Mathematical Model to Study the Influence of Dyrk1A on Spine Maturation

Santiago Acosta Mendoza
Universitat Politecnica de Catalunya, BarcelonaTech

Linus Manubens and Mara Dierssen
Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology
(Supervisors)

(Dated: June 1, 2019)

Down syndrome (DS) is the most frequent genetic cause of cognitive disability. DS abnormal characteristics are caused by the disruptive effect of specific trisomic genes. One of these genes, Dyrk1A (Dual specifity tyrosine phosphorylation-regulated kinase) plays a significant role in signalling pathways regulating the cell proliferation and brain development. Changes in Dyrk1A gene dosage, regardless the direction of the change, are sufficient to produce neuronal alterations observed in DS patients. Specifically, it has been proved that Dyrk1A inhibits NWASP (Neural Wiskott-Aldrich syndrome protein), a protein that stimulates the formation of dendritic spines. Despite this, only one model for the mechanism by which Dyrk1A inactivates this signalling protein has been proposed. Here, we demonstrate that this model cannot effectively describe the non-linear relation of Dyrk1A in NWASP activation. We propose a new model that could effectively account for such mon-monotic behaviour.

I. INTRODUCTION

Down Syndrome is the most common genetic form of intellectual disability. This order results from the presence of an extra copy or major portion of human chromosome 21. Cognitive dysfunction in these patients is correlated with reduced neuronal branching and complexity, along with fewer spines with abnormal shape [1]. Spines are small actin-rich protrusions from neuronal dendrites that form the post-synaptic part of most excitatory synapses and are major sites of information processing and storage in the brain.

DS characteristics are caused by the disruptive effect of specific trisomic genes. It has been reported [2] that overexpression of dual-specifity tyrosine phosphorylation regulated kinase 1A, DYRK1A, a gene located in chromosome 21, is sufficient to produce the spines alteration observed in DS patients. Actually, neurons in Dyrk1A+/- mouse, a model lacking one copy of Dyrk1A, and also in TgDyrk1A, overexpressing 1.5 times the kinase, were less branched and less spinous than those of wild-type. These results indicate that Dyrk1A is affecting cellular pathways involved in dendritic spines development and plasticity in a way that dosage changes lead to similar alteration regardless the direction of this change.

In this regard, it has been shown by Partk *et al.* [3]. that DYRK1A interacts with Neural Wiskott-Aldrich protein (N-WASP). N-WASP regulates the formation of dendritic spines and synapses in neurons by activating the protein complex Arp2/3, being this complex essential for spine maturation [4]. In fact, DYRK1A negatively regulates the activity of N-WASP, inducing changes in its structure that lead to the inactivation of this protein. Hence, by inactivating N-WASP, DYRK1a is able to inhibit dendritic spine formation and maturation.

Although a model for this mechanism has been proposed by Park *et al.*, it cannot effectively account for the non-monotonic behaviour observed in the influence of the dosage of DYRK1A with respect to the complexity of spines (Fig.1). We address this issue, first by demonstrating analytically why such model is not able to characterise this kind of response. Then, we present a model that could explain such behaviour. Our model points that there is an optimum amount of DYRK1A that maximises the amount of active N-WASP.

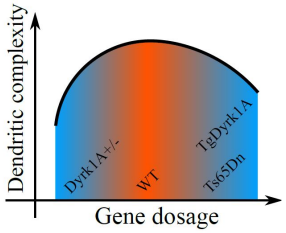

FIG. 1. **Schematic diagram on how Dyrk1A influences dendritic properties** Neurons in Dyrk1A+/- mouse, a model lacking one copy of Dyrk1A, and also in TgDyrk1A and Ts65Dn, overexpressing 1.5 times the kinase, were less less spinous than those of with no alteration

II. MATERIALS AND METHODS

A. DYRK1A regulates N-WASP through multi-site phosphorylation

Protein phosphorylation is a reversible mechanism by which the addition of a phosphate group to a protein is able to change its conformation when interacting with other molecules. It is an important cellular regulatory mechanism as many enzymes and receptors are activated/deactivated by phosphorylation and dephospho-

rylation events, by means of kinases and phosphatases [5]. DYRK1A directly phosphorylates N-WASP and promotes an intramolecular interaction that blocks the interaction of N-WASP with the protein complex Arp2/3, responsible, among other proteins, in dendritic spine formation and maturation. Thus, N-WASP can be in two states: aunto-inhibited 'closed state' and the active state called 'open state'.

