Science Signaling
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Wei Wang (5 February 2013)

Science Signaling **6** (261), pe5. [DOI: 10.1126/scisignal.2003820]

Science Signaling (ISSN 1937-9145) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005.

COMPUTATIONAL BIOLOGY

Therapeutic Hints from Analyzing the Attractor Landscape of the p53 Regulatory Circuit

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Genes are interconnected in the cell to form a genetic network that regulates cell fate. Targeting multiple genes is expected to be more effective in developing therapeutics than targeting single genes. A recent study demonstrated the possibility of systematically searching for such combinatorial treatments by characterizing the attractor landscape of the p53 regulatory circuit.

More than 50 years ago, Conrad Waddington proposed the concept of the "epigenetic landscape" to describe the developmental process (*1*). To summarize, in the cell state space, undifferentiated cells can be viewed as marbles sitting on the top of a hill, and upon differentiation, they roll down the hill to rest on the energetically stable valleys. A mature cell type corresponds to a local minimum of the epigenetic landscape, dubbed as an attractor, which is separated from other attractors by barriers. With the discovery that mature cells can be reprogrammed to be pluripotent (*2, 3*), a finding that was awarded a 2012 Nobel Prize, the landscape idea provides an intuitive and conceptual understanding of how cell fate may be altered by noise or reprogrammed by design (*4–6*). The landscape concept is general and applicable to characterizing any cell state, such as normal and disease states.

To capitalize on the theoretical insights from the epigenetic landscape in manipulating biological systems or developing therapeutic treatments, it is critical to fill in the molecular details that capture the underlying biology (*7*). Previous attempts have been made in this direction (*8, 9*). The overall strategy includes the following steps: First, a genetic network is established, either from literature or statistical learning; this network represents the relationship between the genes and proteins that are critical for specifying cell states, such as a differentiated or a pluripotent cell. Next, a mathematical model is exploited to delineate the potential landscape of the network and identify attractors in the cell state space. Using the activities of marker genes, the cell states corresponding to the attractors are determined. Then, computational predictions can be made for how the cell state is changed by perturbations to the network, such as deletion, knockdown, or overexpression of single or multiple genes (Fig. 1). The computational model can also illuminate the paths of the cell fate change and uncover important transition cell states.

Such a strategy is promising but still at its infant stage. Choi *et al*. demonstrate this promise in their study (*10*), in which they applied landscape analysis to understand how p53 dynamics regulate cell fate. Choi *et al*. used the rich knowledge available in the literature to construct a genetic circuit around p53, which includes feedback loops that are critical in the response to DNA damage. For example, the loop from p53 stimulates the phosphatase Wip1, which inhibits the kinase ataxia telangiectasia mutated (ATM), leading to inhibition of the E3 ubiquitin ligase Mdm2, which, in turn, inhibits p53. The p53-centered genetic circuit also included cell cycle and cell death modules, and this network allowed representation of different cell fates (cell proliferation, cell cycle arrest, cell death, and cell senescence) in the cell state space. A key step in constructing the circuit is to trim down the number of genes and links while keeping the important nodes and regulatory links essential to the cell states of interest (*11*). For this purpose, Choi *et al*. employed several strategies, such as representing an entire pathway or complex with one single node to simplify the network. As a result, the final circuit included 16 nodes with 160 negative and 218 positive links, producing a densely linked circuit. The presence or absence of a DNA damage signal is indicated as an input to the node representing the kinase ATM, which collectively represents the DNA damage response components.

Given the size and complexity of the circuit, Choi *et al*. chose to represent the gene or protein activity with binary variables (on or off) and used a probabilistic Boolean network (*12*) to compute the probability of every possible cell state. Several attractors are identified through such analysis, including a point attractor (a fixed point) and a cyclic attractor (a set of cyclic cell states) (Fig. 1). The cell fate represented by each attractor is indicated by the activities of key regulators. Namely, the point attractors of cell proliferation and cell death are respectively marked by persistent activation of the cyclin E node [representing the complex of cyclin E and cyclin-dependent kinase 2 (CDK2)] and the caspase node (a collective representation of caspase 3, 6, 7, and 9). Persistent p53 activation without caspase activation indicates senescence; a cyclic attractor representing cell cycle arrest is characterized by oscillatory activation of p53 and p21.

Choi *et al*. compared the potential landscapes of two cell types, normal cells and MCF7 cancer cells, in the absence and presence (reparable and irreparable) of DNA damage. In the Boolean network model, the distinction between normal and cancer cells is represented by fixing the activities of the lipid and protein phosphatase PTEN and the cell cycle regulator $p14^{ARF}$ to inactive ("0") and cyclin G to active ("1") in MCF7 cells and leaving all the nodes free to evolve in the normal cells. Both normal and MCF7 cells are attracted to point attractors of cell proliferation in the absence of DNA damage. Similar to an independent study by Purvis *et al*. (*13*), the behavior of the MCF7 cells depends on the dynamics of p53 activity: Pulsatile p53 activity drives cells to a state of proliferation and prolonged p53 activity, which is achieved by inhibition of the interaction between Mdm2 and p53 with the small molecule Nutlin-3, induces senescence or cell death.

