Cascading failure and robustness in metabolic networks

Ashley G. Smart[†], Luis A. N. Amaral, and Julio M. Ottino

Department of Chemical and Biological Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208

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We investigate the relationship between structure and robustness in the metabolic networks of *Escherichia coli*, *Methanosarcina barkeri*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae*, using a cascading failure model based on a topological flux balance criterion. We find that, compared to appropriate null models, the metabolic networks are exceptionally robust. Furthermore, by decomposing each network into rigid clusters and branched metabolites, we demonstrate that the enhanced robustness is related to the organization of branched metabolites, as rigid cluster formations in the metabolic networks appear to be consistent with null model behavior. Finally, we show that cascading in the metabolic networks can be described as a percolation process.

complex networks | metabolism | percolation | topological flux balance

A single overloaded line in a power transmission network can lead to a blackout spanning millions of homes (1), congestion on a single router can lead to large-scale internet collapse (2), and the removal of a single enzyme from a metabolic network—and consequently, its corresponding reaction(s)—can cause the "knockout" of several additional reactions (3–6). In each case, the relationship between structure and function is central: How have these systems balanced the need for robustness against perturbations while being adaptable in the presence of dramatic changes? In man-made systems, this balance may be the culmination of concerted human intervention; in natural systems such as metabolic networks, it is the work of evolution.

Fundamental understanding of structure–function relationships in metabolic networks is particularly elusive. On the one hand, metabolic structure has been characterized extensively—metabolic networks are known to exhibit broad-tailed degree distribution [though they are not scale-free (7)], and are in–out degree correlated (8), degree–degree correlated (9), modular (10), heirarchical (11, 12), and self-similar (13). On the other hand, numerical techniques in biology permit remarkably accurate predictions of metabolic function (14–18). Yet, connecting the two areas remains a major challenge. For example, a deceptively simple question—"Are metabolic networks robust, and if so, why?"—does not yet have a definitive answer.[‡]

Here, we use a topology-based cascading failure algorithm to probe structure–function relationships in *Escherichia coli*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, and *Methanosarcina barkeri* metabolic networks.[§] We show by comparison to appropriate null models (i.e., rewired networks that preserve degree distribution yet lack organizational motifs) that these metabolic networks are robust—their structural features reduce the likelihood of large failure cascades. By decomposing each network into rigid clusters and nonrigid branches, we show that enhanced network robustness is related to the organization of nonrigid, branched elements. We also show that cascading can be described as a percolation-like process that is, in the null model case, subcritical with respect to rigid cluster formation and supercritical with respect to branching.

Analysis

We represent the cellular metabolism as a directed, bipartite graph, with two types of nodes—reaction nodes and metabolite nodes. A directed edge connects a metabolite node to a reaction node if the metabolite participates in the reaction as a reactant (directed toward the reaction node) or product (directed toward the metabolite node). Each node can then be characterized by an incoming degree, k_{in}, outgoing degree, k_{out}, and total degree, $k = k_{\text{in}} + k_{\text{out}}$, indicating the incoming, outgoing, and total number of edges.

The Topological Flux Balance (TFB) Criterion. We define a viable metabolite as one that can be maintained at a steady, nonzero concentration in a metabolic steady state [this is essentially the flux balance requirement imposed in flux balance analysis, a numerical method commonly used to study metabolic robustness (14, 24, 25)]. It follows that—as a minimum requirement for viability—each metabolite must participate in at least one generating and one consuming reaction; the concentration of a metabolite that is consumed but not generated quickly diminishes to zero, whereas the concentration of a metabolite that is generated but not consumed grows infinitely. The topological equivalent is that each metabolite node must have at least one incoming and one outgoing edge. Formally, we say that metabolite node i is viable if and only if $k_{i,\text{in}}$ and $k_{i,\text{out}} \geq 1$.

