Gene regulatory network attractor selection and cell fate decision: insights into cancer multi-targeting

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***Abstract*—Cell fate decision processes in intracellular network biology have been traditionally associated with the analysis of linear pathways and correlated with the activity of specific components. Here we review some of the concepts and formalisms in discussion in the literature of cell fate decision and defend an integrative approach to modeling intracellular processes. This approach is based on the concept of attractor selection that could inform on cancer targeting strategies such as small molecule kinase inhibitors.**

***Index Terms*— Multi-targeting therapy, attractor selection, cell fate decision, synergy, high-order dimensional system, cancer**

I. Introduction

Proteins synthesized from genes may function as transcription factors binding to regulatory sites of other genes, as enzymes catalyzing metabolic reactions, or as components of signal transduction pathways processing extracellular messages. To understand how genes are implicated in the control of intracellular and intercellular processes, a systemic integrative approach is necessary to go from sequences of nucleotides coding for proteins to regulatory systems determining expression patterns of genes. Here we will focus on signalling-transcription integrative networks. The integration of cue signals is both performed by the signalling system, whose function is akin to a multi-layer perceptron or combinatorial decoder[1], and the transcriptional machine[2]. The signalling module generates a classification of the combination of inputs (activated receptors) based on their level (dosage) and timing[1], recurring to the multiple interlinked chemical processes (most commonly Kinase/Phosphatase reactions). The transformation outcome is a combination of activation concentration profiles of the output nodes (S_1 and S_2 in Fig. 1), whose shape (signal duration, signal amplitude, signalling rising and relaxation times) has been correlated through combined experimental and simulation studies with the induced genetic programs. For example, the temporal control of the signaling module that contains IkB kinase (IKK), its substrate inhibitor of NF-kB (IkB), and the key inflammatory transcription factor NF-kB can allow for selective gene activation by Tumour Necrosis Factor Receptor (TNFR) and other concurrent pathways[3]. Other pathway systems such as the Epidermal Growth Factor Receptor (EGFR) have also been the focus of extensive experimental studies associating signalling output activation (e.g.MAPK/Erk kinase in double phosphorylated state) with cell phenotype. Transient versus sustained activation of the output node indicates the competition between two mutually exclusive cell phenotypes, proliferation and differentiation[4], respectively, in some cell lines.

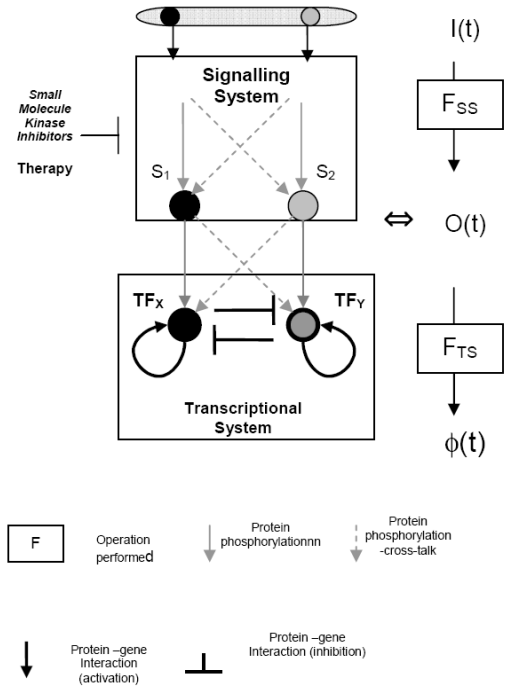

Fig. 1. Simple representation of the two modules involved in intracellular signal integration. Signalling is usually associated roughly with a combination of 3 layer cascades (only he final layer is represented here).The transcriptional system represents a common motif used in gene regulatory network, toggle or decision switch[5]. I(t) stands for the set of inputs. O(t) represents the set of outputs. ϕ(t) represents the phenotype or gene expression pattern. Phosphorylation reaction motifs seen in this diagram are known as BIFAN motifs, one of the most common motifs in signalling networks[6]. The multilayer perceptron commonly used in classification tasks may be seen as a generalization of the BIFAN motif to higher dimensions[6].

