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Review

Medium-dependent control of the bacterial growth rate

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ABSTRACT

By combining results from previous studies of nutritional up-shifts we here re-investigate how bacteria adapt to different nutritional environments by adjusting their macromolecular composition for optimal growth. We demonstrate that, in contrast to a commonly held view the macromolecular composition of bacteria does not depend on the growth rate as an independent variable, but on three factors: (i) the genetic background (i.e. the strain used), (ii) the physiological history of the bacteria used for inoculation of a given growth medium, and (iii) the kind of nutrients in the growth medium. These factors determine the ribosome concentration and the average rate of protein synthesis per ribosome, and thus the growth rate. Immediately after a nutritional up-shift, the average number of ribosomes in the bacterial population increases exponentially with time at a rate which eventually is attained as the final post-shift growth rate of all cell components. After a nutritional up-shift from one minimal medium to another minimal medium of higher nutritional quality, ribosome and RNA polymerase syntheses are co-regulated and immediately increase by the same factor equal to the increase in the final growth rate. However, after an up-shift from a minimal medium to a medium containing all 20 amino acids, RNA polymerase and ribosome syntheses are no longer coregulated; a smaller rate of synthesis of RNA polymerase is compensated by a gradual increase in the fraction of free RNA polymerase, possibly due to a gradual saturation of mRNA promoters. We have also analyzed data from a recent publication, in which it was concluded that the macromolecular composition in terms of RNA/protein and RNA/DNA ratios is solely determined by the effector molecule ppGpp. Our analysis indicates that this is true only in special cases and that, in general, medium adaptation also depends on factors other than ppGpp.

1. Introduction

Understanding how bacteria adapt to different nutritional environments and achieve an optimization of their growth rate remains a major challenge in microbial physiology. Previously we used a systems biology approach to analyze the control of ribosomal RNA (rRNA) synthesis and its relation to growth rate in the model bacterium *Escherichia coli* [1]. In that review we described the factors and effectors that interact with the rRNA (*rrn*) promoter region or the RNA polymerase to affect the rate of rRNA synthesis. However, the mechanisms whereby the bacteria sense the nutritional content of the growth medium and connect this to the activity of the factors and effectors regulating the synthesis of ribosomes are complex and remain partly obscure. One way to interrogate the connections within this sensing and control network is to monitor over time the macromolecular adjustments that occur as bacteria transit from one particular nutritional environment to a second that causes a change in their rate of growth. The responses to the nutrient changes that occur during this transition begin immediately upon entering the new environment and continue over an extended period of time until a new steady-state of exponential growth is achieved. Below we describe and analyze these macromolecular adjustments following a *nutritional up-shift* into a growth medium with higher nutritional quality to gain a deeper understanding of the mechanisms that allow bacteria to adapt to different nutrient environments.

2. Relationship between macromolecular cell composition and bacterial growth rate

A widely accepted concept of bacterial physiology, known as “growth rate-dependent control” of physiological parameters was first introduced 40 years ago by Maaløe [2]. Recently this concept

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has been redefined as "growth medium-dependent control" and expanded to incorporate the observation that macromolecular composition and growth rate are also affected by the physiological history of a bacterial culture [3]. In the following, the major parameters and relationships affecting bacterial growth are explained within the context of these new concepts.

2.1. Definition of balanced, nonsynchronous, steady-state exponential growth

When bacteria of a given genetic background are brought into a medium containing all nutrients including oxygen (provided by aeration or shaking) at saturating, non-limiting concentrations, the culture gradually assumes a constant rate of nonsynchronous exponential growth, which can continue indefinitely as long as the culture is periodically diluted into fresh medium. Under such conditions, defined as "balanced, steady-state exponential growth" [2], the amount of every cellular component per unit volume of culture, measured in samples containing a large number of cells at varying stages of the cell cycle, increases with the same exponential function of time. This function defines the growth rate, given either as μ in doublings per hour, or by its reciprocal, the doubling time τ expressed in minutes ($\mu = 60/\tau$).

