ABSTRACT

Recent genomic analyses on the cellular metabolic network show that reaction flux across enzymes are diverse and exhibit power-law behavior in its distribution. While one may guess that the reactions with larger fluxes are more likely to be lethal under the blockade of its catalyzing gene products or gene knockouts, we find, by in silico flux analysis, that the lethality rarely has correlations with the flux level owing to the widespread backup pathways innate in the genome-wide metabolism of *Escherichia coli*. Lethal reactions, of which the deletion generates cascading failure of following reactions up to the biomass reaction, are identified in terms of the Boolean network scheme as well as the flux balance analysis. The avalanche size of a reaction, defined as the number of subsequently blocked reactions after its removal, turns out to be a useful measure of lethality. As a means to elucidate phenotypic robustness to a single deletion, we investigate synthetic lethality in reaction level, where simultaneous deletion of a pair of nonlethal reactions leads to the failure of the biomass reaction. Synthetic lethals identified via flux balance and Boolean scheme are consistently shown to act in parallel pathways, working in such a way that the backup machinery is compromised.

Keywords: metabolic network, flux balance analysis, synthetic lethality
1 Introduction

Complex machinery of cellular metabolism occurring in a living organism makes up a part of autocatalytic network of biochemical reaction pathways. The reaction network in itself constitutes an intricate web in such a way as sharing intermediates. Yet another dimension of complexity comes from the tight control of reactions by functional proteins which are again under transcriptional, translational control as well as degradative and other inductive regulations, making even a single pathway analysis formidable task. Only recently, advances in high-throughput experiments and the computing power incorporating diverse data sets collected in genomic research make it possible to construct cellular networks of metabolism in genome-wide perspectives. At the same time, many quantitative theoretical methods including graph theories and other mathematical tools developed from diverse disciplines attract much attention to tackle the large-scale networks (Xia et al., 2004; Barabási & Oltvai, 2004).

In the early graph-theoretic approaches to the metabolic network, attention has been paid to the so-called scale-free feature of topological structure (Jeong et al., 2000), small-world-ness (Wagner and Fell, 2001), modularity (Girvan and Newmann, 2002) and hierarchical organization (Ravasz et al., 2002). Despite the immanent specificity in cellular functions of various organisms, the connectivity, or number of connections each node (metabolite or associated reactions) has, is generally far from homogeneous. In particular, this connectivity distribution of the metabolic network, as shared by many naturally occurring complex networks, follows a power law, meaning large deviations in spite of well defined average value. It is this context that borrows the term scale-free network, where hubs, nodes with large number of connections, play essential roles. When such hubs are removed or turned off, the whole system becomes vulnerable. Indeed, it was found (Jeong et al., 2001) that, for the yeast protein interaction network, hub proteins are more likely to be lethal than the others.

In the framework of networks, metabolic reactions and participating metabolites can be mapped into alternating nodes, where the outward(inward) connections from a reaction node indicate that those metabolites are produced(consumed) as a result of the reaction. Once constructing a directed bipartite graph in this way, we calculate graph-theoretic quantities that characterize the global topology and give a clue to assessing lethality of metabolic reactions. Then, we examine the correlations between the metabolic flux level and the lethality of each metabolic reaction using the flux balance analysis[FBA] (Edwards and Pålsson, 2000). Here, by lethal, we mean the organism could rarely synthesize the indispensable biomass, or the flux of the biomass reaction is significantly reduced when that reaction is blocked or removed from the network,
mimicking gene knockout experiments*. One seemingly counterintuitive result is the absence of correlation between flux level and lethality (Fig. 2), which is related with the fact that the high-flux reactions have abundant bypasses or backup pathways.

We also invoke the Boolean network scheme, an idealization of the metabolic network as a wiring of binary logic gates to elucidate the pathway structure of the network on the logical basis. Considering the knockout and consequent cascading failure in the metabolic reaction network as an avalanche, we investigate the distribution of avalanche size defined as the number of reactions subsequently turned off on account of the removal of a target reaction to find it a good measure of lethality. The distribution follows a power law with the characteristic exponent around 2.5, pervasive throughout disparate model systems having self-organized criticality (Bak, 1996).

In the latter part, we investigate the effects of simultaneous deletion of multiple reactions with a view to elucidating the interaction between them. Though only a small portion of reactions lead to distinctive phenotype under their single deletion, it is non-trivial to make a prediction on how destructive a dual deletion of nonlethal reactions. Actually, the effect can be aggravating or alleviating as well as simple sum of each, depending on their role played in the otherwise intact metabolic network. In particular, when a pair of nonlethal reactions are deleted to make no growth of cell, we call it synthetic lethal, metabolic homologue of the same term in genetics. Synthetic lethality in metabolic network is a manifestation of their complementary nature responsible for the buffering between alternative parallel pathways. We show the synthetic lethal pairs are distributed over distinct the avalanche size of a pair of reactions is strongly correlated with its synthetic lethality.