Exceptions to the TFB criterion are (*i*) external metabolite nodes—which represent extracellular compounds such as nutrients and end-products—and (*ii*) dead-end metabolite nodes—which represent metabolites that, because of incomplete *in silico* reconstructions, appear to participate in either no generating or no consuming reactions. We treat external and dead-end metabolite nodes as infinite reservoirs; they are the only metabolite nodes allowed to exist in the network with either k_{in} or $k_{\text{out}} = 0$.[¶]

Before node deletions, all metabolite nodes (excluding external and dead-end nodes) meet the TFB criterion. However, the removal of a reaction node, along with its associated edges, may leave a neighboring metabolite node with either k_{in} or $k_{\text{out}} = 0$. Such a node is said to be nonoperational and is subsequently deleted from the network along with each reaction in which it participates (a reaction is viable—i.e., can maintain a steady, nonzero flux—if and only if each of its reactants and products are viable).

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[†]To whom correspondence should be addressed. E-mail: a-smart@u.northwestern.edu.

[‡]A previous finding by Jeong *et al.* (8) that metabolic networks are error-tolerant because of their scale-free construction—although potentially important to regulation—is not considered to be relevant to the robustness of synthetic pathways (19).

[§]We have used *in silico* reconstructions compiled by the Palsson group (20–23) and made publicly available at the BiGG database (http://systemsbiology.ucsd.edu).

[¶]Transport, including diffusion, across cell membranes is treated as a reaction, such that extra- and intracellular versions of the same metabolite are distinguished as separate nodes.

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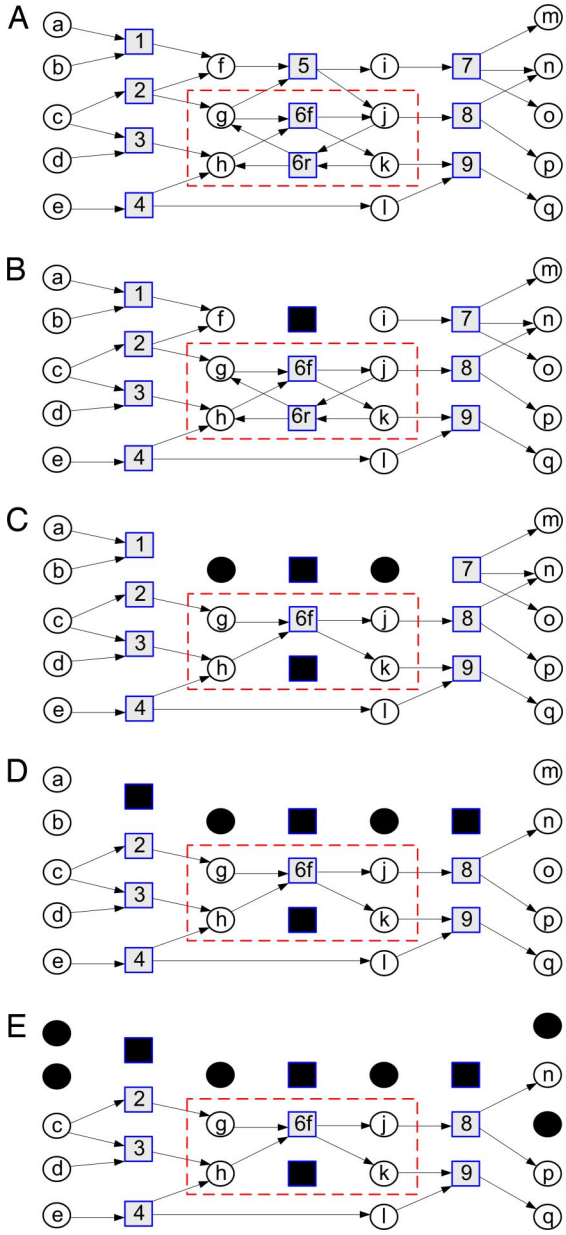

Fig. 1. The failure algorithm. (*A*) We represent a metabolic network as a bipartite graph, with directed edges connecting metabolite nodes (circles) to reaction nodes (squares). Note that reaction 6 is reversible and thus represented as a coupled pair of reaction half nodes, 6f and 6r. (*B*) In this example, we initiate a cascade by removing reaction 5 and its edges. This removal leaves metabolites f and i nonoperational, i.e., with no outgoing and no incoming edges, respectively. (*C*) Metabolites f and j must be removed, along with their edges. Since the lone incoming edge of metabolite i comes from the reaction half node 6f, reaction 6 is fixed in the forward direction, and thus 6r is deleted, along with its edges. (*D*) The reactions associated with the deleted metabolites (1 and 7) are removed, along with their edges. (*E*) Removal of reactions 1 and 7 leave metabolites a, b, m, and o nonoperational.

