JOURNAL OF BACTERIOLOGY, Oct. 1978, p. 55-62
0021-9193/78/0136-0055$02.00/0

Vol. 136, No. 1
Printed in U.S.A.

Effect of Mutation in the Aromatic Amino Acid Pathway on Sporulation of *Saccharomyces cerevisiae*

G. LUCCHINI,* A. BIRAGHI, M. L. CARBONE, A. DE SCRILLI, AND G. E. MAGNI
Istituto di Genetica, Università di Milano, Milan, Italy

Received for publication 8 August 1978

Mutations in *ARO1* and *ARO2* genes coding for enzymes involved in the common part of the aromatic amino acid pathway completely block the sporulation of *Saccharomyces cerevisiae* when in a homozygous state, whereas mutations in all the other genes of the same pathway do not. This effect is not due to the lack of any intermediate metabolite but rather to the accumulation of a metabolite preceding chorismic acid. Shikimic acid or one of its precursors was identified as the possible inhibitor. The presence of the three aromatic amino acids in the sporulation medium restores the ability to undergo meiosis. This seems not to be due to a feedback inhibition of the first enzymes of the pathway but rather to a competition between aromatic amino acids and the inhibitor on a site specific for the meiotic process. The inhibition of sporulation seems to occur at a very early step in meiosis, as indicated by the lack of premeiotic DNA synthesis in *aro1* and *aro2* mutants.

Numerous investigations have been undertaken to gain a better understanding of the biochemical and genetic mechanisms underlying the meiotic process in *Saccharomyces cerevisiae*. Since the pioneering work of Miller and coworkers (18, 21), a good deal of information has been accumulated on the major patterns of macromolecular synthesis during sporulation (9, 25). These comprise studies on the effect of specific functions needed for sporulation such as respiration (20, 28), mitochondrial protein synthesis (10, 22), and cytoplasmic protein synthesis (12), as well as on the effect of carbohydrate and nitrogen suppply (3, 17, 24, 26), on the timing of the meiotic process itself (2, 25), and on macromolecular synthesis during sporulation (9, 25).

More recently, a substantial contribution to this problem was provided by the isolation of *spo* (4, 5) and *mei* (23) mutants. Temperature-sensitive mutations blocking meiosis and/or sporulation only at nonpermissive temperature permitted the characterization of some meiotic functions and their timing (5, 6). The logical background for these approaches is that there should exist in the yeast cells some functions essential for meiosis and nonessential for mitosis and vice versa. This seems to be indicated also by differences in the range of permissive temperatures and pH's for the two processes in wild-type strains.

A great variety of mechanisms could be invoked to explain differences in essential functions for mitosis and meiosis in the same type of cells. Among others, the question could be raised whether intermediate metabolites of some biosynthetic pathways, whose final product is the only compound required for vegetative growth, could play a more critical role in starting or carrying out the meiotic process. Some indications in this direction were presented by Wejksnora and Haber (29), who found that methionine-requiring diploids homozygous for *met2* or *met13* genes are unable to sporulate in the absence of methionine. Their experiments have shown that in such strains an imbalance of different species of rRNA occurs during sporulation in the absence of methionine, namely, the lack of 26S and 5.8S rRNA. The authors concluded that a reduced methylation of rRNA or other methionine-dependent event can account for rRNA imbalance and the lack of sporulation, but no explanation was provided for the perfect sporulation observed in the absence of methionine in strains homozygous for other genes in the same pathway (in our hands, many alleles of *met4* and *met5* sporulate normally). It appears therefore more probable that the lack or the accumulation of some intermediate metabolites, rather than the absence of methionine itself, is the first cause of the observed phenomena.

We thought it would be interesting to investigate whether other pathways show the same pattern described for methionine, i.e., that some mutants do not allow sporulation in the absence of the specific end product, while others do. The aromatic amino acid pathway was chosen for our investigations, because previous experiments, carried out for quite different purposes,

indicated a certain variability in sporulation of homozygous strains. The pathway of aromatic amino acids has been widely investigated in *Escherichia coli* (8, 27) and in *Neurospora* (7, 8, 27). In *S. cerevisiae*, all its major interrelationships have been confirmed (8, 27; A. De Leeuw, Ph.D. Thesis, Yale University, New Haven, Conn., 1968); this is shown schematically in Fig. 1. The first step is catalyzed by two isofunctional enzymes, coded by two independent genes, *ARO4* and *ARO3*, which are respectively sensitive to feedback inhibition by tyrosine (3-deoxy-D-arabino-heptulosonic acid 7-phosphate [DAHP] synthetase [tyr]) and by phenylalanine (DAHP synthetase [phe]) (15, 16). The next five steps are catalyzed by five enzymes that form an enzyme aggregate (molecular weight, 250,000) and are encoded by a cluster of five structural genes (*ARO1* region). The last step of the common part of the pathway is catalyzed by chorismate synthetase, encoded by gene *ARO2* (De Leeuw, Ph.D. Thesis).

