Effect of the MAPK cascade structure, nuclear translocation and regulation of transcription factors on gene expression

Vivek K. Mutalik, K.V. Venkatesh *

Department of Chemical Engineering and School of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai 400076, India

Abstract

The mitogen activated protein kinase (MAPK/ERK) cascade system represents a highly conserved prototype of signal transduction by enzyme cascades. One of the best-studied properties of the MAPK system is its ability to convert graded input stimulus to switch-like all-or-none responses. Previous theoretical studies have centered on quantifying dual phosphorylated MAPK as a final output response and have not incorporated its influence on the regulation of gene expression. The main objective of the current work is to understand the regulatory effect of positive feedback loop embedded in the MAPK cascade, nuclear translocation of active MAPK, phosphorylation and activation of nuclear target proteins on the regulation of specific gene expression. To achieve this objective, we have simulated the MAPK cascade system, which resembles Hog1p activation pathway in yeast, at steady state. Thus, the input signal to the MAPK system is correlated with gene expression as a final system-level output response. The steady-state simulation results suggest that other than regulating the signal propagation through cascades, the nuclear translocation of activated MAPK and subsequent regulation of gene expression represent one of the key modes to control the threshold level of response. This work proposes that, it is essential to consider the compartmental distributions of signaling species and the corresponding regulatory mechanisms of gene expression to study the system-level performance of signaling modules such as the MAPK cascade. Such an analysis will relate the extracellular cues to the final phenotypic response by capturing the mechanistic details of the signaling pathway.

Keywords: MAPK cascade; Switch-like response; Ultrasensitivity; Systems biology; Theoretical modeling; Regulatory design principles

Cellular signal transduction networks continuously sense extracellular cues and transduce signals from the cell surface to interior of the cell. The cell responds to this signal through change in protein activity and gene expression to yield a specific phenotype. In eukaryotic cells, the MAPK cascade system represents a highly conserved regulatory element mediating the transduction of signals from the cell surface to the nucleus (Widmann et al., 1999; Pearson et al., 2001). MAPK cascades are known to be activated by a wide array of signals ranging from growth factors, hormones, stress signals, cytokines and neurotransmitters perceived by different types of receptors (Schaeffer and Weber, 1999; Widmann et al., 1999; Pearson et al., 2001; Kyriakis and Avruch, 2002). MAPK cascades have been shown to regulate numerous cellular processes including cell differentiation, movement, division and cell death. Because of its highly conserved architecture and biological significance, the MAPK cascade system has been a prototype
of signal transduction by enzyme cascades and is a very well studied biological system (Gustin et al., 1998; Bannett, 1998; Schaeffer and Weber, 1999; Lengeler et al., 2000; Pearson et al., 2001; Kyriakis and Avruch, 2002; Holmann, 2002).

The basic assembly of MAPK system consists of three sequentially activating enzyme cascades of phosphorylation–dephosphorylation reaction such that an active MAPK kinase (MAPKKK, e.g., Raf, mos) activates MAPK kinase (MAPKK, e.g., Mek, MKK), which further activates a specific MAPK (e.g., ERK, JNK, P38, P42). The terminal MAPK acts as an effector of a unique pathway, by regulating the gene expression and protein activity to elicit an appropriate physiological response. MAPKK and MAPK are activated by double phosphorylation on conserved serine/threonine and tyrosine residues and are inactivated by dephosphorylation reaction catalyzed by specific phosphatases (Widmann et al., 1999; Pearson et al., 2001). Some of the intriguing aspects of the MAPK cascade system that have made both experimental and theoretical biologists to take a keen interest in the MAPK cascade signaling are, its highly conserved three tier regulatory architecture, retaining specificity even when cascade components are shared between different pathways, ability of a particular pathway structure to elicit diverse responses depending on the type and/or amplitude of input signal, the possibility of signal and sensitivity amplification and the intricate feedback loops embedded in the signaling pathway (Madhani and Fink, 1998; Ferrell and Machleder, 1998; Schaeffer and Weber, 1999; Widmann et al., 1999; Pearson et al., 2001; Breitkreutz and Tyers, 2002).