2 Materials and Methods

We use, with minor curation, the recent revision of in silico model E. coli (Reed et al., 2003), which was obtained by searching databases, such as LIGAND (http://www.genome.jp/kegg/ligand.html), EcoCyc (http://www.ecocyc.org), TC-DB (http://tcdb.ucsd.edu), and referring to updated literatures on sequence annotation (Serres et al., 2001). To mimic random or targeted mutation strains, a specific reaction is removed from the network and the result-

*More recently, Segrè et al. proposed alternative scheme phrased as “minimization of metabolic adjustment[MOMA]” for the phenotypic prediction of deletion mutants. Instead of assuming optimality in growth yield of deletion mutants, MOMA approximates metabolic phenotype by performing distance minimization in flux space, whereby the correlation with experimental results are improved. See Segrè 2002.
tant metabolic capabilities are to be assessed. For this purpose, we introduce a single pivotal reaction, the biomass reaction, originally formulated as a linear combination of essential metabolic reactions giving rise to the growth of the organism (Neidhardt and Umbarger, 1996). Throughout the study, lethality of a certain reaction is determined by the flux of this biomass production, which is contingent to the ansatz of optimality that the selection pressure has imposed in the long history of evolution.

2.1 Metabolic Network as a Graph

The overall map of metabolic reactions we study is a bipartite graph, composed of two different types of nodes, 627 metabolites and their participating 1074 metabolic reactions including transport and exchange events. One type of nodes connect only to the other type of nodes in the networks†. Each link between a pair of a metabolite and a reaction is directed, reflecting the metabolite is either consumed (substrate) or produced (product) or both in reactions. Of the 1074 reactions, 254 reversible reactions are decomposed into two separate reactions catalyzed by the same enzyme, and possible multiple connections between metabolites via isozymes are taken as a single directed edge in the graph representation. 627 distinct metabolites have either their intracellular or extracellular version or both, which sum into 761 distinct nodes of metabolites. Once the metabolic network is reconstructed as a graph, we quantify, by various numerical measures, the lethality of each node in the wild-type strain and compare them with those of knockout mutants.

2.2 Flux Balance Analysis (FBA)

For each metabolite in the metabolic reaction network, dynamic flux balance condition on the concentration $X_i$ can be written as

$$\frac{\partial X_i}{\partial t} = -\sum_{j=1}^{n} S_{ij} \phi_j,$$

where $\phi_j$ is the corresponding reaction rate (outward flux), and $S_{ij}$ are stoichiometric coefficients involved in the metabolite $i \in \{1, 2, \cdots, m\}$ participating in the reaction $j \in \{1, 2, \cdots, n\}$ including transport and exchange reactions also. As an alternative

†Depending upon the objectives, it can be projected to recover either of the single-mode metabolite network or reaction (enzyme) network.
to yet intractable kinetic models in genome-wide perspectives, mass conservation can be applied in the balanced state to give the stationary condition \( \sum_j S_{ij} \phi_j = 0 \) for all \( i \). Stoichiometric matrix encodes the topology of the metabolic network and plays the role of weighted adjacency matrix in the corresponding graph. Once the stoichiometric coefficients are given for all reactions, we have, in general, under-determined situation \( m < n \) where huge degeneracy in the null space of Eq. (1) is unavoidable. Since, in reality, enzymatic capacity cannot be arbitrarily large, flux values are also constrained to vary in limited range, which can be estimated via, say, the Michaelis-Menten type kinetics. Actually, this multiplicity in the feasible metabolic state can be considered a manifestation of the capability of a metabolic genotype. It makes physiological sense in that cells are expected to adapt themselves responding to different stresses and growth conditions. Among those feasible metabolic phenotypes, FBA assumes the existence of optimized point(s) of a certain objective function, called biomass reaction or growth flux, by utilizing further constraints based on the thermodynamic irreversibility of reactions.

