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Stochastic modulation evidences a transitory EGF-Ras-ERK MAPK activity induced by PRMT5

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Abstract.

The extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) pathway involves a three-step cascade of kinases that transduce signals and promote processes such as cell growth, development, and apoptosis. An aberrant response of this pathway is related to the proliferation of cell diseases and tumors. By using simulation modeling, we document that the protein arginine methyltransferase 5 (PRMT5) modulates the MAPK pathway and thus avoids an aberrant behavior. PRMT5 methylates the Raf kinase, reducing its catalytic activity and thereby, reducing the activation of ERK in time and amplitude.

Two minimal computational models of the epidermal growth factor (EGF)-Ras-ERK MAPK pathway influenced by PRMT5 were proposed: a first model in which PRMT5 is activated by EGF and a second one in which PRMT5 is stimulated by the cascade response. The reported results show that PRMT5 reduces the time duration and the expression of the activated ERK in both cases, but only in the first model PRMT5 limits the EGF range that generates an ERK activation. Based on our data, we propose the protein PRMT5 as a regulatory factor to develop strategies to fight against an excessive activity of the MAPK pathway, which could be of use in chronic diseases and cancer.

Keywords.

Cell response modulation, cancer, MAPK pathway, EGF-Ras-ERK signaling route, PRMT5, methylation, computational model.

1. Introduction.

Biological processes occurring in the microscale, or even in the nanoscale, play key roles in cells and living organisms. A great amount of those processes drive different behaviors' patterns, which can be altered by local shifts induced by individual molecules. In such a scenario, cell signaling pathways are responsible for the transmembrane signal transduction. Those pathways facilitate the sensing, integration and processing of extracellular information through the plasma membrane, generating robust high-fidelity responses [1, 2].

A common motif of such signaling pathways is represented by a cascade structure of a sequential activation of three mitogen-activated protein kinases (MAPKKK, MAPKK and MAPK) that act as messengers, driving extracellular signals to the inner of the cell [3-7]. The active MAPK is delivered to the cytosol, reaching the nucleus of the cell, for further activation of transcription factors, collaborating in this way in the regulation of the cell activity [8-10]. In detail, the extracellular signal-regulated kinase 1/2 MAPK (ERK1/2 MAPK) pathway features K-Ras as the G-protein, Raf-1 as the MAPKKK, MEK1/2 as the MAPKK, and ERK1/2 as the MAPK [10, 11] (Suppl. Fig. S1). The response of such a signaling pathway to the epidermal growth factor (EGF) is the expression of the double phosphorylated ERK1/2 ($_{pp}$ERK1/2) (Suppl. Fig. S2).

The ERK MAPK pathway generates different response behaviors, including grading, oscillation, bistability (switching between alternate states), and ultrasensitivity, and such behaviors can be influenced by the type and activity of the cell. Thus, an all-or-none switch-like response of the ERK MAPK route was reported in Xenopus oocytes, whilst a graded response of this pathway was observed in mammalian cells. Such data suggests the existence of mechanisms different from phosphorylation that promote the high-fidelity in ERK signals, such as positive and negative feedback loops, cross-talk between signaling pathways, and associations between protein partners [2, 3, 12-15]. In addition, different types of stimuli (such as EGF and NGF) induce both graded ERK phosphorylation and switch-like ERK nuclar translocation. Thereby, the conversion of graded ERK phosphorylation into a switch-like ERK nuclear translocation may be a property in growth factor-induced ERK signaling [15]. The sensitivity of the ERK MAPK signaling pathway is a key factor in the cell fate decision, but also the pattern of its temporal response is involved [16, 17].

In detail, in the EGF-Ras-ERK1/2 MAPK pathway, a maximum response sustained in time can lead to undesirable cell behaviors [18-20]. In biological microenvironments, many molecules interact with each other in the framework of this signaling pathway, establishing sophisticated dynamics that regulate specific response patterns, affecting different cell processes such as migration, proliferation, and differentiation. However, this fragile network can be easily disturbed by small and local alterations, resulting in aberrant responses related to the proliferation of cell diseases and tumors.

Hence, specific molecular motifs are capable of modifying the dynamics of some kinases, modulating the time response of the MAPK pathway, altering the overall cell response [21-23]. This is the case of protein arginine methyltransferases (PRMTs), a family of intracellular enzymes localized in mammalian cells [24]. PRMTs play important roles in regulating signal transduction, promoted by different growth factors affecting distinct cell types [23, 25, 26]. The protein PRMT5, one of the nine components (from PRMT1 to PRMT9) of the PRMTs family in humans, achieves great shifts in the pattern of the EGF-Ras-ERK1/2 MAPK response, avoiding inappropriate cell behaviors such as the tumorigenesis. However, literature so far does not recognize PRMT5 as a tumor suppressor gene, but a key factor for regulating the activity of several tumor suppressors in distinct cancer cell lines. Indeed, PRMT5 is an epigenetic enzyme involved in different physiological processes, including transcriptional regulation, RNA metabolism and cell cycle regulation [27]. Increasing evidence suggests that PRMT5 plays a complex role in oncogenesis, controlling the expression of genes implicated in both tumor promotion and suppression. Mainly, cellular context, tissue dependence and the localization within the cell determine what function the protein PRMT5 performs [28-30]. Thereby, a large number of studies give evidences of PRMT5 overexpression in different cancer types and its role as a potential oncoprotein involved in tumorigenesis by promoting the proliferation, differentiation, invasion, and migration of cancer cells, being able of silencing its target tumor suppressor genes [31-35]. Nevertheless, other studies had showed opposite effects of PRMT5, being an important regulatory factor of cancer progression, inhibiting tumor cell proliferation, migration and invasion. Therefore, oncogenic properties of PRMT5 trigger shifts required for reprogramming normal cell growth to malignant cell activity and metastasis. But PRMT5 is also needed for normal development and differentiation. Then, reported data suggests that PRMT5 may become a potential diagnostic biomarker and a therapeutic target for various cancers, but no references recognize PRMT5 as a cancer suppressor gene.

The action of PRMT5 over the EGF-Ras_ERK1/2 MAPK cascade consists of the methylation of the active Raf kinase, reducing its catalytic activity and, thereby, limiting the $_{pp}$ERK1/2 expression. The activated Raf methylation through PRMT5 is comparable to the DNA methylation. Both processes are driven by the methylation of arginine residues. PRMTs catalyze the methylation of

arginine residues of a diverse group of proteins that can be monomethylated, symmetrically dimethylated, or asymmetrically dimethylated at the terminal nitrogen atoms of the guanidinium side chain of the arginine residue [36]. One clear example is the methylation on histone arginine residues, which can promote DNA replication, DNA damage response, and the activation or repression of gene transcription. In detail, PRMT5 catalyzes monomethylation and symmetric dimethylation of arginine residues in histones H2A and H4 at residue R3, and histone H3 at residues R2 and R8 [37]. Through histone methylation, PRMT5 is involved in different cellular processes, including transcriptional regulation, germ cell development and gene expression regulation [36, 38-40]. In addition, PRMTs promote one of the most extensive protein methylation modification in mammalian cells: the modification of arginine chain side guanidine groups (NH_2). Arginine is an amino acid that its guanidine group contains five potential hydrogen bond donors, which are able of interacting with hydrogen bond acceptors [23]. In detail, the arginine side chain presents two terminal guanidine groups that can be subjected methylation (Suppl. Fig. S3). And PRMTs remove one methyl group from the donor molecule S-adenosyl-L-methionine (AdoMet) and they transfer it to the terminal nitrogen of the guanidine group of an arginine residue in the target protein [41]. Although some research has reported that PRMT-mediates methylation of proteins impacts on stability, the mechanisms responsible may differ from case to case. Thus, it has been reported that PRMT5 up-regulated the protein levels of the anti-apoptotic protein CFLARL by decreasing the ubiquitination and increasing its protein level [42]. Alternatively, it has been reported that arginine methylation modulates autophagic degradation of PGC granules in C. elegans [43]. In this connection, the mechanisms by which PRMT5 impacts on CRaf and BRaf stability remain unknown. What is known so far is that PRMT5 triggers a symmetric dimethylation of arginine residues $BRaf^{R671}$ and $CRaf^{R563}$ within the -Gly-Arg-Gly- (-GRG-) motif upon growth factor stimulation, which is highly conserved in all the Raf isoforms [44, 45] and where two terminal guanidine groups (NH_2) susceptible to being methylated are found (see Suppl. Fig. S4 with the crystal structure of CRaf and its highlighted Arg^{563} residue). In fact, the Raf kinase domain consists of an amino-terminal lobe (N-lobe) and a carboxy-terminal lobe (C-lobe) that are connected through a flexible hinge region [46]. The C-lobe is comprised primarily of α-helices and contains the activation segment. In this C-terminal catalytic domain, the arginine residue where the protein PRMT5 binds to further methylate the Raf kinase is found. The addition of methyl groups do not change the charge of proteins but mask polar arginines, removing amino hydrogens and further blocking the ability to form hydrogen bonds, promoting inhibition of some binding partners. Such blocked hydrogen-bonding groups become more hydrophobic, directly altering protein-protein, protein-DNA, and protein-RNA interactions [47-49]. Therefore, the methylation of arginine residues in proteins can modulate their binding capabilities, decreasing the stability of Raf proteins

and targeting them to the proteasome [50-52]. Otherwise, inhibition of PRMT5 increased the activity of Raf and enhanced the phosphorylation of CRaf^{Ser621}, which may be required to avoid proteasome-mediated CRaf degradation [53]. In this way, the PRMT5 has the potential to reduce an important source of cellular dysfunction and disease: the persistence in time of a maximum inappropriate level of the EGF-Ras-ERK1/2 MAPK output response [18-20, 40]. This regulatory effect of PRMT5 on the $_{pp}$ERK1/2 output response is similar to the role of the mitogen-activated protein (MAP) kinase phosphatases (MKPs or DUSPs), which dephosphorylate MAPK, reducing its activity and switching from a sustained response to a more transitory one [54, 55]. One example is MKP-3 (also known as DUSP6), an ERK target gene that forms autoregulatory loops in response to sustained $_{pp}$ERK signals, attenuating its activity and yielding to a more transient response [56, 57]. Furthermore, there are evidences of decreased MKP expression in malignant disease, suggesting that these phosphatases play key roles in cancer regulation [54]. Thereby, the loss, deletion or inhibition of MKPs is related to a sustained activation of MAPKs [54, 58, 59].

The purpose of the present work is to develop a model approach of the EGF-Ras-ERK1/2 MAPK signaling pathway. We want to shed light on the influence of the PRMT5 on the regulation and tuning of the pathway response through a methylation process of the Raf-1, which modulates the time pattern of the $_{pp}$ERK1/2 and triggers the final cell fate decision [45]. Unfortunately, there is a lack of knowledge on the misfit of the PRMT5 protein to the signaling cascade that we aim to investigate. It is experimentally observed that the PRMT5 activity increases upon activation of the signaling cascade [45, 60]. But, it is not possible to differentiate if the PRMT5 activation is promoted either by the same input stimulus or by the pathway output response. To explore this challenge, the time output responses of two different configurations of the EGF-Ras-ERK1/2 MAPK cascade influenced by the activity of PRMT5 are analyzed: 1) a model with the PRMT5 activity regulated by EGF and 2) another one with the PRMT5 activity regulated by $_{pp}$ERK1/2. In addition, the reported results are aimed to clarify if $_{pp}$ERK1/2 response patterns belonging to experimental research can be modulated by the PRMT5 protein and, moreover, to identify if its activity is controlled either by the input stimulus or by the output response of this signaling pathway. Here we provide computational evidence for the regulatory role of the PRMT5 on the ERK-1/2 MAPK activity by limiting its amplitude and duration, as well as the range of EGF stimulus able of generating MAPK response. Thereby, to identify activators of this methyltransferase should be of interest as drug targets for MAPK-related indications.

PRMT5 methylates Ras effectors BRaf and CRaf [45], which in turn bind to HRas [61], KRas [45, 62-64] and NRas [65-67]. So, from now on, to simplify the nomenclature, any of the three isoforms of Ras will be named simply as Ras, the two isoforms of Raf that are methylated by PRMT5 (BRaf and CRaf or Raf-1) will be named as Raf, MEK1/2 as MEK, and ERK1/2 as ERK.

2. Methods.

2.1. Models

The proposed model for the present study (called from now as reference model) corresponds to a simplified version of the ERK MAPK signaling pathway defined by *Sturm OE et al* [3] (Fig. 1A). Briefly, it consists of the three-level kinase Raf-MEK-ERK MAPK cascade with a regulatory negative feedback loop from the output response ($_{pp}$ERK) to the Raf activation process (Fig. 1B), which is defined in a comparable way to the system of the two different feedback loops described in the model of *Sturm OE et al* [3], but starting at the level of MAPKKK instead of at the upper level of the signaling pathway (the obtained responses of both models can be compared in Suppl. Fig. S5).

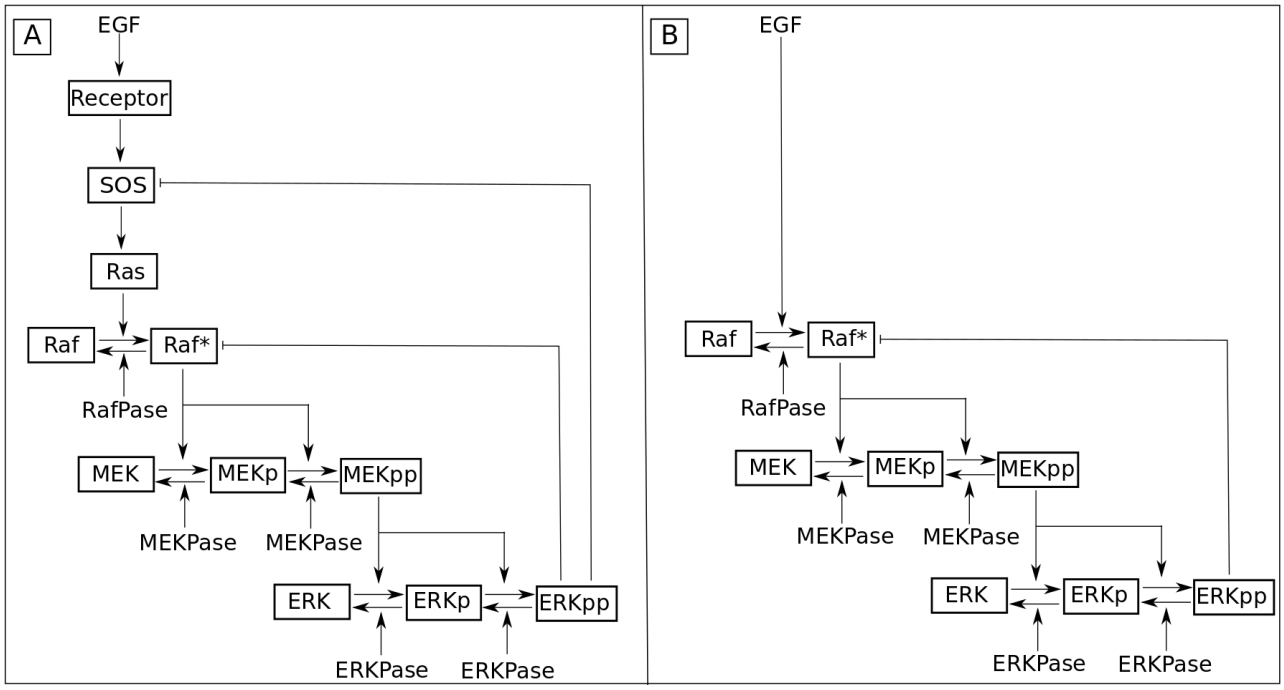

FIGURE 1: **Schematic representations of the Ras-ERK MAPK signaling pathway.**
(A) Schematic representation of the Ras-ERK MAPK signaling pathway with the three levels of kinases (Raf, MEK and ERK) proposed by *Sturm OE et al* [3], presenting two regulatory feedbacks from the output $_{pp}$ERK to SOS and Raf. **(B)** Schematic representation of the reference model, formed by the Ras-ERK MAPK signaling pathway with the three levels of kinases (Raf, MEK and ERK) and one negative feedback from the output $_{pp}$ERK to Raf, being equivalent to both negative feedbacks of the previous model (Fig. 1A). In both representations, the corresponding kinases phosphatases of Raf, MEK and ERK, as well as the input stimulus EGF are defined.

The implemented feedback loop from $_{pp}$ERK promotes a hyperphosphorylation of multiple serine sites of Raf-1 (S29, S289, S296, S301, and S642) after mitogen stimulation, in a manner

similar to Raf-1 inactivation, but rendering an inactive and desensitized Raf-1 that presents reduced affinity for Ras-GTP and low response to subsequnt activation processes [68]. This feedback loop is defined within the whole pathway by a Michaelis-Menten process through next reactions (Eq. 1):

$$Raf^{*} + {}_{pp}ERK \leftrightarrow Raf^{*}-{}_{pp}ERK \rightarrow Raf + {}_{pp}ERK \qquad \text{(Eq. 1)}$$

where Raf^{*} is the activated state of the Raf isoform. The reaction rates of this regulatory mechanism have been selected following those found in the literature [3, 68-71], where a strong negative feedback loop from ERK to Raf was reported.

In addition to this regulatory mechanism, we have considered that the phosphatases of the kinases Raf, MEK, and ERK (RafPase, MEKPase and ERKPase) deactivate each corresponding kinase in all its possible activation states.

The experimental values of the concentrations of the components and the cascade parameters correspond to those described by *Schoeberl B et al* [72], in which *Sturm OE et al* [3] model is mainly based from a parametric point of view [Suppl. Tables I and II]. Regarding the reaction rates, they were defined from other experimental scientific publications that reported real measures for the parameters or, failing that, suggested experimental estimations or biochemical quantifications. As to the cascade's components, their concentrations were expressed by the number of signaling proteins per cell, which were determined by biochemical quantification through western blotting.

Then, the proposed reference model considered in our study (Schema B of Figure 1) only presents two possible variable parameters: the concentration of the input stimuli *EGF* and the forward reaction constant of the negative feedback loop from ${}_{pp}ERK$ to Raf, defined as a_{11} and related to the impact of the negative feedback loop. Therefore, to avoid uncertainties in the validation of the proposed computational model for the EGF-Ras-ERK MAPK signaling pathway (Schema B of Figure 1), simulations varying these two parameters were carried out (Suppl. Fig. S6). Then, the model was simulated considering:

a) different values of *EGF* Є [2414, $1e^{6}$] molecules/cell (no EGF was not considered since no ERK signal is triggered)

b) three different values of a_{11}: 0 , $1e^{-5}$ and $1e^{-6}$ $(molecules/cell)^{-1}\cdot s^{-1}$

Regarding the input stimulus EGF, shifts in its values triggered shifts in the ${}_{pp}ERK$ response, regardless the value of the parameter a_{11}. The same way, changes in the reaction constant a_{11}

promoted modifications in the amplitude of the $_{pp}$ERK signal. Despite these variability in the results, regardless the conditions of EGF and a_{11}, all $_{pp}$ERK responses are ranged between a minimum and a maximum response, without presenting uncontrolled values. Moreover, notice that for very high values of EGF, the $_{pp}$ERK response was practically unchanged because a saturation state was reached (Suppl. Fig. S6).

In detail, simulations (Suppl. Fig. S5) reveal that the reference model proposed for the present study approaches to the response of the Ras-ERK MAPK model defined by *Sturm OE et al* [3], to a greater or lesser extent depending on the value of the parameter a_{11}. Indeed, these results suggest that the effect of the negative feedback loop of the *Sturm OE et al*'s model [3] is comprised between the results reported by the reference model for both values of a_{11}: $1e^{-6}$ (molecules/cell)$^{-1}$ $\cdot s^{-1}$ and $1e^{-5}$ (molecules/cell)$^{-1}$ $\cdot s^{-1}$. Thereby, considering the evidence of a strong negative feedback loop from ERK to Raf [3, 68-71], the reference model with a_{11} = $1e^{-5}$ (molecules/cell)$^{-1}$ $\cdot s^{-1}$ is selected to develop the simulations of the present study.

Furthermore, reported results indicated that the response patterns of our proposed model, with or without the $_{pp}$ERK negative feedback loop, fit qualitatively with the published results of *Sturm OE et al* [3] (Suppl. Fig. S5), even when this last model presents more cascade levels, more feedback loops and, thereby, more reactions (Schema A of Figure 1). In fact, these results revealed that the $_{pp}$ERK negative feedback acts as an inhibitor of the activated Raf, reducing the stepness and the ultrasensitivity of the output signal of the Ras-ERK MAPK cascade [3], increasing its negative effect on the output signal as its forward reaction (a_{11}) increases, since greater extension of activated Raf was inhibited.

All these data suggest that the pattern of the ERK response upon EGF stimulation obtained with the proposed minimum model of the EGF-Ras-ERK MAPK cascade was qualitatively similar to those of published studies that pointed to the sustained activation of ERK as responsible for specific cellular processes [73-76]. Then, the three-step of the signaling cascade and the $_{pp}$ERK-induced negative feedback loop on Raf may be considered as the main players responsible of the sustained $_{pp}$ERK response of this signaling route. Therefore, we suggest that our proposed minimum model of the EGF-Ras-ERK MAPK cascade is valid to perform computational studies of this signaling route.

To this reference model, the protein PRMT5 proposed by *Andreu-Pérez P et al* [45] has been added as an inhibitor of the activated Raf (Raf*), degrading its expression through the next reaction velocity (Eq. 2):

$$a_{PRMT5} = [PRMT5]\cdot[\,Raf^*]\cdot k_{PRMT5}\cdot \alpha_{PRMT5} \qquad \text{(Eq.2)}$$

where [PRMT5] and [Raf*] are the concentrations of the PRMT5 and the activated Raf respectively,

and k_{PRMT5} is considered to be the catalytic rate constant of the reaction that promotes the degradation of activated Raf through the methylation process exerted by the protein PRMT5, defining the rate-limiting step of this conversion process. This process is described as a biomolecular reaction involving te collision of these two molecules (activated Raf and PRMT5), and the rate of the reaction depends on the product of the concentrations of both involved reactants, triggering a second-order reaction. This reaction rate is not necessary to be constant, and there are different factors that can affect it, such as the reactant concentrations, cell type and context, pressure, temperature, chemical nature of reacting substances, physical state of reactants and their dispersion, surface areas of reactants, among others. And such variability is aimed to be reflected in the parameter k_{PRMT5}, which can take different values and defines the strength of the PRMT5 action. Regarding the protein PRMT5, its concentration was considered as the same of the Raf, in order to establish a relation 1:1 and ensure that no Raf could not be methylated. In addition, the PRMT5 activity is considered to be regulated either by the input stimulus EGF or by the current $_{pp}$ERK output. And the parameter α_{PRMT5} has been defined to expressed such dependence. Therefore, two different models have been described: the model A (Fig. 2, red dashed lines) where the activity of the PRMT5 depends positively on EGF, defining $\alpha_{PRMT5(EGF)}$ as the ratio between the applied EGF (EGF_t) and the maximum considered one (EGF_{max}); and the model B (Fig. 2, blue dashed lines) where the activity of the PRMT5 depends positively on the $_{pp}$ERK output, with $\alpha_{PRMT5(ppERK)}$ defined as the ratio of the current $_{pp}$ERK concentration ($_{pp}ERK(t)$) over its maximum possible value, corresponding to the total amount of the initial ERK ($_{pp}ERK_{max}$). Then, the effective constant rate of the Raf^* degradation exerted by PRMT5 is defined as $k_{PRMT5} \cdot \alpha_{PRMT5}$.

The reference model consists of 33 reactions and the activity of the PRMT5 involves one more reaction. These reactions are described in Suppl. Table III.