DYRK1A phosphorylates N-WASP at three different molecular places called Thr196, Thr202 and Thr259. The sole phosphorylation of Thr202 and Thr259 alone doesn't change its conformation to the close state, while the phosphorylation of both of them is sufficient to induce it. The phosphorylation of Thr196 alone is enough to induce the closed state. When all three sites are phosphorylated the protein is in a more stable closed state. This mechanism by which multiple phosphorylations of the same substrate can occur is called multi-site phosphorylation. The underlying chemical modifications mechanism are typically assume to be either processive or distributive. Processive multi-phosphorylation occurs when a single kinase phosphorylates multiple places at the same time, while for distributive phosphorylation to happen one kinase for each place is required [6].

In his study, Park *et al.* proves that phosphorylation occurs distributively such that one phosphate group at a time is added. However, since there is no available data for the dephosphorylation mechanism, he proposes that it occurs processively so a single phosphatase dephosphorylates all three phosphate groups at the same time.

We will consider an ordered mechanism (in front of a random mechanism), where the first site to be phosphorylated is always the one being able to induce the close state, Thr196. We won't make a distinction between Thr202 and Thr256, given that their phosphorylation has the same behavior. Hence, we will have four states, one open state, where no phosphate group is attached to N-WASP and three closed states, each with one up to three phosphates. DYRK1A and the unknown phosphatase directly regulate the transitions. An schematic representation of such model is shown in Fig.2.

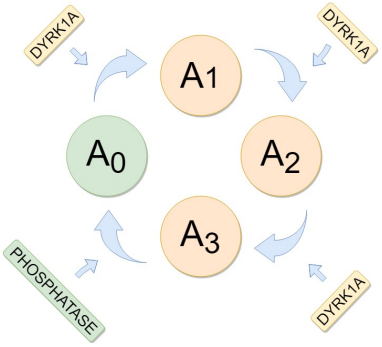

FIG. 2. **Proposed model** N-WASP is represented by A_i where $i = 0, 1, 2, 3$ are the phosphorylated places. We have two possible states, open (depicted in green, when no phosphate is added) and closed (depicted in orange, where at least one phosphate is added). The transition between the different species is regulated by DYRK1A and a phosphatase.

B. Enzyme kinetics

Enzymatic reactions are two-step reactions in which the action of one molecule, the enzyme (E), results in a substrate (S) being converted into a product (P) via a reversible reaction that produces a complex (ES). The enzyme itself is not consumed. This kind of reaction was first described by Michaelis and Menten [8] as the reaction sequence:

$$\mathrm{E + S \underset{k_-}{\overset{k^+}{\rightleftharpoons}} ES \xrightarrow{r} E + P}$$

In the model proposed, the enzymes are the kinase DYRK1A (P) and the unknown phosphatase (P). Each N-WASP species is denoted by B_i, where $i = 0, 1, 2, 3$ are the phosphates added. The rate by which they binding and unbinding rates are denoted by k_+,k_- and r. Remembering that phosphorylation is considered as distributive and dephosphorylation as processive, we will have the following set of reactions:

$$\mathrm{A + K \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AK \xrightarrow{k_3} K + A_1 \underset{k_5}{\overset{k_4}{\rightleftharpoons}} A_1K \xrightarrow{k_6} K + A_2 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} A_2K \xrightarrow{k_9} A_3 + K}$$

$$\mathrm{A_3 + P \underset{k_{11}}{\overset{k_{10}}{\rightleftharpoons}} A_3P \xrightarrow{k_{12}} A_2P \xrightarrow{k_{13}} A_1P \xrightarrow{k_{14}} A + P}$$

III. RESULTS AND DISCUSSION

A. Distributive phosphorylation and processive dephosphorylation cannot account for a non-monotonic behaviour

Taking into account the set of reactions given in Sec. II B with the respective rate constants, we will have the system of Ordinary Differential Equations given in Fig.3.