When biological problems are analyzed today from a biophysical perspective, i.e. "using mathematical tools to integrate experimental data into a logically consistent framework" [1], it is sometimes preferred to substitute the definitions of τ and μ, introduced by Maaløe, with τ_e and μ_e, respectively, where τ_e is the time required for an e-fold (2.71-fold) increase and the growth rate μ_e is defined as the reciprocal, $1/\tau_e$. Similarly, one might use τ_2 and μ_2 to represent the 2-fold increase that defines bacterial growth; $\tau_e = \tau_2/\ln 2$ and $\mu_e = \mu_2 \cdot (\ln 2/60)$. In the following whenever τ and μ are used without subscript (e.g., in Table 1 below), it always refers to the standard τ_2 and μ_2.

During exponential growth, the fractional increase per unit of time remains constant for any cell component X in the culture and equals μ_e, i.e. $(dX/dt)/X = \mu_e$. For example, if time is measured in minutes, then $100\mu_e$ represents the percent increase per minute of

Table 1
Parameters pertaining to the synthesis rates of ribosomes and RNA polymerase in exponentially growing *E. coli* B/r as a function of growth rate at 37 °C.

At τ (min) and μ (doublings/h):										
Parameter	Symbol	Units	$\tau \rightarrow$ 100	60	40	30	24	20	Observed parameter(s)	Footnote
			$\mu \rightarrow$ 0.6	1.0	1.5	2.0	2.5	3.0		
RNAP synthesizing stable RNA	Ψ_s	%	24	36	56	69	79	86	r_s/r_t, c_s, c_m	[a]
RNAP synthesizing rRNA	Ψ_r	%	21	31	48	59	68	74	Ψ_s, f_t	[b]
rRNA chain elong.	c_r	Nucl./s	85	85	85	85	85	85	Indirect	[c]
RNAP activity	β_p	%	15.5	16.8	17.6	21.9	28.2	36.2	r_s, r_m, c_s, c_m, N_p	[d]
RNAP/total protein	α_p	%	0.90	1.10	1.30	1.45	1.55	1.60	α_p	[e]
Peptide chain elong.	c_p	aa resid./s	13	18	21	22	22	22	Indirect	[f]
Ribosome activity	β_r	%	85	85	85	85	85	85	Indirect	[g]
Ribos. prot/tot. prot.	α_r	%	7.7	9.2	11.6	15.0	18.8	22.7	α_r	[h]
Ribosomes/cell	N_r	10^3 Ribos./cell	8.0	14.9	25.9	43.9	61.4	72.9	R_C, f_s, f_t	[i]
RNAP molec./cell	N_p	10^3 RNAP/cell	1.8	3.5	5.7	8.4	10.0	10.2	α_p, P_C	[j]
RNAP/ribosome	N_p/N_r	Factor	0.23	0.24	0.22	0.19	0.16	0.14	N_p, N_r	[k]
RNAP prot/rib.prot.	α_p/α_r	Factor	0.12	0.12	0.11	0.10	0.08	0.07	α_p, α_r	[l]
Factor (Equation (1a))	a	See text	0.030	0.049	0.078	0.121	0.177	0.248	Ψ_r, c_r, β_p	[m]
Factor (Equation (2a))	b	See text	0.0016	0.0027	0.0038	0.0044	0.0047	0.0048	α_p, c_p, β_r	[n]
Calc. growth rate	μ	Doublings/h	0.6	1.0	1.5	2.0	2.5	3.0	a, b	[o]
Change in a	f_a	Factor	1.0	1.6	2.6	4.0	5.9	8.3	a, a_1	[p]
Change in b	f_b	Factor	1.0	1.7	2.4	2.8	2.9	3.0	b, b_1	[q]
Change in μ	f_μ	Factor	1.0	1.7	2.5	3.3	4.2	5.0	μ, μ_1	[r]

[a] Fraction of active RNA polymerase synthesizing stable RNA (from Table 3 in [3], originally calculated: $\Psi_s = 1/\{1 + [1/(r_s/r_t) - 1]\ (c_s/c_m)\}$, using values for r_s/r_t, c_s and c_m shown in the same Table).

