ISSN No:-2456-2165

An Attractor Analysis of mTOR Signaling Pathway Synchronous Update of One Specific Initial State

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Abstract:- The study of biological systems is drawing the attention of many scientists giving a description of their behavior based on mathematical modeling and numerical approaches. Most of the time, this pathway is followed either when experimental data for a given network is missing or a prediction of the system evolution is made. In both cases, the states of each element of the network as well as the interactions between them are important for modeling the biological system. Here we use a discrete model such as Boolean modeling for making a prediction of the evolution of mTOR signaling pathway based on different initial states of the system and different ways of interactions between elements. We focus on synchronous update of the nodes' states in order to find and analyze the fixed points of the system. It is shown that the system reaches different stable states represented in each case by a fixed point, or it enters in a cycle limit, depending on the initial state of the system and on the way of the interactions between elements, as well. In all cases we see that mTORC1, in which we are mainly focused, becomes inactive. Although this study is limited, we aim to generalize this case of study to other similar cases which can lead us to other in-depth analysis.

Keywords:- *Boolean Model, Synchronous Update, Nodes, Network, Dynamical Evolution.*

I. INTRODUCTION

Knowing the dynamics of intracellular regulation networks is very crucial in systems biology as it gives us not only the information about the interactions between components but also the pathway that this information follows to approach other cells inside and outside the network too. Most of the qualitative information about the cell – cell interactions is given by experimental data, although it is shown that the behavior of a system is often modeled using some mathematical models developed theoretically based on these experimental data and some predictions made on this occasion [1]. Here, we present a study of modeling the biological network which is focused on the signaling pathway of mTOR affecting cancer cells. The mammalian target of rapamycin (mTOR) is a master regulator of cell growth and division that responds to a variety of stimuli, including nutrient, energy and growth factors. During the last years, significant experimental results have been discovered to understand how mTOR coordinates and executes its functions [2-6]. It forms two different protein complexes mTORC1 and mTORC2 defined by the proteins to which it is composed and exerting different but related functions. There is a lack of experimental evidence about mTORC2 whereas mTORC1 is a well-known protein complex and its effects as well as its important role on several diseases have increased the interest of scientists to investigate more [2,4,5]. For this reason we express our interest to make an attractor analysis of the biological network compiled from A. Efeyan and D. M. Sabatini [2] in which it is shown the impact of mTOR in cancer through many loops in one pathway. This network is composed of many elements which interact with each other in different ways, where mTOR regulation involves a series of feedback loops triggered by mTORC1/2 components. In this network, beside the elements interacting with each-other, an important attention is given to the inputs and outputs of the system. Inputs such as growth factors, hypoxia, low energy and amino acids affect directly or indirectly mTORC1 and the whole system as a consequence, whereas the outputs of the system such as autophagy, protein synthesis and proliferation and survival are the results we want to get through each the system will affect the cancer cells and the entire organism. Thus, the pathway that the genetic information flows from inputs to outputs and then to all the possible cells outside this network can be understood not only by experimental data but also from numerical simulation supported by a mathematical model designed in this case.

There might be several mathematical models describing biological systems but here we focus on Boolean modeling which is a qualitative representation of a biological system in which the elements are presented by nodes and the interactions between them are presented by links [7-10]. The nodes of the network can take only two possible values determined by 1 (ON/Active) or 0 (OFF/Inactive). The future state of each node is determined by some logic functions applied on current states of the nodes. These logic functions, known as Boolean functions are expressed by logic operators such as AND, OR and NOT. Operator NOT is used when a node is regulated by an inhibitor. In this paper we use synchronous update of the nodes which is a simple and an attractive way to find and analyze the fixed points of the system. We see the dynamical behavior simulated by BooleSim [11] starting from only one initial state. The system is simulated several times starting from different initial state of the nodes and in each case the system reaches different stable states represented by one fixed point, but we repeat the same simulations even after changing Boolean functions (rules) for mTORC1 and TSC1/2 in order to see if the dynamic evolution changes or not. Indeed, when the Boolean functions of these two nodes change the system changes its behavior as well. It is shown that the system starting from an

initial state where all nodes are active (ON) or when only the inputs are active (ON) enters in a cycle limit with length four. In all cases we see that mTORC1, in which we are mainly focused, evolves toward an inactive state and this is a result which requires further analysis in order to understand the effect of this state in the organism. Although this study is limited, we aim to generalize it to other similar cases of study which can lead us to other in-depth analysis.