Regarding targeting strategies of complex networks, the question arises of how to modulate the biochemical processes as to induce the correct combination of concentration profiles of output nodes and how to interpret this combination. Targeting single nodes belonging to the central processing core of the signalling information processing layer has not been as fruitful as desired[7]. The multiple mutation scenario that characterizes cancer associated networks[8], and turns these abnormal cells into very robust systems[7], working with the inherent redundancy of molecules in cell signalling exhibiting extensive cross-talk[9], renders the development of targeting strategies a highly complex endeavour. In Fig. 1 we represent a paradigmatic global system and the drugs (small molecule

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kinase inhibitor therapies) to be considered for tuning or modulating the selection of gene expression.

The function performed by each of the modules (F_{SS} signalling and F_{TS} transcription) is dependent on the wiring of the network, W_{SS} (1) and W_{TS} (2)[10], and the set of inputs to the module over time. Here the wiring is not only associated with the connectivity matrix between system's nodes but also with the actual parameters (kinetic parameters such as phosphorylation, transcription and degradation rates) determining nuances in the dynamical regimes.

$$O(t) = F_{SS}(I(t), W_{SS}) \quad (1)$$

$$\phi(t) = F_{TS}(O(t), W_{TS}) \quad (2)$$

Composit motifs[6] involving feedback loops between the transcriptional and the signalling systems will not be considered here. They can give rise to much more complex behaviours such as damped sustained oscillations as well as differentiation[11].

II. Deterministic and Stochastic Gene Regulatory Network Attractor Selection

The concept of attractor landscapes has been extensively explored in cell differentiation studies. Deterministic studies of low order gene regulatory networks such as bistable switches have been given a thorough theoretical investigation and constructed de novo[12]. Conditions for multistability are naturally linked to parameters controlling self-stimulation and cross-stimulation[13] (Fig. 1) and are thought to be a clear framework to understand selective expression in different cell types. Bistability in stochastic gene regulatory networks has been studied in several systems,e.g. λ phage decision switch in Escherichia coli coupled or uncoupled to the quorum-sensing signalling pathway or the SOS signalling pathway[14]. Noise is essential for switching from attractor to attractor when the system has an interface with a signalling module. Generalizations to high dimensional switches have been carried out both in deterministic systems[15] and in noisy ones[16]. Kaneko and coworkers[16] showed that the strength and duration of noise in globally couple map lattices is sufficient for transition from attractor to attractor in a system capable of exhibiting ordered phases, synchronized phases and turbulent phases. In the same work, it was also shown that in the absence of noise, efficient attractor selection is also possible by variations in the rate of sweeping of the single parameter coupling each node to the mean-field activity. Noise is also essential for optimal attraction selection in evolutionary systems coupling metabolic activity to gene pattern expression in the absence of any adequate signalling external inputs[17]. The gene expression pattern selected in integrated metabolic-transcription network simulations corresponds to the maximal cell growth controlled by metabolic network activity. Cell fate being associated with a high-dimensional attractor or gene expression pattern, as first proposed by Kauffman, was confirmed experimentally in neutrophil differentiation[18] and in a hematopoietic cell line with convergence of high-dimensional trajectories[19]. Return of noise-induced deviations of the transcriptome from the border of the basin of attraction back to the attractor state have also reinforced the idea of high-dimensional attractor[20].

Generalizations of the attractor landscape to dynamic cyclic attractors representing for example the cell cycle would entail having a sequence of states with a privileged transition sequence between each of the stages/attractors (G0,G1,S,M) of the cell cycle[21]. Consider the following potential landscape (Fig. 1) as a paradigmatic example representing point attractor selection by external signals in intracellular network dynamics.

$$U = \Delta r^2 \times (\Delta r^2 - 2) + (1 - S_1(t)) \times (\Delta r - 1)^2 + (1 - S_2(t)) \times (\Delta r + 1)^2 \quad (3)$$

$$\Delta r = [TF_y] - [TF_x] \quad (4)$$

Evolution of the system could be modelled in a Langevin type of approach by differentiating the potential with respect to Δr. A remark should be done about the sensitivity of the dynamics of the gene regulatory system, here represented by the potential landscape, to the external signals. Input functions to the gene promoter regions can often show extreme complexity due to cooperativity between binding sites and to the presence of multimers necessary for initiation of transcription[22]. Moreover, since the chosen mechanism for transcription factor activation in this paper is phosphorylation the type of dynamics considered in this reaction, mass action or Michaelis Menten kinetics, will function as a filter to $S_1(t)$ and $S_2(t)$ dynamics.

