Incorporation and fate of *N*-acetyl-D-glucosamine during hyphal growth in *Streptomyces*

ELISA M. MIGUÉLEZ, CARLOS HARDISSON and MANUEL B. MANZANAL*

Laboratorio de Microbiología, Facultad de Medicina, Universidad de Oviedo, c/ Julián Clavería s/n, 33006 Oviedo, Spain

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N-Acetyl-D-[1-¹⁴C]glucosamine ([¹⁴C]GlcNAc) was used to label specifically the cell wall in *Streptomyces antibioticus*. Lysozyme solubilized 86.5% of the radioactivity present in the trichloroacetic acid-precipitated and RNAase plus trypsin-digested fraction of hyphae pulse-labelled with [¹⁴C]GlcNAc for 3 min in a medium containing glucose, yeast extract and a mixture of nine amino acids. Experiments with [¹⁴C]GlcNAc-labelled hyphae revealed that growth of *Streptomyces* occurs without turnover of peptidoglycan. Autoradiographic analysis of *S. antibioticus* hyphae indicated that cell wall elongation occurs by intercalation of newly synthesized wall polymers at the tip, but also over a relatively broad zone that, depending on the hyphal length, extends up to 6–10 µm from the tip.

Introduction

The processes of cell wall growth and morphogenesis in bacteria have been the subject of many investigations. Most of our knowledge on these topics was obtained from only a few selected bacteria such as *Escherichia coli* and members of the genera *Bacillus* and *Streptococcus*. However, the morphogenesis of the cell walls of the streptomycetes remains almost unexplored.

These mycelial bacteria grow through the formation of long multinuclear hyphae, which branch repeatedly as they elongate to form firstly a substrate mycelium that grows deeply into the medium and then an aerial mycelium that grows into the air and forms spores. Because of this fungus-like growth and differentiation, it is generally assumed that streptomycete hyphae elongate by apical extension, that is, by deposition of wall polymers only at the very tip of the hypha (Schuhmann & Bergter, 1976; Kretschmer, 1982). However, autoradiographic studies carried out on hyphae pulse-labelled with *N*-acetyl-D-[1-³H]glucosamine seemed to indicate that wall synthesis was not restricted to the hyphal tip. Although there was a preferential incorporation of the label at the tip, a relatively broad segment of the hypha also appeared labelled. The length of the labelled segment varied from 4 to 20 µm, depending on the strain and labelling conditions (Braña *et al.*, 1982; Gray *et al.*,

1990). The presence of radioactivity behind the tip has been attributed to the unspecific incorporation of the label into macromolecules other than peptidoglycan, or to some kind of wall metabolism induced by a process of turnover (Gray *et al.*, 1990). However, in the above experiments, the specificity of radioactive GlcNAc incorporation into the cell wall was not assessed. Moreover, whereas cell wall turnover has been widely studied in other bacteria (Doyle *et al.*, 1988), the possible existence of this process in streptomycetes has not yet been investigated. This reveals how little we know about the process of cell wall growth in *Streptomyces*.

To provide answers to these questions, in the present study we investigated the fate of GlcNAc incorporated into the hyphae of *Streptomyces antibioticus* during growth in different media and labelling conditions. Secondly, we examined whether the cell wall is subject to an active process of turnover during growth. Finally, the pattern of cell wall synthesis was re-examined by autoradiography under conditions in which the wall material was specifically labelled.

Methods

Bacterial strains and media. *S. antibioticus* ATCC 11891 was used throughout the work. *S. coelicolor* A3(2) (kindly provided by Professor D. A. Hopwood from the culture collection of the John Innes Institute, Norwich, UK) and *S. glaucescens* ATCC 23622 were also used in some experiments. To determine the efficacy of *N*-acetyl-D-[1-¹⁴C]glucosamine ([¹⁴C]GlcNAc) as a cell wall label, hyphae of *S. antibioticus* were grown in either minimal salts medium (MSM)

* Author for correspondence. Tel. 8 5103559; fax 8 5103534.

containing 50 mM-glucose, 15 mM-ammonium sulphate, 3 mM- K_2HPO_4 , 1 mM- Mg_2SO_4 , 0.4 mM- $MnSO_4$, 5 μ M- $FeSO_4$, 3.4 μ M- $ZnSO_4$ and 100 mM-MOPS buffer (pH 7.0) or in MSM supplemented with (i) 0.2% (w/v) yeast extract, (ii) 0.5% (w/v) glutamate, (iii) an amino acid mixture formed by L-lysine, L-alanine, L-arginine, L-valine, L-threonine, L-leucine, L-isoleucine, L-proline and L-serine (100 μ g ml⁻¹ each, final concentration), and (iv) an amino acid mixture plus 0.2% (w/v) yeast extract. For turnover and autoradiographic experiments the hyphae were grown in MSM supplemented with 0.2% (w/v) yeast extract and the amino acid mixture as above. This medium will hereafter be termed GYA medium.

