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# Developmental Control of Glucosamine and Galactosamine Levels During Conidiation in *Neurospora crassa*

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The glucosamine and galactosamine content of mycelia was measured in cultures of *Neurospora crassa* grown on the surface of dialysis membranes. The glucosamine content was relatively constant throughout the different regions of the mycelial mat. The galactosamine content, however, was always lower in the growing-front region of the mycelial mat than in the older regions. At most, only low levels of galactosamine were necessary for the formation of hyphae at the growing front of a mycelial mat. Thus, galactosamine-containing polymers cannot be a major shape-determining component of the cell walls of these hyphae in *Neurospora*. The effect of conidiation on the amino sugar content was determined by using the *bd* (band) strain of *N. crassa*. When grown on the surface of dialysis membranes, this strain rhythmically produced regions of conidiating and non-conidiating growth. With this strain, it was concluded that conidiation did not affect the amino sugar levels. Since conidia that contained only very low levels of galactosamine were produced from regions of the mycelial mat that contained much higher levels of this amino sugar, there must be some mechanism of spatial differentiation that prevented the accumulation of galactosamine-containing polymers in conidia.

Most of the glucosamine and galactosamine in *Neurospora crassa* hyphae is located in the cell wall as high-molecular-weight components (7, 8, 12). The glucosamine is predominantly in chitin microfibrils (6, 8), and much of the galactosamine is in high-molecular-weight homopolymers of partially acetylated galactosamine (9). Galactosamine polymers are also excreted into the medium under some growth conditions (9). The location of these polymers in the cell wall and their possible structural roles are reviewed briefly in the accompanying paper (12).

The amino sugar content of mycelia from various regions of a culture grown on the surface of dialysis membranes was measured to answer the following four questions. (i) Would the amino sugar content of mycelia from cultures grown on the surface of dialysis membranes differ from that obtained for mycelia from cultures grown in liquid media? (ii) Would the amino sugar content of mycelia from the growing front of the mycelial mat differ from that of older regions of the same mycelial mat? (iii) Would the amino sugar content of any particular region of the mycelial mat change as that particular region aged? (iv) Since conidia contained very little galactosamine (5, 12), would regions of the mycelial mat that are producing conidia also be devoid of this amino sugar?

## MATERIALS AND METHODS

**Neurospora strains.** A wild-type strain, RL3-8A (FGSC 2218), and a mutant strain, *bd* (band, FGSC 1858 [11]), were used in these studies. Both of these strains can be obtained from the Fungal Genetics Stock Center, Humboldt State College, Arcata, Calif.

**Growth on dialysis membrane and sample preparation.** The wild-type stain was grown on dialysis tubing (7.6-cm flat width, Will Scientific) in petri dishes (15 by 150 mm) as described previously (1), except that the media contained 2% glucose instead of maltose as the carbon source and did not contain arginine. A small inoculum was placed in the center of each plate. The plates were incubated at 22 C with constant light.

Mycelia were removed with a polyproplyene spatula, from various regions of the mycelial mats from sets of plates that had been growing for 24, 40, and 48 h. In this case, the samples were dried, weighed, ground to fine powder, and hydrolyzed. The total amino sugar content was determined. The wild-type strain did not conidiate under these growth conditions until the mycelia reached the edge of the petri dish.

The *bd* mutant strain was grown at 22 C on the top of dialysis membrane (1) that was overlaid on Vogel minimal medium (13) containing 2% agar, 0.5% maltose, and 100 μg of arginine per ml (10). For large-scale growth, Pyrex baking dishes (8 by 12 inches [ca. 20.3 by 30.5 cm]) were used instead of petri dishes. Each baking dish was filled with about 300 ml of medium. Cotton was taped to the sides of the baking dishes to provide a sterile seal with the plate glass covers. The dishes were inoculated with 0.3 ml of

a dense conidial suspension (*bd* strain) in a trough that had been cut into the center of the agar surface before the dialysis tubing was applied. The inoculated dishes were exposed to fluorescent light for 12 h to synchronize the formation of conidiating bands (1). The dishes were then incubated in constant darkness. The appropriate sections of the mycelial mat were removed from the dialysis tubing by scraping with a flat-ended polypropylene spatula. As soon as the samples were removed from the dishes, they were plunged into boiling 80% ethanol. The samples were then treated as described for liquid shake cultures (12). The corresponding conidiating (band) and non-conidiating (interband) regions of three baking dish cultures were combined for each time point, and the amino sugar content of the alcohol-insoluble and alcohol-soluble fractions was determined.