One of the best-studied properties of signal transduction through the MAPK system is its ability to convert the graded input stimulus to switch-like all-or-none responses (Ferrell, 1996, 1997, 1998, 2002; Ferrell and Machleder, 1998). Such ultrasensitive switch-like responses display steeply sigmoidal stimulus–response curves. Mutisite phosphorylation, zero order effects and cascade structure appear to contribute to such molecular switch phenomenon (Ferrell, 1996, 1997). The presence of positive feedback loops or double negative feedback loops further impart extreme ultrasensitivity to the system in which signaling species switch between two stable states without settling in an unstable intermediate state (bistable response). However, these system-level responses appear to be specific to a cell type or are dependent on a particular signaling milieu. For example, the switch-like performance of the MAPK cascade has been shown in Xenopus oocytes (Ferrell and Machleder, 1998), whereas it has been implicated to display a graded output response in mammalian cells (Whitehurst et al., 2004; MacKeigan et al., 2005) and budding yeast mating pheromone pathway (Poritz et al., 2001) at the level of individual cells. Thus, in such cases graded output of the MAPK cascade system displays rheostat like performance and may be the all-or-none regulatory phenomenon imposed somewhere downstream of the MAPK cascade such as nuclear translocation or regulatory mechanisms of gene expression (Ferrell, 2002). Studies have indicated that, sub-cellular translocation of proteins can also exhibit switch-like outputs (Ferrell, 1998; Teruel and Meyer, 2002). It can be argued that, such all-or-none responses may not be always required, for example, when the target protein has to sense wide range of change in the input signal (Poritz et al., 2001; Sauro and Khodolenko, 2004). Typically, there are several target proteins of active MAPK in the cytoplasm and in the nucleus. This action of MAPK is dependent on many other aspects such as phosphatase activity, interaction with adaptors and scaffold proteins and nuclear translocation of active MAPK.

Several theoretical models of the MAPK cascade system are available which have focused on its different regulatory facets (reviewed in Sauro and Khodolenko, 2004; Kolch et al., 2005; Oriti et al., 2005). Most of the previous theoretical studies have centered on uncovering the design features of MAPK cascades with dual phosphorylated MAPK as a final output response and have not been studied in the context of gene expression (Huang and Ferrell, 1996; Brightman and Fell, 2000; Khodolenko, 2000; Asthagiri and Lauffenburger, 2001; Bhalia et al., 2002; Schoeberl et al., 2002; Harakeyama et al., 2003; Chapman and Asthagiri, 2004; Nakabayashi and Sasaki, 2005). Recently, Hazzalin and Mahadevan (2002) proposed that, the MAPK activation and its nuclear translocation may yield a continuously variable switch at the level of gene expression. They related each cycle of transcriptional initiation to a single event of phosphorylation and dephosphorylation. Thus, as transcription factors shuttle between phosphorylated and dephosphorylated states, the genes switch on and off in a periodic way rather than a stable on and off state (Hazzalin and Mahadevan, 2002).

The main objective of the present work is to understand the regulatory effect of nuclear translocation of active MAPK, phosphorylation and activation of nuclear target proteins leading to regulation of specific gene expression. To achieve this objective, we have simulated a MAPK cascade system, which resembles Hog1p activation pathway in yeast, at steady state. The MAPK pathway was quantified at system-level by incorporating the regulatory mechanisms such as, activation and
nuclear translocation of MAPK, activation of transcription factors and regulation of gene expression. Thus, the input signal to the MAPK system is correlated with gene expression as a final system-level output response. The effect of positive feedback on the MAPK translocation and gene activation is also analyzed. The steady state simulation results suggest that, other than regulating the signal propagation through cascades, nuclear translocation of activated MAPK and subsequent regulation of gene expression represent one of the key modes of regulation by means of controlling the threshold level of activation. This work proposes that, it is essential to consider the compartmental distributions of signaling species and the corresponding regulatory mechanisms of gene expression to study the system-level performance of signaling modules such as the MAPK cascade system.

