Predicted stimulus/response curves in the mitogen-activated protein kinase cascade

Javier Castillo Uviña
*Facultat de Física, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain.**

Cells, as living units, have the ability to communicate with other living organisms. In this article is explained the different mechanisms to carry out this task. We focus on how the cell receive and process the information. Besides, we will study the MAPK cascade, a process through an external stimulus triggers a series of chemical reactions.
The focus on this cascade remains on the activated kinases production, the robustness and the sensitivity of the kinases to the external stimulus. We characterize the chemical reactions involved in the cascade in order to get the differential equations so we can numerically obtain the concentrations with a Runge-Kutta 4 algorithm.
A graphic is found where the normalized behaviour of the concentration in the steady state are plotted versus the E1 in multiples of the EC50 and we can compare they all have different shapes. The earlier the activated kinase appears in the cascade the sensitive it is, on the other hand as they appear they become more robust to variations in E1.

I. INTRODUCTION

A cell is the smallest unit of life that can replicate independently, and it is the basic structural, functional, and biological unit of all known living organisms. There are two cell types, prokaryotes and eukaryotes. Both types has the transcription-translation system in order to create proteins. For the prokaryote case both processes are carried out inside the cell membrane, so they can be triggered simultaneously. It has a rigid wall and doesn't have organelles or internal structure. On the other hand the eukaryote cells are larger and more complex. A flexible membrane surrounds the cytoplasm and the organelles, so this type of cell has compartments, the most important is the nucleus where the transcription is conducted. Then the mRNA has to leave the nucleus to be transcripted into a protein in the cytosol thank to the ribosomes. Then this proteins are packed and stored in the Golgi body. After this process there exists the possibility for the protein to become the cell communication media.

Cells have four different communication ways [3], as it is represented in FIG 6. The first one is the self communication by the autocrine signaling, it emits signals (hormones or chemicals messenger) so called autocrine agents which will be collected by itself. The second way is called juxtacrine signaling, this type of signaling binds the cells by the contact of the extracellular matrix (ECM) protein with the target cell receptor, is a very short range of interaction (for example in the tissue growth in embryo development). A third communication is also known, the paracrine signaling. This type is a short range communication but the cells are not bound so the emitter cell can affect all its immediate surroundings. The last one is endocrine signaling, here the signals are called hormones and they travel through the blood vessel to the whole body, where are collected by other cells. These signals reception can cause cell division, specialization, shape changes, death...

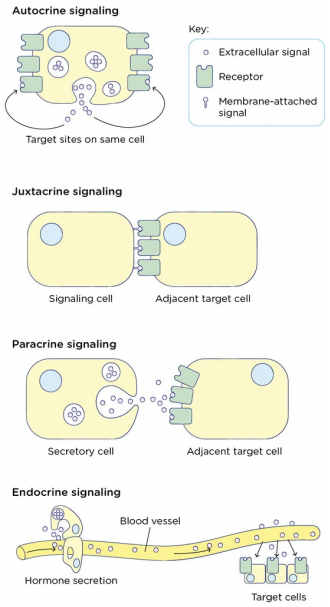

FIG. 1: Four type cell signaling representation, this figure is from [1]

The cell has a whole network of chemical reactions before it responds to a signal. This network can be divided in pathways, which are the simplest set of reactions in order to do a specific action or in order to obtain a specific product (for example the MAPK cascade, a process we will study below). This pathways are a set of motifs, which are the simplest two (or one) chemical specie interaction between them or each other. In a pathway once the cell receives a signal it catalyzes a reaction this is well-known as Michaelis-Menten reaction:

$$E + S \underset{k_b}{\overset{k_f}{\rightleftharpoons}} E \cdot S \underset{k_{cat}}{\rightarrow} E + P \tag{1}$$

Where S is an inactive protein, E is the enzyme and P is the phosphated protein (the new enzyme). Each k_i

*Electronic address: jcastiuv7@alumnes.ub.edu

corresponds to the constant associated to the reaction steps: f forward, b backward and cat catalyzation.

