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Changes in Regulation of Ribosomal Protein Synthesis During Vegetative Growth and Sporulation of *Saccharomyces cerevisiae*

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When diploid *Saccharomyces cerevisiae* cells logarithmically growing in acetate medium were placed in sporulation medium, the relative rates of synthesis of 40 or more individual ribosomal proteins (r-proteins) were coordinately depressed to approximately 20% of those of growing cells. These new depressed rates remained constant for at least 10 h into sporulation. If yeast nitrogen base was added 4 h after the beginning of sporulation to shift the cells back to vegetative growth, the original relative rates of r-protein synthesis were rapidly reestablished. This upshift in the rates occurred even in diploids homozygous for the regulatory mutation *rna2* at the restrictive temperature for this mutation (34°C). However, once these mutant cells began to bud and grow at 34°C, the phenotype of *rna2* was expressed and the syntheses of r-proteins were again coordinately depressed. At least one protein whose rate of synthesis was not depressed by *rna2* in vegetative cells did have a decreased rate of synthesis during sporulation. Another r-protein whose synthesis was depressed by *rna2* maintained a high rate of synthesis at the beginning of sporulation. These data suggest that the mechanism responsible for coordinate control of r-protein synthesis during sporulation does not require the gene product of *RNA2* and thus defines a separate mechanism by which r-proteins are coordinately controlled in *S. cerevisiae*.

Several stimuli will induce a coordinate depression in the relative rate of synthesis of ribosomal proteins (r-proteins) in the eucaryote *Saccharomyces cerevisiae*. For example, this response occurs under the influence of several temperature-sensitive *rna* mutations (3), during amino acid starvation (11), and transiently when growing cells are shifted from 23 to 36°C (10). The latter two phenomena are remarkably similar to observations in the procaryote *Escherichia coli* (1, 4). Unlike the situation in *E. coli*, little is known about the mechanism of this coordinate inhibition in yeasts or possible effector molecules involved.

All of these conditions cause a very similar coordinate depression of r-protein synthesis measured both by in vivo protein synthesis and by in vitro translation of r-protein mRNA (10, 11). However, there are qualitative and quantitative differences among the responses to suggest that the regulation of the level of r-protein synthesis is under very complex control. For example, when cells undergo a temperature shift, all the r-proteins show a transient inhibition. The relative rates of r-protein synthesis drop to less than 40% of original values within 20 min, but recover by 1 h (10). When cells carrying such mutations as *rna2* or *rna6* are raised to the restrictive temperature of 36°C, almost all r-proteins are also coordinately inhibited. Their relative rates of synthesis drop to approximately 20% of normal within 30 min and do not recover. There are, however, at least three proteins that are not inhibited by the *rna* mutants. Protein 14 from the 40S ribosomal subunit and proteins 25 and 39 from the 60S ribosomal subunit show a high relative rate of synthesis even after 1 h at the restrictive temperature (3). In another situation, during acid starvation, protein 14 is not coordinately inhibited, whereas protein 39 is inhibited (10). Thus, at least some r-proteins are differently affected under these several inhibitory conditions, but most r-proteins are indeed coordinately controlled.

This report describes another condition under which depression of the rate of r-protein synthesis occurs, namely, during sporulation. Diploid yeast cells are induced to sporulate when starved for nitrogen in the presence of an oxidizable carbon source. This set of conditions activates a series of developmental steps culminating in the production of four haploid ascospores. These differentiating cells continue actively to metab-

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olize despite nitrogen starvation. For example, premeiotic DNA replication occurs, followed by two typical meiotic divisions. Furthermore, in response to sporulation conditions, new proteins are synthesized not characteristic of vegetative growth (9; E. Kraig and J. E. Haber, submitted for publication). There is, however, a significant decrease in total protein synthetic activity, as measured by a large decrease in the fraction of ribosomes on polysomes (Kraig and Haber, submitted). These changes in protein synthesis are also found in diploids homozygous for *MATα* which do not carry out premeiotic DNA synthesis or other meiotic events leading to the actual production of spores.

If sporulating cells are given a nitrogen source before commitment to ascospore formation, they revert to a mitotic, vegetative growth cycle (2). We wished to know whether this represented a condition which signaled an acceleration in the relative rates of synthesis of r-proteins. Our data show that the relative rates of synthesis of r-proteins do increase coordinately during this transition and reach a maximum level before the first cell division.

Based on the results presented here and the previous studies by Warner and Gorenstein (10, 11) mentioned above, it is obvious that there are a number of conditions which selectively stimulate or depress the rate of synthesis of r-proteins in *S. cerevisiae*. It is not known whether the same control mechanism is responsible for these changes in each case. The data presented here suggest that there is more than one regulatory circuit which coordinates the rate of synthesis of r-proteins in *S. cerevisiae*.