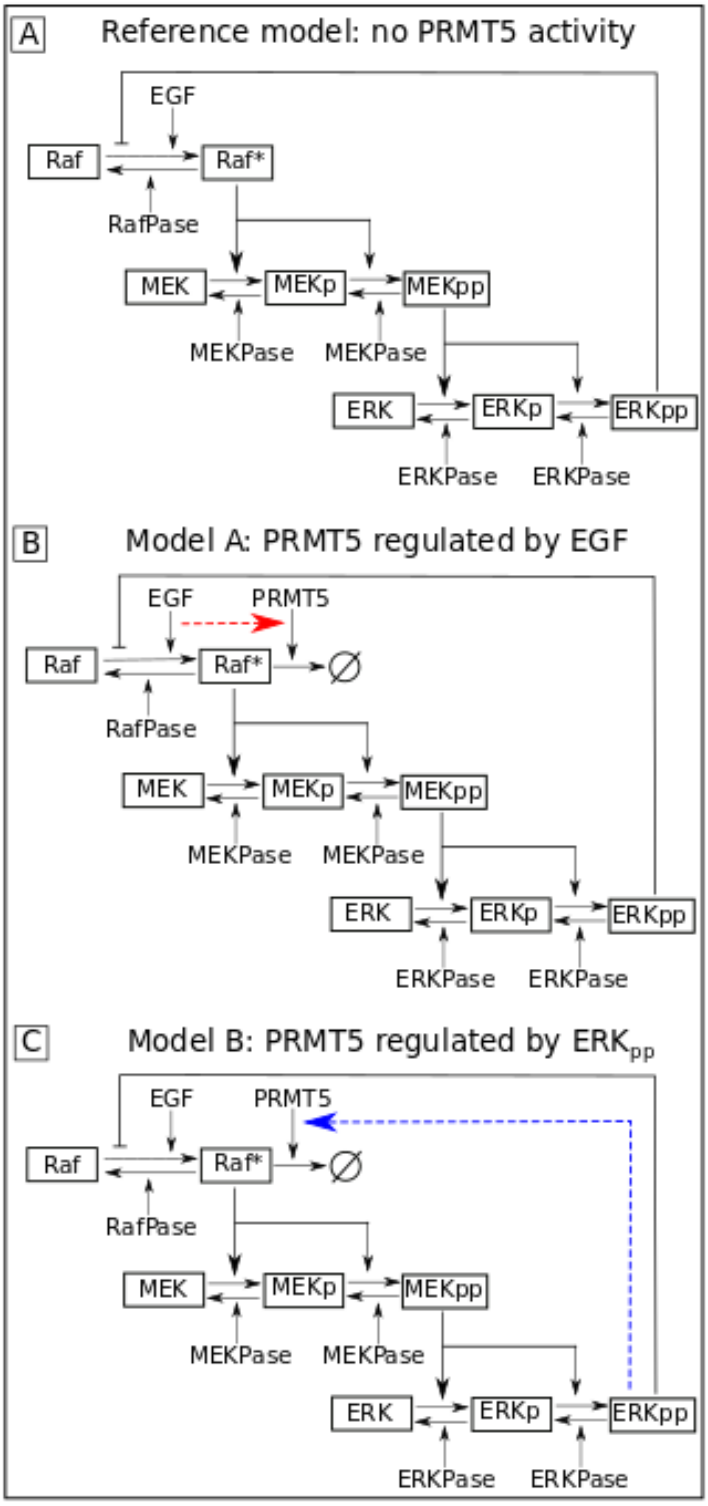

FIGURE 2 (color version required for printing): **Schematic representation of the Ras-ERK MAPK signaling pathway for each considered models.**

(A) Reference model: schematic view of the Ras-ERK MAPK signaling pathway with the three levels of kinases (Raf, MEK and ERK) and the $_{pp}$ERK negative feedback. No PRMT5 activity is present. **(B)** Model A: reference model presenting a regulatory mechanism of the $_{pp}$ERK response through the Raf* degradation process caused by the PRMT5 controlled by the EGF (red dashed line). **(C)** Model B: reference model presenting a regulatory mechanism of the $_{pp}$ERK response through the Raf* methylation exerted by the PRMT5 controlled by the $_{pp}$ERK (blue dashed line). In these two last models, the protein PRMT5 acts as inhibitor of the active Raf (Raf*).

2.2. Simulations

All the model simulations have been developed with a stochastic approach similar to Gillespie's Stochastic Simulation Algorithm (SSA), treating the cascade system as a group of stochastic events [77-79]. The different signaling events have been defined as Poissonian processes with a frequency characterized by the corresponding reaction rates. And all the models have been implemented in the Fortran programming language (Force 2.0 + GNU Fortran 77).

The concentration of the input stimulus EGF and the parameter k_{PRMT5} that regulates the Raf^* degradation activity exerted by the PRMT5 were the only parameters that were changed in the study. The values of the rest of the components concentrations and reaction rates belonging to the considered models have been fixed (summarized in Suppl. Tables I and II, respectively). Thus, with these two variable parameters is possible to regulate the dynamics of the EGF-Ras-ERK MAPK response, acting on the first level of the cascade.

In short, the input stimulus EGF has been gradually increased from 0 to 10^6 molecules, presenting a wide range of values that allows us to define accurately the response behavior of the signaling cascade. There is a common use in developing molecular simulations defining the actors of the computational model with its molar concentration (M = mol/L of dissolution). This option is valid for a deterministic model since it is based on the concentrations of the involved species. However, a stochastic model presents a factor representing the number of molecules of the species, an aspect that gives a higher resolution of the simulations at low species' concentrations [80]. Furthermore, if units of species are expressed in M (mol/L), the simulations become dependent on a factor that is not relevant in the present study, the amount of dissolution (liters), which is a more important factor in experimental studies. Thereby, in the computational models used in the present study, all species (including kinases, phosphatases, and input stimuli) are expressed in molecules rather than M (mol/L).

Three different values of k_{PRMT5} have been selected to modulate the effect of the PRMT5 in the proposed signaling models A and B: 0.0001, 0.0002, and 0.0006 (cell/molecules)$\cdot s^{-1}$. In this way, the effect of the strength of the Raf^* methylation process on the $_{pp}$ERK expression can be analysed. Such values have been selected presenting a similar magnitude order to the other constant rates involved in the first step of the cascade, where the PRMT5 exerts its action (since no experimental evidences of such reaction rate have been reported so far).

The reference model was simulated for enough time to ensure that the system reaches a steady-state (about 2000 seconds). However, the models A and B were simulated until the $_{pp}$ERK response was extinguished.

2.3. Analysis

To characterize the response of the EGF-Ras-ERK MAPK cascade, the time responses ($_{pp}$ERK vs. time), and the maxima amplitudes of the $_{pp}$ERK response as a function of EGF ($_{pp}ERK_{max}$ vs. EGF) were analyzed for the three models (reference model, model A and model B). However, the $_{pp}$ERK response areas and the $_{pp}$ERK time durations as a function of EGF were plotted for both models A and B, since their output responses expired in time. Alternatively for the reference model, the steady-state $_{pp}$ERK response depending on EGF was represented, due to that its responses are not finite in time. To clarify, the response area was defined for the present research as the integration of the $_{pp}$ERK amplitude along the time at which the output signal is maintained over the threshold of the 10% of its maximum value, for each input stimulus. This consideration is related with two different topics: i) the requirement of a minimum $_{pp}$ERK level to consider a functional cell activation [81-83] and ii) the consideration that the stimulus/response curve of a Michaelis-Menten enzyme, such as the case of the $_{pp}$ERK response, is hyperbolic and, thereby, its sensitivity is measured with the Hill coefficient, which quantifies the fold change in the input stimuli (EGF) required to drive the response ($_{pp}$ERK) from 10% activation to 90% activation of its maximum value [7, 84].

3. **Results.**

Time evolutions of the EGF-Ras-ERK MAPK cascade responses ($_{pp}$ERK vs time) have been analysed either in the presence or absence of PRMT5, in order to highlight its role in the regulation of the signaling cascade output. The main obtained evidences are that the protein PRMT5 allows to modulate the cascade response reducing its time duration and amplitude, as well as limiting the range of input stimuli able of generating an output response of the pathway.

3.1. MAPK activity in the absence of PRMT5 (reference model)

In the reference model, the response reaches a steady-state for any input stimulus value (Fig. 3A and Suppl. Fig. S8A).

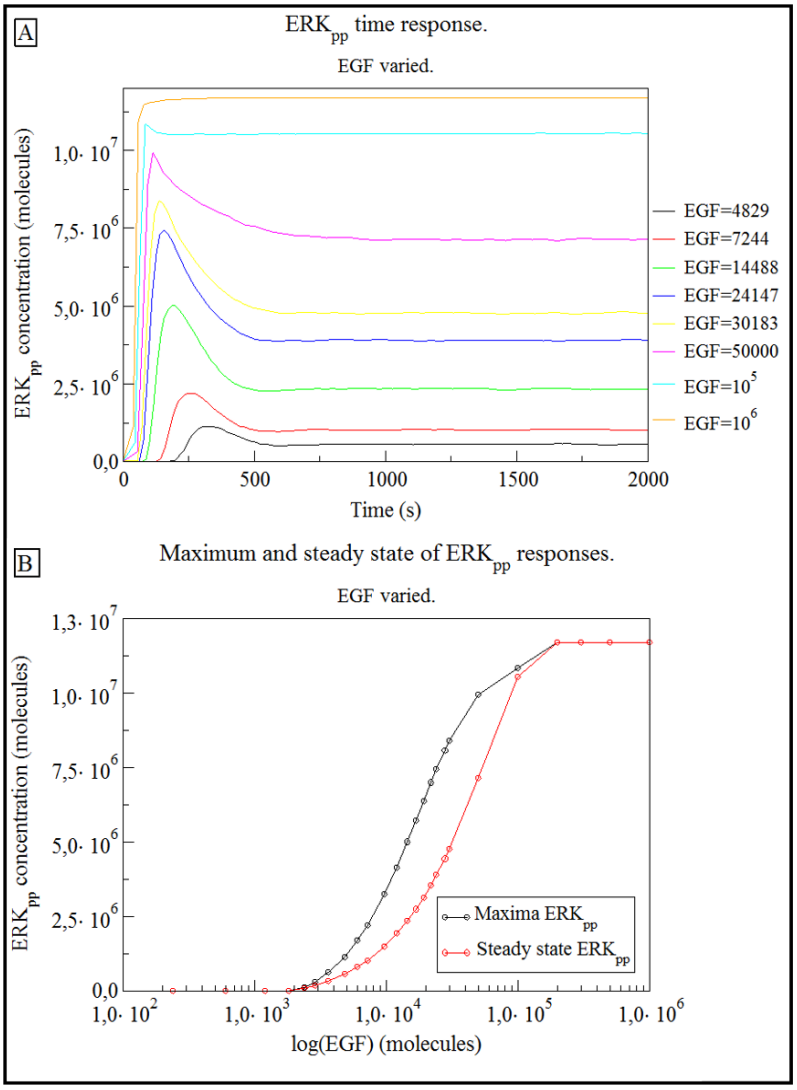

FIGURE 3 (color version required for printing): **Time responses, maxima amplitudes and steady state of the ppERK response in the reference model.**

(A) ppERK time responses of the reference model. Each colour line represents the time evolution of the ppERK concentration for each different EGF value. **(B)** The maximum concentrations (black line) and the steady state values (red line) of the ppERK responses of the reference model as functions of EGF (represented in a semilogarithmic scale). For both figures, EGF is defined from 0 to 10^6 molecules (the considered values of EGF to simulate the output response are summarized in the graph legend). The components concentrations and the reaction rates used in simulations are described in Suppl. Tables I and II.

The model does not present any mechanism of degradation of any of the cascade's components, which could result in the extinction of the output response (there are only deactivation processes of kinases species). For each different EGF value, the ppERK expression starts to increase until reaching a maximum value, promoted by Raf*, which is responsible for activating the downstream cascade levels of MEK and ERK. Such Raf* activation depends directly on the input stimulus (Fig.

3A). Next, when the $_{pp}$ERK response reaches enough high level, the deactivation of Raf* induced by the negative feedback takes relevance over the Raf activation process exerted by EGF, causing, in turn, a decrease in the production of $_{pp}$ERK. In addition, the phosphatases within the MAPK cascade (RafPase, MEKPase, and ERKPase) deactivate the corresponding kinases Raf, MEK, and ERK in all its possible activation states, inducing a slowdown in the production of $_{pp}$ERK. Thereby, the output response decreases except for very high EGF values where saturated levels of Raf* are reached (Fig. 3A). Finally, the $_{pp}$ERK response reaches a steady-state, which is a result from the balance between the Raf activation induced by EGF, the Raf* deactivation process performed by the $_{pp}$ERK negative feedback, and the deactivation of the active species of Raf, MEK, and ERK through the corresponding phosphatases (Fig. 3A and Suppl. Fig. S8A).

To expand the analysis of the reference model, both maximum and steady-state $_{pp}$ERK responses as functions of EGF have been analysed (Fig. 3B). Such responses increase with the stimulus, until reaching maxima values for EGF $\geq$ EGF_{Th}, where EGF_{Th} is defined as the threshold value of EGF required to obtain the same maximum output signal, that is when the maximum value of $_{pp}$ERK is equal to its steady-state value (Fig. 3B). However, these metrics are different for the values of EGF < EGF_{Th}, and such differences are due to the role of the $_{pp}$ERK negative feedback on the deactivation of Raf*.

3.2. MAPK activity in presence of PRMT5

The protein PRMT5 switches the steady-state of the EGF-Ras-ERK MAPK output response into a time-limited pattern, through a methylation process that promotes the degradation of the Raf*, limiting the further activation of its downstream substrates of the cascade (MEK and ERK) [45], avoiding an uncontrolled activation of the signaling pathway.

One aspect to keep in mind is the different effects on the $_{pp}$ERK response caused by the PRMT5 activity depending on its regulation, either by the input stimulus (model A) or by the cascade response (model B).

Case A: PRMT5 activity regulated by EGF (model A)

In the model A, for a fixed EGF value, the time response of the Ras-ERK MAPK cascade becomes time-limited, describing a normal or Gaussian pattern (Fig. 4 and Suppl. Fig. S8B).

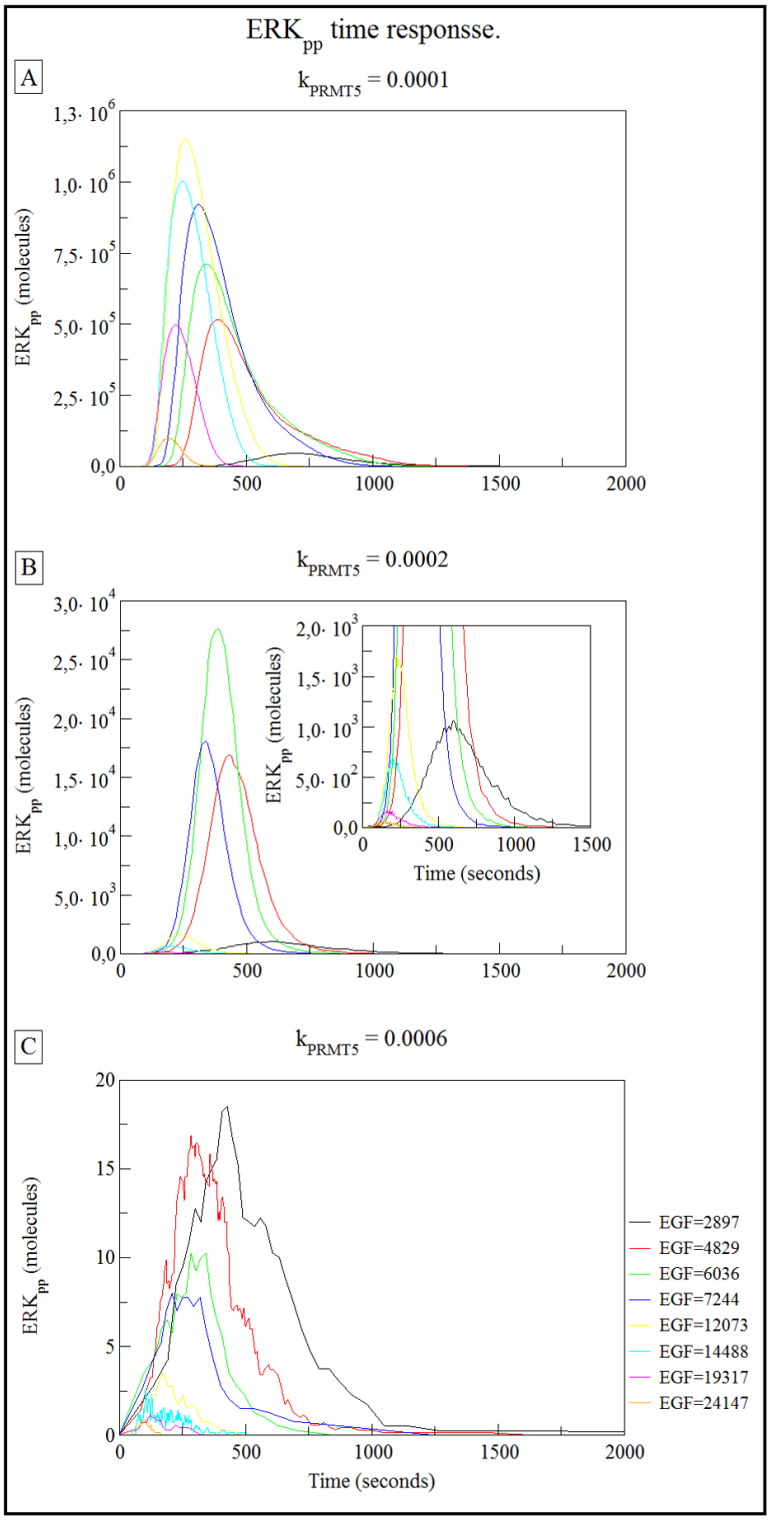

FIGURE 4 (color version required for printing): **$_{pp}$ERK time responses of the model A for different k_{PRMT5} values.** Time responses of $_{pp}$ERK of the model A for different values of k_{PRMT5}: **graph A** for k_{PRMT5} = 0.0001 (cell/molecules)·s^{-1}, **graph B** for k_{PRMT5} = 0.0002 (cell/molecules)·s^{-1} and **graph C** for k_{PRMT5} = 0.0006 (cell/molecules)·s^{-1}. Each colour line represents the Ras-ERK MAPK cascade time response for each different EGF value. EGF is defined from 0 to 10^6 molecules. The considered values of EGF to simulate the output response are summarized in the graph legend. The components concentrations and the reaction rates used in simulations are described in Suppl. Tables I and II.

At initial times, the output response presents weak values since EGF requires some time to activate Raf. Next, the Raf^* expression increases with time and, thereby, the $_{pp}$ERK time response dramatically increases until reaching a maximum value ($_{pp}ERK_{max}$). After that, the response decreases to extinction due to the degradation of Raf^* exerted by the PRMT5 methylation. This process becomes relevant just when there is enough Raf^* expression to compete with the Raf activation process. To this Raf^* degradation process, the actions of, on one hand, the $_{pp}$ERK negative feedback and, on the other hand, the phosphatases (RafPase, MEKPase, and ERKPase) act as complementary mechanisms for the output response regulation.

The simulations reveal that there is a dependence correlation between the values of the $_{pp}$ERK response and the values of the parameter k_{PRMT5}, the higher the amplitude attenuation and the time limitation, the higher of the k_{PRMT5} values (Fig. 4, black and green results from Suppl. Fig. S7, and Suppl. Fig. S8B). The reason is, as well, the higher k_{PRMT5}, the faster the Raf^* degradation. Therefore, there is a lower expression of Raf^* capable of activating its substrate MEK, which in turn activates ERK. Consequently, both the amplitude of the $_{pp}$ERK response (compare Fig. 4A, 4B, and 4C) and the time wherein the $_{pp}$ERK response is non zero decrease (Fig. 4, black and green results from Suppl. Fig. S7, and Suppl. Fig. S8B).

Furthermore, both the time duration and the amplitude of the response vary with the values of EGF (Fig. 4 and Appendix B Suppl. Fig. S8B) because of both the Raf activation process and the activity of the PRMT5 depend directly on the input stimulus.

The PRMT5 acts as a tuner: the higher k_{PRMT5}, the lower range of EGF capable of generating enough relevant $_{pp}$ERK response, since higher EGF expression is required to maintain a similar level of Raf^* (Fig. 4). Simulations reveal that the maxima amplitudes of the $_{pp}$ERK response and the $_{pp}$ERK response areas decrease with k_{PRMT5} because of the increasing effect of the Raf^* methylation process (Fig. 5A and 5B respectively).

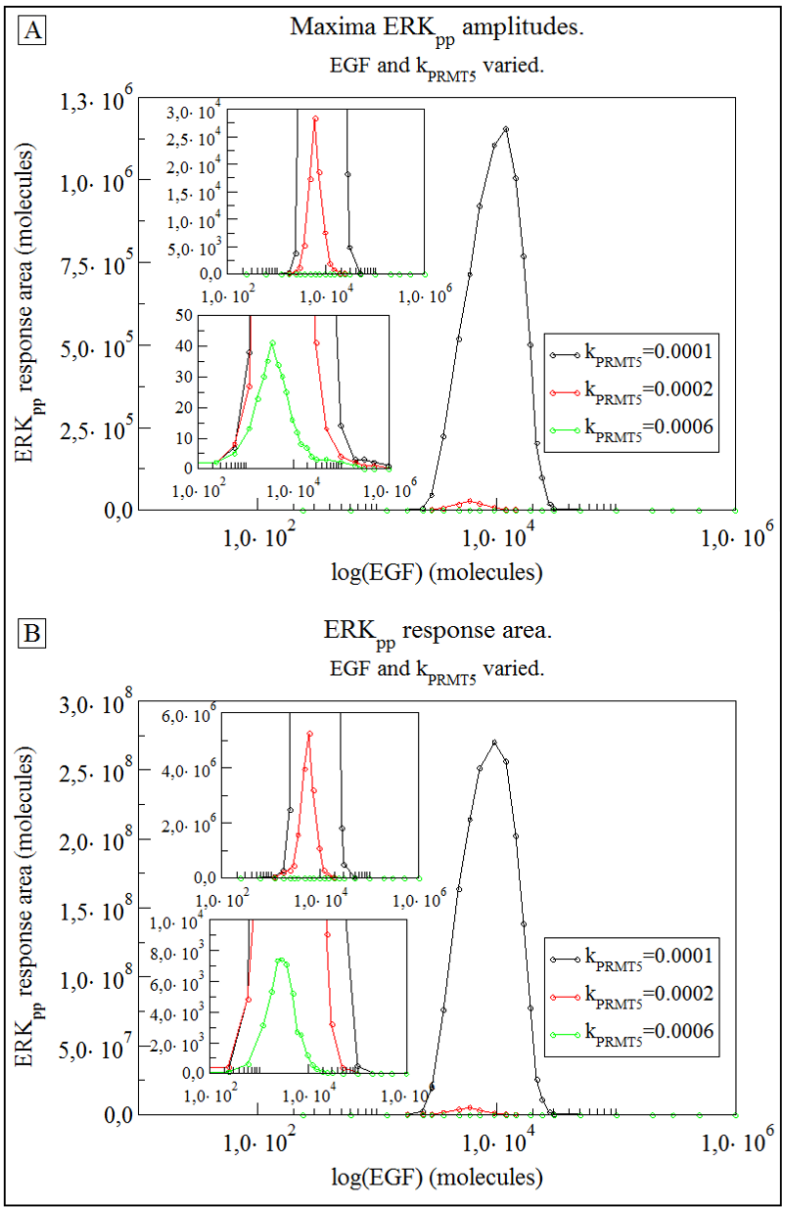

FIGURE 5 (color version required for printing): **Maxima amplitudes and responses areas of the $_{pp}$ERK in the model A.**

(A) Representation of the maxima amplitudes of the $_{pp}$ERK time responses in the model A as function of EGF (represented in a semilogarithmic scale). **(B)** Representation of the response areas of the $_{pp}$ERK concentration in the model A as function of EGF (represented in a semilogarithmic scale). These both responses have been represented for the three values of k_{PRMT5}: 0.0001 (cell/molecules)·s^{-1} (black lines), 0.0002 (cell/molecules)·s^{-1} (red lines) and 0.0006 (cell/molecules)·s^{-1} (green lines). EGF is defined from 0 to 10^6 molecules.

Both the maxima amplitudes of the $_{pp}$ERK response and the $_{pp}$ERK response areas describe a pass-band pattern for a fixed k_{PRMT5} (Fig. 5A and 5B respectively), demonstrating that the EGF-Ras-ERK MAPK cascade with the presence of EGF-dependent PRMT5 activity limits the range of the EGF values that provide non-zero responses (or enough response levels to be considered as relevant

for this study). First, there is a short-range of initial EGF values that are not capable of generating relevant $_{pp}$ERK response. Next, there is a medium-range of EGF values where the $_{pp}$ERK expression increases until reaching a maximum value. At this point, the activity of the PRMT5 increases promoted by high EGF and Raf* levels, starting to speed up the Raf* degradation process. Next, higher EGF values increase the PRMT5 activity, which takes more relevance than the activation of downstream substrates (MEK and ERK), resulting in weaker and shorter $_{pp}$ERK responses. And finally, there is a very high range of EGF values where the Raf* degradation is so fast that there is no time for the signaling cascade to generate any ERK$_{pp}$ response (Fig. 5A and 5B).

When PRMT5 is regulated by EGF, obtained results give evidences for its regulatory role on the time duration and amplitude of the ERK-1/2 MAPK activity, as well as in limiting the range of EGF able of generating a nonzero $_{pp}$ERK response.

Case B: PRMT5 activity regulated by $_{pp}$ERK (model B)

In the model B, considering a fixed EGF, the response of the EGF-Ras-ERK MAPK cascade becomes time-limited, but describing a lognormal pattern (Fig. 6 and Suppl. Fig. S8C). At starter times, the cascade response presents weak values, as there is not enough input stimuli to activate a consistent Raf* concentration capable of activating relevant ERK. Next, the Raf* expression increases due to the action of EGF, so that the response of the signaling cascade grows with time until reaching a maximum value (ERK$_{pp(max)}$) (Fig. 6). Consequently, the methylation process exerted by the PRMT5 becomes faster, reducing the Raf* expression. At this point, both the deactivation of kinases through the corresponding phosphatases and the deactivation of Raf* through the $_{pp}$ERK negative feedback loop help the Raf* methylation process to diminish the ERK$_{pp}$ response until its extinction, avoiding an inappropriate steady-state response (Fig. 6 and Suppl. Fig. S8C).

The simulations reveal that there is no correlation between the k_{PRMT5} value and the $_{pp}$ERK amplitude. But there is a slight dependence between the k_{PRMT5} and the $_{pp}$ERK time duration and, thereby, with the $_{pp}$ERK response areas. Both the maxima amplitudes of the $_{pp}$ERK response and the $_{pp}$ERK response areas describe a switch-like pattern for a fixed k_{PRMT5} (Fig. 7A and Suppl. Fig. S10A, and Fig. 7B and Suppl. Fig. S10B, respectively).