It will be shown that mutations in genes *ARO1* and *ARO2*, and only in these two genes, block almost completely the ability of homozygous strains to sporulate and that this inhibition can be attributed to the accumulation of an intermediate metabolite of the aromatic pathway.

MATERIALS AND METHODS

Genetic markers and yeast strains. Two wild-type strains were used: the diploid strain 5300 as a standard for sporulation, and the haploid strain 5834/1c for the induction of new mutants in the aromatic pathway. The standard mutations in genes involved in the aromatic pathway have the following origins: *aro1-B*, *aro2-1*, *trp2*, *trp4*, *trp1*, *trp3*, *trp5*, and *tyr7* were derived from strains obtained from R. K. Mortimer; *tyr1-1* came from D. C. Hawthorne; *aro3* and *aro4* were from P. Meuris; and *phe2* came from our collection. New alleles were obtained for some of the above genes by mutagenizing with UV and ethyl methane sulfonate.

Our new *aro1* alleles were identified by a complementation test, and we showed that they belong to at least two complementation groups, one of which contains all but one of the new alleles and the other one the allele *aro1-102*. Further distinction between the alleles was achieved by means of X-ray-induced mitotic mapping according to the procedure developed by Manney and Mortimer (14). From their position on the map relative to the mutant *aro1-1*, which belongs, according to De Leeuw (Ph.D. Thesis), to the gene *ARO1-B*, we concluded that allele *aro1-108* is very probably a mutation in gene *ARO1-B*; alleles *aro1-100*, *aro1-101*, and *aro1-102* are mutations in gene *ARO1-A*; and allele *aro1-104* is probably located between *ARO1-B* and *ARO1-A*, while the alleles *aro1-103* and *aro1-105* could be mutations in another gene.

Diploid strains homozygous for any particular mutation were obtained by crossing to strains of identical phenotypes derived from the original mutation through at least two backcrosses. Their collection numbers are indicated in the Tables.

The double mutant strain *aro1 aro3* needed for constructing the diploid 6089 (see Table 5) was obtained by meiotic segregation from a heterozygous diploid $\frac{ARO1}{aro1}\frac{aro3}{ARO3}$. Each one of the $Phe^- Tyr^- Trp^-$ segregants was crossed to a strain *ARO1 aro3*, and the diploid was tested for feedback inhibition by tyrosine.

Media. YEPD, minimal medium, and VB medium for sporulation were as described in reference 13.

Cross procedure. Mass crosses, isolation of diploids, and dissection of tetrads were done by the standard techniques.

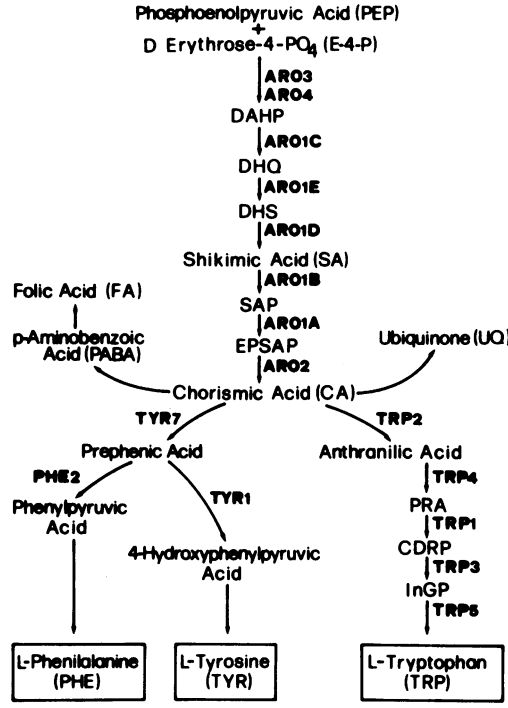

FIG. 1. *Aromatic amino acid pathway. Abbreviations: DAHP, 3-deoxy-D-arabino-heptulosonic acid 7-phosphate; DHQ, 5-dehydroquinic acid; DHS, 5-dehydroshikimic acid; SAP, shikimic acid 5-phosphate; EPSAP, 3-enolpyruvylshikimic acid 5-phosphate; PRA, N-(5'-phosphoribosyl)-anthranilic acid; CDRP, 1-(o-carboxyphenylamino)-1-deoxyribulose 5-phosphate; InGP, indole glycerol phosphate. The genes marked at each step code the following enzymes: ARO3, DAHP synthetase (phe); ARO4, DAHP synthetase (tyr); ARO1C, DHQ synthetase; ARO1E, dehydroquinase; ARO1D, DHS reductase; ARO1B, shikimic acid kinase; ARO1A, EPSAP synthetase; ARO2, chorismic acid synthetase; TYR7, chorismic acid mutase; TYR1, prephenic acid dehydrogenase; PHE2, prephenic acid dehydratase; TRP2, anthranilic acid synthetase; TRP4, PRA transferase; TRP1, PRA isomerase; TRP3, InGP synthetase; TRP5, TRP synthetase.*

