A random Boolean network (RBN) is a collection of \( N \) binary logic gates, or nodes, wired together in a random fashion, with each node implementing a randomly chosen logical function of its inputs. RBNs are paradigms for systems in which excitatory and inhibitory interactions occur among a large set of interacting elements. One example of great current interest is the regulatory network that governs gene expression in a cell. It has been suggested that the distinct dynamical attractors of a single RBN be interpreted as distinct cell types carrying the same genetic information. Surprisingly, RBN attractors can exhibit many features of biological cells, including stability against random external perturbations, qualitative change in response to special perturbations, and plausible scaling laws for numbers of attractors and attractor cycle lengths. It therefore appears important to understand the behavior of RBNs as a first step in determining relevant global properties that might be probed in real gene expression experiments.

Even very simply constructed RBNs with deterministic updating rules can exhibit a rich set of dynamical behaviors. We focus here on the case in which each node has the same number of inputs, \( K \). Fig. 1 shows an example with \( K = 2 \). Each node \( i \) implements a truth function \( F_i \) (e.g. AND, XOR, etc. for \( K=2 \)) that is chosen at random from a weighted distribution of all of the \( 2^K \) possible truth functions on \( K \) binary inputs. On each discrete time step, the outputs are updated synchronously. Since the number of states of the system is finite (equal to \( 2^N \)) and the system is deterministic, for any initial condition the network must eventually settle into a periodic attractor. We are interested in the behavior of large \( N \) networks. How many attractors do they have? How many nodes typically participate in the attractor dynamics?

It is well known that tuning the probabilities of different \( F \)'s can produce an order-chaos transition. (See [1] for a thorough review.) In the ordered regime, almost all nodes are frozen and attractor cycles are short. In the chaotic regime, on the other hand, the number of fluctuating nodes is a finite fraction of \( N \) and attractor cycles can be quite long. For large \( N \), there is a narrow critical regime between these phases. The networks of greatest interest for biological systems are conjectured to lie near the critical regime, on the ordered side.

In this Letter, we present numerical and analytic results that clarify the dynamical structure of RBNs in the ordered and critical regimes, revealing several surprising features: (1) for RBNs in the ordered regime the average number of relevant nodes (defined below) remains finite for \( N \to \infty \) and they are organized into trivial loops; (2) in critical RBNs, the average number of fluctuating nodes grows like \( N^{2/3} \); (3) the system sizes required to observe the asymptotic regime can be extremely large, especially for \( K = 2 \); and (4) the median number of attractors in critical RBNs grows faster than linearly with \( N \), at least for \( N \) up to 1200. Both (2) and (4) contradict previous claims ([2] and [3], respectively) which we believe to have been based on studies that did not consider sufficiently large \( N \). (4) also supersedes an old claim by one of us that the median number of attractors grows like \( \sqrt{N} \) in critical networks.

The concepts of “relevant” nodes and “canalizing inputs” are essential to our analysis. In any given network, there may be nodes whose outputs are frozen at the same value on every attractor. Such nodes serve only to fix inputs to other nodes and are otherwise “irrelevant”. There may also be nodes whose outputs go only to irrelevant nodes. These are also classified as irrelevant. Though they may fluctuate, they act merely as slaves to
the nodes that determine the attractor cycle.

Almost all the irrelevant nodes can be found as follows. One first identifies “fixed” nodes whose outputs are entirely independent of their inputs. One then uses an iterative procedure to identify nodes that must be frozen because their outputs depend only on inputs from other frozen nodes. We call all frozen nodes identified this way “clamped”, let \( s \) denote their number, and define \( u \equiv N - s. \) A similar iterative procedure is then used to remove (or “prune”) nodes with no relevant outputs. For example, in Fig. 1, even if nodes 1 - 5 are all unclamped, nodes 6 - 8 can be pruned. For the purposes of this paper we designate all nodes that are neither clamped nor pruned via the described procedure as “relevant”, and let \( r \) denote their number. (Additional nodes may be frozen due to correlations between two or more unclamped inputs \([5]\), so \( r \) is greater than or equal to the number of truly relevant nodes.)

A canalizing input to a given node is one that can be set to a value that determines the output, independent of the other inputs. (For example, if either input to an OR function is set to 1, the output is determined.) Following \([5]\), we define a set of parameters \( p_k \) as follows. For a randomly selected \( F \), fix a randomly selected \( K-k \) inputs at arbitrarily chosen values. \( p_k \) is the probability that those input values are collectively canalizing; i.e., that \( F \) is independent of the \( k \) remaining inputs. Note that \( p_0 = 1 \) (fixing all the inputs certainly determines the output) and \( p_K \) is the probability that a node is fixed (i.e., that its output is independent of all of its inputs).