**Hydrolysis and amino sugar determinations.** The amino sugar content of the total mycelia, the ethanol-insoluble cell wall-containing fractions, and the alcohol-soluble fraction containing the nucleotide sugars uridine diphospho-2-acetamido-2-deoxy-D-glucose (UDP-GlcNAc) and uridine diphospho-2-acetamido-2-deoxy-D-galactose (UDP-GalNAc) were measured after acid hydrolysis on a Beckman 120C amino acid analyzer as described previously (12).

## RESULTS

**Amino sugar content of surface-grown cultures of wild-type N. crassa as a function of time and position.** The wild-type strain RL-38A was grown on dialysis membranes as described above. Samples of mycelia were removed from sets of plates as a function of time and position (Table 1). A single sample containing all of the mycelia was removed from the first set of plates after 24 h of growth (6-cm diameter disk). The second set of plates was harvested after 40 h of growth. Two samples were taken: the first corresponding to the area of the mycelial mat at 24 h (6-cm disk) and the second corresponding to new growth that had occurred between 24 and 40 h (6- to 10-cm ring). The last set of plates was harvested after 48 h of growth. Three samples were removed from each plate; the first corresponded to the area of the mycelial mat at 24 h, the second corresponded to the ring of mycelia produced between 24 and 40 h, and the third corresponded to the new growing-front region. The amino sugar concentrations given in Table 1 are the total amount of amino sugar obtained by hydrolysis of the entire sample (no alcohol fractionation).

TABLE 1. *Amino sugar concentration of mycelia from wild-type Neurospora grown on the surface of dialysis membrane*

| Period of growth (h) | Amino sugar concn (μmol/g total dry wt) | | | | | |
|---|---|---|---|---|---|---|
| | 6-cm disk[a] | | 6- to 10-cm ring | | 10- to 12-cm ring | |
| | GlcN[b] | GalN[b] | GlcN | GalN | GlcN | GalN |
| 24 | 64 | 3.4 | | | | |
| 40 | 79 | 18 | 73 | 9.9 | | |
| 48 | 67 | 16 | 71 | 16 | 56 | 8.6 |

[a] The mycelial mat was about 6 cm in diameter after 24 h of growth, 10 cm after 40 h, and 12 cm after 48 h.

[b] GlcN, Glucosamine; GalN, galactosamine.

The glucosamine concentrations were similar in samples taken from different regions of the mycelial mat, and there were no apparent differences in the glucosamine content that could be correlated with the period of growth (Table 1). The average concentration of glucosamine was 68 μmol/g of total dry weight. This was equivalent to a concentration of 85 μmol/g of residual dry weight (residual dry weight = 80% total dry weight) and was within the range of levels obtained for mycelia from liquid-shake cultures (12).

In contrast to the glucosamine concentration, which was relatively constant throughout the culture, the galactosamine concentration was always lowest in mycelia from the growing-front region of the mycelial mat (Table 1). At 48 h, the sample from the growing front contained 8.6 μmol of galactosamine per g (dry weight), while in the center of the culture the galactosamine concentration was 16 μmol/g. The levels of galactosamine also increased within a particular area of the mycelial mat as the culture aged (Table 1). Since the dry weight of this central region increased during this time, it is not known whether the increased levels of galactosamine were due to the accumulation of galactosamine in the old hyphae or in the new hyphae that had been formed within the last 24 h.

**Effect of conidiation on the levels of the amino sugars.** Since conidia contained very little galactosamine (5, 12), it was of interest to determine whether there were any differences in the galactosamine content of mycelia from conidiating and non-conidiating regions of a culture. That is, were regions of a mycelial mat that were producing conidia also devoid of this amino sugar?

The *bd* strain of *Neurospora* was used because conidiating (band) and non-conidiating (interband) regions could be harvested separately. When grown in the dark on solid media, the *bd* strain produced a band of aerial hyphae and conidia for 11 h followed by 11 h of non-conidiating growth (1, 11; Fig. 1). Thus, the effect of conidiation on amino sugar concentration could be determined.