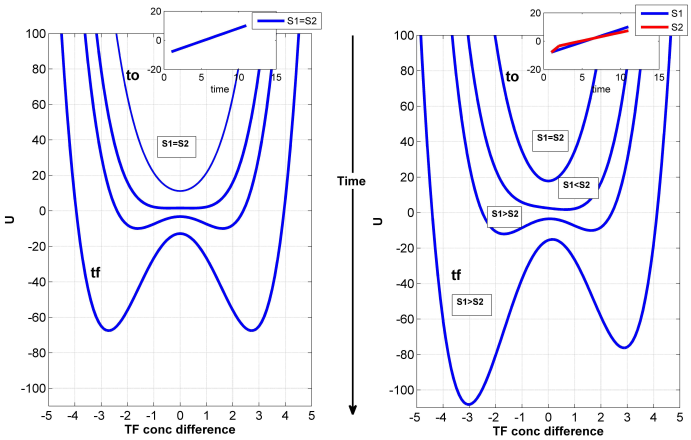

Fig. 2. Potential for hypothetical gene regulatory decision switch (Fig. 1) driven by external signals leading to attractor selection. Left – signals have equal profiles. Right-signals change differently with time. The differences are integrated by the transcriptional system. U, potential function. TF conc difference = Δr = $[TF_y]$-$[TF_x]$

The potential shape represented in (3) and Fig. 2 shows that external signals may exert an asymmetric change in parameters ending in a state space with only one terminal attractor (Fig. 2, right). This particular "deterministic-instructive" setting is also known as a progression switch[5]. Another avenue for understanding the action of signals brings a higher contribution to noise. A symmetric change (see Fig. 2,left), also known as "deterministic-selective", forces a bistable regime, through a pitchfork bifurcation, with equally large attractor basins for each of the attractor states with the end attractors being

populated as a result of stochasticity. The system at time t_f obeys a fixed potential landscape and the distribution of trajectories over each attractor is sensitive only to variations in the position in the basin of attraction at instant t_0, if the potential barrier is sufficiently large at time t_f, hence inducing memory effects. The speed with which the critical zone is crossed may be used to trap the system in one of the basins of attraction[23]. The slight variations in position at time t_0 inducing asymmetries at time t_f will only be dismissed as simulation artifacts under extensive simulations. The mechanism involving state space structure changes, e.g. bifurcation (Fig. 2), has been proven to be more efficient in selecting attractors with slightly larger basins of attractions in comparison to the system not undergoing bifurcation and exhibiting the same asymmetries[24]. The difference in basin size and depth of the attractors can be intrinsic, e.g. entry point G1 of the proliferation set of attractors in cancer cells would be associated with larger basin of attraction, or time dependent following roughly the asymmetry between external signals (Fig. 2, right). Combination of symmetry breaking by external signals and noise constitute a viable mechanism for cell fate decision where combination of N external signal characteristics are clustered by the transcription factor network attractors (N.R. Nene and A. Zaikin, in preparation).

III. Synergistic effects of high-order targeting strategies: developing targeting signatures inducing the appropriate expression patterns by multi-parametric stochastic optimization methods

In many cancer treatments multi-component combinations have become the main strategy. The development of combinatorial targeting strategies may take two avenues: combination of multiple agents with high specificity or development of an agent with multiple targets[25]. Development of a single agent with multiple targets might overcome molecular heterogeneity and have a better chance of success if tumour markers and selection criteria are uncertain. On the other hand, the mechanisms of action are difficult to understand and the development of a drug with optimal potency against several relevant targets is complex. Another problematic aspect with combinatorial approaches stems from the additional targets (non-specific interactions) which may or may not be relevant in a particular tumour molecular profile increasing the probability for toxicity. Binding affinity studies against panels of kinases[26] have revealed that a wealth of additional targets are inhibited by currently used small molecule kinase inhibitors. Additional inhibited kinases often show structural similarities (conserved ATP binding site) that enhance the probability of binding of the inhibitor. Studies of additional effects of non-specific interactions on the EGFR pathway dynamics have shown that Gefitinib, a drug already approved for Non Small Cell Lung Carcinoma (NSCLC) treatment, has a conflicting action on output (Erk) activation. Gefitinib additionally perturbs a kinase (Gyclin G associated kinase (GAK)) which is involved in internalization of vesicles and consequently of receptors. The EGFR internalization pathway under normal concentration of ligand protects the cell from excessive signalling. GAK additional inhibition perturbs this protective pathway, counterbalancing the intended positive inhibition, that of EGF receptors (N.R.Nene et al, in preparation). Development of kinase inhibitors with a specific kinase-inhibitory profile showing optimal potency and therapeutic index for each of the chosen multiple targets is still not possible. Hence, even in a scenario where specific multi-targeting is intended, the side-effects may not be as predictable as one hoped.