The Algorithm. To investigate the robustness of metabolic networks, we use an iterative algorithm where the removal of a single reaction node can lead to large-scale cascading failure:

1. Initiate failure by deleting a reaction node and each of its connecting edges (both incoming and outgoing edges are removed).
2. Delete any metabolite node for which k_{in} or $k_{\text{out}} = 0$, along with each of its remaining edges.

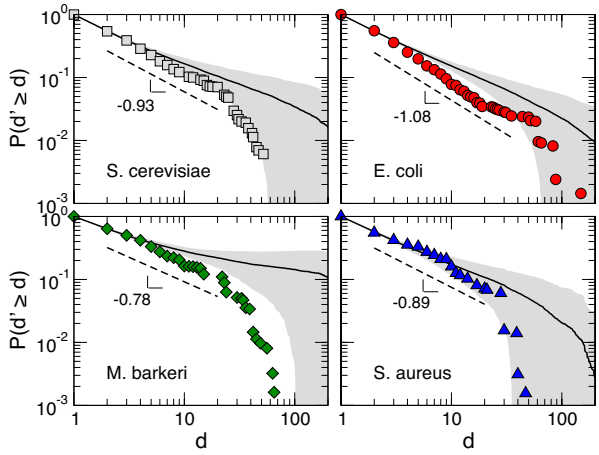

Fig. 2. Damage distributions for cascading events in metabolic networks. The cumulative distribution function, $P(d' \geq d)$, for each species is shown on log–log axes (data points). All species have similar, broad-tailed distributions, decaying at a rate close to power-law for small d but tailing off at a rate closer to an exponential decay at large d. Randomization typically results in networks with an increased susceptibility for large cascades, suggesting that organizational features in the metabolic networks act to enhance robustness. The solid curve indicates $P(d' \geq d)$ for an ensemble of 100 randomly rewired networks, with the shaded area denoting the 95% confidence interval.

3. Delete any reaction node that shares an edge with a nonoperational metabolite, along with each of its remaining edges.

Steps 2 and 3 are iterated until all remaining reaction nodes and metabolite nodes meet the TFB criterion (see Fig. 1). This algorithm is similar, in spirit, to that used in ref. 3 and the "synthetic accessibility" algorithm used in ref. 17.

A subtle yet significant feature of the TFB failure algorithm is that it permits bi-directional cascades. A reaction node deletion affects both its product and reactant metabolites, so that failure can cascade both forward (toward end-products) and backward (toward nutrients) along the network. This has biological relevance—assuming that there are no alternative pathways, pathways that "funnel" into a removed node are rendered nonoperational, as are those that emanate from it.

Reversible Reactions. Reversible reactions require particular attention. Consider a metabolite that is both fed and consumed exclusively by a single reversible reaction. Although the metabolite meets the TFB criterion, one can see that it and its associated reaction are effectively nonoperational, since the reversible reaction must have zero net flux. Approaches found in the literature to handle these situations are inadequate; they either treat reversible reactions as two separate reaction nodes (which gives rise to the same problem) or treat reversible reactions as directed reactions (which yields a less robust network) (3).

We resolve the reversibility anomaly by representing each reversible reaction as a coupled pair of reaction half-nodes, where one half-node is deleted if the topology of a neighboring node fixes the direction of positive reaction flux (Fig. 1). For example, if the lone incoming edge of a metabolite node is connected to the forward half-node of a reversible reaction, then the reaction is necessarily fixed in the forward direction and the corresponding reverse half-node is deleted. Our approach thus precludes the possibility for self-producing, self-consuming metabolites in the network.