MATERIALS AND METHODS

Strains. Haploid *S. cerevisiae* strains carrying the temperature-sensitive mutation *rna2* were obtained from C. McLaughlin. All other strains used to construct diploids were obtained from the Berkeley Stock Collection. Two closely related diploids were used in this study, one homozygous for *rna2* and temperature sensitive for both growth and sporulation and the other heterozygous for *rna2* and capable of growing and sporulating at both 25 and 34°C. Their genotypes are:

NP1: $\frac{MAT\mathbf{a}\ rna2\ tyr1\ ura1}{MAT\alpha\ rna2} \frac{+}{+} \frac{+}{ade1} \frac{+}{ade2} \frac{+}{lys2} \frac{+}{his7} \frac{+}{thr4}$

NP2: $\frac{MAT\mathbf{a}\ rna2\ ade1\ ade2\ ura1\ his7\ lys2\ tyr1}{MAT\alpha} \frac{+}{+} \frac{+}{+} \frac{+}{+} \frac{+}{+} \frac{+}{+} \frac{+}{+} \frac{+}{thr4}$

In addition, a nonsporulating *MATα/MATα* diploid strain was also used (7):

NP3: $\frac{MAT\alpha\ rna2\ ade1\ thr4}{MAT\alpha\ rna2} \frac{+}{+} \frac{+}{tyr1} \frac{+}{ura1} \frac{+}{his7}$

Media. Presporulation medium (YEPA) contained 10 g of yeast extract, 20 g of peptone (Difco), and 10 g of potassium acetate per liter. To induce sporulation, logarithmically growing cells in YEPA were harvested, washed twice with sporulation medium, and then diluted 1:4 in sporulation medium. Cells were sporulated in 1% potassium acetate buffered to pH 5.5 with succinic acid (6). In some experiments yeast nitrogen base (YNB) without amino acids (Difco) was added to sporulating cultures to a final concentration of 1.34% in order to convert sporulation medium back to a growth medium. Addition of the YNB did not affect the pH of the buffered sporulation medium.

For steady-state labeling, cells were grown in minimal medium (0.67% YNB without amino acids, 2% glucose). The prototrophic diploids used in this study have approximately the same generation time (3 h) whether grown in minimal medium or YEPA.

Separation and quantitation of r-proteins. The two-dimensional electrophoresis method used to separate yeast r-proteins and quantitate their relative rates of synthesis was that devised by Gorenstein and Warner (3). In brief, 5 ml (10^7 cells per ml) of growing or sporulating cells was pulse-labeled for 5 min with [^{3}H]leucine (Amersham, >100 Ci/mmol) at 100 μCi/ml. After the pulse, cells were broken in ice-cold distilled water containing 30 μg of phenylmethylsulfonyl fluoride per ml by blending them in a Vortex mixer with glass beads. The protein from this broken-cell suspension was acid extracted and dialyzed as described previously (3). The ^{3}H-labeled protein was then mixed with a portion of protein extracted from logarithmically growing cells labeled for 6 h with [^{14}C]leucine (New England Nuclear, >270 mCi/mmol) at 10 μCi/ml. A 0.1-ml amount of this mixture was removed to determine the ^{3}H/^{14}C ratio of total protein, and the rest was lyophilized, dissolved in 0.1 ml of sample buffer, and applied to a tube gel. After separating proteins in the second dimension, the gel was stained with 0.2% Coomassie brilliant blue in 50% methanol–7% acetic acid and destained in 30% methanol. Each r-protein spot was then identified and punched out with a cork borer. The acrylamide was dissolved in 30% H_2O_2 in a sealed glass scintillation vial at 65°C overnight. The 0.1 ml removed previously to be used as a total protein standard received the same treatment. Each sample was then cooled, mixed with 10 ml of ACS scintillation fluid (Amersham), and counted in a Beckman scintillation counter. The relative rates of synthesis of r-proteins and other proteins were then expressed as:

$$\frac{^3H/^{14}C \text{ for each spot}}{^3H/^{14}C \text{ for total protein}}$$

This ratio has been denoted Ai by Gorenstein and Warner (3). The numbering of individual ribosomal spots was the same as that used by Gorenstein and Warner (3).

RESULTS

Transition from growth to sporulation. When cells entered sporulation from YEPA, there was a coordinate decline in the relative rates of synthesis of r-proteins (Fig. 1 and Table

