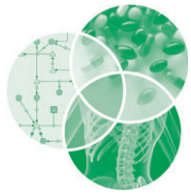
Opinion



The reverse control of irreversible biological processes

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Most biological processes have been considered to be irreversible for a long time, but some recent studies have shown the possibility of their reversion at a cellular level. How can we then understand the reversion of such biological processes? We introduce a unified conceptual framework based on the attractor landscape, a molecular phase portrait describing the dynamics of a molecular regulatory network, and the phenotype landscape, a map of phenotypes determined by the steady states of particular output molecules in the attractor landscape. In this framework, irreversible processes involve reshaping of the phenotype landscape, and the landscape reshaping causes the irreversibility of processes. We suggest reverse control by network rewiring which changes network dynamics with constant perturbation, resulting in the restoration of the original phenotype landscape. The proposed framework provides a conceptual basis for the reverse control of irreversible biological processes through network rewiring.

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INTRODUCTION

For decades, biological processes such as differentiation, tumorigenesis, and cellular aging have been thought to be irreversible. A stem cell loses its stemness during differentiation, while a differentiated cell cannot regain the stemness spontaneously. A non-tumor cell becomes a tumor cell by genetic mutations or epigenetic events but a tumor cell cannot return to a non-tumor cell naturally. An aged cell meanwhile cannot independently recover youth. However, some exceptional cases and recent researches have shown the possibility of their reversion. Budding yeast restores its youth when it undergoes meiosis.[1] Dedifferentiation, a phenomenon where terminally differentiated cells revert back to a less-differentiated state, is observed in tissue repair and regeneration.[2–4] Even in mammalian cells including human cells, gametogenesis and fertilization can cause reversion of both differentiation and aging. Yamanaka and others showed that reprogramming of a differentiated cell to a pluripotent stem cell is possible.[5–7] It has also been shown that aged liver stem cells and muscle stem cells can be rejuvenated by changing the cellular environment based on heterochronic parabiosis.[8,9] Moreover, tumorigenesis can be reversed through the targeted inactivation of oncogenes.[10] This body of evidence naturally raises questions on how we can understand the reversion of such irreversible biological processes, what the specific requirement for the reversion is, and how we can control the irreversible biological processes reversely to obtain their original state.

To answer these questions, we assumed that, although differentiation, tumorigenesis, and cellular aging are disparate processes, their irreversibility and possibility of reversion would share a common principle. Moreover, we found that the processes are regulated by molecular regulatory networks, and that each process transforms these networks into different structures. Therefore, in this study, we suggest a unified conceptual framework for the irreversible biological processes based on the 'phenotype landscape' of a molecular regulatory network. The proposed

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framework can describe the origin of irreversibility of various biological processes and identify the conditions for reversion and reverse control.

A UNIFIED CONCEPTUAL FRAMEWORK

Most biological processes are governed by physical and chemical interactions between proteins (Figure 1(a)). Cells handle environmental changes through various signal transduction pathways, which forms a signaling network. In addition, proteins are expressed from genes, and genes are regulated by proteins such as transcription factors. The relationship between proteins and genes can therefore be abstracted as a gene regulatory network. Components (nodes) of these networks are activated/inactivated or increased/decreased in amount according to their interactions (links) over time; this is called network dynamics (Figure 1(b) and (c)).

To trace the dynamics of a network, we can define a 'network state' as a tuple of values of network components at a specific time point. When there is no change in the input signal of the network system, the network state will follow the inherent network dynamics determined by interactions between network components (Figure 1(c)). Eventually, the network state without any input signal will converge to a steady state called an attractor state while the network state with sustained input signals will converge to another steady state called a pseudo-attractor state. In this paper, we do not distinguish between an attractor and a pseudo-attractor as there is no meaningful biological difference. The trajectories from all the initial states to attractor states form an 'attractor landscape' (Figure 1(d), Box 1).

Recent studies have shown that the attractor states correspond to the cellular phenotypes in response to external stimuli. Huang et al. reported that a particular cell type corresponds to each stable attractor during the differentiation of HL60 cells under various conditions, and another group showed that blood stem cells and blood cell types are attractor states by analyzing the dynamics of the regulatory networks based on the Boolean network formalism.[11,12] Phenotypes of pancreatic cells were found to be well represented by attractors from the analysis of an ordinary differential equation (ODE) model.[13] Moreover, cellular responses of cancer cells such as proliferation, apoptosis, or senescence were represented by attractors.[14–17]

