Results: additional illustrations and analysis.

Functional context analysis of essentiality data.

Amino Acid metabolism (AAM): Lysine biosynthesis is discussed in detail in the manuscript. Many of the other essential genes involved in amino acid metabolism appear to be involved in redox related reactions. The essentiality of *gshB* suggests that glycine is consumed to reduce glutathione, although the rest of the γ-glutamyl cycle (catalyzed by the products of the *pepN* and *ggt* genes) is nonessential. However, glutathione reductase (the product of the *gor* gene) is also essential and so is *zwf*, encoding glucose-6-phosphate dehydrogenase, which plays a key role in balancing the oxidation and reduction of NADP necessary for glutathione reduction and oxidation. The role of *nrdH*, which produces a glutaredoxin-like protein is less certain, but the fact that it is essential suggests that it plays a significant role in regulating the oxidation and reduction status of the cell.

Nucleotide and cofactor metabolism (NCM): purine biosynthesis. The rich medium used in our study is likely to supplement any auxotrophies related to nucleoside biosynthesis, and most of the corresponding genes are expected to be dispensable. On the other hand, all of the phosphorylated derivatives (nucleoside mono-, di- and triphosphates) must be synthesized inside the cell, since they cannot be transported across the cell membrane. This means the enzymes involved in production of the phosphorylated compounds are predicted to be essential. A similar pattern was hypothesized and confirmed for the biosynthesis of the nucleotide-related cofactors NAD, CoA, and FAD in vitamin-enriched medium (Gerdes et al., 2002). Here we present a brief analysis of gene essentiality in purine nucleotide metabolism. In agreement with our expectations, most of the genes involved with *de novo* biosynthesis, interconversion, and salvage of purines are dispensable. Of the ~30 genes representing this highly redundant metabolic system, only 4 were found essential, all of them directly related to the formation of nucleoside di- and tri-phosphates: *gmk*, *ndk*, *apt*, and *adk*. Although these observations are completely consistent with the anticipated essentiality of enzymes producing impermeable phosphorylated metabolites, two discrepancies with other reports are apparent. *E. coli ndk* (nucleoside diphosphate kinase) mutants were reported as viable and possessing a normal growth rate (Lu and Inouye, 1996; Lu et al., 1995), due to functional complementation by adenylate kinase (gene *adk*) (Lu and Inouye, 1996), and possibly by polyphosphate kinase (gene *ppk*) (Kuroda and Kornberg, 1997). The second discrepancy is related

to the *apt* gene, encoding adenine phosphoribosyltransferase thought to be dispensable due to the existence of alternative routes of AMP biosynthesis. The *apt*-independent pathway from adenine to AMP via adenosine involves the *deoD* and *ushA* gene products, both of which are dispensable in our data. The observed essentiality of *apt* may indicate that in our conditions the adenine phosphoribosyltransferase reaction is the main route from adenine to AMP, and that flux through the alternative *apt*-independent pathway is insufficient to support competitive cell growth.

Lipids, lipopolysaccharides, lipoproteins, peptidoglycan, cell wall (LPC): fatty acids biosynthesis. The fatty acid and phospholipid biosynthetic pathways provide an example of a metabolic system in which almost all of the genes are known to be essential (reviewed in (Magnuson et al., 1993) (Rock and Cronan, 1996)). The intermediates of the bacterial fatty acid biosynthetic pathway are covalently linked to acyl carrier protein (ACP) and cannot be exogenously provided. Previous work has shown that many of the genes involved in fatty acid biosynthesis are essential and only conditional mutants of the genes encoding these proteins are available. The data presented here clearly reflect this situation. With the exception of a single transposon insertion within the *accD* gene, the genetic footprinting data accurately differentiate essential and non-essential genes within this system (see **Table S3**). Of the three β-ketoacyl ACP synthetases (KAS's) the transposon data correctly identifies KAS I (encoded by *fabB*) as essential, and KAS II (produced by the *fabF* gene) as nonessential. The third KAS enzyme (the *fabH* gene product) is identified as essential, as are most of the genes within the fatty acid biosynthetic cluster. Within the phospholipid biosynthetic genes the data offers no surprises except *gpsA* (encoding glycerol-3-phosphate dehydrogenase) was found to be nonessential. Mutants lacking *gpsA* require high levels of glycerol, which is not likely to be abundant in the media used in our experiment. Therefore, we expected this gene to be essential. The *acpS* gene encoding holo-[ACP] synthase required for fatty acid and phospholipid biosynthesis, appears essential in our study as well as the *pdxJ* gene, which is located immediately upstream from *acpS* and cotranscribed with it (Matsunaga et al., 1996). The latter gene is involved in pyridoxine metabolism, which is dispensable in its entirety due to the abundance of vitamin B_6 in the medium. Previously it was shown that transposon insertions in the *pdxJ* gene block transcription of the downstream *acpS* gene (Takiff et al., 1992), therefore, this is one of a few cases of transposon polar effects encountered in this study.

