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# Carbohydrate Metabolism During Ascospore Development in Yeast

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Carbohydrate metabolism, under sporulation conditions, was compared in sporulating and non-sporulating diploids of *Saccharomyces cerevisiae*. Total carbohydrate was fractionated into trehalose, glycogen, mannan, and an alkali-insoluble fraction composed of glucan and insoluble glycogen. The behavior of three fractions was essentially the same in both sporulating and non-sporulating strains; trehalose, mannan, and the insoluble fraction were all synthesized to about the same extent regardless of a strain's ability to undergo meiosis or sporulation. In contrast, aspects of soluble glycogen metabolism depended on sporulation. Although glycogen synthesis took place in both sporulating and non-sporulating strains, only sporulating strains exhibited a period of glycogen degradation, which coincided with the final maturation of ascospores. We also determined the carbohydrate composition of spores isolated from mature asci. Spores contained all components present in vegetative cells, but in different proportions. In cells, the most abundant carbohydrate was mannan, followed by glycogen, then trehalose, and finally the alkali-insoluble fraction; in spores, trehalose was most abundant, followed by the alkali-insoluble fraction, glycogen, and mannan in that order.

In *Saccharomyces cerevisiae*, sporulation is induced by transferring growing cells to a simple nitrogen-free, acetate-containing medium (3, 15). Under these conditions, the vegetative cells cease dividing and initiate meiosis, which terminates with the conversion of cells into mature asci, each containing four haploid ascospores (4, 19). Meiosis and sporulation are accompanied by an extensive accumulation of intracellular carbohydrate which can account for over two-thirds of the total increase in mass during development (14). The carbohydrates synthesized include trehalose and glycogen (6, 11, 12, 14), which are considered reserve or storage materials, and mannan and glucan, the major carbohydrate components of yeast cell walls (6, 9, 11).

This report had two main objectives. First, we wished to provide a single, unified account of the metabolic behavior of the major yeast carbohydrates during meiosis and sporulation. Secondly, we attempted to distinguish those biochemical events which required meiosis or spore formation from those which did not. To accomplish this, we compared carbohydrate metabolism in sporulating and non-sporulating strains (7).

## MATERIALS AND METHODS

**Strains and culture conditions.** The yeast strains used and their properties are given in Table 1. Strains 11-5B, 11-5D, and 11-35C were provided by H. Roman (5). The procedures for growth, sporulation, cell enumeration, and determination of sporulation percentages have been described (14). At intervals during incubation in sporulation medium (1% potassium acetate), culture samples were rapidly collected by centrifugation; supernatant fractions were saved to determine extracellular carbohydrate. The cell pellets were suspended in 10 mM hydrochloric acid and stored at 5 C prior to analysis.

**Carbohydrate determinations.** All carbohydrate determinations were performed by the anthrone procedure (8) modified so that color development was performed at 100 C for 10 min. Absorbance was determined with a Klett colorimeter by using a 620-nm filter. Carbohydrate concentrations (expressed as glucose equivalents) were determined by reference to glucose standards run in each experiment. All values represent the average of duplicate determinations.

**Analysis for total and extracellular carbohydrate.** For analysis of total cellular carbohydrate, unfractionated cell suspensions were diluted with water and assayed directly with the anthrone reagent. Extracellular carbohydrate was determined directly

TABLE 1. *Properties of diploid yeast strains*

| Strain | Genotype for mating type | Characteristics | Doubling time (min) |
|---|---|---|---|
| SK-1 | $a/\alpha$ | Homothallic; rapid and synchronous sporulation | 150 |
| 11-5B | $a/\alpha$ | Heterothallic; normal sporulation | 190 |
| 11-35C | $\alpha/\alpha$ | Heterothallic; non-sporulating | 170 |
| 11-5D | $a/a$ | Heterothallic; non-sporulating | 160 |

in the culture medium saved after harvesting the cells.

