Growth rate control of protein and nucleic acid content in *Streptomyces coelicolor* A3(2) and *Escherichia coli* B/r

Neelam Shahab, Fiona Flett, Stephen G. Oliver and Philip R. Butler

Author for correspondence: Philip R. Butler. Tel: +44 161 200 4226. Fax: +44 161 236 0409.
e-mail: p.butler@umist.ac.uk

Department of Biochemistry
and Applied Molecular
Biology, University of
Manchester Institute of
Science and Technology,
PO Box 88, Manchester
M60 1QD, UK

***Escherichia coli* possesses regulatory mechanisms that coordinate cell growth with the synthesis of essential macromolecules (protein, RNA and DNA). While fundamental differences have been identified in the growth habit and chromosome structure of *E. coli* and *Streptomyces*, little is known about these regulatory mechanisms in filamentous bacteria. This paper reports on the relationship between the macromolecule content of *S. coelicolor* A3(2) and its specific growth rate. The protein, RNA and DNA contents (g per 100 g biomass) of *S. coelicolor* A3(2) grown in steady-state continuous culture over a range of specific growth rates (0.025 – 0.3 h^{-1}) were 31–45, 10–22 and 3.5–4.5% (w/w), respectively. This composition is qualitatively similar to that of other micro-organisms. Changes in the macromolecular content of *S. coelicolor* A3(2) and *E. coli* B/r with specific growth rate appear to be essentially similar. However, the data indicate that the RNA content of *S. coelicolor* A3(2), grown under the conditions used, exceeds that of *E. coli* grown at the same specific growth rate. The data also suggest that overlapping rounds of replication are not a feature of DNA synthesis in *S. coelicolor* A3(2). This may be a function of the organism's low maximum specific growth rate. Alternatively, it may be a consequence of regulatory mechanisms which act to inhibit the initiation of DNA synthesis in a linear chromosome which is already undergoing replication.**

Keywords: *Streptomyces coelicolor*, *Escherichia coli*, continuous culture, macromolecular content, growth control

INTRODUCTION

Streptomyces coelicolor A3(2) is an aerial-mycelium-producing actinomycete (Waksman & Henrici, 1943) and as such is a Gram-positive, soil-dwelling filamentous prokaryote. Many *Streptomyces* species are very important industrially, being responsible for the production of many diverse pharmaceutically active secondary metabolites.

The mechanisms governing the regulation of secondary metabolism in streptomycetes have been the subject of much research (reviewed by Chater, 1992; Champness & Chater, 1994; Chater & Bibb, 1996). As a general rule, secondary metabolism appears to be promoted when conditions restrict growth (Demain *et al.*, 1983; Vining, 1986), one or more nutrients becomes depleted or its

availability is reduced (reviewed by Demain, 1989; Martin, 1989; Shapiro, 1989), and/or when a transient metabolic imbalance occurs (Hobbs *et al.*, 1992). Regulatory mechanisms that have been implicated in controlling secondary metabolism include the stringent response (Ochi, 1987; Strauch *et al.*, 1991; Takano & Bibb, 1994), and the synthesis of threshold levels of small, diffusible signal molecules (Horinouchi & Beppu, 1992).

It appears that the regulation of secondary metabolism is complex. However, either a low specific growth rate and/or a change in this rate appears to be an underlying feature of the majority of factors which have been implicated in this regulation. To adjust for the cell's metabolic demands and to maintain its genetic and structural integrity, regulatory mechanisms that coordinate macromolecule (DNA, RNA and protein) synthesis with the specific growth rate are necessary. These mechanisms have been most studied in *Escherichia coli* (reviewed by Neidhardt *et al.*, 1987). Little is known

Abbreviations: C+D period, period required to replicate and segregate chromosome; HGU, hyphal growth unit; μ , specific growth rate; $\mu_{\max}$, maximum specific growth rate; μ_r , relative specific growth rate ($\mu/\mu_{\max}$).

about how macromolecule synthesis and growth are coordinated in *Streptomyces* species.

Some fundamental differences in both growth habit and genome organization between *S. coelicolor* A3(2) and *E. coli* are apparent. While *E. coli* grows by binary fission, the cell cycle completing with a balanced division, *S. coelicolor* A3(2) grows by apical hyphal extension and branch formation (Prosser & Tough, 1991), which essentially results in unequal cell division (Kretschmer, 1982). Based on pulsed-field gel electrophoresis, the size of the *S. coelicolor* A3(2) chromosome has been estimated to be about 8 Mbp (Kieser *et al.*, 1992), approximately twice that of the *E. coli* chromosome (estimated to be 4.5 Mbp; Cole & Saint Girons, 1994). Finally, recent evidence has indicated that the chromosomal DNA of *Streptomyces* species is linear (Leblond *et al.*, 1993; Lin *et al.*, 1993), which contrasts with the traditional view of a covalently-closed circular bacterial chromosome. Given these differences, and the fact that *S. coelicolor* and *E. coli* have evolved to accommodate quite different ecological niches, it is possible that the regulatory mechanisms coordinating macromolecule synthesis and growth differ in these two prokaryotes. Moreover, considering that the specific growth rate appears to be an important factor determining secondary metabolism, it is worthwhile to examine the nature of these regulatory mechanisms in *S. coelicolor* A3(2). In this paper, as the first step towards understanding how *S. coelicolor* A3(2) coordinates macromolecule synthesis with growth, we report on the relationship between macromolecule content and specific growth rate in this micro-organism.

