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Dynamical Systems

Robustness in biological regulatory networks IV: Application to genetic networks controlling the cell cycle

Robustesse dans les réseaux de régulation biologique IV : Applications biologiques aux réseaux génétiques contrôlant le cycle cellulaire

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ABSTRACT

Various indices of complexity are used in biological regulatory networks like the number n of their components and I of the interactions between these components, their connectance (or connectivity) equal to the ratio I/n , or the number of the strong connected components of their interaction graph. The stability of a biological network corresponds to its ability to recover from dynamical or parametric disturbance. Complexity is here quantified by the evolutionary entropy, which describes the way the asymptotic presence distribution or equilibrium distribution of the corresponding dynamical system is spread over the state space and the stability (or robustness) is characterized by the rate at which the system returns to its equilibrium distribution after a perturbation. This article applies these notions in the case of genetic networks having a getBren structure (*i.e.*, being threshold Boolean random networks) and notably those controlling the cell cycle.

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RÉSUMÉ

De nombreux indices ont été proposés pour quantifier la complexité des réseaux biologiques de régulation, comme le nombre de leurs composants, leur connectivité, ou le nombre des composantes fortement connexes de leur graphe d'interaction. Quant à la stabilité de ces réseaux biologiques, elle correspond à leur capacité à absorber les changements dynamiques ou paramétriques. La complexité est ici mesurée par l'entropie évolutionnaire, qui décrit la manière dont la probabilité de présence asymptotique du système dynamique correspondant est distribuée dans l'espace d'état, et la stabilité est caractérisée par la vitesse de retour à l'équilibre de cette distribution, après perturbation. Cet article utilise ces notions dans le cadre de réseaux génétiques ayant une structure aléatoire booléenne à seuil, et plus particulièrement ceux contrôlant le cycle cellulaire.

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Version française abrégée

La notion de stabilité dans les réseaux de régulation génétique est reliée à la notion de complexité, et l'on peut quantifier ces deux notions en utilisant un même paramètre, l'entropie évolutionnaire H , qui est corrélée, à la fois à la vitesse de retour

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à l'équilibre après perturbation, et au degré de complexité [11–13]. La présente note correspond à l'utilisation de H comme indice de robustesse, et montre sa variation monotone, au cours de l'évolution, pour le réseau de contrôle du cycle cellulaire de différentes espèces.

L'entropie évolutionnaire d'un réseau de n gènes peut être estimée, dans les conditions de la Proposition 1 de [12], par l'égalité : $H = n \log 2 - E_{\text{attracteur}}$, ce qui, pour le réseau de la Fig. 1 en mode de mise à jour séquentiel, correspond à : $H = 12 \times 0,69 - 0,86 = 7,5$, lorsque le paramètre température T est choisi suffisamment large. En mode parallèle, cette quantité passe à 8,3, ce qui montre l'influence de l'implémentation des modes de mise à jour sur la robustesse du réseau, liée à la diminution du nombre des attracteurs de la dynamique du réseau, lorsque l'on passe du séquentiel au parallèle (cf. Fig. 2). Il en est de même lorsque le nombre des gènes sources du graphe d'interaction augmente, au cours de l'évolution, ce qui accroît les contraintes sur la dynamique du réseau (en particulier celles liées à l'existence d'inhibiteurs de type microARNs, cf. Fig. 3). On assiste alors à une diminution du nombre des attracteurs, donc à une diminution de $E_{\text{attracteur}}$, ce qui correspond à une augmentation de H , donc de la robustesse du réseau. C'est en particulier le cas lors de l'évolution d'un réseau dédié au contrôle de la même fonction, ici proliférative, dans différentes espèces (*C. elegans*, *D. melanogaster* et l'homme, cf. Fig. 4), et également dans des réseaux à l'interface de plusieurs fonctions, comme le réseau « Engrailed », qui impacte trois fonctions : prolifération, apoptose et transmission neurale (cf. Fig. 3) comportant de nombreux microARNs de contrôle.

Le réseau « Engrailed » comporte un mécanisme assurant l'équilibre, à l'état adulte, entre prolifération et apoptose, pour éviter les deux écueils de la croissance et de la dégénérescence tissulaire incontrôlées. Pour ce faire, la dynamique du réseau comporte un attracteur cycle-limite, permettant d'activer une fois sur quatre la fonction prolifération (responsable d'une croissance exponentielle, en l'absence de contrôle) et une fois sur deux la fonction apoptose (responsable d'une décroissance linéaire, en l'absence de contrôle), assurant ainsi l'équilibre du nombre des cellules du tissu.