Results

We initiate failure cascades by removing a single reaction node (this could correspond to failure of an enzyme due to knockout,

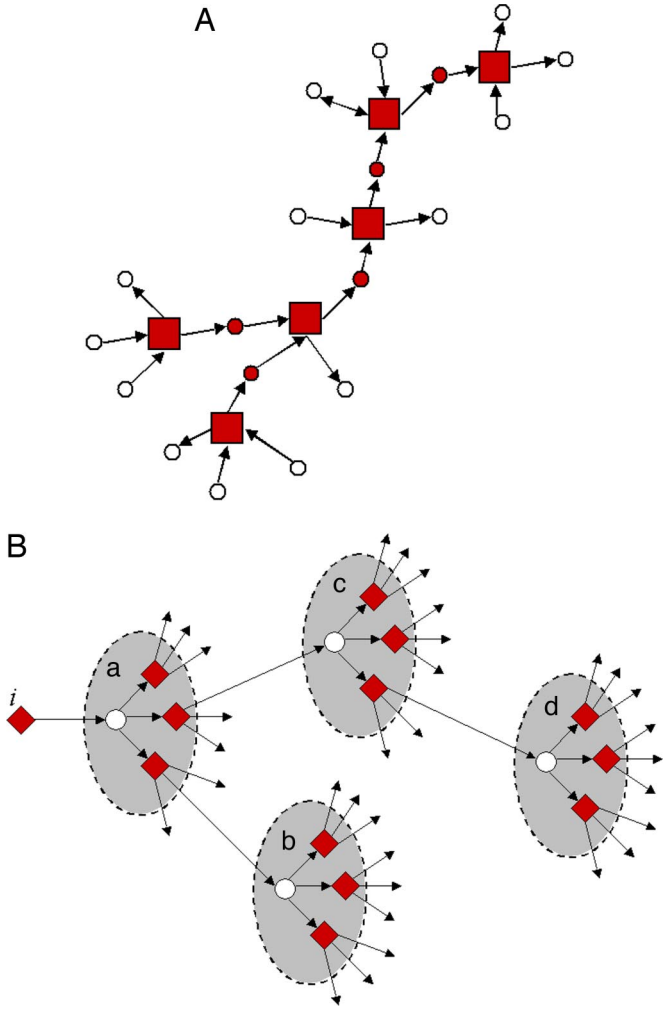

Fig. 3. Rigid clusters and branching in the metabolic network. The metabolic network can be decomposed into rigid and nonrigid elements. (*A*) A rigid cluster, defined as a cluster of contiguous nodes that does not contain a branched metabolite node (squares, reaction nodes; filled circles, uniquely produced–uniquely consumed metabolite nodes; open circles, branched metabolite nodes). The rigid cluster in *A* has size $s_c = 6$, where s_c is the number of reaction nodes contained in the cluster. (*B*) A single failure cascade may comprise multiple rigid clusters. If a rigid cluster is connected to the feeding edge of a branched, susceptible metabolite node, it will produce a failure cascade that propagates to other rigid clusters. In *B*, diamonds represent rigid clusters, and open circles represent branched, susceptible metabolite nodes. The elements inside of each shaded oval constitute a supernode.

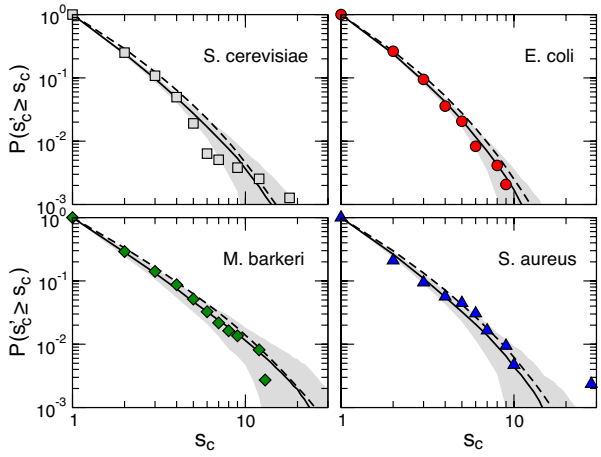

Fig. 4. Rigid cluster size distributions in metabolic networks. The rigid cluster size distribution, $P(s_c \geq s_c)$, for each metabolic network (data points) is close to the expected result for its corresponding randomly wired network [each solid curve indicates $P(s_c \geq s_c)$ for an ensemble of 100 randomly rewired networks, with the shaded area denoting the 95% confidence interval]. The distributions are approximately described by the numerical solution for random percolation on a Bethe lattice (dashed curves). All curves are consistent with subcritical percolation (i.e., $\gamma_c < 1$).

inhibition, or mutation), and characterize the resultant cascade by its total number of reactions deleted, d (we refer to this as the damage). Clearly, d is only a coarse indicator of the deleterious effect of a node removal. A truly accurate assessment should take into account the identity and function of each deleted metabolite and reaction (it is possible for a small cascade to be lethal, while a larger cascade in the same system may be nonlethal). Such detailed analysis, however, is beyond the scope of this work—our aim is to isolate generic trends relating cascade behavior to network structure, and thus we take d as an approximate indicator of network damage.

