THE INTERPLAY OF DOUBLE PHOPHORYLATION AND SCAFFOLDING IN THE MAPK PATHWAYS

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ABSTRACT

The MAPK cascade is a principal kinase transduction pathway in eukaryotic cells. It transmits signals through three layers of sequentially activated kinases, RAF, MEK and ERK. The latter two kinases require dual phosphorylation for activation. Another property of MAPK signalling is its involvement of scaffolds - multidomain proteins that can assemble protein complexes; in this case the three MAPK components. In this study we analyze analytically and numerically four heuristic models of MAPK signalling in the presence or absence of a scaffold considering both real MEK and ERK kinases and their hypothetical isoforms that require only monophosphorylation. Based on this analysis we will demonstrate that double phosphorylation enforces signaling through scaffolds, which increases both versatility and specificity of the regulation.

INTRODUCTION

The Mitogen Activated Protein Kinase (MAPK) cascade transmits plethora of various signals in eukaryotic cells eliciting diverse cellular responses such as proliferation, differentiation, and apoptosis [2]. It transmits signals through three layers of sequentially activated kinases, RAF, MEK and ERK. The latter two kinases require double phosphorylation for activation – this property is perfectly conserved in evolution from yeast to human. Their dephosphorylation at either residue leads to deactivation. The requirement of double phosphorylation of MEK and ERK has important dynamical consequences - activation of MEK and ERK require two collisions with their upstream kinases. As we will show, this introduces fourth order nonlinearity in signal processing: in the limit of low signal S, the response is proportional to S^4 in the case of double phosphorylation. The situation is different, however, when signaling involves scaffolds - multidomain proteins that can assemble protein complexes, in this case the three MAPK components [5]. In such case phosphorylation can be processive - two residues are phosphorylated one by one (effectively in a single step) on the same scaffold molecule. One may thus expect that the response would be a linear function of S in the limit of low S. The above observations lead to conjecture that double-phosphorylation is required for transmission of signals through scaffolds, which results in a better control of signal transmission and the specificity of response. In signalling involving scaffold, the strength of the output is controlled both by the magnitude of signal and the scaffold concentration. We will verify this conjecture by constructing four simple models of MAPK signalling in the presence or absence of the scaffold considering both real MEK and ERK kinases and their hypothetical isoforms that require only mono-phosphorylation. We will then analyze their behavior and performance in terms of signal amplification.
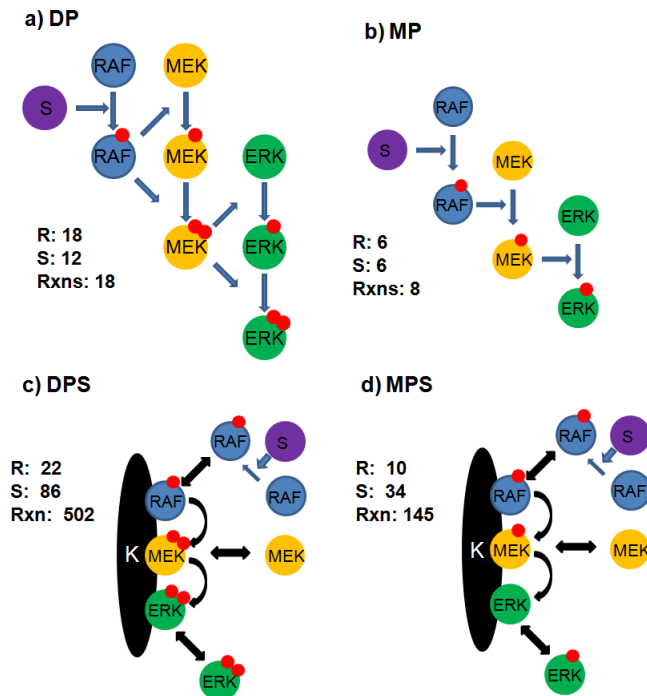
Figure 1. The implemented MAPK cascade models. R – the number of BioNet-Gen rules, S – the total number of generated species, Rxn – the number of generated reactions.

MODELS

We have constructed four models of the MAPK cascade: (1) MP model – where MEK and ERK required monophosphorylation for activation; (2) DP model – where MEK and ERK required double phosphorylation for activation; (3) MPS model – based on MP, with scaffold K; (4) DPS model – based on DP, with scaffold K. The total concentration of each kinase has been set to 10^5. The models have been graphically presented on Fig. 1, the parameters are listed in Table 1, and the equations or equation rules are presented below.

The input Signal (S) controls the phosphorylation rate of the first kinase in the cascade (RAF); biologically, S is the level of an upstream activator RAS. The level of the final MAP kinase (ERK) in its active state is considered the output. In further analysis we will consider the level of activated ERK in the stationary state as a function of S.