Sporulation. Standard conditions of sporulation were as follows: the cells were pregrown in YEPD medium up to the stationary phase (3 to 5 h). The cells were centrifuged and resuspended in VB medium at a concentration of about 2×10^7 cells per ml. After 3 days of incubation on an alternating shaker at 28°C, sporulating cultures were microscopically counted, and the percentage of asci was determined.

DNA synthesis. DNA synthesis was monitored by incorporation of [8-^{14}C]adenine, added at the time of the shift to VB media, into acid-insoluble, alkali-stable material. Samples (2 ml) of sporulating cultures, containing 2×10^7 cells per ml and 0.5 μCi of [8-^{14}C]-adenine per ml, were removed at different times and added to an equal volume of cold 20% trichloroacetic acid. The samples were centrifuged, and the pellet was suspended in 2 ml of 0.5 M NaOH. After overnight incubation at 37°C, DNA was precipitated with cold 10% trichloroacetic acid, collected on glass fiber filters, and washed with 5% trichloroacetic acid and then with a 1:1 (vol/vol) alcohol-ether mixture.

RESULTS AND DISCUSSION

Sporulation of strains homozygous for mutants in the aromatic amino acid pathway. After some preliminary experiments that seemed to indicate a drastic effect of some mutants in the aromatic pathway on sporulation, a detailed investigation was carried out for all the genes. A series of alleles obtained in our laboratory in many genes of the pathway were made homozygous, and sporulation was performed according to the standard procedure without addition of the required amino acids to the sporulation medium. All the genes blocking the aromatic pathway after chorismic acid had no effect on sporulation even in the absence of the required amino acids (Table 1). On the other hand, a mutation in genes *ARO1* and *ARO2*, which are involved in the metabolic steps prior to chorismic acid, seemed to affect drastically the sporulation capacity. The phenomenon appears quite clearly to be gene dependent and not allele dependent.

Certainly the lack of sporulation observed in strains carrying *aro1* and *aro2* mutations is not due to their phenotype for amino acid dependence (Table 2). The same biochemical requirements caused by double mutations after chorismic acid (strains 6093 and D1017) do not significantly affect sporulation.

To prove that the blockage of sporulation is caused by mutations in genes *ARO1* and *ARO2* and not to concomitant factors introduced by chance in our strains, spontaneous revertants of mutants *aro1-104* and *aro2-102* were obtained; and the heterozygous diploids exhibited a significant resumption of sporulation capacity (Table 3). Moreover, a temperature-sensitive revertant (*aro2-102* ts) was obtained, showing in both haploid and heterozygous diploid conditions a normal growth in absence of phenylalanine, tyrosine, and tryptophan at the permissive temperature (23°C). This revertant was fully auxotrophic at the nonpermissive temperature (33°C); sporulation was blocked in the heterozygous diploid at 33°C and was normal at 23°C. We can therefore conclude that the lack of products of the genes *ARO1* and *ARO2* causes a block of sporulation.

TABLE 1. *Effect of different alleles or genes of the aromatic pathway on sporulation*

Gene	Allele	Phenotype	Percent sporulation
trp5	*101*	Trp$^-$	33
trp5	*102*	Trp$^-$	15
trp3	*1*	Trp$^-$	35
trp1	*1*	Trp$^-$	55
trp4	*1*	Trp$^-$	52
trp2	*1*	Trp$^-$	27
phe2	*1*	Phe$^-$	50
phe2	*102*	Phe$^-$	60
tyr1	*1*	Tyr$^-$	17
tyr1	*104*	Tyr$^-$	30
tyr1	*105*	Tyr$^-$	29
tyr1	*106*	Tyr$^-$	24
tyr1	*107*	Tyr$^-$	31
tyr7	*1*	Phe$^-$ Tyr$^-$	51
tyr7	*100*	Phe$^-$ Tyr$^-$	21
tyr7	*101*	Phe$^-$ Tyr$^-$	17
aro2	*1*	Phe$^-$ Tyr$^-$ Trp$^-$	0
aro2	*100*	Phe$^-$ Tyr$^-$ Trp$^-$	0.5
aro2	*102*	Phe$^-$ Tyr$^-$ Trp$^-$	0
aro2	*103*	Phe$^-$ Tyr$^-$ Trp$^-$	0
aro2	*104*	Phe$^-$ Tyr$^-$ Trp$^-$	0.8
aro2	*105*	Phe$^-$ Tyr$^-$ Trp$^-$	1.3
aro2	*108*	Phe$^-$ Tyr$^-$ Trp$^-$	2.0
aro1	*1*	Phe$^-$ Tyr$^-$ Trp$^-$	0
aro1	*108*	Phe$^-$ Tyr$^-$ Trp$^-$	0
aro1	*103*	Phe$^-$ Tyr$^-$ Trp$^-$	1.2
aro1	*105*	Phe$^-$ Tyr$^-$ Trp$^-$	0.8
aro1	*104*	Phe$^-$ Tyr$^-$ Trp$^-$	0
aro1	*100*	Phe$^-$ Tyr$^-$ Trp$^-$	1.7
aro1	*102*	Phe$^-$ Tyr$^-$ Trp$^-$	0
aro1	*101*	Phe$^-$ Tyr$^-$ Trp$^-$	0