1. Materials and methods

The modeling framework considered for the MAPK cascade system, nuclear translocation of activated MAPK, activation and dimerization of transcription factors and subsequent gene expression is schematically shown in Fig. 1. The concentration of metabolites ATP, UTP and PPs are considered to be invariant. The framework of Goldbeter and Koshland (1981) was used to model the system at steady state and accordingly an equivalent rate constant and Michaelis-Menten constant nomenclature scheme for each phosphorylation–dephosphorylation cycle is applied. The forward and reverse covalent modification–demodification reactions are considered to be at steady state, and all protein–protein and protein–DNA interactions are assumed to be at equilibrium. The mass balance equations for total species in each module are taken into account while simulating the network.

The reaction scheme shown in Fig. 1 was represented in the form of a set of steady state algebraic equations instead of writing ordinary differential equations for each species of the signaling pathway. This set of steady state algebraic equations and molar balances for each species was written by neglecting Michaelis-Menten complexes (details given in Appendix A). These equations were then numerically solved to estimate the output response of the system for a given input stimuli. Four different cases were considered for the analysis.

1. The MAPK cascade system without the effect of feedback loop.
2. The MAPK cascade system with positive feedback loop.
3. The MAPK cascade system with nuclear translocation of active MAPK and subsequent regulation of gene expression in absence of feedback loop.
4. The effect of positive feedback loop in the MAPK cascade system on the overall regulation of gene expression.

In the MAPK cascade system, an input signal (a kinase) activates MAPKKK and which further phosphorylates downstream MAPK. The activated MAPKKK phosphorylates terminal MAPK. The input signal is then related to the fractional activation of MAPKKK, MAPK and MAP. The fractional activation of cascade components is given by,

\[
\begin{align*}
\text{f}_1 &= \frac{\text{MAPKK}_p}{\text{MAPKK}_\text{total}} \\
\text{f}_2 &= \frac{\text{MAPK}_p}{\text{MAPK}_\text{total}} \\
\text{f}_3 &= \frac{\text{MAP}}{\text{MAP}_\text{total}}
\end{align*}
\]

where subscript p and total indicate the number of phosphorylated and total concentration of that kinase, respectively. \( f_1, f_2 \) and \( f_3 \) represent the fractional phosphorylation of MAPKKK, MAPKK and MAP, respectively.

The input stimulus to the MAPK cascade in absence of positive feedback loop is defined as, total concentration of kinase at a fixed concentration of phosphatase of MAPKKK cycle. In presence of positive feedback loop, it is assumed that the dual phosphorylated MAPK activates the phosphorylation of MAPKKK. Thus, in presence of positive feedback loop, the input stimulus to MAPKKK is defined as,

\[
\text{input} = K_{I_1} + \frac{v}{H} \left( \frac{\text{MAPK}_\text{total}}{K_n} + K_n \right)
\]

where \( K_{I_1} \) is total concentration of primary kinase, i.e., input stimulus at a fixed concentration of corresponding phosphatase, \( v \) the strength of feedback loop, \( K_n \) the half saturation constant and \( H \) is Hill coefficient quantifying the steepness of MAPK dual phosphorylation against concentration of input stimulus in absence of feedback loop. Here it is assumed that, the dual phosphorylation (activation) of MAPKKK and MAPK occurs by the two-step distributive mechanism (Ferrell and Bhatt, 1997).