In the presence of DNA damage, the landscapes of both normal and MCF7 cells are changed, and a single cyclic attractor representing cell cycle arrest is observed for both cells. The most interesting observations come from the analyses of the cell fates upon inhibition of a protein (deletion of a node in the Boolean network) or blockage of a regulatory interaction (deletion of a link). Choi *et al*. found that inhibition of neither the protein phosphatase Wip1 nor

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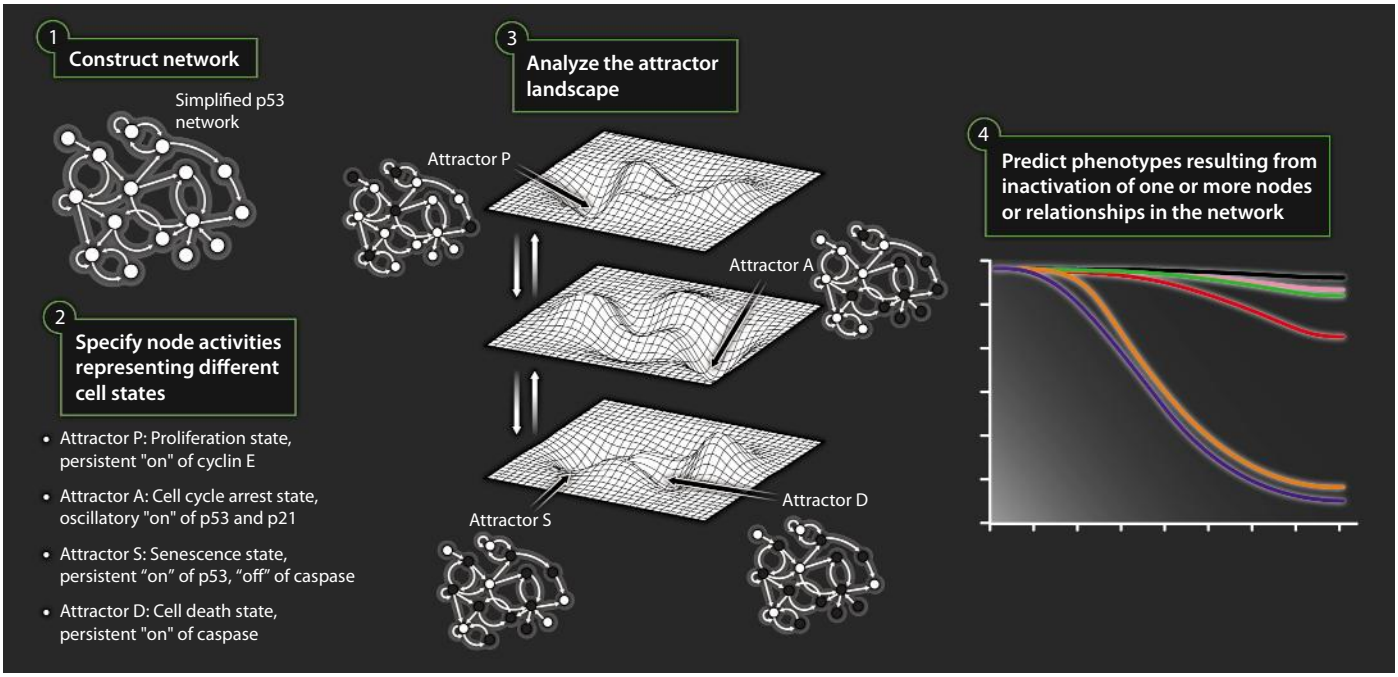

Fig. 1. The four steps to using attractor landscape analysis to predict outcomes of network perturbation experiments.

the Mdm2-p53 regulatory interaction alone effectively induced cell death for MCF7 cells in the presence of DNA damage, because the two landscapes show a cyclic attractor representing cell cycle arrest with a large basin of attraction. The basin of attraction reflects the number of initial states that end up in the same attractor. Thus, a large basin for the cyclic attractor representing cell cycle arrest indicates that most of the initial states do not reach the point attractors of cell death or senescence. However, simultaneous application of these two inhibitions drives MCF7 cells to attractors representing either cell death (~71% of the population) or cell senescence (~29% of the population). This observation suggests a possible therapeutic treatment of cancer, because the double inhibition can kill the majority of the cancer cells.

