3 What controls the growth rate of *Escherichia coli*? Is it transport after all?

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Introduction

An intriguing question is which cellular properties are important in determining the maximal growth rates of microorganisms. Under unrestricted growth conditions, microorganisms exhibit their maximal growth rates; these maxima are dependent on the intrinsic properties of the microbial species in study, on the nature of the nutrients provided to the microbe, and on the external growth conditions. If cell function is organised as a 'democratic' hierarchy, then the regulation of a complex property, in general, occurs simultaneously at the levels of transcription, translation, transport, metabolism, signal transduction, and communication with the environment and with other cells. Therefore, beforehand no simple answer to the above question is to be expected. This study primarily concerns control of the growth rate of *Escherichia coli*. Significant control on the growth rate of *E. coli* could reside in any of the above levels of hierarchy.

Because numerous anabolic reactions require ATP, the H^+-ATPase is expected to be important for the physiology of the normal *E. coli* cell. Indeed, the physiology of a strain which has a deletion of all the genes in the *atp* operon (LM2800) has been shown to be seriously affected [1]. However, experiments in which the cellular concentration of the H^+-ATPase was varied around the normal concentration in the cell revealed that the H^+-ATPase exerts no control at all on the growth rate. The absence of control of the H^+-ATPase on the growth rate has been shown for cells grown with either glucose or succinate as free-energy substrates [2].

Similarly, the growth rate of cells grown with glucose under unrestricted growth conditions was not controlled by the membrane-bound glucose carrier $IICB^{Glc}$ (or any other enzyme) of the PTS system of *E. coli*, although this transporter exerted high control on the glucose uptake process itself [3]. In contrast, when control

was measured under restricted conditions in chemostat cultures, the response coefficient of the growth rate towards the $IICB^{Glc}$ concentration was significant, i.e., it amounted to 0.20–0.35 at 6–15 μM residual glucose levels [4].

E. coli atp mutants, which lack a functional H^+-ATPase complex, are capable of growth on glucose but not on the C_4-dicarboxylates succinate, malate, or fumarate (Suc^- phenotype). The Suc^- phenotype has traditionally been used to distinguish an *atp* deletion strain from the wild type. The *E. coli* C_4-dicarboxylates transporter is encoded by the *dctA* gene [5]. The wild type *dctA* gene was cloned into an IPTG-inducible expression vector, which was subsequently used to transform the *atp* deletion strain. When the *dctA* gene was expressed in *trans*, the *atp* deletion strain turned out to be capable of growth on the C_4-dicarboxylates. This shows that the reason why the *E. coli atp* mutant is unable to grow on C_4-dicarboxylates (Suc^- phenotype) is due to insufficient transport capacity for these substrates [6].

Growth on C_4-dicarboxylates requires active gluconeogenic enzymes. The gluconeogenenic enzyme phosphoenolpyruvate carboxykinase (*pck*) may exert some control on the growth rate of wild type *E. coli* strains on C_4-dicarboxylates [7]. Recently, the membrane-bound transporter for C_4-dicarboxylates has been shown to exert significant control on the growth rate of *atp* deletion strains of *E. coli* growing on plates with succinate or malate as the free-energy source [6].

This study then deals with experiments carried out to investigate whether the C_4-dicarboxylates transporter also exerts some control on the growth rate of *E. coli* strains that are already capable of growing on C_4-dicarboxylates by themselves.

Methods

The strains and plasmids used in this study are listed in Table 3.1. Plasmid pLAC is a pBR322-derived expression vector(pFH2106) that harbours a synthetic *lacUV5* promoter, two *lacO* operator sites, a poly-linker region, and a strong transcriptional terminator. It further carries the *lacI* gene, encoding the *lac*-repressor protein that binds to both *lacO* operators, conferring a tight un-induced repression of the cloned gene of interest.

Bacteria were pre-grown on rich (Luria-Bertani) medium agar plates; single colonies then served as inocula for subsequent experiments. Bacteria were grown on AB minimal medium, pH 7.0, supplemented with thiamine and the indicated carbon source [6]. Plates were incubated at 37°C and contained 2% agarose.

Table 3.1 Relevant characteristics of the strains and plasmids used in this study.

Strain/plasmid	Relevant characteristic
LM2800	*atp*$^-$ LacY$^+$ Suc$^-$
LM3305	Spontaneous Suc$^+$ mutant of LM2800
LM3559	Transposon-induced Suc$^+$ mutant of LM2800
LM3133	*atp*$^+$ LacY$^+$
LM3118	*atp*$^+$ LacY$^-$
LM3330	LM3133 transduced with the Suc$^+$ mutation
pLAC	pFH2106; IPTG-inducible expression vector
pLAC$^-$ *dctA*	pFH2106, carrying the wild type *dctA* gene

Table 3.2 Growth of strain LM3305 (*atp*$^-$ Suc$^+$), harbouring pLAC or pLAC-*dctA*. Colony sizes were scored after 72 h of growth on minimal malate medium plates. The colony size of the pLAC-*dctA* transformant at each IPTG concentration was measured relative to that of the pLAC transformant.

IPTG (μM)	LM3305(pLAC)	LM3305 (pLAC-dctA)
0	1.0	1.0
2	1.0	1.0
4	1.0	1.4
6	1.0	1.7
8	1.0	2.0

Results

Stimulation of growth of Suc$^+$ revertants of *atp*$^-$ strains by induction in *trans* of the *dctA* gene at intermediate IPTG levels

Previously, a spontaneous revertant (LM3305) of the*atp* deletion mutant was described [6]. This double mutant completely lacks the H^+-ATPase, but is capable of growth on C_4-dicarboxylates. Strain LM3305 was transformed with either the empty expression vector pLAC or pLAC harbouring the *dctA* gene and the resulting two transformants were grown on minimal malate medium plates to which varying amounts of IPTG (0, 2, 4, 6, or 8 μM) was added. A typical result is shown in Table 3.2.