TABLE 2. *Sporulation of strains deficient for phenylalanine, tyrosine, and tryptophan*

Strain no.	Genotype	Phenotype	Percent sporulation
5300	Wild type	Prototroph	50
6093	*tyr7-1 trp2-1* / *tyr7-1 trp2-1*	Phe$^-$ Tyr$^-$ Trp$^-$	16
D1017	*tyr7-1 trp1-1* / *tyr7-1 trp1-1*	Phe$^-$ Tyr$^-$ Trp$^-$	60
5911	*aro1-104* / *aro1-104*	Phe$^-$ Tyr$^-$ Trp$^-$	0
5964	*aro2-102* / *aro2-102*	Phe$^-$ Tyr$^-$ Trp$^-$	0

TABLE 3. *Effect of complete and temperature-sensitive reversion of mutants aro1 and aro2 on sporulation*

Strain no.	Genotype	Temp[a] (°C)	Percent sporulation
5911	*aro1-104/aro1-104*	23	0
5937/D6	*aro1-104/ARO1*[b]	23	32
5964	*aro2-102/aro2-102*	23	0
5964/D2	*aro2-102/ARO2*[c]	23	18
5945/D1	*aro2-102/aro2-102* ts	23	31
		33	0.2
5300	Wild type	23	54
		33	48

[a] Temperatures of 23 and 33°C are permissive and nonpermissive, respectively, for growth of temperature-sensitive revertant *aro2-102* ts.

[b] Allele *ARO1* is a reversion to wild type of allele *aro1-104*.

[c] Allele *ARO2* is a reversion to wild type of allele *aro2-102*.

Lack versus accumulation of intermediate metabolite(s) in the aromatic pathway. The observed block of sporulation determined by mutants *aro1* and *aro2* can in principle be caused by a lack in the cells of an intermediate metabolite of the aromatic amino acid pathway located between DAHP and chorismic acid or of a secondary metabolite derived from it. The alternative possibility, on the other hand, is that the accumulation of a metabolite of the common part of the aromatic pathway is interfering with some step of meiosis or sporulation.

The first step in the aromatic amino acid pathway is carried out by the activity of two isoenzymes, DAHP synthetases, encoded, according to Meuris (15, 16), by genes *ARO3* and *ARO4* (see Fig. 1). The block of this step can only be achieved by simultaneous mutations in both genes. The sporulation of strains made homozygous for mutations in the above genes is reported in Table 4. Good sporulation of strains 6085 and 6086 was expected, as the aromatic pathway is not particularly altered in these strains, because the inactivity of either one of the two DAHP synthetases can be replaced by the other isoenzyme. Sporulation within the limits of normality in the two double homozygous strains, 6081 and 6083, answers our question. A block in the first biochemical step should cause a lack of intermediate metabolites and of metabolites derived from chorismic acid as well as mutations in *ARO1* and *ARO2* genes. The clear-cut difference in sporulation capacity between mutants *aro1* and *aro2* on the one hand and the double mutant *aro3 aro4* on the other indicates that the hypothesis of an accumulation of some metabolite as a cause of the sporulation blockage in our mutants should be preferred. As mentioned above, the *aro1* mutants we have tested can be located in *ARO1-B* and *ARO1-A* and, with some doubt, in other genes of the *ARO1* cluster. It looks, therefore, as though the metabolite(s) whose accumulation is responsible for the lack of sporulation should be identified between DAHP and 3-enolpyruvylshikimic acid 5-phosphate, e.g., possibly shikimic acid and/or shikimic 5-phosphate and/or 3-enolpyruvylshikimic acid 5-phosphate.