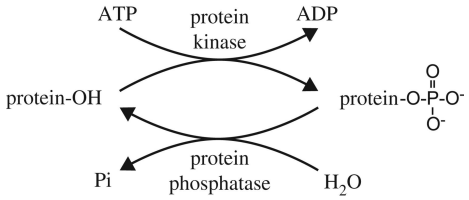

FIG. 2: Here are shown two processes, from left to right is the activation of a protein. On the other hand, from right to left we have the deactivation, this figure is from [2]

Inside the cell there are inactive proteins but they can be activated due to phosphorilation thank to a protein kinase. As we can see in FIG 2 the phosphorylation is the process by which a protein (S) gains a phosphate, from an ATP, to become a kinase (P) thank to the effect of a signal or an enzyme (E) as we can see in 1. There exists the opposite process which is carried out by means of hydrolysis, the deactivation process through an enzyme loses its phosphate and the possibility to act as a catalyze for other reactions, this process is described by a Michaelis-Menten reaction too but S and P switch roles and the catalyzer (E) is a protein phosphatase.
As we saw the signal received in a cell act as a stimulus (E) in a reaction to generate a new enzyme which act as a stimulus for other reaction. The action of consecutive enzyme generation which act as stimulus (catalyzers) for other reactions is known as a cascade. The existence of reaction cascades is needed because they regulate each the involved protein existence. If cascades did not exist, protein concentrations would not saturate at a specific value and there would be no production sensitivity. Proteins would start to be produced uncontrollably until the reactants run out, the complexity on the reactions also lets the cell to make correction on certain processes to get a different global response if something change on the signal it receives.
In this paper we are focusing on the mitogen-activated protein kinase (MAPK) Cascade. The MAP family is important because they are involved in the response to the mitogens, osmotic stress, heat shock and proinflammatory cytokines. They regulate cell functions including proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis [4]. We are about to reproduce the Fig. 2 in the Huang and Ferrell paper [5]. This figure compares how the different kinases steady-state concentration depending on the input stimulus received by the cell (E1) in multiples of he EC50. This EC50 is a treatment we do on the data in order to normalize the saturation curve. The unity of the treated input stimulus has been matched to the 0.5 of each normalized concentration. All the reaction in the cascade has been identified, so we can numerically solve the differential equations for concentration variation related to the chemical reactions.

II. DESCRIPTION OF THE MAPK CASCADE

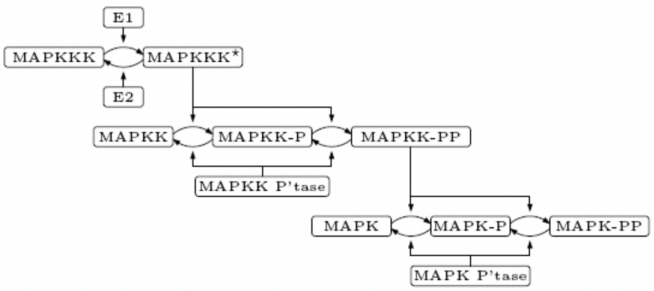

FIG. 3: Here appears the MAPK cascade sketched, including all the chemical species and reactions involved in the process we have studied, this figure is from [6]

On the FIG 3 we can see how an external stimulus E1 act a kinase on the MAPKKK phosphorilation, it is observed an E2 acting as phosphatase for the MAPKKK* hidrolysis. Once the MAPKKK* is generated acts as the kinase that catalyze the MAPKK and MAPKK-P phosphorylation. The MAPKK P'tase is the catalyzer for the reverse reactions. As soon as the MAPKK is doubly phosphated is available to catalyze the MAPK single and double phosphorilation. It exists the phosphatase MAPK P'tase which catalyzes the reverse reactions.
In order to compute numerically the behaviour of the proteins concentration we can stipulate the according Michaelis-Menten reactions. We will show just a sample of the whole pathway. If we start from the top of the cascade until the MAPKKK* generation we obtain the following reactions:

$$\begin{aligned} KKK + E1 &\underset{d_1}{\overset{a_1}{\rightleftharpoons}} KKK \cdot E1 \underset{k_1}{\rightarrow} KKK^* + E1 \\ KKK^* + E2 &\underset{d_2}{\overset{a_2}{\rightleftharpoons}} KKK^* \cdot E2 \underset{k_2}{\rightarrow} KKK + E2 \end{aligned} \tag{2}$$