If active N-WASP, modelled as species A, shall have a non-monotonic relation with respect to DYRK1A K, then we will have that $d[A]/dK_{tot} = 0$, with K_{tot} being the total concentration of kinase. As the concentration of the species is conserved, we will have K_{tot}, A_{tot} and P_{tot} are given by:

$$\begin{aligned} A_{tot} &= [A] + [AK] + [A_1K] + [A_2K] + [A_1] \\ &\quad + [A_2] + [A_3] + [A_3P] + [A_2P] + [A_1P] \end{aligned} \tag{1}$$

$$K_{tot} = [K] + [AK] + [A_1K] + [A_2K] \tag{2}$$

$$P_{tot} = [P] + [A_3P] + [A_2P] + [A_1P] \tag{3}$$

At steady-state conditions we will have for all species that $d[S]/dt = 0$ with $[S]$ being the concentration of a

$$
\begin{aligned}
\frac{d[A]}{dt} &= -k_1[A][K] + k_2[AK] + k_{14}[A_1P] \\
\frac{d[K]}{dt} &= -k_1[A][K] + (k_2+k_3)[AK] - k_4[A_1][K] \\
&\quad + (k_5+k_6)[A_1K] - k_7[A_2K] + (k_8+k_9)[A_2K] \\
\frac{d[P]}{dt} &= -k_{10}[A_3][P] + k_{11}[A_3P] + k_{14}[A_1P] \\
\frac{d[A_1]}{dt} &= k_3[AK] - k_4[A_1][K] + k_5[A_1K] \\
\frac{d[A_2]}{dt} &= k_6[A_1K] - k_7[A_2][K] + k_8[A_2K] \\
\frac{d[A_3]}{dt} &= k_9[A_2K] - k_{10}[A_3][P] + k_{11}[A_3P] \\
\frac{d[AK]}{dt} &= k_1[A][K] - (k_2+k_3)[AK] \\
\frac{d[A_1K]}{dt} &= k_4[A_1][K] - (k_5+k_6)[A_1K] \\
\frac{d[A_2K]}{dt} &= k_7[A_2][K] - (k_8+k_9)[A_2K] \\
\frac{d[A_3P]}{dt} &= k_{10}[A_3][P] - (k_{11}+k_{12})[A_3P] \\
\frac{d[A_2P]}{dt} &= k_{12}[A_3P] - k_{13}[A_2P] \\
\frac{d[A_1P]}{dt} &= k_{13}[A_2P] - k_{14}[A_1P]
\end{aligned}
$$

FIG. 3. **System of Ordinary Differential Equations describing the system**

given species. If we set steady-state conditions to the equations given in Fig.3 we will see that (derivation in Supplemental Information):

$$
\begin{aligned}
&[AK] \propto [A][K] && [A_1] \propto [A] \\
&[A_1K] \propto [A_1][K] \propto [A][K] && [A_3P] \propto [A_3][P] \propto [A][K] \\
&[A_2K] \propto [A_2][K] \propto [A][K] && [A_2P] \propto [A_3][P] \propto [A][K] \\
&[A_3P] \propto [A_3][P] \propto [A][K] && [A_1P] \propto [A_3][P] \propto [A][K] \\
&[A_2] \propto [A] && [A_3] \propto [A][K]/[P]
\end{aligned}
$$

Grouping this relations in Eqs. 1, 2, 3 and solving for $[A]$, $[K]$ and $[P]$:

$$[K] = \frac{K_{tot}}{1 + a[A]} \tag{4}$$

$$[P] = P_{tot} - b[A][K] \tag{5}$$

$$[A] = \frac{A_{tot}}{c + d[K] + e\frac{[K]}{[P]}} \tag{6}$$

Where a, b, c, d, e are the sum of the proportional constants. Given that the concentrations cannot be negative, the proportional constants must be positive and hence also the sum of them.

We see from the equation for $[K]$ that if $d[A]/dK_{tot} = 0$ holds true then:

$$\frac{d[K]}{dK_{tot}} = 1 + a[A] \qquad \frac{d[P]}{dK_{tot}} = -b[A](1 + a[A]) \tag{7}$$

Then, differentiation the equation for $[A]$ with respect to K_{tot}:

$$\frac{d[A]}{dK_{tot}} = -\frac{A_{tot}\frac{d(c+d[K]+e\frac{[K]}{[P]})}{dK_{tot}}}{(c + d[K] + e\frac{[K]}{[P]})^2} \tag{8}$$

So, if $d[A]/dK_{tot} = 0$ is to be true then the numerator of Eq. 8 has to be zero:

$$\frac{d(c + d[K] + e\frac{[K]}{[P]})}{dK_{tot}} = a(1 + a[A]) + e\frac{(1 + a[A])([P] + b[K][A])}{[P]^2} \tag{9}$$

Which can never be zero given that all the quantities: constants and concentrations are positive by definition. So we arrive at the conclusion that such model cannot explain the non-monotonic behaviour observed in the mouse models.

