Stoichiometric Model of *Escherichia coli* Metabolism: Incorporation of Growth-Rate Dependent Biomass Composition and Mechanistic Energy Requirements

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Abstract: A stoichiometric model of metabolism was developed to describe the balance of metabolic reactions during steady-state growth of *Escherichia coli* on glucose (or metabolic intermediates) and mineral salts. The model incorporates 153 reversible and 147 irreversible reactions and 289 metabolites from several metabolic data bases for the biosynthesis of the macromolecular precursors, coenzymes, and prosthetic groups necessary for synthesis of all cellular macromolecules. Correlations describing how the cellular composition changes with growth rate were developed from experimental data and were used to calculate the drain of precursors to macromolecules, coenzymes, and prosthetic groups from the metabolic network for the synthesis of those macromolecules at a specific growth rate. Energy requirements for macromolecular polymerization and proofreading, transport of metabolites, and maintenance of transmembrane gradients were included in the model rather than a lumped maintenance energy term. The underdetermined set of equations was solved using the Simplex algorithm, employing realistic objective functions and constraints; the drain of precursors, coenzymes, and prosthetic groups and the energy requirements for the synthesis of macromolecules served as the primary set of constraints. The model accurately predicted experimentally determined metabolic fluxes for aerobic growth on acetate or acetate plus glucose. In addition, the model predicted the genetic and metabolic regulation that must occur for growth under different conditions, such as the opening of the glyoxylate shunt during growth on acetate and the branching of the tricarboxylic acid cycle under anaerobic growth. Sensitivity analyses were performed to determine the flexibility of pathways and the effects of different rates and growth conditions on the distribution of fluxes. *Biotechnol Bioeng* **56:** 398–421, 1997.
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INTRODUCTION

A central problem in metabolic engineering is understanding how the cell balances its energy and biosynthetic needs (catabolism and anabolism) for optimal growth under various conditions. Simple carbon sources and mineral salts are taken into the cell and transformed into the complex biopolymers and cofactors that compose the cell, while generating the metabolic energy necessary to make these complex biomolecules.

There have been many attempts to study regulation of metabolism using mathematical models. The simplest description of cellular metabolism uses order of magnitude calculations to determine the metabolic yields from cell composition, measured substrate uptake and product synthesis, and growth rates (Blanch and Clark, 1996; Savinell et al., 1989). However, this analysis provides limited formation about the fluxes of intermediary metabolism. A more detailed analysis uses the stoichiometry of biochemical pathways and cell composition data to estimate the steady-state mass and energy distributions (Nissen et al., 1997; Savinell and Palsson, 1992a; Tsai et al., 1988; Vallino and Stephanopoulos, 1993; Varma et al., 1993); experimentally determined intermediary fluxes can be used as constraints to improve the accuracy of calculations. The most detailed level of metabolic pathway analysis examines the dynamic behavior of cell metabolism and requires kinetic and thermodynamic data, most of which is currently unavailable: kinetic parameter measurements are difficult to obtain and kinetic models developed from in vitro measurements may not apply in vivo.

Metabolic modeling, based on the stoichiometry of the reactions, does not require kinetic parameters and information about the kinetic mechanism of each enzyme. The growth kinetics of the cell are incorporated into the model through the energy and biomass requirements, which are functions of the growth rate. The stoichiometry of metabolism is well defined and variations among different cells are limited to a few reactions. There are two different methods of using stoichiometry to study bioreaction networks. The first method reduces the stoichiometric matrix to an overdetermined form and then uses linear regression to find the flux distribution (Tsai et al., 1988; Vallino and Stephanopoulos, 1993). Independent measurements must be added

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or a number of reactions must be removed or constrained to render the matrix nonsingular. Unfortunately, the pathways neglected for mathematical reasons may, in fact, be active. Removing entire pathways may cause large changes in the calculated fluxes. Several previous models solved an overdetermined system by constraining the stoichiometric matrix through inspection (Papoutsakis, 1984, Tsai et al., 1988).

The second method optimizes an underdetermined matrix using different objective functions and allows retention of the entire network. Applications of this approach to a subset of hybridoma, yeast, and *Escherichia coli* metabolism demonstrated the utility of this technique (Majewski and Domach, 1990; Savinell et al., 1989; Savinell and Palsson, 1992a,b; van Gulik and Heijnen, 1995; Varma and Palsson, 1993, 1994a,b; 1995; Varma et al., 1993). Majewski and Domach were able to predict the secretion of acetate during growth of *E. coli* on glucose (Majewski and Domach, 1990). Varma and Palsson showed the effect of oxygen availability on acetate secretion and the metabolic capabilities of *E. coli* to overproduce amino acids and other products (Varma and Palsson, 1993; 1994a,b; 1995; Varma et al., 1993). However, in all of these cases, the metabolic pathways were not complete, numerous reactions were lumped, and there was no accounting for the effects of growth rate on cellular composition and energy requirements.

We developed a detailed stoichiometric model of *E. coli* metabolism that includes a more complete data base of known reactions involved in the catabolism of glucose, acetate, or tricarboxylic acid (TCA) cycle intermediates. None of the pathways are lumped to reduce the matrix so that any future simulations studying deletions or mutations in pathways would not require generation of an entirely new stoichiometric matrix. A detailed stoichiometric matrix allows us to study deletions or mutations of individual enzymes by setting constraints of the flux values for those enzymes. The model uses the precursor requirements (calculated from the known composition of the bacterial cell) (Neidhardt et al., 1990) and solves for the fluxes through the internal metabolic pathways using linear optimization. Rather than include a ''maintenance energy'' term, energy demands for growth are calculated from mechanistic energy requirements for macromolecular polymerization and proofreading, transport of metabolites, and maintenance of transmembrane gradients. Experimental data on nutrient uptake and secretion can be incorporated into the model. The model is solved using linear optimization and predicts the metabolic regulation observed during growth under different conditions and on different carbon sources.

MODEL DEFINITION

The basis for this flux-based model of metabolism is a mass balance on the metabolites in *E. coli*,

$$\frac{d\mathbf{x}}{dt} = \mathbf{S} \cdot \mathbf{v} - \mathbf{b} \tag{1}$$

where **x** is the vector of metabolite concentrations ($n \times 1$ dimension), **S** is the stoichiometric matrix ($n \times m$ dimension), **v** is a vector of reaction rates or fluxes through the metabolic reactions ($m \times 1$ dimension), and **b** is the vector for consumption and secretion rates of metabolites and for biosynthetic requirements for cellular macromolecules ($n \times 1$ dimension). Under balanced growth conditions, the concentrations of intracellular metabolites are constant with time:

$$\mathbf{S} \cdot \mathbf{v} = \mathbf{b}. \tag{2}$$

The goal of this model is to determine how mass and energy is allocated within the network of metabolic reactions **(v).** We begin with descriptions of the composition of the cell (which determines **b**) and of the reactions involved in synthesizing the precursors and energy required for growth (which determine **S**).

Biomass and Energy Requirements

An average *E. coli* B/r cell growing exponentially at 37°C under aerobic conditions in glucose minimal medium with a doubling time of approximately 40 min has a dry weight of 2.8×10^{-13} g. The dry weight is 55% protein, 20.5% RNA, 3.1% DNA, 9.1% lipids, 3.4% lipopolysaccharides, 2.5% peptidoglycan, 2.5% glycogen, 0.4% polyamines, and 3.5% other metabolites, cofactors, and ions (Neidhardt, 1987). The types and amounts of precursors required to synthesize these macromolecules at a given growth rate were determined from the composition of each of the macromolecules: the amino acid composition of proteins and the nucleotide composition of RNA and DNA are listed in Table I, the phospholipid composition in Table II, and the fatty acid composition in Table III. The amounts of cofactors and energy carriers present per gram dry weight (DW) of biomass are listed in Table IV. The **b** vector contains these precursor requirements to account for synthesis of the cellular macromolecules.

In addition to the precursors required to synthesize macromolecules, energy and reducing equivalents are also required for growth. Table V presents the energy requirements for 1 g of *E. coli* B/r cells growing aerobically with a 40-min doubling time at 37°C in glucose minimal medium. The energy requirements for DNA production include that needed by helicase to unwind the helix, the synthesis of the primer RNA to Okazaki fragments and ligation of the fragments, proofreading by DNA polymerase III, adjustment of the torsional tension of each chromosomal domain, and methylation of newly synthesized DNA (Neidhardt et al., 1990). The energy requirements for stable RNA production include that for discarding segments of primary transcripts and that for modifications (Neidhardt et al., 1990). The energy requirements for protein synthesis include that for mRNA synthesis, charging tRNAs with amino acids and incorporation of amino acids into protein, and proofreading, assembly, and modification of the protein (Neidhardt et al.,

Table I. Precursor requirements for synthesis of 40-min cell.

Component	Percent	μmol/g DW
Amino acids		
Ala	9.60	488
Arg	5.53	281
Asn	4.51	229
Asp	4.51	229
Cys	1.71	87
Glu	4.92	250
Gln	4.92	250
Gly	4.92	582
His	1.77	90
Ile	5.43	276
Leu	8.42	428
Lys	6.42	326
Met	2.87	146
Phe	3.46	176
Pro	4.13	210
Ser	4.03	205
Thr	4.74	241
Trp	1.06	54
Tyr	2.58	131
Val	7.91	402
rNTPs		
ATP	26.2	165
GTP	32.2	203
CTP	20.0	126
UTP	21.6	136
dNTPS		
dATP	24.7	24.7
dGTP	25.4	25.4
dCTP	25.4	25.4
TTP	24.7	24.7

Amino acids, ribonucleotide triphosphates (rNTPs), and deoxyribonucleotide triphosphates (dNTPs) are given in percentage of the biopolymer and as micromoles per gram dry weight (μmol/g DW) of cells (Neidhardt, 1987).

1990). These energy requirements were also included in the **b** vector.

Growth-Rate Dependence of Cell Composition

The macromolecular composition and energy requirements listed above are not the same for cells growing at different rates. For example, RNA content increases with growth rate whereas DNA and protein contents decrease with the growth rate (Bremer and Dennis, 1996; Brunschede et al., 1977). To solve for the fluxes through the metabolic reactions for doubling times other than 40 min, correlations were developed from experimental data for RNA, DNA, protein, surface area (for membrane components), and glycogen content as a function of specific growth rate (μ) for exponentially growing cells or dilution rate for continuous cultures (Fig. 1, Table VI). As the macromolecular composition of the cell changes with growth rate, so must the energy requirements to synthesize these macromolecules, which were correlated with the macromolecular needs (Fig. 2). Because protein is one of the most energetically expensive macromolecules and because the relative amount of protein decreases with increasing growth rate, the total energy expended by the cell (per g DW) actually decreases with growth rate.

Metabolic Pathways

The transformation of a simple carbon source and mineral salts to the biomass and energy requirements for growth is facilitated through the metabolic reactions. The stoichiometric matrix **S** contains the stoichiometry of all reactions incorporated into the model. Included in this model were 153 reversible and 147 irreversible reactions (Appendix A) and 289 metabolites (Appendix B) compiled primarily from three sources: the Boehringer–Mannheim wall chart (Michal, 1993), chapters 14–19, 24–41, 44, 48, 49, 67, 69, 72, 75, and 87 of Neidhardt et al. (1996), and the Ecocyc data base (Karp et al., 1996). There are discrepancies between the number of reactions included in this model and the Ecocyc data base because a number of reactions in the Ecocyc data base have the same product but alternative substrates (e.g., NH_4^+ versus glutamine as a source for nitrogen) when only one of these is known to be used under physiological conditions in *E. coli.* Finally, a number of transport reactions were included in the model to account for uptake or secretion of inorganics or metabolites. In many cases, these transport steps deplete or enhance the transmembrane proton gradient.

Table II. Phospholipid composition of *E. coli* strains.

Specific growth rate (h^{-1})	1.3			1.04			0.92		
	PE%	PG%	CL%	PE%	PG%	CL%	PE%	PG%	CL%
Strains									
E. coli B/r				75.0	18.0	5.0			
E. coli B/r	77.0	21.3	1.1				78.0	20.4	1.5
E. coli B/r	74.8	20.6	2.3				75.1	20.3	1.9
E. coli AX14	71.3	19.2	3.4				69.5	19.4	4.6
E. coli K-12	67.1	18.7	5.1				68.8	19.3	5.7
Overall average	73.0	19.7	3.4				72.8	19.8	3.4
Overall SD	3.9	1.0	1.8				4.4	0.6	2.0

Data for a specific growth rate of 1.04 h^{-1} are from Neidhardt (1987). All other data are from Ballesta and Schaechter (1971). PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin.

Table III. Fatty acid composition of *E. coli* lipids.

Fatty acid	Total fatty acid (%)
Myristic acid, 14:00	2.68
Myristoleic acid, 14:01	7.70
Palmitic acid, 16:00	38.23
Palmitoleic acid, 16:01	10.74
Heptadecenoic acid, 17Δ	16.11
cis-Vaccenic acid, 18:00	0.90
Oleic acid, 18:01	17.91
Nonadecenoic acid, 19Δ	5.73

Data from Bright–Gaertner and Proulx (1972), Kanemasa et al. (1967), Mavis and Vagelos (1972), and Neidhardt (1987).

Although the stoichiometry of most biosynthetic reactions is well known, the required cofactor(s) for a particular reaction may not be so well known. When known, correct NADH or NADPH was used in the stoichiometry of a reaction. When it was known that NADP/NADPH and NAD/NADH could be used interchangeably for the same reaction, pathways for both reactions were included; however, if no information was available to determine which electron carrier is used, it was assumed that NAD/NADH was used for anabolism and NADP/NADPH was used for catabolism.