**Carbohydrate fractionation.** Carbohydrates were fractionated into trehalose, glycogen, mannan, and an alkali-insoluble fraction (composed of glucan and insoluble glycogen) by slightly modifying the methods of Berke and Rothstein (2) and Trevelyan and Harrison (20, 21). The stored cells were collected by centrifugation and the supernatant fractions, which contained trehalose, were saved. The cells were suspended in 1 M KOH and treated for 60 min at 100C. Alkali-soluble and -insoluble fractions were separated by centrifugation; the alkali-insoluble material (glucan and insoluble glycogen) was washed, dissolved directly in the acidic anthrone reagent, and analyzed for carbohydrate. The alakli-soluble fraction, which contained trehalose, glycogen, and mannan, was fractionated by the addition of 2 volumes of ethanol. After overnight storage at 5 C, the alcohol-insoluble precipitates (glycogen and mannan) were collected, washed twice with 67% ethanol, and dissolved in water. The glycogen and mannan content of this fraction was evaluated by determining total carbohydrate and then independently determining mannan after its precipitation with Fehling copper reagent (1). Glycogen was taken as total carbohydrate minus the value for mannan. This procedure was used because, as pointed out by Berke and Rothstein (2), addition of Fehling reagent to the combined glycogen-plus-mannan fraction made subsequent separation and carbohydrate determinations of the glycogen difficult to quantitate. To determine mannan content, Fehling reagent (1:1, vol/vol) was added to the combined mannan-plus-glycogen fraction. The resulting mannan precipitate was collected, washed with dilute alkali, and dissolved by the addition of 1 or 2 drops of 6 M hydrochloric acid followed by a small volume of water. Carbohydrate was then determined in the usual way.

The ethanol-soluble fractions, containing trehalose, were pooled with the trehalose-containing supernatant fractions obtained earlier. The combined fractions were neutralized, dried, dissolved in water, and analyzed with anthrone.

To verify the composition of the polysaccharide fractions, samples from both sporulating strains were hydrolyzed, neutralized (20), and assayed for total carbohydrate by the anthrone method, and for glucose with the Glucostat reagent (Worthington Biochemical Corp., Freehold, N.J.). The samples analyzed were obtained from cells just prior to sporulation (12 h for strain 11-5B; 6 h for strain SK-1), a time when all fractions were at a high level. After hydrolysis, the alkali-insoluble fractions, which should only contain glucose residues, yielded between 100 and 111% of the total carbohydrate as glucose. Glycogen was separated from the combined glycogen-plus-mannan fraction by first precipitating mannan with Fehling reagent and then treating the remaining supernatant with 2 volumes of ethanol. After hydrolysis, 91 to 109% of the carbohydrate in this fraction was recovered as glucose. The mannan fractions contained less than 4% of their total carbohydrate as glucose. Chromatography of the hydrolyzed mannan (20) revealed a single spot with the same $R_f$ as purified mannose.

**Isolation and purification of ascospores.** Fully sporulated cultures of 11-5B and SK-1 were collected by centrifugation and washed once with distilled water. Spores were enzymatically released from the asci by treatment with 10% Glusulase (Endo Laboratories, Garden City, N.Y.) as described previously (14). The spores were purified further as described by Rousseau and Halvorson (18).

After purification, spore suspensions were enumerated with a hemacytometer. Samples containing a known number of spores were collected by centrifugation, washed with distilled water, and kept frozen until used for carbohydrate analysis.

## RESULTS

**Sporulation.** In yeast, sporulation is controlled by the mating-type locus; strains heterozygous for the $a$ and $\alpha$ alleles of this locus can sporulate, whereas strains homozygous for either the $a$ or $\alpha$ allele fail to do so (Table 1 and reference 13). The asporogenous nature of homozygous strains results from an early block preventing the initiation of meiosis (16). Nonsporulating diploids were used to determine whether any observed biochemical changes were restricted solely to sporulating strains. The sporulation kinetics of the four strains used (Table 1) are presented in Fig. 1. In strain 11-5B, a typical diploid (15), asci first appeared just prior to 14 h, increased rapidly over the next 16 h, and reached a level of 64% by 30 h. In strain SK-1, a rapid and synchronously sporulating diploid, the percentage of asci rose from 0 to 78% between 7 and 10 h, and reached 94% by 16 h. Strains 11-5D and 11-35C, homozygous for $a$ and $\alpha$, respectively, failed to sporulate.