Not all the molecules of a biological network are crucial in determining the cellular phenotype but rather a limited number of molecules that are characterized as output nodes of the network are rather critical in ruling the cellular phenotype.[18] Therefore, a phenotype of an attractor state can be defined by the states of output nodes. Unfortunately, as a network can have more than one attractor state, we need to define a phenotype space of the network. According to Gupta et al., cancer cell lines SUM159 and SUM149 were shown to have three cell subtypes (stem cell-like, basal, and luminal), and the transitions between cell subtypes were proven to be stochastic.[19] SUM159 cells are mostly basal cell type whereas SUM149 cells are mostly luminal cell type. From this, we infer that these two cancer cell lines have at least three attractors and that the relative dominance of attractors is different depending on cell lines. As more than one attractor can be mapped to a particular phenotype, we can consider a 'phenotype landscape' as being composed of phenotypes and their relative dominance (Figure 1(e), Box 1). This phenotype landscape determines the overall phenotype of a network. The relative dominance of a phenotype can be measured experimentally by analyzing the proportion of cell subtypes and predicted mathematically by calculating basin areas, steady state probabilities, mean first passage time or transition rate of attractors and phenotypes.[14,20,21] A molecular regulatory network uniquely determines the corresponding attractor landscape, and thus, the phenotype landscape can be reshaped by 'network rewiring' in our framework. Network rewiring occurs by perturbations that change the network dynamics or transform the network topology through induction of constant changes of node activity or link connections.

All functional events among natural (epigenetic, genetic, or environmental changes) or artificial (transfection, biomolecule inhibition, or stimulation) perturbations can be considered as network rewiring. For example, epigenetic modification inducing the formation of heterochromatin will prevent the transcription factor and RNA polymerase from binding to DNA. Genetic mutations can also induce overexpression or knock out of a gene and even delete links between proteins by modifying the regulatory domain.[22] In addition, a sustained stimulus can be abstracted as network rewiring because it can pin the state of a node. These perturbations occur during biological process and rewire the network of each process. Therefore, different biological processes including differentiation, tumorigenesis, and cellular aging can be represented by reshaping of the phenotype landscape. By integrating these, we suggest a unified conceptual framework for the biological processes that are caused by network rewiring.