Culture conditions. Spores of *S. antibioticus* and *S. glaucescens* were obtained from well-sporulated cultures after 7 d incubation at 28 °C in solid glucose/asparagine/yeast extract medium, as previously described (Hardisson *et al.*, 1978). Spores of *S. coelicolor* were obtained after 12 d incubation at 30 °C in oatmeal agar plates (Shirling & Gottlieb, 1966). In all cases freshly harvested spores were suspended in growth medium to an OD₅₈₀ of 0.1 and incubated in 30 ml glass tubes each containing 15 ml of culture. The tubes were placed in a water bath at 35 °C and aerated by bubbling air through the culture. The course of germination and growth was followed by measuring changes in the OD₅₈₀ in a Perkin Elmer spectrophotometer. Under these conditions, the germination of *S. antibioticus* in GYA medium was complete in 130 min and after 3 h incubation most of the hyphae were between 5 and 8 μ m in length.

Labelling conditions. Young hyphae were labelled as follows. In continuous labelling experiments the spores were germinated in culture medium containing 0.1 μ Ci [¹⁴C]GlcNAc ml⁻¹ [specific activity 58.7 mCi mmol⁻¹ (2.2 GBq mmol⁻¹); Amersham] and supplemented with 0.015% (w/v) of non-radioactive GlcNAc. After 3 h incubation the walls of the recently formed hyphae were uniformly labelled because, as previously observed, the rates of increase of cell wall radioactivity and OD₅₈₀ were identical (data not shown). In pulse-labelling experiments the spores were germinated in culture medium without carrier GlcNAc. After 3 h incubation the hyphae were quickly filtered through a moist prewarmed cellulose acetate membrane filter (47 mm diameter, 0.45 μ m pore size, Oxoid) and suspended in an equal volume of warm culture medium containing 0.7 μ Ci [¹⁴C]GlcNAc ml⁻¹. After 3 min incubation at 35 °C, under conditions of vigorous agitation, the incorporation was stopped by adding non-radioactive GlcNAc (2 mM, final concentration).

Treatment of [¹⁴C]GlcNAc-labelled hyphae. The incorporation of [¹⁴C]GlcNAc into different fractions of the cell was determined, with some modifications, by the method described by Pooley (1976). Hyphae grown in different media (see Table 1) were continuously or pulse-labelled with [¹⁴C]GlcNAc as above. For each experiment a total of twelve 1 ml samples were employed. The cells were collected on moist glass-fibre filters (25 mm diameter; Whatman GF/C) in a 12-place sampling manifold (Millipore). All filters were washed three times under suction with 5 ml of medium containing 100 mM non-radioactive GlcNAc. Vacuum was released and three of these filters were taken from the manifold and used to estimate the total [¹⁴C]GlcNAc incorporated into the whole cells. The remaining nine filters were treated directly on the manifold for 1 min with 1 ml ice-cold 5% (w/v) trichloroacetic acid (TCA) containing 100 mM-GlcNAc, washed three times with 2 ml distilled water and once more with 2 ml 50 mM-sodium phosphate buffer (pH 8.0), with the aid of intermittent suction. Three samples of the filtrate were taken and used for determination of [¹⁴C]GlcNAc in the pool of soluble precursors (TCA-soluble fraction) and three of the filters were used for determination of [¹⁴C]GlcNAc incorporated into macromolecules (TCA-precipitable fraction).