**Total cellular and extracellular carbohydrate.** Carbohydrate synthesis took place in both sporulating and non-sporulating strains

(Fig. 2). In strain 11-5B, total cellular carbohydrate rose from 0.3 to about 2.0 mg/$10^8$ cells between 0 and 12 h. During spore formation (16 to 30 h), carbohydrate decreased from 2.0 to 1.8 mg/$10^8$ cells. In strain SK-1, carbohydrate accumulation took place more rapidly; between 0 and 6 h, carbohydrate increased from 0.4 to 2.2 mg/$10^8$ cells. Between 6 and 12 h, when 90% of the asci appeared, carbohydrate decreased to 1.7 mg/$10^8$ cells. After 12 h the carbohydrate content again rose, reaching a level of 2.3 mg/$10^8$ cells by 24 h. This second rise was due almost entirely to trehalose synthesis. In both non-sporulating strains, carbohydrate (Fig. 2B) increased from 0.4 to about 2.0 mg/$10^8$ cells between 0 and 36 h. In sporulating and non-sporulating strains, small amounts of carbohydrate appeared in the sporulation medium (Fig. 2).

These initial results showed that bulk carbohydrate synthesis was not restricted to sporulating strains. To examine the individual carbohydrates, we fractionated total carbohydrate into trehalose, soluble glycogen, mannan, and an insoluble fraction consisting of glucan and insoluble glycogen (2, 20, 21).

**Soluble glycogen and alkali-insoluble carbohydrate.** In strain 11-5B (Fig. 3A), soluble glycogen increased ninefold from 0.08 to 0.7 mg/$10^8$ cells between 0 and 16 h; between 16 and 24 h, i.e., during sporulation, glycogen dropped from 0.7 to 0.3 mg/$10^8$ cells. Carbohydrate in the alkali-insoluble fraction rose from 0.03 to 0.45 mg/$10^8$ cells between 0 and 12 h, and then declined to 0.3 mg/$10^8$ cells by 30 h. In strain SK-1 (Fig. 3B), glycogen accumulated about twice as fast as in strain 11-5B; from 0 to 6 h it rose 14-fold—0.07 to 0.95 mg/$10^8$ cells. Between 6 and 10 h, soluble glycogen decreased from 0.9 to 0.3 mg/$10^8$ cells. As in strain 11-5B, the period of glycogen disappearance coincided with the appearance of mature ascospores and with the loss of total carbohydrate shown in Fig. 2. In strain SK-1, insoluble carbohydrate rose slowly from 0.04 to 0.1 mg/$10^8$ cells between 0 and 6 h. From 6 to 10 h, insoluble carbohydrate rose more rapidly from 0.1 to 0.35 mg/$10^8$ cells and remained constant thereafter. Thus, the period of spore maturation in strain SK-1 was characterized by a large decrease in soluble glycogen accompanied by a smaller increase in the insoluble fraction. Part of the loss in soluble glycogen could result from its conversion to an insoluble form; nevertheless, the decline in glycogen represents a net loss since the decrease in soluble glycogen exceeded the rise in the insoluble fraction by 0.4 mg/$10^8$ cells.

In both strains 11-5B and SK-1, the glycogen lost during sporulation cannot be accounted for by conversion to insolube material, mannan, or trehalose (Fig. 4 and 5). Glycogen is either converted to material not reacting with the anthrone reagent or is lost.