METHODS

Strain. The original spore stock of *Streptomyces coelicolor* A3(2) strain 1147 (Hopwood, 1959) was found to be heterogeneous; five distinct colony morphologies and associated colours (red, dark red, blue, cream, and cream with red tinge) were noted on R5 solid medium. From colony hybridization experiments (to investigate plasmid content), a single red isolate considered most representative of the wild-type strain was chosen, cultured on mannitol-soya (MS) solid medium, and used throughout this study. This wild-type isolate was a prototroph, SCP1⁺ SCP2⁺ and produced, under suitable conditions, the secondary metabolites actinorhodin, undecylprodigiosin and methylenomycin. The strain was not tested for the production of A-factor or calcium-dependent-antibiotic. A spore stock of this isolate was prepared according to Hopwood *et al.* (1985), and stored at -20 °C; the isolate was also stored at 4 °C as single colonies on MS solid medium.

Media. MS agar had the following composition per litre; mannitol 20 g, soya bean meal/flour 20 g, Difco Bacto agar 16 g, tap water to 1 l. Regeneration medium (R5) and yeast extract-malt extract (YEME) medium were prepared according to Hopwood *et al.* (1985). A modified YEME medium (containing 100 instead of 340 g sucrose l⁻¹) was used in continuous culture experiments. It was prepared by autoclaving glucose and sucrose separately from yeast extract, Bacto peptone and malt extract for 1 h at 121 °C and combining the two mixtures aseptically when cool.

Growth conditions. Both solid and liquid cultures of *S. coelicolor* A3(2) were grown at 30 °C. Small batch cultures were grown in

flasks containing stainless steel springs (12.7 mm × 6 turns in⁻¹; Alliance Spring Co) to improve aeration and aid dispersed growth. Shake-flask cultures were incubated in an orbital incubator shaking at 200 r.p.m.

Twenty millilitres (1%, v/v, of the final culture volume) of a 48 h shake-flask culture in YEME was used as a seed inoculum in all continuous culture experiments. These were carried out in an LH2000 fermenter (LH Engineering) with a total capacity of 3 l and a working volume of 2 l. Oxygen-sufficient conditions were achieved by aeration at a rate of 0.5 v.v.m. (1.0 l min⁻¹). The aeration rate was maintained constant with a mass-flow valve. To prevent loss of culture volume via evaporation, the air outflow was passed through a water-cooled condenser. The temperature, monitored using a platinum thermocouple, was maintained at 30 °C using a heating element and a water-cooled heat exchanger. The pH was monitored using a combined pH electrode and controlled at 7.0 ± 0.1 by automatic additions of 5 M NaOH. Stirrer speed was 1000 r.p.m. Continuous culture was established by first growing a batch culture until mid- to late-exponential phase and then switching on medium inlet and outlet peristaltic pumps at the desired flow rates. The working culture volume was maintained at 2 l by an overflow weir.

Sampling and storage of samples. During continuous culture experiments, at least three samples were collected from the fermenter over two to three retention times, once a steady-state had been achieved. A steady-state was assumed when a constant biomass concentration was attained, usually after four culture volumes of medium had passed through. At each sampling time, 15 ml of the initial sample was discarded to remove accumulated cells in the sampling port. A suitable volume of sample was subsequently collected and immediately placed on ice. Samples (10 ml) were pipetted into 50 ml centrifuge tubes containing 5 ml distilled water which had been previously frozen. The ice was allowed to thaw and the contents of the tube were centrifuged at 4500 r.p.m. for 5 min. The supernatant was discarded and the pellet resuspended in an equal volume of ice-cold water and re-centrifuged for a further 5 min. The washed pellet was then frozen rapidly in an ethanol/dry ice mixture and stored at -20 °C. No significant difference was found in the macromolecule content of biomass analysed immediately after sampling and after storage for up to one month. Each sample was individually examined for macromolecule content. For any individual dilution rate, the mean macromolecule content was calculated from at least three samples.

Analytical methods. Biomass was determined as dry weight of washed cells. Individual samples were vacuum filtered onto pre-weighed Whatman filter discs (GF/C, 4.7 cm). Filters and biomass were washed at least five times, each time with 10 ml distilled water. They were then dried to constant weight in a vacuum oven at 80 °C. The biomass dry weight was taken to be the difference in the two weights. A biomass dry weight measurement was made for each sample analysed for macromolecule content (see above, Sampling and storage of samples).

The Lowry method was used to determine the protein content of *S. coelicolor* A3(2). The procedure for extraction of nucleic acids was as described by Herbert *et al.* (1971). Total cellular DNA was determined by the method of Burton (1956). RNA was determined by the orcinol method (Herbert *et al.*, 1971).

Calculations. Values for the macromolecular content of *E. coli* B/r, grown in batch cultures at 37 °C for specific growth rates of 0.42–1.73 h⁻¹, were reported by Bremer & Dennis (1987). Values reported were expressed in units of amino acids per OD₄₆₀, nucleotides per OD₄₆₀ and genomes per OD₄₆₀. To enable a comparison of these data with those for *S. coelicolor* A3(2) obtained in this work, it was necessary to recalculate the

E. coli values so that they were expressed in units of g per 100 g dry weight. This was done assuming the following: the molecular masses for the average amino acid residue, average RNA nucleotide residue, and average base pair were 108 Da (composition of *E. coli* protein taken from Spahr, 1962), 324 Da (composition of *E. coli* stable RNA taken from Nierlich, 1972) and 618 Da (for a GC content of 50 mol %), respectively. The *E. coli* genome size was taken to be 4.5 Mbp (Cole & Saint Girons, 1994).

Protein and DNA content as reported by Bremer & Dennis (1987) were from the experimental work of Churchward *et al.* (1981), who used similar assays to those used in this work. RNA content was from Churchward *et al.* (1982). Bremer & Dennis (1987) stated that values for macromolecule content were accurate to better than 10% and representative for the specific growth rate. In this work a standard error of the mean of better than 10% was achieved for all assays (mean standard error = 2.43%).

All errors were treated using the Gaussian law of error propagation (Weltner *et al.*, 1986).