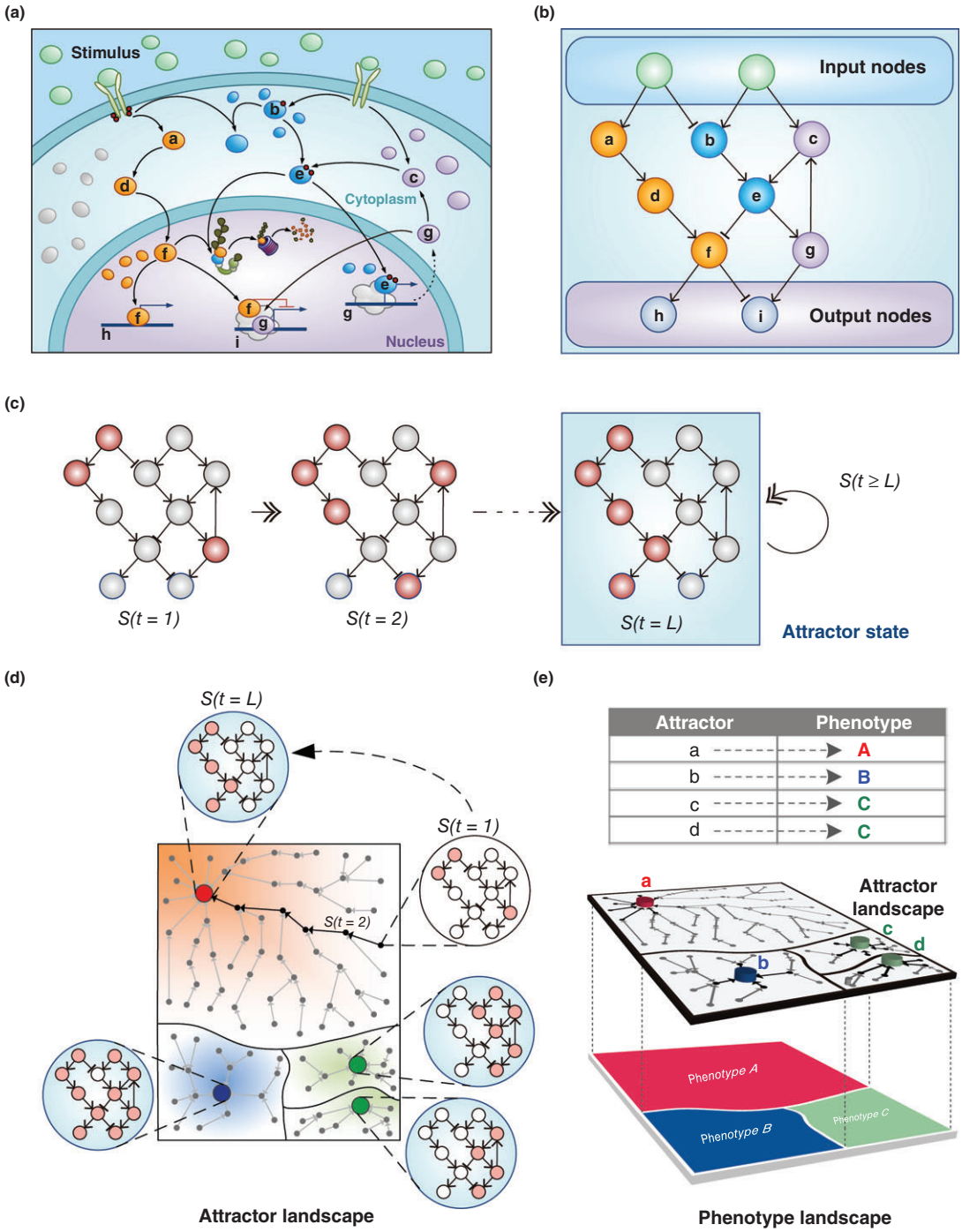
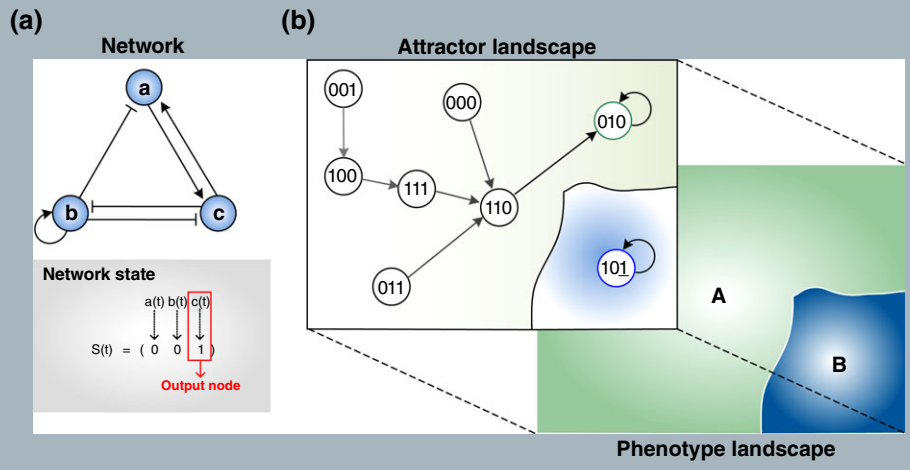


FIGURE 1 | A unified conceptual framework. (a) The cell has a signaling network and a gene regulatory network. Each protein and corresponding node of the network share the same alphabetic notation. (b) The cellular networks can be represented as nodes (circles) and links (arrows). Sharp and blunted arrows indicate 'activation' and 'repression', respectively. Input nodes receive an external stimulus and output nodes determine the phenotypic response of the cell. (c) The dynamics of the network evolves by interaction among nodes over time. Red circles represent active nodes and white circles inactive nodes. We can define a network state from the activities of nodes. The network states at time L and $L + 1$ are the same; such a network state that is not changed over time is called an attractor. (d) From all possible network states, we can obtain the attractor landscape. Output nodes of the attractor state determine the phenotype (color) of a basin. (e) The phenotype landscape is obtained from the attractor landscape. Note that the relative dominance of green phenotype is represented as a green area and basins of two green attractors are projected onto the green area.

BOX 1

ATTRACTOR LANDSCAPE AND PHENOTYPE LANDSCAPE FOR A BOOLEAN NETWORK MODEL

In this box, we explain how the attractor landscape and phenotype landscape are obtained with the Boolean network model. In the Boolean network model, each node has two discrete states: turned ON (denoted as 1) or turned OFF (denoted as 0). We consider a toy example with three nodes: a, b, and c. The Boolean function for each node is defined as: $a(t+1) = \neg b(t) \vee c(t)$, $b(t+1) = b(t) \vee \neg c(t)$, $c(t+1) = a(t) \wedge \neg b(t)$. For all possible initial states $S(t) = [a(t), b(t), c(t)]$, we can calculate the next states $S(t+1) = [a(t+1), b(t+1), c(t+1)]$. In this example, we can find two attractor states, [010] and [101], that satisfy $S(t) = S(t+1)$. Finally, we can obtain an attractor landscape for the example (Box Figure (a)). Note that attractor states have self-targeting arrows, and the basin of [010] is larger than the basin of [101]. If we assume that node c is an output node, then we can define a phenotype for each basin and attractor. An attractor [010] can be mapped into the phenotype A (green in the Box Figure (b)) according to the value of node c, whereas another attractor [101] corresponds to another phenotype B (blue in the Box Figure (b)). Therefore, the phenotype landscape is composed of phenotype A, which has seven states of basin size, and phenotype B, which has one state of basin size.