II. BOOLEAN NETWORK ANALYSIS: A SYNCHRONOUS UPDATE

A. Boolean model

The dynamical analyses of synchronous Boolean networks provided by protein-protein interactions are well known [7-10]. The goal of Boolean models is to find fixed points of a network starting from all initial states of the system which are expressed with binary numbers 0 and 1, related to an inactive and an active state, respectively. Synchronous update, which we use here for our study, is performed by a simultaneous change of all first conditions of the elements composing the system. This means that all nodes change their states, from active (1/ON) to passive (0/OFF) or vice versa, at the same time. The future state of each node is determined by some logic functions applied on current states of the nodes. These logic functions, known as Boolean functions (or Boolean rules) are expressed by logic operators such as AND, OR and NOT and show the way that nodes interact with each other. Operator AND is used when a node is regulated by two (or more) other nodes, both needed in the same time to regulate (activating or inactivating) the target node, while operator OR instead is used when the target node is regulated just by one of the nodes needed for that action. Meanwhile, operator NOT is set when a node is regulated by an inhibitor. Before applying the Boolean rules the network is usually reduced making some corresponding assumptions [7,12] and on the other hand this process doesn't cause any important loss of the genetic information of the original biological system. Furthermore, Boolean models generated by synchronous updates are deterministic, meaning that if the system starts at a given initial condition it always converges to the same state after the same number of time steps [11]. Although this is not a realistic model, we believe that this is a good approach to make a first prediction on how a system could be updated at a point of time (t+1).

B. Boolean Network of mTOR signaling pathway

As mentioned above, before studying the network it is recommended to reduce the original network first, in order to have it as simple as possible to model. This happens usually when one has to deal with a very big network composed of many nodes and edges, because the number of binary states that the system will evolve with time is equal to 2N; where N – represents the number of nodes of the network. In other cases, where the network is not considered a big one, it can remain the same as the original. Considering this, as we study the network given in Fig. 1 we don't reduce it because it is composed of just 13 elements so that it is considered a small network. Thus, we take into account all the elements of the network but it is important to emphasize that four of the elements are considered as inputs and three of them as outputs. This means that only six of the elements are really operating between them, the others serve to send the genetic information inside and outside the network. In such cases, inputs and outputs deserve a big attention because they are considered as entry and exit gates, respectively. The genetic information enters the system and affects it entirely through the input elements such as, in this case, low energy, hypoxia, amino acids and growth factors. After following its path, this genetic information reaches the peripheral elements of the network such as autophagy, protein synthesis and proliferation and survival probably affecting other cells outside this network.