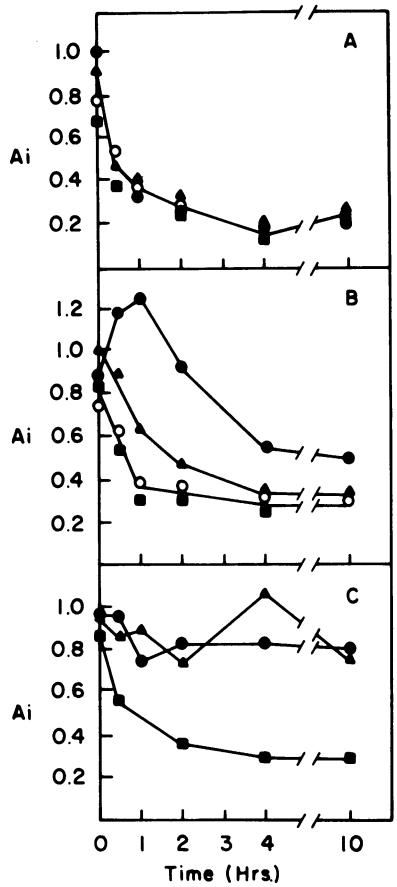

FIG. 1. *Changes in relative rates of synthesis (Ai) of r-proteins after transfer of cells from growth medium to sporulation medium. (A) Ai values from r-proteins 5 (○), 8 (■), 11 (▲), and 12 (●). (B) Ai values for r-proteins 50 (■), 51 (▲), 52 (○), and 59 (●). (C) Ai values for r-proteins 14 (▲), 25 (●), and 39 (■); these three proteins are not inhibited during growth at 34°C in strains carrying rna2 (3).*

1). The decrease occurred rapidly, within the first hour in sporulation medium (T0 to T1). By T0.5 the relative rates of synthesis of these proteins were already depressed 50%, and by T1 most had approached an Ai (see Materials and Methods) of 0.2 to 0.3, which was maintained until at least T10. It should be noted that the Ai values for r-proteins in these experiments were normalized by using ^{14}C-labeled r-proteins from glucose-grown cells and that the relative rates of r-protein synthesis in YEPA were only about 90% of those of the glucose-grown cells. Most non-r-proteins did not show any significant changes in relative rates of synthesis during sporulation, with the exception of two notable proteins which appeared on these gels, designated X and Y, that exhibited very high rates of synthesis only during sporulation. Both the coordinate decrease in r-protein synthesis and the increase in synthesis of proteins X and Y could be seen in fluorographs of two-dimensional gel separations of basic proteins pulse-labeled with [^3H]leucine during growth and at T4 (Fig. 2).

Since yeasts sporulate under nitrogen starvation conditions, it was not surprising that r-protein synthesis was selectively depressed, as this also occurs during amino acid starvation. Certain r-proteins escaped the inhibition (Fig. 1C), including proteins 14 and 25, two proteins that also escape inhibition by *rna2* at the restrictive temperature (3). In contrast, protein 39 was inhibited during sporulation, as it is during amino acid starvation, but not in *rna2* strains at 34°C. r-Protein 59 also consistently showed different behavior during sporulation. Although its synthesis was depressed under sporulating conditions, the kinetics of its inhibition appeared to differ from those of other r-proteins. It maintained a high rate of synthesis for several hours into sporulation before its relative rate declined (see Table 1). Protein 59 is inhibited by *rna2* in growing cells with the same kinetics as those of other r-proteins (3).

An equivalent decline in r-protein synthesis was seen even in asporogenous α/α stains under nitrogen starvation conditions (Fig. 3), which indicated that the change in rates of synthesis of r-proteins was most likely a response to nitrogen starvation and was not necessarily a sporulation-specific event.

Transitions from sporulation back to vegetative growth. Cells which have initiated meiosis can return to a mitotic cycle during early stages of sporulation if transferred to growth medium (2). We wished to know how rapidly the rates of r-protein synthesis would increase to vegetative levels after such a transfer. When nitrogen in the form of YNB was added to cells at T4, there was an immediate increase in the relative rates of synthesis of r-proteins (Fig. 4). The Ai values increased reached maximums before cell division took place. Cells began to grow and divide about 2 h after the addition of nitrogen, as measured by the appearance of buds.

Effect of *rna2* on r-protein synthesis during the transition from sporulation to vegetative growth. Having defined a condition which signals an acceleration in the relative rates of synthesis of r-proteins, we wished to determine whether the temperature-sensitive mutation *rna2* interfered with this nitrogen-induced coordinate increase in r-protein synthesis. Since mutations in this gene (and genes *rna3-rna11*) selectively depress the synthesis of r-proteins during vegetative growth, its gene product may be a positive regulatory element necessary to

TABLE 1. *Changes in relative rates of synthesis (Ai) of r-proteins during transition from exponential growth to sporulation*[a]