The remaining six filters were removed from the manifold, placed separately in scintillation glass vials and incubated with 1 ml RNAase

(1 mg ml⁻¹) in 50 mM-sodium phosphate buffer (pH 8.0) for 45 min at 37 °C. Then, 1 ml trypsin (1 mg ml⁻¹) in the above buffer was added to each vial, and all samples were further incubated at 37 °C. After 90 min incubation, the glass-fibre filters were placed in the manifold on top of a cellulose acetate membrane filter (25 mm diameter, 0.45 μ m pore size; Oxoid). Vacuum was applied and after washing with 5 ml distilled water, the RNAase plus trypsin-soluble filtrate from three samples was collected. Three of the six sets of filters were removed from the manifold and used to estimate the [¹⁴C]GlcNAc present in the RNAase plus trypsin-insoluble fraction (presumably cell wall fraction) and the last three sets of filters were placed again in scintillation vials with a drop of 0.1 M-sodium azide. These filters were incubated overnight at 37 °C with 2 ml lysozyme (100 μ g ml⁻¹) in 50 mM-sodium phosphate buffer (pH 8.0) containing 5 mM-sodium citrate. The above procedure was repeated; the filtrates were used to determine the [¹⁴C]GlcNAc solubilized by lysozyme, and the filters to determine the [¹⁴C]GlcNAc remaining in the lysozyme-resistant fraction.

Measurement of wall turnover. Spores germinated in GYA medium were either continuously or pulse-labelled with [¹⁴C]GlcNAc as indicated above. After labelling, the young hyphae were collected by rapid filtration through a 47 mm membrane filter prewashed with non-radioactive GYA medium at 35 °C. The filters were rapidly washed twice with 10 ml warm (35 °C) GYA medium containing 10 mM-GlcNAc and immediately suspended in fresh prewarmed GYA medium containing 2 mM-GlcNAc. During continuous growth, samples were taken for measurements of optical density, radioactivity in bacteria and radioactivity in the culture supernatants as follows. Every 10 min, four 1 ml samples were filtered, each through a glass-fibre filter placed on top of a membrane filter in the manifold, and washed with 10 ml distilled water. The filters were then treated in two ways. Two sets of filters were dried and used to estimate the radioactivity in whole cells. The remaining sets of filters were treated directly in the manifold with 1 ml ice-cold 5% (w/v) TCA for 1 min, washed with 10 ml distilled water, dried and used to estimate the radioactivity incorporated into macromolecules. The filtrates from samples treated by the first method were collected in scintillation vials and used to estimate the radioactivity present in the culture medium.

Measurement of radioactivity. After drying at 80 °C the filters were placed in scintillation vials containing 5 ml of a toluene-based scintillation fluid. Samples of culture supernatants were added to scintillation vials, evaporated to a final volume of 1 ml and mixed with 10 ml of a toluene/Triton X-100-based scintillation fluid. Radioactivity was measured in a Beckman LS 100C liquid scintillation counter.

Chromatography. Thin-layer chromatography (Silicagel IB) was done with aliquots of the filtrate in parallel to samples of [³H]GlcNAc using n-butanol/acetone/water (4:5:1, by vol.) as an ascending mobile phase. After 5 h, the chromatograms were air dried, cut into 2 × 1 cm strips, and counted in toluene-based scintillation fluid.

Autoradiography. Spores of *S. antibioticus* were incubated in GYA medium until the young hyphae were between 15 and 25 μ m in length (about 5 h incubation). The culture was then filtered and the hyphae were suspended in GYA medium containing 50 μ Ci [³H]GlcNAc ml⁻¹ [specific activity 2.84 Ci mmol⁻¹ (105.1 GBq mmol⁻¹); Amersham]. After 1 min incubation at 35 °C, incorporation was stopped by adding an equal volume of ice-cold 10% (w/v) TCA containing 100 mM-GlcNAc. After 5 min at 0 °C the samples were centrifuged, washed three times with 50 mM-sodium phosphate buffer (pH 8.0), and finally digested with RNAase plus trypsin as above. Samples (0.1 ml) were spread on collodion-coated slides and covered with a monolayer of Ilford L4 nuclear emulsion by the loop method. After 4–7 days exposure, the slides were developed for 1 min at 20 °C in the physical developer of Caro & Van Tubergen (1962), fixed and washed with

Table 1. Incorporation of [^{14}C]GlcNAc into cell fractions of *S. antibioticus*

Spores of *S. antibioticus* were germinated in different media and the young hyphae were continuously or pulse labelled with [^{14}C]GlcNAc. Labelled hyphae were collected by filtration, washed and treated sequentially with trichloroacetic acid (TCA) and different enzymes (see Methods for details). The radioactivity in the resulting fractions was determined in triplicate.