The MP and DP models assumed that the reactions occur in the well-mixed cytoplasm and that all MAPK components were phosphorylated and dephosphorylated with the same rates.

In the MPS and DPS models, additional rules have been introduced to describe the interactions of the MAPK components with molecules of the scaffold. The MAPK components (except RAF) can freely associate and dissociate with and from the scaffold molecules with the same effective rates, independent of their phosphorylation status. Since RAF association with scaffold is signal-dependent, unphosphorylated RAF cannot bind the scaffold. If it is already bound, it dissociates with the rate 10 times greater than phosphorylated RAF. RAF can be phosphorylated only in its unbound state while the phosphorylation of MEK and ERK can occur exclusively on the scaffold. The dephosphorylation rates for all the MAPK components are equal and are independent of their association with the scaffold.

Table 1. The List of Reactions, and the Values and Ranges of Associated Rate Constants. The parameters in bold face were varied jointly. In the specified range the qualitative behavior of the models does not change.

Reactions	Parameter	Value	Range
Association of MEK, unphosphorylated ERK and phosphorylated RAF with scaffold	S_f	$2{*}10^{-6}$ $(\text{mol}{*}\,\text{s})^{-1}$	$0-2.5{*}10^{-6}$
Dissociation of MEK, unphosphorylated ERK and phosphorylated RAF with scaffold	S_r	0.01 $(\text{mol}{*}\,\text{s})^{-1}$	0–13
Dissociation of unphosphorylated RAF from scaffold	S_{r_raf}	100 s^{-1}	$0.1-\infty$
Dissociation of phosphorylated ERK from scaffold	S_{r_erk}	10	$0.59-\infty$
Phosphorylation of RAF by RAS, MEK by RAF, and ERK by MEK (cytoplasm)	K_p	$2{*}10^{-6}$ $(\text{mol}{*}\,\text{s})^{-1}$	$0-2.5{*}10^{-6}$
Phosphorylation of MEK by RAF, and ERK by MEK (scaffold)	S_p	100 s^{-1}	$3.4-\infty$
Dephosphorylation of RAF, MEK, and ERK	K_d	0.1 s^{-1}	$0.084-0.57$

The models have been implemented using BioNetGen, a rule-based modeling environment. In BioNetGen models are constructed by specifying rules that describe allowed protein-protein interactions, processes, and covalent modifications. Based on the rules, the reaction network is automatically generated along with the system of ODEs. The advantage of this approach is that it often allows for concise definition of models with large numbers of reactions. See e.g. [9].

Monophosphorylation Model — MP

R_p, M_p, E_p represent the concentrations of phosphorylated RAF, MEK, and ERK. K_p and K_d represent the rate of phosphorylation and dephosphorylation, respectively.

$$\frac{dR_p}{dt} = K_p S (R_{\text{tot}} - R_p) - K_d R_p$$
$$\frac{dM_p}{dt} = K_p R_p (M_{\text{tot}} - M_p) - K_d M_p \tag{1}$$
$$\frac{dE_p}{dt} = K_p M_p (E_{\text{tot}} - E_p) - K_d E_p$$

Double Phosphorylation Model — DP

R_p, M_p, E_p, M_{pp}, E_{pp} respectively represent the concentrations of mono- and double phosphorylated RAF, MEK, and ERK. K_p and K_d represent the rate of phosphorylation and dephosphorylation, respectively.

$$\frac{dR_p}{dt} = K_p S (R_{\text{tot}} - R_p) - K_d R_p$$
$$\frac{dM_p}{dt} = 2K_p R_p (M_{\text{tot}} - M_p - M_{pp}) + 2K_d M_{pp} - K_d M_p - K_p R_p M_p$$
$$\frac{dM_{pp}}{dt} = 2K_p R_p M_p - 2K_d M_{pp} \tag{2}$$
$$\frac{dE_p}{dt} = 2K_p M_{pp} (E_{\text{tot}} - E_p - E_{pp}) + 2K_d E_{pp} - K_d E_p - K_p M_{pp} E_p$$
$$\frac{dE_{pp}}{dt} = 2K_p M_{pp} E_p - 2K_d E_{pp}$$

Monophosphorylation with Scaffold Model — MPS

Both MPS and DPS models have been expressed in the BioNetGen formalism. The rules of the MPS model are presented (Fig. 1d):

