Funneled Landscape Leads to Robustness of Cellular Networks: MAPK Signal Transduction

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ABSTRACT We uncover the underlying potential energy landscape for a cellular network. We find that the potential energy landscape of the mitogen-activated protein-kinase signal transduction network is funneled toward the global minimum. The funneled landscape is quite robust against random perturbations. This naturally explains robustness from a physical point of view. The ratio of slope versus roughness of the landscape becomes a quantitative measure of robustness of the network. Funneled landscape is a realization of the Darwinian principle of natural selection at the cellular network level. It provides an optimal criterion for network connections and design. Our approach is general and can be applied to other cellular networks.

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Cellular networks are in general quite robust against environmental perturbations (1–3). Recently, there have been increasing numbers of studies on the global topological structures of the networks (4). However, there are so far very few studies of why the network should be robust and perform the biological function from the physical point of view (5–10). We will explore the nature of the network from physical perspectives, formulating the problem in terms of the potential energy landscape (11). Global robustness and the function of the network can then be explored when knowing the underlying potential energy landscape. However, one cannot in general directly extract the potential energy from the typical average description of the chemical reaction network. In the cell, statistical fluctuations from a finite number of molecules provide the source of intrinsic noise, and highly dynamical and inhomogeneous interior environments provide the source of external noise for the networks (12). In general, one should study the chemical reaction network equations in noisy conditions to model realistically the cellular environments. One can also study the steady-state properties of the network under noisy environments. The generalized potential energy for the steady state of the network has been mathematically shown to exist (6,8–10,13). Here we show that the potential energy landscape of the MAP-kinase signal transduction network is funneled toward the global minimum. The funneled landscape is quite robust against random perturbations. This naturally explains robustness from the physical point of view. The ratio of slope versus roughness of the landscape becomes a quantitative measure of the robustness of the network. The funneled landscape is a realization of the Darwinian principle of natural selection at the cellular network level. It provides an optimal criterion for network connections and artificial network design.

To explore the nature of the underlying potential energy landscape of the cellular network, we will study a relatively simple yet important example of the MAP-kinase signal transduction network (Fig. 1).

Mitogen-activated protein kinases (MAPK) belong to a family of serine/threonine protein kinases that are widely conserved among eukaryotes and are involved in many cellular programs such as proliferation, differentiation, movement, and death of the cell. MAPK signaling cascades are organized hierarchically into three-tiered modules. MAPKs are phosphorylated and activated by MAPK-kinases (MAPKKs), which in turn are phosphorylated and activated by MAPKK-kinases (MAPKKKs). The MAPKKK is in turn activated by interaction with a family of small GTPases and/or other protein kinases connecting the MAPK module to the cell surface receptor or external stimuli (2,3) (Fig. 1). We will study the global stability by exploring the underlying potential energy landscape for MAP-kinase network.

Let us study the global robustness of the network by starting from the network of chemical reactions in noisy fluctuating environments: $\dot{x} = F(x) + \zeta$ where $x = \{x_1(t), x_2(t), \ldots, x_n(t)\}$ are the concentrations of the different proteins in the network and $F(x)$ are the ''forces or chemical reaction fluxes'' of nonlinear in protein concentrations x. Here $\dot{x} = F(x)$ describes the averaged dynamical evolution of the chemical reaction network. In the cell, as mentioned, the fluctuations can be very significant from both internal and external sources and in general cannot be ignored (12). The ζ is added as the noise mimicking these fluctuations.

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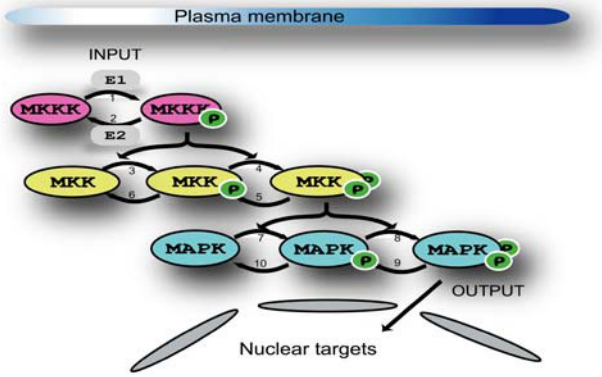

FIGURE 1 Map-kinase reaction network scheme.