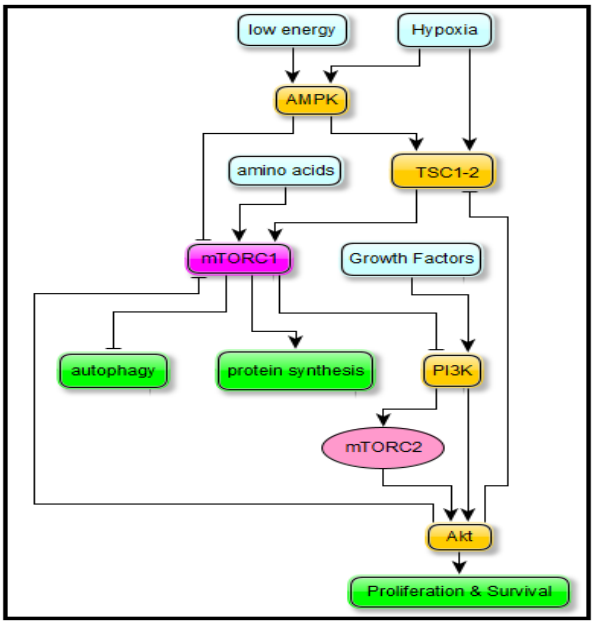


Fig. 1. mTOR signaling pathway. Network edited by yEd Graph Editor [13]. Original network and all the biological information related to it can be found in [2]. Here, nodes are divided by colors depending on the category and the input information they receive.

Considering the network above we write Boolean functions for each element following a simple rule: when two elements acting on another element are independent of each other then the logic operator used in this case will be AND, in other cases, when an element act on another element directly and through another element as well then the logic operator used will be OR. Being more specific, let us take into consideration two elements AMPK and TSC1/2. As we see AMPK is affected by low energy and Hypoxia, which both act independently from each other. Thus, the logic operator used in this case is AND because the future state of AMPK is regulated by both of them at the same time. Differently, we see that TSC1/2 is affected by Hypoxia in two ways, directly and through AMPK. In this way, the logic operator used is OR. Nevertheless, if there is enough information from experimental data then writing Boolean functions is just a straightforward path to follow, but in other cases when data is missing functions are written according to logical assumptions made. The Boolean functions for our network (Fig. 1) are written as it is shown in Table 1 and Table 2. Here, we write sets of different functions because we want to

see the difference it makes in the system evolution if we substitute AND with OR or vice versa. In Table 2 we write only the rules that are different from Table 1.

TABLE I. BOOLEAN FUNCTIONS (RULES) APPLIED FOR 13 ELEMENTS. AS IT IS SHOWN, INPUT ELEMENTS DEPEND ONLY BY THEMSELVES, SO THAT NO LOGIC OPERATOR IS USED FOR THEIR FUTURE STATES. ALTHOUGH IT IS NOT SPECIFIED, THEIR STATES DEPEND ON OTHER EXTERNAL FACTORS THAT ARE OUT OF THE PURPOSE OF THIS ARTICLE.

Nodes	Boolean Rules
low energy **Hypoxia** **amino acids** **Growth Factors**	low energy* = low energy Hypoxia* = Hypoxia amino acids* = amino acids Growth Factors* = Growth Factors
AMPK **PI3K** **Akt**	AMPK* = low energy **AND** Hypoxia PI3K* = Growth Factors **AND** (**NOT** mTORC1) Akt* = PI3K **OR** mTORC2
TSC1-2	TSC1-2* = AMPK **OR** Hypoxia **OR** (**NOT** Akt)
mTORC2	mTORC2* = PI3K
mTORC1	mTORC1* = amino acids **AND** (**NOT** Akt) **AND** ((**NOT** AMPK) **OR** TSC1-2)
autophagy **protein synthesis** **Proliferation & Survival**	autophagy* = **NOT** mTORC1 protein synthesis* = mTORC1 Proliferation & Survival* = Akt

TABLE II. BOOLEAN FUNCTIONS (RULES) APPLIED ONLY FOR TRC1/2 AND MTORC1. THESE RULES ARE DIFFERENT FOR THE ONES WRITTEN IN TABLE 1 BECAUSE HERE IT IS CONSIDERED A DIFFERENT WAY OF STATE EVOLUTION OF THESE TWO ELEMENTS. FOR ALL OTHER ELEMENTS BOOLEAN RULES REMAIN THE SAME AS IN TABLE 1.