(1) RAF(s,S1~U) + RAS(GDP~P) → RAF(s,S1~P) + RAS(GDP~P) K_p

(2) RAF(s!1,S1~U).S(raf!1) → RAF(s,S1~U) + S(raf) S_{r_raf}

(3) RAF(s,S1~P) + S(raf) ↔ RAF(s!1,S1~P).S(raf!1) S_f, S_r

(4) MEK(s) + S(mek) ↔ MEK(s!1).S(mek!1) S_f, S_r

(5) ERK(s!1,S1~P).S(erk!1) → ERK(s,S1~P) + S(erk) S_{r_erk}

(6) RAF(s!1,S1~P).S(raf!1,mek!2).MEK(s!2,S1~U) → RAF(s!1,S1~P).S(raf!1,mek!2).MEK(s!2,S1~P)
   S_p

(7) ERK(s!1,d,S1~U).S(erk!1,mek!2).MEK(s!2,S1~P) ERK(s!1,d,S1~P).S(erk!1,mek!2).MEK(s!2,S1~P)
   S_p

(8) RAF(s,d,S1~P) → RAF(s,d,S1~U) K_d

(9) MEK(s,d,S1~P) → MEK(s,d,S1~U) K_d

(10) ERK(s,d,S1~P) → ERK(s,d,S1~U) K_d

The following are three rules representative of the BioNetGen syntax:

a) Rule 1 — the phosphorylation of RAF on site S1 by the RAS phosphorylated on residue, GDP at the rate K_p

b) Rule 3 — the reversible binding of phosphorylated RAF with scaffold S via the domains, "s" and "raf", respectively, at the rates S_p and S_r

c) Rule 6 — the phosphorylation of MEK by RAF, both bound to scaffold S, at the rate S_p

Double Phosphorylation with Scaffold Model — DPS

This model is represented by 22 BioNetGen rules, which generate 502 reactions, involving 86 species (Fig. 1c).

Mixed Monophosphorylation and Double Phosphorylation Models — FMS and FDS

In these models phosphorylation is allowed to occur both on the scaffold and in the cytoplasm, which is a more realistic representation of the processes taking place in the cell.

RESULTS

In the stationary states of systems (1) and (2), the levels of active ERK kinase are respectively:

$$E_p = S\,\frac{K_p^3 R_{\text{tot}} M_{\text{tot}} E_{\text{tot}}}{K_d^3 + K_d^2 K_p S + K_d K_p^2 S R_{\text{tot}} + K_p^3 S R_{\text{tot}} M_{\text{tot}}} \tag{3}$$

$$E_{pp} = S^4\,\frac{K_p^{10} R_{\text{tot}}^4 M_{\text{tot}}^2 E_{\text{tot}}}{\left(K_d^5 + 2K_d^4 K_p S + 2K_d^2 K_p^3 S^2 R_{\text{tot}} + K_d K_p^4 S^2 R_{\text{tot}}^2 + K_p^5 S^2 R_{\text{tot}}^2 M_{\text{tot}} + b\right)^2} \tag{4}$$

where

$$b = K_d^3 K_p^2 S\,(2R_{\text{tot}} + S)$$

The results clearly demonstrate that in case of monophosphorylation, the output depends linearly on the signal S in the limit of $S \to 0$. In contrast, in the distributive phosphorylation model the output depends on S^4 in the limit of $S \to 0$.

In order to determine the models' performances and dynamic characteristics, we analyzed their responses to signal in the range from 10 to 10^6. At the optimal scaffold concentration (i.e. equal to the concentrations of the kinases), the MP model displayed the greatest response among all the models, while the DP model fared last (Fig. 2a). The DPS model responded more strongly than DP, especially at the low signal level in the range of $10^3 - 3\text{x}10^4$. In contrast, the MPS model responded significantly less than MP for the entire signal range, even though it still surpassed the response of DPS. The DP model displayed appreciable response at a higher signal level than the other models (~$3\text{x}10^4$) as opposed to the MP model which exhibited discernible response at the
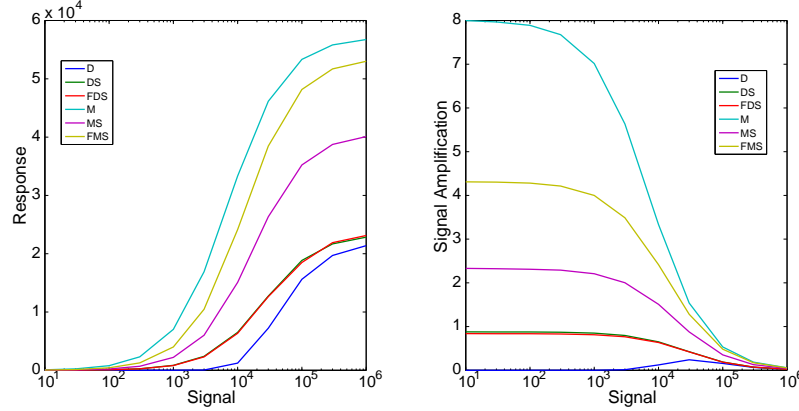
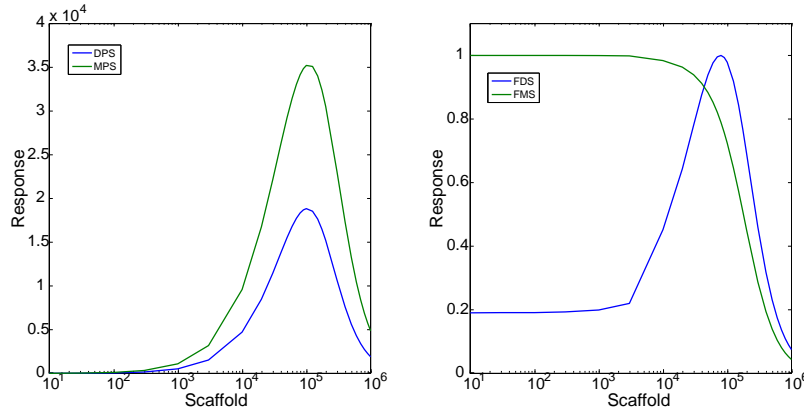Figure 2. Signal Response Relationships of the MAPK Models: (left) Response Magnitude (right) Amplification

