Mathematical Model of the *lac* Operon: Inducer Exclusion, Catabolite Repression, and Diauxic Growth on Glucose and Lactose

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A mathematical model of the lactose (*lac*) operon was developed to study diauxic growth on glucose and lactose. The model includes catabolite repression, inducer exclusion, lactose hydrolysis to glucose and galactose, and synthesis and degradation of allolactose. Two models for catabolite repression were tested: (i) cyclic AMP (cAMP) synthesis inversely correlated with the external glucose concentration and (ii) synthesis inversely correlated with the glucose transport rate. No significant differences in the two models were observed. In addition to synthesis, degradation and secretion of cAMP were also included in the model. Two models for the phosphorylation of the glucose produced from lactose hydrolysis were also tested: (i) phosphorylation by intracellular hexokinase and (ii) secretion of glucose and subsequent phosphorylation upon transport back into the cell. The latter model resulted in weak catabolite repression when the glucose produced from lactose was transported out of the cell, whereas the former model showed no catabolite repression during growth on lactose. Parameter sensitivity analysis indicates the importance of key parameters to *lac* operon expression and cell growth: the lactose and allolactose transformation rates by β-galactosidase and the glucose concentrations that affect catabolite repression and inducer exclusion. Large values of the allolactose hydrolysis rate resulted in low concentrations of allolactose, low-level expression of the *lac* operon, and slow growth due to limited import and metabolism of lactose; small values resulted in a high concentration of allolactose, high-level expression of the *lac* operon, and slow growth due to a limiting concentration of glucose 6-phosphate formed from allolactose. Changes in the rates of all β-galactosidase-catalyzed reactions showed similar behavior, but had more drastic effects on the growth rate. Changes in the glucose concentration that inhibited lactose transport could extend or contract the diauxic growth period during growth in the presence of glucose and lactose. Moreover, changes in the glucose concentration that affected catabolite repression affected the cAMP levels and *lac* operon expression, but had a lesser effect on the growth rate.

Introduction

The lactose (*lac*) operon encodes the genes in the pathway for the import of lactose into the cell and its transformation to glucose and galactose. Due to the large amount of information that has been acquired, this operon has served as a model for genetic control. It has also served as a workhorse for expression of heterologous genes. The use of the *lac* repressor and promoter for heterologous gene expression has motivated the formulation of mathematical models to describe induction of the *lac* operon by the inducer IPTG (Lee and Bailey, 1984a,b; Laffend and Shuler, 1994a). However, relatively few models have been developed to describe induction of the *lac* operon by lactose (Van Dedem and Moo-Young, 1975) or growth on mixed substrates (Straight and Ramkrishna, 1991; Ramakrishna *et al.*, 1996), and no models have considered the effects of catabolite repression and inducer exclusion on *lac* operon induction by lactose. Mathematical models of *lac* operon function during growth on glucose and lactose could give insight into the natural function of the various control mechanisms involved in *lac* operon regulation.

The *lac* operon is induced when lactose is transported into the cell, and a fraction of the lactose is transformed by β-galactosidase into the inducer allolactose. Allolactose binds to the *lac* repressor thereby derepressing expression of the genes for *lac* permease, transacetylase, and β-galactosidase. The remainder of the lactose and the allolactose are hydrolyzed to galactose and glucose, which then enter glycolysis and the Krebs cycle.

The *lac* operon is controlled by glucose at two levels: inducer exclusion and catabolite repression. Transport of lactose by the *lac* permease is inhibited by glucose, a phenomenon known as inducer exclusion. The second mechanism by which glucose affects the lactose operon is through catabolite repression. In the absence of glucose, cyclic AMP (cAMP) accumulates inside the cell where it binds to the cAMP receptor protein (CRP). The cAMP−CRP complex binds to the CRP binding region near the *lac* promoter and enhances transcription. In the absence of binding of the cAMP−CRP complex to the DNA, transcription of genes under control of catabolite repression is significantly reduced (Saier *et al.*, 1996).

We present a model to describe regulation of the *lac* operon in *Escherichia coli* during growth on lactose and glucose. The kinetic information centers on the control of the *lac* operon, the transport of lactose into the cell by *lac* permease, the formation of allolactose, the consumption of lactose and allolactose by β-galactosidase, and cell growth on an intermediate of glucose and lactose catabolism, glucose 6-phosphate (Glu6P). The model incorporates catabolite repression and inducer exclusion. Where possible, literature estimates for model parameters were used.

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Model Definition

Glucose Transport. Extracellular glucose is phosphorylated to Glu6P as it is transported into the cell by the phospho*enol*pyruvate:sugar phosphotransferase system (PTS) (Postma *et al.*, 1996). If one assumes that the number of PTS complexes in the cell membrane is proportional to the biomass,[1] X, and that the rate of glucose transport is dependent on the extracellular glucose concentration, $[Glu_{ext}]$, then the rate of glucose transport per unit biomass

$$V_{t,Glu} = k_{t,Glu}\left(\frac{[Glu_{ext}]}{[Glu_{ext}] + K_{t,Glu}}\right) \quad (1)$$

where $k_{t,Glu}$ is the glucose transport rate constant and $K_{t,Glu}$ is the saturation constant for glucose transport (see Table 1 for a list of parameters and Table 2 for a list of variables).

Catabolite Repression. The metabolism of carbohydrates other than glucose is inhibited when glucose is plentiful, a phenomenon known as catabolite repression (Postma *et al.*, 1996). The primary signal molecule for catabolite repression is cAMP, the concentration of which is modulated by synthesis by adenylate cyclase, degradation, and secretion (Epstein *et al.*, 1975; Fraser and Yamazaki, 1979). In the absence of extracellular glucose, the rate of synthesis (Peterkofsky and Gazdar, 1973; Pastan and Adhya, 1976) and, consequently, the intracellular concentration (Epstein *et al.*, 1975; Grossman *et al.*, 1984) of cAMP increases. It is thought that the phosphorylated form of the PTS enzyme IIA^{Glc} activates adenylate cyclase (Postma *et al.*, 1996; Saier *et al.*, 1996). However, the exact mechanism controlling cAMP synthesis has not been elucidated; therefore, two different mechanisms were examined in this model. For the first mechanism, it was assumed that cAMP synthesis is inhibited by the extracellular glucose concentration

$$V_{cAMP} = \frac{k_{cAMP}}{\rho}\left(\frac{K_{a,cAMP}}{[Glu_{ext}] + K_{a,cAMP}}\right) \quad (2a)$$

where $K_{a,cAMP}$ is the inhibition constant for the effect of glucose on cAMP synthesis, k_{cAMP} is the cAMP synthesis rate constant (Peterkofsky and Gazdar, 1973), and ρ is the density of the cell. For the second mechanism, it was assumed that the rate of glucose transport through the PTS, $V_{t,Glu}$, inhibits cAMP synthesis

$$V_{cAMP} = \frac{k_{cAMP}}{\rho}\left(\frac{K_{b,cAMP}}{V_{t,Glu} + K_{b,cAMP}}\right) \quad (2b)$$

where $K_{b,cAMP}$ is the inhibition constant for the effect of glucose transport on cAMP synthesis. We assumed that cAMP removal (through degradation or transport out of the cell) follows first-order kinetics (Epstein *et al.*, 1975). Since all intracellular concentrations in this model were in moles per gram of dry cell weight (DCW), the density was included to relate them to known constants (generally reported in molarity). The density was assumed to be constant.

The cAMP modulates transcription via a complex formed with CRP (Varmus *et al.*, 1970a,b; Tagami and Aiba, 1995). However, the idea that catabolite repression is controlled solely by cAMP accumulation has been challenged by several experimental results (Saier, 1976; Harman *et al.*, 1986; Ishizuka *et al.*, 1993; Tagami, 1995; Tagami and Aiba, 1995). A recent report concludes that glucose inhibits β-galactosidase synthesis in cells lacking a functional adenylate cyclase (Tagami, 1995). Another report suggests that the CRP concentration is also modulated by the extracellular glucose concentration (Ishizuka *et al.*, 1993). Due to the lack of information about how the CRP concentration affects catabolite repression, it was assumed that catabolite repression was modulated entirely by changes in cAMP levels and that the CRP concentration was constant and nonlimiting. Furthermore, the kinetics of cAMP binding to CRP were assumed to be rapid relative to the time scale of the model.