It has been mathematically shown that there exists a transformation (8) such that the network equations can be written into the form: $(S(x) + A(x))\dot{x} = -\partial U(x) + \xi$,with the following properties: $S(x)$ is a friction term represented by symmetric and semipositive matrix function, $A(x)$ is the transverse force term represented by antisymmetric matrix function, and ξ represents the stochastic force term. U appears in the gradient term; thus $U(x)$ acts as a potential energy function for the network system.

The steady-state distribution function for the state variable can be shown to be exponential in potential energy function $U(x)$ (6,8,10,13): $P_0(x) = 1/Z \exp\{-U(x)\}$ with the partition function $Z = \int d^n x \exp\{-U(x)\}$. The details are given in the Supplementary Material. From the steady-state distribution function, we can therefore identify U as the generalized potential energy function of the network system. In this way, we map out the potential energy landscape. Once we have the potential energy landscape, we can discuss the global stability of the protein cellular networks.

Since the potential energy is a multi-dimensional function in concentration x, it is difficult to visualize $U(x)$. So we do a zero-dimensional (Fig. 2 *A*) and one-dimensional (Fig. 2, *D* and *E*) projection of U and entropy of the configurational states and look at the nature of the underlying potential energy landscape. Fig. 2 *A* shows a histogram of U. We can see that the distribution is approximately Gaussian. The lowest potential energy U is the global minimum of the potential energy landscape. It is important to notice that this global minimum of U is found to be at about the same place (in x) as the steady state or fixed point of the averaged chemical reaction network equations for MAP-kinase (at the end of the signal transduction process) without the noise. Fig. 2 *B* illustrates the potential energy spectrum. It is clear that the global minimum of the potential energy is significantly far from the rest of the potential energy spectrum or distribution.

To quantify this, we define the robustness ratio *RR* for the network as the ratio between the gap δU, the difference between this global minimum $U_{\text{global-minimum}}$, and the average of U, $\langle U \rangle$, versus the spread or the half width of the distribution of U, ΔU. Here $RR = \delta U / \Delta U$. The δU is a measure of the bias or the slope toward the global minimum of the potential energy landscape, whereas ΔU is a measure of the averaged roughness or the local trapping of the potential energy landscape. When *RR* is significantly >1, the gap is significantly larger than the roughness or local trapping of the underlying landscape; then the global minimum is well separated and distinct from the rest of the network potential energy spectrum. Since $P_0(x) = 1/Z \exp\{-U(x)\}$,