Choi *et al*. performed a series of single-cell imaging experiments to test the theoretical predictions in MCF7 cells. They used a fluorescent MCF7 reporter cell line that stably expressed a p53-Venus construct to quantify p53 dynamics under different conditions by measuring the average yellow fluorescence in the nucleus from temporal movies. By counting the fraction of surviving cells under the same conditions, Choi *et al*. confirmed the relationship between p53 dynamics and cell fate predicted by the theoretical study. Intriguingly, they observed that the double inhibition of Wip1 and Mdm2 in the absence and presence of DNA damage induced by a low dose of etoposide drastically reduced the proportion of surviving MCF7 cells. Double inhibition also induced a sustained p53 signal that lasted longer than the prolonged p53 signal generated by continuous exposure of the cells to etoposide (presence of DNA damage) plus Nutlin-3 (inhibition of Mdm2-p53 interaction).

In the independent study of Purvis *et al*., they used transient γ-irradiation to induce DNA damage, and they observed a pulsatile p53 signal (*13*). Assisted by a computational model, they found that three sequential treatments of Nutlin-3 with different concentrations at three time points after γ-irradiation would change the dynamics of p53, from the pulsatile signal to a sustained one with an amplitude equal to that occurring in the pulsatile p53 signal. However, one difference between the two analyses of p53 dynamics was the duration of active p53. Whereas Choi *et al*. reported a sustained p53 signal and cell death in response to DNA damage induced by etopside when both Wip1 and Mdm2 were inhibited, Purvis *et al*. found only a very small amount of cell death under both pulsatile and sustained p53. Instead, when Purvis *et al*. induced DNA damage by transient γ-irradiation in the presence of Nutlin-3, the sustained p53 activity resulted in senescence. Although both studies revealed that changes in p53 dynamics alter cell fate, Purvis *et al*. concluded that the sustained activity of p53 induces cell senescence, and Choi *et al*. found that the sustained p53 activity induces cell senescence and death. These differences in the effect of p53 activity may be due to the different methods for increasing the duration and amplitude of p53 activity.

One of the most interesting implications from the Choi *et al*. study is the prediction of combinatorial effects of double inhibition of Wip1 and Mdm2 on cell fate. However, there are still a few issues to be resolved before we can apply such an approach to complex biological phenotypes or developing treatments for diseases. First, the p53 network analyzed is relatively small, and a legitimate concern is whether all of the essential biology is correctly captured and represented by such a circuitry. Because p53 and its upstream regulatory components and downstream targets have been studied extensively, it is relatively easy to construct a densely connected network around p53 for modeling. For other diseases or phenotypes that are less well understood, there may not be enough information available to construct a sufficiently dense network. For example, in the Choi *et al*. study, there may be other proteins or genes, not included in the network, whose inhibition would better synergize with the inhibition of either Wip1 or Mdm2, but these genes or proteins would not be identified as therapeutic targets be-

cause they were not included in the network. As genomics and epigenomics (such as DNA methylation and histone modifications) data are becoming more commonly available, it is a challenge to incorporate the resulting large-scale data to delineate the landscape of cell fate decisions, but it is critical to successfully extend the usefulness of this approach.

The second possible improvement relates to the computational model and how to represent phenotypes. Although the probabilistic Boolean network is a powerful model in computing the landscape, it greatly simplifies the underlying biology (for example, by representing gene activity using a binary variable and by linearly integrating combinatorial regulations). Methods based on differential equations may not be a suitable choice, because many kinetic rates are unknown and the underlying regulatory mechanisms are unclear. The alternatives include probabilistic graph models (such as dynamic Bayesian networks and random fields) that can consider continuous variables, but customization of models and further algorithm development are required to adapt these methods to this purpose. Computational schemes to predict outcomes of perturbations to the network and the response to the extracellular cues have been developed (*14, 15*), but other algorithms for the purpose of extending these ideas to higher organisms (especially to humans) are still needed. Additionally, in the Boolean model, cell fates are defined by a limited number of cell states, which are derived from prior knowledge. For a less well-studied phenotype, how to accurately represent the phenotypes is a nontrivial question.

Network medicine starts to emerge at the horizon (*16–18*). Approaches that target multiple proteins and/or consider the signaling dynamics guided by systems biology analysis of the regulatory network are gaining excitement (*19*), though many hurdles must still be overcome to achieve this goal. The studies highlighted here demonstrate the promise of developing therapeutic regimes based on the systems-level characterization of the biological mechanisms underlying many diseases. In addition, as routine sequencing of the human genome is within reach, network medicine may become even more powerful when coupled with personal genome sequencing (*20*).

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10.1126/scisignal.2003820

Citation: W. Wang, Therapeutic hints from analyzing the attractor landscape of the p53 regulatory circuit. *Sci. Signal.* **6**, pe5 (2013).