RESULTS AND DISCUSSION

Factors determining the choice of culture conditions

The specific growth rate of a culture (μ) may be varied by changing either the nature of nutrients or the concentration of the growth-rate-limiting substrate. Schaechter *et al.* (1958) first demonstrated that, in *Salmonella typhimurium* grown at a fixed temperature, the cellular content of DNA, RNA and protein depended only on the specific growth rate and not on the nutrient supplement in the culture medium. The dependence of macromolecular content on specific growth rate observed in *S. typhimurium* was subsequently also shown to exist in *E. coli* B/r (Churchward *et al.*, 1981).

Continuous culture allows the experimenter to vary the specific growth rate of a micro-organism to values approaching its maximum specific growth rate ($\mu_{\max}$). Theoretically, the specific growth rate is equal to the dilution rate at a steady-state. Moreover, cells growing at steady-state in continuous culture will, by definition, be in balanced growth, with all the physiological advantages associated with that state (Campbell, 1957). Continuous culture was chosen as the preferred method for varying the specific growth rate of *S. coelicolor* A3(2) in the study reported here.

Continuous culture is best performed in a chemically defined medium where the nature of the growth-rate-limiting substrate is known. However, due to the formation of large, densely packed pellets, the growth of *S. coelicolor* strains in submerged culture on such media is difficult to analyse and results in a potentially physiologically heterogeneous environment. By the addition of polymeric compounds (e.g. Junlon or polyethylene glycol), minimal media have been developed to reduce this problem (Hobbs *et al.*, 1989). However, due to either interference with the assays employed or an inability to completely wash these compounds from biomass samples, thus making dry weight measurements unreliable (unpublished data), such media proved unsuitable. Although

Fig. 1. Influence of dilution rate on biomass dry weight concentration of *S. coelicolor* A3(2). Each point (○) represents the mean of five measurements. Bars represent the standard error.

not ideal, due to its high concentration of sucrose and thus potentially abnormal physiological properties, modified YEME (Hopwood *et al.*, 1985) was chosen as a suitable growth medium for this study. This choice was governed both by the practical advantages YEME provided (i.e. a reasonably well-dispersed culture and reproducible assays) and the observations of Schaechter *et al.* (1958) that the cellular contents of DNA, RNA and protein depended only on the specific growth rate and not on the nature of the culture medium.

In this work, the $\mu_{\max}$ of *S. coelicolor* A3(2) was determined according to the method of Esener *et al.* (1981), from the wash-out kinetics of biomass in continuous culture, to be 0.34 h⁻¹. Therefore, *S. coelicolor* A3(2) was grown at dilution rates ranging from 0.025 to 0.30 h⁻¹. The $\mu_{\max}$ of *S. coelicolor* A3(2) is dependent on the nature of the growth medium and the strain. Values of $\mu_{\max}$ obtained from batch culture reported in the literature, include 0.021 h⁻¹ (Ozergin-Ulgen & Mavituna, 1993), 0.052–0.095 h⁻¹ (Hobbs *et al.*, 1990), 0.025–0.365 h⁻¹ (Hodgson, 1982) and 0.30 h⁻¹ (Strauch *et al.*, 1991; Takano *et al.*, 1992). It appears that the optimal $\mu_{\max}$ of *S. coelicolor* A3(2) is in the range 0.3–0.35 h⁻¹.

To reduce the chances of mutant take-over due to genetic instability and selection, continuous culture was performed for the shortest time feasible, each dilution rate being examined as an independent fermentation. Biomass dry weight concentration was determined (see below) and samples were assayed for protein, RNA and DNA content.

Variation of biomass concentration and cell/hyphal compartment size of *S. coelicolor* A3(2) with dilution rate

Variation of biomass concentration. Biomass dry weight measurements were necessary to calculate the protein, RNA and DNA content of *S. coelicolor* A3(2) (content per cell mass; expressed as g macromolecule per g biomass dry weight). Fig. 1 shows the variation of biomass dry

weight with dilution rate over the range 0.025–0.30 h⁻¹. The biomass dry weight concentration decreased with increasing dilution rate, approaching about 0.05 g l⁻¹ at a dilution rate of 0.30 h⁻¹. Since an undefined medium (modified YEME) was used to grow *S. coelicolor* A3(2), the nature and concentration of the growth-rate-limiting substrate in the inlet was not known. However, it is fair to assume that this concentration was the same for all dilution rates examined. Since no data were acquired about the nature, or residual concentration, of the growth-rate-limiting substrate, no conclusions may be drawn on the cause of the relationship between biomass yield and dilution rate.

Estimation of cell/hyphal compartment size of *S. coelicolor* A3(2) by measurement of the hyphal growth unit. Changes in the macromolecular content (content per cell mass) of *E. coli* B/r with specific growth rate may be explained by considering changes in the mean amount per cell (content per cell) and mass of an average cell (mass per cell) (see below). To gain a complete description of the dependence of macromolecular content on specific growth rate in *S. coelicolor* A3(2), both a measure of content per cell mass and mass of an average cell were deemed necessary. Measurement of the mean macromolecular content per cell mass proved relatively straightforward (given the growth conditions used; see Methods). However, measurements of the mass of an average cell are complicated by the fact that what constitutes a cell in filamentous micro-organisms is not clearly defined.

The hyphal ultrastructure of *Streptomyces* spp. is characterized by the formation of complete septa (Prosser & Tough, 1991). The streptomycete mycelium is made up of three types of cell or hyphal compartment: apical, branched subapical, and unbranched subapical. To allow a complete analysis of the dependence of macromolecular content on specific growth rate in *S. coelicolor* A3(2), both the relative mean mass and the proportion of the total number of compartments that each cell type represents should be known. This information may be obtained by measuring the distance between septa, which have been located by staining. Our inability to reliably stain the septa of mycelial elements from *S. coelicolor* A3(2) grown in submerged culture prevented such an analysis (unpublished data). However, assuming that branched subapical cells have only one branch per hyphal compartment and that the proportion of unbranched subapical cells to other cell types is low (Kretschmer, 1982), the hyphal growth unit (HGU; defined as the total hyphal length divided by the number of apical tips) (Caldwell & Trinci, 1973) may be considered as a measure of cell/hyphal compartment length.