Table S3. Essentiality of genes involved in fatty acids biosynthesis in *E. coli* (this study) and *H. influenzae* (Hutchison et al., 1999).

		E.C. #	*E.coli*		*H.influenzae*		
Acetyl-CoA Carboxylase							
Biotin carboxylase		6.3.4.14	***accC***	X	HI0972	?	
Biotin carboxyl carrier protein (BCCP)			***accB***	X	HI0971	X	
Carboxyl-transferase; subunit a		6.4.1.2					
	subunit α		***accA***	E	HI0406	E	
	subunit β		***accD***	N*	HI1260	E	
Fatty Acid Biosynthesis							
Acetyl carrier protein (ACP)			***acpP***	?	HI0154	?	
Holo-[acyl-carrier protein] synthase		2.7.8.7	***acpS***	E	---	---	
			yhhU	?	HI0152	E	
Malonyl-CoA:ACP transacylase			***fabD***	E	HI0156	E	
3-ketoacyl-ACP-synthase	type I	2.3.1.41	***fabB***	E	HI1533	N	
	type II	2.3.1.39	***fabF***	N	---	---	
	type III	2.3.1.41	***fabH***	E	HI0157	E	
3-Ketoacyl-ACP-reductase		1.1.1.100	***fabG***	E	HI0155	E	
Enoyl-ACP reductase		1.3.1.9	***fabI***	E	HI1734	E	
3-Hydroxyacyl-ACP-dehydratase		4.2.1.-	***fabZ***	?	HI1062	?	
		4.2.1.60	***fabA***	E	HI1325	?	
Phospholipid Biosynthesis							
G-3-P dehydrogenase [NADP+]		1.1.1.94	***gpsA***	N	HI0605	E	G-3-P
G-3-P acyltransferase		2.3.1.15	***plsB***	X	HI0748	?	1-acyl G-3-P
1-acyl-sn-G-3-P acyltransferase		2.3.1.51	***plsC***	E	HI0734	E	diacyl-glycerol phosphate
Phosphatidate cytidylyltransferase		2.7.7.41	***cdsA***	E	HI0919	N	
CDP-diacylglycerol--serine O-phosphatidyltransferase							
		2.7.8.8	***pssA***	E	HI0425	X	
Phosphatidylserine decarboxylase		4.1.1.65	***psd***	X	HI0160	N	PE
CDP-diacylglycerol--G-3-P 3-phosphatidyltransferase							
		2.7.8.5	***pgsA***	E	HI0123	E	PG
Phosphatidylglycerophosphatase A		3.1.3.27	***pgpA***	E	HI1306	E	
Phosphatidylglycerophosphatase B		3.1.3.27	***pgpB***	N	HI0211	?	
Cardiolipin synthetase		2.7.8.-	***cls***	N	---	---	CL

Carbohydrate metabolism (CHM): glycolysis, pentose phosphate pathway, and TCA cycle. Most of the enzymes participating in glycolysis and gluconeogenesis appear to be essential in our study (**Figure S1**) despite the fact that mutants in many of these genes are known to be viable. The gene-products that catalyze the reactions between fructose-1,6-phosphate and phosphoenol-pyruvate are largely essential with the exception of *gpmA* and *gpmB*, which encode two very similar,

phosphoglycerate mutase isoforms (Fraser et al., 1999). The fact that *ppsA* (phosphoenolpyruvate synthase) appears essential suggests that the critical flux within this pathway is directed from the TCA cycle towards glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (see **Figure S1)**. Three genes: *tktA, rpe* and *zwf,* encoding key enzymes in the pentose phosphate pathway also appear essential. The most active isoform of transketolase (*tktA*, (Zhao and Winkler, 1994)) is the only direct route between the pentose phosphate pool and glycolysis in *E. coli*. Ribulose-5-phosphate epimerase (*rpe*) plays a crucial role in connecting the gluconate and ribose branches of the pentose phosphate pathway. Glucose-6-phosphate dehydrogenase (*zwf*), may be critical under competitive outgrowth conditions due to its role in coupling reducing power to carbon catabolism, since viable *zwf* deletion mutants were previously described (Fraenkel, 1996).