Each a is the constant associated to the forward reaction, the b is for the backward reaction and the k is for the catalyzed reaction.
It is said above which component act as stimuli (catalyzer) so obtaining the 10 equations that are carried out in the cascade is trivial.
From this 10 reactions we can obtain 18 differential equations and 4 mass conservation rules to compute the 22 chemical specie concentrations involved in this pathway. An example for the confection of this differential equations, for 2 it is obtained:

$$\begin{aligned} \tfrac{d}{dt}\ & [KKK] = -a_1[KKK][E1] + d_1[KKK \cdot E1] \\ & +k_2[KKK^* \cdot E2] \\ \tfrac{d}{dt}\ & [KKK \cdot E1] = a_1[KKK][E1] - (d_1 + k_1)[KKK \cdot E1] \\ \tfrac{d}{dt}\ & [KKK^*] = -a_2[KKK^*][E2] + d_2[KKK^* \cdot E2] \\ & +k_1[KKK \cdot E1] \\ \tfrac{d}{dt}\ & [KKK^* \cdot E2] = a_2[KKK^*][E2] - (d_2 + k_2)[KKK^* \cdot E2] \end{aligned} \tag{3}$$

As it is mentioned we can use the mass conservation in order to know the real time concentration for the $E1$, $E2$, $KKP'tase(E3)$, $KP'tase(E4)$. The example for the reactions in (2) is:

$$\begin{aligned} [E1]_t &= [E1]_{t=0} - [KKK \cdot E1]_t \\ [E2]_t &= [E2]_{t=0} - [KKK^* \cdot E2]_t \end{aligned} \tag{4}$$

III. SIMULATION METHOD

It has been used a Runge-Kutta 4 in order to solve the differential equations numerically. All the values used for the simulation to run have been $a_i = 1/150$ and $d_i = k_i = 1$ for all the reactions. The stimulus values have been $[E1]_{t=0} = 1nM$, $[E2]_{t=0} = [E3]_{t=0} = 0,3nM$ and $[E4]_{t=0} = 120nM$. The kinases initial concentrations were set up at $[MAPKKK]_{t=0} = 3nM$ and $[MAPKK]_{t=0} = [MAPK]_{t=0} = 1200nM$. The cascade has been simulated for a t = 1000s with a integration step of h = 0.1s.
Besides, it is also studied how the steady state varies with the value of the initial concentration of E1 while maintaining the values for the other components mentioned.

IV. RESULTS

Once the algorithm and the conditions are set up we obtain different graphics for the time evolution of the concentration.

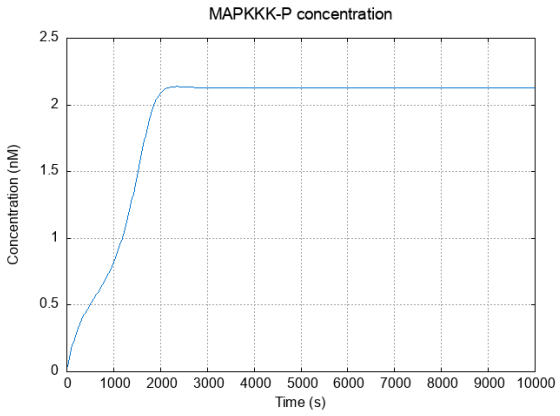

FIG. 4: For a long enough time (t=1000 s) the steady state is reached and we obtain this time development for the MAPKKK-P saturating at 2.125 nM for a E1=1nM.

In order to compare the behaviour of the three components and its robustness and sensitivity to the initial [E1], we normalized the steady state concentrations versus the initial [E1] and it has been plotted in terms of EC50, as it is said in the introduction.