Assuming that the hyphal diameter and the compartment density are approximately uniform along the hypha, and do not vary significantly with specific growth rate, the mean HGU is directly proportional to the mean cell mass. The HGU of *S. coelicolor* A3(2) grown under the conditions used in this study appeared not to vary significantly up to dilution rates of 0.20 h⁻¹ (N. Shahab and others, unpublished). This suggests that the mass of an average *S.*

coelicolor A3(2) cell also does not vary with specific growth rate. However, it must be stated that conclusions based on HGU are tentative, since measurements of this variable are restricted to mycelial elements of a simple structure which, in general, represented only a small proportion of the total population (unpublished). Also, it is not known whether the number of branches per hyphal compartment or the proportion of unbranched subapical cells changes with specific growth rate in *S. coelicolor* A3(2).

Modulation of macromolecular content of *S. coelicolor* A3(2) with specific growth rate and its comparison with *E. coli* B/r

Data for the variation with specific growth rate of the macromolecular (protein, RNA and DNA) content of *S. coelicolor* A3(2) (this work) and *E. coli* B/r (recalculated from published data) are given in Table 1. In *S. coelicolor* A3(2), protein, RNA and DNA represented 31–45, 10–22 and 3.5–4.5 % (w/w) of the biomass, respectively. This composition is qualitatively similar to that of *E. coli* B/r, where protein represents the greatest proportion of the cell mass, followed by RNA, with DNA being the smallest fraction. In total, the macromolecules assayed in *S. coelicolor* A3(2) represented 56–63 % of the mycelial mass. This was significantly less than that observed in *E. coli* B/r (72–85 %), the difference being, in part, due to a lower protein content in *S. coelicolor* A3(2).

Shahab *et al.* (1994) examined the RNA and DNA content of *S. coelicolor* A3(2) grown on cellophane membranes overlaid on solid minimal medium. Cultures grew at a specific growth rate of 0.06 h⁻¹. RNA and DNA content were approximately 10 and 5 % of the mycelial mass, respectively, during the active growth phase. This is in agreement with data presented in this paper for the macromolecular content of *S. coelicolor* A3(2) grown at a dilution rate of 0.05 h⁻¹. This is as would be expected if the macromolecular content was determined by the specific growth rate and not by the nutritional status of the medium (Schaechter *et al.*, 1958) or the growth habit.

Riesenberg & Bergter (1979) examined the macromolecular composition of *Streptomyces hygroscopicus* IMET JA 6599 grown in both batch and continuous culture. The macromolecular composition they reported for *S. hygroscopicus* appears qualitatively similar to that of *S. coelicolor* A3(2), its protein content being, in general, significantly less than that observed in *E. coli*.

Table 1 shows that the highest specific growth rate at which measurements were made in *S. coelicolor* A3(2) (0.30 h⁻¹) was lower than the lowest specific growth rate for which data have been reported on the macromolecular content of *E. coli* B/r (0.42 h⁻¹; Bremer & Dennis, 1987). This made it impossible to compare the macromolecular content of these two micro-organisms at absolute values of the specific growth rate. To allow a meaningful comparison to be made between the two micro-organisms, data for the macromolecular content of *S. coelicolor* A3(2) and *E. coli* B/r were plotted against the relative specific growth rate ($\mu_r = \mu/\mu_{\max}$) of each species

Table 1. Macromolecular content of *E. coli* B/r and *S. coelicolor* A3(2)

Units		<i>E. coli</i> B/r*					<i>S. coelicolor</i> A3(2)						
μ (h^{-1}) ...		0.420	0.690	1.040	1.390	1.730	0.024	0.048	0.109	0.148	0.195	0.240	0.300
Protein content	g g^{-1}	0.670	0.601	0.531	0.529	0.529	0.456	0.432	0.412	0.429	0.416	0.347	0.313
RNA content	g g^{-1}	0.133	0.152	0.174	0.205	0.248	0.098	0.100	0.167	0.171	0.175	0.200	0.216
DNA content	g g^{-1}	0.042	0.029	0.022	0.019	0.017	0.044	0.042	0.036	0.033	0.033	0.036	0.035
DNA content†	10^{12} genomes g^{-1}	9.11	6.21	4.68	4.01	3.78	5.06	4.83	4.14	3.80	3.80	4.14	4.03
Protein per genome†	10^{-13} g genome $^{-1}$	0.74	0.97	1.13	1.32	1.40	0.90	0.89	0.99	1.13	1.10	0.84	0.78
RNA per genome†	10^{-14} g genome $^{-1}$	1.46	2.45	3.73	5.12	6.55	1.94	2.07	4.03	4.50	4.61	4.83	5.36

* Data for the macromolecular content for *E. coli* B/r were taken from Bremer & Dennis (1987), recalculated as described in Methods.

† The molecular masses of the *E. coli* B/r and *S. coelicolor* A3(2) genomes were taken to be 2.41×10^9 and 5.2×10^9 Da, respectively. It was assumed that *E. coli* B/r has a genome size of 4.5 Mb (Cole & Saint Girons, 1994), and that *S. coelicolor* has a GC content of 70 mol% (Gladek & Zakrzewska, 1984; Usdin *et al.*, 1984) and a genome size of about 8.0 Mb (Kieser *et al.*, 1992; Leblond *et al.*, 1993). The molecular masses of the A-T and G-C base pairs were taken to be 653 and 654 Da, respectively.