Induction and Repression of the lac Operon. The *lac* operon is controlled by binding of the tetrameric *lac* repressor to one or more of the three operator regions, of the CRP−cAMP complex to its specific DNA binding site, and of the CRP−cAMP complex to its specific DNA binding site. It has been proposed that binding of RNA polymerase to the nonproductive promoter prevents transcription from the productive promoter (Yu and Reznikoff, 1984, 1985). For the purposes of this model, the effects of the nonproductive promoter were included in the overall transcription rate constant ($k_{mRNA-ZYA}$), and the rate of *lacZYA* mRNA synthesis was assumed to be proportional to the gene concentration [G]

$$V_{mRNA-ZYA} = k_{mRNA-ZYA}\eta_1\eta_2\eta_3[G] \quad (3)$$

where η_1, η_2, and η_3 are the transcription efficiency factors that describe transcriptional control by RNA polymerase, catabolite repression, and the repressor, respectively. These efficiency factors are similar to those developed previously (Lee and Bailey, 1984a,b; Laffend and Shuler, 1994a) and are described below.

The first efficiency factor describes transcription initiation by RNA polymerase from the productive promoter in the *lac* operon and is equal to the fraction of total promoters occuppied by RNA polymerase holoenzyme

$$\eta_1 = \frac{[RNAP{:}\sigma{:}P]}{[P]} \quad (4)$$

where $[RNAP{:}\sigma{:}P]$ is the concentration of the complex of the RNA polymerase holoenzyme ($RNAP{:}\sigma$) and the promoter and [P] is the total concentration of promoters. Mass balances on all complexes involving the promoter, RNA polymerase core enzyme (RNAP), and the sigma factor (σ) were developed using equilibrium relationships (Appendix 1). Due to the complexity of these expressions, a Gauss−Newton algorithm was used to solve for the concentration of promoters occupied by RNA polymerase holoenzyme. This efficiency factor was constant since the concentrations of RNA polymerase, σ factor, and promoter were assumed to be constant.

The second efficiency factor describes the enhancement of transcription initiation by the binding of the CRP−cAMP complex to its binding site near the promoter (Malan *et al.*, 1984; Record *et al.*, 1996) and is equal to the fraction of total binding sites occuppied by the CRP−cAMP complex

$$\eta_2 = \frac{[CRP{:}cAMP{:}E]}{[E]} \quad (5)$$

where [CRP:cAMP:E] is the concentration of CRP:cAMP bound to its binding site in the *lac* operon (E) and [E] is the total concentration of these sites. Again, mass balances on all complexes involving CRP, cAMP, and the

[1] In expontentially-growing cells, the membrane surface area must increase in proportion to biomass.

Table 1. List of Parameters Used in the Model

parameter	definition	value	reference
		Glucose Transport	
$k_{t,Glu}$	influx rate constant for glucose transport	$1-3 \times 10^{-4}$ mol Glu/(g DCW min)	(Neijssel *et al.*, 1980)
$K_{t,Glu}$	saturation constant for glucose transport	15 μM	(Postma and Roseman, 1976)
$k_{out,Glu}$	efflux rate constant for glucose	0.4×10^{-4} L/(g DCW min)	calculated from Kramer (1996)
		Catabolite Repression	
k_{cAMP}	cAMP synthesis rate constant	1 μM cAMP/min	(Fraser and Yamazaki, 1979)
$K_{a,cAMP}$	cAMP synthesis inhibition constant	4×10^{-5} M	(Notley and Ferenci, 1995)
$K_{b,cAMP}$	cAMP synthesis inhibition constant	1×10^{-4} mol Glu/(g DCW min)	assumed
ρ	cell density	0.3×10^{3} g of DCW/L	calculated
k_{ex}	composite cAMP excretion and degradation rate	2.1 min^{-1}	(Epstein *et al.*, 1975)
		Induction and Repression of the *lac* Operon	
$k_{mRNA-Rep}$	repressor transcription rate constant	5.0 M $mRNA_{Rep}$/(M DNA min)	(Chen *et al.*, 1991)
$k_{d,mRNA-Rep}$	repressor mRNA decay rate constant	0.693 min^{-1}	assumed
k_{Rep}	repressor translation rate constant	5.0 M Rep/(M $mRNA_{Rep}$ min)	(Chen *et al.*, 1991)
$k_{d,Rep}$	repressor decay rate constant	0.1 min^{-1}	(Chen *et al.*, 1991)
$k_{mRNA-ZYA}$	$mRNA_{ZYA}$ transcription rate constant	839 mol $mRNA_{ZYA}$/(mol DNA min)	(Kennell and Riezman, 1977)
$k_{d,mRNA-ZYA}$	$mRNA_{ZYA}$ degradation rate constant	0.693 min^{-1}	(Varmus *et al.*, 1970)
$k_{\beta gal}$	βgal translation rate conststant	9.4 M enzyme/(M $mRNA_{ZYA}$ min)	(Kennell and Riezman, 1977)
k_{Perm}	permease translation rate constant	18.8 M enzyme/(M $mRNA_{ZYA}$ min)	(Kennell and Riezman, 1977)
k_d	protein decay rate constant	0.01 min^{-1}	(Kennell and Riezman, 1977)
[G]	*lacZYA* gene concentration	8.47×10^{-12} mol/g DCW	calculated[a]
$[G_{R]}$	*lac* repressor gene concentration	=[G]	calculated
[P]	promoter concentration	=[G]	calculated[b]
[RNAP]	RNA polymerase concentration	5×10^{-6} M	(Lee and Bailey, 1984)
[σ]	σ factor concentration	1×10^{-6} M	(Lee and Bailey, 1984)
[D]	concentration of nonspecific DNA binding sites	0.0118 M	calculated[a]
[E]	CRP:cAMP DNA binding site concentration	=[G]	calculated[b]
[CRP]	cAMP receptor protein concentration	2×10^{-6} M	(Anderson *et al.*, 1971)
[O]	operator concentration	$=3 \times$ [G]	calculated[b]
K_{np}	equilibrium association constant between RNAP and the promoter	1×10^{6} M^{-1}	(Lee and Bailey, 1984)
K_{nd}	equilibrium association constant between RNAP and nonspecific DNA	1×10^{6} M^{-1}	(deHaseth *et al.*, 1978; Lohman *et al.*, 1980; Lee and Bailey, 1984)
K_{ns}	equilibrium association constant between RNAP and σ factor	2×10^{9} M^{-1}	(Record *et al.*, 1996)
K_{nsp}	equilibrium association constant between RNAP:σ and the promoter	1×10^{9} M^{-1}	(Lee and Bailey, 1984)
K_{nsd}	equilibrium association constant between RNAP:σ and nonspecific DNA	1×10^{5} M^{-1}	(Lohman *et al.*, 1980; Record *et al.*, 1996)
K_{ce}	equilibrium association constant between CRP and its DNA binding site	1.1×10^{5} M^{-1}	(Hudson *et al.*, 1990)
K_{cd}	equilibrium association constant between CRP and nonspecific DNA	3.7×10^{3} M^{-1}	(Clore *et al.*, 1983)
K_{ca}	equilibrium association constant between CRP and cAMP	4×10^{4} M^{-1}	(Takahashi *et al.*, 1980)
K_{cae}	equilibrium association constant between CRP:cAMP and its DNA binding site	5×10^{10} M^{-1}	(Fried and Crothers, 1984)
K_{cad}	equilibrium association constant between CRP:cAMP and nonspecific DNA	1×10^{5} M^{-1}	(Kolb *et al.*, 1983; Lee and Bailey, 1984; Takahashi *et al.*, 1989)
K_{ro1}	equilibrium association constant between the repressor and operator 1	1×10^{13} M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)
K_{ro2}	equilibrium association constant between the repressor and operator 2	4×10^{11} M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)
K_{ro3}	equilibrium association constant between the repressor and operator 3	1×10^{10} M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)
λ_{12}	enhancement of binding of repressor to operator 1 byDNA looping of repressor:operator 1 complex	5	(Oehler *et al.*, 1994)[c]
λ_{13}	enhancement of binding of repressor to operator 1 by DNA looping of repressor:operator 3 complex	100	(Oehler *et al.*, 1994)[c]
λ_{23}	enhancement of binding of repressor to operator 2 by DNA looping of repressor:operator 3 complex	2000	(Oehler *et al.*, 1994)[c]
K_{rd}	equilibrium association constant between the repressor and nonspecific DNA binding sites	1×10^{5} M^{-1}	(von Hippel *et al.*, 1974; Lin and Riggs, 1975)
K_{rl}	equilibrium association constant between Rep and allolactose	1×10^{6} M^{-1}	(Jobe and Bourgeois, 1972; von Hippel *et al.*, 1974)
K_{rlo1}	equilibrium association constant between the Rep:Allo complex and the operator	1×10^{10} M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)[d]
K_{rlo2}	equilibrium association constant between the Rep:Allo complex and the operator	4×10^{8} M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)[d]
K_{rlo3}	equilibrium association constant between the Rep:Allo complex and the operator	1×10^{7} M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)[d]
K_{rlo1o2}	equilibrium association constant between the Rep:Allo complex and the operator	5×10^{10} M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)[d]
K_{rlo2o3}	equilibrium association constant between the Rep:Allo complex and the operator	8×10^{11} M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)[d]