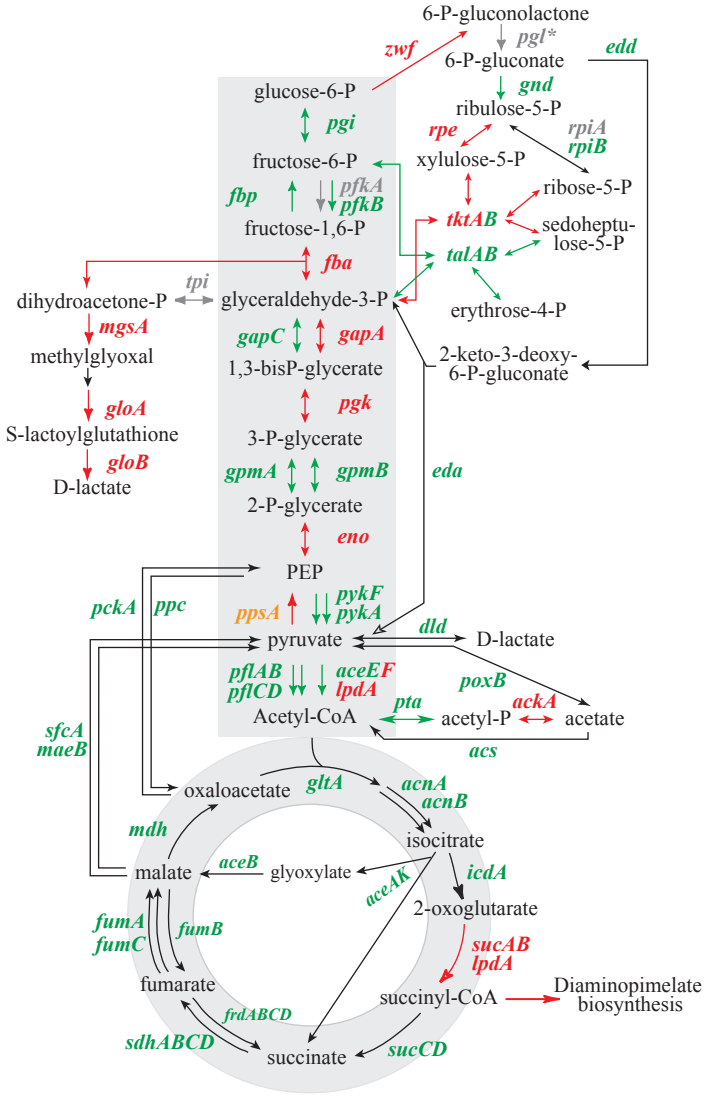
Viable mutants were also described for almost every other gene related to glycolysis or the pentose phosphate pathway (Canonaco et al., 2001; Fuhrman et al., 1998; Sorensen and HoveJensen, 1996), reviewed by (Sprenger, 1995). However, the effect of specific mutations on cell growth was generally analyzed with cultures grown in minimal media supplemented with only one or two carbon sources. Under these conditions, metabolic flux redirection could be tolerated, often at the expense of growth rate (Canonaco et al., 2001; Sauer et al., 1999). The issue becomes more complicated for growth in complex media. For example, *fba, gap, pgk* and *eno* mutants failed to grow in LB medium, although they grew normally in minimal medium supplemented by glycerol and succinate (Irani and Maitra, 1977). This is consistent with the observation that these genes are essential.

Genes involved in methylglyoxal metabolism and detoxification (*mgsA, gloA, gloB*) have been implicated in adaptation to conditions of nutrient imbalance (Ferguson et al., 1998) and also appear essential in our data. Excessive carbon intake (typical of growth in rich medium) was previously shown to lead to intensive production and accumulation of this potentially toxic compound (Totemeyer et al., 1998).

Genes encoding TCA cycle enzymes were found to be largely nonessential, except those encoding all three 2-oxoglutarate dehydrogenase subunits (*sucA*, *sucB* and *lpdA*). This activity is most likely required to produce succinyl-CoA for anabolic purposes, such as production of DAP, an essential intermediate in cell wall biosynthesis. *E. coli* mutants lacking 2-oxoglutarate dehydrogenase are known to grow well in minimal media supplemented with glucose and succinate, but not glucose alone (Creaghan and Guest, 1978).

Figure S1. Essentiality assessments for *E. coli* genes involved in central carbon metabolism. Essential genes are shown in red, non-essentials in green, ambiguous in yellow, and genes for which no data are currently available are in gray.

* The pgl gene has not been yet identified, but the corresponding locus was mapped at 15 min on the *E. coli* K-10 chromosome (Kupor and Fraenkel, 1969).



Protein metabolism and secretion (PMS). Of the twelve functional categories listed in Table 1, this category has the highest percentage of essential genes (48%). We chose three representative functional systems (both metabolic and non-metabolic) within this category to evaluate the consistency of our essentiality assertions produced by genetic footprinting.