The average colony size of the pLAC-*dctA* transformant is larger than that of the pLAC transformant at IPTG concentrations higher than 2 μM. As a control experiment, the two transformants were also grown in the presence or absence of IPTG on pyruvate, a substance that is not taken up via the C_4-dicarboxylates transporter. Even in the presence of 100 μM IPTG, growth of LM3305 harbouring pLAC-*dctA* was neither stimulated nor inhibited compared to the control transformant.

Table 3.3 Growth of strains LM3118(*atp*$^+$LacY$^-$) and LM3133 (*atp*$^+$LacY$^+$), harbouring pLAC or pLAC-*dctA*. Colony sizes were scored after 36 h of growth on minimal malate medium plates. The colony size of the pLAC-dctA transformants at each IPTG concentration was measured relative to that of corresponding pLAC transformants. The abbreviation var. denotes heterogeneity in colony sizes.

IPTG(μM)	LM3118 (pLAC)	LM3118 (pLAC-dctA)	LM3133 (pLAC)	LM3133 (pLAC-dctA)
0	1.0	1.0	1.0	1.0
3	1.0	1.5	1.0	2.0
6	1.0	2.5	1.0	3.0
9	1.0	2.0	1.0	3.0
12	1.0	2.0	1.0	2.0
15	1.0	var.	1.0	var.

Stimulation of growth of *atp*$^+$ strains by induction in *trans* of the *dctA* gene at intermediate IPTG levels

Wild type strains of *E. coli*, which have a functional H$^+$-ATPase, grow readily on C_4-dicarboxylates. Strains LM3133 (LacY$^+$) and LM3118 (LacY$^-$) were both transformed with either pLAC or pLAC-*dctA*. The four transformants were grown on minimal malate medium plates to which varying amounts of IPTG were added (0, 3, 6, 9, 12, or 15 μM). Growth on the plates was checked daily. After 36 h of growth, the average colony size was determined for all four transformants (Table 3.3).

Growth of wild type strains harbouring pLAC-*dctA* is not stimulated in the absence of IPTG, but is clearly stimulated in the range of 3–12 μM IPTG. At the highest IPTG concentration (15 μM), heterogeneity in colony sizes was observed; some colonies were bigger, others were smaller than that of the control transformants (see below).

Besides strain LM3133, strain LM3118, which is devoid of the Lac-permease protein, was used in order to avoid the positive cooperativity in the dependence of expression on the IPTG concentration [8]. However, in these experiments, no clear difference between the LacY$^-$ and the LacY$^+$ strain was observed (Table 3.3).

Strain LM3330 is a wild type strain (LM3133) that has been transduced with the transposon-induced Suc$^+$ mutation from strain LM3559. This strain grows faster in minimal medium containing C_4-dicarboxylates than the wild type strain LM3133. Nevertheless, growth of this strain when transformed with pLAC-*dctA* was even further stimulated by expression in *trans* of the *dctA* gene at intermediate (10 μM) IPTG levels as compared to the control transformant after 24 h of growth (not shown).

Inhibition of growth of *atp*$^+$ and *atp*$^-$ strains by induction in *trans* of the *dctA* gene at high IPTG levels

At the highest IPTG concentration used in Table 3.3 (15μM), heterogeneity in colony sizes was observed for LM3118 and LM3133.At 50 and 100 μM IPTG, growth was even severely inhibited (not shown). The same phenomenon has been observed for the spontaneous (LM3305) and the transposon-induced (LM3559) Suc$^+$ revertant strains, and the wild type strain transduced with the Suc$^+$ mutation (LM3330), provided they were transformed with pLAC-*dctA*. Our interpretation is that in cells harbouring pLAC-*dctA* excessive overproduction of transporter occurs on C_4-dicarboxylates plates in the presence of high levels of IPTG; subsequent incorporation of the transporter proteins in the membrane would then result in loss of structural integrity of the inner membrane.

Discussion

Altogether, the above findings obtained with *atp*$^-$ and *atp*$^+$ strains strongly suggest that the C_4-dicarboxylates transporter seems to control the growth rate to a significant degree in strains that are already able to grow on C_4-dicarboxylates in the absence or presence of H^+-ATPase activities. Growth of the various strains on C_4-dicarboxylates is further enhanced through induction in *trans* of the *dctA* gene at intermediate IPTG concentrations. To carry out control analysis, however, one needs to be able to measure precisely the cellular content of the enzyme of interest. The cellular content (activity) of the transporter was not measured in this study. Moreover, the growth rate should be measured in liquid steady-state cultures. Thus, the data presented in this study cannot be converted into response coefficients quantifying the control of the C_4-dicarboxylates transporter on the growth rate. Currently, a protocol is being developed for the C_4-dicarboxylates transporter on the basis of Western blotting.

An interesting follow-up question to be addressed in strains that grow faster due to a putative increased level of the C_4-dicarboxylates transporter would then be whether the H^+-ATPase has still zero control on the growth rate, as was found for the *E. coli* wild type strain, or whether it has gained significant control in these strains.

One route to gluconeogenesis occurs via PEP carboxykinase and another via malic enzyme and PEP-synthase (*pps*). Moreover, since the *atp* deletion strain grows easily on pyruvate, but not on malate, and both Suc$^+$ revertants grow easily on pyruvate as well as malate [6], the gluconeogenic enzymes operative in between the intermediates malate and pyruvate might also be of vital importance for the expression the Suc$^+$ phenotype of the revertants. Therefore, the control of malic enzyme on the growth rate will also be measured in strains with and without H^+-ATPase.

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