Protein	Ai at:						Protein	Ai at:					
	T0	T0.5	T1	T2	T4	T10		T0	T0.5	T1	T2	T4	T10
r-Protein							40	0.68	0.69	0.25	0.50	0.43	0.38
1	1.08	0.59	0.33	0.30	0.24	0.17	41	0.83	0.48	0.33	0.25	0.24	ND
2	0.85	0.62	0.40	0.28	0.20	0.26	45	0.90	0.40	0.36	ND	ND	ND
5	0.79	0.45	0.33	0.24	0.21	0.26	46	0.83	0.27	0.23	ND	ND	0.14
6	0.90	0.54	0.23	0.24	0.17	0.27	47	0.93	0.26	0.19	ND	ND	ND
8	0.66	0.38	0.37	0.25	0.17	0.25	48	0.68	0.36	0.31	0.29	0.23	0.29
9	0.87	0.54	0.34	0.28	0.20	0.27	49	0.89	0.58	0.53	0.30	0.22	0.34
10	1.08	0.53	0.38	0.28	0.20	0.26	50	0.82	0.53	0.29	0.29	0.23	0.29
11	0.90	0.46	0.41	0.34	0.20	0.26	51	1.00	0.88	0.63	0.47	0.34	0.34
12	1.04	0.55	0.38	0.28	0.16	0.26	52	0.75	0.63	0.38	0.38	0.34	0.32
13	0.97	0.59	0.45	0.33	0.23	0.31	53	0.68	0.50	0.31	0.30	0.25	0.42
14[b]	0.95	0.95	0.73	0.82	0.82	0.78	55	0.83	0.69	0.53	0.50	0.49	0.53
15	0.92	0.59	0.31	0.26	0.27	ND[c]	56	0.71	0.62	0.52	0.58	0.47	0.45
18	0.90	0.47	0.33	0.19	0.18	0.28	59[b]	0.86	1.20	1.25	0.93	0.54	0.50
19	0.89	0.54	0.31	0.19	0.20	ND	61	0.87	0.57	0.31	0.39	0.45	0.44
21	0.68	0.48	0.37	0.26	0.16	0.29	62	0.73	0.61	0.34	ND	ND	0.28
22	1.06	0.48	0.28	0.29	0.19	0.25	63	0.99	0.59	0.31	ND	ND	0.26
23	0.80	0.44	0.33	0.20	0.19	0.30	64	0.94	0.47	0.28	ND	ND	0.24
25[b]	0.94	0.84	0.89	0.72	1.05	0.75	65	1.15	0.59	0.46	ND	ND	0.38
27	0.95	0.59	0.48	0.28	0.19	0.29	Non-r-protein						
28	0.62	0.36	0.22	0.19	0.17	ND	A	0.84	0.83	0.69	0.73	0.93	0.63
29	0.86	0.52	0.28	0.30	0.31	ND	C	0.77	1.26	0.94	1.12	1.30	1.14
30	0.94	0.72	0.39	0.30	0.25	0.26	D	0.89	0.84	0.68	0.67	0.96	1.20
31	1.00	0.61	0.34	0.29	0.25	0.29	D	0.97	0.96	0.98	0.73	1.10	0.78
33	0.83	0.44	0.29	0.32	0.33	0.24	X	0.82	1.10	3.04	1.28	7.33	10.11
38	0.82	0.49	0.37	0.30	0.24	0.28	Y	0.87	6.36	9.76	13.30	10.40	16.32
39[b]	0.83	0.55	0.62	0.36	0.29	0.29							

[a] The relative rates of synthesis (Ai) for a large number of r-proteins during logarithmic growth in YEPA (T0) and at various time points during sporulation were determined by 5-min pulse-labeling with [^{3}H]leucine as described in the text. Strain NP2 was used.

[b] r-Protein showing noncoordinate behavior either in the experiment presented or under other cellular conditions (see text).

[c] ND, Not determined.

maintain synthesis of r-proteins. In this case one might expect *rna2* to prevent the nitrogen-induced coordinate increase in r-protein synthesis when temperature is shifted to 34°C. However, the response of r-protein synthesis to the addition of nitrogen may occur by a pathway independent of *rna2* gene action.

To test these possibilities it was first necessary to determine the effect of temperature shift during sporulation. This was especially important because growing cells exhibit a transient and coordinate decrease in the relative rates of synthesis of r-proteins when the temperature is shifted from 23 to 34°C (10). This transient decrease was also found in vegetative cultures of the (*rna2*/+) diploid used here (data not shown). The diploid strains NP1 and NP2 were grown and sporulated at 25°C. At T4 both cultures were shifted to 34°C. The relative rates of synthesis of r-proteins were then determined at different times by pulse-labeling cells with [^{3}H]leucine as described in Materials and Methods. The results for a number of different r-proteins are presented in Fig. 5. There seemed to be no major effect of raising the temperature in either sporulating culture. Even proteins 14 and 25, which maintained high relative rates of synthesis during sporulation, did not show a temperature-induced transient inhibition as they did in vegetative cells. The only noticeable effect of the temperature shift on r-protein synthesis during sporulation was a slight increase in Ai values, of about 0.1, for most r-proteins.