PMS: aminoacyl-tRNA synthesis. The complete set of 20 aminoacyl-tRNA synthetases (AARSs) is a well-defined metabolic system, intrinsically essential with almost no functional redundancy.

Therefore it constitutes a perfect "positive control" for whole-genome essentiality studies. Additionally, these genes allow side-by-side comparison between whole-genome essentiality data sets. The overwhelming majority of AARS genes are consistently essential in our data as well as in *H. influenzae* (Akerley et al., 2002), *Mycoplasma* spp (Hutchison et al., 1999), and *S. cerevisiae* (http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html) (**Table S4**). In *E. coli* all of the 20 aminoacyl-tRNAs are formed by direct acylation and only two of 25 related genes were defined as dispensable by genetic footprinting: *yadB* and *lysU*. Gene *yadB* encodes a C-terminally truncated paralog of the essential *gltX* gene. This gene is conserved in many bacteria (but not in *H. influenza* or *Mycoplasma* spp), but its biological role is unclear. The *lysU* gene encodes heat inducible LysRS which is functionally redundant in the presence of constitutively active *lysS* (Kawakami et al., 1992). Essentiality of *lysS* is undefined in our study and in *H. influenzae*, while its C-terminal paralog *poxA* (*genX*) is essential in both studies. This is consistent with previous reports in *S. enterica* serovar *typhimurium* that mutations in *poxA* reduced pyruvate oxidase activity and significantly affected growth rate (Kaniga et al., 1998). In both *Mycoplasma* spp and *S. cerevisiae* LysRS is encoded by a single essential gene. In our data each of the two genes (*pheS* and *pheT*), encoding α- and β-subunits of PheRS sustain a single transposon insert, while both subunits are consistently essential in *H. influenzae*, *Mycoplasma* spp, and *S. cerevisiae*. In *E. coli* genes *pheS* and *pheT* form a 3.3 kb operon with a significantly decreased insert density (~0.6/kb compared to 3.2/kb average for the genome). Both the inserts detected in these genes appear as very low intensity bands in the raw data. Assuming that both of these genes are truly essential, these results may represent a local duplication in the *pheST* region of the chromosome within a small subset of the population (Roth et al., 1996).

***PMS: ribosomal proteins*.** Among the 55 genes encoding the protein components of the ribosome 28 genes were found essential in our study, while 20 genes were formally assigned as "ambiguous". The high level of ambiguity is largely due to the fact that most of these genes are shorter than the 240 bp threshold of our data analysis. Reliable transposon inserts were detected in seven genes formally assigned as dispensable in our data (see Table S1). Four occur within a single gene cluster. In another case the *rpsA* contains a single insert (within this >1.6 kb gene) located close to the 3'-end, which is poorly conserved between bacterial species, and may not be critical for the function of this protein.

Table S4. Essentiality of genes encoding aminoacyl-tRNA synthetases in *E. coli* (as determined in this study), *H. influenzae* (Akerley et al., 2002), *Mycoplasma* spp (Hutchison et al., 1999), and *S. cerevisiae* (http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html).

AARS		BACTERIA				EUKARYA	
	Amino acid	gene name (as in *E.coli*)	*E.coli*	*H.influenzae*	*Mycoplasma spp.*	gene name (as in yeast)	*S.cerevisiae*
1	**Ala**	*alaS*	E	E	E	*ALA1*	E
2	**Arg**	*argS*	E	E	E	*YDR341C*	E
3	**Asn**	*asnS*	E	N	E	*DED81*	E
4	**Asp**	*aspS*	E	N	E	*DPS1*	E
5	**Cys**	*cysS*	E	E	E	*YNL247W*	E
6	**Glu**	*gltX*	E	E	E	*MSE1*	N
		yadB	N	E	E		
7	**Gln**	*glnS*	E	E	-*	*GLN4*	E
						YGL245W	E
8	**Gly**	*glyQ* (α)	E	X	E	*GRS1*	E
		glyS (β)	E	E	E	*YPR081*	?
9	**His**	*hisS*	E	X	E	*HTS1*	E
10	**Ile**	*ileS*	E	X	N	*ILS1*	E
11	**Met**	*metG*	E	E	E	*MES1*	E
12	**Leu**	*leuS*	E	E	E	*CDC60*	E
13	**Lys**	*lysS*	X	X	E	*KRS1*	E
		genX (poxA)	E	E			
		lysU	N				
14	**Phe**	*pheS* (α)	?	E	E	*FRS2*	E
		pheT (β)	?	E	E	*FRS1*	E
15	**Pro**	*proS*	E	E	E	*YER087W*	N
						YHR020W	E
16	**Ser**	*serS*	E	X	E	*SES1*	E
17	**Thr**	*thrS*	E	E	E	*THS1*	E
18	**Trp**	*trpS*	E	X	E	*WRS1*	E
19	**Tyr**	*tyrS*	E	E	N	*TYS1*	E
20	**Val**	*valS*	E	E	E	*VAS1*	E

* Mycoplasmas, as well as many other prokaryotes (but not γ-purple bacteria) use transamidation pathway to synthesize GlntRNA^{Gln} instead of direct acylation, therefore they do not contain *glnS* orthologs.