### 2.3 The Boolean Network

Metabolic reactions or genetic switches are seldom turned on or off. Instead, they can change by some fold, either up- or down-regulated, to make a physiological payoff. As a first approximation, Boolean idealization has long been introduced to reconstruct the genetic regulatory network as a directed graph (Kauffman, 1969) and was recently adopted to model the metabolic network (Lemke et al., 2004). In Boolean reconstruction of the metabolic network, each node in the graph is replaced with binary logic gate AND or OR. A metabolite would not cease to be existent in the network until all the reactions having that metabolite as a product are blocked, while a reaction does not take place any more with only a single absence out of substrate metabolites, which renders the metabolites Boolean disjunction, OR, and reactions conjunction, AND as exemplified in Fig. 1. With all nodes given an initial condition, we iteratively relax the network till the network is settled in a fixed point.

### 2.4 Cumulative Lethality Score

As a way to reveal the correlation between avalanche size and lethality, we use the index, cumulative lethality score (CLS), the original version of which was introduced in the context of protein essentiality prediction (Jeong et al, 2003). Once a measure of
lethality, say the flux level of each reaction, is proposed, we can make a serial list of reactions assorted in descending order of the proposed lethality measure. With the lethality criteria determined through the viability under the single deletion of each reaction, we can assign a binary lethality score, one or zero, into each reaction depending on whether it is lethal or not. Summing up the binary scores from the first rank to the \( j \)th rank in the value of the proposed quantity, we get the CLS, say, \( L(j) \). If the proposed quantity is positively(negatively) correlated with the lethality, \( i.e. \) reactions holding high ranks are dominated by the binary lethality score one(zero), the normalized CLS manifests itself as a concave( convex) curve. Otherwise, if it is a random sequence of zero’s and one’s, \( L(j) \) is given by a straight line. That is, the more correlated is the measure with the lethality, the higher curvature \( L(j) \) develops.

3 Results and Discussion

3.1 Flux Level versus Lethality

Under aerobic condition with nine distinct carbon sources, we identify 208 (19.6\%) common lethal reactions, the single blockade of which suffocates the biomass production\(^\dagger\). It is similar in fraction to the essential fraction of \( S. \) cerevisiae genome, 18.7\% (Tong et al., 2001; Winzeler et al., 1999; Giaever et al., 2002).

The flux distribution in the wild-type metabolic network follows a power-law in Pareto’s form, \( P(\phi) \sim (\phi + \phi_0)^{-\alpha} \). Metabolic traffic is concentrated along a few ‘super-highway’ reactions, while the vast majority of reactions are in charge of only a small flux (Almaas et al., 2004). In the meantime, inspired by the roles the main arteries, principal roads, or backbones play in blood circulation, transportation, or data communications, we examine the possibility that the flux level should reflect the lethality of each reaction. Fig. 2 shows that the plausible correspondence between flux level and lethality is a mere conjecture to prove not true. In other words, there is no correlation at all between those quantities, and high flux itself has nothing to do with the lethality of a reaction, which obviously contradicts our intuition. We also investigate the flux redistribution profile upon deletion of a high-flux reaction. Reaction fluxes are redistributed either locally or

\(^\dagger\)Throughout the analysis, acetate, alpha-ketoglutarate, glucose, glycerol, (L-,D-)lactate, L-malate, pyruvate, and succinate are used as the carbon sources. Though the lethality of a reaction does depend on which are used as carbon sources, 208 lethal reactions are shared by every sources and only 22 reactions show nutrient-dependent lethality.
globally. Here, by local, we mean the case that very few reactions, having almost zero flux in the wild type, fully take over the flux of the reaction deleted. However, in the global redistribution, the flux of the removed reaction is shared over a large number of reactions to keep optimal biomass production.

3.2 Avalanche Size versus Lethality

In the Boolean reconstruction of the *E. coli* metabolic network, 41 lethal reactions are identified, which are all lethal in FBA also. It is no wonder, if we consider that lethality in binary scheme is minimal and more stringent than in the weighted version of FBA. To quantify the effect of a single-node deletion, we define the avalanche size of each reaction, in the Boolean scheme, as the number of reactions subsequently turned off on account of the removal of a target reaction.

As shown in the inset of Fig. 3, the avalanche size distribution for *E. coli* metabolic network also displays a power law behavior, implying there exist a few reactions whose deletion triggers a large destructive avalanche cascade in metabolic reaction. In mathematical aspects, at criticality, there exists an infinite spanning cluster enough to reach the ultimate destination, biomass. Thus, avalanche size could be useful to find lethal reaction. Indeed, the reaction which generates a bigger avalanche size is more likely to be lethal as seen in the CLS plot (main frame of Fig. 3).