Table 1 (Continued)

parameter	definition	value	reference
K_{rlo1o3}	equilibrium association constant between the Rep:Allo complex and the operator	1×10^9 M^{-1}	(Winter and von Hippel, 1981; Oehler *et al.*, 1994)[d]
K_{rld}	equilibrium association constant between the Rep:Allo complex and nonspecific DNA binding sites	1×10^5 M^{-1}	(von Hippel *et al.*, 1974)
	Degradation of Lactose and Allolactose		
$k_{cat,Lac-Allo}$	rate constant for transformation of lactose to allolactose	8.46×10^3 min^{-1}	(Huber *et al.*, 1976; Martinez-Bilbao *et al.*, 1991)
$k_{cat,Lac}$	hydrolysis rate for lactose	9.54×10^3 min^{-1}	(Huber *et al.*, 1976; Martinez-Bilbao *et al.*, 1991)
$K_{m,Lac}$	saturation constant for lactose transformation	1.4×10^{-4} M	(Martinez-Bilbao *et al.*, 1991)
$K_{m,Allo}$	saturation constant for allolactose hydrolysis	2.8×10^{-4} M	(Huber *et al.*, 1980)
$k_{cat,Allo}$	allolactose decay rate constant	1.8×10^4 min^{-1}	(Martinez-Bilbao *et al.*, 1991)
	Lactose Transport		
$K_{t,Lac}$	saturation constant for lactose uptake	2.6×10^{-4} M	(Huber *et al.*, 1980; Wright *et al.*, 1981; Page and West, 1984; Lolkema *et al.*, 1991)
$K_{i,Glu}$	lactose transport inhibition constant	2.71×10^{-4} M	(Winkler and Wilson, 1967)
$K_{t,Lac\ int}$	saturation constant for lactose efflux	1.462×10^{-2} M	(Lolkema *et al.*, 1991)
$k_{Lac,in}$	lactose uptake rate constant	2148 mol Lac/(mol permease min)	(Maloney and Wilson, 1978; Wright *et al.*, 1981, 1986; Lolkema *et al.*, 1991)
$k_{Lac,out}$	lactose excretion rate by permease	71.38 mol Lac/(mol permease min)	(Lolkema *et al.*, 1991)
	Cell Growth		
$K_{i,burden}$	constant for burden due to *lac* operon expression	3.18×10^{-9} mol/(g DCW min)	assumed
μ_{max}	maximum growth rate	1.4 h^{-1}	(Blanch and Clark, 1996)
K_s	Monod constant for Glu6P	3.98×10^{-8} M	(Blanch and Clark, 1996)
$Y_{X/Glu6P}$	yield coefficient for Glu6P	30.0 g of DCW/mol glu	(Belaich and Belaich, 1976)

[a] The *lac* operon is located at minute 7 on the chromosome. The average number of genes was calculated according to the equations in Bremer and Dennis (1987) assuming a doubling time of 45 min, a C period of 43 min, and a D period of 21 min. [b] The *lac* operon has two promoters, three repressor binding sites, and one CRP−cAMP binding site. [c] These numbers were calculated assuming that DNA looping decreased the volume space that the repressor needed to search before finding the second operator site. [d] Calculated from data in these references assuming that allolactose reduced the affinity of the repressor for the operator sites 1000-fold.

DNA binding site for the CRP−cAMP complex were developed from equilibrium relationships (Appendix 1).

The third efficiency factor describes the inhibition of transcription by the binding of the *lac* repressor protein to one of the three operator sites near the *lac* promoters and the derepression of transcription by the binding of allolactose to the repressor. It has been proposed that binding of the repressor to two of the operator sites is necessary for tight repression of transcription and that DNA looping between the two operators increases the local repressor concentration and stimulates binding of the repressor to multiple operators (Oehler *et al.*, 1994). Since all three of the operator sites must be free for transcription from the promoter, the third efficiency factor is equal to the product of the probabilities that each operator is free

$$\eta_3 = \left(\frac{[O1_f]}{[O1]}\right)\left(\frac{[O2_f]}{[O2]}\right)\left(\frac{[O3_f]}{[O3]}\right) \tag{6}$$

where [O1] is the total concentration of operator 1 and $[O1_f]$ is the concentration of free operator 1. Again, mass balances for all complexes involving the three operators (O1, O2, and O3), the repressor protein (Rep), and allolactose (Allo) were developed from equilibrium relationships (Appendix 1). The enhancement of repressor binding to two operator sites was also included in the mass balance.

Synthesis of β-galactosidase was assumed to be linearly dependent on the concentration of the *lacZYA* mRNA

$$V_{\beta gal} = k_{\beta gal}[mRNA_{ZYA}] \tag{7}$$

where $k_{\beta gal}$ is the translation rate constant. A similar expression was developed to describe the synthesis of permease

$$V_{Perm} = k_{Perm}[mRNA_{ZYA}] \tag{8}$$

Since β-galactosidase is a tetramer and the relative amount of *lacZ* mRNA is twice that of *lacY* mRNA (Kennell and Riezman, 1977), the rate constant for synthesis of β-galactosidase was assumed to be one-half that for the synthesis of permease to account for differences in productivity. Degradation and dilution (due to cell growth) of *lacZYA* mRNA, β-galactosidase, and permease were assumed to be first-order. Although it has been reported that the efficiency of translation affects the mRNA degradation rate (Iost and Dreyfus, 1995), these effects were not incorporated into the model.

In contrast to the *lacZYA* promoter, the repressor gene is constitutively expressed

$$V_{mRNA-Rep} = k_{mRNA-Rep}\eta_1[G_R] \tag{9}$$

where $k_{mRNA-Rep}$ is the transcription rate constant and

Table 2. List of Variables in the Model

variable	description	units
$[mRNA_{Rep}]$	lac repressor mRNA concentration	mol/g DCW
[Rep]	lac repressor concentration	mol/g DCW
$[mRNA_{ZYA}]$	*lacZYA* mRNA concentration	mol/g DCW
[βgal]	β-galactosidase concentration	mol/g DCW
[Perm]	lactose permease concentration	mol/g DCW
[Allo]	allolactose concentration	mol/g DCW
[cAMP]	internal cAMP concentration	mol/g DCW
X	mass of cells	g DCW
$[Lac_{int}]$	internal lactose concentration	mol/g DCW
$[Lac_{ext}]$	external lactose concentration	M
[Glu6P]	glucose-6-phosphate concentration	mol/g DCW
$[Glu_{ext}]$	external glucose concentration	M
$[Glu_{int}]$	internal glucose concentration	mol/g DCW

$[G_R]$ is the repressor gene concentration. Since there is no regulation of repressor synthesis, only the efficiency factor describing binding of RNA polymerase to the promoter (η_1) is necessary. The rate of translation is dependent on the repressor mRNA concentration $[mRNA_{Rep}]$

$$V_{Rep} = k_{Rep}[mRNA_{Rep}] \quad (10)$$

where k_{Rep} is the translation rate constant. Again, we have assumed first-order degradation and dilution (due to cell growth) of the repressor mRNA and protein.

Lactose Transport. Lactose is cotransported into the cell with a hydrogen ion by the *lac* permease (Wright *et al.*, 1981, 1986; Kaback, 1992). Transport of lactose by the permease is inhibited by extracellular glucose, a phenomenon known as inducer exclusion. Although the exact mechanism of inducer exclusion is not known, it is known that subunit IIA^{Glc} of the PTS interacts with the *lac* permease and prevents lactose transport in the presence of glucose (Osumi and Saier, 1982; Postma *et al.*, 1996; Saier *et al.*, 1996). It has been reported that glucose affects the transport rate constant $k_{Lac,in}$ rather than the saturation constant for lactose transport $K_{t,Lac}$ and does not affect efflux of lactose from the cell (Saier, 1976). For this model, it was assumed that transport of lactose followed saturation kinetics and was inhibited by the concentration of extracellular glucose $[Glu_{ext}]$

$$V_{t,Lac} = k_{Lac,in}\left\{\left(\frac{[Lac_{ext}]}{[Lac_{ext}] + K_{t,Lac}}\right)\left(\frac{K_{i,Glu}}{K_{i,Glu} + [Glu_{ext}]}\right) - \left(\frac{[Lac_{int}]}{[Lac_{int}] + K_{t,Lac}/\rho}\right)\right\}[Perm] \quad (11)$$

where $K_{i,Glu}$ is the lactose transport constant for inhibition by glucose, $[Lac_{ext}]$ is the extracellular lactose concentration, and [Perm] is the *lac* permease concentration. Since lactose transport is reversible, a term was included to account for lactose efflux dependent on the internal lactose concentration; it has been shown that lactose efflux is not affected by extracellular glucose.