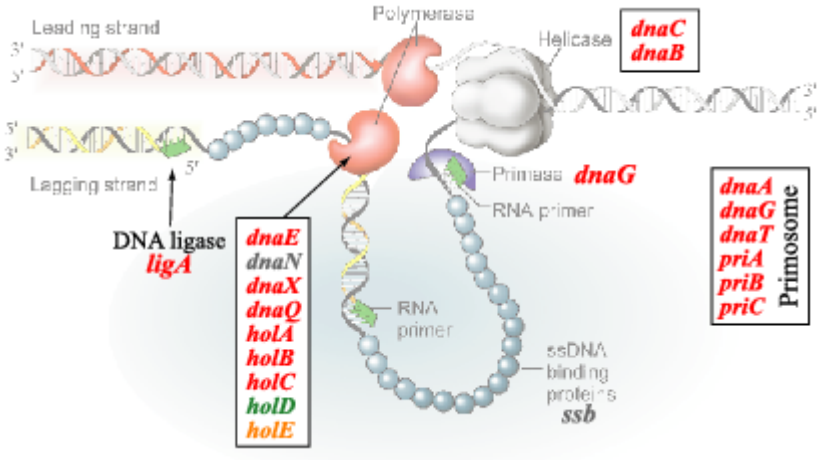
PMS: protein secretory pathways. *E. coli* has several secretory pathways involving: Sec-dependent, Sec-independent (TAT pathway), Type I (translocase mediated uncoupler family), Type II, and Type V (autotransporter). Of the 16 essential genes in this set, 15 genes are involved with the Sec-dependent pathway, which constitutes the sub-system with the highest ratio of essential genes (15 out of 21 genes, or 71%). This observation is in good agreement with previous experimental data (reviewed in (Neidhardt et al., 1996), chapter 63). There are three stages in sec-dependent translocation of proteins from the cytoplasm to periplasmic space: protein targeting, translocation, and protein maturation. All of the genes involved in protein targeting (with a single exception of *dnaJ*) were essential in our study, including those encoding subunits of signal recognition particle (*ybaA* and *ffh*), SecB, and chaperons DnaK, GrpE, GROEL, and GROES. The fact that DnaJ was found dispensable is unexpected, since DnaJ and GrpE jointly stimulate the ATPase activity of DnaK (Liberek et al., 1991), and the two latter subunits were essential. Out of seven proteins involved in the translocation step, four (SecA, SecD, SecF, and SecY) are essential and two (SecE and the trigger factor Tig) are not, while essentiality of SecG remained undetermined. The preexisting data on essentiality of secE is somewhat contradictory: the gene was shown to be essential in vivo (Downing et al., 1990), however subsequent in vitro SecE depletion studies showed that it is enhances, but not required for protein translocation (Yang et al., 1997). As expected, signal peptidases LepB and LspA involved the maturation step (N-terminal leader sequences cleavage) were both found to be essential. In contrast to the Sec-dependent pathway, all of the genes involved with other secretory pathways appear to be nonessential with the single exception of *dsbA*, which encodes a thiol-disulphide interchange protein.

Nucleic acid metabolism (NAM): replication. The essentiality data for genes involved in DNA replication, recombination, and repair are largely consistent with previous reports and expectations based on their specific functional roles (see **Figure S2**). All of the genes encoding proteins involved in initiation of replication were found to be essential, including *dnaA, dnaB, dnaC, dnaG* as well as those participating in primosome assembly. Of the genes encoding subunits of DNA polymerase III, six were assigned as essential and two as ambiguous. Only *holD* was identified as non-essential, but the role of this subunit is not fully understood, and no other data on its essentiality are available. In contrast to the replicative DNA Polymerase III, other *E. coli* DNA polymerases primarily involved in DNA recombination and repair, including Pol I (gene *polA*), Pol II (*polB*), Pol IV (*dinP)* and Pol V (*umuC, umuD*), were found to be dispensable. Almost all of the other *E. coli* enzymes involved with DNA recombination, repair and modification, are also non-essential in our

data. This may reflect the functional redundancy of many of these gene products, while others may be truly dispensable in the absence of extensive selection pressure.