To simulate the effect of activation of MAPK on its nuclear translocation and gene expression, a simple modular steady state approach was used. The MAPK cascade system with or without feedback loop was considered as one module, whereas, the activation of transcription factor, its dimerization and gene expression are considered as another module. The output of the MAPK cascade modules (i.e., the dual phosphorylated MAPK) is fed as an input to the gene expression module. By connecting these modules, the input signal to the MAPK cascade is related to the output of gene expression module. Here, the regulatory structure of Hog1p activation and nuclear translocation in yeast is taken as an example. It is assumed that, dual phosphorylated MAPK translocates into the nucleus and activates phosphorylation of two different transcription factors (TF1 and TF2). The nuclear translocation of MAPK is considered to be in equilibrium and is defined as,

\[
K_i = \frac{\text{MAPK}_\text{nucleus}}{\text{MAPK}_\text{cytoplasm}}
\]

where \( K_i \) is the translocation constant defining the ratio of nuclear to cytoplasmic active MAPK. Phosphorylation and dephosphorylation cycles of both transcription factors are
Fig. 1. Schematic of MAPK signaling network considered in the present work. Number of phosphorylations are indicated by the letter p. Enzyme $E_1$ (a kinase) catalyzes the forward reaction of first cycle, and $E_2$, $E_3$, and $E_4$ are phosphatases of first, second, and third cycle of MAPK system, respectively. $E_5$, and $E_6$ are phosphatases of transcription factor-1 and -2, respectively. $K_m$, $K_d$, and $k$ are Michaelis constant, dissociation constants and rates of the reaction, respectively (subscript $i$ indicate numbers from 1 to 10) as shown in the figure. Output of MAPK cascade system is assumed to be the dual phosphorylated MAPK. Dual phosphorylated MAPK translocates into the nucleus and phosphorylates transcription factor-1 (TF1) and TF2. Activated TF1 and TF2 get dimerized and bind to enhancer sites. Dimerized active form of TF1 activates the expression of gene D1, whereas dimerized active form of TF2 represses the expression of gene D2. The input to this system is the total input kinase concentration of MAPKK cascade and final output response is the fractional gene expression.

Assumed to operate with different properties. Activation of TF1 is considered to operate under zero order effects and that of TF2 is assumed to operate away from zero order. Here the zero order effect is defined as the operation of converter enzymes (kinase/phosphatase) in a region of saturation with respect to their substrates (target proteins). Thus, the cascade parameters are chosen such that, the dose–response curve of TF1 and TF2 are sigmoidal and hyperbolic to input signal, respectively. TF1 is therefore sensitive to broad change in the concentration of nuclear MAPK, whereas TF1 is highly sensitive to levels of nuclear MAPK. The fractional phosphorylation of TF1 and TF2 is given as,

$$f_1 = \frac{TF_1}{TF_{1\text{total}}},$$

$$f_2 = \frac{TF_2}{TF_{2\text{total}}}.$$
where TF1p and TF2p are concentration of phosphorylated TF1 and TF2, and the subscript total represents the total concentration of transcription factors. The phosphorylated TF1 and TF2 further dimerize and regulate the expression of two separate genes D1 and D2, respectively. The phosphorylated TF2 stimulates the expression of gene D1, whereas phosphorylated TF2 represses the gene D2. Thus, the fractional expression of genes D1 and D2 is given as:

\[ f_6 = \frac{D_1TF1p_2}{D_1_{\text{total}}} \]  
\[ f_7 = \frac{D_2_{\text{free}}}{D_2_{\text{total}}} \]

where D1_{total} and D2_{total} are total enhancer sites for binding of TF1 and TF2, respectively. D1_{TF1p_2} represents complex between active dimer TF1p and corresponding enhancer site D1, whereas D2_{free} represents free enhancer site D2.

The steady state equations for covalent modification cycles, equilibrium relationships for allosteric interactions and mass balance equations for total species are listed in Appendix A. These algebraic equations were solved numerically using the fsolve program of Matlab (The MathWorks Inc., USA). The accuracy of the simulation was verified at each stage by numerically checking the mass balance of all species of pathway. The simulations were carried out for estimating the fractions of phosphorylated MAPKK, MAPKK, MAPK (cytoplasmic and nuclear), TF1, TF2 and gene expression. The steepness of the dose–response curves was approximated by the apparent Hill coefficient. The set of parameters and concentrations used in the simulations were taken from literature. These simulation parameters and the solution strategy employed to analyze the system are given in Appendix A.

