A breakdown in macromolecular synthesis preceding differentiation in *Streptomyces coelicolor* A3(2)

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A transitory cessation of growth was recorded in *Streptomyces coelicolor* A3(2) at the end of vegetative mycelium formation on solid medium. In the same phase a striking reduction in protein and nucleic acid synthesis was detected. Growth and macromolecular synthesis resumed, nearly reaching the original values, when morphological differentiation occurred. It is concluded that a physiological stress occurs within the bacterial population just before the onset of the morphological differentiation.

Introduction

The streptomycetes are morphologically among the most highly differentiated prokaryotes. Their colonies on agar consist of a substrate mycelium and an aerial mycelium which produces spores, the latter representing the propagative phase. The growth cycle at 30 °C, from spore to sporulating colony takes about 3–4 d (Hopwood *et al.*, 1973; Chater & Hopwood, 1983). The formation of aerial mycelium occurs about 24 h after seeding spores onto a synthetic medium and coincides with the phase in which secondary metabolites such as pigments and antibiotics are produced. The possibility of common regulatory elements for both morphological and physiological differentiation has been widely investigated in the last few years. The isolation and characterization of genes involved in differentiation and the study of pleiotropic mutants defective in both sporulation and antibiotic production have strongly supported interdependence of the regulatory pathways of these two aspects of differentiation in *Streptomyces coelicolor* (Chater, 1989). It is often asserted that secondary metabolism is initiated by nutrient limitation and a reduction in growth rate (Martin & Demain, 1980).

The aim of the present work was to measure the rate of nucleic acid and protein synthesis in the critical period in which aerial mycelium first appears. This was achieved by following the incorporation of radiolabelled precursors

into macromolecules by *S. coelicolor* A3(2) during its growth cycle on solid media.

Methods

Strains. The mutant strains used throughout the work were derived from the wild-type *Streptomyces coelicolor* A3(2) (Hopwood *et al.*, 1973; Chater & Hopwood, 1983). They were: strain 44 (*hisA1* SCP1⁺SCP2⁺), strain 11 (*uraA1 metA1 strA1* SCP1[−]SCP2⁺) and strain 316 (*hisD3 pheA1 strA1* SCP1[−]SCP2⁺).

Growth conditions. Minimal medium (MM) had the following composition (l^{−1}): NaNO₃, 1 g; MgSO₄·7H₂O, 0.5 g; KCl, 0.5 g; KH₂PO₄, 1 g; trace element solution (FeSO₄·7H₂O, 1%; ZnCl₂, 1%; biotin, 0.1%), 1 ml; agar, 15 g; and glucose (autoclaved separately as 50% w/v solution), 10 g. When required histidine (50 µg ml^{−1}), methionine (37 µg ml^{−1}), phenylalanine (37 µg ml^{−1}) and uracil (7.5 µg ml^{−1}) were added. Complete medium (CM) was as described by Hopwood *et al.* (1985). General procedures were as described by Hopwood & Sermonti (1962) and Hopwood *et al.* (1985). To facilitate harvesting the mycelium and its transfer from one medium to another, the strains were grown on autoclaved cellophane disks (8 cm diam.) lying on solid medium (Sermonti *et al.*, 1971).

Growth curves. Spores of the strains were harvested from slant cultures, filtered through glass funnels containing cotton wool, washed by centrifugation and resuspended at a concentration up to 10⁸ ml^{−1}; 10⁷ spores were placed on each cellophane disk lying on MM supplied with the required amino acids and incubated at 30 °C. After various times the mycelium was scraped from the cellophane and weighed.

Measurement of the rate of DNA, RNA and protein synthesis. Spore suspensions of the strains were seeded on cellophane disks lying on MM and incubated at 30 °C. After various times the cellophane disks with the growing mycelium were transferred onto MM containing: [6-³H]thymidine (30 µCi ml^{−1}; 20–30 Ci mmol^{−1}) for measurement of DNA synthesis; [6-³H]uracil (30 µCi ml^{−1}; 15–30 Ci mmol^{−1}) for

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measurement of RNA synthesis; and [^{35}S]methionine ($30\ \mu\text{Ci ml}^{-1}$; $10\text{--}500\ \text{mCi mmol}^{-1}$) for measurement of protein synthesis. ($1\ \mu\text{Ci} = 37\ \text{kBq}$; all radiochemicals were present without cold carrier.) After 2 h incubation on the labelled substrate the mycelium was harvested from the surface of the cellophane and lysed by freezing in liquid nitrogen and thawing. Samples ($25\ \mu\text{l}$) of each lysate were placed on two filter-paper disks. One of each pair of disks was counted in a scintillation counter to obtain the total radioactivity associated with the mycelium; the other was treated with trichloroacetic acid (TCA), followed by alcohol/ether and ether, according to Bollum (1959) and Greenberg & Rothstein (1957). Synthesis of macromolecules is expressed as the fraction of TCA precipitable counts over the total radioactivity associated with the mycelium.