The order-chaos transition can be observed by tuning the \( p_k \)'s. One simple way to do this is to assign to each \( F \) a probability that depends only on the number of 1 in the output column of its truth table. For \( p \in [0, 1] \), we let the probability that a node has truth function \( F \) be \( p^{k_1}q^{K-k_1} \), where \( q = (1-p) \). It is straightforward to check that this parametrization corresponds to \( p_k = p^k + q^{2^k} \). With this weighting of the \( F \)'s, the transition occurs at \( 2Kpq = 1 \), with \( 2Kpq < 1 \) corresponding to the ordered regime \([4, 6, 8]\). For our numerical studies, we set \( K = 2 \) and vary \( p \).

We have carried out two types of numerical experiment on \( K = 2 \) networks. First, for 1000 networks at each of five \( p \) values, we determine \( u \) and \( r \). Fig. 2(a) shows the measured \( \langle r \rangle \) for \( N \) up to \( 3 \times 10^6 \). It appears that \( \langle r \rangle \) approaches a constant at large \( N \) for \( p \) in the ordered regime. At the critical value \( p = 1/2 \), the data are inconclusive, showing significant curvature on the log-log plot out to the largest \( N \) we have studied. They are consistent, however, with an asymptotic scaling law \( \langle r \rangle \sim N^{1/3} \). Panel (b) shows the \( \langle u \rangle \) for \( p = 1/2 \), indicating a clearer power-law scaling \( \langle u \rangle \sim N^{2/3} \).

Second, for at least 1000 networks at each \( p \), we attempt to measure the number of attractors, \( A \), on the set of relevant nodes. In some networks, however, \( A \) is prohibitively large, making measurements of \( \langle A \rangle \) difficult. It is much easier to measure the median, \( \hat{A} \), since one need not continue to count attractors in a given network after the count has exceeded the median. To count attractors, we repeatedly choose random initial conditions and identify the attractor reached. If 1000 consecutive attempts yield no new attractor, we record the number of attractors found and move on to another network. This gives a lower bound on \( A \) for each network, and hence a lower bound on \( \hat{A} \).

Fig. 3 shows the results for \( N \) up to 1200. We note that in \([4]\), where measurements of \( \langle A \rangle \) were sought, it was not possible to consider nets larger than \( N = 144 \). From Fig. 3, however, it is clear that any extrapolation based on data for \( N \) smaller than about 500 at the critical point is suspect. We see a faster than linear rise in the median \( A \) above \( N \approx 500 \), which almost certainly implies a faster than linear rise in \( \langle A \rangle \) as well. Moreover, Fig. 2(a) strongly suggests that one must study \( N > 10^6 \) to observe the true asymptotic behavior!

A simple calculation provides a rigorous upper bound...
on \( \langle u \rangle \) and explains why asymptotic scaling sets in only for very large \( N \). A technical note: in the networks studied numerically, gates were not permitted to have two inputs from the same node. The analysis below ignores this constraint, which yields \( \mathcal{O}(1/N) \) effects. In all cases, self inputs are allowed.

Let \( P(u) \) be the probability that a randomly selected network has \( u \) unclamped nodes. To compute \( P(u) \), we should count the networks with \( u \) unclamped nodes and divide by the total number of networks \( T = [\tau N^K]^N \), where \( \tau = 2^{K-1} \) is the number of possible truth functions for each node. We begin by considering a quantity \( P'(u) \) that is guaranteed to exceed \( P(u) \):

\[
P'(u) = \frac{1}{T} C(N, u) \left[ \sum_{k=0}^{K} C(K, k) u^k s^{k-1} \tau p_k \right]^u \times \left[ \sum_{k=0}^{K} C(K, k) u^k s^{k-1} \tau q_k \right],
\]

where \( q_k \equiv 1 - p_k \) and \( C(m, n) \) is the number of combinations of \( n \) objects drawn from \( m \). The factor \( C(N, u) \) is the number of ways of having \( u \) unclamped nodes. The first sum counts the ways that a node can be clamped, weighted by the probability of its truth function: the node in question can have up to \( k \) unstable inputs as long as the other \( K - k \) are collectively canalizing, which occurs with probability \( p_k \). Similarly, the second sum counts the ways a node can be unclamped.

\( P'(u) \) overcounts the probability of having \( u \) unclamped nodes because it ignores the constraint that all clamped nodes must be traceable through a sequence of inputs back to a fixed node. That is, the first sum overcounts the number of ways that \( s \) clamped nodes can be wired, as it includes graphs in which a subset of the \( s \) nodes collectively clamp each other without any connection to a fixed node. For example, suppose that in Fig. 1, nodes 1 and 4 are fixed, node 9 implements OR, and the output of 10 is simply equal to its input from 9. Nodes 2, 3, and 5–8, are clearly clamped through inputs that can be traced back to 1 and 4. The sum then counts this network as a possible arrangement of the clamped nodes for \( u = 0 \) because it is self-consistent to assume that both 9 and 10 are clamped. (Note that after a few time steps 9 and 10 will either both be stuck on 0 or both on 1.) However the iterative procedure defined above would (correctly) not identify 9 and 10 as clamped, so its inclusion in \( P'(0) \) constitutes overcounting. Note that the same network is also (properly) counted in \( P'(2) \). Thus \( P'(u) > P(u) \) for all \( u \), implying that \( \sum_u g(u) P'(u) \geq \sum_u g(u) P(u) \) for any \( g(u) \geq 0 \).