It is interesting that the *rna2* homozygote showed no further reduction in the rate of r-protein synthesis at the restrictive temperature, since the strain is temperature sensitive for sporulation due to this mutation (7). We further investigated the effect of *rna2* on r-protein synthesis during sporulation by comparing the Ai values of r-proteins in strains NP1 and NP2 sporulated at 25 and 34°C from T0 to T4 and then pulsed with [^{3}H]leucine. Again, no significant differences were seen between the two spor-

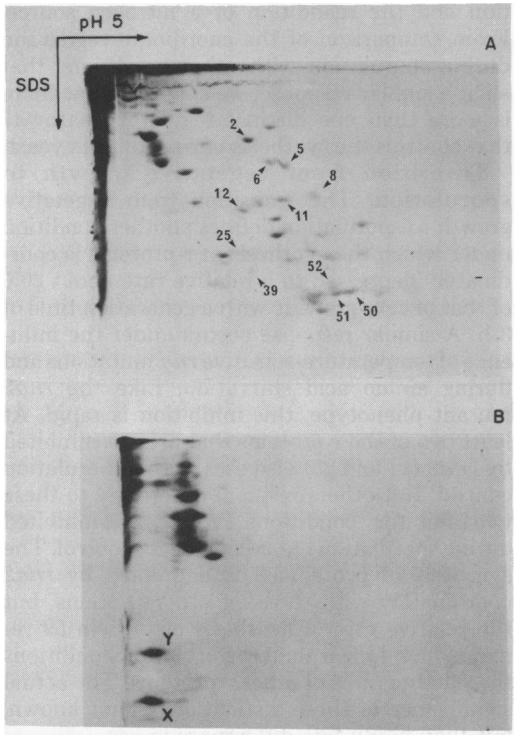

FIG. 2. *Autoradiographs of two-dimensional gel electrophoresis of basic proteins from growing (A) or sporulating (B) cells. Cells were labeled with [^{3}H]-methionine as described in the text, and the proteins were prepared for gel electrophoresis. The r-proteins are readily visible in the center of the gel A. Some r-proteins are designated by numbers assigned by Gorenstein and Warner (3). SDS, Sodium dodecyl sulfate. In gel B, which was exposed for a longer period, the r-proteins are still hardly visible, reflecting a fivefold decrease in their relative synthesis. Two proteins, designated X and Y, are clearly visible among the sporulation proteins, but not among vegetative proteins.*

ulating cultures maintained at either temperature (data not shown). Therefore, whatever the inhibitory effect of *rna2* is on sporulation, it is not associated with in any changes in the relative rates of r-protein synthesis, in contrast to the case during vegetative growth.

Having determined the effect of temperature shift during sporulation on r-protein synthesis in strains NP1 and NP2, we then performed an experiment to find out whether *rna2* interfered with the increase in r-protein synthesis induced by the addition of YNB to sporulating cultures. The diploid strains NP1 and NP2 were induced to sporulate at 25°C. At T4, the cultures were shifted to 34°C, the restrictive temperature for *rna2*, for 1 h. After this period YNB was added. The cells were pulsed with [^{3}H]leucine at various times, and the relative rates of synthesis of r-proteins were determined as described. The temperature was raised before the addition of YNB, to allow sufficient time for any effect of the mutation to occur and to separate the slight increase in Ai values of r-proteins associated with the temperature shift from any change brought about by addition of nitrogen to the culture.

In the *rna2* heterozygote, the addition of YNB to sporulating cells at 34°C stimulated a rapid increase in r-protein synthesis (Fig. 6) similar to that seen before in the same strain at 25°C. In the *rna2* homozygote (Fig. 6) the response was more complex. For the first hour the response was identical to the increase seen in the heterozygote, but after 1.5 h the relative rates of r-protein synthesis began to decline. It seems that the *rna2* mutation did not prevent the increase in Ai during the transitional period from sporulation to growth, but subsequently it inhibited r-protein synthesis when cells were restored to a growth cycle. The exceptional r-proteins behaved as expected in the *rna2* homozygote. Proteins 14 and 25, which were not inhibited by sporulation conditions or *rna2*, remained at high

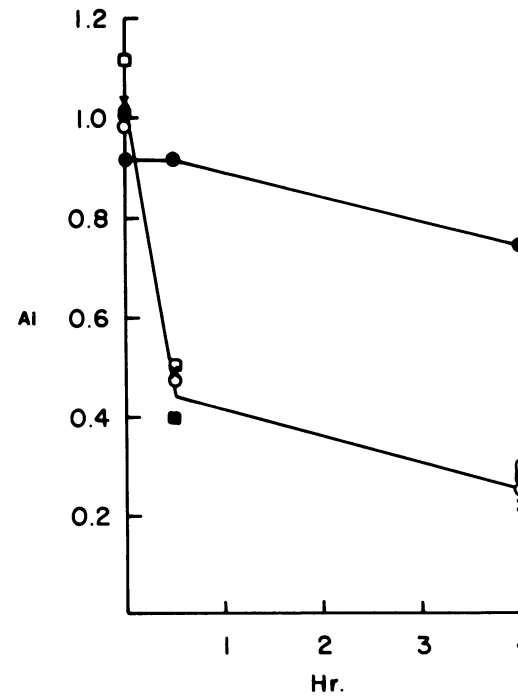

FIG. 3. *Changes in relative rates of synthesis of r-proteins in MATα/MATα cells transferred to sporulation medium. Diploids homozygous for MATα do not enter meiosis or sporulate. r-Proteins 5* (○), *8* (■), *11* (×), *50* (□), *and 25* (●) *are shown.*