In both non-sporulating strains (Fig. 3C), soluble glycogen increased continuously between 0 and 24 h, rising from approximately 0.1

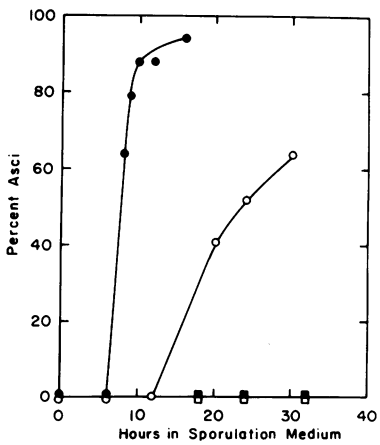


FIG. 1. *Sporulation of diploid yeast strains. At zero hours, logarithmically growing cells were harvested, washed, and resuspended in 1% potassium acetate sporulation medium. At intervals, samples were withdrawn for the determination of the percentage of asci by phase-contrast microscopy. Symbols:* ○, *strain 11-5B;* ●, *SK-1;* □, *11-35C;* ■, *11-5D.*

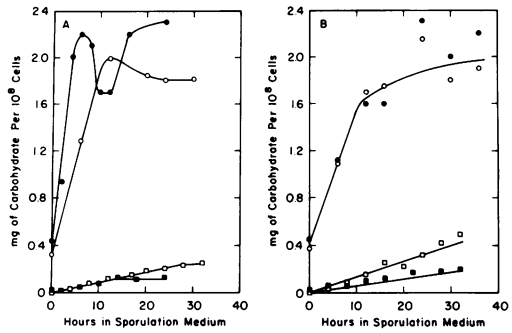


FIG. 2. *Changes in cellular and extracellular carbohydrate during incubation of yeast in sporulation medium. At intervals, samples of cells were harvested from sporulation medium by centrifugation (14). Supernatant fractions were saved to determine extracellular carbohydrate, whereas the cell pellets were used to evaluate total cellular carbohydrate. All carbohydrate values were corrected so as to correspond to $10^8$ cells. Symbols: (A)* ○ *and* □, *strain 11-5B cellular and extracellular carbohydrate, respectively;* ● *and* ■, *SK-1 cellular and extracellular carbohydrate respectively; (B)* ○ *and* □, *strain 11-35C cellular and extracellular carbohydrate, respectively;* ● *and* ■, *strain 11-5D cellular and extracellular carbohydrate, respectively.*

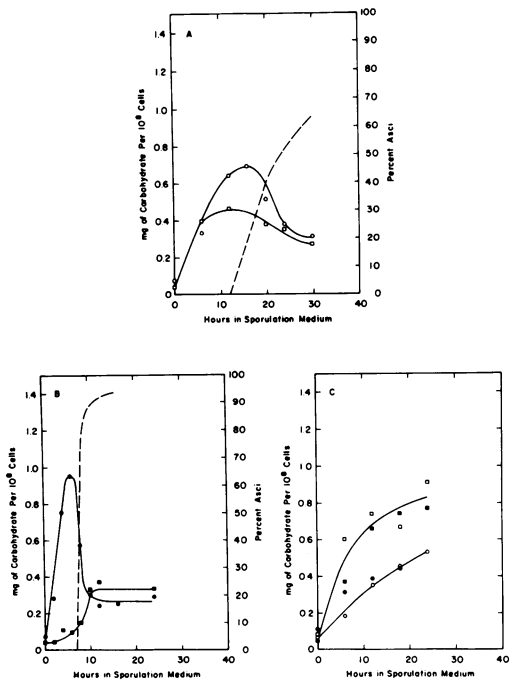


FIG. 3. *Changes in soluble glycogen and alkali-insoluble carbohydrate during incubation of yeast in sporulation-medium. At intervals, cell samples were harvested from sporulation medium as indicated in Fig. 2. Subsequently, the total cellular carbohydrate was fractionated as described in Materials and Methods; the changes in the glycogen and alkali-insoluble fractions are shown here. Symbols: (A: strain 11-5B) O, soluble glycogen; □, alkali-insoluble carbohydrate. The broken line represents the sporulation kinetics as determined in figure 1. Symbols: (B) strain SK-1: ●, soluble glycogen; ■, alkali-insoluble carbohydrate. The broken line shows sporulation. Symbols: (C) O and □, strain 11-35C soluble glycogen and alkali-insoluble carbohydrate, respectively; ● and ■, 11-5D soluble glycogen and alkali-insoluble carbohydrate, respectively.*