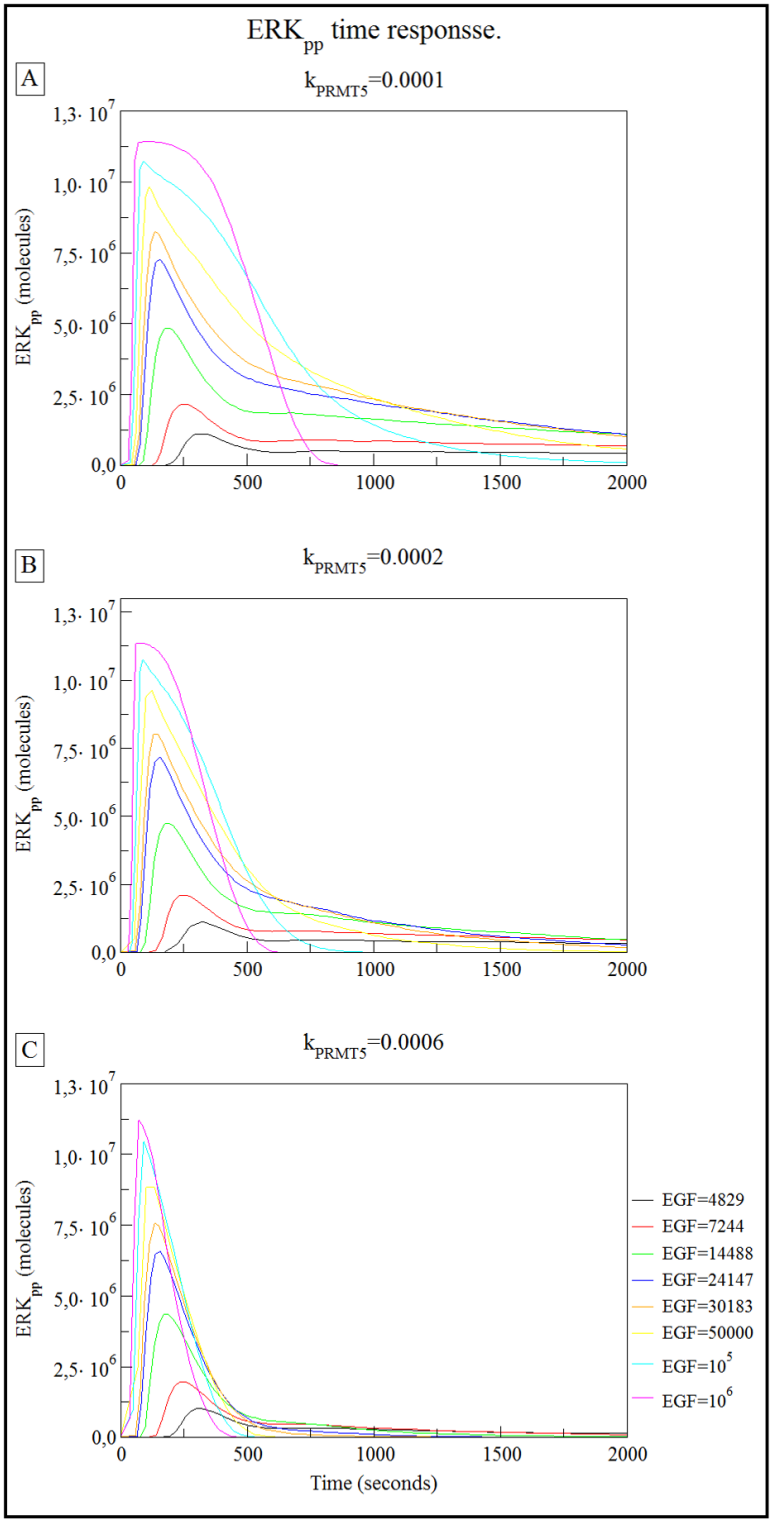

FIGURE 6 (color version required for printing): **$_{pp}$ERK time response of the model B for different k_{PRMT5} values.** Time responses of $_{pp}$ERK of the model A for different values of k_{PRMT5}: **graph A** for k_{PRMT5} = 0.0001 (cell/molecules)·s^{-1}, **graph B** for k_{PRMT5} = 0.0002 (cell/molecules)·s^{-1} and **graph C** for k_{PRMT5} = 0.0006 (cell/molecules)·s^{-1}. Each colour line represents the Ras-ERK MAPK cascade time response for each different EGF value. EGF is defined from 0 to 10^6 molecules. The considered values of EGF to simulate the output response are summarized in the graph legend. The components concentrations and the reaction rates are described in Suppl. Tables I and II.

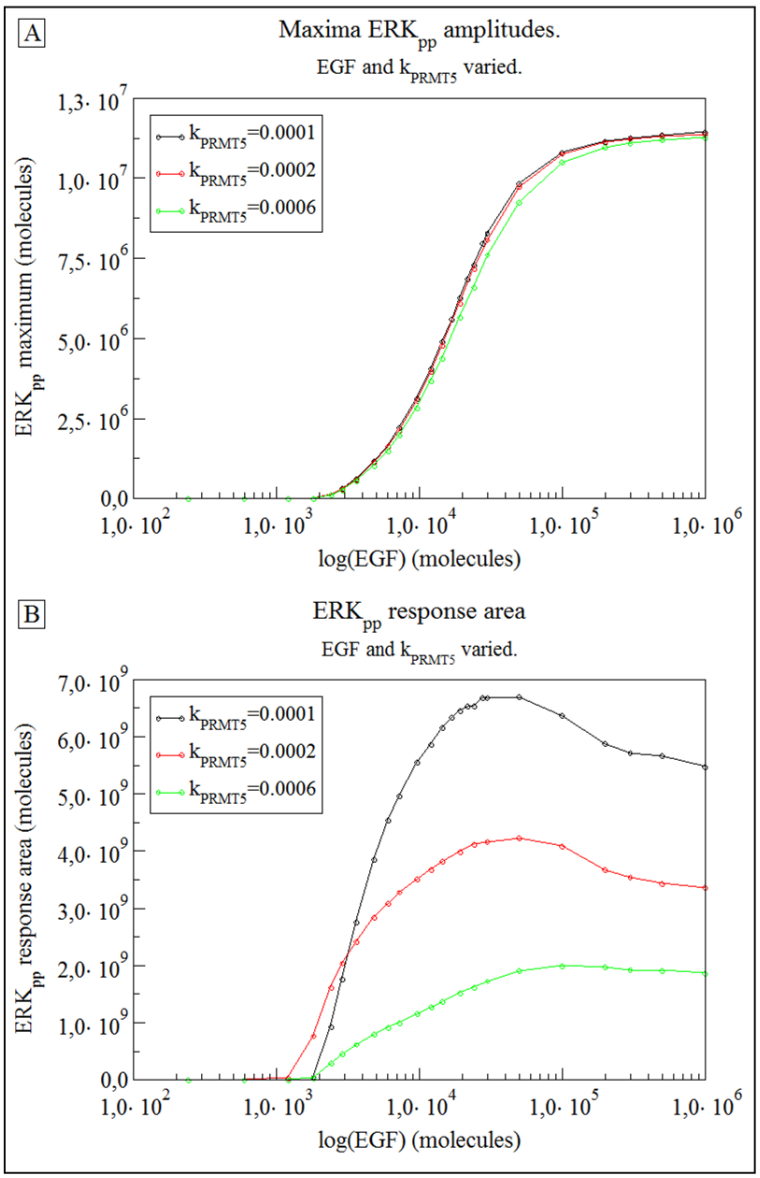

FIGURE 7 (color version required for printing): **Maxima amplitudes and responses areas of the $_{pp}$ERK in the model B.**

(A) Representation of the maxima amplitudes of the $_{pp}$ERK time responses in the model B as function of EGF (represented in a semilogarithmic scale). **(B)** Representation of the response areas of the $_{pp}$ERK concentration in the model B as function of EGF (represented in a semilogarithmic scale). These both responses have been represented for the three values of k_{PRMT5}: 0.0001 (cell/molecules)·s^{-1} (black lines), 0.0002 (cell/molecules)·s^{-1} (red lines) and 0.0006 (cell/molecules)·s^{-1} (green lines). EGF is defined from 0 to 10^6 molecules.

At low EGF values, there is a discrete Raf activation that results in poor output responses. As the Raf* degradation process depends directly on the $_{pp}$ERK expression, its effect is irrelevant against the activation of Raf, thereby the $_{pp}$ERK responses are sustained in time (Fig. 6, and red and blue results from Suppl. Fig. S7). Next, with increasing EGF, the relative and maxima amplitudes of the $_{pp}$ERK response increase until reaching a maximum value when EGF = EGF_{Th}, from 10^7

molecules (Suppl. Fig. S10A and green result from Suppl. Fig. S9). Such $_{pp}$ERK concentration induces a Raf* methylation process through the PRMT5 activity that gains relevance, speeding up the response degradation and reducing its time duration (red and blue graphs from Suppl. Fig. S7). After that, for EGF > EGF_{Th}, the $_{pp}$ERK time responses are controlled by a competition process between a maximum steady state of the $_{pp}$ERK expression (promoted by an overexpressed concentration of EGF) and a constant degradation of Raf*. Under these conditions, the maxima amplitudes of the $_{pp}$ERK responses do not practically change, maintaining a constant value (Suppl. Fig. S10A). Nevertheless, the time duration continues decreasing (red and blue results from Suppl. Fig. S7) because of the $_{pp}$ERK production becomes faster and thereby, the Raf* methylation process. Therefore, the response area of the output signal, after reaching a maximum value, describes a slight decrease as the EGF increases (Fig. 7B and Suppl. Fig. S10B) promoted by the reduction of the response times (red and blue results from Suppl. Fig. S7), and lasts until the differences in the response times are barely noticed.

The obtained results reveal that the time responses of the model B are influenced by the impact of the PRMT5 methylation exerted through the parameter k_{PRMT5}, which positively regulates the velocity of the Raf* degradation. Thereby, as k_{PRMT5} increases, lower Raf* concentration is available and less time is required to become extinct, generating shorter output responses (Fig. 6 and Suppl. Fig. S8C). Even though the amplitude of $_{pp}$ERK does not reveal important shifts due to the k_{PRMT5} effect (a saturated response regime is guaranteed by an overexpressed concentration of EGF, see Fig. 6, 7A and Suppl. Fig. S9A), its response area decreases while k_{PRMT5} increases (Fig. 7B and Suppl. Fig. S10B) due to shorter time responses of the $_{pp}$ERK expression.

So, reported results reveal that the PRMT5 regulated by $_{pp}$ERK modulates the time response of the ERK-1/2 MAPK cascade, limiting its duration and moving its activity away from an aberrant activation state. However, the amplitude of the $_{pp}$ERK response is not influenced by the PRMT5 activity.

4. **Discussion**.

The PRMT5 protein is present in different cell compartments, namely cytoplasm and nucleus, and its functional role seems to depend on its localization. Thereby, PRMT5 acts as oncogene and epigenetic silencer of tumor repressors when is localized in cytoplasm, but PRMT5 inhibits cancer cell proliferation, migration and invasion when it is placed in the cell nucleus [85, 86]. Then, PRMT5 may act as an up-regulating mechanism for cancer proliferation, migration and invasion when it acts in the cytoplasm, whilst it may have a tumor antiproliferative and pro-survival effect when it is activated in the cell nucleus. Such issue can be related with the simulations

performed in our work, where the ERKpp-induced PRMT5 may act in the cell nucleus, regulating the EGF-ERK MAPK activity, avoiding a sustained maximum ERKpp response that can lead to tumorigenesis.

Previous works provided evidences of the regulatory role of PRMT5 in normal cell proliferation and cell survival. *Andreu-Pérez P et al*. [45] determined that the methylation of arginine residue in the GRG motif of BRaf and CRaf is involved in the limitation of these kinases activity and stability, promoting the limitation of ERK1/2 signal output and, thereby, switching from differentiation to proliferation. *Hsu JM et al.* [60] found that EGFR methylated in Arg^{1175} positively modulated EGF-induced EGFR trans-autophosphorylation at Tyr^{1173}, enhancing the binding between EGFR and SHP1 that resulted in suppression of EGFR-mediated ERK activation. In addition, they reported that EGFR Arg^{1175} methylation is not regulated by EGF stimulation, finding that can be related to the results obtained with the EGF-Ras-ERK MAPK model of our research that presents PRMT5 activated by $_{pp}$ERK. *Liu F et al.* [87] reported an increase in colony formation of myeloproliferative neoplasms (MPNs) and erythroid differentiation when knocking down PRMT5 in human CD34+ cells using shRNA, indicating that PRMT5 negatively regulates hematopoietic stem/progenitor cell proliferation and expansion. *DongSheng W et al.* [88] confirmed that PRMT5 can bind with death receptor 4 (DR4) and reduce its expression, providing a molecular mechanism to abolish the inflammatory cytokine expression induced by protein CCL20, inhibiting its expression via inhibiting NF-κB. An study developed by *Yang M et al.* [89] in Caenorhabditis elegans showed that PRMT5 negatively regulates DNA damage-induced apoptosis, triggering a decrease in germ cell apoptosis. Furthermore, an study done by *Scoummane A et al.* [90] manifested that PRMT5 is required for normal cell proliferation and regulates cell-cycle transition from G1 to S phase. They gave evidences that, in response to stress signals, PRMT5 induced sufficient p53 activation to promote pro-survival mechanisms, such as cell-cycle arrest and DNA repair.

There is a large body of evidence on the role of PRMT5 inhibitors, both natural and synthetic, but most of them focus on reversing the activity of PRMT5 when it is related to the positive regulation of tumor proliferation and progression in cancer cell lines [91-94]. However, there are few scientific studies aimed at verifying the effect of inhibiting the activity of PRMT5 when it regulates an aberrant tumor response. *Andreu-Perez P et al.* [45] reported that the silencing of PRMT5 activity through methylthioadenosine (MTA) or its depletion stabilized Raf and positively regulates its activity, not only increasing the amplitude and duration of Raf signal and, so on, the ERK phosphorylation in response to growth factors, but also redirecting the response of PC12 cells to EGF from proliferation to differentiation. Related to this point, it is known that both NGF and EGF stimulate the Ras-ERK-MAPK pathway in PC12 cells, but with different effects on ERK activation: EGF induces a transient phosphorylation of ERK, promoting PC12 cell

differentiation, whereas NGF elicits a sustained activation of ERK, inducing proliferation of PC12 cells [95-97]. Then, it may be assumed that, when knocking down PRMT5, the induced differentiation behavior provoked by a sustained ERK response was triggered by a sustained Raf signal. Apart from *Andreu-Perez et al.* [45], there are other scientific studies aimed at verifying the effect of inhibiting the activity of PRMT5 when it regulates an aberrant tumor response. *Hsu JM et al.* [60] conducted experiments where knockdown of PRMT5 expression by short interfering RNAs (siRNAs) diminished Arg^{1175}, down regulating EGF-induced Tyr^{1173} phosphorylation and enhancing EGF-stimulated ERK activation by reducing SHP1 recruitment to EGFR. As a result, cell proliferation, migration and invasion of EGFR-expressing cells increased. *Jansson M et al.* [98] showed that the depletion of PRMT5 in human osteosarcoma U2OS cells induced p53-dependent apoptosis. And *Yang M et al.* [89] reported in Caenorhabditis elegans that loss of PRMT5 activity promoted specific over upregulation of the cell death initiator EGL-1 in response to DNA damage, which is directly responsible for the excessive germ cell apoptosis.

In our study, the basic structure of the EGF-Ras-ERK MAPK pathway (reference model) describes a switch-like time response, reaching a steady-state condition (Fig. 3A) that results from an equilibrium process between the Raf activation promoted by EGF, the Raf^* attenuation performed by the $_{pp}$ERK negative feedback loop, and the deactivation of the active species of Raf, MEK, and ERK through the corresponding phosphatases (RafPase, MEKPase and ERKPase). Such sustained ERK signaling may be responsible of an aberrant cell response related to tumor differentiation, migration and invasion.

This response can be modulated both in time duration and in amplitude through the simple action of a protein present in the own cell environment: the PRMT5.

This protein acts as an inhibitor of the Raf^*, degrading it through a methylation process, limiting the activation of its downstream substrates (MEK and ERK) and, thereby, converting the steady-state of the output response into a time-limited pattern (Fig. 4, 6, Suppl. Fig. S7, and Suppl. Fig. S8A and S8B), avoiding an uncontrolled activation state of the $_{pp}$ERK response, as the reference model describes.

Both the time limitation and the amplitude attenuation of the EGF-Ras-ERK MAPK response depends on the parameter k_{PRMT5}, which defines the strength of the methylation action. Thereby, the Raf^* expression decreases while k_{PRMT5} increases, resulting in less levels of activation of downstream substrates (MEK and ERK) and, in turn, less output response that expires in less time (Fig. 4, 6, and Suppl. Fig. S8B, S8C and S9). Therefore, the protein PRMT5 constitutes a cell component able of switching between sustained and transient $_{pp}$ERK responses without requiring different types of stimuli and, thereby, provides a mechanism to switch from cell differentiation to cell proliferation [45]. Although further investigation should be required, such switching behavior

appears to be reversible upon removal of PRMT5, as it was reported in the literature related to the application and removal of both EGF and NGF [99-101].

Different effects on the $_{pp}$ERK response patterns arise depending on the mechanism of the PRMT5 activation. For this reason, two different models have been defined to illustrate such differences: the model A where the PRMT5 activity is regulated by the EGF, describing a Gaussian pattern of the $_{pp}$ERK time response (Fig. 4), and the model B where the PRMT5 is regulated by the $_{pp}$ERK, providing a lognormal pattern of the $_{pp}$ERK time response (Fig. 6).

The reduction of the time duration and the amplitude of the $_{pp}$ERK response is greater in the model A than in the model B (Suppl. Fig. S7, S8B and S8C), regardless the k_{PRMT5} value. In short, in the model A, the Raf* methylation exerted by PRMT5 is present from first stages of the stimulation since it is activated by the EGF and thereby, fast rates of Raf* degradation are achieved. As a result, the duration and the amplitude of the $_{pp}$ERK response fall down (Fig. 4 and Suppl. Fig. S8B). Thus, the EGF-dependent PRMT5 shortens the $_{pp}$ERK response time 20-fold more (Suppl. Fig. S7) and can reach attenuations 10-fold greater (Suppl. Fig. S9) than the $_{pp}$ERK-dependent PRMT5, regardless the value of k_{PRMT5}.

Nevertheless, the Raf* methylation process in the model B depends on the output response of the signaling cascade, requiring more time to induce the Raf* methylation effect of the PRMT5. Thereby, the duration of the response is prolonged (Fig. 6, and Suppl. Fig. S7 and S8C). In addition, the attenuation of the $_{pp}$ERK amplitude is low, presenting irrelevant shifts when k_{PRMT5} changes (Fig. 6 and Suppl. Fig. S8C), since a maximum steady state of the $_{pp}$ERK expression (promoted by an overexpressed concentration of EGF) is counteracted by a constant degradation of Raf* (which in turn is controlled by the $_{pp}$ERK expression). Then, the Raf* methylation effect exerted by the PRMT5 requires enough high expression of $_{pp}$ERK to be effective and compete with the Raf activation promoted by EGF.

Furthermore, when the PRMT5 activity depends on the input stimulus (model A), it is possible to tune a specific and delimited range of stimuli able of generating a nonzero response, resulting in a behavior similar to a pass-band filter. Thereby, for low EGF values, there is not enough activation of the signaling cascade to produce an $_{pp}$ERK response, whilst for high EGF values the rate of Raf* methylation is so fast that no $_{pp}$ERK response is generated (Fig. 5A and red result from Suppl. Fig. S9). In contrast, if the PRMT5 activity is controlled by the output $_{pp}$ERK (model B), it is not possible to delimitate the EGF range at which the $_{pp}$ERK response is nonzero, resulting in a switch-like output response because of the sustained maximum value of the $_{pp}$ERK when EGF $\geq$ EGF_{Th} (Suppl. Fig. S10A and green result from Suppl. Fig. S9), similar to the response behavior of the reference model (black results from Fig. 3B and Suppl. Fig. S9).

Our data predict that PRMT5 activity reduces the duration and the amplitude of the EGF

response on ERK activity, and such response can be limited to a specific range of input stimulus. Future studies should validate our prediction through the use of cultured cells. This is favoured by the fact that human cells such as HeLa or A431 express high levels of EGF receptors, components of the MAPK pathway, as well as PRMT5. In consequence, it is feasible to manipulate PRMT5 genetically (overexpression or silencing), and to analyse the time-dependent responses to different concentrations of EGF on ERK activity/phosphorylation.

Weighing all the differences between both models of the Ras-ERK MAPK pathway with PRMT5 activity, their response patterns can be useful, as a guide, to identify if any of both analyzed mechanisms of PRMT5 activation may act as a modulator of the signaling pathway response in experimental research.

The proposed model of the EGF-Ras-ERK MAPK signaling pathway only considers the main components of the cascade directly related in the phosphorylated protein levels, thereby, the different docking and activator factors integrated in the MAPK signalling pathway are not considered in the model, such as KSR1/2, β-arrestin1/2, paxillin, MP1, RKIP, MORG1, FRS2, Shoc2/Sur8, IQGAP, PEB1, or Sef, among others. Although these molecules are required for the compartmentalization of signaling proteins and their spatial arrangement to be activated/deactivated and to interact each others, the proposed model aims to determine only the role of PRMT5 on ERK activity. For instance, presence or knockout of scaffold proteins are related to modifications in the cell response related to ERK MAPK activity. This is the case of the KSR, with evidences in the literature of its role as scaffold, pseudo-kinase or even as a kinase, promoting or inhibiting Raf, MEK or ERK signaling, mainly driving translocation of such kinases to specific sites of the cell, increasing their local concentrations, or forming heterotetramers with Raf and MEK to promote its phosphorylation and, thereby, improving signaling [102, 103]. As a result, knockout of scaffolds can inhibit MAPK in tumor cells and reverse oncogenic transformation of cells, such as the knockout of KSR that can reverse oncogenic transformation in colorectal and pancreatic cancers [104,105]. Moreover, scaffold proteins, as signaling regulators, could help to develop therapeutic strategies against unwanted ERK signaling (i.e., proliferation), while preserving other desired ERK activity (e.g. differentiation or apoptosis) [106-108]. Then, it should be of interest to include the KSR or other scaffold protein related to Ras-ERK MAPK pathway in a further extension of the model to investigate how their role can influence ERK signaling.

In addition, reported results are based in simulations of a model of the EGF-Ras-ERK MAPK route that does not consider other regulations on kinases and PRMT5 activity that could influence the final cell response, caused by cross-links with partner signaling pathways or by different feedback loops from the considered ones. In the latter case, several positive and negative feedback loops have been identified within the EGF-Ras-ERK MAPK pathway: positive feedback loops (such as the

existing one from Ras to SoS) are related to bistability and switch-like responses, whilst negative feedback loops (such as the exising ones from ppERK to either MEK, Ras or Raf) switch pathway response from ultrasensitivity to oscillations, even inhibiting signal via kinases phosphorylation [109, 110].

Such a simplification of the model does not permit to quantify to what extent the activity of MAPK pathway is regulated by PRMT5 in cells under in vivo conditions. In this regard, a further step may be to extend the EGF-Ras-ERK MAPK model including some of the regulatory mechanisms discussed previously, in order to evaluate the extent of Raf methylation and attenuation of ERK activity in a more real approach.

When addressing computational studies of simplified models related to complex biological systems such as the EGF-Ras-ERK MAPK signaling pathway, doubts can arise about the impact and the extent that the proposed regulatory mechanism based on the protin PRMT5 has on the modulation of the ERK signal. Despite there is a lack of quantitative studies about the impact of this regulatory effect of PRMT5 in ERK signal, some publications [45, 60] reported that activating PRMT5 alone, the levels of ERK signal are drastically reduced. And our predictions are in accordance with such published evidences. Therefore, the PRMT5 may be considered an important mechanism in terms of regulating the overexpression of the EGF-Ras-ERK pathway by itself, switching ERK signal from a sustained response to a transient one.

To conclude, regardless the type of regulation of the PRMT5 activation, this small protein opens up great options related to the modulation of the Ras-ERK MAPK cascade response upon EGF stimulation, allowing to limit in a great extent the ERK signal through a methylation process of Raf, which allows to delimit ERK duration over time and to attenuate its amplitude, since an uncontrolled intensity or the persistence in time of an inappropriate response in the Ras-ERK MAPK route can lead to wrong physiological functions of the cell. So, activators of this methyltransferase can be considered as a potential target to develop biological and/or pharmacological strategies to fight against cell diseases and tumorigenesis, such as exposing cells to hypoxic conditions [111] through any hypoxia mimicker such as the cobalt chloride ($CoCl_2$), which is considered to prevent HIF-1α degradation, and ginkgolides [112]; posphorylating MEP50, which builds a complex with PRMT5 and promotes substrate recognition and orientation, as well as stimulation of PRMT5 activity [113, 114]; the growth factor TGFβ [115]; the c-Myc that recruits the PRMT5-MEP50 complex, inducing H4R3 symmetric di-methylation [116]; the cyclin D1/CDK4 that phosphorylates Thr5 on MEP50, activating the methyltransferase activity of PRMT5 [117]; ionizing radiation [118]; and DNA damage that can induce PRMT5-catalyzed methylation of non-histone substrates, promoted by different factors, such as camptothecin, etoposide, hydroxyurea, doxorubicin, and UVB irradiation [90, 119-121].