[b] Fraction of active RNA polymerase synthesizing rRNA, $\Psi_r = (1 - f_t)\ \Psi_s$, where f_t is the fraction of stable RNA that is tRNA = 0.14 (Table 1 in [3]).

[c] Stable RNA (or rRNA) chain elongation rate (from Table 3 in [3]; originally determined from the accumulation *rrn*-terminal 5S-rRNA or tRNA after stopping transcription initiation with rifampicin).

[d] Fraction of total RNA polymerase that is actively transcribing (from Table 3 in [3]; originally calculated using the relationship: $\beta_p = (r_s/c_s + r_m/c_m)/N_p$, using values for r_s, r_m, c_s, c_m, and N_p in the same Table).

[e] Fraction of total protein that is core RNA polymerase (from Table 3 in [3]; determined from the β and β′ subunit content measured after sodium dodecyl sulfate-gel electrophoresis).

[f] Peptide chain elongation rate (from Table 3 in [3]; calculated from the amount of protein per cell, P_C, and the number of active ribosomes per cell, $\beta_r\ N_r$, using the relationship $c_p = (\ln 2/\tau) \cdot P_C/(\beta_r \cdot N_r)$, as explained in the same table).

[g] Fraction of total ribosomes active in polypeptide synthesis (from Table 3 in [3], originally measured as fraction of ribosomes in polysomes, with a correction for active 70S ribosomes, as explained in the same table).

[h] Fraction of total protein that is ribosomal protein (from Table 3 in [3], originally determined as the fraction of labeled protein in 30S and 50S ribosomal particles).

[i] Number of ribosomes per cell (from Table 3 in [3], determined from the amount of total RNA per cell, R_C, the fraction of total RNA that is stable RNA, $f_s = 0.98$, the fraction of stable RNA that is tRNA, $f_t = 0.14$ and the number of RNA nucleotides per 70S ribosome, nucl./rib = 4566: $N_r = R_C f_s\ (1 - f_t)/(\text{nucl./rib})$.

[j] Number of core RNA polymerase per cell [from Table 3 in [3], calculated from the amount of protein per cell, P_C,the fraction of total protein that is RNA polymerase, α_p (this table, footnote [e]), and the number of amino acid residues per core RNA polymerase, aa/pol = 3707: $N_p = P_C\ \alpha_p/(\text{aa/pol})$].

[k] Number of RNA polymerase molecules per ribosome, N_p/N_r, using the values for N_p and N_r in this table (footnotes [i] and [j]).

[l] RNA polymerase protein per ribosomal protein, α_p/α_r, using the values for α_p and α_r in this table (footnotes [e] and [h]).

[m] Factor a in Equation (1a): $a = (\Psi_r\ c_r\ \beta_p)/(\text{nuc/rib})$, using the values for Ψ_r, c_r and β_p in this table (footnotes [b], [c], and [d]) and the number of nucleotides per 70 ribosome, (nuc/rib) = 4566.

[n] Factor b in Equation (2a): $b = (\alpha_p\ c_p\ \beta_r)/(\text{aa/pol})$, using the values for α_p, c_p and β_r in this table (footnotes [e], [f], and [g]) and the number of amino acid residues per core RNA polymerase, (aa/pol) = 3707.

[o] Calculated growth rate (doublings/h), using Equation (7): $\mu = (60/\ln 2)\sqrt{ab}$ with the values for a and b in this table (footnotes [m and n]).

[p] Change in a, $f_a = a(\mu > 0.6)/a\ (\mu = 0.6)$.

[q] Change in b, $f_b = b(\mu > 0.6)/b\ (\mu = 0.6)$.

[r] Change in μ, $f_\mu = (\mu > 0.6)/0.6$.

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