation for the resistance of cdc25(ts) to the inhibition of sporulation by ammonium and methylamine is that the mutant may be less permeable to ammonium at the restrictive temperature. This could account for the cell cycle arrest phenotype of cdc25(ts), i.e. cell cycle arrest could be the result of ammonium starvation. Since methylamine is transported by the ammonium transport system (Roon et al., 1975), we examined $[^{14}\text{C}]$methylamine uptake in X213 and 131 at permissive and restrictive temperatures. Uptake of $[^{14}\text{C}]$methylamine was the same in mutant and wild-type strains at 34 °C (Fig. 1) and at 22 °C (data not shown), and intracellular accumulation was the same in both strains (data not shown). These data showed that cdc25(ts) is not a permeability mutant.

DISCUSSION

Nutritional signals ultimately control two aspects, proliferation (vegetative growth) and differentiation (meiosis and ascospore formation), of the yeast life cycle. Proliferation requires the presence of a few critical nutrient compounds (Johnston et al., 1977; Carter et al., 1983);
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depletion of one or more of these key nutrients provokes a regulated response, resulting in a
distinct form of cell cycle arrest, originally referred to as G1 arrest (Johnston et al., 1977). In
contrast, differentiation is normally triggered by a special type of nutritional deprivation,
ammonium starvation (Dawes, 1983). Previous studies (Miller, 1963; Piñon, 1977; Vezinhet et
al., 1979) have shown that ammonium is an antagonist of differentiation; among the nutritional
ingredients in growth medium, only ammonium acts as a potent inhibitor of meiosis and
sporulation. The non-metabolizable ammonium analogue methylamine, like ammonium, is also
a potent sporulation inhibitor (Piñon, 1977), which indicates that ammonium, and not a
metabolite, is acting as an effector molecule in this respect. These results suggest that
ammonium plays a pivotal role as a signal molecule, providing a positive signal with respect to
proliferation and a negative signal with respect to differentiation. The mechanism by which
ammonium is monitored, and the signal transduced into the appropriate developmental
response, has been unclear. Recent genetic and biochemical studies (Matsumoto et al., 1983)
demonstrate that cAMP-dependent protein phosphorylation plays a positive role in the control
of proliferation, and a negative role in the control of differentiation in yeast. These studies suggest
that the cAMP pathway, defined in part by the mutations cyr1-2(ts) and bcyl, may play a
major role in mediating the developmental response to nutritional information. The data
reported here suggest that CDC25 and the cAMP pathway are distinct components of a
nutritional signal-response system, one of whose roles is to monitor ammonium signals, and
mediate an appropriate developmental response.

Because of the phenotypic similarity of cdc25(ts) to cyr1-2(ts), we set out to determine if
CDC25 is a component of the cAMP pathway. The ability of cdc25(ts) to complement cyr1-2(ts)
as well as the inability of bcyl to suppress the temperature-sensitive phenotype of cdc25(ts)
indicated that CDC25 does not encode the catalytic subunit of adenylyl cyclase. In addition, the
non-temperature-sensitive phenotype of the cdc25(ts) and bcyl heterozygote (Table 2, strain
CR41) showed that CDC25 does not encode the R subunit of the cAMP kinase. Similarly, the
temperature-sensitive phenotype of the double mutant (Table 2, strain CR48) further
demonstrated that CDC25 is not the catalytic subunit of the cAMP kinase. Moreover, the
inability of the cdc25(ts) bcyl double mutant to sporulate suggested that CDC25 controls
sporulation through its regulation of the cAMP kinase, rather than the cAMP kinase controlling
sporulation through CDC25. This conclusion takes into account the observations that (1)
sporulation is triggered in cdc25(ts) homozygous diploids by a shift to the restrictive temperature
(Shilo et al., 1978), and (2) that an active cAMP kinase is sufficient to prevent sporulation [i.e.
that neither bcyl nor cyr1-2(ts) bcyl homozygous diploids sporulate; Matsumoto et al., 1983].
Hence, it follows that inactivation of CDC25 [i.e. cdc25(ts)/cdc25(ts) at the restrictive
temperature] must lead to the inactivation of the cAMP kinase.