One may want to check the possibility that nearer nodes to the biomass reaction should be liable to be lethal. However, of the 374 distinct reactions that produce the substrates of biomass reaction, only 13 of them are lethal in the Boolean scheme, making the identification of lethal reactions nontrivial—proximity to the biomass reaction has nearly nothing to do with the lethality of a reaction.

The notion of the avalanche in the network can be extended to the FBA scheme, where the avalanche size of a reaction is defined as the number of reactions whose flux levels under its knockout differ from those in the wild type. Actually, we used three different criteria of the avalanche: (i) reactions are newly turned on or off, (ii) flux level change exceeds an arbitrary cutoff value, and (iii) fractional changes in flux exceeds the cutoff value. There is, however, little difference among the different counting schemes. Fig. 4, drawn by using the criterion (i) above, reveals the absence of correlation between the flux level and the avalanche size both in the Boolean scheme and in FBA, which is consistent with the fact that flux level is irrelevant to assessing lethality.(Fig. 2)

Due to the small-world-ness of the complex network, local perturbations are liable to propagate to the whole network leading to the sharing of load, which underlies the system-wide high flexibility. At the same time, because of the scale-free-ness, avalanche
cascade can either be long-ranged by making a large number of nodes bear parts of the ‘expenses’ or be absorbed at a short distance from the source of perturbation, depending on the detailed functional characteristics of the reaction. In other words, the response to single deletion perturbation of a single reaction are too diverse to definitely predict how they would be, and so they can be predicted in a probabilistic way (Kim et al., 2003). Identification of lethal reactions in metabolic network can be viewed in the same footing. The effects of a node removal or a gene deletion are largely negligible as a whole, which is manifested by the dominance of nonlethal reactions. However, even if a reaction is not lethal, its potential damage to the network varies. It is this insufficiency in lethality assessing that raises the need for the knowledge of synthetic lethals in the next section.

3.3 Synthetic Lethality in Metabolic Network

The close genetic relationships between genes which underlie the functional buffering has been associated with the notion of synthetic lethality. It has been assessed in a high-throughput manner by the synthetic genetic array (SGA) analysis (Tong et al., 2001). Likewise, analysis of multiple-deletion mutants in genome-scale metabolic network may shed light on novel topological features of backup pathways leading to the robustness. In the restricted level of metabolism, such relationships can be revealed more clearly by performing the double reaction knockout experiments, which can be easily performed in silico. Furthermore, such synthetic lethal pairs identified allow us to track the backup pathways and to visualize the precise genomic origin from which the metabolic phenotypic stability arises.

When the glucose is used as a carbon source in aerobic condition, 55 synthetic lethal pairs are identified out of all possible pairs of reactions (Table I). Relatively small number of synthetic lethal pairs suggests high density of backup pathways for a given specific condition in the metabolic network and is in accordance with the case of the yeast (Papp et al, 2004). Among these, 33 (60%) pairs are involved in the same subdivision of the reaction categories. As expected, most of those homofunctional synthetic lethals usually work as the ‘simple’ backup pathway: One of the pair is never used in the wild type but it almost completely takes over the flux of the blocked reaction, which amounts to 64% of total synthetic lethal pairs, as depicted in Fig. 5(a). We note, however, the greater part of such simple takeover case might be attributed to an artifact of the optimization scheme. Since there can be multiple solutions yielding the same maximized growth rate, and one cannot select a specific solution over the others without relevant physiological constraints, such as regulations in transcription level. Nevertheless, the effect of degeneracy on the (synthetic) lethality assay is not harmful since the deletion
of lethal reaction unambiguously indicates no feasible solutions for nonzero biomass production. Interestingly, the homofunctional synthetic lethals of the other type, for which both the reactions are operational in the wild type, are mostly involved in the two subsystems of the pentose phosphate cycle and threonine-lysine metabolism. Excluding these particular cases, 91% of homofunctional synthetic lethals are simple, while, for the other 22 heterofunctional synthetic lethals, only 9 (41%) of them are the simple backup pathways. In total, 25 pairs were both operational in the wild type and the remaining 30 are paired in one used and one unused in the wild type.

We also investigate the synthetic lethality in the Boolean scheme, where 37 pairs of synthetic-lethal doublets are identified in addition to 41 lethal singlets. Contrary to the lethal singlets which are dominated by cell envelope biosynthesis (78%), synthetic lethals are scattered throughout diverse functional categories (Fig. 7).