Pour finir, le réseau « Engrailed » est analysé sur le plan de la sensibilité en terme de robustesse. Cette sensibilité est mesurée par la dérivée de l'entropie évolutionnaire par rapport au poids d'interaction (supposé avoir la même valeur absolue, pour toutes les arêtes du graphe d'interaction), suivant la Proposition 2 de [12]. Elle peut être ainsi estimée par la variance de la frustration du réseau, montrant une diminution de la robustesse, lorsque le poids augmente : ceci est en accord avec le fait que la diminution du poids, analogue à une augmentation de la température du réseau [12], entraîne une convergence de la mesure invariante vers la mesure uniforme sur les états du réseau, correspondant toujours à un maximum de son entropie évolutionnaire.

1. Introduction

The notion of stability in genetic regulatory networks is related to the notion of complexity of such networks. We quantify these two notions by using only one parameter, the evolutionary entropy H , which is correlated with both the rate of return to equilibrium after perturbations, and the degree of complexity [11–13]. The application concerns the use of this parameter H as robustness index, and its monotonic variation during the evolution, for the same type of networks devoted to the control of the cell cycle, in different species.

2. Genetic network ruling the cell cycle

We will study a simple example of sub-network extracted from the genetic regulatory network dedicated to an important function, the control of the cell cycle in mammals, in particular the control of the cell proliferation at the G1/S transition check point [20,21]. We give respectively in Figs. 1 and 2 its interaction graph G and describe its attractors in both parallel and sequential mode of updating, with temperature T equal to 0 [13].

The evolutionary entropy H of the network pictured in Fig. 1 may be calculated for a sequential updating, under assumptions of Proposition 1 of [12], using the equation relating H and $E_{\text{attracteur}}$ from calculations of ABRS's: $H = n \log 2 - E_{\text{attracteur}} = 12 \times 0,69 - 0,86 = 7,5$, when the temperature parameter T is supposed to be sufficiently large. H increases from sequential to parallel updating (from 7.5 to 8.3), showing that the updating mode influences the robustness of the network. For the network of Fig. 1, representing the core of the regulation of the cell cycle in mammals [20], the evolutionary entropy H decreases when boundary constraints grow (cf. Fig. 2 bottom right), showing that the inhibition by miRNA 159 contributes to diminish the robustness of the genetic network. But, more generally, the introduction of an important inhibition noise from miRNAs (cf. Figs. 1 and 2) causes a huge reduction of the attractor number, hence an increase of H . It is the case in the control network of Cdk2 in Fig. 2 bottom, which shows the complex role played by several miRNAs (through the protein p53) in the expression of the Cdk's, the most important proteins controlling the G1/S transition in cell cycle.

Let us consider now a hybrid network, mixing neural and genetic sub-networks: the top of Fig. 3 exhibits the neural part of the Engrailed network, under the control of the gene Elk whose expressed protein is necessary for the correct functioning of the K^+ ion-channels in excitable cells. Indeed, Elk controls positively the ability of CA3 cells (denoted Y_1 and Y_2 on the interaction graph of Fig. 3) to express their negative feedback upon the CA1 cells (denoted X_1 and X_2 in Fig. 3) inside the hippocampus [26].

The Elk gene is activated by the Erk gene depending positively on MEK, an intermediary gene in the pathway from the gene Egfr to the gene MAPK [20]. In the centre of the Engrailed network of Fig. 3, the genes GATA-6 and c-Myc are