Restoration of sporulation in *aro1* and *aro2* strains by aromatic amino acids. To better understand the physiology of sporulation blockage in mutants *aro1* and *aro2*, some trials of resumption of sporulation capacity were made by addition of the aromatic amino acids to the sporulation medium. The presence of the aromatic amino acids in the sporulation medium (Fig. 2) restored to a certain degree the sporulation capacity of strain *aro1*, with a good dose-effect relation up to 10 μg of each amino acid per ml. The effect was, in addition, under the influence of preculture conditions: the higher the concentration of the aromatic amino acids in the vegetative medium, the better the sporulation at any given amino acid dosage when added to the sporulation medium. Comparable results were obtained with the *aro2* mutant.

The addition of a single amino acid or of any combination two by two, except phenylalanine + tyrosine, was completely ineffective (Fig. 3). In principle, two mechanisms can be involved in the restoration of sporulating capacity determined by the aromatic amino acids, as follows. (i) The amount of intermediate metabolite accumulated in strains *aro1* and *aro2* during vegetative growth is not sufficient to block sporulation: the block is achieved by the cumulative action of the intermediate metabolite previously accumulated plus the one accumulated during

TABLE 4. *Effect of mutations in the first gene of the aromatic pathway on sporulation*

Strain no.	Genotype	Phenotype	Percent sporulation
5300	Wild type	Prototroph	55
6085	$\frac{aro3\ ARO4}{aro3\ ARO4}$	Prototroph	46
6086	$\frac{ARO3\ aro4}{ARO3\ aro4}$	Prototroph	28
6081	$\frac{aro3\ aro4}{aro3\ aro4}$	$Phe^-\ Tyr^-\ Trp^-$	31
6083	$\frac{aro3\ aro4}{aro3\ aro4}$	$Phe^-\ Tyr^-\ Trp^-$	20
5911	$\frac{aro1}{aro1}$	$Phe^-\ Tyr^-\ Trp^-$	0

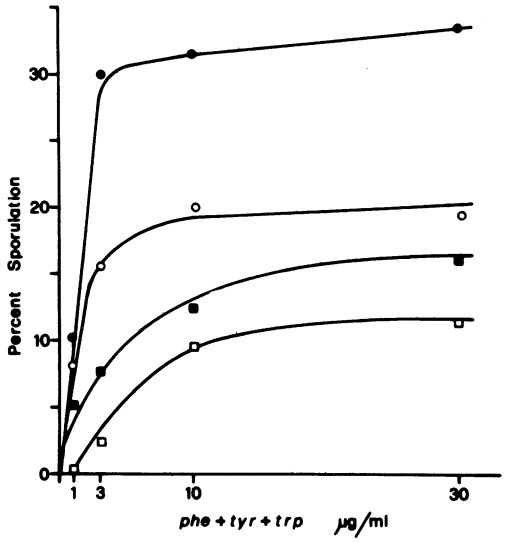

FIG. 2. *Sporulation of strain 5911 (aro1-104/aro1-104) in the presence of the aromatic amino acids. Vegetative growth was permitted to stationary phase (5 h) in YEPD* (○); *YEPD + aromatic amino acids (75 µg of each per ml)* (●); *minimal medium + aromatic amino acids (100 µg of each per ml)* (□); *minimal medium + aromatic amino acids (300 µg of each per ml)* (■).

the sporulation process. A feedback inhibition of DAHP synthetases caused by the presence of phenylalanine plus tyrosine in sporulation medium does not allow the intermediate metabolite to reach the minimal level needed for the blocking of sporulation. In other words, the effect of phenylalanine plus tyrosine during sporulation is based on a feedback inhibition mechanism that could in some cases be magnified by the concomitant presence of tryptophan. (ii) The amount of intermediate metabolite accumulated during vegetative growth is sufficient to block sporulation: the effect of phenylalanine plus tyrosine (+tryptophan) can be in this case accounted for by a mechanism of competition between the two (or three) amino acids and the intermediate metabolite on a site whose activity is specifically required for sporulation.

A strain with the following genotype was prepared (see Material and Methods): 6089 $\frac{aro1}{aro1}$ $\frac{aro3}{aro3}$ $\frac{ARO4}{ARO4}$. Although it cannot be demonstrated directly, this strain must be sensitive to feedback by tyrosine and one should therefore expect that, if the feedback hypothesis is correct, sporulation should be restored in strain 6089 by tyrosine alone at the same level observed in *aro1* strains by phenylalanine plus tyrosine. The feedback of the product of the gene *ARO4*, when *aro3* is present, did not restore sporulation, whereas the strain sporulated in presence of phenylalanine + tyrosine + tryptophan (Table 5). This fact seems to contradict the feedback hypothesis.