Box Figure Attractor landscape of a three nodes example. (a) The topology of an example network. Circles represent nodes of the network and sharp/blunt arrows show activating/repressing interaction between nodes. The network state is represented by three digit values. (b) Attractor landscape and phenotype landscape. Each circle represents a network state and arrows show relationships between precursor and successor states. The state of output node determines a particular phenotype and distinct colors show different phenotypes.

How can we then define the irreversibility of a biological process? From the perspective of our unified framework, a reversible process can be defined by a process in which a phenotype landscape of a network remains unchanged. Although the system undergoes a state transition from an original phenotype to another one, a transient stimulus can return the system to the original phenotype. On the other hand, an irreversible process can be characterized by a deformation of a phenotype landscape. Therefore, the original phenotype may no longer be on the reshaped phenotype landscape. To reverse the irreversible process, we need to reshape the deformed phenotype landscape to the original phenotype landscape.

THE REVERSE CONTROL

How can we restore the original phenotype by reverse control? Within the network, the effect of controlling a node is different depending on the network wiring and dynamics of the node. Identifying essential nodes for controlling network states has become a recently highlighted topic in network

science.[23–25] Most studies have investigated structural controllability by assuming linear dynamics of the given network structure and time-varying kinetics of inputs. Such approaches have limitations in application to biological systems whose dynamics are inherently nonlinear and allow only some restricted inputs under experimental conditions. Only few studies have considered such nonlinear dynamics of biological networks with attractors and realistic inputs.[26–28] Cornelius et al. investigated the state transition from an attractor to another attractor with a limited number of nodes that can be controlled simultaneously or a limited range of control effect.[26] Kim et al. introduced a 'control kernel' of a network on the basis of the attractor landscape, which is the minimal set of network nodes required to be controlled for transition from any state to the desired attractor, by pinning the state of nodes in the control kernel.[27] Zañudo and Albert developed an algorithm for predicting control targets in a logical dynamic scheme by identifying the stable motifs of a network.[28] However, most studies only considered state transitions in a fixed attractor landscape. Because the original attractor and its relevant phenotype can be removed in the reshaped phenotype landscape during irreversible processes, those approaches will have limited applicability for reverse control. To achieve the reverse control by rewiring a network, a novel control strategy for reshaping an attractor landscape is required. For example, Kim et al. found that the human signaling network can be divided into an evolvable core and a robust neighbor depending on whether or not, respectively, perturbations change the attractor landscape.[29] Nodes in the evolvable core of a network may be the target for the reverse control. By perturbing the target nodes, we might be able to induce network rewiring for the reverse control. Continuous drug administration or genome editing technology can be utilized for implementing such control strategies.[30]

If we control the rewired network to have exactly the same attractor landscape as the original one, then the original phenotype could be restored (Figure 2, top panel). For this strategy, we need to