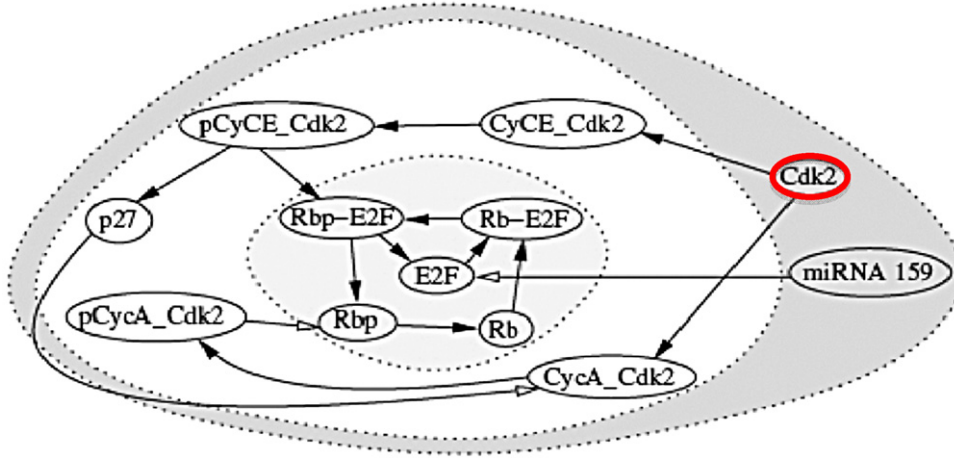
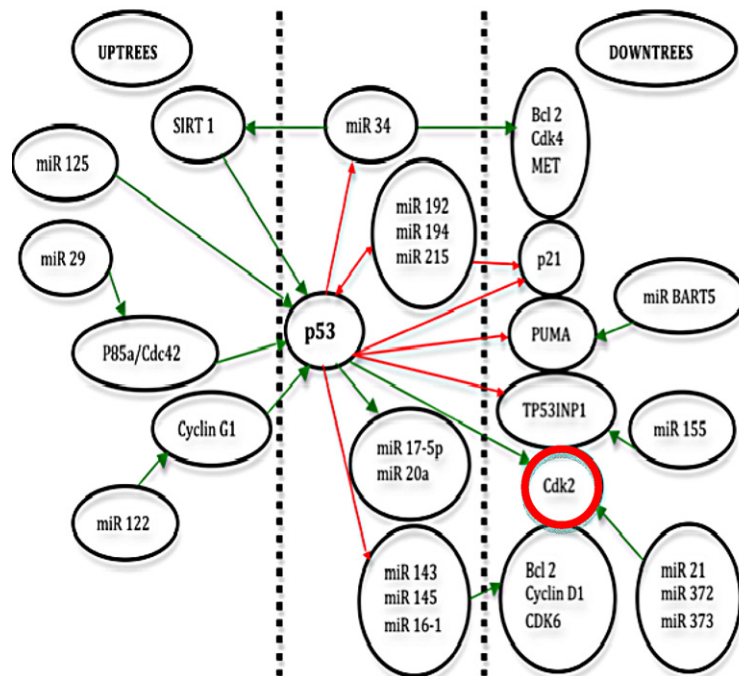


Fig. 1. Interaction signed digraph modelling the genetic regulation network of the cell cycle in mammals [20]. Black (resp. white) arrows represent activations (resp. inhibitions).

| miRNA 159=0 | Sequential updating | | Parallel updating | | |
|---------------|------------------------------|-----------|-------------------|-----------|------|
| | Nature | Attractor | ABRS | Attractor | ABRS |
| Fixed point 1 | 000000000000 | 6.25% | 000000000000 | 0.5% | 1.45 |
| Fixed point 2 | 000000011111 | 56.25% | 000000011111 | 99.5% | 5.40 |
| Limit cycle 1 | 000000001000 000000010111 | 37.5% | None | | |



miRNA159=1 Parallel updating

| Nature | Attractor | ABRS |
|---------------|--|-------|
| Fixed point 1 | 000010000000 | 0.12% |
| Fixed point 2 | 000010011011 | 0.54% |
| Limit cycle 1 | 000010001010 000010010001 | 10.6% |
| Limit cycle 2 | 000010010011 000010001011 000010011001 000010011010 | 26.6% |
| Limit cycle 3 | 000010010010 000010000011 000010001001 000010011000 | 32.4% |
| Limit cycle 4 | 000010000010 000010000001 000010001000 000010010000 | 23.7% |

Fig. 2. Top: Attractors and attraction basins relative sizes (ABRS) of the network drawn in Fig. 2, when miRNA 159 is fixed to 0, in case of sequential and parallel schedules, with $T = 0$. The nodes are ordered as follows: p27, Cdk2, pCyCE_Cdk2, CyCE_Cdk2, miRNA 159, pCycA_Cdk2, CycA_Cdk2, Rbp-E2F, Rb-E2F, E2F, Rbp and Rb. Bottom right: Attractors and attraction basins relative sizes (ABRS), when miRNA 159 is fixed to 1, in case of parallel schedule, with $T = 0$. Bottom left: Control network of Cdk2 (after [19]).