Figure S2. Essentiality of genes encoding selected components of the *E. coli* chromosomal DNA replication machinery.

A model of the DNA replication fork, reproduced with permission from the Annual Review of Biochemistry, Volume 70 (Frick and Richardson, 2001) ©2001 by Annual Reviews (www.AnnualReviews.org) with kind permission of Dr. David Frick. Color coding of gene names is the same as in Figure S2.



Expression regulators and cell cycle/division (RCD): transcription factors. The RCD functional category combines two very distinct groups of genes with an overall ratio of essential genes of 17% (Table 1). The smaller group is comprised of 22 cell division/cell cycle proteins and includes 12 (>50%) essential genes. The larger group consists of >150 mostly dispensable genes encoding a variety of transcription regulatory factors. Only 12% of these were found essential in our data. Among them: DNA-binding proteins H-NS and FIS; secretion regulator SecM; MarA involved in both activation and repression of transcription of many stress-related genes; and several RNA polymerase sigma subunits. In agreement with previous reports, the main σ^{70} subunit (*rpoD*) required for most exponential phase transcription and the gene encoding its regulator (*rsd*) were essential, as well as σ^{54} controlling nitrogen metabolism, formate degradation and many other regulons. As expected for cells harvested in log-phase, the major sigma factor σ^{38} of stationary

phase expression (*rpoS)* was dispensable. Interestingly, σ^{32} (*rpoN*) required for transcription of heat shock and other stress proteins appeared essential. Many other observations related to transcriptional regulators may not be easily interpreted without in-depth analysis of convoluted regulatory networks.

Membrane transporters (MTR): cation uptake. More than 90% of *E. coli* proteins involved in transport across the cellular membrane are dispensable in our data. This is consistent with the overall prototrophic nature of the *E. coli* strain used in this work. In general, the *de novo* biosynthetic pathways for multiple metabolites (such as amino acids and vitamins) compensate for mutations occurring in the corresponding transport pathways. A percentage of essential transporters is substantially higher in *H. influenzae*, which lacks many of the *de novo* pathways found in *E. coli*. For example, 32% of genes within a common reference set of ~ 70 orthologous transporter genes (with defined essentiality in both *E. coli* and *H. infuenzae*) are essential in *H. infuenzae*, compared to only 12% in *E.coli*. In addition, many transporters in *E.coli* are functionally redundant due to the existence of multiple paralogs (families) with overlapping specificities. The few that were found essential despite this redundancy might be preferentially expressed under these conditions or possess additional indispensable functions. For example of the two Mg^{2+}- transporter genes, *corA* was found essential but *mgtA* dispensable in our study. This may be due to preferential *phoPQ*-mediated repression of the latter in the presence of high Mg^{2+} concentration in the medium (for the review see (Moncrief and Maguire, 1999)). Alternatively, the observed essentiality of the *corA* gene may originate from its apparently non-redundant role in cobalt uptake (Smith and Maguire, 1998).

Phylogenetic analysis of essentiality data within functional groups

Table S5. Essentiality of the putative orthologs of the universally essential *E. coli* genes in *M. pneumoniae, M. genitalium, H. influenzae, S. aureus,* and *S. pneumoniae.*