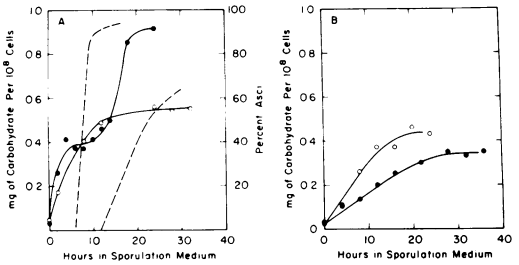


FIG. 4. *Trehalose synthesis during incubation of yeast in sporulation medium. Cells were harvested and the carbohydrates were fractionated as described in Fig. 2 and 3. The changes in the trehalose fraction are shown here. Symbols: (A) O, strain 11-5B; ●, strain SK-1. The dashed lines depict the sporulation kinetics for the two strains as determined in Fig. 1. Symbols: (B) O, strain 11-35C; ●, strain 11-5D.*

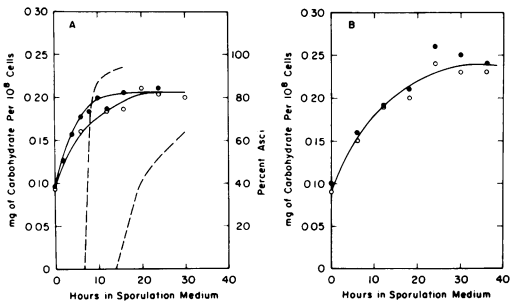


FIG. 5. *Mannan synthesis during incubation of yeast in sporulation medium. Cells were harvested and the carbohydrates were fractionated as described in Fig. 2 and 3. The changes in the mannan fraction are shown here. Symbols: (A) O, strain 11-5B; ●, strain SK-1. The dashed lines represent the sporulation kinetics of the two strains, as determined in figure 1. Symbols: (B) O, strain 11-35C; ●, strain 11-5D.*

mg to about 0.5 mg/$10^8$ cells. During the same interval, insoluble carbohydrate rose from 0.07 to 0.9 mg/$10^8$ cells in strain 11-35C and from 0.04 to 0.8 mg/$10^8$ cells in strain 11-5D.

Thus, both sporulating and non-sporulating strains synthesized considerable quantities of glycogen during incubation in sporulation medium, but glycogen degradation was restricted to sporulating strains and occurred coincidently with ascus maturation.

**Trehalose.** Trehalose accumulated in all four strains, increasing at least 10-fold over the levels present in vegetative cells (Fig. 4). In strain 11-5B (Fig. 4A), trehalose rose rapidly between 0 and 12 h, increasing from 0.04 to 0.5 mg/$10^8$ cells; after 12 h, there was only a slight additional increase. In strain SK-1 (Fig. 4A), trehalose increased from 0.03 to 0.4 mg/$10^8$ cells between 0 and 6 h, remained constant between 6 and 10 h (when asci were maturing), and then rose again between 10 and 24 h, reaching 0.9 mg/$10^8$ cells. Trehalose was also synthesized by both non-sporulating diploids (Fig. 4B); in strain 11-35C, trehalose rose from 0.02 to 0.46 mg/$10^8$ cells between 0 and 20 h; in strain 11-5D, it increased from 0.03 to 0.35 mg/$10^8$ cells between 0 and 28 h.