If \( u \) is small compared to \( N \), a useful approximation to Eq. (1) can be obtained. Using \( s = N - u \) and using Stirling’s formula to simplify the binomial coefficients, together with the identity \((1 + x/n)^n = \exp[x(1 - (x/n))/2 + (x/n)^2/3 + \ldots] \), we find

\[
P'(u) \simeq \frac{1}{\sqrt{2\pi u(1-u/N)}} \exp \left[ -\theta_1 u - \theta_2 u^2/N - \theta_3 u^3/N^2 \right],
\]

where

\[
\theta_1 = Q - 1 - \ln Q, \quad \theta_2 = (1 - Q)(1 + Q - 2K + 2Q_2)/2, \quad \theta_3 = -\theta_2 - P_3 - Q_3 + (K - Q)P_2 + \frac{1}{2}Q_2^2 \left[-(K - 1 + 2(K - Q)^3)/6, \right.
\]

with \( Q \equiv K q_1, Q_n \equiv C(K, n) q_n / Q, \) and \( P_n \equiv C(K, n) p_n \). The calculation involves only straightforward algebra and the assumption that terms of order \( u^3/N^3 \) can be neglected in the exponent, which is justified whenever \( \theta_1, \theta_2, \) and \( \theta_3 \) are all positive and at least one of them is nonzero. Under such circumstances, and in the limit of large \( N \), \( P'(u) \) is exponentially strongly suppressed for \( u \)’s larger than order \( N^{2/3} \), whereas the neglected terms would only become relevant for \( u \)’s of order \( N^{3/4} \).

\( Q \) is equivalent to the order parameter defined by Flyvbjerg in [1], where it was also argued that \( Q = 1 \) marks the critical boundary. Eq. (1) proves that for all \( Q < 1 \) the \( P(u) \) decays exponentially with a decay length independent of \( N \). It also strongly suggests that this is not the case at \( Q = 1 \), though the computation only gives an upper bound. The fact that \( \theta_1 \) and \( \theta_2 \) both vanish at \( Q = 1 \) is a surprising result that affects the scaling of \( \langle r \rangle \) in critical networks, as we shall see below.

In the ordered regime \( Q < 1 \), we have \( \theta_1 > 0 \), so higher order terms are irrelevant at sufficiently large \( N \) and \( P'(u) \) becomes independent of \( N \). Thus \( \langle u \rangle \) asymptotically large \( N \) is bounded above by a constant. For the case where \( p_k \) is determined simply by the one parameter \( p \), we have \( q_1 = 2p(1-p) \) and the critical \( p \) satisfies \( 2K p_c (1-p_c) = 1 \), consistent with previous studies of the effect of \( p \). For \( p \) near \( p_c \) we get

\[
\theta_1 \simeq \left\{ \begin{array}{ll} 8(p-p_c)^4 & \text{for } K = 2 \\ 2K(K-2)(p-p_c)^2 & \text{for } K > 2. \end{array} \right.
\]

For \( K = 2 \), then, \( \theta_1 \) is quite small even for \( p \) relatively far from \( p_c = 1/2 \). For example, \( p = 0.55 \) gives \( \theta_1 \approx 5 \times 10^{-5} \). The asymptotic behavior of the system should only be apparent when \( u \) is bigger than \( 1/\theta_1 \) and hence when \( N \) is substantially larger than that. The vertical dashed lines in Fig. 1(a) are drawn at \( N = 3/\theta_1 \), which is seen to give an accurate indication of where effects associated with the ordered regime become important.