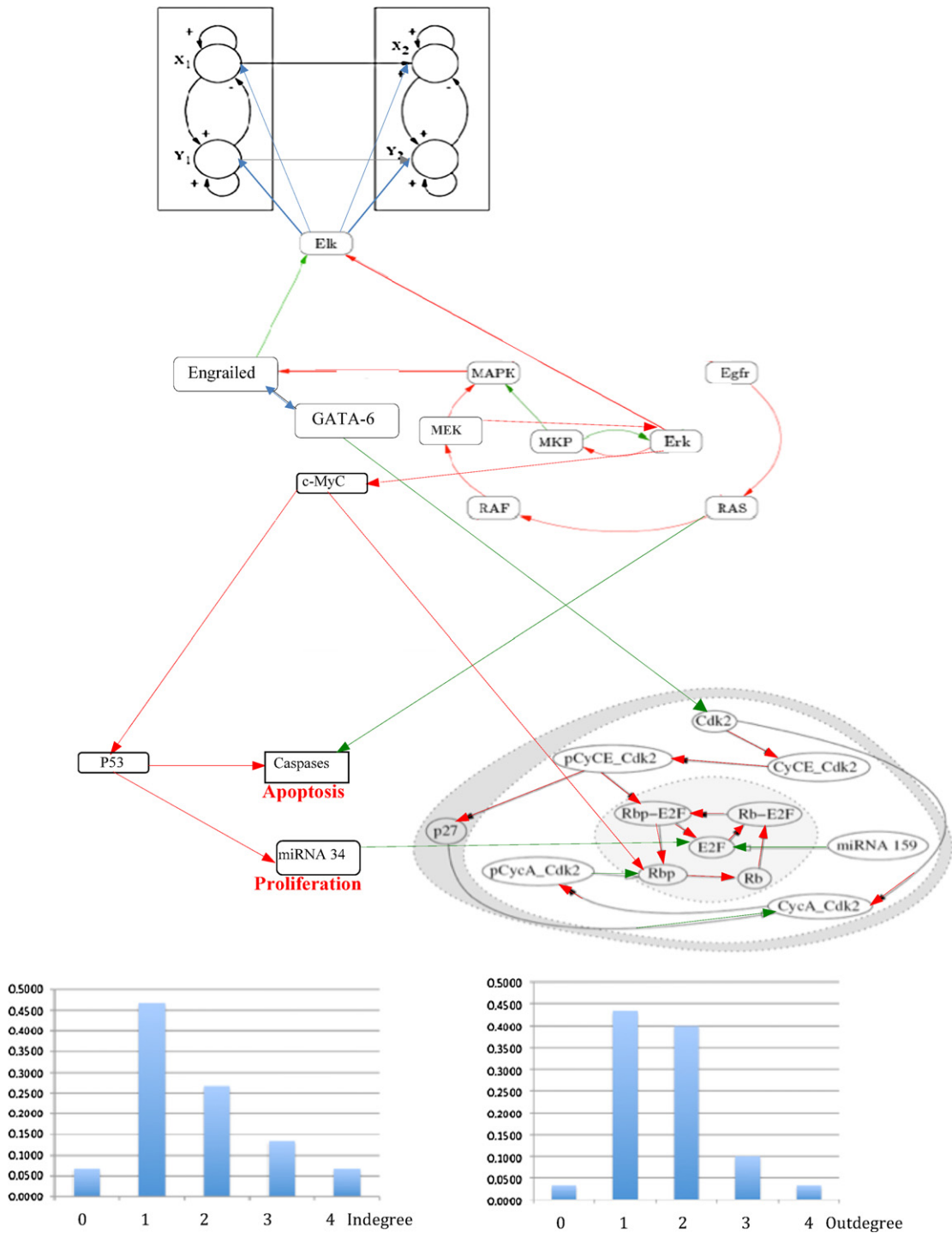


Fig. 3. Top: Engrailed hybrid network with multiple connections to apoptosis and proliferation control boxes, as well as to the hippocampus neural network. The activation (resp. inhibition) arrows are red (resp. green). Black arrows correspond to neural activations and inhibitions, and blue arrows correspond to activation links toward other functions than the strict Engrailed homeobox control. Bottom: histograms of the indegrees (left) and outdegrees (right) of the 50 interactions of the Engrailed network.

regulating the proliferation through both positive and negative actions on E2F expression in the mitotic regulatory box [15, 6,18–20,23], as well as the apoptosis through p53 [4,17,21,24,25].