In contrast to most of the biosynthetic reactions that have well-known stoichiometry, the pathways involved in electron transport and oxidative phosphorylation have variable stoichiometry due to the use of different dehydrogenases and cytochromes: the NADH dehydrogenases NDH-I and NDH-II transport 2 H^+/e^- and 0 H^+/e^-, respectively (Gennis and Stewart, 1996); the cytochromes cyt bd and cyt bo3 transport 1 H^+/e^- and 2 H^+/e^-, respectively (Gennis and Stewart, 1996); the number of H^+ transported into the cell by the membrane bound H^+-ATPase to phosphorylate ADP has been estimated as 2–4, with 3 being the most likely (Harold and Maloney, 1996). Pathways for all possible stoichiometries were incorporated into the model. Thus, the P/O ratio can be a noninteger value (Neidhardt et al., 1990), because it is a function of multiple enzymes being used in parallel for respiration. The model simulates the noninteger P/O ratios by incorporating all known respiration pathways into the stoichiometric matrix and allowing fluxes through multiple pathways simultaneously.

Table IV. Amounts of cofactors, energy carriers, and other molecules in *E. coli*.

Metabolite	μmol/g DW
5-Methyl-THF	50.0
Putrescine	35.0
Spermidine	7.0
NAD	2.15
NADH	0.05
NADP	0.13
NADPH	0.4
UDP-Glucose (soluble pool)	3.0
ATP (soluble pool)	4.0
ADP (soluble pool)	2.0
AMP (soluble pool)	1.0

Data from Lowry et al. (1971) and Penfound and Foster (1996).

Table V. Energy requirements for polymerization and processing of macromolecules.

Process	Energy required	Reference
Protein synthesis and processing[a]		
Activation and incorporation	4.0	Neidhardt et al., 1990
mRNA synthesis	0.2	Neidhardt et al., 1990
Proofreading	0.1	Neidhardt et al., 1990
Assembly and modification	0.006	Neidhardt et al., 1990
RNA synthesis and processing[b]		
Discarding segments	0.38	Neidhardt et al., 1990
Modification	0.02	Neidhardt et al., 1990
DNA synthesis and processing[b]		
Unwinding helix	1.0	Neidhardt et al., 1990
Proofreading	0.36	Neidhardt et al., 1990
Discontinuous synthesis	0.006	Neidhardt et al., 1990
Negative supercoiling	0.005	Neidhardt et al., 1990
Methylation	0.001	Neidhardt et al., 1990
Membrane processes[c]		
Proton leakage	62.9	Maloney, 1987

Data from Neidhardt et al. (1990).
[a] μmol ATP/μmol amino acid.
[b] μmol ATP/μmol nucleotide.
[c] μmol H^+/g DW h.

Solution Method

For the system described above, the number of reactions (m) is greater than the number of metabolites (n). Because multiple solutions exist, linear optimization was used to determine the fluxes **(v).** Linear optimization requires objective functions for solution:

$$\text{minimize/maximize:} \quad Z = \sum_i c_i v_i \tag{3}$$

where c_i are the weights and v_i are the elements of the flux vector. Some of the objective functions that were used included minimization or maximization of ATP usage, substrate uptake, growth rate, and product synthesis. Typically, maximization of growth rate and minimization or maximization of metabolite secretion were used as objective functions.

For the objective of maximizing growth rate, the flux of precursors for a biomass composition corresponding to a particular growth rate was calculated using the relationships in Table VI. The amount of each precursor per unit time necessary for synthesis of biomass at a particular growth rate was used as the upper bound for the flux of each precursor, and the lower bound was set to zero. Then the model was forced to maximize the synthesis rate of each precursor for biomass composition, such as individual amino acids, nucleotides, and glycogen monomer units. If the model was unable to match the necessary flux of one or more of the precursors for a biomass composition consistent with a particular growth rate, the biomass composition was recalculated at another growth rate based on the precursor metabo-

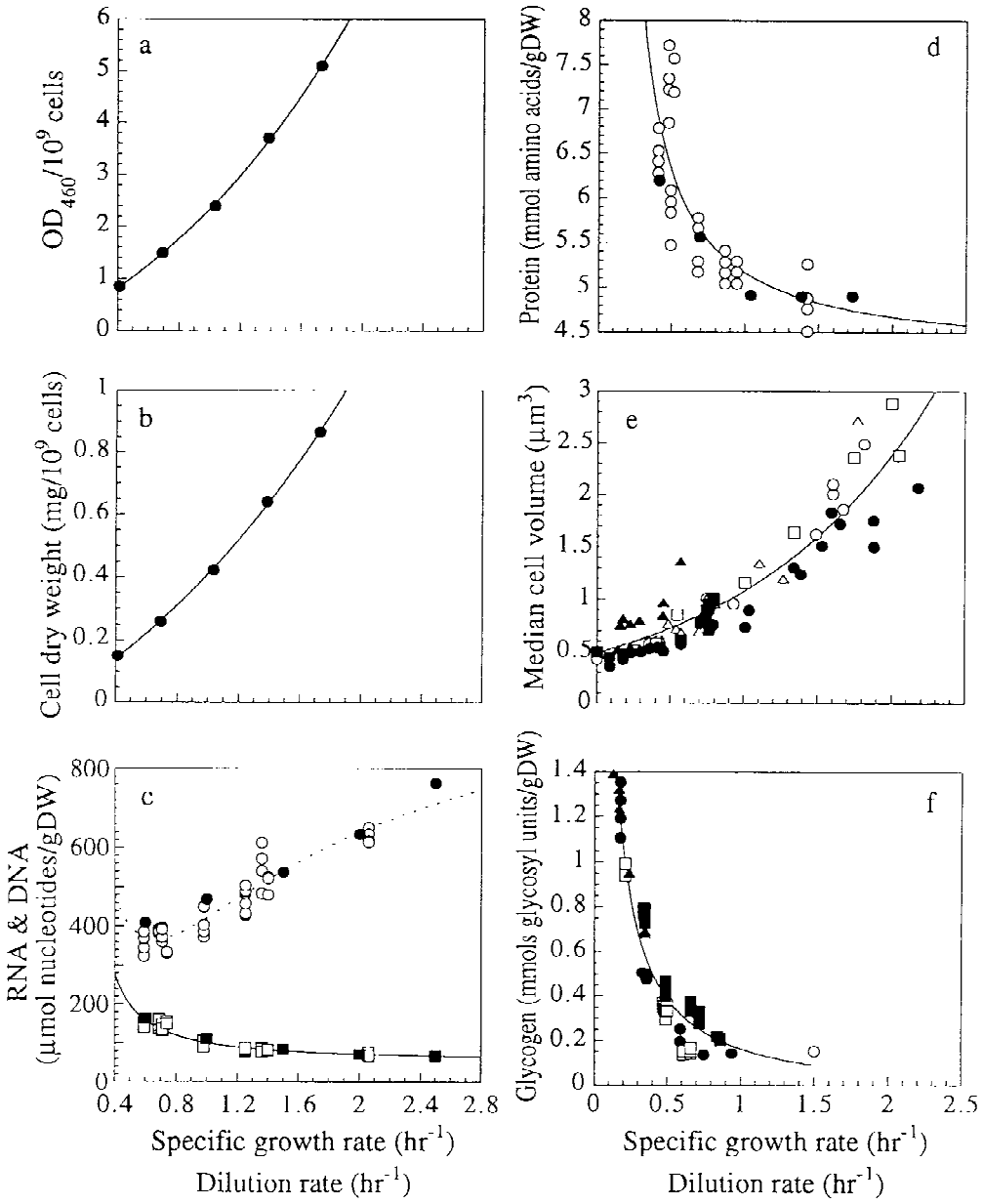

Figure 1. Variation in macromolecular composition and size of *E. coli* with growth rate. The equations in Table VI were fit to the data (lines in each plot). (a) Optical density per 10^9 cells as a function of specific growth rate (Bremer and Dennis, 1987). (b) Dry cell weight per 10^9 cells as a function of specific growth rate (Bremer and Dennis, 1987). (c) RNA and DNA (μmol per g DW) as a function of specific growth rate. Circles: RNA. Squares: DNA. Open symbols are from Brunschede et al. (1977). Closed symbols are from Bremer and Dennis (1987). (d) Protein (mmol per g DW) as a function of specific growth rate. Open symbols are from Brunschede et al. (1977). Closed symbols are from Bremer and Dennis (1987). (e) Median cell volume (μm^3) as a function of specific growth rate (Bremer and Dennis, 1987; Eckert and Schaechter, 1965; Shehata and Marr, 1971). (f) Glycosyl units (mmol per g DW) as a function of dilution rate. Circles are from Neidhardt (1987). All other data are from Holme (1957).

lite that was not synthesized at the rate calculated from the correlations. The model was resolved with the new biomass composition. If the synthesis fluxes were consistent with biomass composition at that growth rate, then the case was considered biologically feasible. Otherwise, the iterative procedure was repeated until the appropriate biomass requirements could be balanced for a particular carbon and energy source and growth rate.

In addition to the objective function, optimization requires constraints. Equation (2) served as one set of constraints. This constraint states that the mass balance must be satisfied and no accumulation of metabolites is allowed. Another set of constraints included the minimum (α_i) and/or maximum (β_i) of allowable fluxes:

$$\alpha_i < v_i < \beta_i \quad i = 1, 2, 3, \ldots, m. \tag{4}$$

Because fluxes were defined as positive values only, the individual fluxes had a lower limit of zero and an upper limit of infinity. Reversible reactions were divided into two positive reactions in opposite directions. Experimental data for individual fluxes can be used as either lower and/or

Table VI. Equations for growth-rate dependent biomass composition.

Component	Correlation	Reference
Optical density ($OD_{460}/10^9$ cells)	$-2.688 + 2.748 \cdot 2^{0.869\,\mu}$	Calculated from data in Figure 1(a)
Cell mass (10^{-12} g DW)	$-0.636 + 0.635 \cdot 2^{0.718\,\mu}$	Calculated from data in Figure 1(b)
RNA[a,b] (μmol NTPs/g DW)	$1139.5 - \frac{966.5}{\mu} \cdot 2^{-0.665/\mu}$	Calculated from data in Figure 1(c)
DNA[b] (μmol dNTPs/g DW)	$\frac{100 \cdot \mu}{0.023}(2^{(0.017+0.663)/\mu} - 2^{0.663/\mu})$	Calculated from data in Figure 1(c)
Protein[b] (mmol AAs/g DW)	$4.228 \cdot 2^{0.288/\mu}$	Calculated from data in Figure 1(d)
Cell volume (μm^3)	$0.486 \cdot 2^{1.144\mu}$	Calculated from data in Figure 1(e)
Cell radius (R) (μm)	$0.293 \cdot 2^{0.41\mu}$	
Cell length (L) (μm)	$2 \cdot 2^{0.333\mu}$	Donachie and Robinson, 1987
Surface area[c] (μm^2)	$2\pi R\,(L - 2R) + 4\pi R^2$	
Glycogen (glycosyl units)	$10^3(1-2^{-3.24 \cdot 10^4/\mu})$	Calculated from data in Figure 1f

[a]It has been speculated that the increase in the RNA content with growth rate is due to the larger fraction of stable RNA (rRNA, tRNA) necessary for the increase in protein production rate.

[b]Although the amino acid and nucleotide compositions of protein and DNA and RNA, respectively, may vary under different growth conditions, the amino acid and nucleotide fractions were available for only a cell with a 40-min doubling time (Fig. 1); it was assumed that this composition was maintained at all growth rates.

[c]The radius and length are then used to calculate the surface area (A) of the cell, assuming that the cell is a cylinder with hemispherical caps. The lipid composition was calculated from the surface area. For a cell with a 40-min doubling time, phosphatidylethanolamine makes up 75% of these lipids, phosphatidylglycerol 18%, and cardiolipin 5% with only trace amounts of phosphatidylserine (Table II) (Ballesta and Schaechter, 1971; Neidhardt, 1987). The lipopolysaccharide content of the 40-min cell is 8.4 μmol/g DW and the peptidoglycan content is 8.4 μmol/g DW (Neidhardt, 1987). The fatty acid composition found in total lipids is presented in Bright–Gaertner and Proulx (1972), Kanemasa et al. (1967), Mavis and Vagelos (1972), and Neidhardt (1987). Because the lipid and fatty acid composition was available for only a cell with 40-min doubling time, it was assumed that the composition did not change with growth rate (data from Table III averaged).

upper constraints, or the flux value can be set to the value of the experimental data. Because a range of values is allowed for the individual flux constraints, variability in experimental data can be incorporated into the model. In this model the internal fluxes were not constrained because the goal was to predict the fluxes and compare them with experimental data.

The model was solved using the Simplex subroutines in the OSL package (IBM, 1992). This package has a number of important features: it has several alternative solution methods, it has been optimized for very large problems, and it enables the performance of a extensive sensitivity analysis.

Sensitivity Analysis

The basis matrix for each case **(B)** is a nonsingular subset of the stoichiometric matrix corresponding to the elements of **v** **($\mathbf{v_B}$),** which uniquely solve the equation.

$$\mathbf{B} \cdot \mathbf{v_B} = \mathbf{b}. \tag{5}$$

Two types of sensitivity analysis can be performed on the solution. The first type determines what changes are required in the row and column bounds to cause the optimum solution to occur with a different basis. That is, it examines how constrained a given reaction is in order to achieve a given objective using the same basis matrix. If a reaction is bounded tightly with respect to the basis matrix, it means that the basis matrix is very dependent on the flux value through that reaction and so is the simulation solution. The second type of analysis determines how large a change is required in the objective function coefficients to cause the optimum solution to occur with a different basis. This type of analysis gives rise to ''reduced costs'' (Luenberger, 1984).