Treatment	Fraction	$10^{-3} \times [^{14}\text{C}]\text{GlcNAc incorporation [c.p.m.} \pm \text{SD} (\% \text{ of total})]$					
		Continuously labelled cells					Pulse-labelled cells
		Minimal salts medium (MSM)	MSM plus glutamate (0.5%)	MSM plus yeast extract (0.2%)	MSM plus amino acid mixture	MSM plus amino acid mixture and yeast extract (0.5%)	MSM plus amino acid mixture and yeast extract (0.5%)
Whole washed cells		31.0 (100)	45.3 (100)	38.5 (100)	40.1 (100)	54.6 (100)	642.1 (100)
Trichloroacetic acid	Soluble	10.0 $\pm$ 0.9 (31.7)	7.5 $\pm$ 0.6 (18.0)	5.6 $\pm$ 0.4 (15.1)	6.0 $\pm$ 0.6 (16.0)	8.7 $\pm$ 0.5 (17.2)	152.3 $\pm$ 9.8 (25)
	Insoluble	21.5 $\pm$ 1.7 (68.3)	34.6 $\pm$ 2.0 (82.0)	30.5 $\pm$ 2.1 (84.9)	31.6 $\pm$ 1.8 (84.0)	42.3 $\pm$ 1.6 (82.8)	458.8 $\pm$ 20.7 (75)
RNAase plus trypsin	Soluble	13.2 $\pm$ 1.1 (48.5)	9.5 $\pm$ 1.1 (28.6)	7.4 $\pm$ 0.6 (23.5)	6.4 $\pm$ 0.6 (20.9)	6.3 $\pm$ 0.4 (16.0)	39.7 $\pm$ 1.0 (8.2)
	Insoluble	14.0 $\pm$ 0.8 (51.5)	23.7 $\pm$ 1.9 (71.4)	24.3 $\pm$ 1.3 (77.5)	24.5 $\pm$ 0.9 (79.1)	33.3 $\pm$ 1.2 (84.0)	446.9 $\pm$ 15.2 (91.8)
Lysozyme	Soluble	9.3 $\pm$ 0.7 (74.4)	16.0 $\pm$ 1.2 (78.1)	15.0 $\pm$ 0.9 (75.5)	18.2 $\pm$ 1.0 (80.9)	24.8 $\pm$ 1.2 (79.9)	401.9 $\pm$ 10.6 (86.5)
	Insoluble	3.2 $\pm$ 0.2 (25.6)	4.4 $\pm$ 0.3 (21.9)	4.9 $\pm$ 0.3 (24.5)	4.4 $\pm$ 0.2 (19.1)	6.3 $\pm$ 0.4 (20.1)	62.6 $\pm$ 2.1 (13.5)

distilled water. The samples were observed and photographed in a Philips EM-300 electron microscope at 60 kV. Distribution of silver grains was determined on enlarged micrographs by counting the number of grains contained in successive 1 μm hyphal segments from the tip. The number of grains per segment is represented as a percentage of the total number of grains per hypha.

Results

Efficacy of GlcNAc as a cell wall label in *S. antibioticus*

Spores of *S. antibioticus* were germinated in different media and the young hyphae were labelled continuously or pulse-labelled with [^{14}C]GlcNAc. The fate of the [^{14}C]GlcNAc was determined by sequential treatment of the samples with TCA and different lytic enzymes. When *S. antibioticus* grown in MSM and continuously labelled with [^{14}C]GlcNAc was subjected to chemical fractionation, about 68% of the radioactivity present in whole biomass was precipitated by TCA (Table 1). The remainder was TCA-soluble and presumably contained the pool of low-molecular-mass precursors of the wall. RNAase plus trypsin released over 48.5% of the radioactivity present in the TCA-insoluble fraction, which indicates that a substantial part of the radioactivity present in the TCA-precipitable fraction does not correspond to wall material.

The specificity of labelling with [^{14}C]GlcNAc was improved by modifying the conditions of incubation. The best results (Table 1) were obtained by adding yeast extract and a mixture of amino acids to the culture medium (GYA medium). Under these conditions of growth and in continuous labelling experiments, the amount of radioactivity released from the TCA-treated cells by RNAase plus trypsin decreased to 16% and of

the remaining fraction about 80% of the radioactivity was solubilized by lysozyme. In pulse-labelling experiments 8.2% of the radioactivity present in the TCA-treated cells was incorporated into RNAase plus trypsin-soluble material while lysozyme released 86.5% of the radioactivity remaining in the RNAase plus trypsin-digested fraction.