Nodes	Boolean Rules
TSC1-2	TSC1-2* = (AMPK **OR** Hypoxia) **AND** (**NOT** Akt)
mTORC1	mTORC1* = amino acids **AND** ((**NOT** Akt) **OR** ((**NOT** AMPK) **OR** TSC1-2)

III. RESULTS AND DISCUSSION

As mentioned before, writing Boolean Functions is very challenging for everyone because in case when the information from experiments is missing one should be careful in order to make the suitable assumptions. There is always the risk that those rules lead us to wrong modeling and results as well but in any case the idea is to give a theoretical description of the dynamical evolution of the system. Once the rules are written, the next step is to apply them in a numerical simulation tool and to get the results aimed. The main purpose is to find the final state that reaches the system which corresponds to the stable state as well. There might be more than one stable state but there is the possibility for a system to have not stable states at all [14]. In some other cases the system enters a cycle limit composed by several states. The number of states inside a cycle limit defines the length of the cycle limit. Once that the system enters in a cycle limit it remains there forever and has no chance to exit it [15]. It is important to emphasize that the final state usually is considered the states generated when all the possible initial states of the system are taken into account. This is possible to be found by using BooleanNet [16,17], but this is out of the focus of this article.

Here we aim to see the dynamical evolution of the system by starting from just one initial state of the system. For this we use simulation results generated by BooleSim [11] and see the time transition graph for different initial states which are considered separately from one other. The reason why we do this way is to know where does the system tend to go, or which stable state reaches when we know exactly what is the initial state of that system.

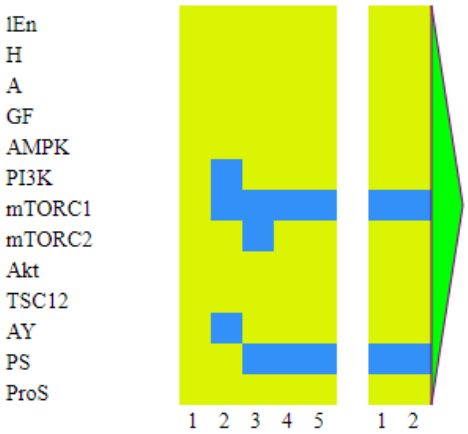


Fig. 2. State transition graph of the system starting from an initial state where all the nodes are active (ON). The system reaches the stable state after four steps. The fixed point, representing the stable state here, expressed in binary is (1111110111101). It seems that the system in this state has all nodes active except mTORC1 and the output Proliferation & Survival (PS) that are inactive.

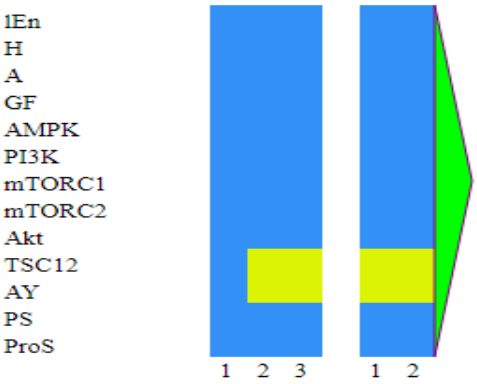
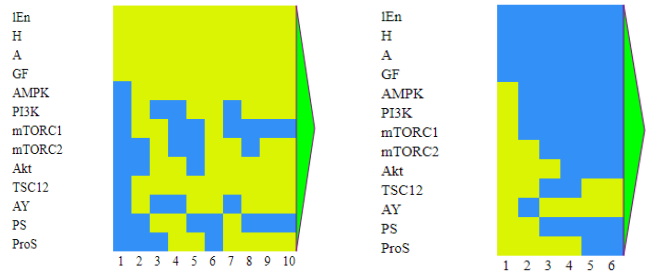
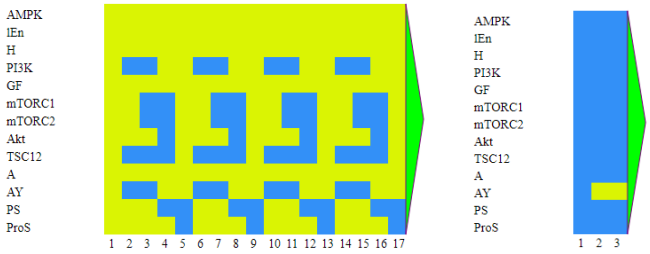


Fig. 3. State transition graph where all nodes in the initial state are inactive. The stable state is reached immediately after 2 steps. The fixed point here expressed in binary is (0000000001100). It seems that the system in the steady state has all nodes inactive except TSC1/2 and AY that are active.