RESULTS

Model Predictions Compared to Experimental Data

To determine the validity of the model, model predictions were compared to experimental data for two different growth conditions: aerobic growth on acetate plus glucose with a doubling time of 70 min and aerobic growth on acetate with a doubling time of 145 min (Walsh and Koshland, 1985b). The experimental flux measurements from Walsh and Koshland (1985b) were originally in units of

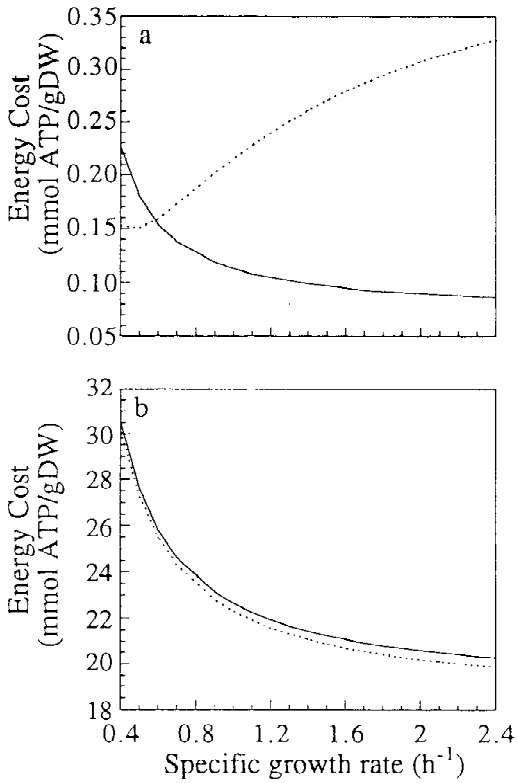

Figure 2. Growth rate dependence of energy requirements. (a) RNA and DNA polymerization energy requirements (Neidhardt et al., 1990). (---) RNA, (—) DNA. (b) Total energy and protein polymerization requirements (Neidhardt et al., 1990). (---) Protein polymerization requirements. (—) Total energy requirements.

millimoles of substrate consumed per minute per liter of cytoplasmic volume. The experimental data was converted to units of millimoles of substrate consumed per hour per gram DW, assuming that cytoplasmic volume of a cell is 75% of the total volume, and the correlations for cell mass and cell volume (Table VI) were used to convert from a volume basis flux to a gram DW basis flux. The lower bound for the carbon dioxide secretion rate was set at the experimentally determined value, and the values predicted for oxygen uptake rates were in the range of experimental values (Andersen and von Meyenburg, 1980; Harrison and Loveless, 1971; Hempfling and Mainzer, 1975; Marr, 1991; Schulze and Lipe, 1964). The constraints and objective function for each case are presented in Table VII.

For aerobic growth on acetate plus glucose, the model predictions were very similar to experimental data for fluxes through the reactions of glycolysis and the TCA cycle (Fig. 3). The experimental glucose uptake rate for this case (7 mmol glucose/g DW h) for a doubling time of 70 min was taken from Herbert and Kornberg (1976), Schulze and Lipe (1964), and Tempest and Neijssel (1987). The average difference between the experimental values and the simulation results was 16%. In addition, the model was able to predict several levels of genetic regulation; for example, the glyoxylate shunt was not functional for growth on glucose and the flux through PEP carboxykinase was toward oxaloacetate (Walsh and Koshland, 1985b).

For aerobic growth on acetate only, the model predictions were also very similar to experimentally determined fluxes (Fig. 4). The average difference between the experimental values and the simulation results for this case was 17%. The model was able to predict that the glyoxylate shunt must be active during growth on acetate to generate sufficient precursors for macromolecule synthesis. The model was also able to predict the correct directions for reactions catalyzed by PEP carboxykinase and malic enzyme (Fraenkel, 1996).

The elemental compositions for bacteria were determined experimentally to be $CN_{0.2}O_{0.27}$, $CN_{0.25}O_{0.5}$, and $CN_{0.24}O_{0.49}$ (Blanch and Clark, 1996; Characklis and Marshall, 1989) and our biomass composition data predicted elemental balances of $CN_{0.31}O_{0.2}$ for aerobic growth on acetate plus glucose and $CN_{0.3}O_{0.58}$ for aerobic growth on acetate. Because the model only accounts for a proton balance across the cell membrane and there is no accounting of protons in the cell moving between metabolites, it is not possible to calculate an intracellular proton composition necessary for the protons in the elemental composition. The model predictions of $Y_{x/s}$ (g g^{-1}) = 0.53 (neglecting the acetate consumed) or $Y_{x/s}$ (g g^{-1}) = 0.52 (including acetate and assuming that the acetate has half of the carbon value of glucose) compares favorably with the experimentally determined yield coefficient of $Y_{x/s}$ (g g^{-1}) = 0.53 (Characklis and Marshall, 1989). If the acetate and glucose consumed are normalized per carbon, the model predicts $Y_{x/s}$ [g(g carbon)$^{-1}$] = 0.086 compared to experimental data of $Y_{x/s}$ [g(g carbon)$^{-1}$] = 0.088 (Characklis and Marshall, 1989). The oxygen uptake rate for a 70-min doubling time was reported as 11–33 mmol of O_2/g DW h and the model predicted a flux of 12.6 mmol of O_2/g DW h. For a 145-min doubling time the oxygen uptake was reported as 8–29 mmol of O_2/g DW h (Andersen and von Meyenburg, 1980; Harrison and Loveless, 1971; Hempfling and Mainzer, 1975; Marr, 1991; Schulze and Lipe, 1964) and the model predicted a flux of 16 mmol of O_2/g DW h. It was observed experimentally that the oxygen consumption rate decreases by 22% for growth on acetate plus glucose relative to growth on only acetate, even though the growth rate increases (Walsh and Koshland, 1985a), and the model predicted a 21% decrease in oxygen consumption.

Anaerobic growth on glucose with a 70-min doubling time was simulated to determine if the TCA cycle would branch as observed experimentally (Fig. 5) (Cronan and LaPorte, 1996; Nimmo, 1987). The glucose uptake rate was set to 3 times the aerobic rate (Smith and Neidhardt, 1983), and secretion rates for organic acids were set to experimentally determined values (Tempest and Neijssel, 1987). The model predicted the experimental observation that the TCA cycle is not a cycle but rather branches into a reductive pathway that produces succinyl-CoA and an oxidative pathway that produces α-ketoglutarate (Cronan and LaPorte, 1996). The model was also able to predict that α-ketoglutarate dehydrogenase is not expressed and that the glyoxylate shunt is closed during anaerobic growth. Unfortunately, there were no available experimental data to validate the flux values.

Table VII. Simulation parameters: Constraints on fluxes and criteria for maximization and minimization.

Growth condition	Lower bound (mmol/g DW h)	Upper bound (mmol/g DW h)	Objective function criterion	Note
Aerobic, glucose + acetate				
Glucose uptake	0	7	Maximize	Measured[a]
Acetate uptake	0	11.94	Maximize	Measured[b]
Carbon dioxide secretion	14	∞	Minimize	
Acetate secretion	0	11.14	Maximize	Measured[b]
Precursor production rate	μ dependent		Maximize	Calculated[c]
Aerobic, acetate				
Acetate uptake	0	33.42	Maximize	Uptake[d]
Carbon dioxide secretion	45	∞	Minimize	Measured[b]
Precursor production rate	μ dependent		Maximize	Calculated[c]
Anaerobic, glucose				
Glucose uptake	0	21	Maximize	Measured[e]
Oxygen uptake	0	0		
Carbon dioxide secretion	18.48	∞	Minimize	Measured[f]
Acetate secretion	0	7.67	Maximize	Measured[e]
Lactate secretion	0	16.69	Maximize	Measured[e]
Formate secretion	0	0.50	Maximize	Measured[e]
Succinate secretion	0	2.25	Maximize	Measured[e]
Ethanol secretion	0	10.46	Maximize	Measured[e]
Precursor production rate	μ dependent		Maximize	Calculated[c]

[a]The values present in the literature are in the same range as the value used as a lower bound for the simulation (Herbert and Kornberg, 1976; Schulze and Lipe, 1964; Tempest and Neijssel, 1987).

[b]Data from Walsh and Koshland (1985b).

[c]Calculated from correlations in Table VI and information presented in Tables I–V.

[d]The acetate uptake rate from Walsh and Koshland (1985b) was 41.4 mmol/g DW h but the model predicted an acetate uptake rate of 33.42 mmol/g DW h. Any amount of acetate provided in surplus resulted in acetate secretion.

[e]Data from Smith and Neidhardt (1983).

[f]Data from Bock and Sawers, (1996).

Sensitivity to Biomass Composition

To determine the sensitivity of the solutions to the biomass composition, the three cases described above were simulated for incorrect biomass compositions: the cell composition corresponding to a 145-min doubling time (rather than the experimentally determined 70-min doubling time) was used to solve for the fluxes for aerobic growth on glucose and acetate and for anaerobic growth on glucose, and the cell composition corresponding to a 70-min doubling time (rather than the experimentally determined 145-min doubling time) was used to solve for the fluxes for aerobic growth on acetate. The constraints and objective functions remained unchanged. For growth on glucose plus acetate, the correct biomass composition gave rise to an average error of 16% between the experimental data and model predictions for fluxes through the TCA cycle and glycolytic pathway, whereas the incorrect biomass composition increased the average error to 80%. Similarly, for growth on only acetate the correct biomass composition gave rise to an average error of 17%, whereas the incorrect biomass composition increased the average error to 32%.

The results for the two aerobic cases were significantly affected by changing the biomass compositions (Table VIII). The flux distribution predicted by the model for aerobic growth on acetate with the incorrect biomass composition resulted in no flux through PEP carboxykinase, contrary to experimental observations (Walsh and Koshland, 1985b). For anaerobic growth on glucose, the incorrect biomass composition gave rise to incorrect branching of the TCA cycle with fumarase and malate dehydrogenase catalyzing reactions in directions that were not observed experimentally (Cronan and LaPorte, 1996). Further, the simulation suggested that there would be flux through the glyoxylate shunt, which was not observed experimentally (Nimmo, 1987). The sensitivity analysis of the anaerobic case was not performed because there were no available experimental data for comparisons.

Sensitivity Analysis on Flux Constraints

Sensitivity analysis was performed on the three cases to determine which reactions were the most constrained; that is, which reactions had the least amount of flexibility in flux values for which the solution will not change. The flexibility in the flux for a reaction is the range of flux values (represented as a percentage) that can occur without changing the basis matrix (Figs. 3–5).

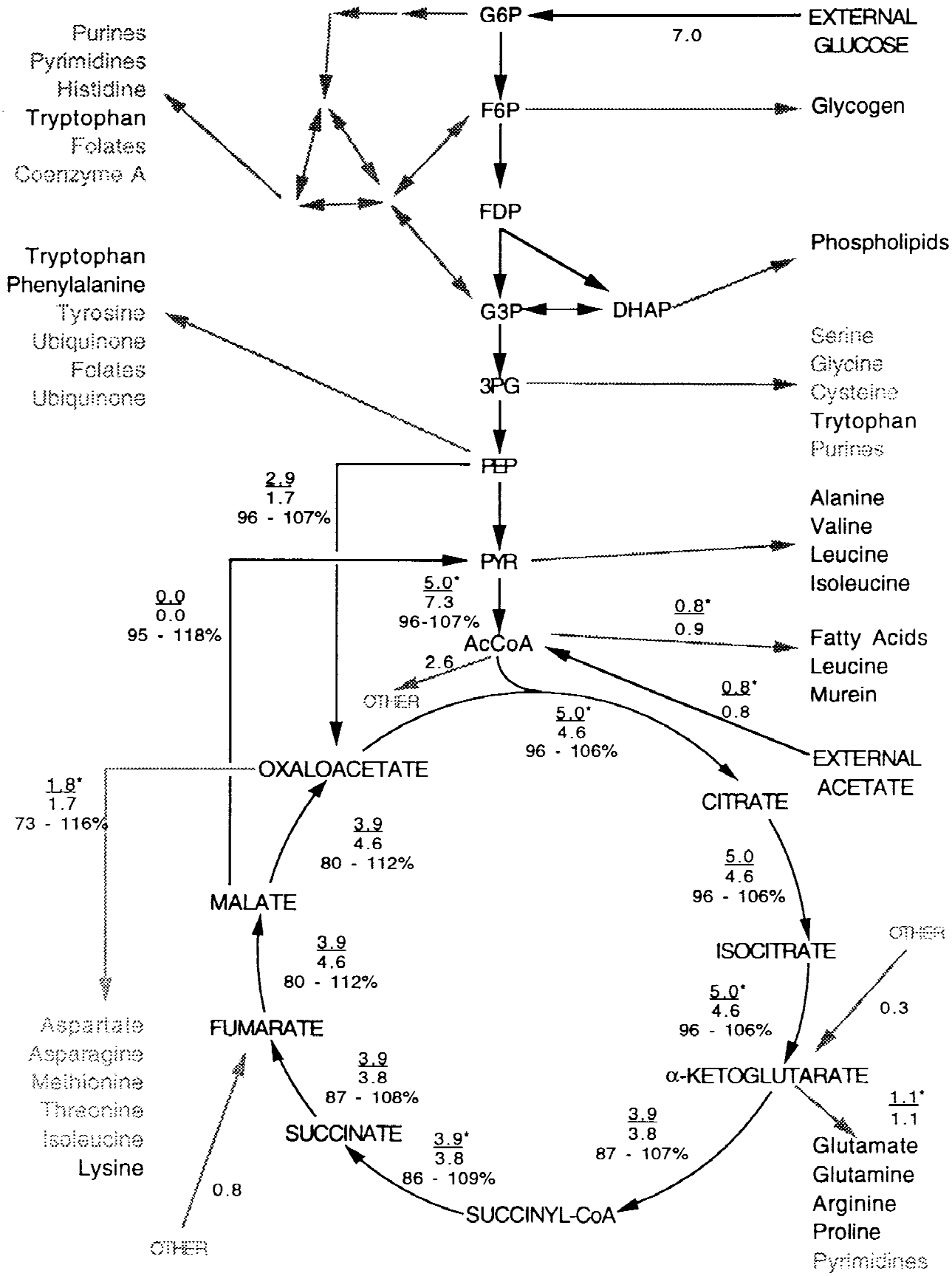

Figure 3. Fluxes through glycolysis and TCA cycle during aerobic growth on glucose plus acetate. The doubling time was 70 min. Experimental data are underlined (Walsh and Koshland, 1985b), the simulation results are the center values, and the flexibility of the reactions (as a percent of the simulation results) are on the bottom. Solid lines and words indicate highly constrained reactions and precursors. Stippled lines and words indicate less constrained reactions and precursors. The flux values measured experimentally are marked with an asterisk, and the flux values calculated from the experimental data using a simpler model are unmarked (Walsh and Koshland, 1985b). The ''other'' on the figure means synthesis of the metabolite due to the presence of other enzymes not depicted in the figure.