B. Double phosphorylation as a possible distributive mechanism

So far we have demonstrated that a pure distributive phosphorylation and processive dephosphorylation alone cannot account for a non-monotonic behaviour. So we can either modify the phosphorylation cycle or the dephosphorylation cycle. It has been observed in other reactants that phosphorylation can occur in two consecutive steps, so that when the kinase phosphorylates one site the energy required to phosphorylate the other is much lesser. Taking into account that Thr259 phosphorylation alone is enough to deactivate N-WASP but Thr202 and Thr196 phosphorylation have to be both phosphorylated in order to deactivate N-WASP, we propose that double phosphorylation occurs in the second step, so that:

$$
\begin{aligned}
\mathrm{A + K} &\underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{AK} \xrightarrow{\mathrm{k_3}} \mathrm{A_1 + K} \\
\mathrm{A_1 + 2\,K} &\underset{\mathrm{k_5}}{\overset{\mathrm{k_4}}{\rightleftharpoons}} \mathrm{A_1K} \xrightarrow{\mathrm{k_6}} \mathrm{A_3 + 2\,K} \\
\mathrm{A_3 + P} &\underset{\mathrm{k_8}}{\overset{\mathrm{k_7}}{\rightleftharpoons}} \mathrm{A_3P} \xrightarrow{\mathrm{k_9}} \mathrm{A + P}
\end{aligned}
$$

We note that this is not a processive step, given that two kinases are required to double-phosphorylate the substrate. As dephosphorylation is processive and we have already shown that each phospatase complex is proportional to the previous one, we can consider the dephosphorylation as being in one single step.

It is usual to assume that the system is almost in equilibrium with respect to the complexes ($[AK]$, $[A_1K]$ and $[A_3P]$), being its rates very fast, so the system spends little time in this state [7]. This approximation, however is only valid when the concentration of substrates is larger

than that of enzymes, which seems legit given that N-WASP is involved in far more processes than DYRK1A [9]. Then, the enzymatic reaction introduced in Sec. II B can be approximated as:

$$\mathrm{E} + \mathrm{S} \xrightarrow{\mathrm{k}} \mathrm{E} + \mathrm{P}$$

with $k = \frac{rk_+}{k_- + r}$. Thus we can approximate our set of reactions as:

$$\begin{aligned} \mathrm{A} + \mathrm{K} &\xrightarrow{\mathrm{k_1}} \mathrm{A_1} + \mathrm{K} \\ \mathrm{A_1} + 2\,\mathrm{K} &\xrightarrow{\mathrm{k_2}} \mathrm{A_3} + 2\,\mathrm{K} \\ \mathrm{A_3} + \mathrm{P} &\xrightarrow{\mathrm{k_3}} \mathrm{A} + \mathrm{P} \end{aligned}$$

Now we will have the following set of ODEs:

$$\frac{d[A]}{dt} = -k_1[A][K] + k_3[A_3][P] \tag{10}$$

$$\frac{d[A_1]}{dt} = k_1[A][K] - k_2[A_1][K]^2 \tag{11}$$

$$\frac{d[A_3]}{dt} = k_2[A_1][K]^2 - k_3[A_3][P] \tag{12}$$

At steady state, the fraction of active N-WASP will be given by:

$$\frac{[A]}{[A_{tot}]} = \frac{k_2k_3[K][P]}{k_2k_3[K][P] + k_1k_3[P] + k_2k_1[K]^2} \tag{13}$$

C. System Behaviour

Given that there is no experimental evidence for the value of the constants, we shall analytically see the conditions that fix the set of constants k_1, k_2, k_3. First, we will assume $[P] = 1$ and set the optimum level of DYRK1A as $[K] = 2$. Deriving Eq.13 with respect to $[K]$ and setting it to zero when $[K] = 2$ together with setting the concentration of active N-WASP as 0.5 when the latter condition is imposed gives us the relation of constants given in Eq.14:

$$k_2 = \frac{k_3}{4} \qquad\qquad k_2 = k_1 \tag{14}$$

With this assumptions now we are able to see how the system behaves as the concentration of $[K]$ and $[P]$ varies (Fig.4 & Fig.5).