**Mannan.** Since trehalose and glycogen are storage carbohydrates, their synthesis might be expected in any strain incubated in nitrogen-free medium (10, 21). In contrast, extensive formation of structural carbohydrates, such as mannan and glucan, might be expected only in strains undergoing spore wall construction (2). Thus, we anticipated that mannan or glucan

synthesis would be a useful biochemical marker dependent on ascospore development. Of the two wall components, mannan was chosen for detailed analysis since it has a unique composition and could be easily isolated in relatively pure form. To our surprise, mannan synthesis occurred in both sporulating and non-sporulating strains (Fig. 5). In addition, mannan accumulation began immediately after introduction of cells into sporulation medium and was largely completed prior to the first appearance of mature spores. In strain 11-5B (Fig. 5A), mannan rose from 0.1 to 0.2 mg/$10^8$ cells between 0 and 20 h and remained constant thereafter; at least 75% of the rise preceded the initial appearance of mature asci. In strain SK-1 (Fig. 5A), mannan also increased from 0.1 to 0.2 mg/$10^8$ cells, but this occurred between 0 and 10 h; again, about 75% of the mannan was made before any mature spores were detectable. In both non-sporulating strains (Fig. 5B), mannan increased from 0.1 to 0.24 mg/$10^8$ cells between 0 and 24 h; the kinetics of accumulation were similar to the sporulating strains, except that synthesis continued for a longer period.

**Carbohydrate analysis of purified ascospores.** The extensive accumulation of mannan prior to sporulation in both strains 11-5B and SK-1 prompted us to question whether mannan was actually found in mature spores. Since a detailed carbohydrate analysis of purified spores was not available, it was conceivable that spores were very deficient, or even free of mannan; this might explain our failure to detect extensive mannan synthesis during spore maturation. Spores isolated from mature asci of strains 11-5B and SK-1 were fractionated into the components described previously; for comparison we analyzed vegetative cells (Table 2). Spores contained all carbohydrates present in vegetative cells, but displayed major differences in the relative proportions of each fraction. In vegatative cells, the most abundant carbohydrate was mannan, followed by soluble glycogen, then trehalose, and finally the alkali-insoluble fraction. In spores, trehalose was most abundant, followed by the alkali-insoluble fraction, the soluble glycogen, and finally mannan. Assuming each ascus contained four spores reveals that total spore-associated mannan in strain SK-1 was 0.048 mg/$10^8$ asci (0.012 mg $\times$ 4), whereas in strain 11-5B it was 0.12 mg/$10^8$ asci. Thus, our failure to detect extensive mannan synthesis coincident with ascus maturation does not reflect its absence from mature spores, but indicated that much of the spore-associated mannan is made prior to the visible appearance of mature asci.

Analysis of purified spores also showed that much of the trehalose made during development (Fig. 4A) became localized in the mature spores. Assuming four spores per ascus revealed that spore associated trehalose in strain 11-5B was 0.52 mg/$10^8$ asci, whereas in strain SK-1 the value was 0.48 mg/$10^8$ asci.

## DISCUSSION

Carbohydrate formation, which accounts for at least $^2/_3$ of the total increase in mass during sporulation, results in mature asci containing five times more anthrone-positive material than the vegetative cells which initiate development (Fig. 2 and reference 14). Previously, it was shown that the newly synthesized carbohydrates consisted of both trehalose and polysaccharides. For this report we analyzed the polysaccharide fraction and re-examined trehalose in order to provide a single, unified account of carbohydrate metabolism during development.

Total carbohydrate was fractionated into four components: trehalose, glycogen, mannan, and an alkali-insoluble fraction (composed of glucan and insoluble glycogen). The behavior of three fractions was essentially the same in both sporulating and non-sporulating strains: trehalose, mannan, and the insoluble fraction were all synthesized to about the same extent regardless of a strain's ability to undergo meiosis or sporulation. In contrast, aspects of soluble glycogen metabolism depended on sporulation. Although glycogen synthesis took place in both sporulating and non-sporulating strains, only