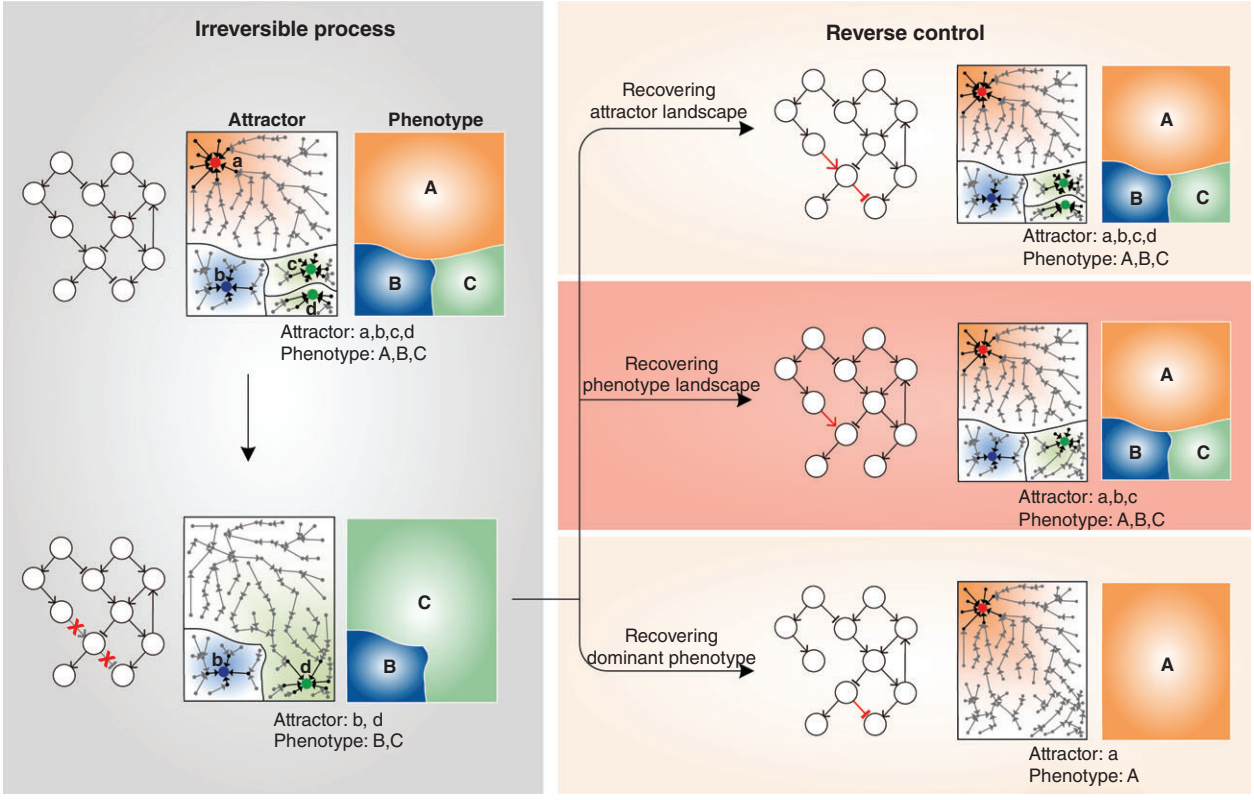


FIGURE 2 | Three strategies for the reverse control of an irreversible biological process. The original phenotype landscape is lost by network rewiring during the irreversible process. To recover the original phenotype, we can consider three strategies: recovering the original attractor landscape, the original phenotype landscape, or a landscape having the same dominant phenotype. Red X marks represent deletion of links during the irreversible process, whereas red arrows indicate the recovered links as possible means for the reverse control. Note that the strategy of recovering the original phenotype landscape is highlighted with a red background.

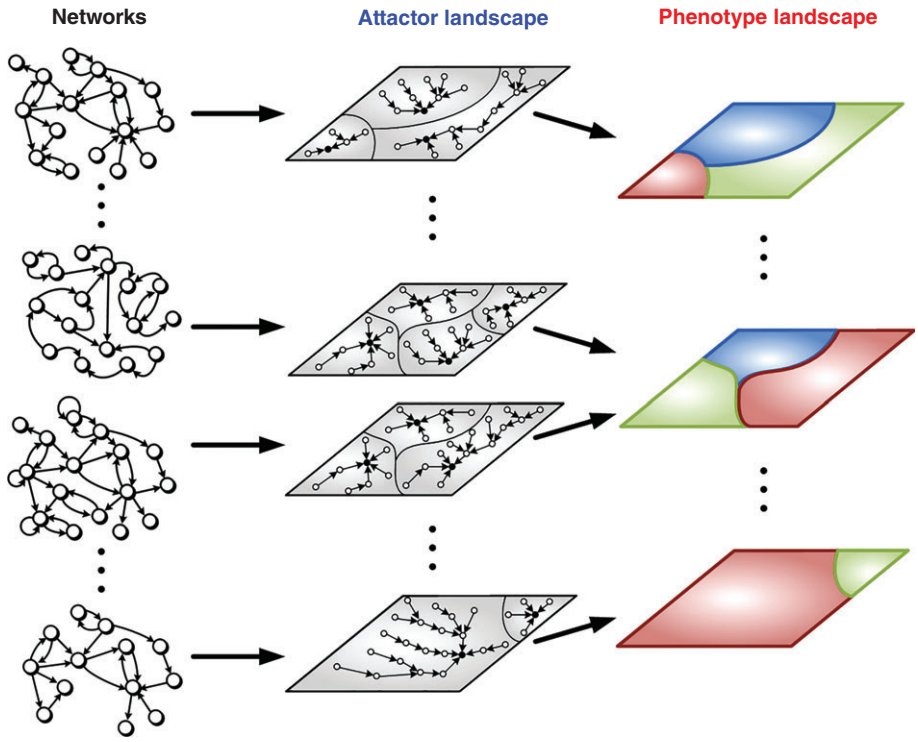


FIGURE 3 | The relationship among networks, attractor landscape, and phenotype landscape. Networks and attractor landscapes show a one-to-one relationship. However, more than one attractor landscape can be projected to one phenotype landscape. Therefore, although two network topologies are different, they can be functionally equivalent in terms of the phenotype landscape.