Acknowledgments.

This study was supported by grants from the MICINN (SAF2016-75246R and RTI2018-097038-B-C22), the Generalitat de Catalunya (Grants 2017 SGR 1015, ICREA Acadèmia), and CIBERDEM. We gratefully acknowledge institutional funding from the MICINN through the Centres of Excellence Severo Ochoa Award, and from the CERCA Programme of the Generalitat de Catalunya. M.J. thanks MICINN for the FPI contract BES-2011-043331- M.J. also thanks Ramón Reigada and Mª Ángeles Serrano their contribution in the conception of the initial idea of the present study. In addition, I gratefully acknowledge Juan Recio his support in understanding the experimental bases of the present investigation.

Conflict of interests.

Authors declare no conflict of interests.

References.

[1] Bhalla US, Iyengar R. 1999. *Emergent properties of networks of biological signaling pathways*. Science 283, 381-387.

[2] Serrano MA, Juardo M, Reigada R. 2013. *Negative feedback self-regulation contributes to robust and high-fidelity transmembrane signal transduction*. J R Soc Interface;10(88):20130581.

[3] Sturm OE et al. 2010. *The mammalian MAPK/ERK pathway exhibits properties of a negative feedback amplifier*. Sci. Signal. 3, ra90.

[4] Lewis TS, Shapiro PS, Ahn NG. 1998. *Signal transduction through MAP kinase cascades*. Adv Cancer Res 74, 49139.

[5] Errede B et al. 1995. *Dynamics and organization of MAP kinase signal pathways*. Mol Reprod Dev 42, 477485.

[6] Pearson G et al. 2001. *Mitogen-Activated Protein (MAP) Kinase Pathways: Regulation and Physiological Functions*. Endocrine Reviews 22(2), 153183.

[7] Huang CY, Ferrell JE. 1996. *Ultrasensitivity in the mitogen-activated protein kinase cascade*. Proc. Natl. Acad. Sci. USA 93.

[8] Cargnello M and Roux P P. 2011. *Activation and function of the MAPKs and their substrates, the MAPK-Activated Protein Kinases*. Microbiology and Molecular Biology Reviews, 5083.

[9] Inder K et al. 2008. A*ctivation of the MAPK Module from Different Spatial Locations Generates Distinct System Outputs*. Molecular Biology of the Cell Vol. 19, 47764784.

[10] Roux PP et Blenis J. 2004. *ERK and p38 MAPK-Activated Protein Kinases: a Family of Protein Kinases with Diverse Biological Functions*. Microbiology and molecular biology reviews, June 2004, 320344.

[11] Campbell SL et al. 1998. *Increasing complexity of Ras signaling*. Oncogene 17: 13951413.

[12] Kholodenko BN. 2000. *Negative feedback and ultrasensitivity can bring about oscillations in MAPK cascades*. Eur. J. Biochem. 267, 1583-1588.

[13] Fey D, Croucher DR, Kolch W, Kholodenko BN. 2012. *Crosstalk and signaling switches in mitogen-activated protein kinase cascades*. Frontiers in Physiology, Vol. 3, Article 355, 1-21.

[14] Caunt CJ and McArdle CA. 2012. *ERK phosphorylation and nuclear accumulation: insights from single-cell imaging*. Biochem Soc Trans 40(1):224-229.

[15] Shindo Y et al. 2016. *Conversion of graded phosphorylation into switch-like nuclear translocation via autoregulatory mechanisms in ERK signalling*. Nature Communications 7:10485.

[16] Behar M and Hoffmann A. 2010. *Understanding the temporal codes of intra-cellular signals*. Curr. Opin. Genet. Dev. 20, 684-693.

[17] Purvis JE and Lahav G. 2013. *Encoding and decoding cellular information through signaling dynamics*. Cell 152, 945-956.

[18] Yee KL, Weaver VM and Hammer DA. 2008. *Integrin-mediated signalling through the MAP-kinase pathway*. IET Systems Biology, Volume 2, Issue 1, pages 8-15.

[19] Grandis JR, Sok JC. 2004. *Signaling through the epidermal growth factor receptor during the development of malignancy*. Pharmacol. Ther. 102, 37-46.

[20] Downward, J. 2003. *Targeting RAS signalling pathways in cancer therapy*. Nat. Rev. Cancer 3, 1122.

[21] McBride AE, Silver PA. 2001. *State of the Arg: Protein methylation at arginine comes of age*. Cell 106,5-8.

[22] Bedford MT, Richard S. 2005. *Arginine methylation an emerging regulator of protein function*. Mol. Cell 18, 263-272.

[23] Bedford MT, Clarke SG. 2009. *Protein arginine methylation in mammals: Who, what, and why*. Mol. Cell 33,1-13.

[24] Bedford MT. 2007. *Arginine methylation at a glance*. J Cell Sci. 20, 4243- 4246.

[25] Branscombe TL et al. 2001. *PRMT5 (Janus kinase-binding protein 1) catalyzes the formation of symmetric dimethylarginine residues in proteins*. J. Biol. Chem. 276, 32971-32976.

[26] Lee YH, Stallcup MR. 2009. *Minireview: Protein arginine methylation of non histone proteins in transcriptional regulation*. Mol. Endocrinol. 23, 425-433.

[27] Wei T-Y W et al. 2012. *Protein arginine methyltransferase 5 is a potential oncoprotein that upregulates G1 cyclins/cyclin-dependent kinases and the phosphoinositide 3-kinase/AKT signaling cascade*. Cancer Sci. 103(9):1640-50.

[28] Kim H and Ronai ZA. 2020. *PRMT5 function and targeting in cancer*. Cell Stress, Vol. 4, No. 8, 199-215.

[29] Lattouf H et al. 2019. *LKB1 regulates PRMT5 activity in breast cancer*. Int. J. Cancer: 144, 595-606.

[30] Gu Z, Li Y, Lee P, Liu T, Wan Ch, Wang Z. 2012. *Protein Arginine Methyltransferase 5 Functions in Opposite Ways in the Cytoplasm and Nucleus of Prostate Cancer Cells*. PloS ONE 7(8): e44033.

[31] Mundade R, Wei H and Lu T. 2014. *PRMT5, A Pivotal Player in Cancer*. Austin J Pharmacol Ther. 2 (2).4

32[6] Xiao W, Chen X, Liu L, Shu Y, Zhang M, Zhong Y. 2019. *Role of protein arginine methyltransferase 5 in human cancers*. Biomedicine & Pharmacotherapy 114, 108790.

[33] Wang L, Pal S and Sif S. 2008. *Protein Arginine Methyltransferase 5 Suppresses the Transcription of the RB Family of Tumor Suppressors in Leukemia and Lymphoma Cells*. Mol. Cell. Biol. Vol. 28, No. 20, 6262-6277.

[34] Pal S, Vishwanath SN, Erdjument-Bromage H, Tempst P, Sif S. 2004. *Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes*. Mol Cell Biol. 24(21):9630-45.

[35] Alinari L et al. 2015. *Selective inhibition of protein arginine methyltransferase 5 blocks initiation and maintenance of B-cell transformation*. Blood 125(16):2530-43.

[36] Majumder S et al. 2010. *Methylation of Histone H3 and H4 by PRMT5 Regulates Ribosomal RNA Gene Transcription*. J Cell Biochem.; 109(3): 553-563.

[37] Liu F et al. 2020. *PRMT5-mediated histone arginine methylation antagonizes transcriptional repression by polycomb complex PRC2*. Nucleic Acids Research, 2020, Vol. 48, No. 6, 2956-2968.

[38] Richard S, Morel M, Cleroux P. 2005. *Arginine methylation regulates IL-2 gene expression: A role for protein arginine methyltransferase 5 (PRMT5)*. Biochem. J. 388, 379-386.

[39] Greenblatt SM, Liu F and Nimer SD. 2016. *Arginine methyltransferases in normal and malignant hematopoiesis*. Exp. Hematol., 44, 435–441.

[40] Richters A. 2017. T*argeting protein arginine methyltransferase 5 in disease*. Future Medical Chemistry, 9(17), 20812098.

[41] Gary JD and Clarke S. 1998. *RNA and protein interactions modulated by protein arginine methylation*. Prog Nucleic Acid Res Mol Biol. 61:65-131.

[42] Li M, An W, Xu L, Lin Y, Su L, and Liu X. 2019. *The arginine methyltransferase PRMT5 and PRMT1 distinctly regulate the degradation of anti-apoptotic protein CFLARL in human lung cancer cells.* J. Exp. Clin. Cancer Res. 38:64.

[43] Li S, Yang P, Tian E, and Zhang H. 2013. *Arginine Methylation Modulates Autophagic Degradation of PGL Granules in C. elegans*. Molecular Cell 52, 421-433.

[44] Meister G, Eggert C, Bühler D, Brahms H, Kambach C, Fischer U. 2001. *Methylation of Sm proteins by a complex containing PRMT5 and the putative U snRNP assembly factor pICln*. Curr Biol. 11(24):1990-4.

[45] Andreu-Pérez P et al. 2011. *Protein Arginine Methyltransferase 5 Regulates ERK1/2 Signal Transduction Amplitude and Cell Fate Through CRAF*. Sci Signal. 13; 4(190): ra58.

[46] Shaw AS et al. 2014. *Kinases and Pseudokinases: Lessons from Raf.* Molecular and Cellular Biology, Vol. 34, No. 9, 1538-1546.

[47] Wolf SS. 2009. *The protein arginine methyltransferase family: an update about function, new perspectives and the physiological role in humans*. Cell MolcLife Sci. 66(13):2109-21.

[48] Zhu F and Rui L. 2019. *PRMT5 in gene regulation and hematologic malignancies*. Genes & Diseases 6, 247-257.

[49] Tsai C-J, Lin SL, Wolfson HJ, Nussinov R. 1997. *Studies of protein-protein interfaces: A statistical analysis of the hydrophobic effect*. Protein Science, 6:53-64.

[50] Fan Q, Wang Q, Cai R, Yuan H, Xu M. 2020. *The ubiquitin system: orchestrating cellular signals in non-small-cell lung cancer*. Cellular & Molecular Biology Letters 25:1.

[51] Nguyen LK, Kolch W and Kholodenko BN. 2013. *When ubiquitination meets phosphorylation: a systems biology perspective of EGFR/MAPK signalling*. Cell Communication and Signaling 11:52, 1-15.

[52] Grbovic OM, Basso AD, Sawai A, Ye Q, Friedlander P, Slit D, Rosen N. 2005. *V600E B-Raf requires the Hsp90 chaperone for stability and is degraded in response to Hsp90 inhibitors*. PNAS, Vol. 103, No. 1, 57-62.

[53] Noble C et al. 2008. *CRAF autophosphorylation of serine 621 is required to prevent its proteasome-mediated degradation*. Mol Cell. 31(6):862-72.

[54] Kidger AM and Keyse SM. 2016. *The regulation of oncogenic Ras/ERK signalling by dual-specificity mitogen activated protein kinase phosphatases (MKPs).* Seminars in Cell & Developmental Biology, Volume 50, 125-132.

[55] Zhao Y and Zhang Z-Y. 2001. *The Mechanism of Dephosphorylation of Extracellular Signal-regulated Kinase 2 by Mitogen-activated Protein Kinase Phosphatase 3*. The journal of biological chemistry, Vol. 276, No. 34, pp. 32382–32391.

[56] Caunt CJ and Keyse M. 2012. *Dual-specificity MAP kinase phosphatases (MKPs) Shaping the outcome of MAP kinase signalling*. FEBS Journal 280 (2013) 489–504.

[57] Avraham R & Yarden Y. 2011. *Feedback regulation of EGFR signalling: decision making by early and delayed loops.* Nat Rev Mol Cell Biol 12, 104–117.

[58] Mizuno R et al. 2004. *Inhibition of MKP-1 expression potentiates JNK related apoptosis in renal cancer cells*. Journal of Urology, Volume 172, Issue 2, 723-727.

[59] Liu L, Doran S, Xu Y, Manwani B, Ritzel R, Benashski S, McCullough L, Li J. 2014. *Inhibition of mitogen-activated protein kinase phosphatase-1 (MKP-1) increases experimental stroke injury*. Exp Neurol.; 261:404-11.

[60] Hsu JM et al. 2011. *Crosstalk between Arg 1175 methylation and Tyr 1173 phosphorylation negatively modulates EGFR-mediated ERK activation*. Nat. Cell. Biol., 13(2):174-181.

[61] Fischer A, Hekman M, Kuhlmann J, Rubio I, Wlese S, Rapp UR. 2006. *B- and C-RAF Display Essential Differences in Their Binding to Ras*. J. Biol. Chem. Vol. 282, No. 36, 26503-26516.

[62] Li S, Jang H, Zhang J, Nussinov R. 2018. *Raf-1 Cysteine-Rich Domain Increases the Affinity of*

K-Ras/Raf at the Membrane, Promoting MAPK Signaling. Structure, 26(3):513-525.

[63] Berg M and Soreide K. 2012. *EGFR and Downstream Genetic Alterations in KRAS/BRAF and PI3K/AKT Pathways in Colorectal Cancer - Implications for Targeted Therapy*. Discov. Med. 14(76):207-14.

[64] Lamba S et al. 2014. *RAF Suppression Synergizes with MEK Inhibition in KRAS Mutant Cancer Cells*. Cell Reports 8, 1475-1483.

[65] Gorden A et al. 2003. *Analysis of BRAF and N-RAS Mutations in Metastatic Melanoma Tissues*. Cancer Research 63, 3955-3957.

[66] Goydos JS et al. 2003. *Detection of B-RAF and N-RAS mutations in human melanoma*. Journal of the American College of Surgeons, Vol. 200, Issue 3, 362-370.

[67] Maurer G, Tarkowski B and Baccarini M. 2011. *Raf kinases in cancer - roles and therapeutic opportunities*. Oncogene 30, 3477-3488.

[68] Dougherty MK et al. 2005. *Regulation of Raf-1 by direct feedback phosphorylation*. Mol. Cell 17, 215-224.

[69] Kamioka Y, Yasuda S, Yoshihisa F, Kazuhiro A, and Michiyuki M. 2010. *Multiple decisive phosphorylation sites for the negative feedback regulation of SOS1 via ERK*. J. Biol. Chem. 285, 33540-33548.

[70] Kochaczyk M et al. 2017. *Relaxation oscillations and hierarchy of feedbacks in MAPK signaling*. Sci. Rep. 7, 38244.

[71] Cirit M, Wang C and Haugh JM. 2010. *Systematic quantification of negative feedback mechanisms in the extracellular signal-regulated kinase (ERK) signaling network*. J. Biol. Chem. 285, 36736-36744.

[72] Schoeberl B, Eichler-Jonsson C, Gilles ED, Muller G. 2002. *Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors*. Nature Biotechnology 20, 370-375.

[73] Sharrocks DA. 2006. *Cell Cycle: Sustained ERK Signalling Represses the Inhibitors*. Curr Biol. 16(14):R540-2.

[74] Ebisuya M, Kondoh K and Nishida E. 2005. *The duration, magnitude and compartmentalization of ERK MAP kinase activity: mechanisms for providing signaling specificity.* Journal of Cell Science 118: 2997-3002.

[75] Sasagawa S, Ozaki Y-i, Fujita K, Kuroda S. 2005. *Prediction and validation of the distinct dynamics of transient and sustained ERK activation*. Nature Cell Biology 7, 365-373.

[76] Keyes J et al. 2020. *Signaling diversity enabled by Rap1-regulated plasma membrane ERK with distinct temporal dynamics*. eLife 9:e57410.

[77] Gillespie DT. 1976. *General method for numerically simulating stochastic time evolution of coupled chemical-reactions*. Journ. Comput. Phys., 22:403434.

[78] Gillespie DT. 1977. *Exact stochastic simulation of coupled chemical reactions*. Journ. Phys. Chem., 81(25):23402361.

[79] Gillespie DT. 2007. *Stochastic simulation of chemical kinetics*. Annu. Rev. Phys. Chem., 58:3555.

[80] Lecca P. 2013. Stochastic chemical kinetics. *A review of the modelling and simulation approaches*. Biophys Rev (2013) 5:323–345.

[81] Pettigrew DB, Smolen P, Baxter DA, Byrne JH. 2005. *Dynamic Properties of Regulatory Motifs Associated with Induction of Three Temporal Domains of Memory in Aplysia*. Journal of Computational Neuroscience 18, 163–181.

[82] Barbot B, Haddad S, Picaronny C. 2015. *Rare Event Handling in Signalling Cascades*. INT. J. Adv. Syst. Meas. 8(1-2), 69-79.

[83] Tian T, Harding A, Inder K, Plowman S, Parton RG, Hancock JF. 2007. *Plasma membrane nanoswitches generate high-fidelity Ras signal transduction*. Nature Cell Biology 9, Number 8, 905914.

[84] Vinod PK and Venkatesh KV. 2008. *Quantification of Signaling Networks*. Journal of the Indian Institute of Science, Vol. 88:1.

[85] Pal S, Baiocchi RA, Byrd JC, Grever MR, Jacob ST, Sif S. 2007. *Low levels of miR-92b/96 induce PRMT5 translation and H3R8/H4R3 methylation in mantle cell lymphoma*. The EMBO Journal 26, 3558-3569.

[86] Liang JJ et al. 2007. *The expression and function of androgen receptor coactivator p44 and protein arginine methyltransferase 5 in the developing testis and testicular tumors*. J Urol. 177(5):1918-22.

[87] Liu F et al. 2011. *JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation*. Cancer Cell 19(2): 283-294.

[88] DongSheng W et al. 2012. *PRMT5 suppresses DR4-mediated CCL20 release via NF-κB pathway*. Chin. Sci. Bull., Vol. 57, No. 33, 4351-4355.

[89] Yang M, Sun J, Sun X, Shen Q, Gao Z, Yang C. 2009. *Caenorhabditis elegans protein arginine methyltransferase PRMT-5 negatively regulates DNA damage induced apoptosis*. PloS Genet 5(6): e1000514.

[90] Scoummane A, Zhang J and Chen X. 2009. *PRMT5 is required for cell-cycle progression and p53 tumor suppressor function*. Nucleic Acids Research, Vol. 37, No. 15, 4965-4976.

[91] Calabretta S et al. 2018. *Loss of PRMT5 Promotes PDGFRα Degradation during Oligodendrocyte Differentiation and Myelination*. Dev Cell. 46(4):426-440.e5.

[92] Zhang B et al. 2015. *Targeting protein arginine methyltransferase 5 inhibits colorectal cancer growth by decreasing arginine methylation of eIF4E and FGFR3*. Oncotarget 6(26):22799-811.

[93] Strobl CD et al. 2019. *Selective PRMT5 Inhibitors Suppress Human CD8β T Cells by Upregulation of p53 and Impairment of the AKT Pathway Similar to the Tumor Metabolite MTA*. Mol Cancer Ther 19(2), 409-419.

[94] Lin H et al. 2019. *Discovery of Potent and Selective Covalent Protein Arginine*

Methyltransferase 5 (PRMT5) Inhibitors. ACS Med. Chem. Lett., 10, 7, 1033-1038.

[95] Brightman FA, Fell DA. 2000. *Dfferential feedback regulation of the MAPK cascade underlies the quantitative differences in EGF and NGF signaling in PC12 cells*. FEBS Letters 482, 169-174.

[96] Traverse S, Gomez N, Paterson H, Marshall C, Cohen P. 1992. *Sustained activation of the mitogenactivated protein (MAP) kinase cascade may be required for differentiation of PC12 cells. Comparison of the effects of nerve growth factor and epidermal growth factor.* Biochem. J 288, 351–355 (1992).

[97] Kao S, Jaiswal RK, Kolch W, Landreth GE. 2001. *Identification of the mechanisms regulating the differential activation of the MAPK cascade by epidermal growth factor and nerve growth factor in PC12 cells*. J. Biol. Chem 276, 18169–18177.

[98] Jansson M et al. 2008. *Arginine methylation regulates the p53 response*. Nat. Cell Biol. 10(12), 1431–1439.

[99] Lazarovici P, Dickens G, Kuzuya H, and Guroff G. 1987. *Long-Term, Heterologous Down-Regulation of the Epidermal Growth Factor Receptor in PC12 Cells by Nerve Growth Factor.* The Journal of Cell Biology, Volume 104, 1611-1621.

[100] Hondermarck H, Mclaughlin CS, Patrersont SD, and Bradshaw RA. 1994. *Early changes in protein synthesis induced by basic fibroblast growth factor, nerve growth factor, and epidermal growth factor in PC12 pheochromocytoma cells.* Proc. Nati. Acad. Sci. USA, Vol. 91, 9377-9381.

[101] Mouri K, Sako Y. 2013. *Optimality conditions for cell-fate heterogeneity that maximize the effects of growth factors in PC12 cells.* PLoS Comput Biol 9(11): e1003320.

[102] Brennan DF, Dar AC, Hertz NT, Chao WCH, Burlingame AL, Shokat KM, Barford D. 2020. *A Raf-induced allosteric transition of KSR stimulates phosphorylation of MEK*. Nature, Vol. 472, 366-371.

[103] Hymowitz SG and Malek S. 2018. *Targeting the MAPK Pathway in RAS Mutant Cancers*. Cold Spring Harb Perspect Med;8:a031492.

[104] Fisher KW et al. 2015. *AMPK promotes aberrant PGC1beta expression to support human colon tumor cell survival*. Mol Cell Biol. 35:3866–3879.

[105] Zhang J et al. 2008. *Downregulation of KSR1 in pancreatic cancer xenografts by antisense oligonucleotide correlates with tumor drug uptake*. Cancer Biol Ther. 7:1490–1495.

[106] Witzel F, Maddison L and Blüthgen N. 2012. *How scaffolds shape MAPK signaling: what we know and opportunities for systems approaches*. Frontiers in Physiology 3:475.

[107] Vetterkind S, Poythress RH, Lin QQ, Morgan KG. 2013. *Hierarchical scaffolding of an ERK1/2 activation pathway*. Cell Communication and Signaling 11:65.

[108] Casar B and Crespo P. 2016. ERK Signals: Scaffolding Scaffolds? Front. Cell. Dev. Biol. 4:49.

[109] Shin S-Y et al. 2009. *Positive- and negative-feedback regulations coordinate the dynamic behavior of the Ras-Raf-MEK-ERK signal transduction pathway*. Journal of Cell Science 122, 425-435.

[110] Kochanczyk M et al. 2017. *Relaxation oscillations and hierarchy of feedbacks in MAPK signaling*. Scientific Reports 7:38244.

[111] Lim SK et al. 2013. *Activation of PRMT1 and PRMT5 mediates hypoxia- and ischemia-induced apoptosis in human lung epithelial cells and the lung of miniature pigs: the role of p38 and JNK mitogen-activated protein kinases*. Biochem. Biophys. Res. Commun. Vol. 44, Issue 4, 707-713.

[112] He W et al. 2008. *Ginkgolides mimic the effects of hypoxic preconditioning to protect C6 cells against ischemic injury by up-regulation of hypoxia-inducible factor-1 alpha and erythropoietin*. Int. J. Biochem. Cell Biol. 40(4):651-62.

[113] Ho M-C et al. 2013. *Structure of the Arginine Methyltransferase PRMT5-MEP50 Reveals a Mechanism for Substrate Specificity*. PLoS ONE 8(8).

[114] Burgos ES et al. 2015. *Histone H2A and H4 N-terminal Tails Are Positioned by the MEP50*

WD Repeat Protein for Efficient Methylation by the PRMT5 Arginine Methyltransferase. J. Biol. Chem. 290(15):9674-89.

[115] Chen H, Lorton B, Gupta V, Shechter D. 2017. *A TGFβ-PRMT5-MEP50 axis regulates cancer cell invasion through histone H3 and H4 arginine methylation coupled transcriptional activation and repression*. Oncogen, 36(3):373-386.

[116] Mongiardi MP et al. 2015. *Myc and Omomyc functionally associate with the Protein Arginine Methyltransferase 5 (PRMT5) in glioblastoma cells*. Scientific Reports 5(1):15494.

[117] Aggarwal P et al. 2010. *Nuclear Cyclin D1/CDK4 Kinase Regulates CUL4 Expression and Triggers Neoplastic Growth via Activation of the PRMT5 Methyltransferase*. Cancer Cell 18(4): 329-340.

[118] Owens JL et al. 2020. *PRMT5 Cooperates with pICln to Function as a Master Epigenetic Activator of DNA Double-Strand Break Repair Genes*. Iscience 23, 100750.

[119] Rehman I et al. 2018. *PRMT5-mediated arginine methylation of TDP1 for the repair of topoisomerase I covalent complexes*. Nucleic Acids Research, Vol. 46, No. 11, 5601-5617.

[120] Hwang JW et al. 2020. *PRMT5 promotes DNA repair through methylation of 53BP1 and is regulated by Src-mediated phosphorylation*. Communications Biology, 3:428.

[121] He W, Ma X, Yang X, Zhao Y, Qiu J, and Hang H. 2011. *A role for the arginine methylation of Rad9 in checkpoint control and cellular sensitivity to DNA damage*. Nucleic Acids Research, Vol. 39, No. 11, 4719-4727.

[122] Berman HM et al. 2020. *The Protein Data Bank. Nucleic Acids Research,* Volume 28, Issue 1, Pages 235–242.

[123] Pettersen EF et al. 2021. *UCSF ChimeraX: Structure visualization for researchers, educators, and developers*. Protein Sci. 30(1):70-82.