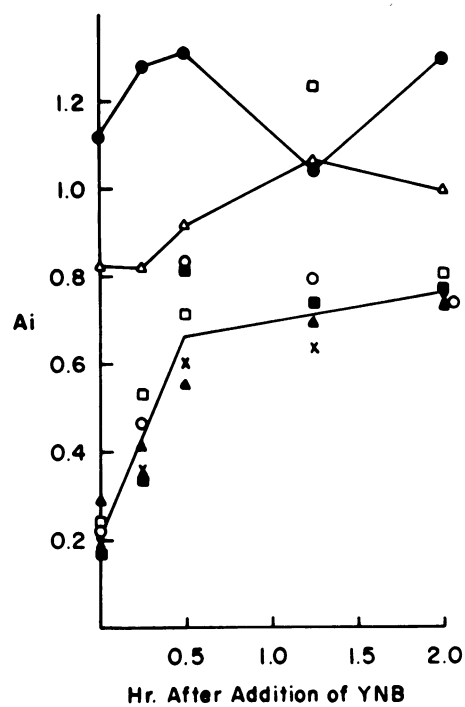

FIG. 4. *Effect on r-protein synthesis of adding YNB to sporulating cells. Cells were incubated in nitrogen-free sporulation medium for 4 h. YNB was then added to the sporulation medium to a final concentration of 1.34% to convert it to a growth medium. The relative rates of r-protein synthesis were then measured at intervals. Ai values for r-proteins 5 (○), 8 (■), 11 (×), 14 (△), 50 (□), 25 (●), and 39 (▲) are shown.*

levels of synthesis throughout. Protein 39 had a low Ai in the sporulating culture, which increased when YNB was added. The Ai for 39 remained at this high value even when the relative rate of synthesis of other r-proteins declined in this strain (NP1). This is consistent with the finding that the synthesis of r-protein 39 was depressed by sporulating conditions but escaped inhibition by *rna2* during vegetative growth. The decline in r-protein synthesis began at about the time cells started to grow (Fig. 6). After one generation, growth was arrested in the *rna2* homozygote, characteristic of vegetative *rna2* cells at the restrictive temperature. The growth of the *rna2* heterozygous strain continued for several generations.

DISCUSSION

The aim of this study was to determine how r-proteins are regulated during transition from vegetative growth to sporulation. The data show that these proteins as a class are coordinately regulated in response to both sporulation induction and the readdition of a nitrogen source. From comparison of the coordinate regulation during sporulation with other conditions that elicit a similar response, we conclude that there is more than one distinct regulatory pathway that controls the synthesis of r-proteins in yeast.

Transition from vegetative growth to sporulation. The transition from vegetative growth to sporulation defines another condition under which the synthesis of r-proteins is coordinately depressed to a relative rate about 20% of that of cells growing with a generation time of 3 h. A similar response occurs under the influence of temperature-sensitive *rna* mutations and during amino acid starvation. Like the *rna2* mutant phenotype, this inhibition is rapid. At least two of the r-proteins that are not inhibited by *rna2* (14 and 25) also escape this sporulation control. But others respond differently to these two inhibitory conditions. Protein 39 is inhibited during sporulation but escapes *rna2* control. The synthesis of protein 59 is depressed by *rna2* coordinately with those of other proteins, but the relative rate of synthesis of protein 59 remains high longer under sporulation conditions than do the rates of other r-proteins. The actual significance of these distinctions is not known, but they imply fine differences in regulation of r-proteins during sporulation as compared with the nonpermissive conditions for the *rna2* mutation during vegetative growth.

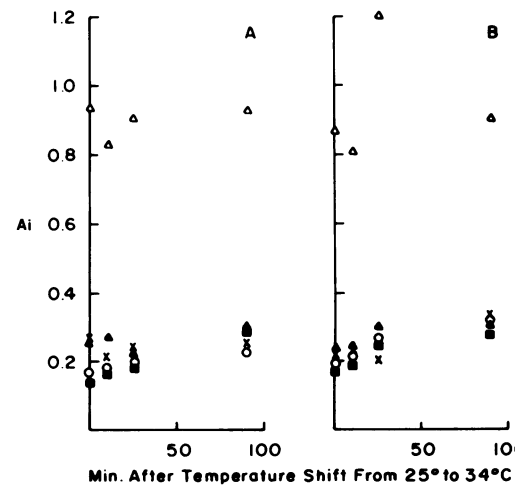

FIG. 5. *Effect of shifting temperature from 25 to 34°C on the synthesis of r-proteins in diploids either heterozygous or homozygous for rna2. Cells of strains NP2 (A) and NP1 (B) were grown to stationary phase in YEPA and transferred to sporulation medium at 25°C. At T4 cells were shifted to 34°C, and the relative rates of more than 40 r-proteins were determined as described in the text. Ai values for r-proteins 5 (○), 8 (■), 11 (×), 14 (△), and 39 (▲) are shown.*