Degradation of Lactose. Once inside the cell, a fraction of the lactose is transformed by β-galactosidase to allolactose and the remainder is hydrolyzed to glucose and galactose. Formation of allolactose from lactose was assumed to follow Michaelis−Menten kinetics (Huber *et al.*, 1976)

$$V_{Lac-Allo} = k_{Lac-Allo}\left(\frac{[Lac_{int}]}{[Lac_{int}] + K_{m,Lac}/\rho}\right)[\beta gal] \quad (12)$$

where $[Lac_{int}]$ is the intracellular lactose concentration, $[\beta gal]$ is the β-galactosidase concentration, $K_{m,Lac}$ is the saturation constant for lactose transformation, and $k_{Lac-Allo}$ is the rate constant for transformation of lactose to allolactose.

The hydrolysis of lactose to glucose and galactose by β-galactosidase was also assumed to follow Michaelis−Menten kinetics (Martinez-Bilbao *et al.*, 1991)

$$V_{cat,Lac} = k_{cat,Lac}\left(\frac{[Lac_{int}]}{[Lac_{int}] + K_{m,Lac}/\rho}\right)[\beta gal] \quad (13)$$

where $K_{m,Lac}$ is the saturation constant for lactose degradation (same as above) and $k_{cat,Lac}$ is the rate constant for transformation of lactose to glucose and galactose. It was also assumed that the kinetics for hydrolysis of allolactose is similar to that of lactose

$$V_{cat,Allo} = k_{cat,Allo}\left(\frac{[Allo]}{[Allo] + K_{m,Allo}/\rho}\right)[\beta gal] \quad (14)$$

where [Allo] is the intracellular lactose concentration, $K_{m,Allo}$ is the saturation constant for allolactose degradation, and $k_{cat,Allo}$ is the rate constant for hydrolysis of allolactose to glucose and galactose. The galactose is then converted to Glu6P by the enzymes of the Leloir pathway (Adhya, 1987); we neglected the kinetics for the enzymes of the Leloir and any other pathways beyond lactose degradation and assumed that the galactose formed from lactose was instantaneously transformed to Glu6P.

It is not known exactly what pathways the cell uses to consume the glucose formed from lactose and allolactose. One possibility is that the glucose is phosphorylated by hexokinase to Glu6P (Curtis and Epstein, 1975; Roehl and Vinopal, 1976; Fukuda *et al.*, 1983; Fraenkel, 1996). For this case, it was assumed again that the kinetics of phosphorylation were not limiting, such that the glucose was immediately phosphorylated to Glu6P.[2]

Yet another possibility is that the glucose diffuses out of the cell (Kramer, 1996)

$$V_{out,Glu} = PA(\rho[Glu_{int}] - [Glu_{ext}]) \quad (15)$$

where P is the membrane permeability, A is the membrane area, $[Glu_{int}]$ is the intracellular concentration of glucose, and $[Glu_{ext}]$ is the extracellular glucose concentration. During exponential growth of a culture, the total surface area of all cells is proportional to biomass. Thus, the amount of glucose transported out of the cell per unit biomass is

$$V_{out,Glu} = k_{out,Glu}(\rho[Glu_{int}] - [Glu_{ext}]) \quad (16)$$

where $k_{out,Glu}$ is the rate constant for glucose secretion. The glucose that diffuses out of the cell would then be transported back into the cell by the PTS and phosphorylated to Glu6P. For the case where the intracellular glucose formed by hydrolysis of lactose is transported out of the cell rather than phosphorylated inside the cell, $V_{t,Glu}$ and $V_{out,Glu}$ are nonzero. In contrast, when the intracellular glucose formed from lactose hydrolysis is phosphorylated by hexokinase inside the cell, $V_{t,Glu}$ and $V_{out,Glu}$ are zero during growth on lactose.

Cell Growth. It was assumed that the cell growth rate was dependent on the intracellular Glu6P concentration, whether that be generated from the hydrolysis of lactose to glucose and galactose and their subsequent transformation to Glu6P or by phosphorylation of glucose during transport into the cell by the PTS

$$\mu = \mu_{max}\left(\frac{[Glu6P]}{[Glu6P] + K_s/\rho}\right)\left(\frac{K_{i,burden}}{K_{i,burden} + V_{mRNA-ZYA}}\right) \quad (17)$$

where K_s is the Monod constant for growth on Glu6P and μ_{max} is the maximum specific growth rate. Thus, the rate of Glu6P utilization lumps both the biosynthetic requirements for Glu6P and the reactions to glycolysis. A term was included to account for the additional burden on cell growth caused by expression of the *lac* operon; $K_{i,burden}$ is the inhibition constant for the metabolic burden due to the *lac* operon expression rate $V_{mRNA-ZYA}$. The effect of *lac* operon expression on the growth rate was expected to be minimal, since a single chromosomal copy of the

[2] There is no evidence that this assumption is necessarily correct. However, it allows the use of a common intermediate, Glu6P, for glucose and galactose processing.

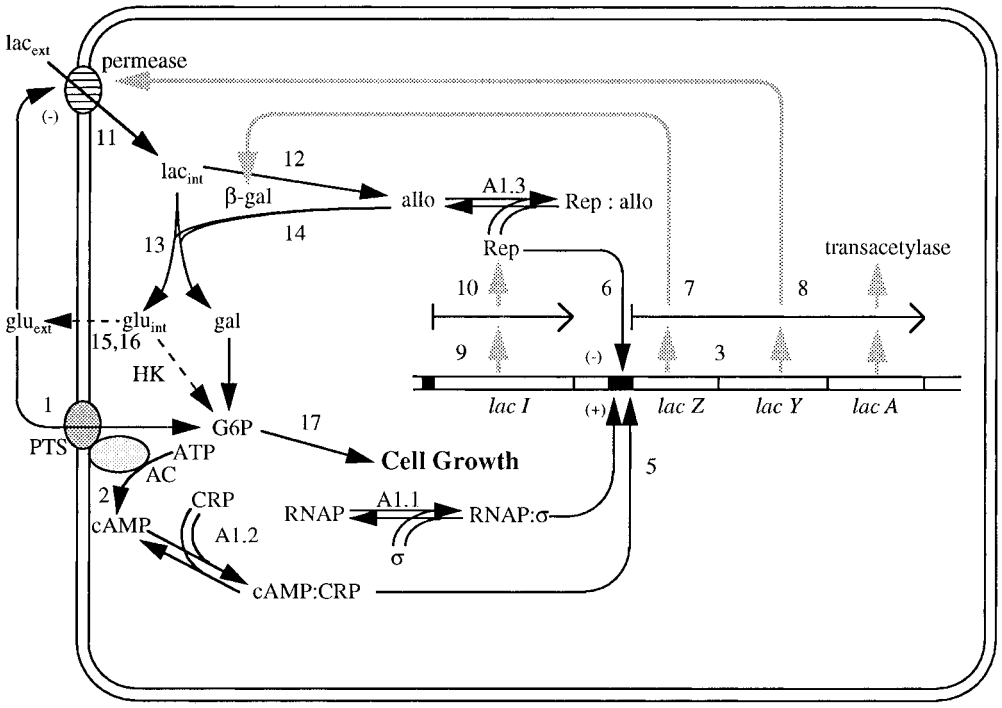

Figure 1. A diagram of the *lac* operon and PTS. See the description in the text. Shaded lines indicate transcription or translation. Dashed lines around glucose indicate two possible mechanisms of glucose phosphorylation. The numbers beside the arrows indicate the corresponding equation in the text or in Appendix 1. AC: adenylate cyclase. PTS: phospho*enol*pyruvate:sugar phosphotransferase system.

gene was expressed. The yield for growth on Glu6P was chosen to be the same as the literature values for the yield on glucose (Belaich and Belaich, 1976).

The reaction rates described above were incorporated into mass balances that are presented in Appendix 1. A diagram of the aspects of the *lac* operon that were incorporated into the model are shown in Figure 1. To analyze the transient response of the system, the equations were integrated using an algorithm suitable for stiff equations (Shampine and Reichelt, 1995). Initial conditions were selected for exponential growth on glucose. To determine the importance of various parameters in the model the extracellular glucose and lactose concentrations and the biomass were set constant and the steady-state solutions to the remaining equations were determined using a Gauss−Newton algorithm.