Fig. 2. Comparison of the dependence of macromolecular content on relative specific growth rate in *S. coelicolor* A3(2) and *E. coli* B/r. Protein ($\square$, $\blacksquare$), RNA ($\circ$, $\bullet$) and DNA ($\triangle$, $\blacktriangle$) contents of *S. coelicolor* A3(2) (open symbols) and of *E. coli* B/r (filled symbols) are plotted against relative specific growth rate, μ_r . Data for the macromolecular content of *S. coelicolor* A3(2) are the means of at least three measurements; bars indicate the standard error. Data for *E. coli* B/r are derived from Bremer & Dennis (1987). μ_r was determined assuming $\mu_{\max}$ for *S. coelicolor* A3(2) and *E. coli* B/r to be 0.34 and 1.73 h^{-1} , respectively.

(Fig. 2). The protein content of *S. coelicolor* A3(2) decreased with increasing specific growth rate. In *E. coli* B/r, protein content decreased up to a μ_r of about 0.6, above which it remained constant. Most strikingly, the RNA content of *S. coelicolor* A3(2) and *E. coli* B/r varied in an almost identical way, increasing proportionately with specific growth rate. The DNA content of both *S. coelicolor* A3(2) and *E. coli* B/r decreased with increasing specific growth rate.

In *E. coli* B/r, the protein and RNA content per cell increase with specific growth rate (Bremer & Dennis, 1987). This reflects the increase in the cell's demands for

these components at high specific growth rates and results in an increase in the average mass of a cell. Also, at high specific growth rates, the DNA content per cell increases with specific growth rate as a result of overlapping rounds of DNA replication. However, the DNA content per cell mass decreases because the average mass of a cell increases faster than the mass of DNA in the cell. The proportion of the cell's mass represented by protein remains approximately constant since the mass of protein per cell increases in direct proportion with the average mass of the cell. In contrast, the RNA content per cell mass increases with specific growth rate, because the RNA portion increases faster than the average cell mass.

Trends in the proportions of protein, RNA and DNA per cell mass in *S. coelicolor* A3(2) appear to be similar to those observed in *E. coli* B/r. However, since the HGU (and thus hyphal compartment size, accepting the above-stated assumptions) appears approximately constant up to a relative specific growth rate of about 0.7 (N. Shahab and others, unpublished), it appears that changes in the proportions of each macromolecule in *S. coelicolor* A3(2) probably do not arise from an overall increase in cell size.

Indicators of the coordination of macromolecular synthesis and growth in *S. coelicolor* A3(2) and *E. coli* B/r

Coordination of RNA synthesis with growth. The rate of synthesis of ribosomes in *E. coli* B/r is coordinated with growth (reviewed by Jinks-Robertson & Nomura, 1987). At high specific growth rates, rRNA represents the greatest single portion of the cellular RNA content. The suggestion that changes in the demand for protein synthesis are met by changes in the number of ribosomes, and not by changes in the catalytic activity of individual ribosomes, has been made to explain the observed proportionality between RNA per genome and specific growth rate in *E. coli* (Maaløe, 1969).

Fig. 3. Comparison of the dependence of protein and RNA per genome on relative specific growth rate in *S. coelicolor* A3(2) and *E. coli* B/r. Protein (□, ■) and RNA (○, ●) per genome of *S. coelicolor* A3(2) (open symbols) and of *E. coli* B/r (filled symbols) are plotted against relative specific growth rate, μ_r . Data for the macromolecular content of *S. coelicolor* A3(2) are the means of at least three measurements; bars indicate the standard error. Data for *E. coli* B/r are derived from Bremer & Dennis (1987). μ_r was determined assuming $\mu_{\max}$ for *S. coelicolor* A3(2) and *E. coli* B/r to be 0.34 and 1.73 h⁻¹, respectively.

The linear relationship between RNA content per genome and specific growth rate observed for *S. coelicolor* A3(2) (Fig. 3) suggests that, as with *E. coli* B/r, the demands of growth for protein synthesis in this micro-organism are met by changes in the number of ribosomes. However, it should be noted that the maximum specific growth rate of *S. coelicolor* A3(2) is five times less than that of *E. coli* B/r. Thus, it might be argued that the demand for protein synthesis is considerably less in *S. coelicolor* A3(2). Assuming that the observed RNA content is mostly rRNA, and that ribosome structure is similar in the two organisms, the apparently higher RNA content of *S. coelicolor* A3(2) hyphae might be due to a low maximum specific ribosome activity, ribosomes operating submaximally, a higher proportion of ribosomes being present in an inactive form, and/or a higher turnover rate of rRNA. A relatively high ribosomal content may have evolved in streptomycetes to support the high proportion of extra-cellular protein synthesized by these organisms as an adaptation to growth on solid substrata (Shahab *et al.*, 1994).

Coordination of DNA synthesis with growth. Taking into account the differences in size and GC content of the *S. coelicolor* A3(2) and *E. coli* B/r genomes, the protein content per genome and number of genomes per cell mass were calculated (Table 1). These data are plotted against μ_r in Figs 3 and 4. The dependence of the number of genomes per cell mass on specific growth rate was far greater in *E. coli* B/r than in *S. coelicolor* A3(2) (Fig. 4). However, it is of interest that the concentration of genomes in the two organisms approaches approximately the same value, about 4×10^{12} genomes g⁻¹, when they are growing at their maximum specific rates. Values for

Fig. 4. Comparison of the dependence of number of genomes per cell mass on relative specific growth rate in *S. coelicolor* A3(2) and *E. coli* B/r. Number of genomes of *S. coelicolor* A3(2) (□) and of *E. coli* B/r (■) are plotted against relative specific growth rate, μ_r . Data for the macromolecular content of *S. coelicolor* A3(2) are the means of at least three measurements. Data for *E. coli* B/r are derived from Bremer & Dennis (1987). μ_r was determined assuming $\mu_{\max}$ for *S. coelicolor* A3(2) and *E. coli* B/r to be 0.34 and 1.73 h⁻¹, respectively.

protein per genome in *S. coelicolor* A3(2) were of a similar magnitude to those reported for *E. coli* B/r (Table 1) but appeared to vary independently of the specific growth rate (Fig. 3).