recover all the rewired links and nodes because a network and the corresponding attractor landscape have a one-to-one relationship (Figure 3). However, such exact control might be unrealistic in practice because it requires controlling the dynamics of all the molecules within a cell including the mutated genes. Therefore, we can choose an alternative strategy to obtain a phenotype landscape that contains the original dominant phenotype, the largest portion of the phenotype landscape, because a dominant phenotype will be the most observable and robust phenotype of a cell (Figure 2, bottom panel). Means for recovering the original dominant phenotype is more realistic because it needs to recover only a part of the rewired components or to control other components that have not been rewired during the irreversible process. The aforementioned studies controlling the phenotype of networks adopt the strategy of recovering the dominant phenotype; however, as not only the dominant phenotype but also other phenotypes are important, recovering only the dominant phenotype might induce some unexpected consequences.[31]

As the phenotypes of a cellular system are usually determined by a small subnetwork or several output nodes instead of all the nodes, there exist a vast number of different network structures having a common phenotype landscape. In other words, networks and phenotype landscapes have a many-to-one relationship (Figure 3). Therefore, we can restore the original phenotype landscape by rewiring a network even though we cannot exactly restore the original network topology (Figure 2, middle panel). Such a strategy would be more practical than the strategy of recovering the attractor landscape and also be less destructive to minor phenotypes than the strategy of recovering the dominant phenotype.

There are many examples that already imply a kind of reverse control through network rewiring and thereby restoring the original phenotype of irreversible processes in differentiation, tumorigenesis, and cellular aging as described in the following sections.

DIFFERENTIATION

In differentiation, a stem cell and a differentiated cell have different distributions of DNA methylation and histone modification.[5,32] Pluripotency genes and stemness genes are locked epigenetically in the differentiated cell but unlocked in the stem cell.[33] Such differences in network topology can be understood by a

spontaneous rewiring of the network, which results in different phenotype landscapes of the stem cell and differentiated cell, and eventually the irreversibility of differentiation. A stem cell activates epigenetic regulation constantly to maintain pluripotency and stemness, whereas a differentiated cell should lock the genes that involve pluripotency and stemness epigenetically. The mechanisms of how the cell sophisticatedly maintains and changes the epigenetic regulation are still unknown.[33]

Nuclear transfer stem cells (NTScs) and induced pluripotent stem cells (iPScs) are well-defined examples for reverse control of differentiation by network rewiring through perturbation. An NTSc is obtained by exchanging a nucleus of an egg or oocyte and that of a somatic cell.[34] The nucleus of a somatic cell has the phenotype landscape of a differentiated cell. However, cytosol of the egg or oocyte contains abundant epigenetic regulatory factors.[35] Takahashi et al. produced iPScs by introducing Yamanaka's four factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*), and these factors guided remodeling of the epigenetic pattern of stem cell genes.[5,33] These epigenetic factors induce network rewiring and reshape the phenotype landscape.

TUMORIGENESIS

Cancer arises by accumulation of genetic and epigenetic alterations that trigger inappropriate activation or inactivation of specific genes that are critical for tumorigenesis.[36–38] These sequential alterations cause, at a system level, a dynamic rewiring of the molecular interaction network and thus, cancer cells have different network dynamics from normal cells. In other words, the network rewiring by genetic and epigenetic alterations allows normal cells to transform to cancer cells and consequently leads cancer cells to have characteristic phenotypes such as uncontrolled cell proliferation.