For each network, we generate a cumulative distribution function, $P(d' \geq d)$—the probability that a reaction node removal will yield a damage greater than or equal to d (Fig. 2). The distributions $P(d' \geq d)$ have a similar form for the species we studied: they are broad-tailed, indicating that while most cascades are small (≈90% of cascades have $d \leq 10$, which corresponds to ≈1% or less of the total number of reactions, depending on the species), some are quite large (the largest cascades range from $d \approx 50$ to $d \approx 80$, which is approximately on the order of 10% of the total number of reactions). These large failures represent catastrophic—and likely lethal—events, so that the behavior of $P(d' \geq d)$ at large d is of special interest. We find that $P(d' \geq d)$ initially decays at a rate close to power-law but appears to tail off at a rate closer to exponential decay at large d. We explore the origins of this behavior in greater detail later.

Null Model Comparison. We identify organizational effects on network robustness by comparing metabolic networks to appropriate null models (i.e., networks that preserve identical degree distribution to the original yet lack organizational motifs). The null models are constructed via a random rewiring procedure: starting with the original metabolic network, pairs of edges are randomly switched, or rewired, conserving both node degree and edge orientation (k, k_{in}, and k_{out} for each node are the same as in the original metabolic network, so that in–out degree correlations are preserved). We also impose the constraint that no redundant edges can exist in the network. A minimum of $(50L)$ switching moves are performed to ensure equilibrium, where L is the total number of edges in the network (switches are performed according to the method discussed in refs. 26 and 27). For each metabolic network, we generate an ensemble of 100 rewired networks, each having broad-tailed degree distribution but lacking organizational motifs (26, 27).

We then assess metabolic robustness by comparing the cascading behavior of each metabolic network to its ensemble of rewired networks. We find that, typically, the probability for large cascades is much smaller in the metabolic networks than in the rewired ensembles, especially for $d > 20$ (see Fig. 2). The result strongly suggests that organizational features in metabolic networks act to enhance robustness. In the following sections, we attempt to identify the origins of this robustness.

Rigid Clusters in Metabolic Networks. It is useful to distinguish metabolite nodes according to node degree: uniquely produced, uniquely consumed (UU) nodes have $k_{in} = k_{out} = 1$ (5); branched, susceptible (BS) nodes have either k_{in} or $k_{out} = 1$, but not both; and branched, nonsusceptible (BN) nodes have both k_{in} and $k_{out} > 1$.

Table 1. Metabolic network information and percolation parameters

Species	N_r	N_m (UU, BS, BN)	κ_r	ω_c	ω_c^*	γ_c	λ	κ_{sn}	ω_b	ω_b^*	γ_b
E. coli	2,082	1,669 (618, 322, 308)	4.81	0.14	0.26	0.53	0.29	39.6	0.032	0.026	1.25
S. cerevisiae	1,149	1,061 (367, 157, 227)	5.12	0.14	0.24	0.59	0.21	70.6	0.021	0.014	1.45
S. aureus	644	644 (223, 70, 113)	5.30	0.14	0.23	0.62	0.18	62.0	0.021	0.016	1.29
M. barkeri	619	628 (254, 83, 92)	5.22	0.17	0.24	0.73	0.09	86.6	0.024	0.012	2.09

For each species we show the number of reactions (N_r); the number of metabolites (N_m, with UU, BS, and BN metabolites in parentheses); effective mean degree (κ) of reaction nodes and supernodes (denoted by subscripts r and sn, respectively); bond probability (ω), critical probability (ω^*), and critical parameter (γ) for the rigid cluster and branching percolation models (denoted by subscripts c and b, respectively); and the rigid cluster size scaling exponent (λ).

We can use this formulation to further analyze the cascading behavior of metabolic networks.