Results

Two models for the phosphorylation of the glucose generated from lactose hydrolysis and two models for the effect of glucose on the cAMP accumulation rate were developed, as described above, and tested. In the first model (model I, Appendix 1), it was assumed that the rate of cAMP production depended on the level of glucose in the medium and that the glucose formed from the hydrolysis of lactose was phosphorylated intracellularly by hexokinase. In this model, cells exhibited exponential growth on glucose and lactose and diauxic growth during the transition between glucose and lactose utilization (Figure 2a), consuming nearly all of the glucose before consuming the lactose (Figure 2b). During growth on glucose, the concentrations of cAMP (Figure 2c), *lacZYA* mRNA, β-galactosidase, and permease (Figure 2f) were low but nonzero, as a basal level of transcription of *lac* operon was maintained even when glucose was present. When all external glucose had been consumed, the Glu6P level dropped significantly (Figure 2d) and cAMP was produced. Lactose, which had been thus far excluded from entering the cell through the lactose permease by glucose in the medium, was also taken up and hydrolyzed to glucose and galactose (and subsequently transformed to Glu6P) or transformed to allolactose (Figure 2d). The increased allolactose and cAMP levels induced expression of the *lac* operon and produced *lacZYA* mRNA, β-galactosidase, and permease (Figure 2f). The predicted concentrations of these intermediates are similar to literature values (Epstein *et al.*, 1975; Kolb *et al.*, 1983). The observed changes in the repressor mRNA and protein levels (Figure 2e) were due to changes in growth rate (dilution) rather than to changes in gene expression or degradation. The doubling time for growth on lactose is slightly longer than that for growth on glucose. The slower growth rate on lactose reflects, primarily, the lower concentration of intracellular Glu6P than during growth on glucose and, secondarily, the burden of producing the additional enzymes for lactose degradation.

A second mechanism was postulated for phosphorylation of the glucose formed from the hydrolysis of lactose (model II, Appendix 1). It was assumed that the glucose diffused out of the cell (eq 16) and was phosphorylated to Glu6P during transport back into the cell by the PTS (eq 1). For this case, a low concentration of extracellular glucose (generated by lactose hydrolysis and secreted from the cell) was observed during growth on lactose (Figure 3b, time $\geq$ 550 min). This low concentration of glucose resulted in a slight decrease in the cAMP concentration (Figure 3c), an increase in catabolite repression, a subsequent decrease in *lacZYA* expression (Figure 3f), and much slower growth on lactose (Figure 3a) than in the prior case (Figure 2).

A second mechanism for cAMP synthesis was postulated in which the rate of cAMP synthesis was dependent on the glucose transport rate (eq 2b) rather than the extracellular glucose concentration (eq 2a). The simulation results were virtually identical to those cases in which cAMP synthesis was inversely correlated with the extracellular glucose concentration. The results are not shown.

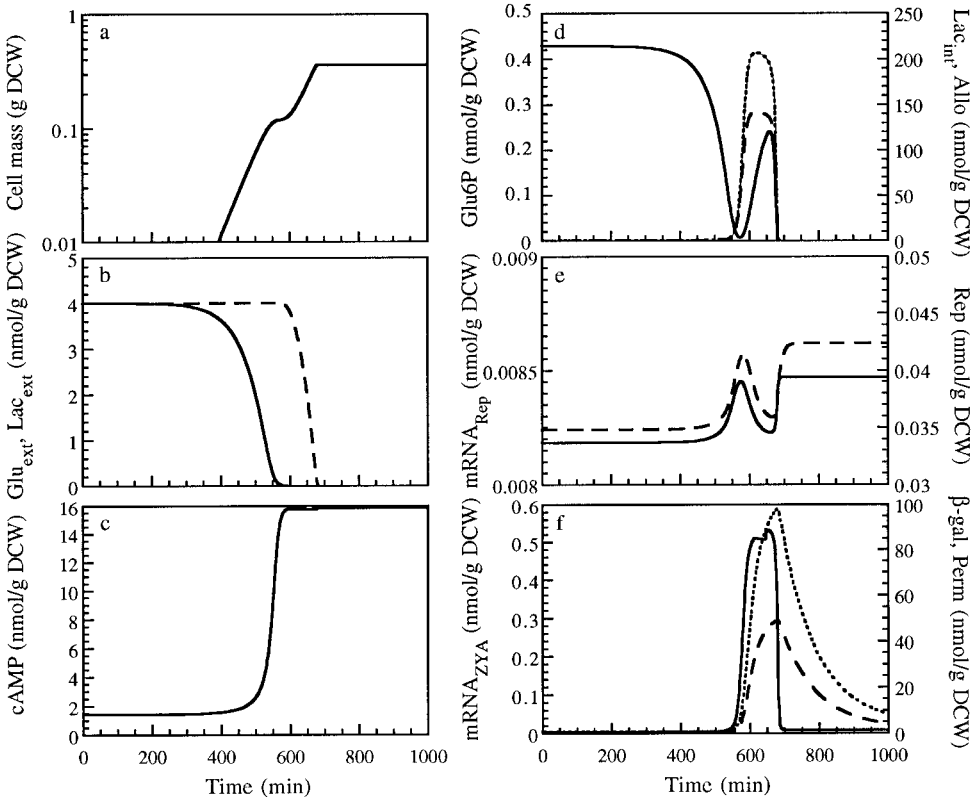

Figure 2. Diauxic growth on glucose and lactose. In this model, it has been assumed that glucose formed from the hydrolysis of lactose is phosphorylated internally by hexokinase. (a) Cell mass. (b) External glucose, solid line. External lactose, dashed line. (c) Intracellular cAMP concentration. (d) Intracellular glucose 6-phosphate concentration, solid line. Intracellular lactose concentration, dashed line. Intracellular allolactose concentration, dotted line. (e) $mRNA_{Rep}$ concentration, solid line. Repressor protein concentration, dashed line. (f) $mRNA_{ZYA}$ concentration, solid line. β-Galactosidase concentration, dashed line. Permease concentration, dotted line.

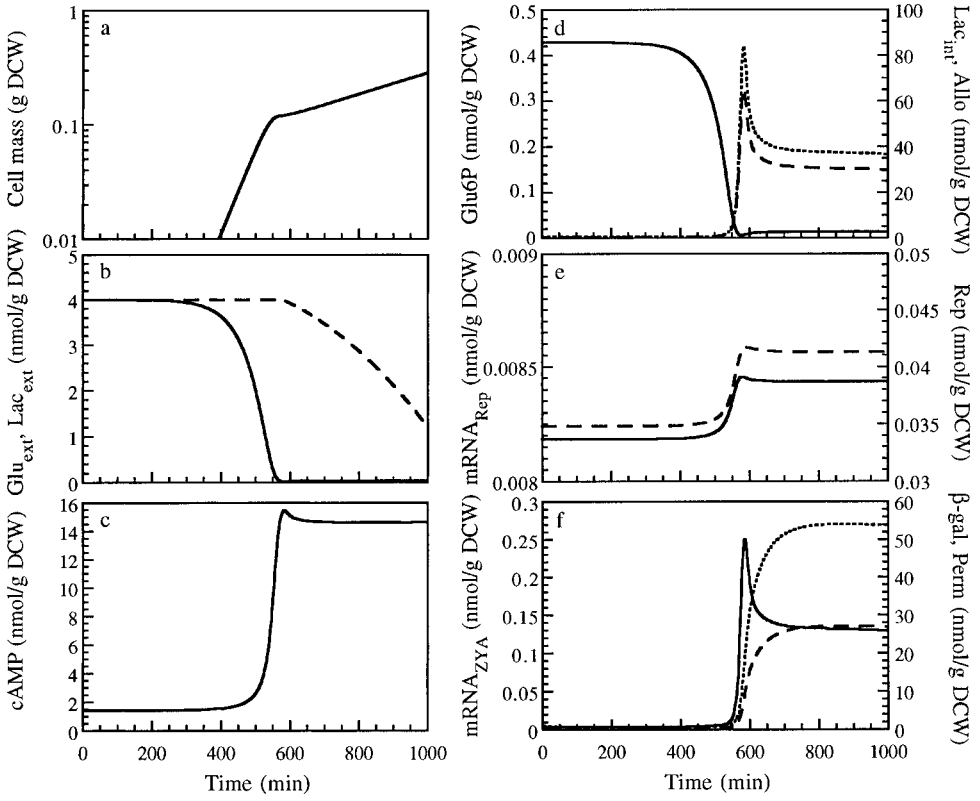

Figure 3. Responses of a batch culture growing on glucose and lactose. In this model, it has been assumed that glucose formed internally from the hydrolysis of lactose is secreted from the cell and phosphorylated during uptake. (a) Cell mass. (b) External glucose, solid line. External lactose, dashed line. (c) Intracellular cAMP concentration. (d) Intracellular glucose 6-phosphate concentration, solid line. Intracellular lactose concentration, dashed line. Intracellular allolactose concentration, dotted line. (e) $mRNA_{Rep}$ concentration, solid line. Repressor protein concentration, dashed line. (f) $mRNA_{ZYA}$ concentration, solid line. β-Galactosidase concentration, dashed line. Permease concentration, dotted line.