The triple action (accelerate, stop and slow down the cell cycle) on proliferation process is exerted negatively by the gene GATA-6, which is inhibited 1 time out of 4 by MAPK, and successively positively and negatively by the gene c-MyC which

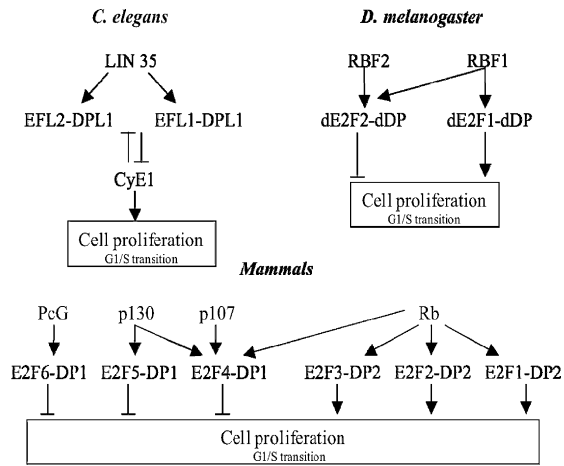


Fig. 4. (After [21]) Evolution of the structure of control of the cell proliferation box (Fig. 1) for different species, *Cænorhabditis elegans*, *Drosophila melanogaster* and mammals.

is activated 1 time out of 2 by Erk. The limit cycle of order 4 brought by the negative circuit of size 2 (MKP/Erk) leads the genes MKP, Erk, MAPK, Engrailed, GATA-6, c-Myc, p53, miRNA34, Cdk2, E2F, RAS and caspases to enter in the limit cycle:

$$(000000110110, 011000010010, 111101000011, 100111100011).$$

Then, the E2F/proliferation box is activated 1 time out of 4 and the apoptosis box 1 time out of 2, such as we observe the following cyclic behavior: 4 cells, 4 cells, 3 cells, 2 cells, 4 cells, ... This dynamical behavior allows the exponential growth of proliferation to be compensated in a tissue by the linear growth of apoptosis, 2 daughter cells replacing 2 dead cells during one period of the limit cycle, hence ensuring the conservation of the tissue volume and function.

Any disequilibrium of this balance leads either to a tumor growth or to a tissue rarefaction. Finally, concerning the network of Fig. 3, we can exploit the conclusion of Proposition 2 of [12]. We have:

$$Q_+(N) = 39, \quad Q_-(N) = 11, \quad F(00\dots 0) = Q_+(N) \quad \text{and} \quad F(11\dots 1) = Q_-(N),$$

all other values of $F(x)$ falling inside the interval $[0, \sup(Q_-(N), Q_+(N))]$. If we consider that the distribution of the values of $F(x)$ is uniform over this interval, then we get: $\text{Var } U = \text{Var } F = q^2/2 - (q/2)^2 = q^2/4$, where q denotes the value $\sup(Q_-(N), Q_+(N)) = 39$. Then, for the network of Fig. 4 where all of its weights are supposed to have the same absolute value 1, we get: $\text{Var } U = 39^2/4 \approx 380$ and its robustness rate can be quantified by the value -380 .

A systematic calculation of $E_{\text{attractor}}$ allows to quantify the complexification and the robustness of the networks ensuring dedicated regulatory functions in different species: for example, the increase of the number of sources in up-trees converging on a conserved (among species during evolution) sub-graph of a genetic network, e.g., on the core regulating the cell cycle in *C. elegans*, *D. melanogaster* and mammals (cf. Fig. 4), causes a decrease of its attractor number, hence an increase of its evolutionary entropy and of its robustness.

The progressive appearance during the evolution of many upstream controllers as the mammals microRNAs inhibiting p27 [23] and p53 (cf. Fig. 2 bottom left and [4,17,21,24,25]) gives a robust control [20] of Rb-E2F, ensuring the integrity of a crucial cellular function: the cell proliferation at the G1/S transition [15,20,21].

Another example of the crucial role of the microRNAs in the robustness of genetic regulatory network [7–9] lies in the appearance in the not-coding genome of the mammals of the inhibition by microRNA miR-17-5p-20a-106a of human myeloid cells on the gene RUNX1, involved in the control of the TCR α recombination in immunology [3,21,26] or in the control by several microRNAs including miR-150, miR-155 and let-7 family of the memory T cell fate determination [2,27].

3. Conclusion

The quantification of the complexity in biological regulatory networks allows us to explain in which way they have been adapted during the evolution, e.g., by exploiting the opportunities offered by the increasing number of small RNAs [7,8,22] as translation factors inhibiting connected components, in particular circuits, of the getBrens [9–16], resulting in an increase of the efficiency of their own robustness mechanisms.

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