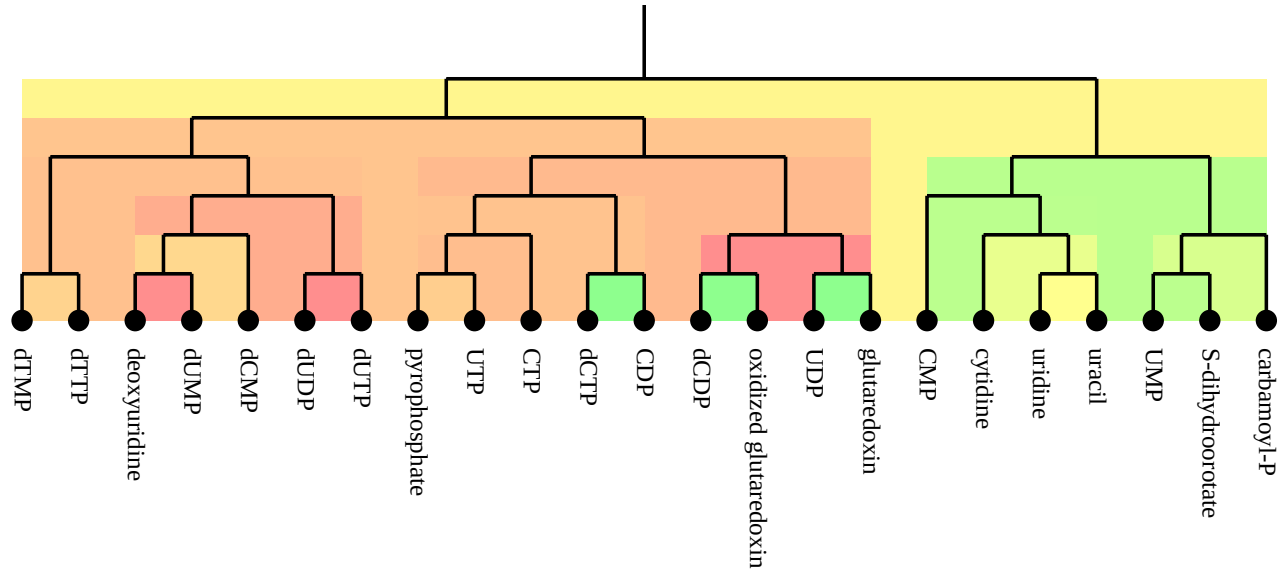
Organism	*H. influenzae*	*M. genitalium*	*M. pneumoniae*	*S. pneumoniae*	*S. aureus**
Source of essentiality data	(Akerley et al., 2002)	(Hutchison et al., 1999)	(Hutchison et al., 1999)	(Thanassi et al., 2002)	(Forsyth et al., 2002)
Number of universally essential *E. coli* genes for which orthologs could be identified	145	107	104	145	96
Orthologs with unambiguously asserted essentiality/nonessentiality	86	107	104	22	68
Orthologs found essential in each organism	60	93	90	16	68
Fraction (%) of essential orthologs among those with unambiguously asserted (non)essentiality	70%	87%	87%	73%	100%
Total number of ORFs in a genome	1288	517	756	2304	2595
Total number of essential ORFs	536	est. 265 - 350	n. d.	n.d.	658
Essential ORFs (%)	42%	51% - 68%	n. d.	n.d.	25%

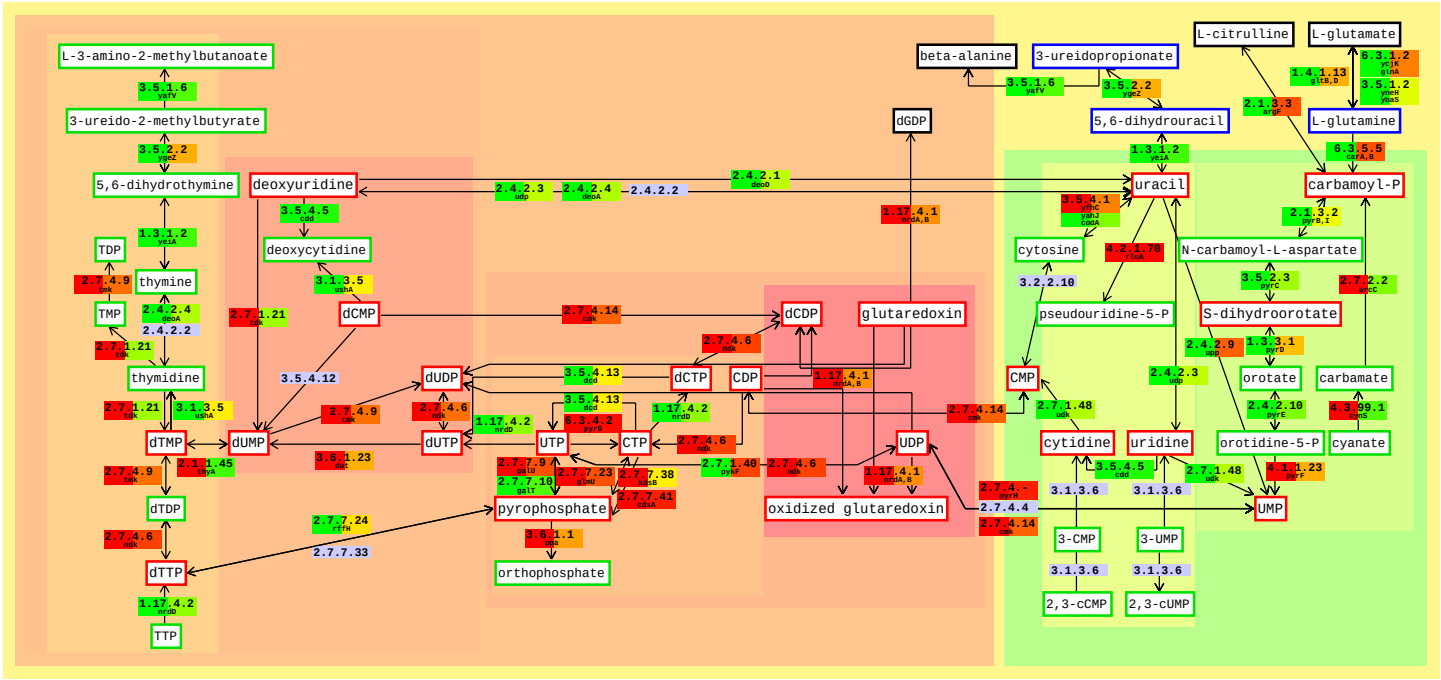
*Out of 658 genes found essential in S.aureus, only a sub-set of 158 genes conserved in M.genitalum was reported. No data are currently available for nonessensial genes. Therefore, absolute (100%) essentiality of all orthologs in this organism is misleading.