In *E. coli* B/r, the rate of DNA replication is controlled not by the rate of chain elongation, but by the rate of initiation from the origin of replication, *oriC*. Growth at doubling times below the minimum C+D period (the time required to replicate and segregate the chromosome) requires overlapping rounds of replication and results in the presence of partially replicated chromosomes at cell division. Thus the number of origins per genome increases with specific growth rate. Given this and the fact that the protein content per origin is relatively constant (reviewed by von Meyenburg & Hansen, 1987; Bremer & Churchward, 1991; Zyskind & Smith, 1992), the protein content per genome must increase proportionally with the number of origins per genome. Thus, in *E. coli* B/r growing at short doubling times, protein content per genome increases with specific growth rate (Fig. 3). At the doubling times at which *S. coelicolor* A3(2) grows this appears not to be the case (Fig. 3), implying that the number of origins per genome does not change significantly with specific growth rate.

Based on measurements of HGU, estimates of the mean size of *S. coelicolor* A3(2) cells indicate that cell size does not vary appreciably below specific growth rates of about 0.25 h⁻¹ (N. Shahab and others, unpublished). Changes in the size of *E. coli* B/r cells are most significant when the doubling time is less than the minimum C+D period of 60 min (Bremer & Dennis, 1987). Estimates of the C+D period in streptomycetes are rare. Miguélez *et al.* (1988) reported a C period for *S. antibioticus* of 90 min but did not indicate at what specific growth rate this was measured. The doubling times of *S. coelicolor* A3(2) in our experi-

ments ranged from 140 to 1730 min. Based on the observation that the number of genomes per cell mass is relatively constant (Fig. 4) and assuming that the mean mass of a *S. coelicolor* A3(2) cell does not change with specific growth rate, the number of genomes per cell should be constant. If this is the case it would indicate that, under the conditions used, the doubling time is larger than the C + D period and that overlapping rounds of DNA replication are not necessary for duplication of the *S. coelicolor* A3(2) chromosome.

The absence of overlapping rounds of DNA replication in *S. coelicolor* A3(2) may simply be a consequence of this organism's low maximum specific growth rate (as compared with *E. coli*). An alternative explanation is that mechanisms coordinating DNA replication and growth in *S. coelicolor* A3(2) may act to prevent re-initiation of chromosome replication during an ongoing round. This certainly appears to be the case in eukaryotic cells, which normally replicate their DNA only once between mitoses, re-initiation within existing replication bubbles being blocked (reviewed by Li & Deshaies, 1993; Coverly & Laskey, 1994). Regulatory mechanisms that block the segregation of replicating DNA molecules have presumably evolved to compensate for difficulties associated with the duplication of large, structurally complex genomes. The presence of such mechanisms in *S. coelicolor* A3(2) would have to be reconciled with the existence of a *dnaA* gene, DnaA boxes at *oriC* (Calcult & Schmidt, 1992; Calcult, 1994) and a single principal origin of replication (Musialowski *et al.*, 1994). Certainly a level of post-initiation control appears to exist in *E. coli* and *Bacillus subtilis* (Levine *et al.*, 1991, 1995), and might also be active in *S. coelicolor* A3(2). If eukaryotic-like regulatory mechanisms operate in *S. coelicolor* A3(2), the rate of DNA replication might be a significant determinant of the maximum specific growth rate of this organism. This would contrast with *E. coli*, where the maximum specific growth rate is determined mainly by the metabolic flux of substrates through primary and intermediary metabolism (Jensen & Pederson, 1990; Marr, 1991).

Conclusions

Qualitatively, modulation of macromolecular content by specific growth rate in *S. coelicolor* A3(2) appears to be similar to that of *E. coli* B/r in the following respects: (i) the majority of the biomass is made up of macromolecules, with protein representing the largest portion, followed by RNA, and DNA being the smallest; (ii) the relative proportions of the macromolecules changes with specific growth rate, the protein concentration decreasing, RNA concentration increasing and DNA concentration decreasing with an increase in the specific growth rate; (iii) the greatest relative change with specific growth rate is observed in the RNA portion.

The most striking differences between *S. coelicolor* A3(2), under the culture conditions used in this study, and *E. coli* B/r are that the RNA content of *S. coelicolor* A3(2) exceeds that of *E. coli* B/r at the same specific growth rate, and *S. coelicolor* A3(2) does not require or lacks the capability

to generate overlapping rounds of DNA synthesis to replicate its genome.