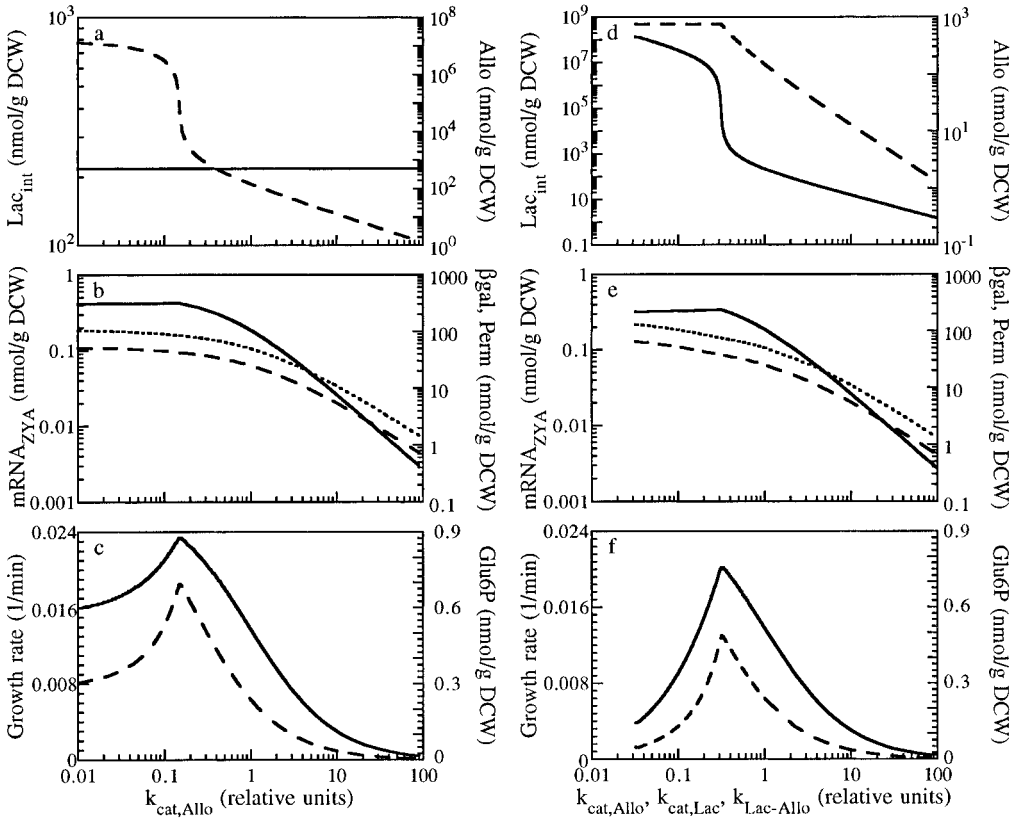

Figure 4. Effect of the lactose and allolactose hydrolysis rates and lactose−allolactose convervion rate by β-galactosidase on growth and induction of the *lac* operon. (a−c) Effect of variation in $k_{cat,Allo}$. (d−f) Effect of variation in $k_{cat,Allo}$, $k_{cat,Lac}$, and $k_{Lac-Allo}$. (a and d) Intracellular lactose concentration, solid line. Allolactose concentration, dashed line. (b and e) mRNA$_{ZYA}$ concentration, solid line. β-Galactosidase concentration, dashed line. Permease concentration, dotted line. (c and f) Growth rate, solid line. Glu6P concentration, dashed line.

Parameter Sensitivity Analysis. Parameter sensitivity was performed to determine the importance of key parameters. Some of the key parameters include the rate constants for processing of lactose and allolactose by β-galactosidase. With increasing allolactose hydrolysis rate ($k_{cat,Allo}$), the steady-state allolactose concentration decreased (Figure 4a), giving rise to decreased induction of the *lac* operon (Figure 4b) and slower growth (Figure 4c). In contrast, decreasing the allolactose hydrolysis rate gave rise to a high concentration of intracellular allolactose and subsequently increased induction of the *lac* operon. A maximum in the intracellular Glu6P concentration and the growth rate is found at approximately 20% of the reported allolactose hydrolysis rate, due to a large change in the allolactose concentration. The effect of the simultaneous changes in the lactose and allolactose hydrolysis rates had more drastic consequences, as very slow and very fast lactose and allolactose hydrolysis rates led to complete inhibition of growth (Figure 4d−f).

The extracellular glucose concentration affects lactose utilization via two mechanisms: catabolite repression and inducer exclusion. The two parameters that determine the glucose concentrations at which catabolite repression and inducer exclusion are modulated are $K_{i,cAMP}$ (the glucose concentration or transport rate at which the cAMP synthesis rate is at one-half of the maximum) and $K_{i,Glu}$ (the glucose concentration at which the lactose transport rate is at one-half of the maximum). The effect of the extracellular glucose concentration on cell growth and *lac* operon expression was determined for three values of these parameters. For large $K_{i,Glu}$ (100× the nominal value), low to moderate levels of extracellular glucose did not inhibit lactose import (Figure 5a, dashed line), the *lac* operon was induced (Figure 5c), and there was very little slowing of the growth rate during the transition from glucose to lactose utilization (Figure 5d). One would predict that there would be only a slight diauxic growth period for this case. For small $K_{i,Glu}$ (1/100 of the nominal value, dotted line), low to moderate extracellular glucose concentrations inhibited *lac* operon expression and significantly slowed growth during the transition from glucose to lactose utilization.

Changes in $K_{i,cAMP}$ had a significant impact on the cAMP concentration for a given extracellular glucose concentration (Figure 6b) but had no effect on the intracellular lactose or allolactose concentrations (Figure 6a). For small $K_{i,cAMP}$ (1/100 of the nominal value, dotted line), the growth rate was significantly slower during the period between lactose and glucose utilization than for the nominal value of $K_{i,cAMP}$ (Figure 6d). For large $K_{i,cAMP}$ (100× the nominal value, dashed line) had a significant effect on the cAMP concentration for a given glucose concentration but had no effect on the minimum growth rate during diauxic growth.

Discussion

A mathematical model has been developed to describe induction of the *lac* operon during diauxic growth of *E. coli* on glucose and lactose. Twelve or thirteen ordinary differential equations (depending on the model) describe cell growth, transport and utilization of glucose and lactose, catabolite repression, inducer exclusion, and induction and repression of the *lac* operon. The transient responses showed similar behavior to experimental data: glucose utilization prior to lactose utilization, slow growth between glucose and lactose utilization, and repression and induction of the *lac* operon.

Lactose is cotransported into the cell with a hydrogen ion. In this model, the effect of a changing transmem-

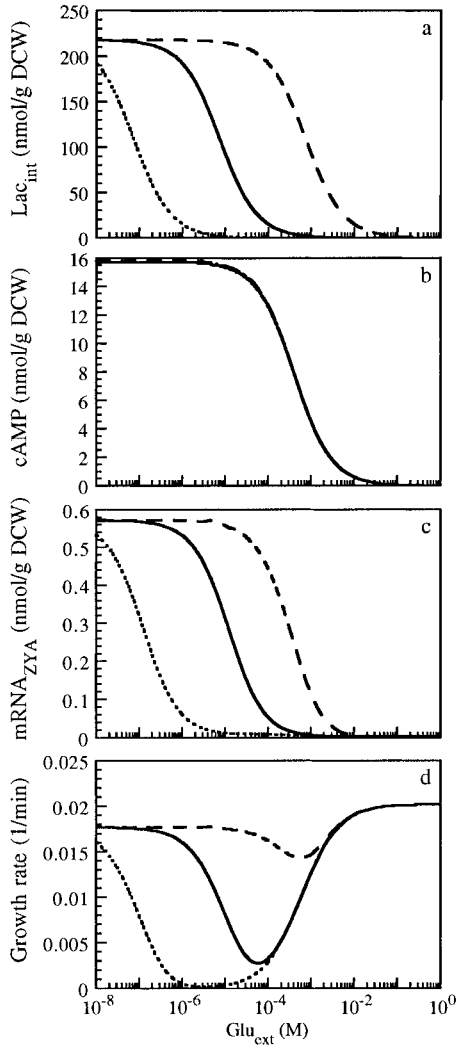

Figure 5. Effect of $K_{i,Glu}$ on lactose uptake and growth. Solid line, $1K_{i,Glu}$. Dashed line, $100K_{i,Glu}$. Dotted line, $0.01K_{i,Glu}$. (a) Intracellular lactose concentration. (b) cAMP concentration. (c) $mRNA_{ZYA}$ concentration. (d) Growth rate.