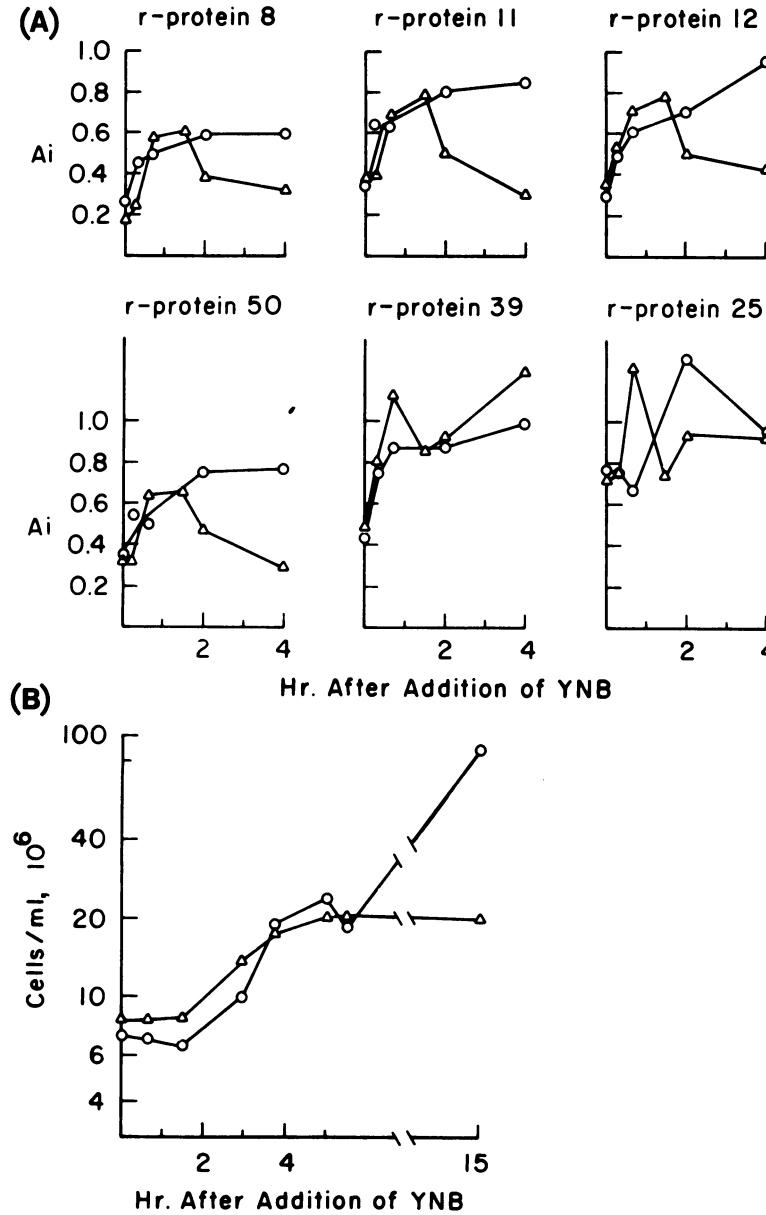

FIG. 6. *(A) Effect of rna2 on the Ai values of r-proteins when YNB has been added to sporulating cells. As described in the text cells of strains NP1 (rna2/rna2) and NP2 (rna2/+) were sporulated for 5 h, the last hour of which was at 34°C. YNB was then added to the cultures to shift the cells back to growth at 34°C. (B) Effect of adding YNB to cells on cell growth (cell number). The decrease in Ai values of r-proteins began about the time that eells begin to divide. The rna2/rna2 cells stopped growing after about one generation, as expected for vegetative cells at the restrictive temperature. Symbols: △, strain NP1; ○, strain NP2.*

The synthesis of r-protein 39 also appears to be depressed with those of other r-proteins during amino acid starvation. It is quite likely that there may be similarities between the regulation of r-proteins during amino acid starvation and their regulation during nitrogen starvation used to induce sporulation. Both conditions reduce total protein synthesis (11; Kraig and Haber, submitted for publication). However, the kinetics of inhibition of r-protein synthesis differ under these two conditions. In transitions from vegetative growth to sporulation, the depression

in rate of synthesis occurs immediately, whereas the response is delayed for 1 to 2 h after amino acid starvation (11), presumably because amino acid pools must first be depleted before a large change in the rate of synthesis of r-proteins occurs. This implies that the regulation of r-protein synthesis that occurs immediately in response to sporulation induction does not require the secondary event that delays the depression of r-protein synthesis in response to amino acid starvation. It should also be noted that other metabolic changes that occur in the cell in response to sporulation, such as the synthesis of many new proteins (9) and the induction of premeiotic DNA synthesis, do not occur at all during amino acid starvation.