Conserved across both the network scheme is the proximity of the two reactions constituting a synthetic-lethal pair. Fig. 6 illustrates the distance distribution for the synthetic-lethal reaction pairs. As shown in the inset of Fig. 6, 94.5% (97.3%) of synthetic-lethal pairs in FBA(Boolean) scheme are apart in three steps and below, which stands out sharp contrast to the fraction 42.4% for all pair of metabolites. If we eliminate, to make better sense of biochemical reaction pathways, currency metabolites or cofactors from the network, the fraction of synthetic-lethal pairs whose separation is three steps and below are 96.2% and 91.1% in FBA and Boolean scheme, respectively (main plot). Whether the analytical scheme is Boolean or FBA, long-ranged complicity of synthetic lethals through the intermediary of a ‘filamentary’ single pathway like in Fig. 5(b) comprises only a small fraction(<5%) of synthetic-lethal pairs, and is rather an exception.

Another important outcome regarding synthetic lethality is already shown in Fig. 3, where we measure the avalanche size of the synthetically lethal doublets and triplets in addition to that of the singlets. Synthetic lethal multiplets give rise to even higher correlation of their avalanche size with the lethality. In effect, robustness in metabolic network stems from redundancy in branched and parallel pathways. Conversely, lack of reaction pathways, whether it is due to the innate biochemical nature or to the incompleteness of pathway database, lead to vulnerability. Hence, the more we know about a reaction pathway, the less probable it should contain lethal reactions. In particular, we cannot completely rule out the latter possibility: A quarter of the E. coli genome is yet to be functionally assigned. Accumulated bias in molecular biology research, if any, might be considerable to our result that central pathway across the species, such as the citrate cycle or the glycolysis pathways, have very few lethal reactions (Fig. 7). However, at least for E. coli, one of the best known bacteria yet studied, there are no good reasons to
suspect such a bias. Moreover, our analytical results are compatible with the fact that a wide-spread strategy of antimicrobials is acting against cell wall synthesis (fosfomycin, cycloserine) or integrity (lysozyme). Rather to be supposed is that the more important a reaction is, the better facilitated its backup pathways have been during evolution.

4 Summary and Outlook

Systematic deletion study in a genome-wide view of model organisms help reveal the organizing principles of the metabolic network and may shed light on how the selection has been embodied at the network levels, and especially the recent controversy surrounding the causes and evolution of the enzyme dispensability (Papp et al., 2004). As an index quantifying lethality in the graph-reconstructed metabolic reaction network, we propose the avalanche size of each reaction, the number of ‘dead’ reactions due to the knockout of that reaction or its related gene products to show an even more remarkable interdependence than various measures yet proposed. By identifying synthetic lethals or lethal multiplets in the genome-scale metabolic network under controlled environments, we see the emergence of phenotypic stability supported by rich backup pathways, which is shared by diverse realization of complex networks with scale-free nature. Studies on multiple deletion mutations in metabolomic interaction network can also be applied to natural metabolomic variations, reminiscent of single-nucleotide polymorphism, giving rise to practical buffering or phenotypic robustness under targeted mutations (Tong et al., 2001; Tucker and Fields, 2003; Ooi et al., 2003). Furthermore, if we incorporate network analyses on metabolism with the gene-protein-reaction associations by including the other sectors of intra- and inter-cellular networks, it can be used in designing new microorganismal strains on the computer truly beyond the reductionist perspectives.

Note Added at Proof

After completion of the present study, we learned of the work by Burgard et al. (2004), who introduced seemingly parallel concept of flux coupling analysis to our avalanche analysis in FBA scheme. Originally, the metabolic flux $\phi_1$ was defined as (directionally) ’coupled’ to $\phi_2$ when a nonzero flux value $\phi_2$ implies a nonzero flux value $\phi_1$ in any stationary states of the system. Taking the contraposition, so as to interpret it in the context of the lethality, $\phi_1$ is coupled to $\phi_2$. Then the zero flux of $\phi_1$ implies the zero flux of $\phi_2$. While the avalanche size is correlated with the number of fluxes coupled with
the biomass reaction, they are not mathematically identical, because the former refers only to the optimal flux distribution, whereas the latter covers all possible stationary states.