Oncogenes play a key role in initiating a program of neoplasia by inducing genomic instability, thereby contributing to sustained expression of genes involved in cell proliferation. Although oncogenes may no longer be needed after the initiation of tumorigenesis, the inactivation of such oncogenes is expected to suppress the development of cancer. Many recent reports have demonstrated that the inactivation of oncogenes *in vivo* in transgenic models causes oncogene-induced tumorigenesis to become reversible.[10] These results confirmed that tumorigenesis induced by several oncogenes, such as *MYC* and *RAS*, could be reversible in various types of cancer including leukemia and breast cancer by driving cancerous states into an anti-proliferative state such as apoptosis and cell cycle arrest. In many cases, however, there could be no effect or limited efficacy in reversibility of cancer when only one or few oncogenes are inactivated.[39] There have been different approaches that have adopted an alternative strategy for reversing cancers into another normal state, rather than the exact original state. The treatment of cancer cell lines with H1 parvovirus isolated revertant cells by preferentially killing tumor cells. These revertants have significantly fewer tumor characteristics than the parental cells, and have several reversion genes, such as *SIAH1*, *PS1* (presenilin 1), *TSAP6*, and *TCTP*. Considerable evidence has shown that the activation of reversion genes by perturbing signaling pathways involving such genes could drive a cancer cell to lose its malignant phenotype and halt the tumor progression.[40–42] Recent reports on direct reprogramming of cancer cells have shown that human cancer cells can be reprogrammed and finally differentiated with reduced tumorigenic potential.[43–45] Ohnishi et al. showed that the transient induction of reprogramming factors by the withdrawal of doxycycline (Dox) results in kidney tumor development through epigenetic regulation, and further reported that no tumor formation was observed in the kidney of mice generated with iPSCs derived from the Dox-withdrawn tumor cells. These findings indicate that epigenetic regulation can contribute to the reversion of tumor cells to nonneoplastic cells.[46] These results further imply that network rewiring by inactivating specific oncogenes, activating reversion genes, or epigenetic regulation can lead the cancer system to have a phenotype landscape that corresponds to a normal system.

CELLULAR AGING

While cells divide, various types of damage inevitably accumulate in the process of responding to external stimuli. Genomic instability, telomere attrition, epigenetic alteration, and loss of proteostasis are representative cellular damage and hence cause cellular aging.[47] Once cellular damage has accumulated to some extent, a cell stops dividing and enters another cellular state called senescence. The increment of senescent cells in older organisms is the major cause of organismal aging.[48] Once a cell enters a senescent state, it generates a huge number of different molecular profiles compared with normal cellular states including cell cycle-related molecules and extracellular signaling molecules that regulate the cellular

micro-environments.[49] Several different signaling pathways including p21/p53 and pINK4A/pRb pathways are activated in senescent cells.[50] It was recently reported that the non-canonical WNT pathway is activated while the canonical WNT pathway is inhibited during cellular aging in skin cells. When the non-canonical WNT pathway is artificially activated, accelerated cellular aging was observed.[51] Therefore, the cellular aging can be controlled by activation of specific cellular pathways and such reshaping of the phenotype landscape because of different input signals or cellular damage can induce senescence.

There have been also several recent reports that even aged cells can be rejuvenated. First, aged cells can be rejuvenated by injection of specific factors of young cells. For example, in mouse muscle cells, growth differentiation factor 11 (GDF11) is decreased during aging and the aged cells can be rejuvenated by injection of GDF11.[52] In the mouse brain, retinoblastoma binding protein 4 (RbAp48), a histone modifier, is decreased during aging and the memory performance is recovered by injection of RbAp48. By injecting RbAp48 in an old mouse, the differential histone acetylation and activity of protein kinase A (PKA)/cAMP responsive element binding protein 1 (CREB1) pathway, central molecular signaling molecules of memory and synaptic plasticity phenotype, are observed at the cellular level.[53] Moreover, this pathway activation by a certain systemic factor from young mouse blood can reverse cognitive function of an old mouse.[54] Therefore, it is strongly suggested that PKA/CREB1 pathway activation can lead to cellular rejuvenation in the mouse brain. In addition to this cellular rejuvenation, aged cells can be reprogrammed by ectopic activation of six transcription factors including Yamanaka's four cellular reprogramming factors plus *Nanog* and *Lin28*.[55] Such an aging reversion mechanism can be explained by cellular reprogramming. Likewise, gametogenesis, a sporulation process, can reverse cellular aging and the transient activation of *NDT80* leads to cellular rejuvenation in yeast.[1] They showed that the rejuvenation process can eliminate various age-associated damage such as extrachromosomal ribosomal DNA (rDNA) and aggregated proteins associated with heat shock protein 104 (Hsp104) foci which are common age-dependent damage during aging in yeast. This is an experimental evidence that age-dependent cellular damage can be repaired, in other words, cellular aging can be reversed. Overall, these results suggest that cellular network rewiring by cellular environmental or genetic perturbation, which reshapes the phenotype landscape, can reversibly control cellular aging.