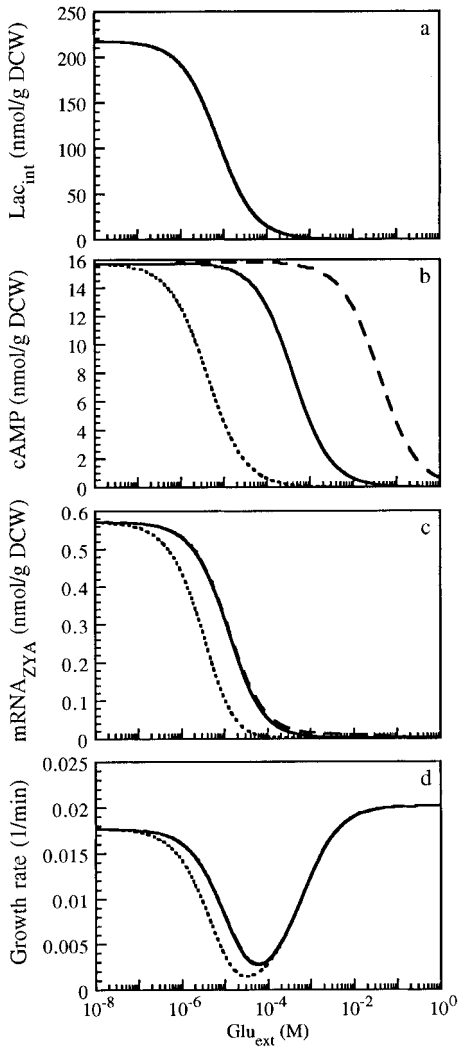

Figure 6. Effect of $K_{i,cAMP}$ on lactose uptake and growth. Solid line, $1K_{i,cAMP}$. Dashed line, $100K_{i,cAMP}$. Dotted line, $0.01K_{i,cAMP}$. (a) Intracellular lactose concentration. (b) cAMP concentration. (c) $mRNA_{ZYA}$ concentration. (d) Growth rate.

brane potential (electric potential and proton gradient) was neglected; it was assumed that this potential was constant. Although this is an appropriate assumption during exponential growth on glucose or lactose, it is probably not correct during the transition from one carbon source to another. Nonetheless, the effect of the transmembrane potential on the rate of lactose transport into the cell may be secondary to the effects of inducer exclusion and the production of permease during induction of the *lac* operon. In general, a decrease in the transmembrane potential during diauxic growth would decrease the rate of lactose import and slow growth during the exit from diauxic growth (Wright *et al.*, 1981).

In this study, two possible models for phosphorylation of internal glucose were investigated. The first model assumed that glucose formed from the hydrolysis of lactose was phosphorylated via hexokinase inside the cell. Enterobacteria are known to contain hexokinase (Curtis and Epstein, 1975; Roehl and Vinopal, 1976; Fukuda *et al.*, 1983; Fraenkel, 1996). Although its function in lactose metabolism has not been documented, it is possible that a primary function of hexokinase would be to phosphorylate glucose formed from the hydrolysis of lactose or other carbohydrates.

The second model assumed that the glucose resulting from lactose hydrolysis was secreted from the cell (actively or passively) and became phosphorylated upon reentry into the cell through the PTS. The diffusion of glucose from the cell during lactose metabolism limited the growth rate on lactose and resulted in a small increase in the extracellular glucose concentration and a subsequent increase in catabolite repression. Although this effect was noticeable in the simulation, it is doubtful that this phenomenon would be observable experimentally given the sensitivity and time scales necessary to detect it. Furthermore, it is not clear why the cell would choose to secrete such a valuable substrate into the medium where a competing organism could consume it.

Two models for catabolite repression were also investigated; both of these models assumed that changes in the cAMP concentration were responsible for catabolite repression. The first model assumed that the cAMP synthesis rate was inversely correlated with the extracellular glucose concentration. The second model assumed that the flux of glucose into the cell through the PTS determined the cAMP synthesis rate (Dumay and Danchin, 1996). There were no qualitative differences between these two models. Since the rate of glucose transport is linear with the glucose concentration (for low concentrations), the two models should exhibit similar behavior under conditions where the glucose concentration is low. It has also been reported that extracellular glucose stimulates the efflux of cAMP from the cell (Saier *et al.*, 1996). Although this mechanism might decrease

the amount of cAMP in the cell during the metabolism of lactose and subsequent efflux of glucose from the cell (model II), it should have no effect on model I in which glucose is phosphorylated intracellularly.

Both models incorporated highly simplified versions of catabolite repression. For example, it has been shown that the CRP concentration changes during changes in the extracellular glucose concentration (Ishizuka *et al.*, 1993) and that CRP can modulate catabolite repression in the absence of a functional adenylate cyclase gene (Tagami, 1995). Unfortunately, there was too little information about these aspects of catabolite repression to incorporate them into this mathematical model. Given that changes in protein levels would take place on time scales much slower than the synthesis and degradation of cAMP, the qualitative nature of the response of a model that incorporated CRP dynamics as well as those of cAMP may not be significantly different than that reported here.

In this model, we assumed that the concentrations of RNA polymerase, ribosomes, and any other proteins involved in protein synthesis were constant. This assumption is valid during exponential growth, but may be inadequate during diauxic growth. Inclusion of changes in the protein biosynthetic capacity of the cell would necessitate a significantly more complex model (Laffend and Shuler, 1994b). However, such increased complexity may only change the length of the diauxic growth phase and may not affect the qualitative nature of the response.

Parameter sensitivity analysis indicates the importance of the lactose and allolactose transformation rates by β-galactosidase. The maximum in the growth rate as a function of the transformation rate implies that the *lac* operon, in particular β-galactosidase, is critically balanced for maximal growth on lactose. That the peak in growth rate did not occur at the literature values for these constants may reflect discrepencies in system parameters or in the mathematical model itself. Given that the growth rate changes relatively little for large changes in the transformation rates, the control mechanisms for the *lac* operon must also be quite robust. However, for very low lactose and allolactose transformation rates, *lac* operon expression was maximal and could not be enhanced further to negate the effects of the low transformation rate.

Parameter sensitivity analysis also indicates that the glucose concentration that inhibits lactose transport is critically important to the growth rate during the transition from growth on glucose to growth on lactose. Increasing $K_{i,Glu}$ by 2 orders of magnitude resulted in a much smaller decrease in the growth rate during the transition, and decreasing $K_{i,Glu}$ caused a significant decrease in the growth rate during the transition. Thus, one would predict from the model results that any mutations that would result in a loss of inducer exclusion would result in a faster transition from growth on glucose to growth on lactose.

Acknowledgment

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Appendix 1

Mass balances for protein (RNA polymerase, repressor, and CRP), effectors (σ factor, allolactose, and cAMP), and specific binding sites (promoter, operator, and CRP−cAMP site) are given below. Binding of proteins to nonspecific sites (D) on the chromosome was also included. Equilibrium association constant were found in the literature and are listed in Table 1. Intracellular volume was assumed to be constant.

1. RNA polymerase, sigma factor, promoter interactions.

$$[\mathrm{RNAP}] = [\mathrm{RNAP_f}] + [\mathrm{RNAP:P}] + [\mathrm{RNAP}:\sigma] + [\mathrm{RNAP:D}] + [\mathrm{RNAP}:\sigma:\mathrm{P}] + [\mathrm{RNAP}:\sigma:\mathrm{D}]$$

$$[\sigma] = [\sigma_f] + [\mathrm{RNAP}:\sigma] + [\mathrm{RNAP}:\sigma:\mathrm{P}] + [\mathrm{RNAP}:\sigma:\mathrm{D}]$$

$$[\mathrm{P}] = [\mathrm{P_f}] + [\mathrm{RNAP:P}] + [\mathrm{RNAP}:\sigma:\mathrm{P}]$$

where

$$[\mathrm{RNAP}:\sigma] = K_{ns}\cdot[\mathrm{RNAP_f}]\cdot[\sigma_f]$$

$$[\mathrm{RNAP:P}] = K_{np}\cdot[\mathrm{RNAP_f}]\cdot[\mathrm{P_f}]$$

$$[\mathrm{RNAP:D}] = K_{nd}\cdot[\mathrm{RNAP_f}]\cdot[\mathrm{D}]$$

$$[\mathrm{RNAP}:\sigma:\mathrm{P}] = K_{nsp}\cdot[\mathrm{RNAP}:\sigma]\cdot[\mathrm{P_f}]$$

$$[\mathrm{RNAP}:\sigma:\mathrm{D}] = K_{nsd}\cdot[\mathrm{RNAP}:\sigma]\cdot[\mathrm{D_f}]$$