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Stochastic modulation evidences a transitory EGF-Ras-ERK MAPK activity induced by PRMT5

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Abstract.

The extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) pathway involves a three-step cascade of kinases that transduce signals and promote processes such as cell growth, development, and apoptosis. An aberrant response of this pathway is related to the proliferation of cell diseases and tumors. By using simulation modeling, we document that the protein arginine methyltransferase 5 (PRMT5) modulates the MAPK pathway and thus avoids an aberrant behavior. PRMT5 methylates the Raf kinase, reducing its catalytic activity and thereby, reducing the activation of ERK in time and amplitude.

Two minimal computational models of the epidermal growth factor (EGF)-Ras-ERK MAPK pathway influenced by PRMT5 were proposed: a first model in which PRMT5 is activated by EGF and a second one in which PRMT5 is stimulated by the cascade response. The reported results show that PRMT5 reduces the time duration and the expression of the activated ERK in both cases, but only in the first model PRMT5 limits the EGF range that generates an ERK activation. Based on our data, we propose the protein PRMT5 as a regulatory factor to develop strategies to fight against an excessive activity of the MAPK pathway, which could be of use in chronic diseases and cancer.

Keywords.

Cell response modulation, cancer, MAPK pathway, EGF-Ras-ERK signaling route, PRMT5, methylation, computational model.

1. Introduction.

Biological processes occurring in the microscale, or even in the nanoscale, play key roles in cells and living organisms. A great amount of those processes drive different behaviors' patterns, which can be altered by local shifts induced by individual molecules. In such a scenario, cell signaling pathways are responsible for the transmembrane signal transduction. Those pathways facilitate the sensing, integration and processing of extracellular information through the plasma membrane, generating robust high-fidelity responses [1, 2].

A common motif of such signaling pathways is represented by a cascade structure of a sequential activation of three mitogen-activated protein kinases (MAPKKK, MAPKK and MAPK) that act as messengers, driving extracellular signals to the inner of the cell [3-7]. The active MAPK is delivered to the cytosol, reaching the nucleus of the cell, for further activation of transcription factors, collaborating in this way in the regulation of the cell activity [8-10]. In detail, the extracellular signal-regulated kinase 1/2 MAPK (ERK1/2 MAPK) pathway features K-Ras as the G-protein, Raf-1 as the MAPKKK, MEK1/2 as the MAPKK, and ERK1/2 as the MAPK [10, 11] (Suppl. Fig. S1). The response of such a signaling pathway to the epidermal growth factor (EGF) is the expression of the double phosphorylated ERK1/2 ($_{pp}$ERK1/2) (Suppl. Fig. S2).

The ERK MAPK pathway generates different response behaviors, including grading, oscillation, bistability (switching between alternate states), and ultrasensitivity, and such behaviors can be influenced by the type and activity of the cell. Thus, an all-or-none switch-like response of the ERK MAPK route was reported in Xenopus oocytes, whilst a graded response of this pathway was observed in mammalian cells. Such data suggests the existence of mechanisms different from phosphorylation that promote the high-fidelity in ERK signals, such as positive and negative feedback loops, cross-talk between signaling pathways, and associations between protein partners [2, 3, 12-15]. In addition, different types of stimuli (such as EGF and NGF) induce both graded ERK phosphorylation and switch-like ERK nuclar translocation. Thereby, the conversion of graded ERK phosphorylation into a switch-like ERK nuclear translocation may be a property in growth factor-induced ERK signaling [15]. The sensitivity of the ERK MAPK signaling pathway is a key factor in the cell fate decision, but also the pattern of its temporal response is involved [16, 17].

In detail, in the EGF-Ras-ERK1/2 MAPK pathway, a maximum response sustained in time can lead to undesirable cell behaviors [18-20]. In biological microenvironments, many molecules interact with each other in the framework of this signaling pathway, establishing sophisticated dynamics that regulate specific response patterns, affecting different cell processes such as migration, proliferation, and differentiation. However, this fragile network can be easily disturbed by small and local alterations, resulting in aberrant responses related to the proliferation of cell diseases and tumors.

Hence, specific molecular motifs are capable of modifying the dynamics of some kinases, modulating the time response of the MAPK pathway, altering the overall cell response [21-23]. This is the case of protein arginine methyltransferases (PRMTs), a family of intracellular enzymes localized in mammalian cells [24]. PRMTs play important roles in regulating signal transduction, promoted by different growth factors affecting distinct cell types [23, 25, 26]. The protein PRMT5, one of the nine components (from PRMT1 to PRMT9) of the PRMTs family in humans, achieves great shifts in the pattern of the EGF-Ras-ERK1/2 MAPK response, avoiding inappropriate cell behaviors such as the tumorigenesis. However, literature so far does not recognize PRMT5 as a tumor suppressor gene, but a key factor for regulating the activity of several tumor suppressors in distinct cancer cell lines. Indeed, PRMT5 is an epigenetic enzyme involved in different physiological processes, including transcriptional regulation, RNA metabolism and cell cycle regulation [27]. Increasing evidence suggests that PRMT5 plays a complex role in oncogenesis, controlling the expression of genes implicated in both tumor promotion and suppression. Mainly, cellular context, tissue dependence and the localization within the cell determine what function the protein PRMT5 performs [28-30]. Thereby, a large number of studies give evidences of PRMT5 overexpression in different cancer types and its role as a potential oncoprotein involved in tumorigenesis by promoting the proliferation, differentiation, invasion, and migration of cancer cells, being able of silencing its target tumor suppressor genes [31-35]. Nevertheless, other studies had showed opposite effects of PRMT5, being an important regulatory factor of cancer progression, inhibiting tumor cell proliferation, migration and invasion. Therefore, oncogenic properties of PRMT5 trigger shifts required for reprogramming normal cell growth to malignant cell activity and metastasis. But PRMT5 is also needed for normal development and differentiation. Then, reported data suggests that PRMT5 may become a potential diagnostic biomarker and a therapeutic target for various cancers, but no references recognize PRMT5 as a cancer suppressor gene.

The action of PRMT5 over the EGF-Ras_ERK1/2 MAPK cascade consists of the methylation of the active Raf kinase, reducing its catalytic activity and, thereby, limiting the $_{pp}$ERK1/2 expression. The activated Raf methylation through PRMT5 is comparable to the DNA methylation. Both processes are driven by the methylation of arginine residues. PRMTs catalyze the methylation of

arginine residues of a diverse group of proteins that can be monomethylated, symmetrically dimethylated, or asymmetrically dimethylated at the terminal nitrogen atoms of the guanidinium side chain of the arginine residue [36]. One clear example is the methylation on histone arginine residues, which can promote DNA replication, DNA damage response, and the activation or repression of gene transcription. In detail, PRMT5 catalyzes monomethylation and symmetric dimethylation of arginine residues in histones H2A and H4 at residue R3, and histone H3 at residues R2 and R8 [37]. Through histone methylation, PRMT5 is involved in different cellular processes, including transcriptional regulation, germ cell development and gene expression regulation [36, 38-40]. In addition, PRMTs promote one of the most extensive protein methylation modification in mammalian cells: the modification of arginine chain side guanidine groups (NH_2). Arginine is an amino acid that its guanidine group contains five potential hydrogen bond donors, which are able of interacting with hydrogen bond acceptors [23]. In detail, the arginine side chain presents two terminal guanidine groups that can be subjected methylation (Suppl. Fig. S3). And PRMTs remove one methyl group from the donor molecule S-adenosyl-L-methionine (AdoMet) and they transfer it to the terminal nitrogen of the guanidine group of an arginine residue in the target protein [41]. Although some research has reported that PRMT-mediates methylation of proteins impacts on stability, the mechanisms responsible may differ from case to case. Thus, it has been reported that PRMT5 up-regulated the protein levels of the anti-apoptotic protein CFLARL by decreasing the ubiquitination and increasing its protein level [42]. Alternatively, it has been reported that arginine methylation modulates autophagic degradation of PGC granules in C. elegans [43]. In this connection, the mechanisms by which PRMT5 impacts on CRaf and BRaf stability remain unknown. What is known so far is that PRMT5 triggers a symmetric dimethylation of arginine residues $BRaf^{R671}$ and $CRaf^{R563}$ within the -Gly-Arg-Gly- (-GRG-) motif upon growth factor stimulation, which is highly conserved in all the Raf isoforms [44, 45] and where two terminal guanidine groups (NH_2) susceptible to being methylated are found (see Suppl. Fig. S4 with the crystal structure of CRaf and its highlighted Arg^{563} residue). In fact, the Raf kinase domain consists of an amino-terminal lobe (N-lobe) and a carboxy-terminal lobe (C-lobe) that are connected through a flexible hinge region [46]. The C-lobe is comprised primarily of α-helices and contains the activation segment. In this C-terminal catalytic domain, the arginine residue where the protein PRMT5 binds to further methylate the Raf kinase is found. The addition of methyl groups do not change the charge of proteins but mask polar arginines, removing amino hydrogens and further blocking the ability to form hydrogen bonds, promoting inhibition of some binding partners. Such blocked hydrogen-bonding groups become more hydrophobic, directly altering protein-protein, protein-DNA, and protein-RNA interactions [47-49]. Therefore, the methylation of arginine residues in proteins can modulate their binding capabilities, decreasing the stability of Raf proteins

and targeting them to the proteasome [50-52]. Otherwise, inhibition of PRMT5 increased the activity of Raf and enhanced the phosphorylation of $CRaf^{Ser621}$, which may be required to avoid proteasome-mediated CRaf degradation [53]. In this way, the PRMT5 has the potential to reduce an important source of cellular dysfunction and disease: the persistence in time of a maximum inappropriate level of the EGF-Ras-ERK1/2 MAPK output response [18-20, 40]. This regulatory effect of PRMT5 on the $_{pp}$ERK1/2 output response is similar to the role of the mitogen-activated protein (MAP) kinase phosphatases (MKPs or DUSPs), which dephosphorylate MAPK, reducing its activity and switching from a sustained response to a more transitory one [54, 55]. One example is MKP-3 (also known as DUSP6), an ERK target gene that forms autoregulatory loops in response to sustained $_{pp}$ERK signals, attenuating its activity and yielding to a more transient response [56, 57]. Furthermore, there are evidences of decreased MKP expression in malignant disease, suggesting that these phosphatases play key roles in cancer regulation [54]. Thereby, the loss, deletion or inhibition of MKPs is related to a sustained activation of MAPKs [54, 58, 59].

The purpose of the present work is to develop a model approach of the EGF-Ras-ERK1/2 MAPK signaling pathway. We want to shed light on the influence of the PRMT5 on the regulation and tuning of the pathway response through a methylation process of the Raf-1, which modulates the time pattern of the $_{pp}$ERK1/2 and triggers the final cell fate decision [45]. Unfortunately, there is a lack of knowledge on the misfit of the PRMT5 protein to the signaling cascade that we aim to investigate. It is experimentally observed that the PRMT5 activity increases upon activation of the signaling cascade [45, 60]. But, it is not possible to differentiate if the PRMT5 activation is promoted either by the same input stimulus or by the pathway output response. To explore this challenge, the time output responses of two different configurations of the EGF-Ras-ERK1/2 MAPK cascade influenced by the activity of PRMT5 are analyzed: 1) a model with the PRMT5 activity regulated by EGF and 2) another one with the PRMT5 activity regulated by $_{pp}$ERK1/2. In addition, the reported results are aimed to clarify if $_{pp}$ERK1/2 response patterns belonging to experimental research can be modulated by the PRMT5 protein and, moreover, to identify if its activity is controlled either by the input stimulus or by the output response of this signaling pathway. Here we provide computational evidence for the regulatory role of the PRMT5 on the ERK-1/2 MAPK activity by limiting its amplitude and duration, as well as the range of EGF stimulus able of generating MAPK response. Thereby, to identify activators of this methyltransferase should be of interest as drug targets for MAPK-related indications.

PRMT5 methylates Ras effectors BRaf and CRaf [45], which in turn bind to HRas [61], KRas [45, 62-64] and NRas [65-67]. So, from now on, to simplify the nomenclature, any of the three isoforms of Ras will be named simply as Ras, the two isoforms of Raf that are methylated by PRMT5 (BRaf and CRaf or Raf-1) will be named as Raf, MEK1/2 as MEK, and ERK1/2 as ERK.

2. Methods.

2.1. Models

The proposed model for the present study (called from now as reference model) corresponds to a simplified version of the ERK MAPK signaling pathway defined by *Sturm OE et al* [3] (Fig. 1A). Briefly, it consists of the three-level kinase Raf-MEK-ERK MAPK cascade with a regulatory negative feedback loop from the output response ($_{pp}$ERK) to the Raf activation process (Fig. 1B), which is defined in a comparable way to the system of the two different feedback loops described in the model of *Sturm OE et al* [3], but starting at the level of MAPKKK instead of at the upper level of the signaling pathway (the obtained responses of both models can be compared in Suppl. Fig. S5).

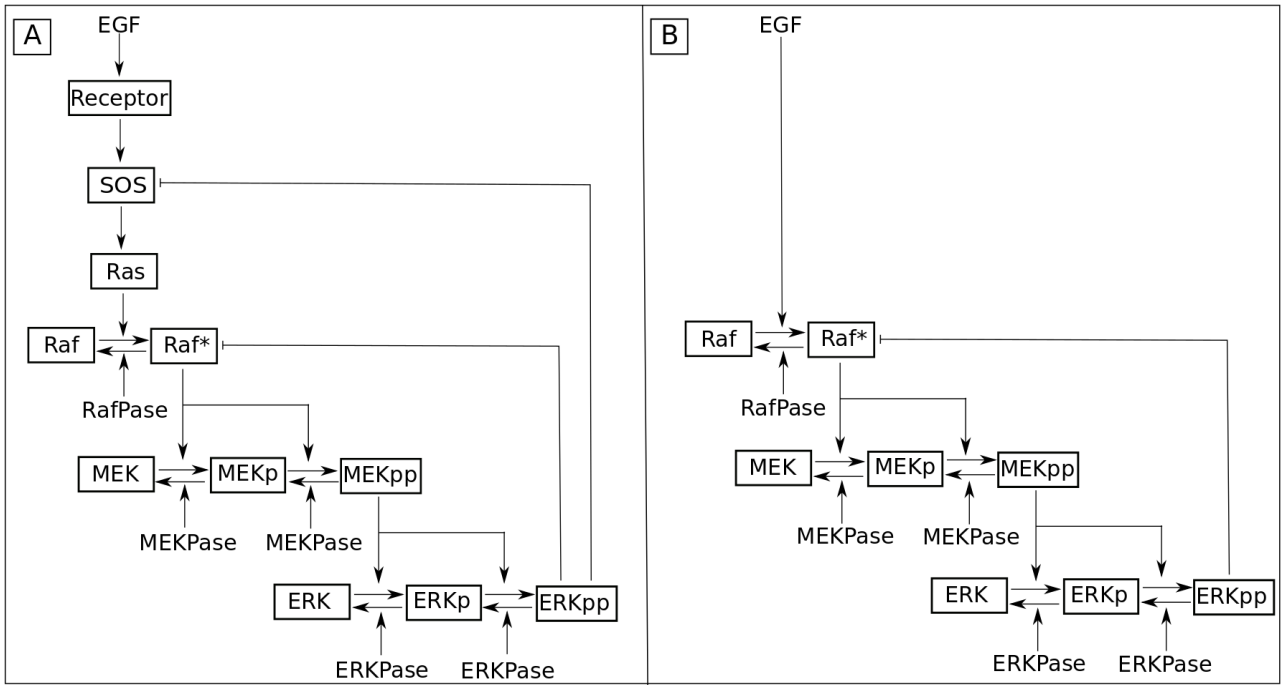

FIGURE 1: **Schematic representations of the Ras-ERK MAPK signaling pathway.**
(A) Schematic representation of the Ras-ERK MAPK signaling pathway with the three levels of kinases (Raf, MEK and ERK) proposed by *Sturm OE et al* [3], presenting two regulatory feedbacks from the output $_{pp}$ERK to SOS and Raf. **(B)** Schematic representation of the reference model, formed by the Ras-ERK MAPK signaling pathway with the three levels of kinases (Raf, MEK and ERK) and one negative feedback from the output $_{pp}$ERK to Raf, being equivalent to both negative feedbacks of the previous model (Fig. 1A). In both representations, the corresponding kinases phosphatases of Raf, MEK and ERK, as well as the input stimulus EGF are defined.

The implemented feedback loop from $_{pp}$ERK promotes a hyperphosphorylation of multiple serine sites of Raf-1 (S29, S289, S296, S301, and S642) after mitogen stimulation, in a manner

similar to Raf-1 inactivation, but rendering an inactive and desensitized Raf-1 that presents reduced affinity for Ras-GTP and low response to subsequnt activation processes [68]. This feedback loop is defined within the whole pathway by a Michaelis-Menten process through next reactions (Eq. 1):

$$Raf^{*} + {}_{pp}ERK \leftrightarrow Raf^{*}-{}_{pp}ERK \rightarrow Raf + {}_{pp}ERK \qquad \text{(Eq. 1)}$$

where Raf^{*} is the activated state of the Raf isoform. The reaction rates of this regulatory mechanism have been selected following those found in the literature [3, 68-71], where a strong negative feedback loop from ERK to Raf was reported.

In addition to this regulatory mechanism, we have considered that the phosphatases of the kinases Raf, MEK, and ERK (RafPase, MEKPase and ERKPase) deactivate each corresponding kinase in all its possible activation states.

The experimental values of the concentrations of the components and the cascade parameters correspond to those described by *Schoeberl B et al* [72], in which *Sturm OE et al* [3] model is mainly based from a parametric point of view [Suppl. Tables I and II]. Regarding the reaction rates, they were defined from other experimental scientific publications that reported real measures for the parameters or, failing that, suggested experimental estimations or biochemical quantifications. As to the cascade's components, their concentrations were expressed by the number of signaling proteins per cell, which were determined by biochemical quantification through western blotting.

Then, the proposed reference model considered in our study (Schema B of Figure 1) only presents two possible variable parameters: the concentration of the input stimuli *EGF* and the forward reaction constant of the negative feedback loop from ${}_{pp}ERK$ to Raf, defined as a_{11} and related to the impact of the negative feedback loop. Therefore, to avoid uncertainties in the validation of the proposed computational model for the EGF-Ras-ERK MAPK signaling pathway (Schema B of Figure 1), simulations varying these two parameters were carried out (Suppl. Fig. S6). Then, the model was simulated considering:

a) different values of *EGF* Є [2414, $1e^{6}$] molecules/cell (no EGF was not considered since no ERK signal is triggered)

b) three different values of a_{11}: 0 , $1e^{-5}$ and $1e^{-6}$ $(molecules/cell)^{-1}\cdot s^{-1}$

Regarding the input stimulus EGF, shifts in its values triggered shifts in the ${}_{pp}ERK$ response, regardless the value of the parameter a_{11}. The same way, changes in the reaction constant a_{11}

promoted modifications in the amplitude of the $_{pp}$ERK signal. Despite these variability in the results, regardless the conditions of EGF and a_{11}, all $_{pp}$ERK responses are ranged between a minimum and a maximum response, without presenting uncontrolled values. Moreover, notice that for very high values of EGF, the $_{pp}$ERK response was practically unchanged because a saturation state was reached (Suppl. Fig. S6).

In detail, simulations (Suppl. Fig. S5) reveal that the reference model proposed for the present study approaches to the response of the Ras-ERK MAPK model defined by *Sturm OE et al* [3], to a greater or lesser extent depending on the value of the parameter a_{11}. Indeed, these results suggest that the effect of the negative feedback loop of the *Sturm OE et al*'s model [3] is comprised between the results reported by the reference model for both values of a_{11}: $1e^{-6}$ (molecules/cell)$^{-1}$ $\cdot s^{-1}$ and $1e^{-5}$ (molecules/cell)$^{-1}$ $\cdot s^{-1}$. Thereby, considering the evidence of a strong negative feedback loop from ERK to Raf [3, 68-71], the reference model with a_{11} = $1e^{-5}$ (molecules/cell)$^{-1}$ $\cdot s^{-1}$ is selected to develop the simulations of the present study.

Furthermore, reported results indicated that the response patterns of our proposed model, with or without the $_{pp}$ERK negative feedback loop, fit qualitatively with the published results of *Sturm OE et al* [3] (Suppl. Fig. S5), even when this last model presents more cascade levels, more feedback loops and, thereby, more reactions (Schema A of Figure 1). In fact, these results revealed that the $_{pp}$ERK negative feedback acts as an inhibitor of the activated Raf, reducing the stepness and the ultrasensitivity of the output signal of the Ras-ERK MAPK cascade [3], increasing its negative effect on the output signal as its forward reaction (a_{11}) increases, since greater extension of activated Raf was inhibited.

All these data suggest that the pattern of the ERK response upon EGF stimulation obtained with the proposed minimum model of the EGF-Ras-ERK MAPK cascade was qualitatively similar to those of published studies that pointed to the sustained activation of ERK as responsible for specific cellular processes [73-76]. Then, the three-step of the signaling cascade and the $_{pp}$ERK-induced negative feedback loop on Raf may be considered as the main players responsible of the sustained $_{pp}$ERK response of this signaling route. Therefore, we suggest that our proposed minimum model of the EGF-Ras-ERK MAPK cascade is valid to perform computational studies of this signaling route.

To this reference model, the protein PRMT5 proposed by *Andreu-Pérez P et al* [45] has been added as an inhibitor of the activated Raf (Raf^*), degrading its expression through the next reaction velocity (Eq. 2):

$$a_{PRMT5} = [PRMT5]\cdot[Raf^*]\cdot k_{PRMT5}\cdot \alpha_{PRMT5} \quad \text{(Eq.2)}$$

where [PRMT5] and [Raf^*] are the concentrations of the PRMT5 and the activated Raf respectively,

and k_{PRMT5} is considered to be the catalytic rate constant of the reaction that promotes the degradation of activated Raf through the methylation process exerted by the protein PRMT5, defining the rate-limiting step of this conversion process. This process is described as a biomolecular reaction involving te collision of these two molecules (activated Raf and PRMT5), and the rate of the reaction depends on the product of the concentrations of both involved reactants, triggering a second-order reaction. This reaction rate is not necessary to be constant, and there are different factors that can affect it, such as the reactant concentrations, cell type and context, pressure, temperature, chemical nature of reacting substances, physical state of reactants and their dispersion, surface areas of reactants, among others. And such variability is aimed to be reflected in the parameter k_{PRMT5}, which can take different values and defines the strength of the PRMT5 action. Regarding the protein PRMT5, its concentration was considered as the same of the Raf, in order to establish a relation 1:1 and ensure that no Raf could not be methylated. In addition, the PRMT5 activity is considered to be regulated either by the input stimulus EGF or by the current $_{pp}$ERK output. And the parameter α_{PRMT5} has been defined to expressed such dependence. Therefore, two different models have been described: the model A (Fig. 2, red dashed lines) where the activity of the PRMT5 depends positively on EGF, defining $\alpha_{PRMT5(EGF)}$ as the ratio between the applied EGF (EGF_t) and the maximum considered one (EGF_{max}); and the model B (Fig. 2, blue dashed lines) where the activity of the PRMT5 depends positively on the $_{pp}$ERK output, with $\alpha_{PRMT5(ppERK)}$ defined as the ratio of the current $_{pp}$ERK concentration ($_{pp}ERK(t)$) over its maximum possible value, corresponding to the total amount of the initial ERK ($_{pp}ERK_{max}$). Then, the effective constant rate of the Raf^* degradation exerted by PRMT5 is defined as $k_{PRMT5} \cdot \alpha_{PRMT5}$.

The reference model consists of 33 reactions and the activity of the PRMT5 involves one more reaction. These reactions are described in Suppl. Table III.