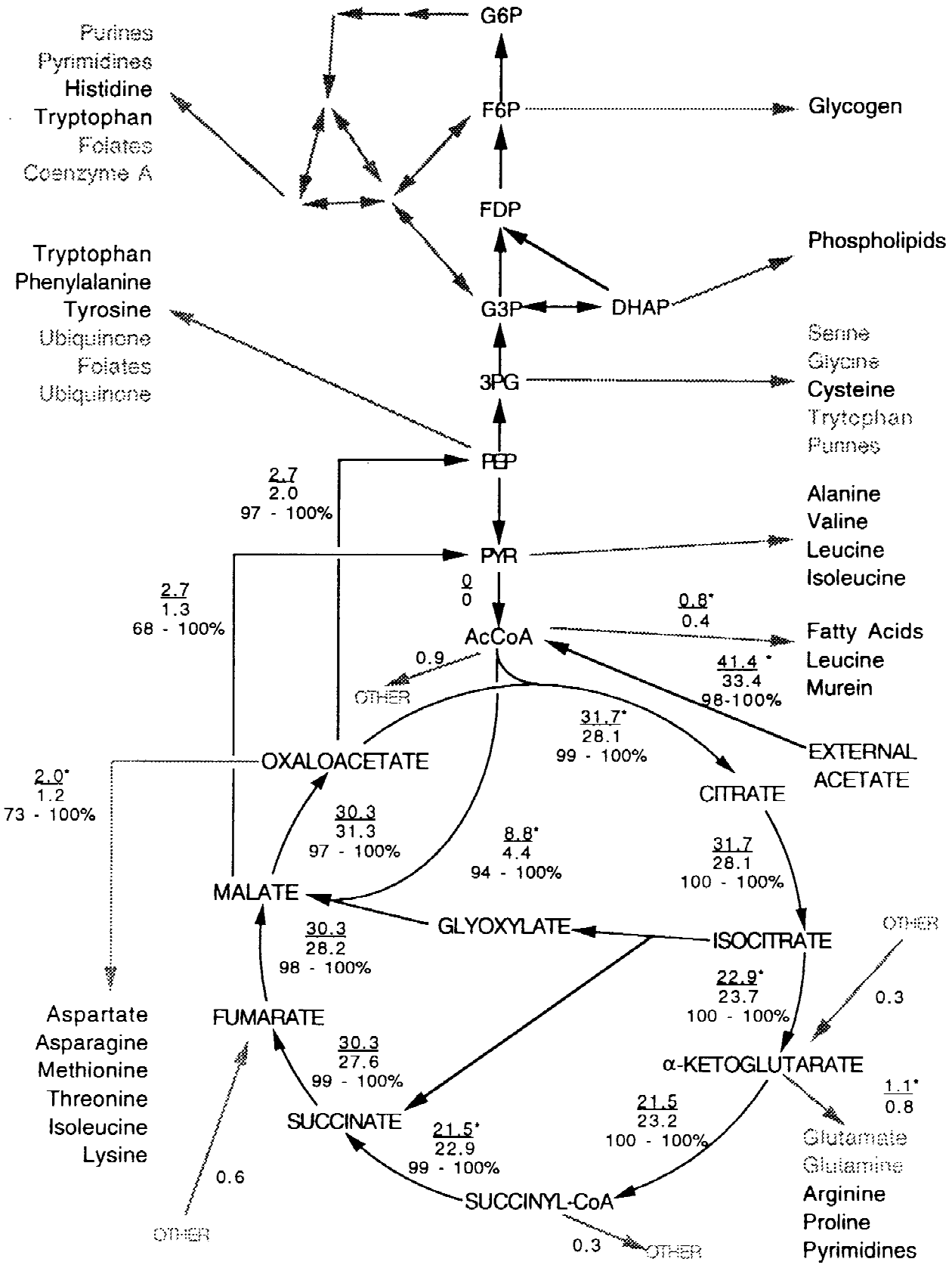

Figure 4. Fluxes through glycolysis and TCA cycle during aerobic growth on acetate. The doubling time was 145 min. Experimental data are underlined (Walsh and Koshland, 1985b), the simulation results are the center values, and the flexibility of the reactions (as a percent of the simulation results) are on the bottom. Solid lines and words indicate highly constrained reactions and precursors. Stippled lines and words indicate less constrained reactions and precursors. The flux values experimentally measured are marked with an asterisk, and the flux values calculated from the experimental data using a simpler model are unmarked (Walsh and Koshland, 1985b). The ''other'' on the figure means synthesis of the metabolite due to the presence of other enzymes not depicted in the figure.

Table VIII. Effect of biomass composition on the simulation results. The simulation results for fluxes through key reactions of TCA cycle and glycolysis for two different biomass compositions are compared to experimental data. The error is relative to experimental data (Walsh and Koshland, 1985b). Only the flux values presented in Table 8 were used to determine average error percentages.

	Aerobic, glucose + acetate			Aerobic acetate		
	Predictions		Experiment[a]	Predictions		Experiment[a]
	$\tau_D = 70$	$\tau_D = 145$	$\tau_D = 70$	$\tau_D = 70$	$\tau_D = 145$	$\tau_D = 145$
PEP carboxykinase	0	0	0	0 (100%)	2.0 (28%)	2.71
PEP carboxylase	1.7 (41%)	0.8 (71%)	2.9	0	0	0
Pyruvate dehydrogenase	7.3 (45%)	7.5 (50%)	5.0	0	0	0
Citrate synthase	4.6 (8%)	1.0 (79%)	5.0	25.1 (21%)	28.1 (11%)	31.7
Aconitase	4.6 (8%)	1.0 (79%)	5.0	25.1 (21%)	28.1 (11%)	31.7
Isocitrate dehydrogenase	4.6 (8%)	0.6 (87%)	5.0	18.5 (19%)	23.7 (3%)	22.9
2-Ketoglutarate dehydrogenase	3.8 (3%)	0 (100%)	3.87	17.7 (18%)	23.2 (8%)	21.5
Succinate thiokinase	3.8 (3%)	0 (100%)	3.9	17.2 (20%)	22.9 (7%)	21.5
Succinate dehydrogenase	3.8 (3%)	0.4 (90%)	3.9	24.3 (20%)	27.6 (9%)	30.3
Fumarase	4.6 (20%)	1.1 (72%)	39	25.2 (17%)	28.2 (7%)	30.3
Malate dehydrogenase	4.6 (20%)	1.1 (72%)	3.87	26.9 (11%)	31.3 (3%)	30.3
Malic enzyme	0	0	0	4.9 (80%)	1.3 (52%)	2.7
Isocitrate lyase	0	0.4	0	6.6 (25%)	4.4 (50%)	8.8
Overall error[b]	16%	80%		32%	17%	

[a]Data from Walsh and Koshland (1985b).
[b]The error is calculated as (|experiment – prediction|)/experiment.

In general, the sensitivity analysis indicated that glycolysis was the most constrained pathway during aerobic and anaerobic growth on glucose, whereas the TCA cycle was the most constrained pathway during growth on acetate (Table IX). The rigid constraints on glycolysis during growth on glucose are due, in part, to the sensitivity of the leucine and valine biosynthetic pathways, which diverge from pyruvate. For growth on acetate, the rigid constraints on the TCA cycle are partly due to the rigidity in isocitrate dehydrogenase and in the cysteine and methionine synthesis rates. In the anaerobic case, the TCA cycle reactions had a lower bound of 59% of the flux value and no upper bound except for reactions catalyzed by fumarase and malate dehydrogenase, which had bounds of 92–142%; these are the two enzymes responsible for the TCA cycle branching under anaerobic growth conditions by changing direction of the reactions.

DISCUSSION

A steady-state, flux-based model was developed to study the distribution of mass and energy fluxes through the *E. coli* metabolic reaction network. The contributions of this model are threefold: a relatively complete data base of reactions from glucose to precursors, coenzymes, and prosthetic groups was used; correlations for experimentally observed changes in biomass composition with growth rate were included; and energy requirements for growth were based on mechanistic requirements rather than on a lumped maintenance energy requirement.

The model incorporated 153 reversible and 147 irreversible reactions using 289 metabolites. The relatively complete nature of the reactions will allow one to examine the effect of mutations in specific genes on fluxes through pathways by constraining fluxes through relevant enzymes. It will also allow more flexibility in choosing which metabolites or pathways might be most amenable to alterations to engineer metabolism. In genetically manipulating cells for the production of a desired compound or protein, the growth rate of the cell may change significantly. Therefore, incorporation of growth-rate dependent biomass composition as well as energy requirements in such a model would allow one to accurately simulate the effect of the growth rate changes on the distribution of resources throughout the cell.

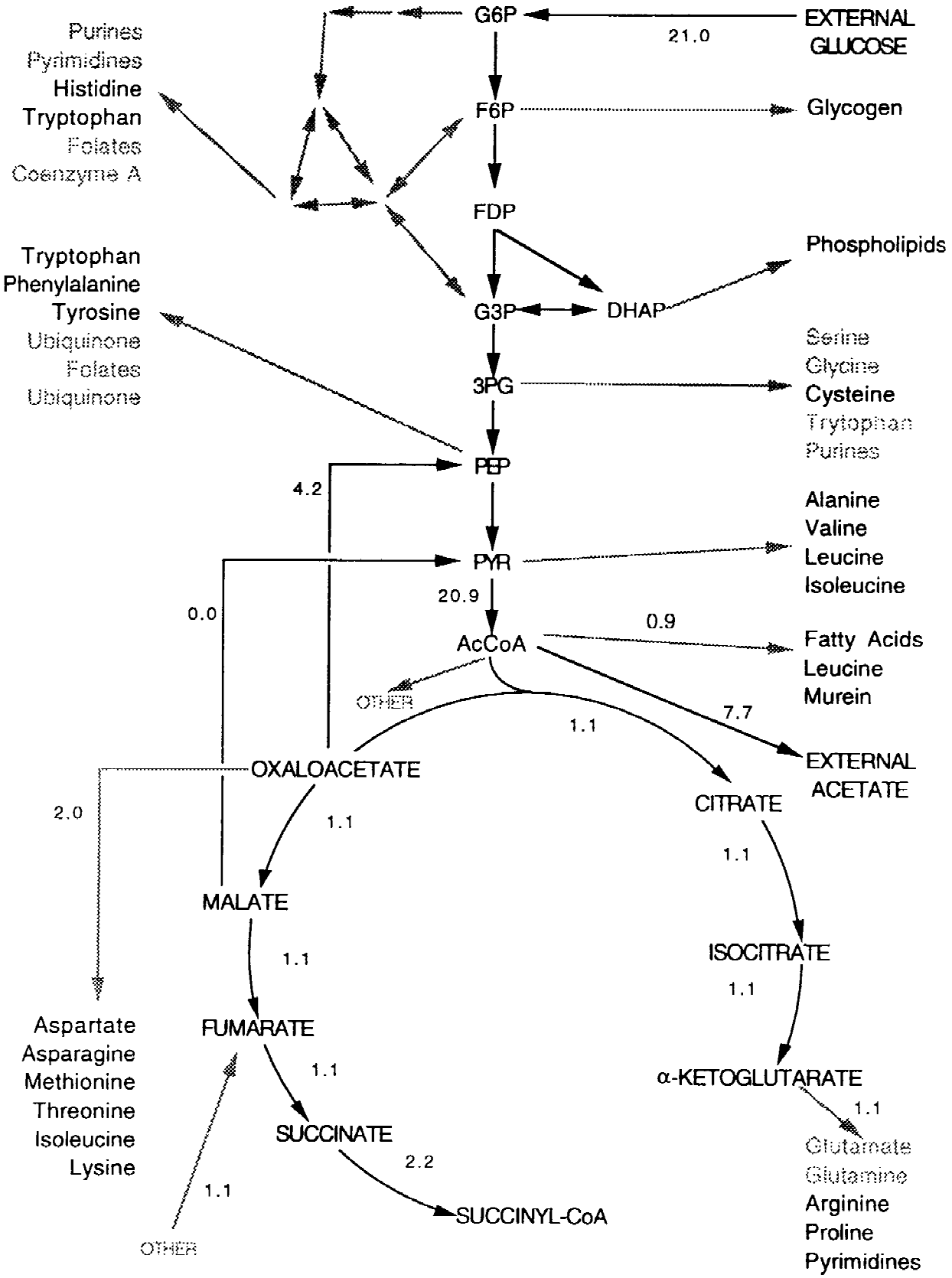

Figure 5. Fluxes through glycolysis and TCA cycle during anaerobic growth on glucose. The doubling time was 70 min. Solid lines and words indicate highly constrained reactions and precursors. Stippled lines and words indicate less constrained reactions and precursors. The ''other'' on the figure means synthesis of the metabolite due to the presence of other enzymes not depicted in the figure.