IV. CONCLUSIONS

We study the mechanism by which DYRK1A is able to deactivate N-WASP. In particular, we show that this mechanism cannot arise com a purely distributive phosphorylation and a processive dephosphorylation. We propose a mechanism by which the first phosphorylation occurs individually and the latter two simultaneously. Using realistic approximations, we then show that this mechanism produces a non-monotonic behaviour, such as the one observed in the phenotype of Dyrk1A+/- mouse, a model lacking one copy of Dyrk1A, and also in TgDyrk1A, over-expressing the kinase, thus indicating that there is an optimum amount of DYRK1A that maximises active N-WASP. Here, we have just provided a formalism but experimental data is required in order to validate the model.

Further steps should be in the direction of obtaining this experimental evidence. Also, additional pathways to include spine formation should be modelled in order to study the interplay between DYRK1A and spine morphology.

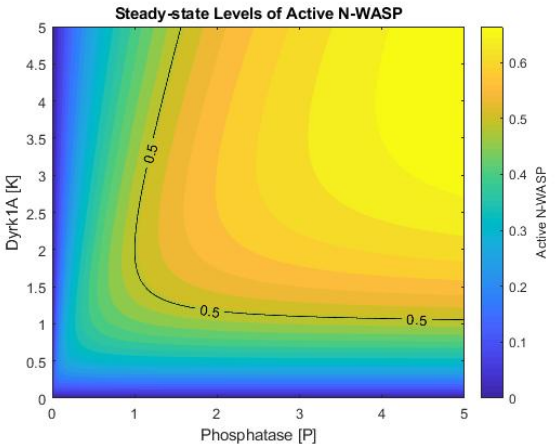

FIG. 4. **Contour plot of active N-WASP with respect to DYRK1A and Phosphatase concentrations** The black line is the contour line of the surface where active N-WASP is equal to 0.5.

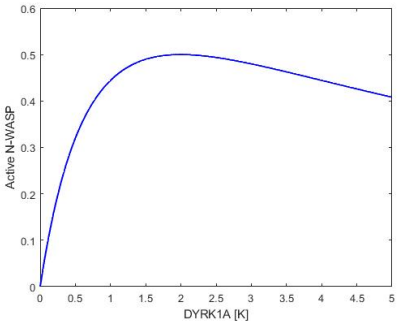

FIG. 5. **Active N-WASP as function of DYRK1A dosage** Setting $[P] = 1$ we are able so see that a non-monotonic behaviour. A maximum active N-WASP concentration is achieved when the concentration of DYRK1A is $[K] = 2$

[1] Dierssen, M. (2012). Down syndrome: the brain in trisomic mode. *Nature Reviews Neuroscience*, 13(12), 844858

[2] Martinez de Lagran, M., Benavides-Piccione, R., Ballesteros-Yanez, I., Calvo, M., Morales, M., Fillat, C., Dierssen, M. (2012). Dyrk1A Influences Neuronal Morphogenesis Through Regulation of Cytoskeletal Dynamics in Mammalian Cortical Neurons. *Cerebral Cortex*, 22(12), 28672877.

[3] Park, J., Sung, J. Y., Park, J., Song, W.-J., Chang, S., Chung, K. C. (2012). Dyrk1A negatively regulates the actin cytoskeleton through threonine phosphorylation of N-WASP. *Journal of Cell Science*, 125(1), 6780.

[4] Spence, E. F., Kanak, D. J., Carlson, B. R., Soderling, S. H. (2016). The Arp2/3 Complex Is Essential for Distinct Stages of Spine Synapse Maturation, Including Synapse Unsilencing. *Journal of Neuroscience*, 36(37), 96969709.

[5] Ardito, F., Giuliani, M., Perrone, D., Troiano, G., Muzio, L. L. (2017). The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review). *International Journal of Molecular Medicine*, 40(2), 271280

[6] Suwanmajo, T., Krishnan, J. (2015). Mixed mechanisms of multi-site phosphorylation. *Journal of The Royal Society Interface*, 12(107).

[7] Blas Echebarria (2019). Notes on biophysics. *Edicions UPC*.

[8] Michaelis, L., and Menten, M. L. (1913) Die Kinetik der Invertinwirkung. *Biochem. Z.* 49, 333-369.

[9] Takenawa, T., Suetsugu, S. (2007). The WASPWAVE protein network: connecting the membrane to the cytoskeleton. *Nature Reviews Molecular Cell Biology*, 8(1), 3748