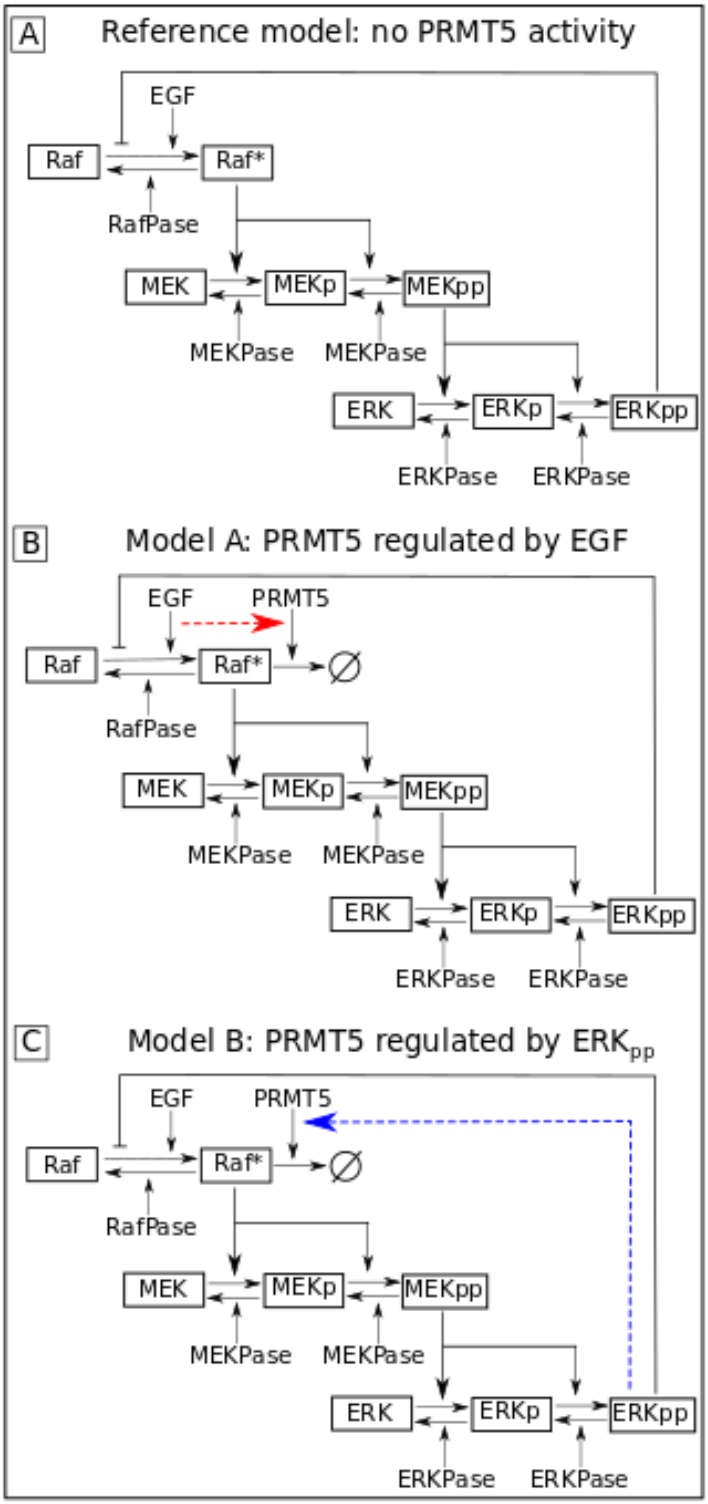

FIGURE 2 (color version required for printing): **Schematic representation of the Ras-ERK MAPK signaling pathway for each considered models.**

(A) Reference model: schematic view of the Ras-ERK MAPK signaling pathway with the three levels of kinases (Raf, MEK and ERK) and the $_{pp}$ERK negative feedback. No PRMT5 activity is present. **(B)** Model A: reference model presenting a regulatory mechanism of the $_{pp}$ERK response through the Raf* degradation process caused by the PRMT5 controlled by the EGF (red dashed line). **(C)** Model B: reference model presenting a regulatory mechanism of the $_{pp}$ERK response through the Raf* methylation exerted by the PRMT5 controlled by the $_{pp}$ERK (blue dashed line). In these two last models, the protein PRMT5 acts as inhibitor of the active Raf (Raf*).

2.2. Simulations

All the model simulations have been developed with a stochastic approach similar to Gillespie's Stochastic Simulation Algorithm (SSA), treating the cascade system as a group of stochastic events [77-79]. The different signaling events have been defined as Poissonian processes with a frequency characterized by the corresponding reaction rates. And all the models have been implemented in the Fortran programming language (Force 2.0 + GNU Fortran 77).

The concentration of the input stimulus EGF and the parameter k_{PRMT5} that regulates the Raf^* degradation activity exerted by the PRMT5 were the only parameters that were changed in the study. The values of the rest of the components concentrations and reaction rates belonging to the considered models have been fixed (summarized in Suppl. Tables I and II, respectively). Thus, with these two variable parameters is possible to regulate the dynamics of the EGF-Ras-ERK MAPK response, acting on the first level of the cascade.

In short, the input stimulus EGF has been gradually increased from 0 to 10^6 molecules, presenting a wide range of values that allows us to define accurately the response behavior of the signaling cascade. There is a common use in developing molecular simulations defining the actors of the computational model with its molar concentration (M = mol/L of dissolution). This option is valid for a deterministic model since it is based on the concentrations of the involved species. However, a stochastic model presents a factor representing the number of molecules of the species, an aspect that gives a higher resolution of the simulations at low species' concentrations [80]. Furthermore, if units of species are expressed in M (mol/L), the simulations become dependent on a factor that is not relevant in the present study, the amount of dissolution (liters), which is a more important factor in experimental studies. Thereby, in the computational models used in the present study, all species (including kinases, phosphatases, and input stimuli) are expressed in molecules rather than M (mol/L).

Three different values of k_{PRMT5} have been selected to modulate the effect of the PRMT5 in the proposed signaling models A and B: 0.0001, 0.0002, and 0.0006 (cell/molecules)$\cdot s^{-1}$. In this way, the effect of the strength of the Raf^* methylation process on the $_{pp}$ERK expression can be analysed. Such values have been selected presenting a similar magnitude order to the other constant rates involved in the first step of the cascade, where the PRMT5 exerts its action (since no experimental evidences of such reaction rate have been reported so far).

The reference model was simulated for enough time to ensure that the system reaches a steady-state (about 2000 seconds). However, the models A and B were simulated until the $_{pp}$ERK response was extinguished.

2.3. Analysis

To characterize the response of the EGF-Ras-ERK MAPK cascade, the time responses ($_{pp}$ERK vs. time), and the maxima amplitudes of the $_{pp}$ERK response as a function of EGF ($_{pp}ERK_{max}$ vs. EGF) were analyzed for the three models (reference model, model A and model B). However, the $_{pp}$ERK response areas and the $_{pp}$ERK time durations as a function of EGF were plotted for both models A and B, since their output responses expired in time. Alternatively for the reference model, the steady-state $_{pp}$ERK response depending on EGF was represented, due to that its responses are not finite in time. To clarify, the response area was defined for the present research as the integration of the $_{pp}$ERK amplitude along the time at which the output signal is maintained over the threshold of the 10% of its maximum value, for each input stimulus. This consideration is related with two different topics: i) the requirement of a minimum $_{pp}$ERK level to consider a functional cell activation [81-83] and ii) the consideration that the stimulus/response curve of a Michaelis-Menten enzyme, such as the case of the $_{pp}$ERK response, is hyperbolic and, thereby, its sensitivity is measured with the Hill coefficient, which quantifies the fold change in the input stimuli (EGF) required to drive the response ($_{pp}$ERK) from 10% activation to 90% activation of its maximum value [7, 84].

3. **Results.**

Time evolutions of the EGF-Ras-ERK MAPK cascade responses ($_{pp}$ERK vs time) have been analysed either in the presence or absence of PRMT5, in order to highlight its role in the regulation of the signaling cascade output. The main obtained evidences are that the protein PRMT5 allows to modulate the cascade response reducing its time duration and amplitude, as well as limiting the range of input stimuli able of generating an output response of the pathway.

3.1. MAPK activity in the absence of PRMT5 (reference model)

In the reference model, the response reaches a steady-state for any input stimulus value (Fig. 3A and Suppl. Fig. S8A).

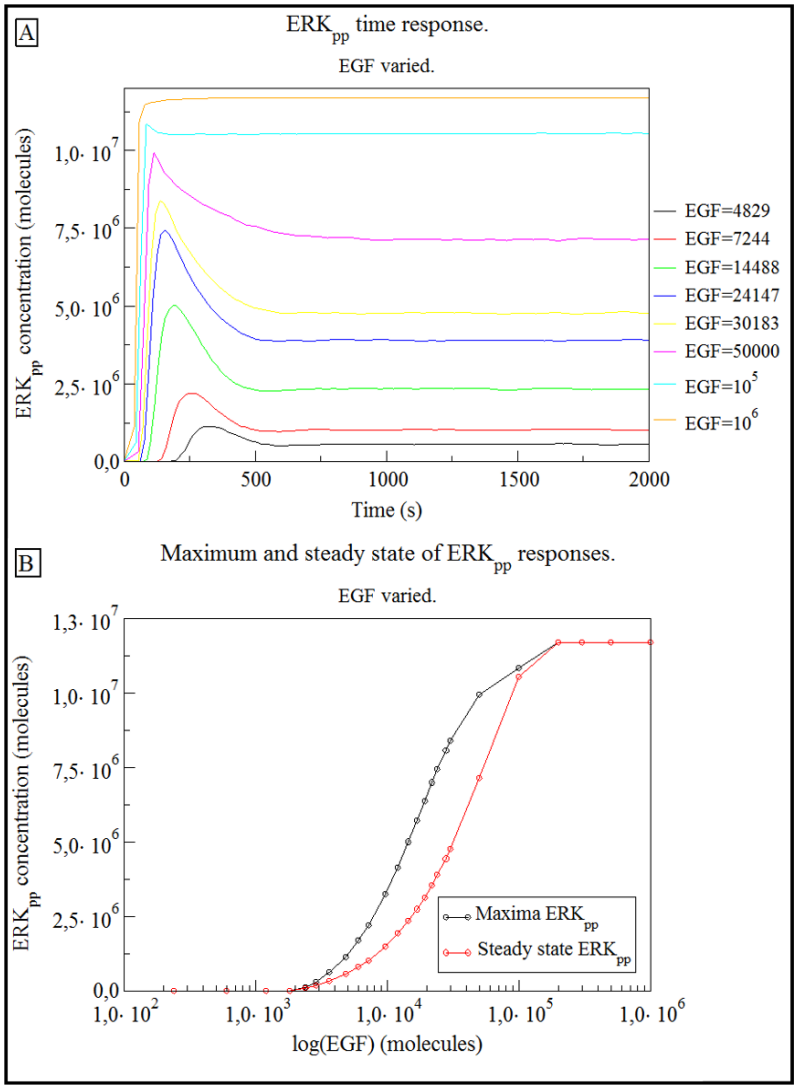

FIGURE 3 (color version required for printing): **Time responses, maxima amplitudes and steady state of the ppERK response in the reference model.**

(A) ppERK time responses of the reference model. Each colour line represents the time evolution of the ppERK concentration for each different EGF value. **(B)** The maximum concentrations (black line) and the steady state values (red line) of the ppERK responses of the reference model as functions of EGF (represented in a semilogarithmic scale). For both figures, EGF is defined from 0 to 10^6 molecules (the considered values of EGF to simulate the output response are summarized in the graph legend). The components concentrations and the reaction rates used in simulations are described in Suppl. Tables I and II.

The model does not present any mechanism of degradation of any of the cascade's components, which could result in the extinction of the output response (there are only deactivation processes of kinases species). For each different EGF value, the ppERK expression starts to increase until reaching a maximum value, promoted by Raf*, which is responsible for activating the downstream cascade levels of MEK and ERK. Such Raf* activation depends directly on the input stimulus (Fig.

3A). Next, when the $_{pp}$ERK response reaches enough high level, the deactivation of Raf* induced by the negative feedback takes relevance over the Raf activation process exerted by EGF, causing, in turn, a decrease in the production of $_{pp}$ERK. In addition, the phosphatases within the MAPK cascade (RafPase, MEKPase, and ERKPase) deactivate the corresponding kinases Raf, MEK, and ERK in all its possible activation states, inducing a slowdown in the production of $_{pp}$ERK. Thereby, the output response decreases except for very high EGF values where saturated levels of Raf* are reached (Fig. 3A). Finally, the $_{pp}$ERK response reaches a steady-state, which is a result from the balance between the Raf activation induced by EGF, the Raf* deactivation process performed by the $_{pp}$ERK negative feedback, and the deactivation of the active species of Raf, MEK, and ERK through the corresponding phosphatases (Fig. 3A and Suppl. Fig. S8A).

To expand the analysis of the reference model, both maximum and steady-state $_{pp}$ERK responses as functions of EGF have been analysed (Fig. 3B). Such responses increase with the stimulus, until reaching maxima values for EGF $\geq$ EGF$_{Th}$, where EGF$_{Th}$ is defined as the threshold value of EGF required to obtain the same maximum output signal, that is when the maximum value of $_{pp}$ERK is equal to its steady-state value (Fig. 3B). However, these metrics are different for the values of EGF < EGF$_{Th}$, and such differences are due to the role of the $_{pp}$ERK negative feedback on the deactivation of Raf*.

3.2. MAPK activity in presence of PRMT5

The protein PRMT5 switches the steady-state of the EGF-Ras-ERK MAPK output response into a time-limited pattern, through a methylation process that promotes the degradation of the Raf*, limiting the further activation of its downstream substrates of the cascade (MEK and ERK) [45], avoiding an uncontrolled activation of the signaling pathway.

One aspect to keep in mind is the different effects on the $_{pp}$ERK response caused by the PRMT5 activity depending on its regulation, either by the input stimulus (model A) or by the cascade response (model B).

Case A: PRMT5 activity regulated by EGF (model A)

In the model A, for a fixed EGF value, the time response of the Ras-ERK MAPK cascade becomes time-limited, describing a normal or Gaussian pattern (Fig. 4 and Suppl. Fig. S8B).

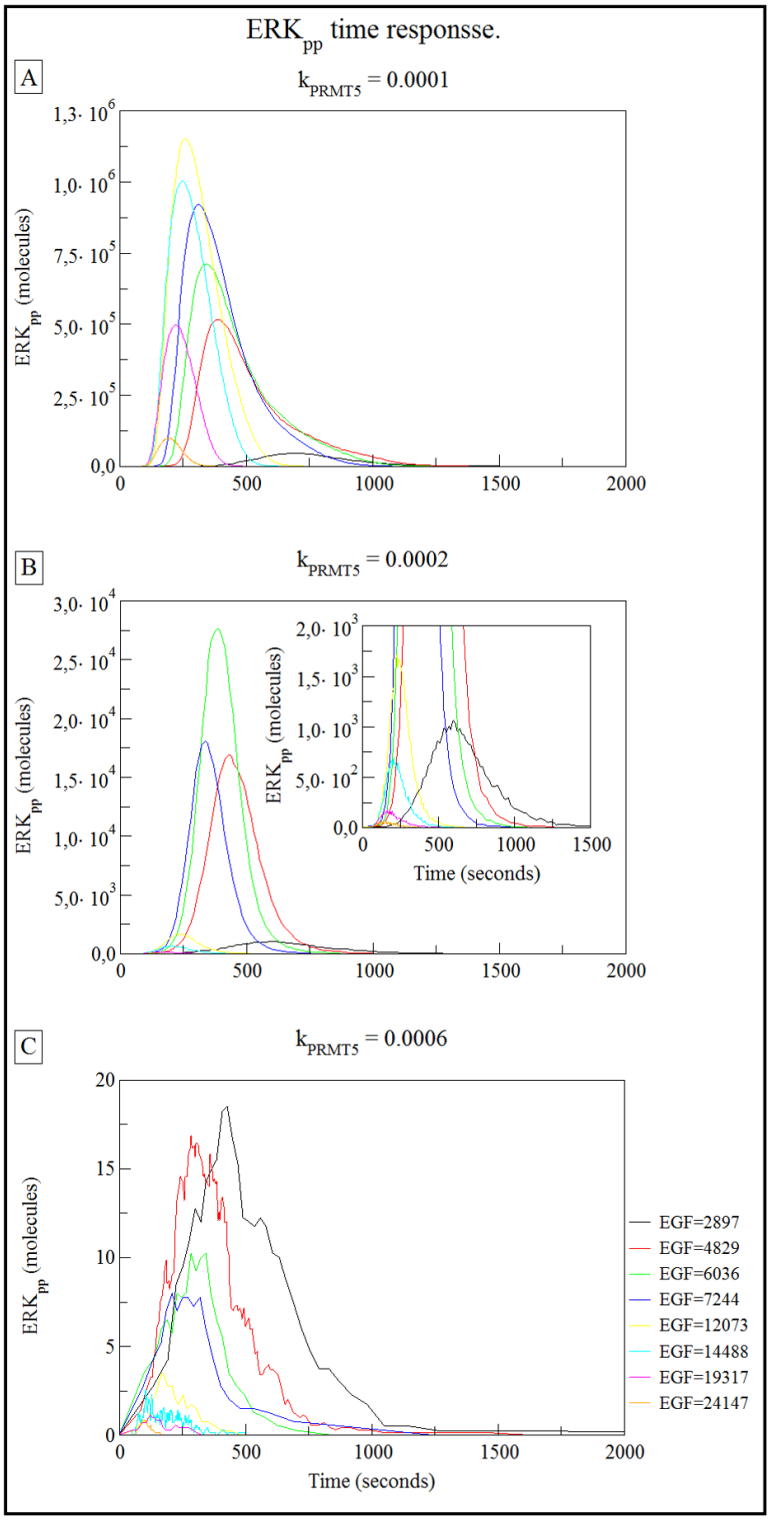

FIGURE 4 (color version required for printing): **$_{pp}$ERK time responses of the model A for different k_{PRMT5} values.** Time responses of $_{pp}$ERK of the model A for different values of k_{PRMT5}: **graph A** for k_{PRMT5} = 0.0001 (cell/molecules)·s^{-1}, **graph B** for k_{PRMT5} = 0.0002 (cell/molecules)·s^{-1} and **graph C** for k_{PRMT5} = 0.0006 (cell/molecules)·s^{-1}. Each colour line represents the Ras-ERK MAPK cascade time response for each different EGF value. EGF is defined from 0 to 10^6 molecules. The considered values of EGF to simulate the output response are summarized in the graph legend. The components concentrations and the reaction rates used in simulations are described in Suppl. Tables I and II.

At initial times, the output response presents weak values since EGF requires some time to activate Raf. Next, the Raf^* expression increases with time and, thereby, the $_{pp}$ERK time response dramatically increases until reaching a maximum value ($_{pp}ERK_{max}$). After that, the response decreases to extinction due to the degradation of Raf^* exerted by the PRMT5 methylation. This process becomes relevant just when there is enough Raf^* expression to compete with the Raf activation process. To this Raf^* degradation process, the actions of, on one hand, the $_{pp}$ERK negative feedback and, on the other hand, the phosphatases (RafPase, MEKPase, and ERKPase) act as complementary mechanisms for the output response regulation.

The simulations reveal that there is a dependence correlation between the values of the $_{pp}$ERK response and the values of the parameter k_{PRMT5}, the higher the amplitude attenuation and the time limitation, the higher of the k_{PRMT5} values (Fig. 4, black and green results from Suppl. Fig. S7, and Suppl. Fig. S8B). The reason is, as well, the higher k_{PRMT5}, the faster the Raf^* degradation. Therefore, there is a lower expression of Raf^* capable of activating its substrate MEK, which in turn activates ERK. Consequently, both the amplitude of the $_{pp}$ERK response (compare Fig. 4A, 4B, and 4C) and the time wherein the $_{pp}$ERK response is non zero decrease (Fig. 4, black and green results from Suppl. Fig. S7, and Suppl. Fig. S8B).

Furthermore, both the time duration and the amplitude of the response vary with the values of EGF (Fig. 4 and Appendix B Suppl. Fig. S8B) because of both the Raf activation process and the activity of the PRMT5 depend directly on the input stimulus.

The PRMT5 acts as a tuner: the higher k_{PRMT5}, the lower range of EGF capable of generating enough relevant $_{pp}$ERK response, since higher EGF expression is required to maintain a similar level of Raf^* (Fig. 4). Simulations reveal that the maxima amplitudes of the $_{pp}$ERK response and the $_{pp}$ERK response areas decrease with k_{PRMT5} because of the increasing effect of the Raf^* methylation process (Fig. 5A and 5B respectively).

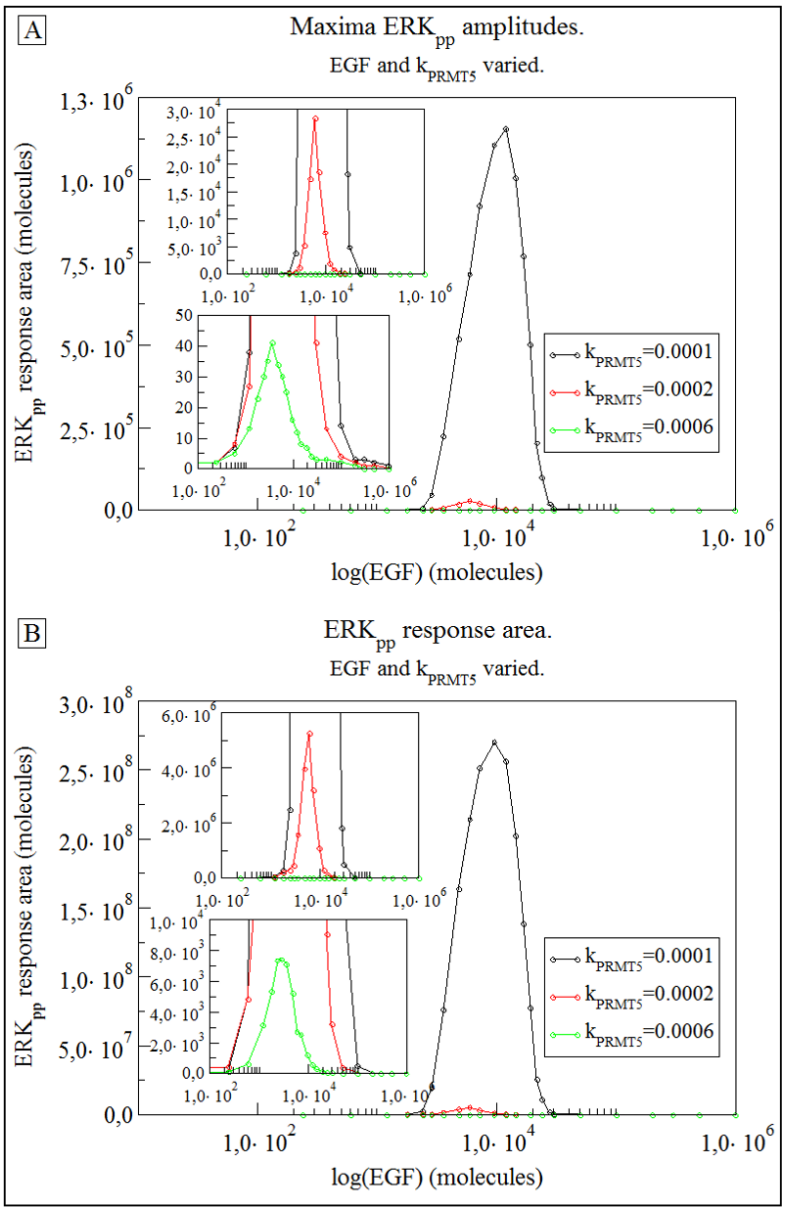

FIGURE 5 (color version required for printing): **Maxima amplitudes and responses areas of the ppERK in the model A.**

(A) Representation of the maxima amplitudes of the ppERK time responses in the model A as function of EGF (represented in a semilogarithmic scale). **(B)** Representation of the response areas of the ppERK concentration in the model A as function of EGF (represented in a semilogarithmic scale). These both responses have been represented for the three values of k_{PRMT5}: 0.0001 (cell/molecules)·s^{-1} (black lines), 0.0002 (cell/molecules)·s^{-1} (red lines) and 0.0006 (cell/molecules)·s^{-1} (green lines). EGF is defined from 0 to 10^6 molecules.

Both the maxima amplitudes of the ppERK response and the ppERK response areas describe a pass-band pattern for a fixed k_{PRMT5} (Fig. 5A and 5B respectively), demonstrating that the EGF-Ras-ERK MAPK cascade with the presence of EGF-dependent PRMT5 activity limits the range of the EGF values that provide non-zero responses (or enough response levels to be considered as relevant

for this study). First, there is a short-range of initial EGF values that are not capable of generating relevant $_{pp}$ERK response. Next, there is a medium-range of EGF values where the $_{pp}$ERK expression increases until reaching a maximum value. At this point, the activity of the PRMT5 increases promoted by high EGF and Raf* levels, starting to speed up the Raf* degradation process. Next, higher EGF values increase the PRMT5 activity, which takes more relevance than the activation of downstream substrates (MEK and ERK), resulting in weaker and shorter $_{pp}$ERK responses. And finally, there is a very high range of EGF values where the Raf* degradation is so fast that there is no time for the signaling cascade to generate any ERK$_{pp}$ response (Fig. 5A and 5B).

When PRMT5 is regulated by EGF, obtained results give evidences for its regulatory role on the time duration and amplitude of the ERK-1/2 MAPK activity, as well as in limiting the range of EGF able of generating a nonzero $_{pp}$ERK response.

Case B: PRMT5 activity regulated by $_{pp}$ERK (model B)

In the model B, considering a fixed EGF, the response of the EGF-Ras-ERK MAPK cascade becomes time-limited, but describing a lognormal pattern (Fig. 6 and Suppl. Fig. S8C). At starter times, the cascade response presents weak values, as there is not enough input stimuli to activate a consistent Raf* concentration capable of activating relevant ERK. Next, the Raf* expression increases due to the action of EGF, so that the response of the signaling cascade grows with time until reaching a maximum value (ERK$_{pp(max)}$) (Fig. 6). Consequently, the methylation process exerted by the PRMT5 becomes faster, reducing the Raf* expression. At this point, both the deactivation of kinases through the corresponding phosphatases and the deactivation of Raf* through the $_{pp}$ERK negative feedback loop help the Raf* methylation process to diminish the ERK$_{pp}$ response until its extinction, avoiding an inappropriate steady-state response (Fig. 6 and Suppl. Fig. S8C).

The simulations reveal that there is no correlation between the k_{PRMT5} value and the $_{pp}$ERK amplitude. But there is a slight dependence between the k_{PRMT5} and the $_{pp}$ERK time duration and, thereby, with the $_{pp}$ERK response areas. Both the maxima amplitudes of the $_{pp}$ERK response and the $_{pp}$ERK response areas describe a switch-like pattern for a fixed k_{PRMT5} (Fig. 7A and Suppl. Fig. S10A, and Fig. 7B and Suppl. Fig. S10B, respectively).