The results of the analysis of the amino sugar content of mycelia from band and interband

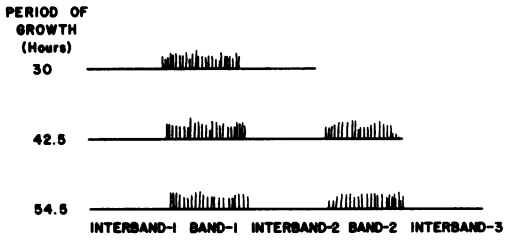


FIG. 1. *Representation of the rhythmic conidiation pattern of a culture of the bd strain growing on the surface of dialysis membrane. The conidiation pattern is the same as in Table 2.*

regions of a surface-grown culture of *bd* strain are given in Table 2. After 30 h, the growing front had completed the second interband and was about to start forming the second band (Fig. 1 and Table 2). At this time, both of these regions were harvested separately from a set of cultures. The first interband was not harvested because it contained the conidial inoculum. At 42.5 h the second conidiating region, band 2, had just been completed, and the second set of cultures was harvested. At this time samples that corresponded to band 1, interband 2, and band 2 were taken for analysis. After 54.5 h, the growth on the remaining three baking dish cultures had nearly reached the edge of the dialysis membrane. The growing front of the mycelial mat had just completed forming the third interband. Three samples that corresponded to interband 2, band 2, and interband 3 were removed.

The amino sugar content of both the alcohol-insoluble and alcohol-soluble fractions was measured (Table 2). The alcohol-insoluble fraction contained most of the high-molecular-weight polymers in the cell, including cell wall and protein. The amino sugars that were released by hydrolysis of this fraction came almost entirely from cell wall components (12). At most, 10% of the amino sugars released from the alcohol-insoluble fraction could have been associated with glycoproteins that were not components of the cell wall. Since most of the amino sugars in the mycelia were in the cell wall, the changes that occurred in the amino sugar levels in the alcohol-insoluble fraction must be due almost entirely to changes in cell wall components. The alcohol-soluble fraction contained amino sugars as the sugar nucleotides UDP-GlcNAc and UDP-GalNAc (12; C. Edson, manuscript in preparation). UDP-GlcNAc has been shown to be the direct precursor of chitin (2–4), the predominant, if not the only, glucosamine-containing cell wall polymer (6, 8). UDP-Gal NAc is thought to be the precursor of galactosamine-containing cell wall polymers (C. Edson, manuscript in preparation). Thus, fractionation by hot alcohol was a convenient procedure for measuring the levels of the amino sugar-containing polymers (for the most part, cell wall components) and the levels of the soluble precursors of the amino sugars in these polymers (sugar nucleotides).

The glucosamine levels in all of the samples was within ± 10% of 135 μmol/g of residual dry weight (Table 2). Thus, the glucosamine levels in the alcohol-insoluble fraction did not vary significantly as a function of time, position, or conidiation. This confirms the results obtained with the wild-type strain (Table 1).

The galactosamine levels in the alcohol-insoluble fraction varied from less than 1.7 μmol/g of residual dry weight in the second interband at 30 h to 11.6 μmol/g in the same area at 54.5 h (Table 2). The galactosamine

TABLE 2. *Amino sugar concentration of alcohol-insoluble and alcohol-soluble fractions from surface-grown cultures of the bd strain*

| Period of growth (h) | Amino sugar concn (μmol/g residual dry wt) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Interband 1[a] | Band 1 | | Interband 2 | | Band 2 | | Interband 3 | |
| | — | GlcN | GalN | GlcN | GalN | GlcN | GalN | GlcN | GalN |
| 30[b] | | 135 (10.0)[c] | 8.7 (0.28) | 147 (17.8) | <1.7 (0.68) | | | | |
| 42.5 | | 136 (5.8) | 10.8 (0.42) | 149 (8.1) | 8.3 (<0.1) | 126 (10.6) | 2.0 (<0.1) | | |
| 54.5 | | | | 126 (8.2) | 11.6 (0.29) | 125 (7.9) | 8.4 (0.36) | 138 (13.0) | 0.8 (0.64) |

[a] Interbands are non-conidiating regions and bands are conidiating regions. Interband 1 was not harvested since it contained the inoculum.

[b] After 30 h the second interband was just completed (see Fig. 1).

[c] Values in parenthesis are the amino sugar concentration of the alcohol-soluble fractions.

content of the alcohol-insoluble fraction was always lowest in the growing front and highest in the older regions of the mycelial mat. This confirms the results obtained with the wild-type strain, i.e., that galactosamine content varied as a function of time and position (Table 1). Furthermore, the galactosamine gradient was not disturbed by the conidiation rhythm of the *bd* strain. Whether the leading edge of the mycelial mat was in its conidiating (42.5 h, band 2) or non-conidiating (30 h, interband 2) state of growth, it always contained less galactosamine than the older regions of the mycelial mat.