Experimental data were used to develop correlations for biomass composition at different growth rates. The biomass sensitivity analysis indicates the importance of incorporating changes in biomass composition with growth rate. For growth on acetate plus glucose, the biomass composition greatly affected the fluxes. Using the correct composition, the predicted fluxes differed from experimental measurements by 16%. Using an incorrect composition, the predicted fluxes differed from experimental measurements by 80%. For growth on acetate the predicted fluxes using a correct composition differed from experimental measurements by 17%, and for the incorrect composition the predicted fluxes differed from experimental measurements by 32%.

Maintenance energy terms have often been incorporated into models to balance energy requirements that could not be accounted for through biomass synthesis requirements (Pirt, 1965, 1982). However, the use of the maintenance energy term does not account mechanistically for this energy drain. To improve the predictive power of stoichiometric models, we accounted for the energy drain using a mechanistic approach and correlated this with growth rate. Besides incorporating the obvious energy requirements, such as those for polymerization of biopolymers, we also incorporated energy requirements, such as those for proofreading of DNA and protein, RNA processing, and proton leakage across membranes. Because the composition of the cell changes with growth rate, the energy requirements were scaled with cell composition. In a similar manner, proton leakage was scaled with the cell's surface area, which increases with growth rate.

There was close agreement between the predicted and experimentally determined flux values, and the solutions agreed with observed regulation under the different growth conditions. The model was able to predict the opening and closing of the glyoxylate shunt in the presence and absence of acetate as the sole carbon source. The O_2 uptake rate under aerobic growth conditions predicted by the model agreed with experimental data (Andersen and von Meyenburg, 1980; Harrison and Loveless, 1971; Hempfling and Mainzer, 1975; Marr, 1991; Schulze and Lipe, 1964). The model also predicted the branching of the TCA cycle with no flux through α-ketoglutarate dehydrogenase under anaerobic growth conditions. The largest errors occurred at the branch points of glycolysis and the TCA cycle, where there were drains for the synthesis of cellular constituents. Because the data used to develop the correlations for the biomass components came from many different experiments conducted under various growth conditions using various strains of *E. coli*, differences in biomass composition could be responsible for these discrepancies. A more complete set of experimental data is required to improve model predictions.

Sensitivity analysis was also performed to determine how changes in the fluxes would affect the basis matrix that was used to arrive at a given solution. This type of analysis indicates how much the flux through a given reaction can change and still allow the model to arrive at the optimal solution using the same basis matrix. This analysis indicates that during growth on glucose the reactions of glycolysis are the most constrained, with the TCA cycle and the pentose phosphate shunt reactions close behind. In contrast, the reactions of the TCA cycle are the most highly constrained during growth on acetate. Because the glycolysis and the TCA cycle reactions are involved in both energy and precursor production, one would expect them to be the most highly constrained reactions.

This metabolic model should be a useful tool for studying the effects of reengineering pathways. It can provide information about how the overall flux distribution will be affected if an organism is forced to synthesize a product or to degrade a pollutant. The model can also elucidate cases under which it will not be possible to satisfy growth demands and secrete a product of interest at a desired level. The sensitivity analysis can be used to study the stiffness of the solutions and the regions where bottlenecks may form under certain growth conditions. The model shows a high degree of sensitivity to the biomass information, and therefore the dependence of biomass composition on growth rates is an important aspect of a flux-based metabolic model.

Table IX. Sensitivity analysis of basis matrix with respect to flux constraints.

	Allowable fluxes[a] (% solution value)		
Pathway/enzyme	Glucose + acetate, aerobic	Acetate, aerobic	Glucose, anaerobic
Glycolysis	94–103	84–100	100–101
Pentose phosphate	95–109	81–100	94–101
TCA cycle	90–106	100–100	92–142
Carbon dioxide secretion	68–108	50–100	72–106
Ammonia uptake	85–109	85–100	87–103
Oxygen uptake	87–108	96–100	
Acetate secretion	48–116		46–117
Precursors tightly constrained	Phosphatidylethanolamine Arginine Lysine Leucine Valine	Phosphatidylethanolamine Cardiolipin Cysteine Methionine	Phosphatidylethanolamine

[a]Values refer to the amount the flux value can change without changing the basis matrix. The most tightly bounded reaction in a particular pathway determines the bounds on that pathway.

APPENDIX A: METABOLIC PATHWAYS IN STOICHIOMETRIC MATRIX GENE NAMES ARE SUPPLIED WHERE POSSIBLE.

Enzyme	Gene	Pathway	Reference
Glycolysis			
Phosphotransferase system	*ptsI, ptsH*	GLC + PEP → PYR + G6P	Fraenkel, 1996; Postma et al., 1996
Phosphoglucose isomerase	*pgi*	G6P ↔ F6P	Fraenkel, 1996
Phosphofructokinase	*pfk*	F6P + ATP ↔ ADP + F16P	Fraenkel, 1996
Fructose-1,6-bisphosphate aldolase	*fbp*	F16P + PI → F6P	Fraenkel, 1996
Fructose-1,6-bisphosphatase	*fba*	F16P ↔ T3P1 + T3P2	Fraenkel, 1996
Triosphosphate isomerase	*tpi*	T3P1 ↔ T3P2	Fraenkel, 1996
Glyceraldehyde-3-phosphate dehydrogenase	*gap*	T3P1 + PI + NAD ↔ NADH + 13P2DG	Fraenkel, 1996
Phosphoglycerate kinase	*pgk*	13P2DG + ADP ↔ ATP + 3PDGL	Fraenkel, 1996
Phosphoglycerate mutase	*gpm*	3PDGL ↔ 2PDGL	Fraenkel, 1996
Enolase	*eno*	2PDGL ↔ PEP	Fraenkel, 1996
Pyruvate kinase	*pyk*	PEP + ADP → ATP + PYR	Fraenkel, 1996
PEP carboxykinase	*pck*	OA + ATP ↔ PEP + CO2 + ADP	Fraenkel, 1996
PEP carboxylase	*ppc*	PEP + CO2 → OA + PI	Fraenkel, 1996
Pyruvate dehydrogenase	*pdh*	PYR + COA + NAD → NADH + CO2 + ACCOA	Fraenkel, 1996
PEP synthase	*pps*	PYR + ATP → PEP + AMP + PI	Fraenkel, 1996
Pentose phosphate shunt			
Glucose-6-phosphate dehydrogenase	*zwf*	G6P + NADP ↔ D6PGL + NADPH	Fraenkel, 1996
6-Phosphogluconolactonase	*pgl*	D6PGL → D6PGC	Fraenkel, 1996
6-Phosphogluconate dehydrogenase	*gnd*	D6PGC + NADP ↔ NADPH + CO2 + RL5P	Fraenkel, 1996
Ribose-5-phosphate isomerase	*rpi*	RL5P ↔ R5P	Fraenkel, 1996
Ribose-5-phosphate epimerase	*rpe*	RL5P ↔ X5P	Fraenkel, 1996
Transketolase 1	*tktA*	R5P + X5P ↔ T3P1 + S7P	Fraenkel, 1996
Transaldolase	*tal*	T3P1 + S7P ↔ E4P + F6P	Fraenkel, 1996
Transketolase 2	*tktB*	X5P + E4P ↔ F6P + T3P1	Fraenkel, 1996
6-Phosphogluconate dehydrase	*edd*	D6PGC → 2K3D6PG	Fraenkel, 1996
2-Keto-3-deoxy-6-phospho-gluconate aldolase	*eda*	2K3D6PG → T3P1 + PYR	Fraenkel, 1996
Glycogen metabolism			
Phosphogluconomutase	*pgm*	G6P ↔ G1P	Fraenkel, 1996
Glycogen synthase	*glgA*	G1P + ATP → ADP + PPI + GLYCOGEN	Preiss, 1996
Glycogen phosphorylase	*glgP*	GLYCOGEN + PI → G1P	Preiss, 1996
Dissimilation of pyruvate			
Lactate dehydrogenase	*ldh*	PYR + NADH ↔ NAD + LAC	Bock and Sawers, 1996; Kessler and Knappe, 1996
Alcohol dehydrogenase	*adh*	ACAL + NADH ↔ ETHANOL + NAD	Bock and Sawers, 1996; Kessler and Knappe, 1996
Acetaldehyde dehydrogenase	*adh*	AC + NADH ↔ NAD + ACAL	Bock and Sawers, 1996; Kessler and Knappe, 1996
Pyruvate formate lyase	*pfl*	PYR + COA → ACCOA + FORMATE	Bock and Sawers, 1996; Kessler and Knappe, 1996
Phosphotransacetylase	*pta*	ACCOA + PI ↔ ACTP + COA	Bock and Sawers, 1996; Kessler and Knappe, 1996
Acetate kinase	*ackA*	ACTP + ADP ↔ ATP + AC	Bock and Sawers, 1996; Kessler and Knappe, 1996
Formate hydrogen lyase	*fhl*	FORMATE → CO2	Bock and Sawers, 1996; Kessler and Knappe, 1996
TCA cycle and glyoxylate bypass			
Citrate synthase	*gltA*	ACCOA + OA ↔ COA + CIT	Cronan and LaPorte, 1996
Aconitase	*acn*	CIT ↔ ICIT	Cronan and LaPorte, 1996
Isocitrate dehydrogenase	*idh*	ICIT + NAD ↔ CO2 + NADH + AKG	Cronan and LaPorte, 1996
2-Ketoglutarate dehydrogenase	*sucAB*	AKG + NAD + COA ↔ CO2 + NADH + SUCCOA	Cronan and LaPorte, 1996
Succinate thiokinase	*sucCD*	SUCCOA + GDP + PI ↔ GTP + COA + SUCC	Cronan and LaPorte, 1996
Succinate dehydrogenase	*sdhABCD*	SUCC + FAD → FADH2 + FUM	Cronan and LaPorte, 1996
Fumurate reductase	*frdABCD*	FUM + FADH2 → SUCC + FAD	Cronan and LaPorte, 1996
Fumarase	*fumAB*	FUM ↔ MAL	Cronan and LaPorte, 1996
Malate dehydrogenase	*mdh*	MAL + NAD ↔ NADH + OA	Cronan and LaPorte, 1996
Malic enzyme	*mez*	MAL + NADP → CO2 + NADPH + PYR	Cronan and LaPorte, 1996
Malic enzyme	*mez*	MAL + NAD → CO2 + NADH + PYR	Cronan and LaPorte, 1996