It is not clear whether the depression in rate of synthesis of r-proteins is necessary for the events characteristic of the sporulation process to occur or whether this inhibition is simply a consequence of nitrogen starvation conditions. The fact that we found a similar depression in the relative rates of synthesis of r-proteins in α/α nonsporulating diploids under sporulation conditions rules out the possibility that depression of r-protein synthesis alone is sufficient to allow cells to sporulate; however, it still seems likely that a conservation of materials and decrease in synthesis of ribosomes might well be necessary in order for cells to complete sporulation under nitrogen starvation conditions. Recently, Rhaese and his colleagues have discovered that sporulating yeasts accumulate several highly phosphorylated adenine molecules not found in growing cells (8). It is not known if these molecules are important in the coordinate regulation of r-proteins during sporulation, as highly phosphorylated guanosine molecules appear to be in the regulation of *E. coli* r-proteins (5).

It should be pointed out that the significant relative decline in r-protein synthesis occurs in all cells in the population, including the 20 to 30% of the cells that do not complete ascus formation. In other experiments we have shown that the incorporation of radioactive methionine into protein early in sporulation (T4 to T6) occurs equivalently both in cells that eventually complete ascus formation and in those that do not (Kraig and Haber, submitted for publication).

Effect of temperature shift (25 to 34°C) during sporulation. During vegetative growth a shift in temperature from 25 to 34°C causes a transient inhibition of r-protein synthesis, which returns to normal after about 60 min (10). No such transient inhibition can be detected during sporulation. Even r-proteins 14 and 25, which maintain high relative rates of synthesis, show no transient inhibition in response to temperature shift. One can only speculate why this is so. The signal which causes this response may require a high rate of protein synthesis. The initiation of protein synthesis appears to be substantially depressed during sporulation (Kraig and Haber, submitted for publication). Alternatively, the transcription of r-proteins during sporulation may use a different RNA polymerase or other factors which are not sensitive to temperature change.

Transition from sporulation to vegetative growth in mutant (*rna2*) and nonmutant strains. When YNB is added to sporulating cells not yet committed to ascospore formation, a rapid coordinate increase in the relative rate of synthesis of r-proteins occurs. The sequence of events, a rise in synthesis of r-proteins to near vegetative levels followed by cell division a short time afterwards, implies that the cell must accumulate a certain number of ribosomes or reach a certain volume before cytokinesis will occur.

In previous studies we could find no effect of *rna2* on ribosome function during sporulation or on meiotic DNA synthesis and nuclear division (7). This report shows that the rate of synthesis of r-proteins is depressed during sporulation even in wild-type strains. However, in parallel experiments with closely related diploid strains, *rna2* does not appear to depress this level of r-protein synthesis any further even after sporulating cells have been maintained at the restrictive temperature up to 4 to 5 h, despite the fact that these cells are temperature sensitive for sporulation. We suspect that *rna2* must affect other cell functions necessary for both sporulation and vegetative growth.

When YNB is added to sporulating cells homozygous for *rna2* which have been maintained for 1 h at the restrictive temperature, these cells begin to divide, just like wild-type cells. The same rise in the relative rates of synthesis of r-proteins during this transition from sporulation to cell growth is seen. However, after about 1.5 h these rates begin to decline. This is the most direct evidence that the gene product of *rna2* does not function in the regulation of r-proteins during the transition from sporulation to vegetative growth and possibly not during the process of sporulation itself. It may be that synthesis of the *rna2* gene product is also depressed during sporulation and is not present in sufficient concentration to have any effect immediately after the addition of YNB. Alternatively, it may be that the mutant gene product is present but does not interfere with

the coordinate upshift in rate of r-protein synthesis. In either case, we conclude that the *RNA2* gene product is not an essential component in the regulation of r-protein synthesis in transitions from sporulation to vegetative growth.

Behavior of non-r-proteins during these transitions. The two-dimensional gels which resolve most of the r-proteins also display a number of other basic proteins. The relative rates of synthesis of most of these non-r-proteins do not change significantly during transitions from growth to sporulation or vice versa. However, there were at least two basic, non-r-proteins on these gels that had greatly increased relative rates of synthesis during sporulation. One of these, protein Y, is synthesized at least 20 times more in sporulating cells than in cells grown with either glucose or acetate as a carbon source. Upon the addition of YNB to sporulating cells, the rate of synthesis of protein Y decreases rapidly, with about the same kinetics as r-protein increase (Table 1). Protein X, on the other hand, continues to be synthesized at a high relative rate even 2 h after the addition of nitrogen. We are currently studying these proteins to determine whether they may have a function during the sporulation process.

In summary, we found that the r-proteins as a class respond in a coordinate fashion to conditions which induce diploid cells to enter meiosis or to revert to a mitotic growth cycle. The regulation of these proteins during the transition back to a vegetative growth cycle occurs independently of the *RNA2* gene product. This leads us to the conclusion that the coordinate control of r-protein synthesis is mediated by more than one regulatory pathway in *S. cerevisiae*.

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