**Acknowledgements**

The authors thank J. L. Reed and B. Ø. Pålsson for helpful comments. This work is supported by the KOSEF grants No. R14-2002-059-01000-0 in the ABRL program and the MOST grant No. M1 03B500000110.
Abbreviation lists for metabolites and reactions (enzymes) in Fig. 1, 5 and Table I.

| Abbreviation | Full Name | Information |
|--------------|-----------|-------------|
| 4r5au | 4-(1-D-Ribitylamino)-5-aminouracil | h, H²⁻ |
| adp | ADP | h₂o, H₂O |
| alg | 2-Oxogluturate | icit, Icitrate |
| atp | ATP | mal-L, L-L-malate |
| cdp | CDP | nadB, Nicotinamide adenine dinucleotide, reduced |
| cit | Citrate | nadph, Nicotinamide adenine dinucleotide phosphate, reduced |
| co2 | CO₂ | oaa, Oxaloacetate |
| coa | Coenzyme A | p, Phosphate |
| ctp | CTP | ppi, Diphosphate |
| db4p | 3,4-dihydroxy-2-butanone-4-phosphate | q₈, Ubiquinone-8 |
| dcdp | dCDP | q₈h₂, Ubiquinol-8 |
| dctp | dCTP | ribfv, Riboflavin |
| dmzl | 6,7-Dimethyl-8-(1-D-ribityl)lumazine | succ, Succinate |
| fad | FAD | succoa, Succinyl-CoA |
| fadH2 | FADH₂ | trdox, Oxidized thioredoxin |
| fam | FMN | trrd, reduced thioredoxin |
| acon | Aconitase | MTHFC, methenyltetrahydrofolate cyclohydrolase |
| ADHFr | Aldehyde dehydrogenase | MTHFR2, 5,10-methylenetetrahydrofolate dehydrogenase (NADP) |
| ADK | Adenylate kinase | NADH₆, NADH dehydrogenase (ubiquinone-8) |
| ADK3 | Adenylate kinase (GTP) | NDPK, nucleoside-diphosphate kinase |
| AKGDH | 2-Oxoglutarate dehydrogenase | NDPK₃, nucleoside-diphosphate kinase (ATP,CDP) |
| ALAR | Alanine racemase | NDPK₇, nucleoside-diphosphate kinase (ATP,CDP) |
| ALARi | Alanine racemase (irreversible) | NDPR, nucleoside-diphosphate reductase (ADP) |
| ARGDC | Arginine decarboxylase | NDPR₂, nucleoside-diphosphate reductase (GDP) |
| ASNS | Asparagine synthase (glutamine-hydrolysing) | NDPR₃, nucleoside-diphosphate reductase (CTP) |
| ASNS2 | Asparagine synthase | NDPR₄, nucleoside-diphosphate reductase (CTP) |
| ASPTA | Aspartate transaminase | NDPR₅, nucleoside-diphosphate reductase (ATP) |
| BIOMASS | Biomass reaction | NDPK₇, nucleoside-diphosphate kinase (ATP,dCDP) |
| CBM | Carbamate kinase | NDPK₈, nucleoside-diphosphate kinase (ATP,dADP) |
| CBPS | Carboxyl-phosphate synthase (glutamine-hydrolysing) | NDPR₉, nucleoside-diphosphate kinase (ATP,dCDP) |
| CO₂ | Carbon dioxide transport | O₂, O₂ transport (diffusion) |
| CS | Citrate synthase | O₂t, O₂ transport (diffusion) |
| DH4PS | 3.4-Dihydroxy-2-butanone-4-phosphate | O₃, O₃ transport via ABC system |
| DHORD2 | Dihydronicotinic acid dehydrogenase | O₅t, O₅ transport via ABC system |
| DHORD5 | Dihydronicotinic acid (menaquinone-8) | O₅t, O₅ transport via ABC system |
| DKMPD | 2,3-diketo-5-methylthio-1-phosphopentane degradation reaction | O₅t, O₅ transport via ABC system |
| DKMPD2 | 2,3-diketo-5-methylthio-1-phosphopentane degradation reaction | O₂t, O₂ transport via ABC system |
| EXₐ₅ra | Trans-system-boundary (irreversible) | O₂t, O₂ transport via ABC system |
| FMₐ₅AT | FMN adenyltransferase | NDPR₉, nucleoside-triphosphate reductase (ATP) |
| FM₃ | Fumarate reductase | NDPR₁₀, nucleoside-triphosphate reductase (CTP) |
| FM₅ | Fumarase | NDPR₁₀, nucleoside-triphosphate reductase (CTP) |
| GAL/Ru | UTP-glucose-1-phosphate uridylyltransferase | SUCCo₂b, Succinate efflux via proton symport |
| GALU | UTP-Glc-1-phosphate uridylyltransferase | SUCCo₂b, Succinate efflux via proton symport |
| GARD | GAR transformylase-T | SUCD₄₁, succinate dehydrogenase |
| GHMT2 | Glycine hydroxymethyltransferase | SUC₃, succinate dehydrogenase |
| GLUDy | Glutamate dehydrogenase (NADH) | SUC₃, succinate dehydrogenase |
| GLUSy | Glutamate synthase | SUC₃, succinate dehydrogenase |
| HNS | Homoserine kinase | SUC₃, succinate dehydrogenase |
| HSST | Homoserine O-succinyltransferase | SUC₃, succinate dehydrogenase |
| HSK | Homoserine kinase | SUC₃, succinate dehydrogenase |
| ICL | Isocitrate lyase | TKT, Transketolase |
| KAS14 | b-Ketoacyl synthase | TRPS₃, tryptophan synthase (indoleglycerol phosphate) |
| KAS15 | b-Ketoacyl synthase | TRPS₃, tryptophan synthase (indoleglycerol phosphate) |
| MALS | Malate synthase | VAL₄, Valine transaminase |
| MDH | Malate dehydrogenase | VPAMT, Valine-pyruvate aminotransferase |
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Figure Legends