DISCUSSION

Our framework was inspired by the theoretical studies of development. For instance, Waddington introduced a multidimensional phase space to explain exaggeration of initial differences and robustness of developmental trajectories.[56] In the phase space, a group of initial points converge to an end point (robustness of developmental trajectories) whereas other groups converge to a quite different point (exaggeration of initial differences). He figured out, however, that such a multidimensional phase space is not familiar to most biologists, so introduced a more intuitive three-dimensional 'epigenetic landscape' metaphor. The convergence of initial points in the phase space was metaphorically explained by a ball rolling down to a valley upon the epigenetic landscape, and the exaggeration of initial differences was described by an increased number of separate valleys at the bottom of the landscape. He claimed that the chemical tendencies of genes shape the courses and slopes of the epigenetic landscape. In other words, the network of genes determines the robustness and direction of developmental processes. Kauffman also inspired the conceptual formation of this study. He introduced Boolean network modeling into biology, and investigated attractors (cycles in his original article) of random Boolean networks.[57] He described cellular differentiation as a transition between attractors by 'noise', and assumed that the noise should be 'sharply biased' for stable and irreversible differentiation. However, the roles of histone modification and DNA methylation were not known at that time, and therefore the network rewiring during a developmental process had not been considered. On account of this, many biologists inspired by Waddington and Kauffman believed that state perturbation could be enough to control biological systems. On the other hand, we suggest in this framework that state perturbation is not enough to reverse control complex biological systems but rather the landscape reshaping by network rewiring should be considered for reverse control.

In the proposed framework, we can also consider the epigenetic change as network rewiring even though epigenetic regulation actually happens by influencing the control of some nodes in the molecular regulatory network. Such epigenetic regulation takes much longer time while the genetic regulation happens in a relatively short period of time in pluripotent stem cells.[58] Therefore, we could assume that the epigenetic factors have no change during genetic regulation. Moreover, as the epigenetic regulation is much global and its regulatory mechanism is only

partially known, it is difficult to exactly reflect the epigenetic regulation in the network model. For these reasons, we should consider the epigenetic regulation as a stationary feature in the network rewiring. However, once considerable (if not all) details of epigenetic regulation are unraveled, we can extend the proposed framework to multi-layer or multi-scale network models to include such epigenetic regulation.

The biggest challenge in applying the proposed framework to real biological systems is constructing a meaningful dynamic network model that can realistically reproduce the previously observed biological phenomena. A sufficiently accurate network model is already available for a small-scale circuit that has been well studied (e.g., p53 regulatory network),[14] however, such a model is not currently available for a large-scale molecular regulatory network in a way to be directly used to implement the proposed strategy for reversely controlling the cellular phenotypes; there are still many genes and proteins whose functions, interactions, kinetic parameters, and regulatory logics have not been discovered yet. This means that the network model is not complete and contain many uncertainties. Nevertheless, we can assume that a large portion of key molecules are available and that more information is becoming available in an accelerated way these days. On the other hand, control of systems with uncertainties (called robust control) has been well studied in control engineering. Recently, robust control theory was applied to controlling complex networks with unreliable components.[59] Interestingly, in case of the structural controllability, it was found that the order of required number of driver nodes for robust control was same as that for control of a system without uncertainty. So, the ideas obtained from robust control theory can help us control uncertain biological network systems which still contain some dark matters in terms of the underlying molecular details. In addition, much more biological data are becoming accumulated and shared by many research groups because of high-throughput measurement technologies and many promising algorithms for data-driven network modeling are being developed, so the current uncertainties of network model will be filled out in the near future.[18,60–64] In the recent study,[14] a novel therapeutic target for breast cancer was identified through dynamics analysis of a small-scale p53 regulatory network model on the basis of its attractor landscape. This can be an example illustrating the potential application of the proposed theoretical framework to finding an actionable target for network rewiring that can induce a desired biological phenotype. We expect many other novel therapeutic targets for tumor reversion, rejuvenation, or regenerative medicine can be found similarly by applying the proposed framework to various network models in the near future.

CONCLUSION

Differentiation, tumorigenesis, and aging are representative examples of irreversible biological processes. We suggested a unified conceptual framework in which the irreversible processes caused by network rewiring could be reversed by a different way of network rewiring. In this framework, the network rewiring induces reshaping of the attractor landscape and the corresponding phenotype landscape, consequently allowing the reverse control of irreversible biological processes.

Predicting phenotype from genotype is one of the most important issues in understanding phenotypic differences of individuals such as disease susceptibility, rate of aging, and life span.[65] The relationship between phenotype and genotype is very complicated because they are intercorrelated by dynamic interactions amongst thousands of proteins that are expressed by genes (genotype) and regulate cellular functions (phenotype). As a result, dynamic characteristics of a cellular network and its stable states, namely the attractor landscape, are key mediators between genotype and phenotype. Therefore, the proposed conceptual framework which represents cellular phenotype through an attractor landscape and allows controlling phenotypes by network rewiring, can also be useful in predicting phenotype from genotype.

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