APPENDIX A: CONTINUED

Enzyme	Gene	Pathway	Reference
Isocitrate lyase	*aceA*	ICIT → GLX + SUCC	Cronan and LaPorte, 1996
Malate synthase	*aceB*	ACCOA + GLX → COA + MAL	Cronan and LaPorte, 1996
Respiration			
NADH dehydrogenase II	*ndh*	NADH + Q → NAD + QH2	Gennis and Stewart, 1996
NADH dehydrogenase I	*ndh*	NADH + Q → NAD + QH2 + 4 HEXT	Gennis and Stewart, 1996
Formate dehydrogenase		FORMATE + Q → 2 HEXT + QH2 + CO2	Gennis and Stewart, 1996
Cytochrome oxidase bo3		QH2 + 1/2 O2 → Q + 4 HEXT	Gennis and Stewart, 1996
Cytochrome oxidase bd		QH2 + 1/2 O2 → Q + 2 HEXT	Gennis and Stewart, 1996
Succinate dehydrogenase complex		FADH2 + Q ↔ FAD + QH2	Gennis and Stewart, 1996
ATP synthesis			
F0F1-ATPase	*unc*	ATP ↔ ADP + PI + 3 HEXT	Harold and Maloney, 1996
Biosynthesis of aspartate			
Aspartate transaminase	*aspC*	OA + GLU ↔ ASP + AKG	Reitzer, 1996
Biosynthesis of asparagine			
Glutamine-dependent asparagine synthetase	*asnB*	ASP + ATP + GLN → GLU + ASN + AMP + PPI	Reitzer, 1996
Ammonia-dependent asparagine synthetase	*asnA*	ASP + ATP + NH3 → ASN + AMP + PPI	Reitzer, 1996
Biosynthesis of glutamate			
Glutamate dehydrogenase	*gdhA*	AKG + NH3 + NADPH → GLU + NADP	Reitzer, 1996
Glutamine synthatase	*glnA*	GLU + NH3 + ATP → GLN + ADP + PI	Reitzer, 1996
Glutamate synthase	*gltBD*	AKG + GLN + NADPH → NADP + 2 GLU	Reitzer, 1996
Biosynthesis of alanine			
Glutamic-pyruvic transaminase		PYR + GLU ↔ AKG + ALA	Reitzer, 1996
Biosynthesis of arginine, putrescine, and spermidine			
N-Acetylglutamate synthase	*argA*	GLU + ACCOA → COA + NAGLU	Glansdorf, 1996
N-Acetylglutamate kinase	*argB*	NAGLU + ATP → ADP + NAGLUYP	Glansdorf, 1996
N-Acetylglutamate phosphate reductase	*argC*	NAGLUYP + NADPH ↔ NADP + PI + NAGLUSAL	Glansdorf, 1996
Acetylornithine aminotransferase	*argD*	NAGLUSAL + GLU ↔ AKG + NAARON	Glansdorf, 1996
N-Acetylornithinase	*argE*	NAARON → AC + ORN	Glansdorf, 1996
Carbamoyl phosphate synthetase	*carAB*	GLN + 2 ATP + CO2 → GLU + CAP + 2 ADP + PI	Glansdorf, 1996
Ornithine carbamoyl transferase	*argFI*	ORN + CAP ↔ CITR + PI	Glansdorf, 1996
Argininosuccinate synthase	*argG*	CITR + ASP + ATP ↔ AMP + PPI + ARGSUCC	Glansdorf, 1996
Argininosuccinase	*argH*	ARGSUCC ↔ FUM + ARG	Glansdorf, 1996
Ornithine decarboxylase	*speC*	ORN → PTRSC + CO2	Glansdorf, 1996
Spermidine synthase	*spE*	PTRSC + DSAM → SPRMD + 5MTA	Glansdorf, 1996
Adenosylmethionine decarboxylase	*speD*	SAM ↔ DSAM + CO2	Glansdorf, 1996; Greene, 1996; Karp et al., 1996
Unknown pathway		5MTA → ADN + MET	Greene, 1996
Agmatine decarboxylase	*speA*	ARG → CO2 + AGM	Glansdorf, 1996
Agmatine ureohydrolase	*speB*	AGM → UREA + PTRSC	Glansdorf, 1996
Biosynthesis of proline			
Glutamyl kinase	*proB*	GLU + ATP → ADP + GLUP	Leisinger, 1996
Glutamate-5-semialdehyde dehydrogenase	*proA*	GLUP + NADPH ↔ NADP + PI + GLUGSAL	Leisinger, 1996
Pyrroline-5-carboxylate reductase	*proC*	GLUGSAL + NADPH ↔ PRO + NADP	Leisinger, 1996
Ornithine oxoacid transaminase		ORN + AKG ↔ GLU + GLUGSAL	Leisinger, 1996
Biosynthesis of branched-chain amino acids			
Isopropylmalate synthase	*leuA*	ACCOA + OIVAL → COA + CBHCAP	Umbarger, 1996
Isopropylmalate isomerase	*leuCD*	CBHCAP ↔ IPPMAL	Umbarger, 1996
3-Isopropylmalate dehydrogenase	*leuB*	IPPMAL + NAD → NADH + OICAP + CO2	Umbarger, 1996
Transaminase C	*ilvE*	OICAP + GLU → AKG + LEU	Umbarger, 1996
Acetyohydroxy acid synthase	*ilvB*	2 PYR → CO2 + ACLAC	Umbarger, 1996
Acetohydroxy acid isomeroreductase	*ilvC*	ACLAC + NADPH → NADP + DHVAL	Umbarger, 1996
Dihydroxy acid dehydratase	*ilvD*	DHVAL → OIVAL	Umbarger, 1996
Transaminase C	*ilvE*	OIVAL + GLU ↔ AKG + VAL	Umbarger, 1996
Threonine deaminase	*ilvA*	THR → NH3 + OBUT	Umbarger, 1996
Acetohydroxy acid synthase	*ilvB*	OBUT + PYR → ABUT + CO2	Umbarger, 1996

APPENDIX A: CONTINUED

Enzyme	Gene	Pathway	Reference
Acetohydroxy acid isomeroreductase	*ilvC*	ABUT + NADPH → NADP + DHMVA	Umbarger, 1996
Dihydroxy acid dehydratase	*ilvD*	DHMVA → OMVAL	Umbarger, 1996
Transaminase B	*ilvE*	OMVAL + GLU ↔ AKG + ILE	Umbarger, 1996
Amino acid oxidase		O2 + ILE → OMVAL + NH3	Umbarger, 1996
Biosynthesis of aromatic amino acids			
3-Deoxy-D-arabinoheptulosonate-7-phosphate synthase	*aroFGH*	E4P + PEP → PI + 3DDAH7P	Pittard, 1996
3-Dehydroquinate synthase	*aroB*	3DDAH7P → DQT + PI	Pittard, 1996
3-Dehydroquinate dehydratase	*aroD*	DQT ↔ DHSK	Pittard, 1996
Shikimate dehydrogenase	*aroE*	DHSK + NADPH ↔ SME + NADP	Pittard, 1996
Shikimate kinase	*aroKL*	SME + ATP → ADP + SME5P	Pittard, 1996
5-Enolpyruvoylshikimate-3-phosphate synthase	*aroA*	SME5P + PEP ↔ 3PSME + PI	Pittard, 1996
Chorismate synthase	*aroC*	3PSME → PI + CHOR	Pittard, 1996
Chorismate mutase	*pheA*	CHOR → PHEN	Pittard, 1996
Prephenate dehydratase	*pheA*	PHEN → CO2 + PHPYR	Pittard, 1996
Phenylalanine aminotransferase	*tyrB*	PHPYR + GLU ↔ AKG + PHE	Pittard, 1996
Prephanate dehydrogenase	*tyrA*	PHEN + NADP → HPHPYR + CO2 + NADPH	Pittard, 1996
Tyrosine aminotransferase	*tyrB*	HPHPYR + GLU ↔ AKG + TYR	Pittard, 1996
Anthranilate synthase	*trpDE*	CHOR + GLN → GLU + PYR + AN	Pittard, 1996
Anthranilate phosphoribosyl transferase	*trpD*	AN + PRPP → PPI + NPRAN	Pittard, 1996
Phosphoribosyl anthranilate isomerase	*trpC*	NPRAN → CPAD5P	Pittard, 1996
Indoleglycerol phosphate synthetase	*trpC*	CPAD5P → CO2 + IGP	Pittard, 1996
Tryptophan synthetase	*trpAB*	IGP + SER → T3P1 + TRP	Pittard, 1996
Biosynthesis of histidine synthesis			
Phosphoribosyl pyrophosphate synthetase	*prs*	R5P + ATP ↔ PRPP + AMP	Zalkin and Nygaard, 1996
Phosphoribosyl pyrophosphate	*hisG*	PRPP + ATP → PPI + PRBATP	Winkler, 1996
PR-ATP pyrophosphohydrolase	*hisI*	PRBATP → PPI + PRBAMP	Winkler, 1996
PR-AMP cyclohydrolase	*hisI*	PRBAMP → PRFP	Winkler, 1996
5-ProFAR isomerase	*hisA*	PRFP → PRLP	Winkler, 1996
Imidazoleglycerol phosphate synthase	*hisFH*	PRLP + GLN → GLU + AICAR + DIMGP	Winkler, 1996
IGP dehydratase	*hisB*	DIMGP → IMACP	Winkler, 1996
L-Histidinol phosphate aminotransferase	*hisC*	IMACP + GLU → AKG + HISOLP	Winkler, 1996
Hol-*P*-phosphatase	*hisB*	HISOLP → PI + HISOL	Winkler, 1996
Hol dehydrogenase	*hisD*	HISOL + 2 NAD → 2 NADH + HIS	Winkler, 1996
Biosynthesis of serine, glycine, and 1-carbon units			
3-Phosphoglycerate dehydrogenase	*serA*	3PDGL + NAD → NADH + PHP	Stauffer, 1996
Phosphoserine transaminase	*serC*	PHP + GLU → AKG + 3PSER	Stauffer, 1996
Phosphoserine phosphatase	*serB*	3PSER → PI + SER	Stauffer, 1996
Serine hydroxymethyltransferase	*glyA*	GLY + METTHF ↔ THF + SER	Matthews, 1996; Stauffer, 1996
Glycine cleavage system	*gcvHTP*	GLY + THF + NAD → METTHF + NADH + CO2 + NH3	Matthews, 1996; Stauffer, 1996
Threonine dehydrogenase	*thd*	THR + NAD ↔ AABK + NADH	Matthews, 1996
Amino-*b*-ketobutyrase	*kbl*	AABK + COA ↔ GLY + ACCOA	Matthews, 1996
Formate dehydrogenase		FORMATE + NAD → NADH + CO2	Kadner, 1996
Formate THF ligase		THF + FORMATE + ATP → ADP + PI + FTHF	Michal, 1993
Formyl THF deformylase		FTHF → FORMATE + THF	Michal, 1993
Dihydrofolate reductase (STEP 2)		DHF + NADPH ↔ NADP + THF	Michal, 1993
Biosynthesis of cysteine			
ATP sulfhydrolase	*cysDN*	H2SO4 + ATP → PPI + APS	Kredich, 1996
ATS kinase	*cysC*	APS + ATP → ADP + PAPS	Kredich, 1996
PAPS sulfotransferase	*cysH*	PAPS + NADPH → NADP + H2SO3 + PAP	Kredich, 1996
NAHPH-sulfite reductase	*cysGIJ*	H2SO3 + 3 NADPH ↔ 3 NADP + H2S	Kredich, 1996
Serine transacetylase	*cysE*	SER + ACCOA ↔ COA + ASER	Kredich, 1996
O-Acetylserine (thiol)-lyase	*cysKM*	ASER + H2S → AC + CYS	Kredich, 1996
Sulfotransferase		PAP + H2SO3 ↔ PAPS	
Adenylyl sulfate kinase		PAPS + ADP → ATP + APS	

APPENDIX A: CONTINUED

Enzyme	Gene	Pathway	Reference
Biosynthesis of threonine and lysine			
Aspartate kinase	*thrA*	ASP + ATP ↔ ADP + BASP	Patte, 1996
Aspartate semialdehyde dehydrogenase	*asd*	BASP + NADPH ↔ NADP + PI + ASPSA	Patte, 1996
Homoserine dehydrogenase	*thrA*	ASPSA + NADPH ↔ NADP + HSER	Patte, 1996
Homoserine kinase	*thrB*	HSER + ATP → ADP + PHSER	Patte, 1996
Threonine synthase	*thrC*	PHSER → PI + THR	Patte, 1996
Dihydrodipicolinate synthase	*dapA*	ASPSA + PYR → D23PIC	Patte, 1996
Dihydrodipicolinate reductase	*dapB*	D23PIC + NADPH → NADP + PIP26DX	Patte, 1996
Tetrahydrodipicolinate succinylase	*dapD*	PIP26DX + SUCCOA → COA + NS2A6O	Patte, 1996
Succinyl diaminopimelate aminotransferase	*dapC*	NS2A6O + GLU ↔ AKG + NS26DP	Patte, 1996
Succinyl diaminopimelate desuccinylase	*dapE*	NS26DP → SUCC + D26PIM	Patte, 1996
Diaminopimelate epimerase	*dapF*	D26PIM ↔ MDAP	Patte, 1996
Diaminopimelate decarboxylase	*lysA*	MDAP → CO2 + LYS	Patte, 1996
Biosynthesis of methionine			
Homoserine transsuccinylase	*metA*	HSER + SUCCOA → COA + OSLHSER	Greene, 1996
Cystathionine synthase	*metB*	OSLHSER + CYS → SUCC + HCYS + PYR + NH3	Greene, 1996
Cystathionase	*metC*	HCYS + ADN ↔ SAH	Greene, 1996
Methionine synthase	*metEH*	HCYS + MTHF → MET + THF	Greene, 1996; Matthews, 1996
Methionyl adenosyl transferase	*metK*	MET + ATP → PPI + PI + SAM	Greene, 1996; Matthews, 1996
Biosynthesis of purine nucleotides			
Glutamine PRPP amidotransferase	*purF*	PRPP + GLN → PPI + GLU + PRAM	Zalkin and Nygaard, 1996
GAR synthetase	*purD*	PRAM + ATP + GLY ↔ ADP + PI + GAR	Zalkin and Nygaard, 1996
GAR transformylase	*purNT*	GAR + FTHF → THF + FGAR	Zalkin and Nygaard, 1996
FGAM synthetase	*purL*	FGAR + ATP + GLN → GLU + ADP + PI + FGAM	Zalkin and Nygaard, 1996
AIR synthetase	*purM*	FGAM + ATP → ADP + PI + AIR	Zalkin and Nygaard, 1996
RCAIM synthetase	*purK*	AIR + CO2 ↔ RCAIM	Zalkin and Nygaard, 1996
PRSCAIM synthetase	*purE*	RCAIM + ATP + ASP ↔ ADP + PI + PRSCAIM	Zalkin and Nygaard, 1996
Adenylosuccinate lyase	*purB*	PRSCAIM ↔ FUM + AICAR	Zalkin and Nygaard, 1996
AICAR transformylase	*purH*	AICAR + FTHF ↔ THF + PRFICA	Zalkin and Nygaard, 1996
IMP cyclohydrolase	*purH*	PRFICA ↔ IMP	Zalkin and Nygaard, 1996
Adenylosuccinate synthetase	*purA*	IMP + GTP + ASP → GDP + PI + ASUC	Zalkin and Nygaard, 1996
Adenylosuccinate lyase	*purB*	ASUC ↔ FUM + AMP	Zalkin and Nygaard, 1996
AMP phosphatase		AMP → PI + ADN	Zalkin and Nygaard, 1996
Adenylate kinase	*adk*	ATP + ADN → ADP + AMP	Zalkin and Nygaard, 1996
Adenylate kinase	*adk*	ATP + AMP → 2 ADP	Zalkin and Nygaard, 1996
IMP dehydrogenase	*guaB*	IMP + NAD → NADH + XMP	Zalkin and Nygaard, 1996
GMP synthetase	*guaA*	XMP + ATP + GLN → GLU + AMP + PPI + GMP	Zalkin and Nygaard, 1996
GMP kinase	*gmk*	GMP + ATP ↔ GDP + ADP	Zalkin and Nygaard, 1996
GDP kinase	*gmk*	GDP + ATP ↔ GTP + ADP	Zalkin and Nygaard, 1996
Ribonucleotide reductase (ADP)	*deoD*	ADP + NADPH → DADP + NADP	Zalkin and Nygaard, 1996
Ribonucleotide reductase (GDP)	*deoD*	GDP + NADPH → DGDP + NADP	Zalkin and Nygaard, 1996
Ribonucleotide reductase (ATP)	*deoD*	ATP + NADPH → NADP + DATP	Zalkin and Nygaard, 1996
Ribonucleotide reductase (GTP)	*deoD*	GTP + NADPH → NADP + DGTP	Zalkin and Nygaard, 1996
dADP kinase	*nck*	DADP + ATP ↔ DATP + ADP	Zalkin and Nygaard, 1996
dGDP kinase	*ndk*	DGDP + ATP ↔ DGTP + ADP	Zalkin and Nygaard, 1996
dAMP kinase	*ndk*	DAMP + ATP ↔ ADP + DADP	Zalkin and Nygaard, 1996
dGMP kinase	*ndk*	DGMP + ATP ↔ DGDP + ADP	Zalkin and Nygaard, 1996
Biosynthesis of pyrimidines			
Aspartate carbamoyl transferase	*pyrBI*	CAP + ASP → CAASP + PI	Neuhard and Klein, 1996
Dihydroorotase	*pyrC*	CAASP ↔ DOROA	Neuhard and Klein, 1996
Dihydroorotate dehydrogenase	*pyrD*	DOROA + NAD ↔ NADH + OROA	Neuhard and Klein, 1996
Orotate phosphoribosyl transferase	*pyrE*	OROA + PRPP ↔ PPI + OMP	Neuhard and Klein, 1996
OMP decarboxylase	*pyrF*	OMP → CO2 + UMP	Neuhard and Klein, 1996
UMP kinase	*pyrH*	UMP + ATP ↔ UDP + ADP	Neuhard and Klein, 1996
UDP kinase	*ndk*	UDP + ATP ↔ UTP + ADP	Neuhard and Klein, 1996
CTP synthetase	*pyrG*	UTP + GLN + ATP → GLU + CTP + ADP + PI	Neuhard and Klein, 1996
CMP kinase	*ndk*	CMP + ATP ↔ CDP + ADP	Neuhard and Klein, 1996
CDP kinase	*ndk*	CDP + ATP ↔ CTP + ADP	Neuhard and Klein, 1996
Deoxycytidilate deaminase	*cdd*	DCMP → NH3 + DUMP	Neuhard and Klein, 1996