**Amino sugar content of the alcohol-soluble fractions.** The glucosamine content of the alcohol-soluble fractions (UDP-GlcNAc) was always higher in the growing-front region of the mycelial mat than in older regions (Table 2, numbers in parentheses). Since this region was growing rapidly, the higher chitin precursor concentration might be needed for rapid formation of glucosamine containing polymers.

The galactosamine content of the alcohol-soluble fractions (UDP-GalNAc) ranged from 0.1 to 0.68 μmol/g of residual dry weight (Table 2) in different areas of the culture. These concentrations were less than ½ the maximum concentration found in mycelia from liquid-shake cultures (12). Since mycelia from older regions of the mycelial mat contained alcohol-insoluble galactosamine, it would appear that only a small pool of UDP-GalNAc was required for the synthesis of these polymers.

## DISCUSSION

Four questions were raised at the beginning of this paper. First, would the amino sugar content of mycelia from cultures grown on dialysis membranes differ from that obtained for mycelia from cultures grown in liquid media? The results show that if the amino sugar content of the mycelia from the older regions of the surface-grown cultures is compared to that obtained from mycelia of liquid-shake cultures (12) there are only quantitative differences between the two culture conditions. The surface-grown cultures contained from 85 to 149 μmol of glucosamine per g of residual dry weight, whereas the range obtained for mycelia from liquid-shake cultures was 70 to 200 μmol/g (12). The galactosamine content of older regions of mycelia from surface-grown cultures varied from 11 to 22 μmol/g of residual dry weight, whereas the range for mycelia from liquid shake cultures was from 20 to 75 μmol/g (12).

The second question was: Would the amino sugar content of mycelia from the growing front of the mycelial mat differ from that of the older regions of the same mycelial mat? For glucosamine there was no consistent variation between the growing front and the older regions of the mycelial mat. However, the galactosamine content was always less in the growing-front region of the mycelial mat than in the older regions.

As we demonstrated for liquid-shake cultures (12), galactosamine accumulation in the older regions of the mycelial mat of surface cultures was dependent in some way on mycelial density. The hyphal density at the leading edge of the mycelial mat was much thinner than in older regions and, as a particular region aged, the hyphal density increased. The accumulation of galactosamine in the older regions of the mycelial mat may result from that region having reached a hyphal density at which the formation of galactosamine-containing polymers could occur. This could be the result of cell contact or perhaps something has been added or removed from the medium. The possibility of zymogen activation has been discussed (12).

It has already been shown that the accumulation of galactosamine-containing polymers in germinating conidia was not required for germ tube formation (12). The hyphae at the leading edge of the mycelial mat had very low levels of galactosamine (Tables 1 and 2). Thus, galactosamine-containing polymers were required, at very low levels at most and perhaps not at all, for the formation of hyphae at the growing front of a surface culture.

The third question was: Would the amino sugar content of any particular region of the mycelial mat change as that particular region aged? Again, only the galactosamine content increased as the mycelial mat aged. The glucosamine content remained relatively constant. Since the dry weight of these older regions continued to increase during these experiments, new growth was occurring. The galactosamine could be accumulated in both the old and newly formed hyphae on these older regions, or could be preferentially deposited in the newly formed hyphae.

The fourth question was: Would regions of the mycelial mat that are producing conidia that do not contain galactosamine (5, 12) also be devoid of this amino sugar? The results indicate that there was no correlation between conidiation and the presence or absence of galactosamine. The galactosamine content was always lower in the growing-front region regardless of whether or not that region was conidiating.

Since conidia, which contained less than 1.0 μmol of galactosamine per g (12), were produced

from mycelial mats that contained up to 10 times this amount of galactosamine (Table 2), the accumulation of galactosamine-containing polymers must be spatially regulated during development. Developmental control of galactosamine levels could occur in several ways. The precursor UDP-GalNAc could be excluded from conidia. The enzyme activities required for the formation of galactosamine-containing polymers could be absent or inactive. Also, degradation of galactosamine polymers could occur in conidia. The data presented do not distinguish among these possibilities.

The *bd* strain grown on the surface of dialysis membrane provides a very convenient system for studying conidiation. The main advantage of this system is that conidiation occurs rhythmically at the edge of the growing mycelial mat (Fig. 1) where nutrients are plentiful. Thus, it is not necessary to induce conidiation by starvation, and those changes that are unique to conidiation can be distinguished from those induced by starvation. With this system we have shown that conidiation does not influence galactosamine accumulation (Table 2). In the future, this system should be very useful for other developmental studies in *N. crassa*.

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