TABLE 2. *Carbohydrate content of ascospores and vegetative cells*[a]

| Strain | Sample | Trehalose | Alkali-insoluble carbohydrate | Soluble glycogen | Mannan | Sum of fractions |
|---|---|---|---|---|---|---|
| SK-1 | Vegetative cells | 0.037 | 0.045 | 0.080 | 0.100 | 0.262 |
| | Spores | 0.120 | 0.110 | 0.014 | 0.012 | 0.256 |
| 11-5B | Vegetative cells | 0.038 | 0.020 | 0.080 | 0.090 | 0.228 |
| | Spores | 0.130 | 0.080 | 0.060 | 0.030 | 0.300 |

[a] All values are expressed as milligrams of carbohydrate per $10^8$ cells (glucose equivalents).

sporulating strains exhibited a period of glycogen degradation. By comparing the kinetics of glycogen disappearance in strains with different rates of development, we showed that glycogen utilization coincided with the final maturation of the ascospores. Since glycogen loss was not accompanied by an equivalent formation of any other carbohydrate(s), we conclude that the glycogen was either catabolized or converted to non-carbohydrate material.

Carbohydrate formation in yeast, under conditions similar to those which exist during sporulation, has been examined by a number of authors (1, 2, 6, 10, 14, 17, 21). However, since these previous studies did not include asporogenous controls, it was not clear whether carbohydrate synthesis required concomitant sporulation or could occur independent of development. Since strains which failed to initiate meiosis accumulated carbohydrates as well as the sporulating strains, it is apparent that carbohydrate formation does not require meiotic development. Carbohydrate synthesis, in sporulation medium, is probably a response to the nitrogen-free environment (15) rather than a sporulation-specific event.

The carbohydrates made during development may serve some function(s) in ascospore construction and spore germination. As previously reported (14), we found that much of the trehalose made during development was localized in the mature spores (Table 2 and Fig. 4). This trehalose is consumed during spore germination (18), probably serving as a source of energy or carbon, or both. We anticipated that some of the newly synthesized polysaccharide(s) found in mature asci, like trehalose, would be localized within the spores (as opposed to being found external to the spore but within the ascospore mother cell). Evidence for polysaccharide accumulation in spores was obtained by isolating intact spores and comparing their carbohydrate content (Table 2) with that of whole asci (Fig. 3 and 5). By assuming that each ascus contained four spores, we revealed that (i) essentially all of the insoluble carbohydrate present in mature asci was found in the spores, and (ii) that spore-associated insoluble carbohydrate exceeded, by 10-fold, the insoluble carbohydrate present in vegatative cells. Thus, newly made insoluble carbohydrates become localized in spores, suggesting a role in ascospore construction, perhaps in formation of the ascospore cell wall. The role of mannan made during development is not clear. By assuming that each ascus contained four spores, we showed that total spore-associated mannan did not significantly exceed mannan present in the original vegatative cell. Although it seems highly likely that the mannan present in the spores was derived from newly-made material, we cannot rule out the possibility that a significant fraction of spore mannan is derived from sources pre-existing in the vegatative cell.

Pontefract and Miller (12), by using cytochemical techniques, originally demonstrated that glycogen was synthesized early in development and was degraded during spore maturation; this observation was confirmed chemically (6). Our contribution has been to show that, whereas glycogen synthesis is not dependent on sporulation, glycogen degradation is. In addition, we demonstrated that the temporal coincidence between glycogen utilization and spore maturation is not fortuitous, but occurs even in strains with dramatically different rates of development (Fig. 3). Glycogen utilization may provide a useful and easily quantified biochemical event specific for sporulating yeast.