Effect of shikimic acid on sporulation. It has been mentioned above that any intermediate metabolite of the aromatic pathway preceding chorismic acid could potentially be the inhibitor of sporulation in *aro1* and *aro2* strains. The only two compounds that can be tested are quinic acid and shikimic acid, because the other intermediates are not available. Quinic acid turned out, in our hands, to be completely ineffective at any dose used (from 100 to 2,000 µg/ml) under any experimental condition. As far as shikimic acid is concerned, our results have been quite variable. In some instances, which represent the minority of dozens of experiments, shikimic acid added to the sporulation medium at high dosage was able to partially inhibit sporulation in wild-type strains (Fig. 4A) and in *aro1* strains with sporulation ability restored by phenylalanine + tyrosine + tryptophan (Fig. 4B). This effect turned out, however, to be strain and culture condition dependent, as some other wild-type strains showed very little decrease of sporulation, if any, in presence of shikimic acid. At the present status of our knowledge, it is difficult to say whether the high concentration of shikimic

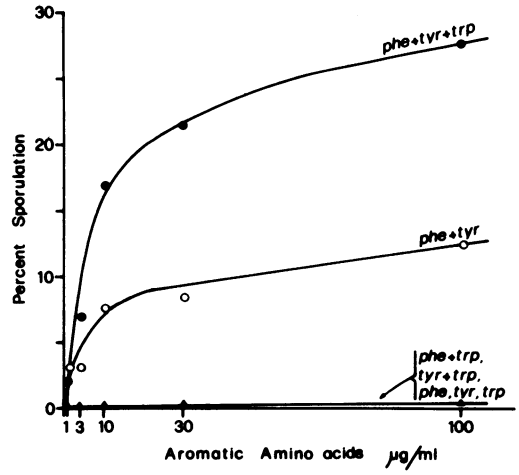

FIG. 3. *Sporulation of strains 5911 (aro1-104/aro1-104) in the presence of different combinations of aromatic amino acids. The cells were pregrown to stationary phase in YEPD. Addition of aromatic amino acids to sporulation medium:* (●) *phenylalanine + tyrosine + tryptophan;* (○) *phenylalanine + tyrosine;* (▲) *tyrosine + tryptophan, or phenylalanine + tryptophan, or phenylalanine, or tyrosine, or tryptophan.*

TABLE 5. *Sporulation under feedback conditions for the aromatic pathway*

Strain no.	Genotype	Supplement to sporulation medium (30 μg/ml)	Percent sporulation	
			Expected[a]	Observed
6089	*aro1 aro3 ARO4* / *aro1 aro3 ARO4*	None	–	0
		Tyr	++	0
		Tyr + Trp	+++	0
		Tyr + Trp + Phe	+++	21
5911	*aro1 ARO3 ARO4* / *aro1 ARO3 ARO4*	None		0
		Tyr		0
		Tyr + Phe		16
		Tyr + Phe + Trp		30

[a] Expectation on the basis of the feedback hypothesis.

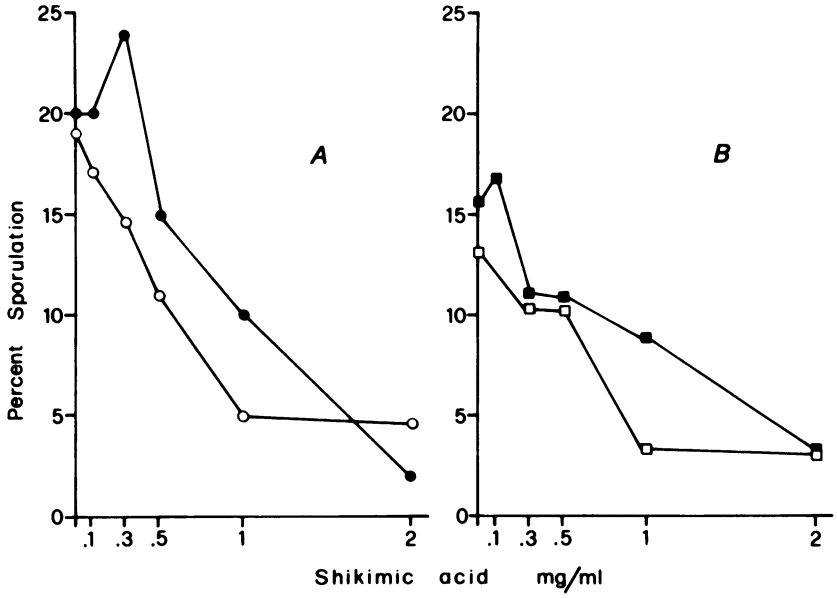

FIG. 4. *Effect of shikimic acid on sporulation. (A)* (●) *Sporulation medium VB, strain 5300 (wild type);* (○) *sporulation medium VB, strain 5937/D6 (aro1-104/ARO1). (B) Strain 5911 (aro1-104/aro1-104);* (□) *sporulation medium VB + 10 μg of phenylalanine + tyrosine + tryptophan per ml;* (■) *sporulation medium VB + 20 μg of the same amino acids per ml. All strains were grown to stationary phase in minimal medium supplemented when needed.*

acid needed and the variability of its action upon sporulation are due either to a poor uptake by the cells or to the fact that it is not the real inhibitor. Possibly it must be transformed into another metabolite.