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REFERENCES

- Bremer, H. & Churchward, G. (1991). Control of cyclic chromosome-replication in *Escherichia coli*. *Microbiol Rev* **55**, 459–475.
- Bremer, H. & Dennis, P. P. (1987). Modulation of chemical composition and other parameters of the cell by growth rate. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 1527–1542. Edited by F. C. Niedhardt, J. L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.
- Burton, K. (1956). A study of the conditions and mechanisms of the diphenylamine reaction for colorimetric estimation of deoxyribonucleic acid. *Biochem J* **62**, 315–322.
- Calcult, M. J. (1994). Gene organization in the *dnaA*–*gyrA* region of the *Streptomyces coelicolor* chromosome. *Gene* **151**, 23–28.
- Calcult, M. J. & Schmidt, F. J. (1992). Conserved gene arrangement in the origin region of the *Streptomyces coelicolor* chromosome. *J Bacteriol* **174**, 3220–3226.
- Caldwell, I. Y. & Trinci, A. P. J. (1973). The growth unit of the mould *Geotrichum candidum*. *Arch Microbiol* **88**, 1–10.
- Campbell, A. (1957). Synchronization of cell division. *Bacteriol Rev* **21**, 263.
- Champness, W. C. & Chater, K. F. (1994). Regulation and integration of antibiotic production and morphological differentiation in *Streptomyces* species. In *Regulation of Bacterial Differentiation*, pp. 61–93. Edited by P. Piggot, C. P. Moran & P. Youngman. Washington, DC: American Society for Microbiology.
- Chater, K. F. (1992). Genetic regulation of secondary metabolic pathways in *Streptomyces*. In *Secondary Metabolites: Their Function and Evolution* (Ciba Foundation Symposium 171), pp. 144–162. Edited by D. J. Chadwick & J. Whelan. Chichester: Wiley.
- Chater, K. F. & Bibb, M. J. (1996). Regulation of bacterial antibiotic production. In *Biotechnology: Products of Secondary Metabolism*, vol. 7. Edited by H. Kleinkauf & H. von Dohren. Weinheim: VCH (in press).
- Churchward, G., Estiva, E. & Bremer, H. (1981). Growth rate dependent control of chromosome replication initiation in *Escherichia coli*. *J Bacteriol* **145**, 1232–1238.
- Churchward, G., Bremer, H. & Young, R. (1982). Macromolecular composition of bacteria. *J Theor Biol* **94**, 651–670.
- Cole, S. T. & Saint Girons, I. (1994). Bacterial genomics. *FEMS Microbiol Rev* **14**, 139–160.
- Coverly, D. & Laskey, R. A. (1994). Regulation of eukaryotic DNA replication. *Annu Rev Biochem* **63**, 745–776.
- Demain, A. L. (1989). Carbon source regulation of idiolite biosynthesis in Actinomycetes. In *Regulation of Secondary Metabolism*, pp. 127–134. Edited by S. Shapiro. Boca Raton, FL: CRC Press.
- Demain, A. L., Aharonowitz, Y. & Martin, J. F. (1983). Metabolic control of secondary metabolite pathways. In *Biochemistry and*