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## LITERATURE CITED

1. Aminoff, D., W. W. Binkley, R. Schaffer, and R. W. Mowry. 1970. Analytical methods for carbohydrates, p. 745–748. *In* W. Pigman, D. Horton, and A. Herp (ed.). The carbohydrates: chemistry and biochemistry, vol. 2B, 2nd ed. Academic Press Inc., New York.
2. Berke, H. L., and A. Rothstein. 1957. The metabolism of storage carbohydrates in yeast studied with glucose-1-$C^{14}$ and dinitrophenol. Arch. Biochem. Biophys. **72**:380–395.
3. Croes, A. F. 1967. Induction of meiosis in yeast. I. Timing of cytological and biochemical events. Planta **76**:209–226.
4. Fowell, R. R. 1969. Sporulation and hybridization of yeasts, p. 303–377. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, vol. 1. Academic Press Inc., New York.
5. Friis, J., and H Roman. 1968. The effects of the mating type alleles on intragenic recombination in yeast. Genetics **59**:33–36.
6. Galzy, P., F. Vezinhet, A. Arnaud, and A. Chassang-Douillet. 1972. Metabolic modifications during sporulation in *Saccharomyces cerevisiae* Hansen, p. 863–866. *In* Fermentation technology today. Proc. 4th Int. Fermentation Symp. Japanese Publications Trading Co., Tokyo.
7. Henry, S. A., and H. O. Halvorson. 1973. Lipid synthesis during sporulation of *Saccharomyces cerevisiae*. J. Bacteriol. **114**:1158–1163.
8. Mokrasch, L. C. 1954. Analysis of hexose phosphate and sugar mixtures with the anthrone reagent. J. Biol. Chem. **208**:55–59.
9. Northcote, D. H., and R. W. Horne. 1952. The chemical composition and structure of the yeast cell wall. Biochem. J. **51**:232–236.

10. Panek, A. 1962. Synthesis of trehalose by bakers' yeast (*Saccharomyces cerevisiae*). Arch. Biochem. Biophys. **98**:349-355.
11. Pazonyi, B., and L. Markus. 1955. The fractionation and measurement of carbohydrates in relation to the life cycle of yeast. Agrokem. Talajtan (In Hungarian). **4**:225-235.
12. Pontefract, R. D., and J. J. Miller. 1962. The metabolism of yeast sporulation. IV. Cytological and physiological changes in sporulating cells. Can. J. Microbiol. **8**:573-584.
13. Roman, H., and S. M. Sands. 1953. Heterogeneity of clones of *Saccharomyces* derived from haploid ascospores. Proc. Nat. Acad. Sci. U.S.A. **39**:171-179.
14. Roth, R. 1970. Carbohydrate accumulation during the sporulation of yeast. J. Bacteriol. **101**:53-57.
15. Roth, R., and H. O. Halvorson. 1969. Sporulation of yeast harvested during logarithmic growth. J. Bacteriol. **98**:831-832.
16. Roth, R., and K. Lusnak. 1970. DNA synthesis during yeast sporulation: genetic control of an early developmental event. Science **168**:493-494.
17. Rothman, L. B., and E. Cabib. 1969. Regulation of glycogen synthesis in the intact yeast cell. Biochemistry **8**:3332-3341.
18. Rousseau, P., and H. O. Halvorson. 1969. Preparation and storage of single spores of *Saccharomyces cerevisiae*. J. Bacteriol. **100**:1426-1427.
19. Tingle, M., A. J. Singh Klar, S. A. Henry, and H. O. Halvorson. 1973. Ascospore formation in yeast, p. 209-243. *In* Microbial differentiation. Symp. Soc. Gen. Microbiol., no. 23. Cambridge University Press, Cambridge, England.
20. Trevelyan, W. E., and J. S. Harrison. 1952. Studies on yeast metabolism. 1. Fractionation and microdetermination of cell carbohydrates. Biochem. J. **50**:298-303.
21. Trevelyan, W. E., and J. S. Harrison. 1956. Studies on yeast metabolism. 7. Yeast carbohydrate fractions. Separation from nucleic acid, analysis, and behavior during anerobic fermentation. Biochem. J. **63**:23-33.
22. Vezinhet, F., A. Arnaud, and P. Galzy. 1971. Etude de la respiration de la levure au cours de la sporulation. Can. J. Microbiol. **17**:1179-1184.