How does CDC25 regulate the cAMP kinase? Possibly the CDC25 function is normally
required to activate the cAMP kinase; e.g. CDC25 may interact with, or covalently modify
(CDC25 may encode a protein kinase or positively control the activity of a cAMP-independent
protein kinase), the R subunit of the cAMP kinase directly. A more likely explanation is that
CDC25 may act indirectly by modulating adenylyl cyclase activity and cAMP levels, the
manner in which the cAMP kinase is normally regulated (Gilman, 1984).

How might CDC25 modulate adenylyl cyclase? One possibility is that CDC25 encodes a
subunit of a G protein and modulates adenylyl cyclase activity as has been proposed for animal
cells (Gilman, 1984). Yeast adenylyl cyclase has been shown to be similar to that of animal cells
in that it is activated by guanine nucleotides (Casperson et al., 1983). Recently, GTP-binding
activity has been shown to be associated with the RAS1 and RAS2 gene products in yeast, and
RAS1 has an intrinsic GTPase activity similar to that of the G subunit of the G protein complex
of higher cells (Temelles et al., 1984; Tamanoi et al., 1984). Although it is not known for certain if
RAS1 and RAS2 are G proteins, the suppression of the ras1 ras2 double mutant by bcyl implicates RAS
in the control of adenylyl cyclase in yeast (Toda et al., 1985). Could CDC25 be
RAS1 or RAS2? CDC25 differs from RAS1 in two significant ways; (1) insertional inactivation of
either RAS1 or RAS2 individually is not lethal (Kataoka et al., 1984; Tatchell et al., 1984); and
(2) cdc25(ts) is not suppressible by bcyl. These differences suggest that CDC25 and RAS1 or
RAS2 may modulate adenylate cyclase differently. Whether CDC25 is a G protein or plays another role in the control of the cAMP pathway awaits further investigation.

The temperature-sensitive phenotype of the cdc25(ts) bcy1 double mutant also demonstrated that, although an active cAMP kinase is sufficient to inactivate meiosis and sporulation, the CDC25 function is an absolute requirement for proliferation. Hence, CDC25 must perform a cell cycle-specific function independent of the cAMP pathway. This observation argues that proliferation is not controlled simply by the regulation of the cAMP kinase, and that CDC25 has at least two distinct functions in the regulation of proliferation. The ability of cdc25(ts) and cyr1-2(ts) diploids to sporulate in nutrient medium when shifted to the restrictive temperature suggested that the ammonium nutritional signal is mediated by CDC25 and/or CYR1. To study this in more detail we examined the sporulation of both mutants in the presence of various concentrations of ammonium and methylamine. As shown in Table 3 and Table 4, the cdc25(ts) and cyr1-2(ts) mutations conferred a high level of resistance to both ammonium and methylamine. In the case of cyr1-2(ts), sporulation was assessed at 28 °C (Table 2) and not at its restrictive temperature of 36 °C (sporulation is inefficient in most strains above 34 °C). Hence, some adenylate cyclase activity (5–10% of wild-type at 26 °C; Matsumoto et al., 1983) was present, and may explain the inhibition observed at high concentrations of ammonium. Similarly, this may explain why the cdc25(ts) strain (34 °C is restrictive for growth) was also not totally refractory to ammonium inhibition of sporulation at 33.5 °C. Alternatively, high levels of ammonium may inhibit sporulation via other pathways.

Other mutants in which sporulation occurs in nutrient medium have been described. The cell division cycle mutant cdc35(ts) has a phenotype very similar to that of cdc25(ts) (Shilo et al., 1978). The spd mutants isolated by Dawes (1975) are impaired for growth on non-fermentable carbon sources (Dawes & Calvert, 1984), and in this sense are similar to RAS2 mutations. More recently, guanine-requiring mutants, gua, have been described, in which high levels of sporulation occur in ammonium-containing medium after guanine deprivation (Varma et al., 1985; M. L. Tripp & R. Piñon, unpublished results). Although the nature of these lesions remains to be defined, the similarity of the spd phenotype to that of RAS2 disruptions, and the reduced GTP levels in the gua mutants, suggests that both the SPD and GUA functions may affect adenylate cyclase activity.

In conclusion, our studies suggest that CDC25 and the cAMP pathway act in concert to mediate nutritional signals which control proliferation and differentiation in yeast. The other important finding was that CDC25 performs a dual function: (1) it regulates cAMP kinase activity directly, or indirectly, through the modulation of adenylate cyclase; and (2) it regulates cell cycle initiation by a mechanism independent of the cAMP pathway.

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Control of the yeast cAMP pathway by CDC25