Fig. 1
A subgraph of the citrate cycle in *E. coli* metabolic network. In Boolean scheme, metabolites (ellipses) are treated as Boolean disjunction (OR), while the reactions (rectangles) as conjunction (AND). If this graph were isolated, though actually not the case, from the other reactions and metabolites, the metabolite $\text{coa}$ would be no more supplied only when both the reactions $\text{SUCCOAS}$ and $\text{CS}$ are blocked. On the other hand, the reaction $\text{AKGDH}$ would not be operational when either of the metabolites $\text{coa}$ or $\text{akg}$ is knocked out. In this hypothetical subnetwork severed from the other part, bold red arrows indicate blocked reaction paths due to the knockout of the reaction $\text{CS}$. Defining avalanche size as the number of reactions subsequently turned off on account of the removal of a target reaction, reaction $\text{CS}$ has avalanche size as big as three.

Fig. 2
Normalized cumulative lethality score (CLS) with respect to the wild-type flux levels grown on distinct carbon sources, where 0(1) in the ordinate corresponds to the highest(lowest) ranker in the flux level. CLS against flux level shows no remarkable convexity, if any, implying they cannot be a lethality measure.

Fig. 3
Normalized cumulative lethality scores drawn for the avalanche size of single and multiple deletion in the Boolean version of the *E. coli* metabolic network, where 0(1) in the ordinate corresponds to the highest(lowest) ranker in the flux level. Inset: Boolean avalanche size distribution under single targeted deletion of each reaction follows a power-law with the (noncumulative) exponent 2.5.

Fig. 4
Predicted lethal (red circles) and nonlethal (blue crosses) reactions in the avalanche size and the wild-type flux level determined by FBA (main frame) and the Boolean scheme (inset). In both the FBA and Boolean schemes, lethal reactions have larger avalanche size and are dispersed along wide range of flux level at the same time. Here, only the reactions being turned on in the wild-type strain are plotted.

Fig. 5
(a) As a consequence of NDPK7(nucleoside-diphosphate kinase, ATP:dCDP) removal, the reaction RNDR3(ribonucleoside-diphosphate reductase) is also blocked(dotted red lines), but RNTR3(ribonucleoside-triphosphate reductase) is newly turned on(solid (sky-)blue lines), with NDPK3(nucleoside-diphosphate kinase, ATP:CDP) flowing more flux to make up for the growth flux. Here, only three reactions are retuned, two of which
depicted by filled blocks constitute a synthetic-lethal pair in both the Boolean and FBA schemes. The flux changes are shown in units of mm/g DW-hr. (b) *E. coli* can subsist without the reaction SUCD4, but, in the absence of SUCD4, all the reactions DB4PS, RBFSa, RBFSb, RBFK, and FMNAT become lethal. In particular, DB4PS has a ‘long-range correlation’ with SUCD4 along with the intermediary lethal reactions. Other parts of the network that the avalanche cascade does not reach are omitted for brevity.

**Fig. 6**
Synthetic lethal pairs tend to be closer neighbors than arbitrary pairs of reactions. Here, ALL refers to all pairs of reactions, while BOOL(FBA) stands for the Boolean(FBA) synthetic-lethal pairs. In the main histogram, distance between a pair of metabolites is measured without counting the connection via currency metabolites or cofactors, while the inset is obtained by counting every connection. Reactions sharing either substrates or products are regarded unit distance apart from each other. Across the analytical schemes, almost all (≥95%) synthetic-lethal pairs are only three steps apart and below, in sharp contrast with the broad distribution of distances between arbitrary pairs. See the text for details.