Figure 3. The Impact of Scaffold. (left) DPS and MPS (right) FDS and FMS (normalized)

signal level as low as 10^3. Lastly, the DP model attained full activation in the more narrow signal range ($10^4 - 3\mathrm{x}10^5$) than the other models.

The MP model provided greatest amplification of the incoming signal in the range of 6–8 for low and moderate signal magnitudes (Fig. 2b). The MPS model's amplification was significantly lower than that of MP, though greater than those of DP and DPS. The amplification provided by MP was relatively uniform at the order of 2 for the signal range from 10 to 10^4. The DPS model yielded no amplification (at the order of 1) while the DP model lead to signal diminution. The amplification of all models deteriorated as the signal approached saturation.

Overall, for the entire signal range the following inequalities held:

$$1 < \frac{A_m}{A_{ms}} > \frac{A_d}{A_{ds}} < 1$$

where A is the amplification of the signal by a specified model. Parameter sensitivity analysis has been performed (Table 1) and its results indicated that the observed qualitatitve behavior occured for a broad range of parameters, being most sensitive to the rate of dephosphorylation, K_d, where it permitted variation by 1.5 orders of magnitude.

The scaffold exerted the canonical influence on DPS and MPS models, yielding the maximal response at its stoichiometric concentration, while leading to the decrease of the response at both lower and higher concentrations (Fig. 3a).

The combined models displayed more complex behavior. In case of FDS, at low scaffold concentration its output was that of DP while displaying significant increase (5 fold) at the optimal scaffold concentration (Fig. 3b). When scaffold was in excess, the response was progressively reduced in the concentration-dependent manner until it was lower than that of DP. As for the FMS model, its response was maximal and equal to that of MP at low scaffold concentrations, and began to decrease, starting at the concentration of 3×10^3; the model finally converged with MPS at saturation. Furthermore, the FMS model exhibited intermediate behavior in regard to MP and MPS, while the behaviors of FDS and DPS were essentially the same (Fig. 2a)

CONCLUSIONS

The presence of the scaffold has opposing effects on signal amplification in the monophosphorylation and double phosphorylation paradigms. In particular, the scaffold diminishes signaling through the MAP kinases that require only monophosphorylation, while augmenting signaling in the situation where diphosphorylation is required. This effect was also exhibited by the mixed models, which are more representative of the cellular environment. In the FMS model, the increasing concentration of the scaffold led to the monotonic decrease in the response. In contrast, in the FDS model, the presence of the scaffold increased the response with the greatest amplification at the stoichiometric concentration, and decreasing it thereafter. We therefore postulate that double phosphorylation favors signaling through scaffolds, especially at low signal levels. Double phosphorylation could therefore functionally cooperate with scaffolding, leading to higher response specificity to general signaling cues. In contrast, at least in terms of amplification similar cooperation could not easily occur within the monophosphorylation paradigm.

In conclusion, our results indicate that while seemingly less efficient, the double-phosphorylation paradigm leads to a wider range of dynamics and is more amenable to regulation and fine-tuning, which can be exemplified by its cooperation with scaffolds. This suggests that in evolution more subtle traits as flexibility and "adjustability" can be more desirable and selected upon rather than simple raw performance.

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