The balance between complete disruptions of a network by a set of specific drugs may not be the most successful strategy. Studies of large networks[27] have shown that multiple weakly modulated nodes have a higher impact on network efficiency, which is a global measure of network integrity related to the shortest path length among each pair of elements within the network. This has motivated therapeutical approaches looking for synergistic effects of drug combinations with reduced toxicity[28]. Systematic biological perturbation approaches call for quantitative phenotypes monitoring system's function. A global phenotype such as cellular proliferation has been frequently used[29]. As in our working hypothesis this phenotype can be identified with specific genetic programs or system's attractors, inducing a particular cellular response can be equated as a pattern selection optimization problem where targeting is induced at the signalling level and response evaluated at transcriptional level. The use of integrative phenotypes allows for simultaneous examination of system's functions with relatively few measurements. Thus for large-scale investigations of high-order combinations, it is likely that global phenotypes will be most practical in the near future. Multi-targeting should be formalized as a basin hopping optimization problem in a multiple fixed attractor landscape or a landscape conformation induction when external inputs force the system to have only one attractor or a reduced number of attractors with noise being crucial for end point attractor populations. The end attractors can be point attractors or dynamic attractors with the initial state determining the accessibility of each of the modes. If the system is initially in attractor P (proliferation in the case of a cancer cell) and we desire to force it into attractor A (apoptosis) or D (differentiated cell), a multiple targeting strategy needs to be developed that induces the appearance of only A and D, erasing completely P or making it highly improbable (small basin of attraction). The problem can be formulated by minimizing the "distance" (5) between the current state, phenotype ϕ^P, and the target states. This can be achieved by simulated annealing approaches minimizing the mean square error or maximizing the mutual information between the initial expression pattern (P) and the target state by targeting the signalling module with small molecule kinase inhibitors (6).

$$d\phi = \phi_{I(t),W_{SS},W_{TS},\Sigma_{Therapy}}{}^{A,D} - \phi_{I(t),W_{SS},W_{TS}}{}^{P} \quad (5)$$

$$\Sigma_{Therapy} = \Sigma_{Therapy}(\varepsilon_1,...,\varepsilon_N,k_{out1},...,k_{outN}) \quad (6)$$

$$\varepsilon_i = \frac{[S_j]/K_M'}{1+[S_j]/K_M'} \ , \ K_M' = K_M(1+\frac{[I]}{K_{d_j}}) \qquad (7)$$

In (6) Σ_{Therapy} represents the "inhibition signature" of the therapy (small molecule kinase inhibitor). In (7), K_d is the binding affinity of the drug to a specific protein network node[26], K_M stands for the Michaelis-Menten constant and ε the inhibition of a node by the administered drug. k_{out} represents the number of output connections of each of the nodes. The drug shifts the threshold (K_M) to higher concentrations, with the increment dependent of the dose I. The optimal path between attractors or landscape configurations induced by the signalling targeting strategy should also take into account the difference in time scales between the two modules composing the integrated system, signalling fast and transcription plus translation slow. Time scale separation could render the perturbations envisaged by the multitargeting therapies irrelevant[30]. Recalling our example of a potential undergoing a bifurcation (Fig. 2), if the time-scale separation between the evolution of signals S_1 and S_2 and transcriptional dynamics is considerable the system is expected to remain near the top of the potential barrier for longer. Consequently, the asymmetries arising from the external signals are not reflected in the final distribution over the attractors since on top of the potential barrier noise has the effect of recovering symmetry.

IV. Conclusion

The combination of cancer mutation scenarios of signalling structures and the transcriptional landscape[8] demand a multi-targeting integrative approach where whole cell phenotypes characterized by gene expression patterns serve as sensors of cell function. Although experimental techniques fully characterizing this integrative approach are still not available, the field of synergistic targeting should benefit from simulation studies with generic dynamic models on networks already constructed[8]. Understanding how information is processed in normal and abnormal network phenotypes and development of inhibition signatures should open avenues for correlating node inhibition, node connectivity and whole network dynamics impact.

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