Results

Growth rate

The growth curve of *S. coelicolor* strain 316, given as mg wet wt cm^{-2} (W) at successive time intervals (n) is shown in Fig. 1; the growth rate, calculated as $(W_n - W_{n-1})/W_n \times 100$ per hour, is shown in Fig. 2. The growth rate reached a maximum at about 20 h, corresponding to the end of vegetative growth. It fell to a minimum at 25 h, and then resumed a high value, probably corresponding to aerial mycelium formation. Eventually, the rate declined at sporulation. Similar results were obtained with either MM or CM and with all the strains: each strain showed a critical phase in which a transitory cessation of growth occurred just after vegetative mycelium formation and before aerial mycelium development.

MM was used in subsequent experiments in which the rate of biosynthesis of macromolecules was determined.

Protein and nucleic acid synthesis

To verify whether there was a reduction in protein synthesis during the critical phase, incorporation of [^{35}S]methionine into protein was determined in the different growth phases (Fig. 3). Incorporation was high during vegetative hyphae formation, fell between 20 and 25 h, and finally increased during the development of aerial hyphae and spores. Uptake of [^{35}S]methionine ($10^6 \times \text{c.p.m. per mg of mycelium}$) was relatively high (0.58) during vegetative growth, then declined during the critical phase (0.17) to stay constant throughout the differentiation phase (0.17–0.19).

There was a decrease in nucleic acid synthesis, particularly in RNA synthesis, at the end of vegetative growth just before aerial mycelium development (Fig. 4). [^3H]Uracil incorporation went from 80% during vegetative growth to about 5% at 25 h; [^3H]thymidine incorporation decreased from 70% in the vegetative mycelium to about 40% at 25 h. When the bacteria

Fig. 1. Growth curve of *S. coelicolor* A3(2) (strain 316) on solid medium. Values at each time-point are the arithmetic mean of the weight of the mycelium from five plates.

Fig. 2. Growth rate of *S. coelicolor* A3(2) (strain 316). Values were derived from those in Fig. 1 using the equation given in Results.

Fig. 3. [^{35}S]Methionine incorporation during the growth cycle of *S. coelicolor* A3(2) (strain 316). Incorporation is expressed as the percentage of total radioactivity found in TCA-precipitable material.

Fig. 4. Incorporation of [6-³H]uracil (○) and [6-³H]thymidine (●) into RNA and DNA respectively by *S. coelicolor* A3(2) (strain 316) in the different stages of its growth cycle. Incorporation is expressed as in Fig. 3.

produced aerial hyphae incorporation increased – [³H]uracil incorporation was about 60% and [³H]thymidine about 80% – then remained constant during sporulation. Uptake of [³H]uracil and [³H]thymidine showed the same pattern as uptake of [³⁵S]methionine.

Figs 3 and 4 show the results obtained using strain 316, but the other strains investigated also showed a reduction in macromolecular synthesis during the critical growth phase.

In order to exclude the possibility that the increase in incorporation of radiolabelled precursors was due to the growth of new vegetative mycelium caused by the transfer to the labelling medium, i.e. to fresh medium, the following experiment was done. *S. coelicolor* spores were seeded onto cellophane disks placed on solid medium and incubated at 30 °C. Every 2 h several disks were transferred onto fresh medium and incubated at 30 °C. Despite the repeated transfer onto fresh medium the cultures produced aerial hyphae and sporulated at the same time as the control cultures which had been kept on the same plate.

Discussion

The data presented point to an overall fall in macromolecular synthesis in the growth phase of *S. coelicolor* A3(2) that separates vegetative growth and aerial mycelium development. Growth almost ceased during this same period. These phenomena are absent in a mutant, isolated in our laboratory, which does not produce aerial mycelium and spores (data not shown).

The synthesis of protein and nucleic acids increased again at the onset of morphological differentiation. This

increase is unlikely to be due to growth of new vegetative mycelium made possible by transfer to the labelling medium since such transfer had no visible effect on the course of morphological differentiation irrespective of the time of transfer. This also shows, in agreement with results obtained by Allan & Prosser (1987), that aerial mycelium formation is not induced by depletion of nutrient in the medium. In contrast to results obtained with *Streptomyces antibioticus* (Mendez *et al.*, 1985), when *S. coelicolor* substrate mycelium is placed on fresh medium, formation of aerial mycelium is not delayed.

Our results suggest that stress takes place within the bacterial population but that it is not induced by depletion of nutrient in the medium. Nevertheless, during the development of a colony, following the increase in cell mass, nutrient transport to parts of the colony could become limiting. This might induce a sort of stress that, mimicking the stringent response, could be transmuted into a signal for the formation of aerial hyphae (Chater, 1989). Ochi (1986*a, b*; 1987) observed that in *Streptomyces griseus* a decrease in the intracellular GTP pool (probably caused by the stringent response) and a coordinated accumulation of intracellular ppGpp initiated (or were needed for) the induction of aerial mycelium formation. Studies of stress responses have indicated that cell division and other metabolic processes in *E. coli* may be negatively controlled by the stringent control regulon (Holland, 1987).

The study of the *Streptomyces* stringent response and its correlation with the major metabolic processes might be extremely useful in order to understand the molecular mechanisms that trigger differentiation in these bacteria.

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