System-level analysis of essentiality data within topologic modules of *E. coli* metabolism.

Figure S3. The ratio of essential enzymes in the pyrimidine topologic module of *E. coli* metabolism (see the manuscript for additional details).

Enlarged view of the substrate module of pyrimidine metabolism. In the *top panel*, the colored boxes denote the various levels of nested modularity suggested by the hierarchical tree according to the fraction of essential enzymes of all biochemical reactions within them. In the *bottom panel* a detailed diagram of the metabolic reactions that surround and incorporate the pyrimidine metabolic module is shown. Red-outlined boxes denote the substrates directly appearing in the tree shown in the top panel. Substrates in green-outlined boxes are internal to pyrimidine metabolism but represent members of nonbranching pathways or end pathways branching from a metabolite with multiple connections. Blue- and black-outlined boxes denote those metabolites that connect pyrimidine metabolism to other parts of the metabolic network. Black-outlined boxes denote core substrates belonging to other branches of the metabolic tree, and blue-outlined boxes denote non-branching pathways (if present) leading to those substrates. The arrows show the direction of the reactions according to the ERGO metabolic maps. The colored boxes around the reactions highlight the modules suggested by the hierarchical tree according to the fraction of essential enzymes of all biochemical reactions within them. The shaded boxes along the links display the enzymes catalyzing the corresponding reactions, their left half denoting their deletion phenotype (red: essential, green: non-essential) and their right half indicating their ERI score (red color indicates 100% evolutionary conservation). Enzymes with unknown/untested phenotype are shaded in blue. Abbreviations are as follows: CDP, cytidine 5'-diphosphate; CMP, cytidine 5'-monophosphate; CTP, cytidine 5'-triphosphate; dCDP, deoxycytidine 5'-diphosphate; dCMP, deoxycytidine 5'-monophosphate; dCTP, deoxycytidine 5'-triphosphate; dUDP, deoxyuridine 5'-diphosphate; dUMP, deoxyuridine 5'-monophosphate; dUTP, deoxyuridine 5'-triphosphate; UTP, uridine 5'-triphosphate, cCMP, cyclic cytidine 5'-monophosphate; cUMP, cyclic uridine 5'-monophosphate; dTDP, deoxythymidine 5'-diphosphate; dTMP, deoxythymidine 5'-monophosphate; dTTP, deoxythymidine 5'-triphosphate; TDP, thymidine diphosphate; TMP, thymidine monophosphate; TTP, thymidine triphosphate.


dTMP
dTTP
deoxyuridine
dUMP
dCMP
dUDP
dUTP
pyrophosphate
UTP
CTP
dCTP
CDP
dCDP
oxidized glutaredoxin
UDP
glutaredoxin
CMP
cytidine
uridine
uracil
UMP
S-dihydroorotate
carbamoyl-P


L-3-amino-2-methylbutanoate
3-ureido-2-methylbutyrate
5,6-dihydrothymine
deoxyuridine
deoxycytidine
TDP
thymine
TMP
thymidine
dTMP
dUMP
dTDP
dTTP
TTP
dCMP
dUDP
dUTP
dCTP
CDP
dCDP
glutaredoxin
UTP
CTP
pyrophosphate
orthophosphate
oxidized glutaredoxin
UDP
dGDP
beta-alanine
3-ureidopropionate
5,6-dihydrouracil
uracil
cytosine
pseudouridine-5-P
CMP
cytidine
3-CMP
2,3-cCMP
3-UMP
2,3-cUMP
UMP
L-citrulline
L-glutamate
L-glutamine
carbamoyl-P
N-carbamoyl-L-aspartate
S-dihydroorotate
orotate
carbamate
orotidine-5-P
cyanate

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