Experiments carried out with *S. coelicolor* and *S. glaucescens* grown in GYA medium also demonstrated the efficacy of [^{14}C]GlcNAc as a cell wall label. Thus, in pulse-labelling experiments the percentages of radioactivity solubilized by RNAase plus trypsin and the percentages of radioactivity solubilized by lysozyme (after all treatments) were respectively 7.1 and 88.4 for *S. glaucescens* and 10.7 and 83.6 for *S. coelicolor*.

Studies on cell wall turnover

Cell wall turnover was assayed by measuring the disappearance of cell-bound radioactivity as well as the appearance of degradation products in the culture medium. Spores of *S. antibioticus* were germinated in GYA medium and continuously labelled with [^{14}C]GlcNAc until the recently formed hyphae were between 5 and 8 μm in length (about 3 h incubation). At this time of growth, the hyphal elongation rate was about 10 $\mu\text{m h}^{-1}$. The hyphae were collected on filters, washed and suspended in non-radioactive GYA medium. Hyphae resumed growth immediately (Fig. 1a), which indicates that they were not affected by washing. As can be seen, the cell bound radioactivity remained constant throughout the period of 'chase' with unlabelled GlcNAc. A similar behaviour was observed when pulse-labelled instead of continuously labelled hyphae were

Fig. 1. Fate of cell wall radioactivity during hyphal growth of *S. antibioticus*. Young hyphae growing in GYA medium were labelled continuously (a) or pulse-labelled (b) with [^{14}C]GlcNAc as described in Methods. After labelling, the cultures were filtered, washed and suspended in fresh non-radioactive medium. At the indicated times following resuspension, 1 ml samples (in triplicate) were harvested by filtration and used to estimate the radioactivity in the cells (○) and in the culture medium (●). □, OD_{580} .

assayed for turnover (Fig. 1b). The same results were obtained when the radioactivity remaining in the TCA-precipitated cells was measured (data not shown).

As Fig. 1 also shows, the culture filtrate contained small amounts of radioactivity which were present from the beginning of the chase and which remained constant throughout the experiment. When samples of the culture medium were analysed by chromatography (see Methods), most of the radioactivity migrated with the standard [^{14}C]GlcNAc, indicating that the soluble radioactivity present in the culture medium was mostly due to carry-over from the labelling medium and not to labelled fragments released from the wall.

It is possible that the lack of turnover could be imposed by the conditions of chase because, as has been described for *Bacillus megaterium* (Frehel & Ryter, 1979), the lytic activity required for wall turnover may be eliminated or diluted during transfer to fresh medium. To investigate whether turnover deficiency in *S. antibioticus* was due to the washing and transfer of the cells to the chase medium, continuously labelled cells were chased directly in the same undisturbed culture tube by adding non-radioactive GlcNAc (2 mM, final concentration). We found this procedure more accurate because it produces the least disturbance to the cultures and ensures that any factor excreted by the cells is present in the culture medium during the chase. As Fig. 2 shows,

Fig. 2. Effect of addition of unlabelled GlcNAc on the incorporation of [^{14}C]GlcNAc during hyphal growth in *S. antibioticus*. Cultures of *S. antibioticus* were grown in GYA medium containing $0.1 \mu\text{Ci}$ [^{14}C]GlcNAc ml^{-1} . After 3 h labelling the culture was divided into two parts. One part was allowed to continue growth in the presence of [^{14}C]GlcNAc (○). To the other part, GlcNAc (2 mM, final concentration) was added (●). At intervals, triplicate 1 ml samples were taken and used to estimate the radioactivity remaining in the cells.

Fig. 3. Autoradiograph of the apical region of an *S. antibioticus* hypha. The hyphae were pulse-labelled for 1 min with [^3H]GlcNAc, treated with TCA and digested with RNAase plus trypsin as described in Methods. After 5 d exposure the samples were developed and observed in the electron microscope. The arrow indicates the hyphal tip. Bar, 2 μm .

addition of GlcNAc immediately stopped incorporation of radioactivity into the wall, which subsequently maintained the same level of radioactivity throughout the experiment.

Similar experiments carried out with *S. coelicolor* and *S. glaucescens* yielded data equivalent to those for *S. antibioticus* (data not shown). This indicates that the absence of cell wall turnover is not a strain-specific event and may be a general feature among the streptomycetes.