- Genetic Regulation of Commercially Important Antibiotics*, pp. 49–72. Edited by L. C. Vining. Reading, MA: Addison-Wesley.
- Esener, A. A., Roels, J. A., Kossen, N. W. F. & Roozenburg, J. W. H. (1981).** Description of microbial growth behaviour during the wash-out phase; determination of the maximum specific growth rate. *Eur J Appl Microbiol Biotechnol* **13**, 141–144.
- Gladek, A. & Zakrzewska, J. (1984).** Genome size of *Streptomyces*. *FEMS Microbiol Lett* **24**, 73–76.
- Herbert, D., Phipps, P. J. & Strange, R. E. (1971).** Chemical analysis of microbial cells. *Methods Microbiol* **5B**, 209–344.
- Hobbs, G., Frazer, C. M., Gardner, D. C. J., Cullum, J. A. & Oliver, S. G. (1989).** Dispersed growth of *Streptomyces* in liquid culture. *Appl Microbiol Biotechnol* **31**, 272–277.
- Hobbs, G., Frazer, C. M., Gardner, D. C. J., Flett, F. & Oliver, S. G. (1990).** Pigmented antibiotic production by *Streptomyces coelicolor* A3(2) – kinetics and the influence of nutrients. *J Gen Microbiol* **136**, 2291–2296.
- Hobbs, G., Obanye, A. I. C., Petty, J., Mason, J. C., Barratt, E., Gardner, D. C. J., Flett, F., Smith, C. P., Broda, P. & Oliver, S. G. (1992).** An integrated approach to studying regulation of production of the antibiotic methylenomycin by *Streptomyces coelicolor* A3(2). *J Bacteriol* **174**, 1487–1494.
- Hodgson, D. A. (1982).** Glucose repression of carbon source uptake and metabolism in *Streptomyces coelicolor* A3(2) and its perturbation in mutants resistant to 2-deoxyglucose. *J Gen Microbiol* **128**, 2417–2430.
- Hopwood, D. A. (1959).** Linkage and the mechanism of recombination in *Streptomyces coelicolor*. *Ann NY Acad Sci* **81**, 887–898.
- Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P., Ward, J. M. & Schrepf, H. (1985).** *Genetic Manipulation of Streptomyces: a Laboratory Manual*. Norwich: John Innes Foundation.
- Horinouchi, S. & Beppu, T. (1992).** Autoregulatory factors and communication in Actinomycetes. *Annu Rev Microbiol* **46**, 377–398.
- Jensen, K. F. & Pederson, S. (1990).** Metabolic growth rate control in *Escherichia coli* may be a consequence of subsaturation of the macromolecular biosynthetic apparatus with substrates and catalytic components. *Microbiol Rev* **54**, 89–100.
- Jinks-Robertson, S. & Nomura, M. (1987).** Ribosomes and tRNA. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 797–806. Edited by F. C. Neidhardt, J. L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.
- Kieser, H. M., Kieser, T. & Hopwood, D. A. (1992).** A combined genetic and physical map of the *Streptomyces coelicolor* A3(2) chromosome. *J Bacteriol* **174**, 5496–5507.
- Kretschmer, S. (1982).** Dependence of the mycelial growth pattern on the individual regulated cell cycle in *Streptomyces granaticolor*. *Z Allg Mikrobiol* **22**, 335–347.
- Leblond, P., Redenbach, M. & Cullum, J. (1993).** Physical map of the *Streptomyces lividans* 66 genome and comparison with that of the related strain. *Streptomyces coelicolor* A3(2). *J Bacteriol* **175**, 3422–3429.
- Levine, A., Vannier, F., Dehbi, M., Henckes, G. & Seror, S. J. (1991).** The stringent response blocks DNA replication outside the ori region in *Bacillus subtilis* and at the origin in *Escherichia coli*. *J Mol Biol* **219**, 605–613.
- Levine, A., Autret, S. & Seror, S. J. (1995).** A checkpoint involving RTP, the replication terminator protein, arrests replication downstream of the origin during the stringent response in *Bacillus subtilis*. *Mol Microbiol* **15**, 287–295.
- Li, J. J. & Deshaies, R. J. (1993).** Exercising self-restraint: discouraging illicit acts of S and M in eukaryotes. *Cell* **74**, 223–226.
- Lin, Y. S., Kiesre, H. M., Hopwood, D. A. & Chen, C. W. (1993).** The chromosomal DNA of *Streptomyces lividans* 66 is linear. *Mol Microbiol* **10**, 923–933.
- Maaløe, O. (1969).** An analysis of bacterial growth. *Dev Biol Suppl* **3**, 33–58.
- Marr, A. G. (1991).** Growth rate of *Escherichia coli*. *Microbiol Rev* **55**, 316–333.
- Martin, J. F. (1989).** Molecular mechanisms for the control by phosphate of the biosynthesis of antibiotics and other secondary metabolites. In *Regulation of Secondary Metabolism*, pp. 213–238. Edited by S. Shapiro. Boca Raton, FL: CRC Press.
- von Meyenburg, K. & Hansen, F. G. (1987).** Regulation of chromosome replication. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 797–806. Edited by F. C. Neidhardt, J. L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington DC: American Society for Microbiology.
- Miguélez, E. M., Martin, M. C., Manzanal, M. B. & Hardisson, C. (1988).** Hyphal growth in *Streptomyces*. In *Biology of Actinomycetes '88*, pp. 490–495. Edited by Y. Okami, T. Beppu & H. Ugawara. Tokyo: Japan Scientific Societies Press.
- Musialowski, M. S., Flett, F., Scott, G. B., Hobbs, G., Smith, C. P. & Oliver, S. G. (1994).** Functional evidence that the principal DNA-replication origin of the *Streptomyces coelicolor* chromosome is close to the *dnaA-gyrB* region. *J Bacteriol* **176**, 5123–5125.
- Neidhardt, F. C., Ingraham, J. L., Brooks Low, K., Magasanik, B., Schaechter, M. & Umbarger, H. E. (1987).** *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*. Washington DC: American Society for Microbiology.
- Nierlich, D. P. (1972).** Regulation of ribonucleic acid synthesis in growing bacterial cells. II. Control over the composition of newly made RNA. *J Mol Biol* **72**, 765–777.
- Ochi, K. (1987).** Metabolic initiation of differentiation and secondary metabolism by *Streptomyces griseus*: significance of the stringent response (ppGpp) and GTP content in relation to A-factor. *J Bacteriol* **169**, 3608–3616.
- Ozergin-Ulgen, K. & Mavituna, F. (1993).** Actinorhodin production by *Streptomyces coelicolor* A3(2) – kinetic-parameters related to growth, substrate uptake and production. *Appl Microbiol Biotechnol* **40**, 457–462.
- Prosser, J. I. & Tough, A. J. (1991).** Growth mechanisms and growth-kinetics of filamentous microorganisms. *Crit Rev Biotechnol* **10**, 253–274.
- Riesenberg, D. & Bergter, F. (1979).** Dependence of macromolecular composition and morphology of *Streptomyces hygroscopicus* on specific growth rate. *Z Allg Mikrobiol* **19**, 415–430.
- Schaechter, E., Maaløe, O. & Kjeldgaard, N. O. (1958).** Dependence on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. *J Gen Microbiol* **19**, 592–606.
- Shahab, N., Kamaruddin, K., Platt, J., Butler, P. R., Oliver, S. G. & Hobbs, G. (1994).** Cell physiology and antibiotic production of *Streptomyces coelicolor* grown on solid medium. *Biotechnol Lett* **16**, 1015–1020.
- Shapiro, S. (1989).** Nitrogen assimilation in Actinomycetes and the influence of nitrogen nutrition on Actinomycete secondary metabolism. In *Regulation of Secondary Metabolism*, pp. 135–212. Edited by S. Shapiro. Boca Raton, FL: CRC Press.

- Spahr, P. F. (1962).** Amino acid composition of ribosomes from *Escherichia coli*. *J Mol Biol* **4**, 395–406.
- Strauch, E., Takano, E., Baylis, H. A. & Bibb, M. J. (1991).** The stringent response in *Streptomyces coelicolor* A3(2). *Mol Microbiol* **5**, 289–298.
- Takano, E. & Bibb, M. J. (1994).** The stringent response, ppGpp and antibiotic production in *Streptomyces coelicolor* A3(2). *Actinomycetologica* **8**, 1–10.
- Takano, E., Gramajo, H. C., Strauch, E., Andres, N., White, J. & Bibb, M. J. (1992).** Transcriptional regulation of the *redD* transcriptional activator gene accounts for growth-phase-dependent production of the antibiotic undecylprodigiosin in *Streptomyces coelicolor* A3(2). *Mol Microbiol* **6**, 2797–2804.
- Usdin, K., Gertsch, K. & Kirby, R. (1984).** Evidence for the wide distribution of repetitive DNA sequences in the genus *Streptomyces*. *J Mol Evol* **20**, 25–30.
- Vining, L. C. (1986).** Secondary metabolism. In *Biotechnology*, pp. 20–38. Edited by E. Rehm & G. Reed. Weinheim: VCH.
- Waksman, S. A. & Henrici, A. T. (1943).** The nomenclature and classification of the Actinomycetes. *J Bacteriol* **46**, 337–341.
- Weltner, K., Grosjean, J., Schuster, P. & Weber, W. J. (1986).** *Mathematics for Engineers and Scientists*. Cheltenham: Stanley Thornes.
- Zyskind, J. W. & Smith, D. W. (1992).** DNA replication, the bacterial cell cycle and cell growth. *Cell* **69**, 5–8.

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