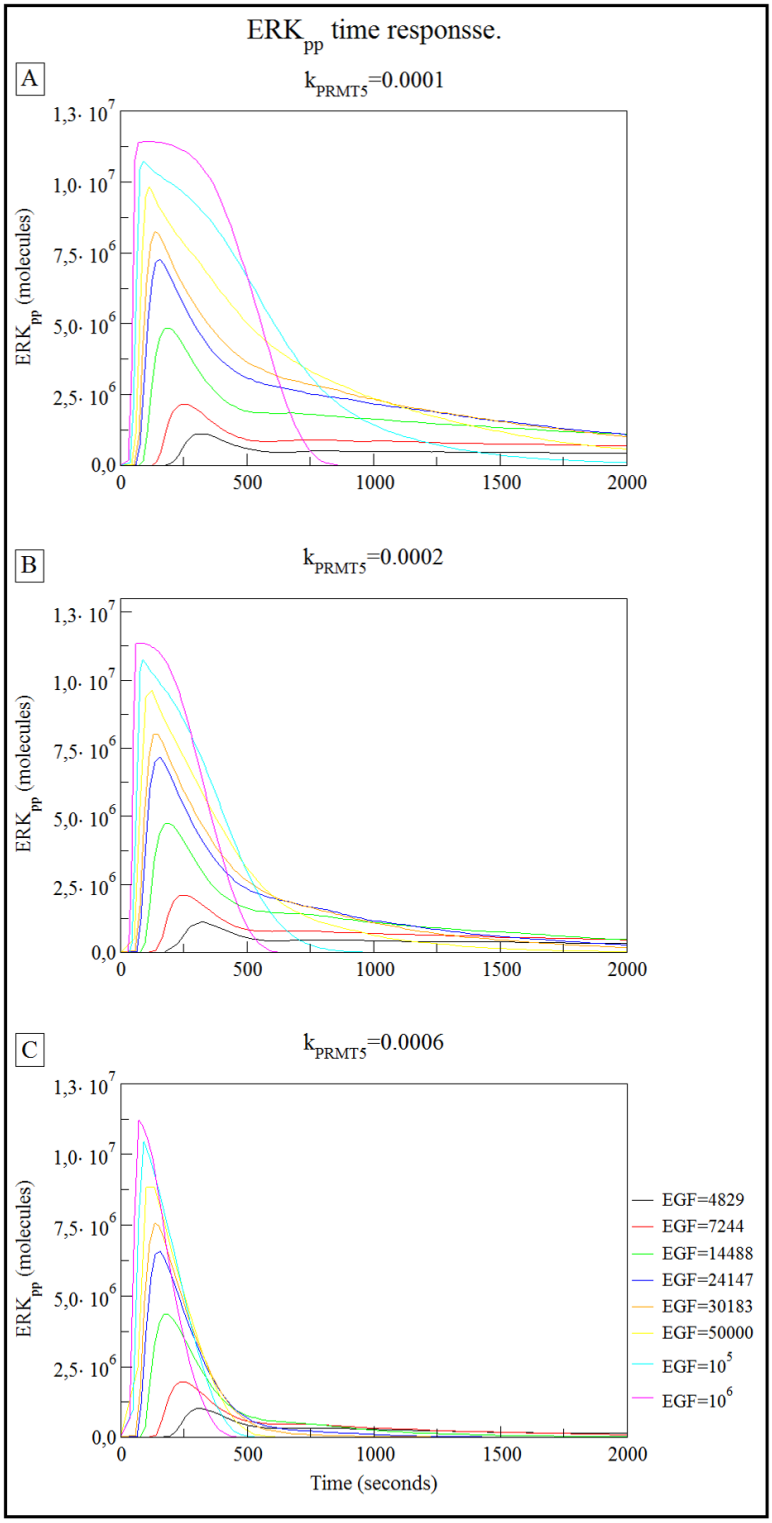

FIGURE 6 (color version required for printing): **$_{pp}$ERK time response of the model B for different k_{PRMT5} values.** Time responses of $_{pp}$ERK of the model A for different values of k_{PRMT5}: **graph A** for k_{PRMT5} = 0.0001 (cell/molecules)·s^{-1}, **graph B** for k_{PRMT5} = 0.0002 (cell/molecules)·s^{-1} and **graph C** for k_{PRMT5} = 0.0006 (cell/molecules)·s^{-1}. Each colour line represents the Ras-ERK MAPK cascade time response for each different EGF value. EGF is defined from 0 to 10^6 molecules. The considered values of EGF to simulate the output response are summarized in the graph legend. The components concentrations and the reaction rates are described in Suppl. Tables I and II.

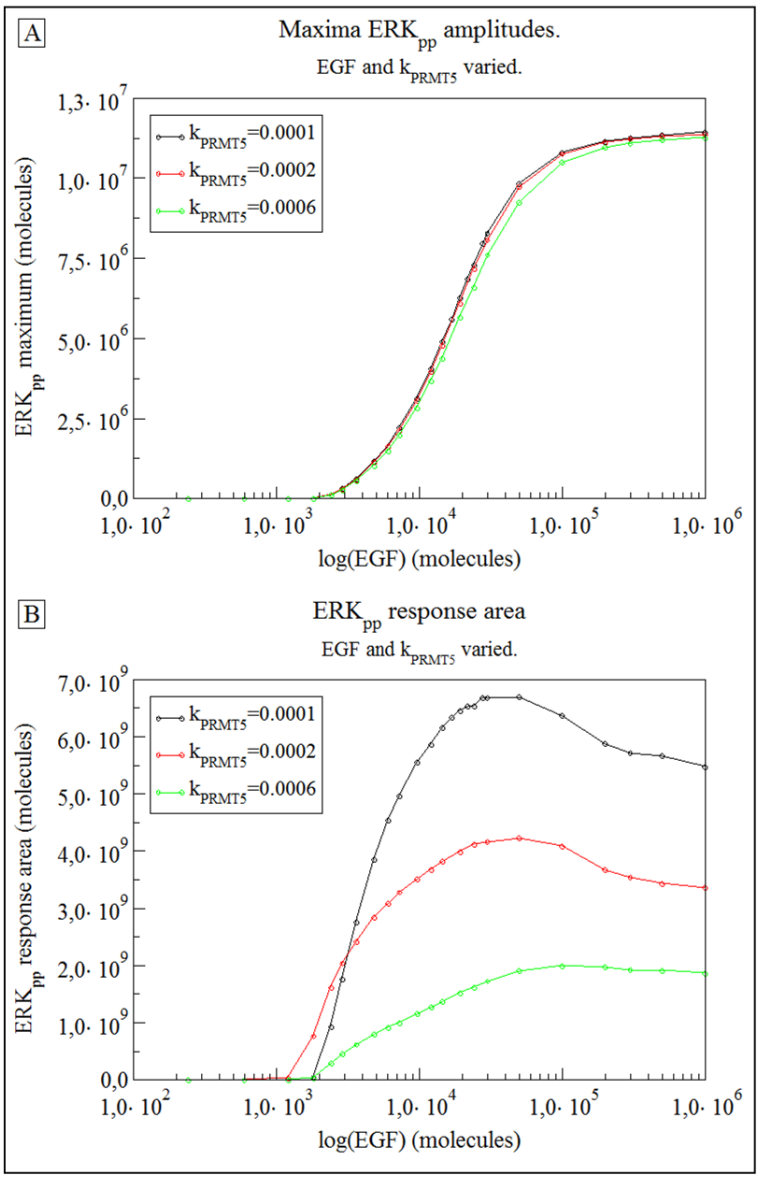

FIGURE 7 (color version required for printing): **Maxima amplitudes and responses areas of the $_{pp}$ERK in the model B.**

(A) Representation of the maxima amplitudes of the $_{pp}$ERK time responses in the model B as function of EGF (represented in a semilogarithmic scale). **(B)** Representation of the response areas of the $_{pp}$ERK concentration in the model B as function of EGF (represented in a semilogarithmic scale). These both responses have been represented for the three values of k_{PRMT5}: 0.0001 (cell/molecules)·s^{-1} (black lines), 0.0002 (cell/molecules)·s^{-1} (red lines) and 0.0006 (cell/molecules)·s^{-1} (green lines). EGF is defined from 0 to 10^6 molecules.

At low EGF values, there is a discrete Raf activation that results in poor output responses. As the Raf* degradation process depends directly on the $_{pp}$ERK expression, its effect is irrelevant against the activation of Raf, thereby the $_{pp}$ERK responses are sustained in time (Fig. 6, and red and blue results from Suppl. Fig. S7). Next, with increasing EGF, the relative and maxima amplitudes of the $_{pp}$ERK response increase until reaching a maximum value when EGF = EGF_{Th}, from 10^7

molecules (Suppl. Fig. S10A and green result from Suppl. Fig. S9). Such $_{pp}$ERK concentration induces a Raf* methylation process through the PRMT5 activity that gains relevance, speeding up the response degradation and reducing its time duration (red and blue graphs from Suppl. Fig. S7). After that, for EGF > EGF_{Th}, the $_{pp}$ERK time responses are controlled by a competition process between a maximum steady state of the $_{pp}$ERK expression (promoted by an overexpressed concentration of EGF) and a constant degradation of Raf*. Under these conditions, the maxima amplitudes of the $_{pp}$ERK responses do not practically change, maintaining a constant value (Suppl. Fig. S10A). Nevertheless, the time duration continues decreasing (red and blue results from Suppl. Fig. S7) because of the $_{pp}$ERK production becomes faster and thereby, the Raf* methylation process. Therefore, the response area of the output signal, after reaching a maximum value, describes a slight decrease as the EGF increases (Fig. 7B and Suppl. Fig. S10B) promoted by the reduction of the response times (red and blue results from Suppl. Fig. S7), and lasts until the differences in the response times are barely noticed.

The obtained results reveal that the time responses of the model B are influenced by the impact of the PRMT5 methylation exerted through the parameter k_{PRMT5}, which positively regulates the velocity of the Raf* degradation. Thereby, as k_{PRMT5} increases, lower Raf* concentration is available and less time is required to become extinct, generating shorter output responses (Fig. 6 and Suppl. Fig. S8C). Even though the amplitude of $_{pp}$ERK does not reveal important shifts due to the k_{PRMT5} effect (a saturated response regime is guaranteed by an overexpressed concentration of EGF, see Fig. 6, 7A and Suppl. Fig. S9A), its response area decreases while k_{PRMT5} increases (Fig. 7B and Suppl. Fig. S10B) due to shorter time responses of the $_{pp}$ERK expression.

So, reported results reveal that the PRMT5 regulated by $_{pp}$ERK modulates the time response of the ERK-1/2 MAPK cascade, limiting its duration and moving its activity away from an aberrant activation state. However, the amplitude of the $_{pp}$ERK response is not influenced by the PRMT5 activity.

4. **Discussion**.

The PRMT5 protein is present in different cell compartments, namely cytoplasm and nucleus, and its functional role seems to depend on its localization. Thereby, PRMT5 acts as oncogene and epigenetic silencer of tumor repressors when is localized in cytoplasm, but PRMT5 inhibits cancer cell proliferation, migration and invasion when it is placed in the cell nucleus [85, 86]. Then, PRMT5 may act as an up-regulating mechanism for cancer proliferation, migration and invasion when it acts in the cytoplasm, whilst it may have a tumor antiproliferative and pro-survival effect when it is activated in the cell nucleus. Such issue can be related with the simulations

performed in our work, where the ERKpp-induced PRMT5 may act in the cell nucleus, regulating the EGF-ERK MAPK activity, avoiding a sustained maximum ERKpp response that can lead to tumorigenesis.

Previous works provided evidences of the regulatory role of PRMT5 in normal cell proliferation and cell survival. *Andreu-Pérez P et al*. [45] determined that the methylation of arginine residue in the GRG motif of BRaf and CRaf is involved in the limitation of these kinases activity and stability, promoting the limitation of ERK1/2 signal output and, thereby, switching from differentiation to proliferation. *Hsu JM et al.* [60] found that EGFR methylated in Arg^{1175} positively modulated EGF-induced EGFR trans-autophosphorylation at Tyr^{1173}, enhancing the binding between EGFR and SHP1 that resulted in suppression of EGFR-mediated ERK activation. In addition, they reported that EGFR Arg^{1175} methylation is not regulated by EGF stimulation, finding that can be related to the results obtained with the EGF-Ras-ERK MAPK model of our research that presents PRMT5 activated by $_{pp}$ERK. *Liu F et al.* [87] reported an increase in colony formation of myeloproliferative neoplasms (MPNs) and erythroid differentiation when knocking down PRMT5 in human CD34+ cells using shRNA, indicating that PRMT5 negatively regulates hematopoietic stem/progenitor cell proliferation and expansion. *DongSheng W et al.* [88] confirmed that PRMT5 can bind with death receptor 4 (DR4) and reduce its expression, providing a molecular mechanism to abolish the inflammatory cytokine expression induced by protein CCL20, inhibiting its expression via inhibiting NF-κB. An study developed by *Yang M et al.* [89] in Caenorhabditis elegans showed that PRMT5 negatively regulates DNA damage-induced apoptosis, triggering a decrease in germ cell apoptosis. Furthermore, an study done by *Scoummane A et al.* [90] manifested that PRMT5 is required for normal cell proliferation and regulates cell-cycle transition from G1 to S phase. They gave evidences that, in response to stress signals, PRMT5 induced sufficient p53 activation to promote pro-survival mechanisms, such as cell-cycle arrest and DNA repair.

There is a large body of evidence on the role of PRMT5 inhibitors, both natural and synthetic, but most of them focus on reversing the activity of PRMT5 when it is related to the positive regulation of tumor proliferation and progression in cancer cell lines [91-94]. However, there are few scientific studies aimed at verifying the effect of inhibiting the activity of PRMT5 when it regulates an aberrant tumor response. *Andreu-Perez P et al.* [45] reported that the silencing of PRMT5 activity through methylthioadenosine (MTA) or its depletion stabilized Raf and positively regulates its activity, not only increasing the amplitude and duration of Raf signal and, so on, the ERK phosphorylation in response to growth factors, but also redirecting the response of PC12 cells to EGF from proliferation to differentiation. Related to this point, it is known that both NGF and EGF stimulate the Ras-ERK-MAPK pathway in PC12 cells, but with different effects on ERK activation: EGF induces a transient phosphorylation of ERK, promoting PC12 cell

differentiation, whereas NGF elicits a sustained activation of ERK, inducing proliferation of PC12 cells [95-97]. Then, it may be assumed that, when knocking down PRMT5, the induced differentiation behavior provoked by a sustained ERK response was triggered by a sustained Raf signal. Apart from *Andreu-Perez et al.* [45], there are other scientific studies aimed at verifying the effect of inhibiting the activity of PRMT5 when it regulates an aberrant tumor response. *Hsu JM et al.* [60] conducted experiments where knockdown of PRMT5 expression by short interfering RNAs (siRNAs) diminished Arg^{1175}, down regulating EGF-induced Tyr^{1173} phosphorylation and enhancing EGF-stimulated ERK activation by reducing SHP1 recruitment to EGFR. As a result, cell proliferation, migration and invasion of EGFR-expressing cells increased. *Jansson M et al.* [98] showed that the depletion of PRMT5 in human osteosarcoma U2OS cells induced p53-dependent apoptosis. And *Yang M et al.* [89] reported in Caenorhabditis elegans that loss of PRMT5 activity promoted specific over upregulation of the cell death initiator EGL-1 in response to DNA damage, which is directly responsible for the excessive germ cell apoptosis.

In our study, the basic structure of the EGF-Ras-ERK MAPK pathway (reference model) describes a switch-like time response, reaching a steady-state condition (Fig. 3A) that results from an equilibrium process between the Raf activation promoted by EGF, the Raf^* attenuation performed by the $_{pp}$ERK negative feedback loop, and the deactivation of the active species of Raf, MEK, and ERK through the corresponding phosphatases (RafPase, MEKPase and ERKPase). Such sustained ERK signaling may be responsible of an aberrant cell response related to tumor differentiation, migration and invasion.

This response can be modulated both in time duration and in amplitude through the simple action of a protein present in the own cell environment: the PRMT5.

This protein acts as an inhibitor of the Raf^*, degrading it through a methylation process, limiting the activation of its downstream substrates (MEK and ERK) and, thereby, converting the steady-state of the output response into a time-limited pattern (Fig. 4, 6, Suppl. Fig. S7, and Suppl. Fig. S8A and S8B), avoiding an uncontrolled activation state of the $_{pp}$ERK response, as the reference model describes.

Both the time limitation and the amplitude attenuation of the EGF-Ras-ERK MAPK response depends on the parameter k_{PRMT5}, which defines the strength of the methylation action. Thereby, the Raf^* expression decreases while k_{PRMT5} increases, resulting in less levels of activation of downstream substrates (MEK and ERK) and, in turn, less output response that expires in less time (Fig. 4, 6, and Suppl. Fig. S8B, S8C and S9). Therefore, the protein PRMT5 constitutes a cell component able of switching between sustained and transient $_{pp}$ERK responses without requiring different types of stimuli and, thereby, provides a mechanism to switch from cell differentiation to cell proliferation [45]. Although further investigation should be required, such switching behavior

appears to be reversible upon removal of PRMT5, as it was reported in the literature related to the application and removal of both EGF and NGF [99-101].

Different effects on the $_{pp}$ERK response patterns arise depending on the mechanism of the PRMT5 activation. For this reason, two different models have been defined to illustrate such differences: the model A where the PRMT5 activity is regulated by the EGF, describing a Gaussian pattern of the $_{pp}$ERK time response (Fig. 4), and the model B where the PRMT5 is regulated by the $_{pp}$ERK, providing a lognormal pattern of the $_{pp}$ERK time response (Fig. 6).

The reduction of the time duration and the amplitude of the $_{pp}$ERK response is greater in the model A than in the model B (Suppl. Fig. S7, S8B and S8C), regardless the k_{PRMT5} value. In short, in the model A, the Raf* methylation exerted by PRMT5 is present from first stages of the stimulation since it is activated by the EGF and thereby, fast rates of Raf* degradation are achieved. As a result, the duration and the amplitude of the $_{pp}$ERK response fall down (Fig. 4 and Suppl. Fig. S8B). Thus, the EGF-dependent PRMT5 shortens the $_{pp}$ERK response time 20-fold more (Suppl. Fig. S7) and can reach attenuations 10-fold greater (Suppl. Fig. S9) than the $_{pp}$ERK-dependent PRMT5, regardless the value of k_{PRMT5}.

Nevertheless, the Raf* methylation process in the model B depends on the output response of the signaling cascade, requiring more time to induce the Raf* methylation effect of the PRMT5. Thereby, the duration of the response is prolonged (Fig. 6, and Suppl. Fig. S7 and S8C). In addition, the attenuation of the $_{pp}$ERK amplitude is low, presenting irrelevant shifts when k_{PRMT5} changes (Fig. 6 and Suppl. Fig. S8C), since a maximum steady state of the $_{pp}$ERK expression (promoted by an overexpressed concentration of EGF) is counteracted by a constant degradation of Raf* (which in turn is controlled by the $_{pp}$ERK expression). Then, the Raf* methylation effect exerted by the PRMT5 requires enough high expression of $_{pp}$ERK to be effective and compete with the Raf activation promoted by EGF.

Furthermore, when the PRMT5 activity depends on the input stimulus (model A), it is possible to tune a specific and delimited range of stimuli able of generating a nonzero response, resulting in a behavior similar to a pass-band filter. Thereby, for low EGF values, there is not enough activation of the signaling cascade to produce an $_{pp}$ERK response, whilst for high EGF values the rate of Raf* methylation is so fast that no $_{pp}$ERK response is generated (Fig. 5A and red result from Suppl. Fig. S9). In contrast, if the PRMT5 activity is controlled by the output $_{pp}$ERK (model B), it is not possible to delimitate the EGF range at which the $_{pp}$ERK response is nonzero, resulting in a switch-like output response because of the sustained maximum value of the $_{pp}$ERK when EGF $\geq$ EGF_{Th} (Suppl. Fig. S10A and green result from Suppl. Fig. S9), similar to the response behavior of the reference model (black results from Fig. 3B and Suppl. Fig. S9).

Our data predict that PRMT5 activity reduces the duration and the amplitude of the EGF

response on ERK activity, and such response can be limited to a specific range of input stimulus. Future studies should validate our prediction through the use of cultured cells. This is favoured by the fact that human cells such as HeLa or A431 express high levels of EGF receptors, components of the MAPK pathway, as well as PRMT5. In consequence, it is feasible to manipulate PRMT5 genetically (overexpression or silencing), and to analyse the time-dependent responses to different concentrations of EGF on ERK activity/phosphorylation.

Weighing all the differences between both models of the Ras-ERK MAPK pathway with PRMT5 activity, their response patterns can be useful, as a guide, to identify if any of both analyzed mechanisms of PRMT5 activation may act as a modulator of the signaling pathway response in experimental research.

The proposed model of the EGF-Ras-ERK MAPK signaling pathway only considers the main components of the cascade directly related in the phosphorylated protein levels, thereby, the different docking and activator factors integrated in the MAPK signalling pathway are not considered in the model, such as KSR1/2, β-arrestin1/2, paxillin, MP1, RKIP, MORG1, FRS2, Shoc2/Sur8, IQGAP, PEB1, or Sef, among others. Although these molecules are required for the compartmentalization of signaling proteins and their spatial arrangement to be activated/deactivated and to interact each others, the proposed model aims to determine only the role of PRMT5 on ERK activity. For instance, presence or knockout of scaffold proteins are related to modifications in the cell response related to ERK MAPK activity. This is the case of the KSR, with evidences in the literature of its role as scaffold, pseudo-kinase or even as a kinase, promoting or inhibiting Raf, MEK or ERK signaling, mainly driving translocation of such kinases to specific sites of the cell, increasing their local concentrations, or forming heterotetramers with Raf and MEK to promote its phosphorylation and, thereby, improving signaling [102, 103]. As a result, knockout of scaffolds can inhibit MAPK in tumor cells and reverse oncogenic transformation of cells, such as the knockout of KSR that can reverse oncogenic transformation in colorectal and pancreatic cancers [104,105]. Moreover, scaffold proteins, as signaling regulators, could help to develop therapeutic strategies against unwanted ERK signaling (i.e., proliferation), while preserving other desired ERK activity (e.g. differentiation or apoptosis) [106-108]. Then, it should be of interest to include the KSR or other scaffold protein related to Ras-ERK MAPK pathway in a further extension of the model to investigate how their role can influence ERK signaling.

In addition, reported results are based in simulations of a model of the EGF-Ras-ERK MAPK route that does not consider other regulations on kinases and PRMT5 activity that could influence the final cell response, caused by cross-links with partner signaling pathways or by different feedback loops from the considered ones. In the latter case, several positive and negative feedback loops have been identified within the EGF-Ras-ERK MAPK pathway: positive feedback loops (such as the

existing one from Ras to SoS) are related to bistability and switch-like responses, whilst negative feedback loops (such as the exising ones from ppERK to either MEK, Ras or Raf) switch pathway response from ultrasensitivity to oscillations, even inhibiting signal via kinases phosphorylation [109, 110].

Such a simplification of the model does not permit to quantify to what extent the activity of MAPK pathway is regulated by PRMT5 in cells under in vivo conditions. In this regard, a further step may be to extend the EGF-Ras-ERK MAPK model including some of the regulatory mechanisms discussed previously, in order to evaluate the extent of Raf methylation and attenuation of ERK activity in a more real approach.

When addressing computational studies of simplified models related to complex biological systems such as the EGF-Ras-ERK MAPK signaling pathway, doubts can arise about the impact and the extent that the proposed regulatory mechanism based on the protin PRMT5 has on the modulation of the ERK signal. Despite there is a lack of quantitative studies about the impact of this regulatory effect of PRMT5 in ERK signal, some publications [45, 60] reported that activating PRMT5 alone, the levels of ERK signal are drastically reduced. And our predictions are in accordance with such published evidences. Therefore, the PRMT5 may be considered an important mechanism in terms of regulating the overexpression of the EGF-Ras-ERK pathway by itself, switching ERK signal from a sustained response to a transient one.

To conclude, regardless the type of regulation of the PRMT5 activation, this small protein opens up great options related to the modulation of the Ras-ERK MAPK cascade response upon EGF stimulation, allowing to limit in a great extent the ERK signal through a methylation process of Raf, which allows to delimit ERK duration over time and to attenuate its amplitude, since an uncontrolled intensity or the persistence in time of an inappropriate response in the Ras-ERK MAPK route can lead to wrong physiological functions of the cell. So, activators of this methyltransferase can be considered as a potential target to develop biological and/or pharmacological strategies to fight against cell diseases and tumorigenesis, such as exposing cells to hypoxic conditions [111] through any hypoxia mimicker such as the cobalt chloride ($CoCl_2$), which is considered to prevent HIF-1α degradation, and ginkgolides [112]; posphorylating MEP50, which builds a complex with PRMT5 and promotes substrate recognition and orientation, as well as stimulation of PRMT5 activity [113, 114]; the growth factor TGFβ [115]; the c-Myc that recruits the PRMT5-MEP50 complex, inducing H4R3 symmetric di-methylation [116]; the cyclin D1/CDK4 that phosphorylates Thr5 on MEP50, activating the methyltransferase activity of PRMT5 [117]; ionizing radiation [118]; and DNA damage that can induce PRMT5-catalyzed methylation of non-histone substrates, promoted by different factors, such as camptothecin, etoposide, hydroxyurea, doxorubicin, and UVB irradiation [90, 119-121].