**Fig. 7**
Classification of lethal and synthetic-lethal reactions according to the functional categories. Frequencies are normalized by total number of lethal singlets and doublets in both the Boolean and FBA scheme, respectively.
Table Legend

Table 1
List of synthetic-lethal reactions. Upper(lower) two cells include synthetic lethals belonging to the same(distinct) functional categories, and the wild-type flux of each reaction is given in units of mm/g DW-hr.
Figure 1: Ghim, Goh & Kahng
Figure 2: Ghim, Goh & Kahng
Figure 3: Ghim, Goh & Kahng
Figure 4: Ghim, Goh & Kahng
Figure 5: Ghim, Goh & Kahng
Figure 6: Ghim, Goh & Kahng
| Reaction I | Reaction II | Category I | Category II | $\phi_{\text{I}}$ | $\phi_{\text{II}}$ |
|------------|-------------|------------|-------------|-----------------|-----------------|
| ASNS2      | ASX5        | Alanine, aspartate metabolism | -            | 0.299933        | 0.000000        |
| ALAR       | ALARi       | Alanine, aspartate metabolism | -            | 0.070257        | 0.000000        |
| PPC        | MAL5        | Anaplerotic reactions | -            | 3.519873        | 0.000000        |
| PPC        | ICL         | Anaplerotic reactions | -            | 3.519873        | 0.000000        |
| DKMPPD2    | DKMPPD     | Aminopterin, Proline Metabolism | -            | 0.009138        | 0.000000        |
| ORNDC      | ARGDC       | Aminopterin, Proline Metabolism | -            | 0.054826        | 0.000000        |
| ORNDC      | AGMT        | Aminopterin, Proline Metabolism | -            | 0.054826        | 0.000000        |
| GLUDy      | GLUSy       | Glutamate metabolism | -            | -10.86474       | 0.000000        |
| KAS15      | KAS14       | Membrane Lipid Metabolism | -            | 0.41942         | 0.000000        |
| ADK        | ADK3        | Nucleotide Salvage Pathways | -            | 3.284564        | 0.000000        |
| RNRTR2     | RNRTR2      | Nucleotide Salvage Pathways | -            | 0.033157        | 0.000000        |
| RNRTR2     | NDPK3       | Nucleotide Salvage Pathways | -            | 0.033157        | 0.000000        |
| RNRTR2     | NDPK8       | Nucleotide Salvage Pathways | -            | 0.0332243       | 0.000000        |
| RNRTR3     | RNRTR3      | Nucleotide Salvage Pathways | -            | 0.033157        | 0.000000        |
| NDPK7      | RNRTR3      | Nucleotide Salvage Pathways | -            | 0.033157        | 0.000000        |
| NDPK       | ADK3        | Nucleotide Salvage Pathways | -            | 1.04572         | 0.000000        |
| GARTF      | GART        | Purine, Pyrimidine Biosynthesis | -            | 0.623856        | 0.000000        |
| DHOIRD5    | DHOIRD2     | Purine, Pyrimidine Biosynthesis | -            | 0.41127         | 0.000000        |
| O2         | SUC2C2n     | Transport, Extracellular | -            | 20.00000        | 0.000000        |
| PI2        | Plabc       | Transport, Extracellular | -            | 1.190038        | 0.000000        |
| TARS       | TRPS        | Tyrosine, Tyrphosph, Phenylalanine Metabolism | -            | 0.0704890       | 0.000000        |
| RNDR       | RNRTR       | Nucleotide Salvage Pathways | -            | 0.269780        | 0.0332243       |
| ADK        | NDPK        | Nucleotide Salvage Pathways | -            | 3.284564        | 1.045572        |
| RPE        | TKT2        | Pentose Phosphate Cycle | -            | 5.716916        | 5.716916        |
| RPE        | TKT         | Pentose Phosphate Cycle | -            | 5.716916        | 3.141363        |
| RPE        | TALA        | Pentose Phosphate Cycle | -            | 5.716916        | 3.110267        |
| TALA       | TKT         | Pentose Phosphate Cycle | -            | 3.110267        | 3.141363        |
| TKT        | TKT2        | Pentose Phosphate Cycle | -            | 3.143163        | 2.573754        |
| GND        | TKT         | Pentose Phosphate Cycle | -            | 10.42057        | 2.573754        |
| GND        | RPE         | Pentose Phosphate Cycle | -            | 10.42057        | 5.716916        |
| THRa       | THRS        | Threonine, Lysine Metabolism | -            | -0.26978        | 0.405103        |
| HSK        | THRS        | Threonine, Lysine Metabolism | -            | -0.26978        | 0.405103        |

**Table 1:** Ghim, Goh & Kahng