Moreover, for very large \( N \), we may obtain an accurate approximation to \( P(u) \) simply by normalizing \( P'(u) \), since the overcounting factor relating the two approaches some nonzero constant at \( u = 0 \). This allows us to compute \( \langle r \rangle \) (not just an upper bound on it) as follows. Consider the unclamped nodes and the links between them.
These form a random graph subject only to the constraint that each node has between 1 and $K$ inputs. But from the second sum in Eq. (6) we know the relative probabilities that an unclamped node will have $k$ unclamped inputs. Specializing to $K = 2$ for simplicity, the average number of inputs per node for fixed $u$ is

$$
\langle z \rangle = \frac{(Qsu + 2q_2u^2)}{(Qsu + q_2u^2)} \simeq 1 + \frac{q_2^2}{Q} u + \left( \frac{q_2^2}{Q} \right) u^2.
$$

(6)

Now in the ordered regime, where the exponential cutoff in $P(u)$ is independent of $N$, we have $\int du P(u)/N \to 0$ for all $u$ as $N \to \infty$ and hence $\langle z \rangle \to 1$. Thus the network of unclamped nodes at large $N$ is just a randomly wired $K = 1$ network with no fixed nodes. Exact combinatorics for $K = 1$ random graphs show that the expected number of loops of size $n$ in a network of size $u$ is $L_u(n) = u/(n(u - n)!)(u - 1)^n$. [10] Since all nodes not in loops can be pruned, we obtain

$$
\langle r \rangle = \sum_{u=0}^{N} \sum_{n=1}^{u} n L_u(n) P(u) \simeq 0.7/\sqrt{u}.
$$

(7)

The horizontal dashed lines on the far right in Fig. 2(a) mark this prediction, which agrees well with the data.

For the critical case $Q = 1$, we have

$$
P'(u) \simeq \left[ 2\pi u(1 - u/N) \right]^{-1/2} \exp \left[ -\theta_2 u^3/N^2 \right],
$$

(8)

which implies that $\langle u \rangle$ cannot grow faster than $N^{2/3}$. This contradicts a previous argument suggesting $\langle u \rangle \sim N^{3/4}$. Note however, that the corrections from terms of order $u^4/N^3$ cannot be neglected unless $(N^{2/3})^4/N^3 = N^{-1/9} << 1$, suggesting that the critical scaling emerges only for $N > 10^9$, as confirmed by Fig. 3.

Naive normalization of $P'(u)$ to get $P(u)$ yields $\langle u \rangle \sim N^{2/3}$ and Fig. 2(b) indicates that this is correct. Given the scaling law and the fact that paths connecting relevant nodes cannot lead to dead ends, the theory of directed random graphs [11] and Eq. (6) can be used to show that $\langle r \rangle \sim N^{1/3}$, consistent with the numerical trend seen in Fig. 2(a). Unfortunately, naive normalization does not yield an accurate prediction for the full form of $P(u)$ because the $u$ dependence of the factor $f(u) \equiv P(u)/P'(u)$ becomes important. Determination of the precise form of $f(u)$ is beyond the scope of this work.

We now address the question of the number of attractors. For the case $Q < 1$ and very large $N$, the relevant nodes form trivial loops with only two possible truth functions: $F(\sigma) = \sigma$ (“identity”) and $F(\sigma) = 1 - \sigma$ (“not”). For $n$ prime, a loop of size $n$ has either $(2^n - 2)/n$ or $(2^n - 2)/2n$ attractors, depending on whether the number of nots in the loop is even or odd. For $n$ not prime, the number of attractors is slightly larger. [10]

Now $L_u(n)(2^n - 2)/n$ is extremely sharply peaked very close to $n = u/2$, $\langle A \rangle_u$ is thus dominated by rare networks that contain a relevant loop of size $u/2$, which gives $\langle A \rangle_u \sim 2^{u/2}$, with $c$ a constant of order unity. Naive averaging over $P(u)$ gives a divergent answer; a consistent calculation would require inclusion of the terms in $P(u)$ that cause rapid decay for $u$ of order $N$.

The median number of attractors, $\tilde{A}$, can be estimated as $2^b/b$, where $b$ is the size of the biggest loop of relevant nodes in a given network. For fixed $u$, the probability of occurrence of $b$ is $P_u(b) = L_u(b)\prod_{b+1}^{n} [1 - L_u(n)]$. To find $\tilde{b}$, we numerically solve $\sum_{b=0}^{\tilde{b}} \sum_{n=0}^{\infty} P(u)P_u(b) = 1/2$. The results for the networks studied in Fig. 3 are $\tilde{A} = 3, 5$, and 14 for $p = 0.7, 0.65,$ and 0.6, respectively. This result and its rough agreement with the data (see Fig. 3) confirms that $\tilde{A}$ approaches a constant at large $N$ for any sub-critical $p$ [12] and illustrates the qualitative distinction between $\langle A \rangle$ and $\tilde{A}$.

In closing, we note that the classification of nodes as relevant is robust across a large class of updating rules. The results on $\langle r \rangle$ reported here apply to asynchronous models and to models in which there is a stochastic time delay in the updating of each node, as long as the update, whenever it occurs, is always accurate. Finally, we note that the numbers of genes in real cells are on the order of $10^4$, which our results show may be too small to exhibit asymptotic large $N$ behavior. Thus, networks with canalization parameters that nominally place them in the ordered regime will exhibit features of critical networks, which may be important for biological function.

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