APPENDIX A: CONTINUED

Enzyme	Gene	Pathway	Reference
Ribonucleotide reductase (CDP)	*nrdAB*	CDP + NADPH → DCDP + NADP	Neuhard and Klein, 1996
Ribonucleotide reductase (UDP)	*nrdAB*	UDP + NADPH → DUDP + NADP	Neuhard and Klein, 1996
Ribonucleotide reductase (CTP)	*nrdAB*	CTP + NADPH → DCTP + NADP	Neuhard and Klein, 1996
Ribonucleotide reductase (UTP)	*nrdAB*	UTP + NADPH → NADP + DUTP	Neuhard and Klein, 1996
dCMP kinase	*ndk*	DCMP + ATP ↔ ADP + DCDP	Neuhard and Klein, 1996
dCDP kinase	*ndk*	DCDP + ATP ↔ DCTP + ADP	Neuhard and Klein, 1996
dUDP kinase	*ndk*	DUDP + ATP ↔ DUTP + ADP	Neuhard and Klein, 1996
dUTP pyrophosphatase	*dut*	DUTP → PPI + DUMP	Neuhard and Klein, 1996
dUMP kinase	*ndk*	DUMP + ATP ↔ ADP + DUDP	Neuhard and Klein, 1996
Thymidilate synthetase	*thyA*	DUMP + METTHF → DHF + TMP	Matthews, 1996; Neuhard and Klein, 1996
TMP kinase	*tmk*	TMP + ATP ↔ ADP + TDP	Neuhard and Klein, 1996
TDP kinase	*ndk*	TDP + ATP ↔ ADP + TTP	Neuhard and Klein, 1996
Biosynthesis of THF			
Methylene THF reductase	*metF*	METTHF + NADH → NAD + MTHF	Matthews, 1996
Methylene THF dehydrogenase	*folD*	METTHF + NADP ↔ METHF + NADPH	Matthews, 1996
Methenyl tetrahydrofolate cyclehydrolase	*folD*	METHF ↔ FTHF	Matthews, 1996
Biosynthesis of membrane lipids			
Acetyl-CoA carboxylase	*acc*	ACCOA + ATP + CO2 ↔ MALCOA + ADP + PI	Cronan and Rock, 1996
Malonyl-CoA:ACP transacylase	*mta*	MALCOA + ACP ↔ MALACP + COA	Cronan and Rock, 1996
b-Ketoacyl-ACP synthase I	*kas1*	MALACP → ACACP + CO2	Cronan and Rock, 1996
Acetyl-CoA:ACP transacylase	*ata*	ACACP + COA ↔ ACCOA + ACP	Cronan and Rock, 1996
b-Ketoacyl-ACP synthase I (C14:0)	*fab*	ACACP + 6 MALACP + 12 NADPH → C14:0ACP + 6 CO2 + 12 NADP + 6 ACP	Cronan and Rock, 1996
b-Ketoacyl-ACP synthase I (C14:1)	*fab*	ACACP + 6 MALACP + 11 NADPH → C14:1ACP + 6 CO2 + 11 NADP + 6 ACP	Cronan and Rock, 1996
b-Ketoacyl-ACP synthase I (C16:0)	*fab*	ACACP + 7 MALACP + 14 NADPH → C16:0ACP + 7 CO2 + 14 NADP + 7 ACP	Cronan and Rock, 1996
b-Ketoacyl-ACP synthase I (C16:1)	*fab*	ACACP + 7 MALACP + 13 NADPH → C16:1ACP + 7 CO2 + 13 NADP + 7 ACP	Cronan and Rock, 1996
b-Ketoacyl-ACP synthase I (C18:1)	*fab*	ACACP + 8 MALACP + 15 NADPH → C18:1ACP + 8 CO2 + 15 NADP + 8 ACP	Cronan and Rock, 1996
Glycerol-3-phosphate dehydrogenase	*gpsA*	NADH + T3P2 ↔ GL3P + NAD	Cronan and Rock, 1996
1-Acyl-G3P acyltransferase	*pls*	GL3P + 0.03 C14:0ACP + 0.086 C14:1ACP + 0.607 C16:0ACP + 0.12 C16:1ACP + 0.85 C18:1ACP → PA + 1.693 ACP	Cronan and Rock, 1996
CDP-Diacylglycerol synthetase	*cdsA*	PA + CTP ↔ CDPDG + PPI	Cronan and Rock, 1996
Phosphatidylserine synthase	*pssA*	CDPDG + SER ↔ CMP + PS	Cronan and Rock, 1996
PS decarboxylase	*psd*	PS → PE + CO2	Cronan and Rock, 1996
Phosphatidylglycerol phosphate synthase	*pgsA*	CDPDG + GL3P ↔ CMP + PGP	Cronan and Rock, 1996
Phosphatidylglycerol phosphate phosphate	*pgpA*	PGP → PI + PG	Cronan and Rock, 1996
Cardiolipin synthase	*cls*	PG + CDPDG ↔ CL + CMP	Cronan and Rock, 1996
Biosynthesis of isoprenoids			
Aldose reductase		GL + NADP ↔ NADPH + GLAL	White, 1996
Glyceraldehyde kinase		GLAL + ATP → ADP + T3P1	White, 1996
Hydroxymethyl-glutaryl-CoA synthase		3 ACCOA → 2 COA + HMGCOA	White, 1996
3-Methyl-glutaconyl-CoA hydratase		HMGCOA ↔ TMGCOA	White, 1996
IPP synthase		HMGCOA + 2 NADPH + 3 ATP → COA + 2 NADP + 3 ADP +1 PI + CO2 + IPPP	White, 1996
GGPP synthase		4 IPPP → 3 PPI + GGPP	White, 1996
Methylcrotonyl-CoA carboxylase		MCCOA + ATP + CO2 ↔ ADP + TMGCOA + PI	Michal, 1996
Acyl-CoA dehydrogenase		ISOVCOA + Q ↔ QH2 + MCCOA	Michal, 1996
2-Keto-isocaproate decarboxylase		OICAP + COA + NADP → NADPH + CO2 + ISOVCOA	Michal, 1996
Biosynthesis of quinone			
Chorismate pyruvate-lyase	*ubiC*	CHOR → 4HBZ + PYR	Meganathan, 1996
Hydroxybenzoate octaprenyl-transferase	*ubiADX*	4HBZ + GGPP → 2PPP + CO2 + PPI	Meganathan, 1996

APPENDIX A: CONTINUED

Enzyme	Gene	Pathway	Reference
2O6H synthetase	*ubiB*	2PPP + O2 → 2O6H	Meganathan, 1996
QH2 synthetase	*ubiEFGH*	2O6H + 2 O2 + 3 SAM → 3 SAH + QH2	Meganathan, 1996
Biosynthesis of riboflavin			
GTP cyclohydrolase	*ribA*	GTP → D6RP5P + CO2 + PPI	Bacher et al., 1996
Pyimidine deaminase	*ribD*	D6RP5P → A6RP5P + NH3	Bacher et al., 1996
Pyrimidine reductase	*ribD*	A6RP5P + NADPH → A6RP5P2 + NADP	Bacher et al., 1996
Phosphatase		A6RP5P2 → A6RP + PI	Bacher et al., 1996
3,4-Dihydroxy-2-butanone-4-phosphate synthase	*ribB*	A6RP → DB4P + FORMATE	Bacher et al., 1996
6,7-Dimethyl-8-ribityllumazine synthase	*ribE*	DB4P + A6RP → D8RL + PI	Bacher et al., 1996
Riboflavin synthase	*ribC*	2 D8RL → RIBOFLAVIN + A6RP	Bacher et al., 1996
Riboflavin kinase	*ribF*	RIBOFLAVIN + ATP → FMN + ADP	Bacher et al., 1996
FAD synthetase	*ribF*	FMN + ATP → FAD + PPI	Bacher et al., 1996
Biosynthesis of folate			
GTP cyclohydrolase	*folE*	GTP → FORMATE + AHTD	Green et al., 1996
H2Neopterin triphosphate pyrophosphatase		AHTD → 3 PI + DHP	Green et al., 1996
H2Neopterin aldolase		DHP → AHHMP + GLAL	Green et al., 1996
6-Hydroxymethyl H2pterin pyrophosphokinase	*folK*	AHHMP + ATP → AMP + AHHMD	Green et al., 1996
H2pteroate synthase	*folP*	AN + AHHMD → PPI + DHD	Green et al., 1996
Dihydrofolate reductase	*folA*	DHD + ATP + GLU → ADP + PI + DHF	Green et al., 1996
Biosynthesis of coenzyme A			
CoA Synthase	*panBCDE*	OIVAL + METTHF + NADPH + ALA + CTP + 4 ATP + CYS → THF + NADP + AMP + 2 PPI + 2 ADP + CO2 + COA	Jackowski, 1996 (Lumped pathway)
ACP Synthase	*acpS*	COA → 35ADP + ACP	Jackowski, 1996
3,5-ADP phosphatase		35ADP → AMP + PI	
Biosynthesis of NAD			
Quinolate synthase	*nadAB*	ASP + FAD + T3P2 → FADH2 + PI + QNL	Penfound and Foster, 1996
Quinolate phosphoribosyl transferase	*nadC*	QNL + PRPP → PPI + NICNT + CO2	Penfound and Foster, 1996
NAMN adenylyl tranferase	*nadD*	NICNT + ATP → PPI + DANAD	Penfound and Foster, 1996
Deamido-NAD ammonia ligase	*nadE*	DANAD + ATP + NH3 → AMP + PPI + NAD	Penfound and Foster, 1996
NAD kinase		NAD + ATP → NADP + ADP	Penfound and Foster, 1996
NADP phosphatase		NADP → NAD + PI	Penfound and Foster, 1996
Biosynthesis of porphyrins and hemes			
GSA synthetase	*gltX, hemA*	GLU + ATP + NADPH → GSA + AMP + PPI + NADP	Beale, 1996
GSA aminotransferase	*hemL*	GSA → ALAV	Beale, 1996
Porphyrinogen synthetase	*hemBCD*	8 ALAV → PORPH + NH3	Beale, 1996
Biosynthesis of lippopolysaccharide and murein			
Glutamine fructose-6-*P* transaminase	*glmS*	F6P + GLN → GLU + GA6P	Raetz, 1996
Glucosamine-*P* acetyl transferase	*glmU*	GA6P + ACCOA → AGA6P + COA	Raetz, 1996
Acetyl glucosamine mutase	*glmU*	AGA6P ↔ AGA1P	Raetz, 1996
UDP *N*-acetylglucosamine pyrophorylase		AGA1P + UTP ↔ PPI + UDPGA	
UDP *N*-acetylglucosamine 4-epimerase		UDPGA ↔ UDPGLN	
N-Acylglucosamine-6-P 2-epimerase	*kdsA*	UDPGA + PEP ↔ PI + UDPGC	Raetz, 1996
UDP-*N*-acetylmuramate dehydrogenase		UDPGC + NADH → UDPAM + NAD	
CMP-2-keto-3-deoxyoctanoate synthase	*kdsB*	R5P + PEP + CTP → 2 PI + PPI + CMPKDO	Raetz, 1996
Isomerase+mutase+pyrophosphorylase+epimerase		S7P + ATP ↔ ADPHEP + PPI	
Ethanolamine phosphotransferase		PE + CMP ↔ CDPETN + DGR	Michal, 1993
Phosphatidate phosphatase		PA ↔ PI + DGR	Michal, 1993
Lyposaccharide synthetase		3 UDPGLN + 5 C14:0 + 2 ATP + 3 CMPKDO + CDPETN + 3 ADPHEP + 2 UDPG + PE+ 2 UDPGAL → 2 AC + UMP + 6 UDP + 5 ADP + 3 CMP + DGR + LPS	