Consider a cluster of UU metabolite nodes adjoined by reaction nodes (i.e., a UU node feeds a reaction that has a UU product node, which in turn feeds another reaction that has a UU product node, and so on; see Fig. 3*A*). This cluster displays the special property that if any of its reaction nodes are removed, the entire cluster will necessarily be removed. At a minimum, a cascade will propagate until it is bounded by branched nodes. For this reason, we say that any cluster of nodes that does not contain a branched metabolite node is rigid. We define the size of a rigid cluster, s_c, as the number of reaction nodes it contains, so that every reaction node is part of a rigid cluster of at least size 1 (a rigid cluster with n reaction nodes—and no loops—has $n - 1$ metabolite nodes).

We find that rigid clusters in the metabolic networks range in size from 1 to about 20 reaction nodes. Specifically, $P(s_c > s_c)$ is broad tailed—most clusters comprise just 1 or 2 reaction nodes, but a few can be large (Fig. 4). Notably, rewiring yields a virtually insignificant change in the cluster size distribution—each metabolic network has a distribution that is very similar to that of its randomly rewired ensemble. In other words, the distribution of rigid clusters in the metabolic networks does not result from any particular organizing principle. Next, we show that such random rigid cluster formation can be described by a percolation model.‖

Random rigid cluster formation can be modeled as bond percolation, where reaction nodes are the vertices and metabolite nodes represent bonds (to avoid confusion, we refer to components of the percolation model using vertex-bond notation, rather than node-edge notation). UU metabolite nodes represent bonds that are turned "on," and branched metabolite nodes (both BS and BN) represent bonds that remain "off," so that each rigid cluster in the metabolic network corresponds to a cluster of vertices connected by "on" bonds.

Cluster formation is then characterized by a critical parameter,

$$\gamma_c = \frac{L_{UU}(\kappa_r - 1)}{L}, \qquad [1]$$

where L_{UU} is the number of edges connected to UU nodes, and κ_r is the effective mean reaction node degree,

$$\kappa_\tau \equiv \langle k_r^2 \rangle / \langle k_r \rangle. \qquad [2]$$

(The derivation of Eq. **1** is discussed in *Methods*.) A network with $\gamma_c = 1$ is critical—it lies at the threshold for infinite percolation of the rigid cluster and yields a rigid cluster size distribution that decays as a power-law; a network with $\gamma_c > 1$ is supercritical, yielding an infinitely percolating rigid cluster; and a network with $\gamma_c < 1$ is subcritical, yielding a rigid cluster size probability curve that decays faster than power-law. From Eq. **1**, we find that each species considered in this study is subcritical ($\gamma_c < 1$) with respect to random rigid cluster formation (Table 1).

Since reactions in a metabolic network tend to have a similar number of edges (roughly 4–6 products and reactants per reaction), the above formulation roughly approximates percolation on a tree of self-similar branches, i.e., a Bethe lattice. The Bethe lattice (also known as a Cayley tree) is a popular model for percolation problems and one of the few for which cluster-size scaling can be solved analytically (28). For each metabolic network, we compare the observed cluster-size distributions (real and rewired) to the numerical solution for percolation on a Bethe lattice, $P_{BL}(s_c > s_c)$, using the γ_c values calculated from Eq. **1** and a branching coefficient, $b = k - 1$, equal to 4.†† The results are in good agreement, with $P_{BL}(s_c > s_c)$ scaling as

$$P_{BL}(s_c' > s_c) \propto s_c^{-3/2} \exp[-\lambda s_c] \qquad [3]$$

(see Table 1 and Fig. 4).

Branching in Metabolic Networks. A single cascade can comprise multiple rigid clusters. If a rigid cluster is connected to the either the lone incoming or lone outgoing edge of a BS metabolite node (we refer to this edge, irrespective of direction, as the feeding edge), it will produce a cascade that propagates to other rigid clusters (see Fig. 3*B*, and recall that a BS metabolite node has $k_{in} = 1$, $k_{out} > 1$, or vice versa). Thus, a failure cascade can be characterized by its branching, i.e., the number of rigid clusters that it contains, s_b. We find that $P(s_b \geq s_b)$, the probability that removal of a single rigid cluster produces a failure cascade containing at least s_b rigid clusters, is broad-tailed for all of the metabolic networks considered—most cascades contain just one or two rigid clusters, but a few contain many (i.e., 20–40 rigid clusters; see Fig. 5). $P(s_b \geq s_b)$ is typically smaller for metabolic networks than in the randomly rewired ensembles, especially at large s_b (rewiring results in a significant probability for cascades with $s_b > 100$). This suggests that the organization of branched metabolites in metabolic networks is nonrandom and has the effect of increasing robustness.