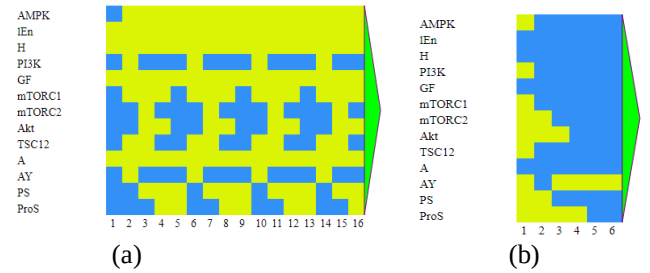
(a) (b)

Fig. 4. (a) State transition graph starting from an initial state when only the inputs are active and all other nodes of the system are inactive. The stable state is obtained after nine steps, where mTORC1 seems to be inactive leading to the output (PS) to be inactive as well. The other nodes of the system remain active. Fixed Point is (1111110111101); (b) State transition graph of the system starting from an initial state when only the input elements are inactive and all other nodes of the system are active. It reaches the stable state after five steps, in which only TSC1/2 remains active affecting also the output node autophagy to be active too. All the other nodes remain inactive. Fixed Point is (0000000001100).



(a) (b)

Fig. 5. (a) State transition graph starting from the initial state where all nodes and inputs and outputs are active. System enters a cycle limit and it remains there forever. This cycle limit has a length four because is composed by four binary states as follow ((1111101110011), (1111100010011), (1111110000101), (1111111111100)); (b) State transition graph starting from the initial state where all nodes and inputs and outputs are inactive. System enters in the stable state immediately after 2 steps. The fixed point is (0000000000100) and it shows that when the system starts from an inactive state of all nodes it remains in the same inactive state except autophagy which turns to be active.



(a) (b)

Fig. 6. (a) State transition graph starting from the initial state where only the inputs are active. System enters a cycle limit and it remains there forever. This cycle limit has a length four because is composed by four binary states as follow ((1111101010011), (111100000011), (1111111001100), (1111101111010); (b) State transition graph starting from the initial state where all nodes and outputs are active whereas only the inputs are inactive. System enters in the stable state after five steps. The fixed point is the binary state (0000000000100) and it shows that when the system starts from an inactive state of all nodes it remains in the same inactive state except autophagy which turns to be active.

As shown above, the system reaches different stable states according to the initial conditions from which it starts evolving. Figures 2, 3 and 4 show simulation of the system related to the Boolean Rules given in Table 1, whereas Figures 5 and 6 are related to the changes in Boolean Rules expressed in Table 2. The stable states in each case refer to the nodes in the following order: (Low Energy, Hypoxia, Amino Acids, Growth Factors, AMPK, PI3K, mTORC1, mTORC2, Akt, TSC1/2, Autophagy, Protein Synthesis, Proliferation & Survival). On the other side, according to BooleSim simulation the state of any node is expressed in two colors: blue and yellow. Corresponding to Boolean Values, 0 and 1, in this simulation app, blue color refers to an inactive state (0) whereas yellow color refers to an active state (1). As we are mainly interested in the evolution of mTORC1 and its effects on other elements we see that despite the initial state of it, mTORC1 tends to enter in an inactive state after some steps. This is a result which we aim to deeper analyze in our future work because the effect that this can cause in the organism might be very interesting for further studies, which can be a collaboration among scientists from different backgrounds.