The behaviour is showed in FIG 7. As we can see we obtain an immediate response for the MAPKKK-P generation. On the other two activated kinases there exist a threshold where there is no generation, what's more,

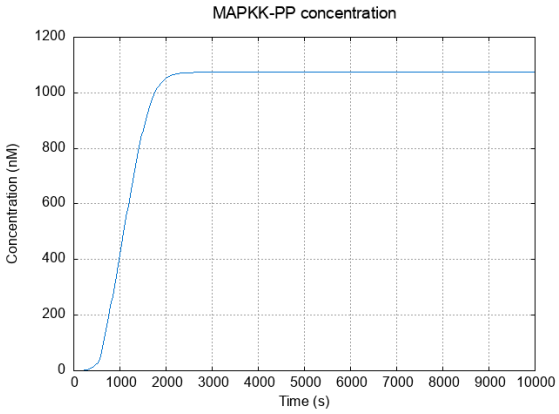

FIG. 5: In this case is plotted the MAPKK-PP time evolution concentration and we can see it also reaches the steady state, in this at 1073.612 nM for E1 = 1nM.

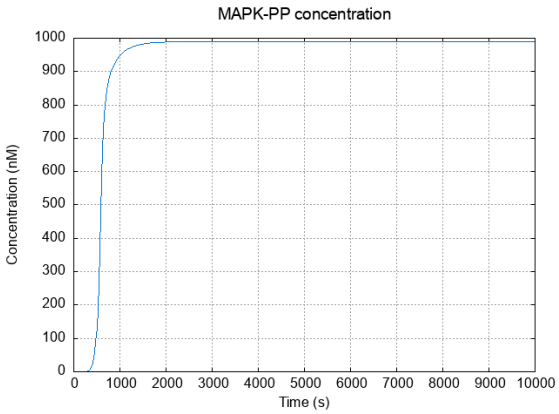

FIG. 6: It is shown the MAPK-PP evolution in time, For this chemical specie the stationary value reached is 988.912 nM for an initial E1 = 1 nM.

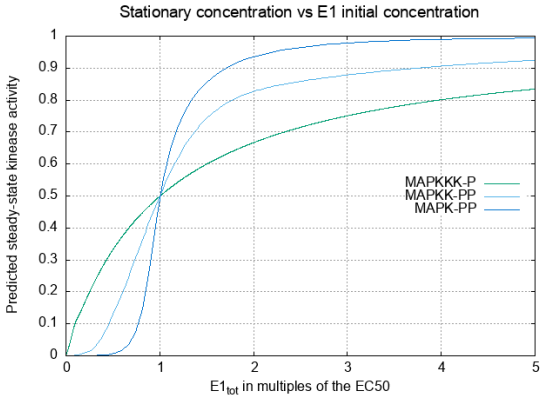

FIG. 7: The graphic shows how the different activated kinases behave at the steady state for the different initial concentrations for the E1. It is expressed in multiples of EC50 so we can see compare all three.

the later the reaction is in the cascade, the higher is the threshold. Once the threshold is exceeded, the maximum steady-state concentration is reached more rapidly the later the chemical compound appears in the cascade. Because the steady state concentration of MAPKK-P varies constantly against E1 fluctuations, we can say that it is the most sensitive of the species we are comparing. On the other hand MAPK-PP is the most robust, there is a threshold to activate it where there is no generation, and after the threshold the maximum is reached quickly so it does not vary even though the initial concentration of E1 increases. The mean chemical species (MAPKKK-PP) is a robust state for small amounts of initial E1, but sensitive for fluctuations in large initial E1.

V. CONCLUSIONS

The cascades are an essential way to control the response given by a cell to a certain stimulus. Cells have the opportunity to react or correct the response if there exists changes in the signal received. The final activated kinasa response becomes sensitive to changes in the initial stimulus but that means we obtain a binary response. Exist a threshold within the response is minimum and beyond the response is maximum.

Acknowledgments

I would like to thank the effort of my professor Dr. Ramon Reigada for teaching me about cell signaling and having a great vision of how this process works. Of course I would like to thank my partner in this project Mireia Olives, it has been easy to learn about this subject with her.

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