Branching in the null model also can be modeled as bond percolation. Here, it is convenient to define a supernode—a cluster comprising a BS metabolite node and the rigid clusters connected to its nonfeeding edges (see Fig. 3*B*). Each supernode is a "vertex" in the percolation model; branched metabolite edges are bonds. Specifically, the feeding edge of a BS metabolite node represents an "on" edge, and all other branched metabolite edges represent "off" edges.

The relevant percolation problem is then similar to that describing rigid cluster formation, and a critical parameter for branching, γ_b, can be calculated for each metabolic network:

‖What follows is a brief overview of the percolation model. Those interested in further background on percolation concepts are referred to excellent texts by Stauffer and Aharony (28), and Bunde and Havlin (29). Readers may also be interested in work by Schwartz *et al.* (30) examining percolation in directed networks, although they use a formulation that differs substantially from that used in our work herein.

††Node degree, k, in the Bethe lattice model corresponds to the effective mean node degree, κ_r, in the rigid cluster model. Since the branching coefficient is, by definition, integer-valued, we round κ_r to the nearest whole number (5) for all species, yielding a branching coefficient of 4. Because the cluster size distribution is a strong function of γ and a weak function of b, this approximation introduces relatively little error.

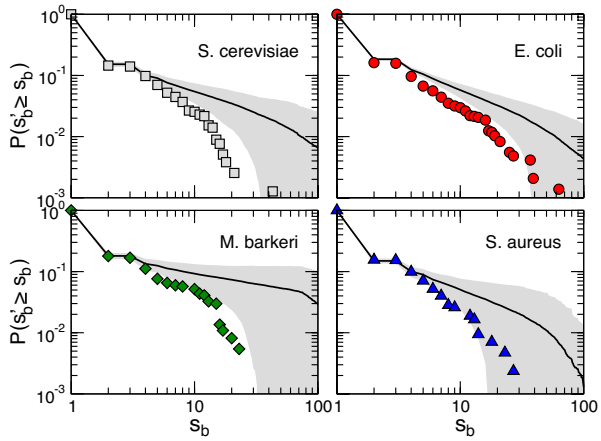

Fig. 5. Branch distributions in metabolic networks. $P(s_b \geq s_b)$, the probability that the removal of a single rigid cluster results in a cascade containing at least s_b rigid clusters, or branches, is shown for each metabolic network (data points). $P(s_b \geq s_b)$ for each metabolic network is typically smaller than the expected result for its randomly rewired ensemble [each solid curve indicates $P(s_b \geq s_b)$ for an ensemble of 100 randomly rewired networks, with the shaded area denoting the 95% confidence interval].

$$\gamma_b = \frac{N_{BS}(\kappa_{sn}-1)}{L-L_{UU}}, \quad [4]$$

where N_{BS} is the number of branched, susceptible metabolite nodes; L_{UU} is the number of edges connected to UU nodes; and κ_{sn}, the effective mean supernode degree, is defined as in Eq. **2**. (The derivation of Eq. **4** is discussed in *Methods*.) From Eq. **4**, we calculate γ_b for the species considered in this study, finding that all species are supercritical ($\gamma_b > 1$) with respect to random branching (see Table 1). This is qualitatively consistent with the observed behavior in the rewired networks, where $p(s_b)$ exhibits a distinct peak at large s_b [see supporting information (SI) Fig. S1]. The result suggests that, in the absence of organizational motifs, single reaction node failures would likely result in network-spanning failure cascades.

Summary. We have used a cascading model—based on a topological flux balance criterion—to describe metabolic network failure as a percolation-like process. The approach yields several valuable insights: we find that the metabolic networks considered are exceptionally robust and that this robustness is not due to the structure of so-called rigid or UU clusters but rather arises from the organization of nonrigid, branched metabolites. Furthermore, the rigid cluster formation and nonrigid branching processes in the null model can be classified as subcritical and supercritical, respectively. That the two types of structures form on different sides of the percolation threshold is particularly intriguing.