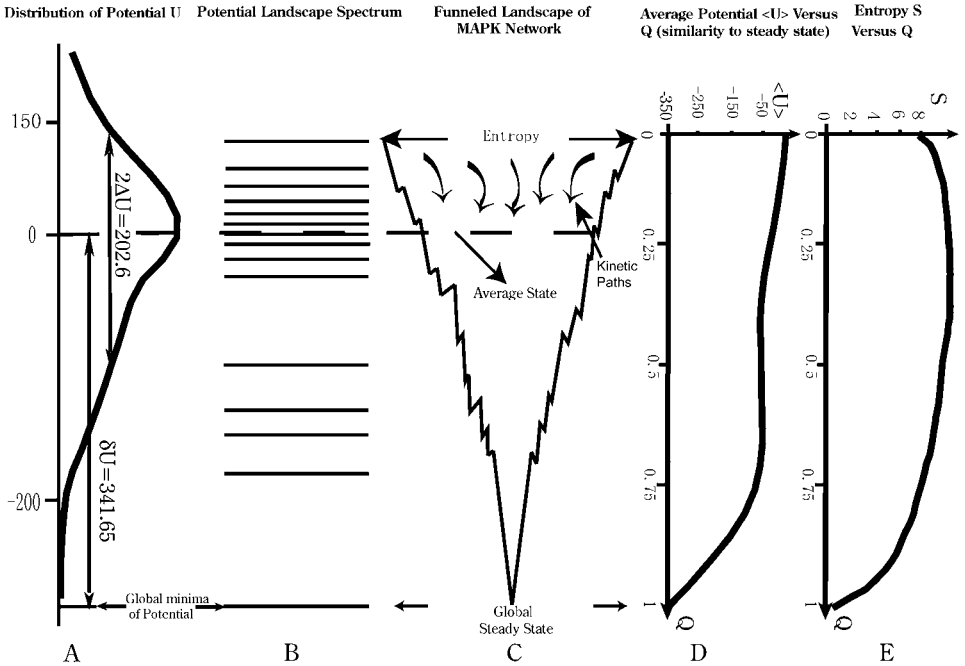

FIGURE 2 Global structures and properties of the underlying funneled potential energy landscape of the MAP-kinase signal transduction network.

the weight or population of the global minimum will be dominated by the one with large *RR*. The populations of the rest of the possible states are much less significant. This leads to the global stability or robustness of the global minimum state discriminating against others. The *RR* value for the MAP-kinase network is $RR = 3.4$, significantly >1 (for typical random networks, we found $RR \sim 1$). So *RR* gives a quantitative measure of the property of the underlying landscape spectrum. Only cellular network landscapes with large *RR* can form a stable global minimum state, be robust, perform biological functions, and survive the natural evolution.

Fig. 2 *D* shows the one-dimensional projection of the averaged U, $\langle U\rangle$ on the overlapping order parameter Q with respect to the global minimum ($Q = \sum_i^N x_i x_i^{\text{global}}/|x||x^{\text{global}}|$). The Q is defined this way so that we can keep track of the degree of ''closeness'' or overlap between an arbitrary state x to the global minimum state x_{global} in the state space of the protein concentrations. $Q = 1$ represents the global minimum state and $Q = 0$ represents the (decorrelated) states with no overlap with the global minimum. We see a downhill slope of the potential energy $\langle U\rangle$ in Q toward the global minimumU_{global}. This shows a funnel of $\langle U\rangle$ along Q toward the global minimum of the potential energy landscape. When the chemical rate coefficients are changed to a moderate degree, the slope of $\langle U\rangle$ changes. The random changes of the rate coefficients correspond to a shallower slope of U in Q toward the global minimum—less funneled. But the degree of the change is mild, so the landscape is still funneled under different conditions. So the network is stable and quite robust.

Fig. 2 *E* shows the configurational entropy S as a function of Q, $S(Q)$. In the cell, there is a finite number of molecules for each type of proteins, so we can divide each protein concentration into finite number of bins. The resulting configurational state space of the network is composed of a finite number of multi-dimensional hypercubes with one state occupying each hypercube. The entropy can thus be estimated by counting the number of the hypercubes. The entropy can be projected to Q to obtain $S(Q)$. As we can see, the entropy is rather smooth at small Q and decays as Q toward the global steady state or minimum. Since the entropy represents the number of states available, this implies that the configurational state space for the network becomes smaller toward the global steady state. Thus entropy can be used as a measure of the radius of the funneled landscape perpendicular to the direction of the funnel toward global steady state (see Fig. 2 *C* that the funnel shrinks toward the global steady state in radial size).

The cellular networks with a rough underlying potential energy landscape can't guarantee robustness or biological function. They are likely to phase out from evolution. Thus, funneled landscape is a realization of the Darwinian principle of natural selection at the cellular network level analogous to protein folding (14). As we see, the funneled landscape provides an optimal criterion for selecting the suitable parameter subspace of cellular networks, guarantees the robustness, and performs biological functions. This leads to an optimal way to network connections and is potentially useful for the network design. Our approach is general and can be applied to other protein networks (2,5,7), with likely one global energy minimum and gene regulatory networks (6,9) with likely several minimum (robustness here might be realized by high barriers among the minimum).

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

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