IV. CONCLUSION

Mammalian target of rapamycin (mTOR) is a master regulator of cell growth and division that responds to a variety of stimuli, including nutrient, energy and growth factors. It is a very important protein kinase that forms two different protein complexes mTORC1 and mTORC2 defined by the proteins to which it is composed and exerting different but related functions. Its important role on several biochemical functions that happen inside the organism makes it very important to study thus we work on mTOR signaling pathway. We use Boolean modeling because it is a suitable method to describe and model a not very well known biological system. By applying Boolean functions following by numerical simulation we find the stable state a specific system reaches after a period of time. Here is used synchronous updating of the nodes in order to see the behavior of the system by updating the nodes in the same time. Simulations with BooleSim help us understand the single steady state that the system reaches starting from one specific initial state. Sometimes, depending on the Boolean rules applied, the system can enter in a cycle limit which is not just a single steady state but it is composed by several states. When a system enters in a cycle limit it remains there forever going around from one state to the following one, continuously. Finally, Boolean modeling is a very good method to approach the reality of a system biology and it gives a better solution than quantitative models such as differential equations, especially when we don't have enough kinetic information of the system and other details which should take into account.

ACKNOWLEDGMENT

Author thanks Dr. Malvina Marku for her time devoted to discussions as well as for her valuable suggestions during the work done.

REFERENCES

[1]. N. Radde, Analyzing fixed points of intracellular regulation networks with interrelated feedback topology, BMC Systems Biology 57, (2012).
[2]. Efeyan, A., D.M.Sabatini, mTOR and cancer: many loops in one pathway, Curr Opin Cell Biol. 22(2): 169-176 (2010).
[3]. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 2002;110:163–175. [PubMed: 12150925].
[4]. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell 2002;10:457–468. [PubMed: 12408816].
[5]. Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA, Sabatini DM. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell 2007;25:903–915. [PubMed: 17386266].
[6]. Tchevkina, E and Komelkov, A. Protein Phosphorylation as a Key Mechanism of mTORC1/2 Signaling Pathways. [ed.] C Huang. Protein Phosphorylation in Human Health. 2012, 1.
[7]. Saadatpour, A., Albert, R., Boolean modeling of biological regulatory networks: A methodology tutorial, Methods 62, 3-12 (2013).
[8]. A.Saadatpour, I. Albert, R. Albert, J. Theor. Biol. 266, 641-656 (2010).
[9]. Wang, R-S., Saadatpour, A., Albert, R., Boolean modeling in system biology: an overview of methodology and applications, Phys. Biol. 9, 055001 (14pp), (2012).
[10]. Helicar, T., et al., Boolean Modeling of Biochemical Networks, 5, 2011, The Open Bioinformatics Journal, pp. 16-25.
[11]. M. Bock, T. Scharp, Ch. Talnikar, E. Klipp, BooleSim: An interactive Boolean Network Simulator, Bioinformatics Advance Access published September 29, 2013.
[12]. Veliz-Kuba, A., Reduction of Boolean network models, J. Theor. Biol. 289, 167-172 (2011).
[13]. https://www.yworks.com/products/yed
[14]. R. Thomas, On the relation between the logical structure of systems and their ability to generate multiple steady states and sustained oscillations, in: J. Della Dora, J. Demongeot, B. Lacolle (Eds.), Numerical Methods in the Study of Critical Phenomena, Springer Verlag, Berlin, 1981, pp. 180–193
[15]. Heidel, J., Maloney, J., Ch, F., J, R., 2003. Finding cycles in synchronous Boolean networks with applications to biochemical systems. Int. J. Bifurcat. Chaos 13, 535–552.
[16]. Albert, I., Thakar, J., Li, S., Zhang, R., Albert, R., Source Code Biol. Med. 3 (2008) 16.
[17]. Kachalo, S., Zhang, R., Sontag, E., Albert, R., DasGupta, B., Bioinformatics 24 (2008) 293–295.