The problem of identifying the exact robustness-enhancing structural motifs in metabolism is formidable and remains open. For example, it is quite plausible—although not obvious—that the robustness of metabolic networks is related to their modular structure (this hypothesis is not inconsistent with our finding that robustness arises from the organization of branched metabolites). However, a crucial hurdle here is the development of a modularity-conserving null model; i.e., an algorithm that randomizes a directed, bipartite network while preserving the degree distribution and modular structure. Similar hurdles are encountered when exploring robustness effects of other structural features (e.g., loop structure, hierarchical organization). As the proper null models are developed, cascading studies may provide even further insight into relationships between structural features and metabolic function.

Methods

Critical Parameter for Rigid Cluster Formation. In the rigid cluster model, reaction nodes represent vertices, UU metabolite nodes represent bonds that are turned ''on,'' and branched metabolite nodes represent bonds that remain ''off.'' The probability, ω_c, that two vertices are connected by an ''on'' bond is equal to the number of edges connected to UU metabolite nodes divided by the total number of edges:

$$\omega_c = L_{UU}/L. \quad [5]$$

Here, we have made the assumption that the probability of forming multiple connections between a single reaction node–metabolite node pair is negligible, so that the percolation model adheres to the constraint prohibiting redundant connections between node pairs. This assumption requires that the product of the relative metabolite node degree, $k_m = k_m/L$, and the reaction node degree, k_r, is small for each metabolite–reaction node pair. Specifically, we say that

$$\xi_{ij} \equiv (k_{m,i}/L)k_{r,j} \ll 1 \quad [6]$$

for all node pair combinations. For the networks considered here, $\xi_{ij} \sim 10^{-3}$. The critical probability, ω_c^*, for percolation is a function of the vertex degree distribution, or—since we have taken reaction nodes as vertices—k_r. Specifically, it can be shown that

$$\omega_c^* = 1/(\kappa_\tau - 1), \quad [7]$$

where

$$\kappa_\tau \equiv \langle k_r^2\rangle/\langle k_r\rangle. \quad [8]$$

(a derivation of Eqs. **7** and **8** can be found in ref. 31). Here, we have assumed that the random network is a tree—i.e., the probability of loop formation is negligible. This assumption holds when the number of vertices (reaction nodes) is much larger than the mean vertex degree (k_r). That is

$$N_r/\langle k_r\rangle \gg 1, \quad [9]$$

where N_r is the total number of reaction nodes. The metabolic networks considered in this work have $N_r/\langle k_r\rangle \sim 10^2$.

The critical parameter is then

$$\gamma_c = \frac{\omega_c}{\omega_c^*} = \frac{L_{UU}(\kappa_\tau - 1)}{L}. \quad [10]$$

Critical Parameter for Branching. In the branching model, each supernode is a ''vertex''; branched metabolite edges are bonds. Specifically, the feeding edge of a BS metabolite node represents an ''on'' bond, and all other branched metabolite edges represent ''off'' bonds. The probability, ω_b, that two vertices are connected by an ''on'' bond (feeding edge of a BS metabolite) is equal to the number of BS feeding edges (or, equivalently, the number of BS nodes, N_{BS}) divided by the total number of edges connected to non-UU nodes (UU nodes are disregarded in the ''supernode'' description):

$$\omega_b = N_{BS}/(L - L_{UU}). \quad [11]$$

Again, we have made the assumption that the probability of forming redundant connections is negligible.

To calculate the critical probability, ω_b^*, for percolation, we first define the degree for any supernode i as

$$k_{i,sn} = \sum_j (k_{c,j} - 1), \quad [12]$$

where j is a rigid cluster in supernode i, and $k_{c,j}$ is the degree of the rigid cluster (i.e., the number of edges in the rigid cluster connected to branched metabolites). Again, we have assumed that the network of supernodes is a tree—i.e., the probability of loop formation is negligible. The critical probability for percolation in the branching model is then

$$\omega_b^* = 1/(\kappa_{sn} - 1), \quad [13]$$

where

$$\kappa_{sn} \equiv \langle k_{sn}^2\rangle/\langle k_{sn}\rangle. \quad [14]$$

The critical parameter is then

$$\gamma_b = \frac{\omega_b}{\omega_b{}^*} = \frac{N_{BS}(\kappa_{sn}-1)}{L - L_{UU}}. \quad [15]$$

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