APPENDIX A: CONTINUED

Enzyme	Gene	Pathway	Reference
UDP glucose synthase		G1P + UTP → PPI + UDPG	
UDP galactose synthase		G1P + UTP ↔ PPI + UDPGAL	
Murein synthetase		UDPGA + UDPAM + 2 ALA + D26PIM + GLU + 5 ATP → PEPTIDO + 5 ADP + 5 PI + 2 UDP	
Polyphosphate and pyrophosphate metabolism			
Pyrophosphatase	*ppa*	PPI → 2 PI	Wanner, 1996
Polyphosphate kinase	*ppk*	1000 ATP ↔ 1000 ADP + POLYP	Wanner, 1996
Polyphosphatase	*ppx*	POLYP → 1000PI	Wanner, 1996
Transport reactions			
Ammonia transport		NH3ext + Hext ↔ NH3	Silver, 1996
Sulfate transport		H2SO4ext ↔ H2SO4	
Phosphate transport	*pit*	PIext + Hext ↔ PI	Wanner, 1996
Acetate transport		ACext + Hext ↔ AC	
Lactate transport		LACext + Hext ↔ LAC	
Formate transport		FORMATEext + Hext ↔ FORMATE	
Ethanol transport		ETHANOLext ↔ ETHANOL	
Succinate transport		SUCCext + Hext ↔ SUCC	
D-Glyceraldehyde transport		GLALext + Hext ↔ GLAL	
Glucose transport		GLCext ↔ GLC	
Carbon dioxide transport		CO_2ext ↔ CO_2	
Oxygen transport		O_2ext ↔ O_2	
Glycerol metabolism			
Glycerol kinase		GL + ATP ↔ GL3P + ADP	Karp et al., 1996
Glycerol-3-phosphate dehydrogenase		GL3P + FAD → T3P2 + FADH2	Karp et al., 1996

APPENDIX B: METABOLITE DEFINITIONS

Abbreviation	Compound
13P2DG	1,3-*P*-D glycerate
2K3D6PG	2-Dehydro-3-deoxy-6-*P*-gluconate
2O6H	2-Octaprenol 6-hydroxyphenol
2PDGL	2-*P*-D glycerate
2PPP	2-Polyprenylphenol
3DDAH7P	3-Deoxy-D-arabinoheptulosonate-7-phosphate
3PDGL	3 *P*-D glycerate
3PSER	3-Phosphoserine
3PSME	*o* (1-Carboxyvinyl)-3-D-shikimate
4HBZ	4-Hydroxybenzoate
5MTA	5′-Methylthioadenosine
A6RP	5-Amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione
A6RP5P	5-Amino-6-ribosylamino-2,4(1H,3H)-pyrimidinedione 5′-phosphate
A6RP5P2	5-Amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione 5′-phosphate
AABK	2-Amino-3-ketobutyrate
ABUT	2-Aceto-2-hydroxybutyrate
AC	Acetate
ACACP	Acetyl-ACP
ACAL	Acetaldehyde
ACCOA	Acetyl-CoA
ACTP	Acetyl-phosphate
ADN	Adenosine
ADP	Adenosine diphosphate
ADPHEP	ADP-mannoheptose
AGA1P	*N*-Acetyl-D-glucosamine-1-phosphate
AGA6P	*N*-Acetyl-D-glucosamine-6-phosphate
AHHMD	2-Amino-4-hydroxy-6-hydroxymethyl dihydropteridine-pyrophosphate
AHHMP	2-Amino-4-hydroxy-6-hydroxymethyl dihydropteridine

APPENDIX B: CONTINUED

Abbreviation	Compound
AHTD	2-Amino-4-hydroxy-6-(erythro-1-2-3-trihydroxypropyl) dihydropteridine-phosphate
AICAR	5-Phosphoribosyl-5-amino-4-imidazole carboxamide
AIR	5-Phosphoribosyl-5-aminoimidazole
AKG	α-Ketoglutarate
ALA	Alanine
ALAV	δ-Amonolevulinate
AMP	Adenosine monophosphate
AN	Anthranilate
APS	Adenylyl sulfate
ARG	Arginine
ARGSUCC	l-Arginiosuccinate
ASER	*o*-Acetylserine
ASN	Asparagine
ASP	Aspartate
ASPSA	Aspartate β-semialdehyde
ASUC	Adenylsuccinate
ATP	Adenosine triphosphate
BASP	β-Aspartyl-phosphate
C14:0ACP	Myristic acid ACP
C14:1ACP	β-Hydroxymyristic acid ACP
C16:0ACP	Palmitic acid ACP
C16:1ACP	Palmitoleic acid ACP
C18:1ACP	*cis*-Vaccenic acid ACP
CAASP	Carbamoyl aspartate
CAP	Carbamoyl phosphate
CBHCAP	3-Carboxy-3-hydroxy-isocaproate
CDP	Cytidine diphosphate
CDPDG	CDP-2-Diacylglycerol
CDPETN	CDP-Ethanolamine
CHOR	Chorismate

APPENDIX B: CONTINUED

Abbreviation	Compound
CITR	Citrulline
CL	Cardiolypin
CMP	Cytidine monophosphate
CMPKDO	CMP-2-Keto-3-deoxyoctanoate
CO2	Carbon dioxide
COA	Coenzyme A-SH
CPAD5P	1-*o*-Carboxyphenylamino 1-deoxyribulose-5-phosphate
CTP	Cytidine triphosphate
CYS	Cysteine
D23PIC	2,3-Dihydrodipicolinate
D26PIM	L,L-2,6-Diaminopimelate
D6PGC	D-6-Phosphoglucono-δ-lactone
D6PGL	D-6-Phosphogluconate
D6RP5P	2,5-Diamino-6-ribosylamino-4(3H)-pyrimidinedione 5′-phosphate
D8RL	6,7-Dimethyl-8-ribityllumazine
DADP	Deoxyadenosine diphosphate
DANAD	Deamido-NAD
DATP	Deoxyadenosine triphosphate
DB4P	L-3,4-Dihydroxy-2-butanone-4-phosphate
DCDP	Deoxycytidine diphosphate
DCTP	Deoxycytidine triphosphate
DGDP	Deoxyguanosine diphosphate
DGMP	2-Deoxy-guanosine-5-phosphate
DGR	D-1,2-Diacylglycerol
DGTP	Deoxyguanosine triphosphate
DHD	7,8-Dihydropteroate
DHF	Dihydrofolate
DHMVA	2,3-Dihydroxy-3-methyl-valerate
DHP	Dihydroneopterin
DHSK	Dehydroshikimate
DHVAL	α,β-Dihydroxy-isovalerate
DIMGP	*d*-Erythroimidazoleglycerol-phosphate
DOROA	Dihydroorotic acid
DQT	3-Dehydroquinate
DSAM	Decarboxylated SAM
DUDP	Deoxyuridine diphosphate
DUMP	Deoxyuridine monophosphate
DUTP	Deoxyuridine triphosphate
E4P	Erythrose 4-phosphate
EFF	Efficiency
ETHANOL	Ethanol
F16P	Fructose 1,6-diphosphate
F6P	Fructose 6-phosphate
FADH	Flavin adenine dinucleotide
FGAM	5-Phosphoribosyl-*N*-formylgycineamidine
FGAR	5-Phosphoribosyl-*N*-formylglycineamide
FMN	Riboflavin 5′-phosphate
FORMATE	Formate
FTHF	10-Formyl-tetrahydrofolate
FUM	Fumarate
G1P	Glucose 1-phosphate
G6P	Glucose 6-phosphate
GA6P	Glucosamine-6-phosphate
GAR	5-Phosphoribosyl glycineamide
GDP	Guanosine diphosphate
GGPP	Geranylgeranyl pyrophosphate
GL	Glycerol
GL3P	Glycoden 3-phosphate
GLAL	D-Glyceraldehyde
GLC	Glucose
GLN	Glutamine
GLU	Glutamate
GLUGSAL	L-Glutamate γ-semialdehyde

APPENDIX B: CONTINUED

Abbreviation	Compound
GLX	Glyoxylate
GLY	Glycine
GLYCOGEN	Glycogen
GMP	Guanosine monophosphate
GSA	Glutamate 1-semialdehyde
GTP	Guanosine triphosphate
H2S	Hydrogen sulfide
H2SO3	Hydrogen sulfite
H2SO4	Hydrogen sulfate
HCYS	Homocysteine
HEXT	External H^+
HIS	Histidine
HISOL	Histidinol
HISOL-P	1-Histidinol-phosphate
HMGCOA	3-Hydroxy-3-methyl-glutaryl CoA
HPHPYR	*para*-Hydroxy phenyl pyruvate
HSER	Homoserine
ICIT	Isocitrate
IGP	Indole glycerol phosphate
ILE	Isoleucine
IMACP	Imidazole acetyl-phosphate
IMP	Inosine monophosphate
IPPP	Isopentyl pyrophosphate
IPPMAL	3-Isopropylmalate
ISOVCOA	Isovaleryl-CoA
LA	Lipoamide
LAC	Lactate
LEU	Leucine
LPS	Lipposaccharide
LYS	*l*-Lysine
MAL	Malate
MALACP	Malonyl-ACP
MALCOA	Malonyl-CoA
MCCOA	3-Methyl crotonyl-CoA
MDAP	*meso*-Diaminopimelate
MET	Methionine
METHF	5,10-Methenyl tetrahydrofolate
METTHF	5,10-Methylene tetrahydrofolate
MTHF	5-Methyl tetrahydrofolate
NAARON	*N*-α-Acetyl ornithine
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NAGLU	*N*-Acetyl glutamate
NAGLUYP	*N*-Acetyl glutamyl-phosphate
NAGLUSAL	*N*-Acetyl glutamate semialdehyde
NH3	Ammonia
NICNT	Nicotinate nucleotide
NPRAN	*N*-5-Phosphoribosyl-antranilate
NS26DP	*N*-Succinyl-1,1-2,6-diaminopimelate
NS2A6O	*N*-Succinyl-2-amino-6-ketopimelate
O2	Oxygen
OA	Oxaloacetate
OBUT	Oxobutyrate
OICAP	2-Oxoisocaproate
OIVAL	Oxoisovalerate
OMP	Orotidylate
OMVAL	Oxomethylvalerate
ORN	Ornithine
OROA	Orotic acid
OSLHSER	*o*-Succinyl-L-homoserine
PA	Phosphatidyl acid
PAP	Adenosine-3,5-diphosphate
PAPS	3-Phosphoadenylyl sulfate
PE	Phosphatidyl ethanolamine

APPENDIX B: CONTINUED

Abbreviation	Compound
PEP	Phosphoenolpyruvate
PEPTIDO	Peptidoglycan
PG	Phosphatidyl glycerol
PGP	1-1-Phosphatidyl-glycerol-phosphate
PHE	Phenylalanine
PHEN	Prephenate
PHP	3-Phosphohydroxypyruvate
PHPYR	Phenyl pyruvate
PHSER	*o*-Phospho-l-homoserine
PI	Phosphate (inorganic)
PIP26DX	δ-Pieperidine-2,6-dicarboxylate
POLYP	Polyphosphate
PORPH	Porphyrinogen
PPI	Pyrophosphate
PRAM	5-Phospho-β-D-ribosyl amine
PRBAMP	Phosphoribosyl-AMP
PRBATP	Phosphoribosyl-ATP
PRFICA	5-Phosphoribosyl-formamido-4-imidazole carboxamide
PRFP	Phosphoribosyl-formimino-AICAR-phosphate
PRLP	Phosphoribulosyl-formimino-AICAR-phosphate
PRO	Proline
PRPP	Phosphoribosyl pyrophosphate
PRSCAIM	5-Phosphoribosy-14-*N*-succinocarboxyamide-5-amino
PS	Phosphatidyl serine
PTRSC	Putrescine
PYR	Pyruvate
Q	Ubiquinone
QH2	Ubiquinol
QNL	Quinolate
R5P	Ribose 5-phosphate
RCAIM	5-*p*-Ribosyl-4-carboxy-5-aminoimidazole
RIBOFLAVIN	Riflavin
RL5P	D-Ribulose 5-phosphate
S7P	D-Sedoheptulose-7-*P*
SAH	*s*-Adenosyl homocystine
SAM	*s*-Adenosyl methionine
SER	Serine
SME	Shikimate
SME5P	Shikimate-5-phosphate
SPRMD	Spermidine
SUCC	Succinate
SUCCOA	Succinyl-CoA
T3P1	Glyceraldehyde-3-phosphate
T3P2	Dihydroxyacetone phosphate
TDP	Thymidine-5-diphosphate
THF	Tetrahydrofolate
THR	Threonine
TMGCOA	*trans*-3-Methyl-glutaconyl-CoA
TMP	Thymidine-5-monophosphate
TRP	Tryptophan
TTP	Thymidine-5-triphosphate
TYR	Tyrosine
UDP	Uridine diphosphate
UDPAM	UDP-Acetylmuramate
UDPGA	UDP-*N*-Acetylglucosamine
UDPGAL	UDP *N*-Acetylgalactosamine
UDPGC	UDP *N*-Acetylglucosamine-enolpyruvate
UDPGLN	UDP *N*-Actylglucosamine
UMP	Uridine monophosphate
UREA	Urea
UTP	Uridine triphosphate
VAL	Valine
X5P	Xylulose-5-phosphate
XMP	Xantosine monophosphate

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