Acknowledgments.

This study was supported by grants from the MICINN (SAF2016-75246R and RTI2018-097038-B-C22), the Generalitat de Catalunya (Grants 2017 SGR 1015, ICREA Acadèmia), and CIBERDEM. We gratefully acknowledge institutional funding from the MICINN through the Centres of Excellence Severo Ochoa Award, and from the CERCA Programme of the Generalitat de Catalunya. M.J. thanks MICINN for the FPI contract BES-2011-043331- M.J. also thanks Ramón Reigada and Mª Ángeles Serrano their contribution in the conception of the initial idea of the present study. In addition, I gratefully acknowledge Juan Recio his support in understanding the experimental bases of the present investigation.

Conflict of interests.

Authors declare no conflict of interests.

References.

[1] Bhalla US, Iyengar R. 1999. *Emergent properties of networks of biological signaling pathways*. Science 283, 381-387.

[2] Serrano MA, Juardo M, Reigada R. 2013. *Negative feedback self-regulation contributes to robust and high-fidelity transmembrane signal transduction*. J R Soc Interface;10(88):20130581.

[3] Sturm OE et al. 2010. *The mammalian MAPK/ERK pathway exhibits properties of a negative feedback amplifier*. Sci. Signal. 3, ra90.

[4] Lewis TS, Shapiro PS, Ahn NG. 1998. *Signal transduction through MAP kinase cascades*. Adv Cancer Res 74, 49139.

[5] Errede B et al. 1995. *Dynamics and organization of MAP kinase signal pathways*. Mol Reprod Dev 42, 477485.

[6] Pearson G et al. 2001. *Mitogen-Activated Protein (MAP) Kinase Pathways: Regulation and Physiological Functions*. Endocrine Reviews 22(2), 153183.

[7] Huang CY, Ferrell JE. 1996. *Ultrasensitivity in the mitogen-activated protein kinase cascade*. Proc. Natl. Acad. Sci. USA 93.

[8] Cargnello M and Roux P P. 2011. *Activation and function of the MAPKs and their substrates, the MAPK-Activated Protein Kinases*. Microbiology and Molecular Biology Reviews, 5083.

[9] Inder K et al. 2008. A*ctivation of the MAPK Module from Different Spatial Locations Generates Distinct System Outputs*. Molecular Biology of the Cell Vol. 19, 47764784.

[10] Roux PP et Blenis J. 2004. *ERK and p38 MAPK-Activated Protein Kinases: a Family of Protein Kinases with Diverse Biological Functions*. Microbiology and molecular biology reviews, June 2004, 320344.

[11] Campbell SL et al. 1998. *Increasing complexity of Ras signaling*. Oncogene 17: 13951413.

[12] Kholodenko BN. 2000. *Negative feedback and ultrasensitivity can bring about oscillations in MAPK cascades*. Eur. J. Biochem. 267, 1583-1588.

[13] Fey D, Croucher DR, Kolch W, Kholodenko BN. 2012. *Crosstalk and signaling switches in mitogen-activated protein kinase cascades*. Frontiers in Physiology, Vol. 3, Article 355, 1-21.

[14] Caunt CJ and McArdle CA. 2012. *ERK phosphorylation and nuclear accumulation: insights from single-cell imaging*. Biochem Soc Trans 40(1):224-229.

[15] Shindo Y et al. 2016. *Conversion of graded phosphorylation into switch-like nuclear translocation via autoregulatory mechanisms in ERK signalling*. Nature Communications 7:10485.

[16] Behar M and Hoffmann A. 2010. *Understanding the temporal codes of intra-cellular signals*. Curr. Opin. Genet. Dev. 20, 684-693.

[17] Purvis JE and Lahav G. 2013. *Encoding and decoding cellular information through signaling dynamics*. Cell 152, 945-956.

[18] Yee KL, Weaver VM and Hammer DA. 2008. *Integrin-mediated signalling through the MAP-kinase pathway*. IET Systems Biology, Volume 2, Issue 1, pages 8-15.

[19] Grandis JR, Sok JC. 2004. *Signaling through the epidermal growth factor receptor during the development of malignancy*. Pharmacol. Ther. 102, 37-46.

[20] Downward, J. 2003. *Targeting RAS signalling pathways in cancer therapy*. Nat. Rev. Cancer 3, 1122.

[21] McBride AE, Silver PA. 2001. *State of the Arg: Protein methylation at arginine comes of age*. Cell 106,5-8.

[22] Bedford MT, Richard S. 2005. *Arginine methylation an emerging regulator of protein function*. Mol. Cell 18, 263-272.

[23] Bedford MT, Clarke SG. 2009. *Protein arginine methylation in mammals: Who, what, and why*. Mol. Cell 33,1-13.

[24] Bedford MT. 2007. *Arginine methylation at a glance*. J Cell Sci. 20, 4243- 4246.

[25] Branscombe TL et al. 2001. *PRMT5 (Janus kinase-binding protein 1) catalyzes the formation of symmetric dimethylarginine residues in proteins*. J. Biol. Chem. 276, 32971-32976.

[26] Lee YH, Stallcup MR. 2009. *Minireview: Protein arginine methylation of non histone proteins in transcriptional regulation*. Mol. Endocrinol. 23, 425-433.

[27] Wei T-Y W et al. 2012. *Protein arginine methyltransferase 5 is a potential oncoprotein that upregulates G1 cyclins/cyclin-dependent kinases and the phosphoinositide 3-kinase/AKT signaling cascade*. Cancer Sci. 103(9):1640-50.

[28] Kim H and Ronai ZA. 2020. *PRMT5 function and targeting in cancer*. Cell Stress, Vol. 4, No. 8, 199-215.

[29] Lattouf H et al. 2019. *LKB1 regulates PRMT5 activity in breast cancer*. Int. J. Cancer: 144, 595-606.

[30] Gu Z, Li Y, Lee P, Liu T, Wan Ch, Wang Z. 2012. *Protein Arginine Methyltransferase 5 Functions in Opposite Ways in the Cytoplasm and Nucleus of Prostate Cancer Cells*. PloS ONE 7(8): e44033.

[31] Mundade R, Wei H and Lu T. 2014. *PRMT5, A Pivotal Player in Cancer*. Austin J Pharmacol Ther. 2 (2).4

32[6] Xiao W, Chen X, Liu L, Shu Y, Zhang M, Zhong Y. 2019. *Role of protein arginine methyltransferase 5 in human cancers*. Biomedicine & Pharmacotherapy 114, 108790.

[33] Wang L, Pal S and Sif S. 2008. *Protein Arginine Methyltransferase 5 Suppresses the Transcription of the RB Family of Tumor Suppressors in Leukemia and Lymphoma Cells*. Mol. Cell. Biol. Vol. 28, No. 20, 6262-6277.

[34] Pal S, Vishwanath SN, Erdjument-Bromage H, Tempst P, Sif S. 2004. *Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes*. Mol Cell Biol. 24(21):9630-45.

[35] Alinari L et al. 2015. *Selective inhibition of protein arginine methyltransferase 5 blocks initiation and maintenance of B-cell transformation*. Blood 125(16):2530-43.

[36] Majumder S et al. 2010. *Methylation of Histone H3 and H4 by PRMT5 Regulates Ribosomal RNA Gene Transcription*. J Cell Biochem.; 109(3): 553-563.

[37] Liu F et al. 2020. *PRMT5-mediated histone arginine methylation antagonizes transcriptional repression by polycomb complex PRC2*. Nucleic Acids Research, 2020, Vol. 48, No. 6, 2956-2968.

[38] Richard S, Morel M, Cleroux P. 2005. *Arginine methylation regulates IL-2 gene expression: A role for protein arginine methyltransferase 5 (PRMT5)*. Biochem. J. 388, 379-386.

[39] Greenblatt SM, Liu F and Nimer SD. 2016. *Arginine methyltransferases in normal and malignant hematopoiesis*. Exp. Hematol., 44, 435–441.

[40] Richters A. 2017. T*argeting protein arginine methyltransferase 5 in disease*. Future Medical Chemistry, 9(17), 20812098.

[41] Gary JD and Clarke S. 1998. *RNA and protein interactions modulated by protein arginine methylation*. Prog Nucleic Acid Res Mol Biol. 61:65-131.

[42] Li M, An W, Xu L, Lin Y, Su L, and Liu X. 2019. *The arginine methyltransferase PRMT5 and PRMT1 distinctly regulate the degradation of anti-apoptotic protein CFLARL in human lung cancer cells.* J. Exp. Clin. Cancer Res. 38:64.

[43] Li S, Yang P, Tian E, and Zhang H. 2013. *Arginine Methylation Modulates Autophagic Degradation of PGL Granules in C. elegans*. Molecular Cell 52, 421-433.

[44] Meister G, Eggert C, Bühler D, Brahms H, Kambach C, Fischer U. 2001. *Methylation of Sm proteins by a complex containing PRMT5 and the putative U snRNP assembly factor pICln*. Curr Biol. 11(24):1990-4.

[45] Andreu-Pérez P et al. 2011. *Protein Arginine Methyltransferase 5 Regulates ERK1/2 Signal Transduction Amplitude and Cell Fate Through CRAF*. Sci Signal. 13; 4(190): ra58.

[46] Shaw AS et al. 2014. *Kinases and Pseudokinases: Lessons from Raf*. Molecular and Cellular Biology, Vol. 34, No. 9, 1538-1546.

[47] Wolf SS. 2009. *The protein arginine methyltransferase family: an update about function, new perspectives and the physiological role in humans*. Cell MolcLife Sci. 66(13):2109-21.

[48] Zhu F and Rui L. 2019. *PRMT5 in gene regulation and hematologic malignancies*. Genes & Diseases 6, 247-257.

[49] Tsai C-J, Lin SL, Wolfson HJ, Nussinov R. 1997. *Studies of protein-protein interfaces: A statistical analysis of the hydrophobic effect*. Protein Science, 6:53-64.

[50] Fan Q, Wang Q, Cai R, Yuan H, Xu M. 2020. *The ubiquitin system: orchestrating cellular signals in non-small-cell lung cancer*. Cellular & Molecular Biology Letters 25:1.

[51] Nguyen LK, Kolch W and Kholodenko BN. 2013. *When ubiquitination meets phosphorylation: a systems biology perspective of EGFR/MAPK signalling*. Cell Communication and Signaling 11:52, 1-15.

[52] Grbovic OM, Basso AD, Sawai A, Ye Q, Friedlander P, Slit D, Rosen N. 2005. *V600E B-Raf requires the Hsp90 chaperone for stability and is degraded in response to Hsp90 inhibitors*. PNAS, Vol. 103, No. 1, 57-62.

[53] Noble C et al. 2008. *CRAF autophosphorylation of serine 621 is required to prevent its proteasome-mediated degradation*. Mol Cell. 31(6):862-72.

[54] Kidger AM and Keyse SM. 2016. *The regulation of oncogenic Ras/ERK signalling by dual-specificity mitogen activated protein kinase phosphatases (MKPs).* Seminars in Cell & Developmental Biology, Volume 50, 125-132.

[55] Zhao Y and Zhang Z-Y. 2001. *The Mechanism of Dephosphorylation of Extracellular Signal-regulated Kinase 2 by Mitogen-activated Protein Kinase Phosphatase 3*. The journal of biological chemistry, Vol. 276, No. 34, pp. 32382–32391.

[56] Caunt CJ and Keyse M. 2012. *Dual-specificity MAP kinase phosphatases (MKPs) Shaping the outcome of MAP kinase signalling*. FEBS Journal 280 (2013) 489–504.

[57] Avraham R & Yarden Y. 2011. *Feedback regulation of EGFR signalling: decision making by early and delayed loops.* Nat Rev Mol Cell Biol 12, 104–117.

[58] Mizuno R et al. 2004. *Inhibition of MKP-1 expression potentiates JNK related apoptosis in renal cancer cells*. Journal of Urology, Volume 172, Issue 2, 723-727.

[59] Liu L, Doran S, Xu Y, Manwani B, Ritzel R, Benashski S, McCullough L, Li J. 2014. *Inhibition of mitogen-activated protein kinase phosphatase-1 (MKP-1) increases experimental stroke injury*. Exp Neurol.; 261:404-11.

[60] Hsu JM et al. 2011. *Crosstalk between Arg 1175 methylation and Tyr 1173 phosphorylation negatively modulates EGFR-mediated ERK activation*. Nat. Cell. Biol., 13(2):174-181.

[61] Fischer A, Hekman M, Kuhlmann J, Rubio I, Wlese S, Rapp UR. 2006. *B- and C-RAF Display Essential Differences in Their Binding to Ras*. J. Biol. Chem. Vol. 282, No. 36, 26503-26516.

[62] Li S, Jang H, Zhang J, Nussinov R. 2018. *Raf-1 Cysteine-Rich Domain Increases the Affinity of*

K-Ras/Raf at the Membrane, Promoting MAPK Signaling. Structure, 26(3):513-525.

[63] Berg M and Soreide K. 2012. *EGFR and Downstream Genetic Alterations in KRAS/BRAF and PI3K/AKT Pathways in Colorectal Cancer - Implications for Targeted Therapy*. Discov. Med. 14(76):207-14.

[64] Lamba S et al. 2014. *RAF Suppression Synergizes with MEK Inhibition in KRAS Mutant Cancer Cells*. Cell Reports 8, 1475-1483.

[65] Gorden A et al. 2003. *Analysis of BRAF and N-RAS Mutations in Metastatic Melanoma Tissues*. Cancer Research 63, 3955-3957.

[66] Goydos JS et al. 2003. *Detection of B-RAF and N-RAS mutations in human melanoma*. Journal of the American College of Surgeons, Vol. 200, Issue 3, 362-370.

[67] Maurer G, Tarkowski B and Baccarini M. 2011. *Raf kinases in cancer - roles and therapeutic opportunities*. Oncogene 30, 3477-3488.

[68] Dougherty MK et al. 2005. *Regulation of Raf-1 by direct feedback phosphorylation*. Mol. Cell 17, 215-224.

[69] Kamioka Y, Yasuda S, Yoshihisa F, Kazuhiro A, and Michiyuki M. 2010. *Multiple decisive phosphorylation sites for the negative feedback regulation of SOS1 via ERK*. J. Biol. Chem. 285, 33540-33548.

[70] Kochaczyk M et al. 2017. *Relaxation oscillations and hierarchy of feedbacks in MAPK signaling*. Sci. Rep. 7, 38244.

[71] Cirit M, Wang C and Haugh JM. 2010. *Systematic quantification of negative feedback mechanisms in the extracellular signal-regulated kinase (ERK) signaling network*. J. Biol. Chem. 285, 36736-36744.

[72] Schoeberl B, Eichler-Jonsson C, Gilles ED, Muller G. 2002. *Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors*. Nature Biotechnology 20, 370-375.

[73] Sharrocks DA. 2006. *Cell Cycle: Sustained ERK Signalling Represses the Inhibitors*. Curr Biol. 16(14):R540-2.

[74] Ebisuya M, Kondoh K and Nishida E. 2005. *The duration, magnitude and compartmentalization of ERK MAP kinase activity: mechanisms for providing signaling specificity*. Journal of Cell Science 118: 2997-3002.

[75] Sasagawa S, Ozaki Y-i, Fujita K, Kuroda S. 2005. *Prediction and validation of the distinct dynamics of transient and sustained ERK activation*. Nature Cell Biology 7, 365-373.

[76] Keyes J et al. 2020. *Signaling diversity enabled by Rap1-regulated plasma membrane ERK with distinct temporal dynamics*. eLife 9:e57410.

[77] Gillespie DT. 1976. *General method for numerically simulating stochastic time evolution of coupled chemical-reactions*. Journ. Comput. Phys., 22:403434.

[78] Gillespie DT. 1977. *Exact stochastic simulation of coupled chemical reactions*. Journ. Phys. Chem., 81(25):23402361.

[79] Gillespie DT. 2007. *Stochastic simulation of chemical kinetics*. Annu. Rev. Phys. Chem., 58:3555.

[80] Lecca P. 2013. Stochastic chemical kinetics. *A review of the modelling and simulation approaches*. Biophys Rev (2013) 5:323–345.

[81] Pettigrew DB, Smolen P, Baxter DA, Byrne JH. 2005. *Dynamic Properties of Regulatory Motifs Associated with Induction of Three Temporal Domains of Memory in Aplysia*. Journal of Computational Neuroscience 18, 163–181.

[82] Barbot B, Haddad S, Picaronny C. 2015. *Rare Event Handling in Signalling Cascades*. INT. J. Adv. Syst. Meas. 8(1-2), 69-79.

[83] Tian T, Harding A, Inder K, Plowman S, Parton RG, Hancock JF. 2007. *Plasma membrane nanoswitches generate high-fidelity Ras signal transduction*. Nature Cell Biology 9, Number 8, 905914.

[84] Vinod PK and Venkatesh KV. 2008. *Quantification of Signaling Networks*. Journal of the Indian Institute of Science, Vol. 88:1.

[85] Pal S, Baiocchi RA, Byrd JC, Grever MR, Jacob ST, Sif S. 2007. *Low levels of miR-92b/96 induce PRMT5 translation and H3R8/H4R3 methylation in mantle cell lymphoma*. The EMBO Journal 26, 3558-3569.

[86] Liang JJ et al. 2007. *The expression and function of androgen receptor coactivator p44 and protein arginine methyltransferase 5 in the developing testis and testicular tumors*. J Urol. 177(5):1918-22.

[87] Liu F et al. 2011. *JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation*. Cancer Cell 19(2): 283-294.

[88] DongSheng W et al. 2012. *PRMT5 suppresses DR4-mediated CCL20 release via NF-κB pathway*. Chin. Sci. Bull., Vol. 57, No. 33, 4351-4355.

[89] Yang M, Sun J, Sun X, Shen Q, Gao Z, Yang C. 2009. *Caenorhabditis elegans protein arginine methyltransferase PRMT-5 negatively regulates DNA damage induced apoptosis*. PloS Genet 5(6): e1000514.

[90] Scoummane A, Zhang J and Chen X. 2009. *PRMT5 is required for cell-cycle progression and p53 tumor suppressor function*. Nucleic Acids Research, Vol. 37, No. 15, 4965-4976.

[91] Calabretta S et al. 2018. *Loss of PRMT5 Promotes PDGFRα Degradation during Oligodendrocyte Differentiation and Myelination*. Dev Cell. 46(4):426-440.e5.

[92] Zhang B et al. 2015. *Targeting protein arginine methyltransferase 5 inhibits colorectal cancer growth by decreasing arginine methylation of eIF4E and FGFR3*. Oncotarget 6(26):22799-811.

[93] Strobl CD et al. 2019. *Selective PRMT5 Inhibitors Suppress Human CD8þ T Cells by Upregulation of p53 and Impairment of the AKT Pathway Similar to the Tumor Metabolite MTA*. Mol Cancer Ther 19(2), 409-419.

[94] Lin H et al. 2019. *Discovery of Potent and Selective Covalent Protein Arginine*

Methyltransferase 5 (PRMT5) Inhibitors. ACS Med. Chem. Lett., 10, 7, 1033-1038.

[95] Brightman FA, Fell DA. 2000. *Dfferential feedback regulation of the MAPK cascade underlies the quantitative differences in EGF and NGF signaling in PC12 cells*. FEBS Letters 482, 169-174.

[96] Traverse S, Gomez N, Paterson H, Marshall C, Cohen P. 1992. *Sustained activation of the mitogenactivated protein (MAP) kinase cascade may be required for differentiation of PC12 cells. Comparison of the effects of nerve growth factor and epidermal growth factor.* Biochem. J 288, 351–355 (1992).

[97] Kao S, Jaiswal RK, Kolch W, Landreth GE. 2001. *Identification of the mechanisms regulating the differential activation of the MAPK cascade by epidermal growth factor and nerve growth factor in PC12 cells*. J. Biol. Chem 276, 18169–18177.

[98] Jansson M et al. 2008. *Arginine methylation regulates the p53 response*. Nat. Cell Biol. 10(12), 1431–1439.

[99] Lazarovici P, Dickens G, Kuzuya H, and Guroff G. 1987. *Long-Term, Heterologous Down-Regulation of the Epidermal Growth Factor Receptor in PC12 Cells by Nerve Growth Factor.* The Journal of Cell Biology, Volume 104, 1611-1621.

[100] Hondermarck H, Mclaughlin CS, Patrersont SD, and Bradshaw RA. 1994. *Early changes in protein synthesis induced by basic fibroblast growth factor, nerve growth factor, and epidermal growth factor in PC12 pheochromocytoma cells.* Proc. Nati. Acad. Sci. USA, Vol. 91, 9377-9381.

[101] Mouri K, Sako Y. 2013. *Optimality conditions for cell-fate heterogeneity that maximize the effects of growth factors in PC12 cells.* PLoS Comput Biol 9(11): e1003320.

[102] Brennan DF, Dar AC, Hertz NT, Chao WCH, Burlingame AL, Shokat KM, Barford D. 2020. *A Raf-induced allosteric transition of KSR stimulates phosphorylation of MEK*. Nature, Vol. 472, 366-371.

[103] Hymowitz SG and Malek S. 2018. *Targeting the MAPK Pathway in RAS Mutant Cancers*. Cold Spring Harb Perspect Med;8:a031492.

[104] Fisher KW et al. 2015. *AMPK promotes aberrant PGC1beta expression to support human colon tumor cell survival*. Mol Cell Biol. 35:3866–3879.

[105] Zhang J et al. 2008. *Downregulation of KSR1 in pancreatic cancer xenografts by antisense oligonucleotide correlates with tumor drug uptake*. Cancer Biol Ther. 7:1490–1495.

[106] Witzel F, Maddison L and Blüthgen N. 2012. *How scaffolds shape MAPK signaling: what we know and opportunities for systems approaches*. Frontiers in Physiology 3:475.

[107] Vetterkind S, Poythress RH, Lin QQ, Morgan KG. 2013. *Hierarchical scaffolding of an ERK1/2 activation pathway*. Cell Communication and Signaling 11:65.

[108] Casar B and Crespo P. 2016. ERK Signals: Scaffolding Scaffolds? Front. Cell. Dev. Biol. 4:49.

[109] Shin S-Y et al. 2009. *Positive- and negative-feedback regulations coordinate the dynamic behavior of the Ras-Raf-MEK-ERK signal transduction pathway*. Journal of Cell Science 122, 425-435.

[110] Kochanczyk M et al. 2017. *Relaxation oscillations and hierarchy of feedbacks in MAPK signaling*. Scientific Reports 7:38244.

[111] Lim SK et al. 2013. *Activation of PRMT1 and PRMT5 mediates hypoxia- and ischemia-induced apoptosis in human lung epithelial cells and the lung of miniature pigs: the role of p38 and JNK mitogen-activated protein kinases*. Biochem. Biophys. Res. Commun. Vol. 44, Issue 4, 707-713.

[112] He W et al. 2008. *Ginkgolides mimic the effects of hypoxic preconditioning to protect C6 cells against ischemic injury by up-regulation of hypoxia-inducible factor-1 alpha and erythropoietin*. Int. J. Biochem. Cell Biol. 40(4):651-62.

[113] Ho M-C et al. 2013. *Structure of the Arginine Methyltransferase PRMT5-MEP50 Reveals a Mechanism for Substrate Specificity*. PLoS ONE 8(8).

[114] Burgos ES et al. 2015. *Histone H2A and H4 N-terminal Tails Are Positioned by the MEP50*

WD Repeat Protein for Efficient Methylation by the PRMT5 Arginine Methyltransferase. J. Biol. Chem. 290(15):9674-89.

[115] Chen H, Lorton B, Gupta V, Shechter D. 2017. *A TGFβ-PRMT5-MEP50 axis regulates cancer cell invasion through histone H3 and H4 arginine methylation coupled transcriptional activation and repression*. Oncogen, 36(3):373-386.

[116] Mongiardi MP et al. 2015. *Myc and Omomyc functionally associate with the Protein Arginine Methyltransferase 5 (PRMT5) in glioblastoma cells*. Scientific Reports 5(1):15494.

[117] Aggarwal P et al. 2010. *Nuclear Cyclin D1/CDK4 Kinase Regulates CUL4 Expression and Triggers Neoplastic Growth via Activation of the PRMT5 Methyltransferase*. Cancer Cell 18(4): 329-340.

[118] Owens JL et al. 2020. *PRMT5 Cooperates with pICln to Function as a Master Epigenetic Activator of DNA Double-Strand Break Repair Genes*. Iscience 23, 100750.

[119] Rehman I et al. 2018. *PRMT5-mediated arginine methylation of TDP1 for the repair of topoisomerase I covalent complexes*. Nucleic Acids Research, Vol. 46, No. 11, 5601-5617.

[120] Hwang JW et al. 2020. *PRMT5 promotes DNA repair through methylation of 53BP1 and is regulated by Src-mediated phosphorylation*. Communications Biology, 3:428.

[121] He W, Ma X, Yang X, Zhao Y, Qiu J, and Hang H. 2011. *A role for the arginine methylation of Rad9 in checkpoint control and cellular sensitivity to DNA damage*. Nucleic Acids Research, Vol. 39, No. 11, 4719-4727.

[122] Berman HM et al. 2020. *The Protein Data Bank*. *Nucleic Acids Research,* Volume 28, Issue 1, Pages 235–242.

[123] Pettersen EF et al. 2021. *UCSF ChimeraX: Structure visualization for researchers, educators, and developers*. Protein Sci. 30(1):70-82.

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