Location of newly inserted wall material

The topological distribution of cell wall synthesis was analysed in *S. antibioticus* by autoradiography. Hyphae grown in GYA medium were pulse-labelled with [^3H]GlcNAc for 1 min and treated sequentially with cold TCA, RNAase and trypsin to remove unspecific labelling. After 5 d exposure the samples were developed and

Fig. 4. Silver grain distribution in [^3H]GlcNAc pulse-labelled hyphae of *S. antibioticus*. Hyphae (about 25 μm long) were labelled and processed for autoradiography as in Fig. 3. Number of grains per μm is expressed as a percentage of the total number of grains per hypha. Bars show the variability in eleven hyphae examined.

observed by electron microscopy. For this experiment a total of 40 hyphae were analysed. The hyphae appeared labelled at the tip (Fig. 3) but many silver grains also appeared distributed in a broader zone that, in the longer hyphae examined (about 25 μm long), extended to a maximum of about 10 μm from the tip (Fig. 4). In hyphae ranging in length from 15 to 25 μm , the mean length of this subapical incorporation was 8 ± 2 μm .

The possibility that the radioactivity located behind the tip could be due to developing septa or to nascent branches was further investigated. In crystal-violet-stained hyphae, septa were not observed at this time of growth and the branches, which start to be observed after about 6 h incubation, appeared near to the spore and in no case were seen on the labelled (about 10 μm long) segment of the hypha (data not shown).

Discussion

In previous experiments (unpublished) we observed that *S. antibioticus* can grow in a minimal medium containing GlcNAc as the only carbon source. This indicates that, as in the case of *Bacillus subtilis* (Mobley *et al.*, 1982, 1984), the GlcNAc molecules can be degraded, thus producing an unspecific labelling of the cell. The results obtained in the present work indicate, however, that under certain conditions of incubation, [^{14}C]GlcNAc can be used to specifically label the cell wall of streptomycetes. Thus, when hyphae of *S. antibioticus* grown in a rich medium containing glucose, yeast extract and a mixture of amino acids were pulse-labelled with [^{14}C]GlcNAc, only 8% of the radioactivity was incorporated into proteins and most of the label present in the TCA-precipitated and RNAase plus trypsin-digested cells (about 86.5%) was solubilized by lysozyme (Table 1). The radioactivity in the lysozyme-resistant fraction

might be attributed to the presence of a chemically modified or highly cross-linked peptidoglycan (Trentini & Murray, 1975). It is also possible that some protein may account for this lysozyme-insoluble fraction since, as previously observed in hyphae of *S. antibioticus* continuously labelled with [^3H]leucine, the trypsin treatment removed 80% of the radioactivity (data not shown).

By using [^{14}C]GlcNAc as label it was possible to demonstrate that in *S. antibioticus*, wall growth takes place without turnover of the peptidoglycan. Preliminary experiments also revealed absence of turnover in *S. coelicolor* and *S. glaucescens*. This seems to indicate that in these micro-organisms, as in other Gram-positive bacteria (Boothby *et al.*, 1973; Frehel & Ryter, 1979; Pooley & Karamata, 1984), turnover is not an obligatory feature for cell wall elongation.

With regard to the pattern of cell wall synthesis, autoradiographic experiments in which the hyphae were labelled with [^3H]GlcNAc and any unspecific incorporation of the label was removed by enzymic treatments confirmed that in *S. antibioticus* wall synthesis is not restricted to the very tip but also occurs at other sites distributed along a relatively broad segment of the hypha that in some cases extends to about 10 μm behind the tip. Obviously, these data alone do not reveal how hyphal growth is accomplished in streptomycetes. An explanation for the autoradiographic results could be a mechanism of growth in which the sites of peptidoglycan incorporation are initially formed as a cluster at the tip. Later, and as a consequence of their biosynthetic activity, the wall expands and the sites, that still remain active, separate from each other and become dispersed behind the tip. However, since [^{14}C]GlcNAc could also be present in wall polymers other than peptidoglycan (the presence of teichoic acids in the wall of streptomycetes has been reported by Naumova *et al.*, 1980), the possibility that new peptidoglycan is incorporated at the tip but that secondary wall polymers are added at sites along the hyphal length can therefore not be ruled out. These data seem to indicate that in streptomycetes the processes of growth and maintenance of the hyphal morphology are more complex than those previously supposed. More experimental data are needed before any model of hyphal growth can be accepted for *Streptomyces*.

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