Effect on premeiotic DNA synthesis of *aro1* mutation. An important effect to be elucidated about the action of *aro1* and *aro2* genes is whether they block only sporulation (i.e., the formation of visible spores) or they act on the meiotic process.

The events that are usually investigated in the time course of sporulation are premeiotic DNA synthesis, the appearance of two nuclei, the end of the meiotic process (four nuclei), and the condensation of cytoplasm to form the four spores. The first parameter to be investigated was premeiotic DNA synthesis. Our results (Fig. 5) indicate quite clearly that the lack of sporulation in *aro1* strains is due to the blockage of premeiotic DNA synthesis when the strains are transferred into sporulation medium without aromatic amino acids. The addition of amino acids to the sporulation medium restored DNA synthesis in a fraction of cells and consequently allowed the same cells to undergo the entire meiotic process and sporulation. These results do not indicate that the proposed inhibitor accumulated in *aro1* mutants acts directly on DNA synthesis. During the first hours immediately

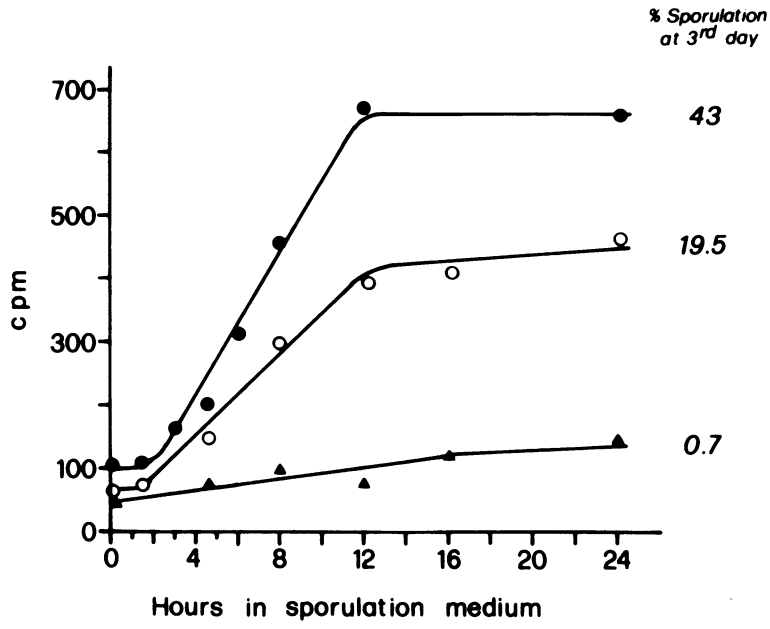

FIG. 5. *Premeiotic DNA synthesis.* (●) *Strain 5300 (wild type) in VB;* (○) *strain 5911 (aro1-104/aro1-104) in VB + aromatic amino acids (100 μg of each per ml);* (▲) *strain 5911 in VB without amino acids.*

preceding premeiotic DNA synthesis, a series of many biochemical events occurs in the cells. Each one is indispensable for the initiation of DNA synthesis. The evidence nearest to our investigation is the fact that sulfanilamide, an inhibitor of tetrahydrofolate dehydrogenase, blocks sporulation, causing a partial premeiotic DNA synthesis inhibition (1).