2. CRP, cAMP, CRP−cAMP binding site interactions.

$$[\mathrm{CRP}] = [\mathrm{CRP_f}] + [\mathrm{CRP:E}] + [\mathrm{CRP:cAMP}] + [\mathrm{CRP:D}] + [\mathrm{CRP:cAMP:E}] + [\mathrm{CRP:cAMP:D}]$$

$$[\mathrm{cAMP}] = [\mathrm{cAMP_f}] + [\mathrm{CRP:cAMP}] + [\mathrm{CRP:cAMP:E}] + [\mathrm{CRP:cAMP:D}]$$

$$[\mathrm{E}] = [\mathrm{E_f}] + [\mathrm{CRP:E}] + [\mathrm{CRP:cAMP:E}]$$

where

$$[\mathrm{CRP:cAMP}] = K_{ns}\cdot[\mathrm{CRP_f}]\cdot[\mathrm{cAMP_f}]$$

$$[\mathrm{CRP:E}] = K_{np}\cdot[\mathrm{CRP_f}]\cdot[\mathrm{E_f}]$$

$$[\mathrm{CRP:D}] = K_{nd}\cdot[\mathrm{CRP_f}]\cdot[\mathrm{D}]$$

$$[\mathrm{CRP:cAMP:E}] = K_{nsp}\cdot[\mathrm{CRP:cAMP}]\cdot[\mathrm{E_f}]$$

$$[\mathrm{CRP:cAMP:D}] = K_{nsd}\cdot[\mathrm{CRP:cAMP}]\cdot[\mathrm{D_f}]$$

3. Repressor, allolactose, operator interactions.

$$[\mathrm{Rep}] = [\mathrm{Rep_f}] + [\mathrm{Rep:Allo}] + [\mathrm{Rep:O1}] + [\mathrm{Rep:O2}] + [\mathrm{Rep:O3}] + [\mathrm{Rep:D}] + [\mathrm{Rep:Allo:O1}] + [\mathrm{Rep:Allo:O2}] + [\mathrm{Rep:Allo:O3}] + [\mathrm{Rep:Allo:D}] + [\mathrm{O1:Rep:Allo:O2}] + [\mathrm{O1:Rep:Allo:O3}] + [\mathrm{O2:Rep:Allo:O3}] + [\mathrm{O1:Rep:O2}] + [\mathrm{O1:Rep:O3}] + [\mathrm{O2:Rep:O3}]$$

$$[\mathrm{Allo}] = [\mathrm{Allo_f}] + [\mathrm{Rep:Allo:O1}] + [\mathrm{Rep:Allo:O2}] + [\mathrm{Rep:Allo:O3}] + [\mathrm{Rep:Allo:D}] + [\mathrm{O1:Rep:Allo:O2}] + [\mathrm{O1:Rep:Allo:O3}] + [\mathrm{O2:Rep:Allo:O3}] + [\mathrm{O1:Rep:O2}] + [\mathrm{O1:Rep:O3}] + [\mathrm{O2:Rep:O3}]$$

$$[\mathrm{O1}] = [\mathrm{O1_f}] + [\mathrm{Rep:O1}] + [\mathrm{Rep:Allo:O1}] + [\mathrm{O1:Rep:O2}] + [\mathrm{O1:Rep:O3}] + [\mathrm{O1:Rep:Allo:O2}] + [\mathrm{O1:Rep:Allo:O3}]$$

$$[\mathrm{O2}] = [\mathrm{O2_f}] + [\mathrm{Rep:O2}] + [\mathrm{Rep:Allo:O2}] + [\mathrm{O1:Rep:O2}] + [\mathrm{O1:Rep:Allo:O2}] + [\mathrm{O2:Rep:O3}] + [\mathrm{O2:Rep:Allo:O3}]$$

$$[\mathrm{O3}] = [\mathrm{O3_f}] + [\mathrm{Rep:O3}] + [\mathrm{Rep:Allo:O3}] + [\mathrm{O1:Rep:O3}] + [\mathrm{O1:Rep:Allo:O3}] + [\mathrm{O2:Rep:O3}] + [\mathrm{O2:Rep:Allo:O3}]$$

where

$$[\mathrm{Rep:Allo}] = K_{rl}\cdot[\mathrm{Rep_f}]\cdot[\mathrm{Allo_f}]$$

$$[\mathrm{Rep:O1}] = K_{ro1}\cdot[\mathrm{Rep_f}]\cdot[\mathrm{O1_f}]$$

$$[\mathrm{Rep:O2}] = K_{ro2}\cdot[\mathrm{Rep_f}]\cdot[\mathrm{O2_f}]$$

$$[\mathrm{Rep:O3}] = K_{ro3}\cdot[\mathrm{Rep_f}]\cdot[\mathrm{O3_f}]$$

$$[\mathrm{Rep:D}] = K_{rd}\cdot[\mathrm{Rep_f}]\cdot[\mathrm{D}]$$

$$[\mathrm{Rep:Allo:O1}] = K_{rlo1}\cdot[\mathrm{Rep:O1}]\cdot[\mathrm{Allo_f}]$$

$$[\mathrm{Rep:Allo:O2}] = K_{rlo2}\cdot[\mathrm{Rep:O2}]\cdot[\mathrm{Allo_f}]$$

$$[\mathrm{Rep:Allo:O3}] = K_{rlo3}\cdot[\mathrm{Rep:O3}]\cdot[\mathrm{Allo_f}]$$

$$[\mathrm{Rep:Allo:D}] = K_{rld}\cdot[\mathrm{Rep:Allo}]\cdot[\mathrm{D}]$$

$$[\mathrm{O1:Rep:O3}] = \lambda_{13}\cdot K_{ro1}\cdot[\mathrm{Rep:O3}]\cdot[\mathrm{O1_f}] = \lambda_{13}\cdot K_{ro3}\cdot[\mathrm{Rep:O1}]\cdot[\mathrm{O3_f}]$$

$$[\mathrm{O1:Rep:O2}] = \lambda_{12}\cdot K_{ro1}\cdot[\mathrm{Rep:O2}]\cdot[\mathrm{O1_f}] = \lambda_{12}\cdot K_{ro2}\cdot[\mathrm{Rep:O1}]\cdot[\mathrm{O2_f}]$$

$$[\mathrm{O2:Rep:O3}] = \lambda_{23}\cdot K_{ro2}\cdot[\mathrm{Rep:O3}]\cdot[\mathrm{O2_f}] = \lambda_{23}\cdot K_{ro3}\cdot[\mathrm{Rep:O2}]\cdot[\mathrm{O3_f}]$$

$$[\mathrm{O1:Rep:Allo:O3}] = K_{rlo1o3}\cdot[\mathrm{O1:Rep:O3}]\cdot[\mathrm{Allo_f}]$$

$$[\mathrm{O1:Rep:Allo:O2}] = K_{rlo1o2}\cdot[\mathrm{O1:Rep:O2}]\cdot[\mathrm{Allo_f}]$$

$$[\mathrm{O2:Rep:Allo:O3}] = K_{ro2o3}\cdot[\mathrm{O2:Rep:O3}]\cdot[\mathrm{Allo_f}]$$

Appendix 2

$$\frac{d[mRNA_{Rep}]}{dt} = V_{mRNA-Rep} - (k_{d,mRNA-Rep} + \mu)\cdot[mRNA_{Rep}]$$

$$\frac{d[Rep]}{dt} = V_{Rep} - (k_{d,Rep} + \mu)\cdot[Rep]$$

$$\frac{d[mRNA_{ZYA}]}{dt} = V_{mRNA-ZYA} - (k_{d,mRNA-ZYA} + \mu)\cdot[mRNA_{ZYA}]$$

$$\frac{d[\beta gal]}{dt} = V_{\beta gal} - (k_d + \mu)\cdot[\beta gal]$$

$$\frac{d[Perm]}{dt} = V_{Perm} - (k_d + \mu)\cdot[Perm]$$

$$\frac{d[Lac_{int}]}{dt} = V_{t,Lac} - V_{cat,Lac} - V_{Lac-Allo} - \mu\cdot[Lac_{int}]$$

$$\frac{d[Allo]}{dt} = V_{Lac-Allo} - V_{cat,Allo} - \mu\cdot[Allo]$$

$$\frac{d[cAMP]}{dt} = V_{cAMP} - (k_{ex} + \mu)\cdot[cAMP]$$

$$\frac{d[Glu_{ext}]}{dt} = (V_{out,Glu} - V_{t,Glu})\cdot X$$

$$\frac{d[Lac_{ext}]}{dt} = -V_{t,Lac}\cdot X$$

$$\frac{dX}{dt} = \mu X$$

$$\frac{d[Glu6P]}{dt} = V_{t,Glu} + 2\cdot(V_{cat,Lac} + V_{cat,Allo}) - \frac{\mu}{Y_{X/Glu6P}} - \mu\cdot[Glu6P] \quad \text{(model I only)}$$

$$= V_{t,Glu} + V_{cat,Lac} + V_{cat,Allo} - \frac{\mu}{Y_{X/Glu6P}} - \mu\cdot[Glu6P] \quad \text{(model II only)}$$

$$\frac{d[Glu_{int}]}{dt} = -V_{out,Glu} + V_{cat,Lac} + V_{cat,Allo} \quad \text{(model II only)}$$

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