LITERATURE CITED

1. **Colonna, J., J. M. Gentile, and P. T. Mayer.** 1977. Inhibition by sulfanilamide of sporulation in Saccharomyces cerevisiae. Can. J. Microbiol. **23**:659–671.
2. **Croes, A. F.** 1967. Induction of meiosis in yeast. I. Timing of cytological and biochemical events. Planta **76**: 209–226.
3. **Croes, A. F.** 1967. Induction of meiosis in yeast. II. Metabolic factors leading to meiosis. Planta **76**:227–237.
4. **Esposito, M. S., and R. E. Esposito.** 1969. The genetic control of sporulation in *Saccharomyces*. I. The isolation of temperature sensitive sporulation deficient mutants. Genetics **61**:79–89.
5. **Esposito, M. S., and R. E. Esposito.** 1974. Genes controlling meiosis and spore formation in yeast. Genetics **78**:215–225.
6. **Esposito, M. S., R. E. Esposito, M. Arnaud, and H. O. Halvorson.** 1970. Conditional mutants of meiosis in yeast. J. Bacteriol. **104**:202–210.
7. **Gaertner, F. H., and K. W. Cole.** 1977. A cluster gene: evidence for one gene, one polypeptide, five enzymes. Biochem. Biophys. Res. Commun. **75**:259–264.
8. **Gibson, F., and J. Pittard.** 1968. Pathways of biosynthesis of aromatic amino acids and vitamins and their control in microorganisms. Bacteriol. Rev. **32**:465–492.
9. **Hopper, A. K., P. T. Magee, S. K. Welck, M. Friedman, and B. D. Hall.** 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:619–628.
10. **Küenzi, M. T., M. A. Tingle, and H. O. Halvorson.** 1974. Sporulation of *Saccharomyces cerevisiae* in the absence of a functional mitochondrial genome. J. Bacteriol. **117**:80–88.
11. **Lawrence, J., G. B. Cox, and F. Gibson.** 1974. Biosynthesis of ubiquinone in *Escherichia coli* K-12: biochemical and genetic characterization of a mutant unable to convert chorismate into 4-hydroxybenzoate. J. Bacteriol. **118**:41–45.
12. **Magee, P. T., and A. K. Hopper.** 1974. Protein synthesis in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:952–960.
13. **Magni, G. E., and R. G. Von Borstel.** 1962. Different rates of spontaneous mutation during mitosis and meiosis in yeast. Genetics **47**:1097–1108.
14. **Manney, T. R., and R. K. Mortimer.** 1964. Allelic mapping in yeast by X-ray-induced mitotic reversion. Science **143**:581–583.
15. **Meuris, P.** 1967. Mise en evidence et séparation génétique de deux DOPH aldolases sommises á la rétroinhibition chez *Saccharomyces cerevisiae*. C. R. Acad. Sci. Paris **264**:1197–1199.
16. **Meuris, P.** 1973. Feedback-inhibition of the DAHP synthetase by tRNA in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **121**:207–218.
17. **Miller, J. J.** 1963. The metabolism of yeast sporulation: stimulation and inhibition of sporulation and growth by nitrogen compounds. Can. J. Microbiol. **9**:260–277.
18. **Miller, J. J., J. Calvin, and J. M. Tremaine.** 1955. A study of certain factors influencing sporulation of *Saccharomyces cerevisiae*. Can. J. Microbiol. **1**:560–573.
19. **Miller, J. J., and O. Hoffman-Ostenhof.** 1964. Spore formation and germination in *Saccharomyces*. Z. Allg. Mikrobiol. **4**:273–294.
20. **Miller, J. J., O. Hoffmann-Osternhof, E. Scheiber, and O. Gabriel.** 1959. The metabolism of yeast sporulation. III. Respiration of sporulating and growing cells. Can. J. Microbiol. **5**:153–159.

21. **Pontefront, R. D., and J. J. Miller.** 1962. The metabolism of yeast sporulation. IV. Cytological and physiological changes in sporulating cells. Can. J. Microbiol. **8**:573–574.
22. **Puglisi, P. P., and E. Zennaro.** 1971. Erythromycin inhibition of sporulation in *Saccharomyces cerevisiae*. Experientia **27**:963–967.
23. **Roth, R.** 1973. Chromosome replication during meiosis: identification of gene functions required for premeiotic DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **70**: 3087–3091.
24. **Roth, R., and H. O. Halvorson.** 1969. Sporulation of yeast harvested during logarithmic growth. J. Bacteriol. **98**:831–832.
25. **Tingle, M., A. J. Singhklar, S. A. Henry, and H. O. Halvorson.** 1973. Microbial identification: Ascospore formation in yeast. Symp. Soc. Gen. Microbiol. **23**: 209–243.
26. **Tsuboi, M., K. Seiichiro, and N. Yanagishima.** 1972. Effect of cyclic 3′-5′-adenosinemonophosphate on the sporulation in *Saccharomyces cerevisiae*. Plant Cell Physiol. **13**:585–588.
27. **Umbarger, M. E.** 1969. Regulation of amino acid metabolism. Annu. Rev. Biochem. **38**:323–370.
28. **Vezinhet, F., M. Roger, M. Pellequer, and P. Galzy.** 1974. Genetic control of some metabolic modifications during the sporulation of *Saccharomyces cerevisiae* Hansen. J. Gen. Microbiol. **81**:373–382.
29. **Wejksnora, P. J., and J. E. Haber.** 1974. Methionine-dependent synthesis of ribosomal ribonucleic acid during sporulation and vegetative growth of *Saccharomyces cerevisiae*. J. Bacteriol. **120**:1344–1355.