### 2. Results

The steady state model was used to obtain the dose–response curves of fractional activation of MAPK, its nuclear translocation, activation of transcription factors and gene expression in absence and presence of positive feedback loop. To analyze the effect of positive feedback on the output response of the MAPK cascade system, simulations were performed in presence and absence of positive feedback. Fig. 2A shows the effect of input signal on the fractional activation of each of MAPK cascade component in absence of feedback loop. Results indicate that the steepness of the dose–response curve for MAPKK is hyperbolic with Hill coefficient of one, whereas the predicted response curves for the activation of MAPKK and MAPK are sigmoidal with Hill coefficient of 1.65 and 4.8, respectively. The predicted response curves indicate that, as signal propagates down the MAPK cascade, it gets amplified requiring very less input concentration to activate the terminal kinase. These results are in agreement with that of Huang and Ferrell (1996) and validate the methodology adopted in the present work.

Fig. 2B shows the dose–response curves of MAPK cascade system components in presence of a positive feedback loop at various input concentrations. Results indicate that, the output response of MAPK cascade (i.e., dual phosphorylated MAPK) is of all-or-none nature with discrete dose–response curve. This is a characteristic of a binary response seen in presence of positive feedback loop, in which signaling molecules switch between two stable states without settling in an intermediate unstable state. For the set of parameters used, activation of MAPKK also exhibits partial binary response. By comparing Fig. 2A and B, it is clear that, presence of positive feedback effects not only imparts binary characteristics to the entire system, but also illustrates significant amplification of the input signal. It was observed that, this binary response in MAPK cascade is strongly dependent on the strength of feedback loop defined by the term v (Eq. (4)). To analyze its effect on the overall output
Fig. 3. Predicated dose–response curves of MAPK cascade components in presence of positive feedback loop. (A–C) The effect of feedback strength on the response curves for MAPKKK, MAPKK and MAPK modifications. The different feedback strengths are indicated as, curve (a) no feedback, (b) $10^{-6}$, (c) $10^{-5}$, (d) $10^{-3}$ and (e) 1. Simulation was carried out with a Hill coefficient of 5 and half saturation constant of $10^{-5}$ M. Binary response of MAPK is more sensitive than MAPKK and MAPKKK to feedback strength. Dashed lines indicate the discrete response of cascade components between stable states. Response of MAPK cascade, simulations were done with different values of feedback strengths. Fig. 3 shows the effect of different feedback strengths on the steady state response of MAPKKK, MAPKK and MAPK phosphorylation.

The dose–response curves in Fig. 3 demonstrate that, as the feedback strength is increased the fractional phosphorylation of all three kinases exhibit true switch (binary) characteristics. The transition of MAPK phosphorylation from monostable to binary behavior requires much lower feedback strength as compared to that for MAPKK and MAPKKK modification (curve b in Fig. 3). It can be noted that, as feedback strength is increased, the signal propagates down the cascade with high amplification. This signal amplification and binary characteristic are not much affected by changes in the nonlinearity embedded in the (quantified as Hill coefficient) of feedback loop in Eq. (4). The basic characteristics of the response remain same, even if the feedback loop is defined with Hill coefficient one. However, changes in the Hill coefficient do affect the threshold of the response.

Once MAPK is activated in response to a particular stimulus, it phosphorylates several target proteins with in the cytoplasm as well as in the nucleus. Most of the nuclear target proteins are transcription factors, which either activate a particular gene expression or repress it by repression (Widmann et al., 1999; Pearson et al., 2001). In the present work, it was assumed that, active MAPK translocates into the nucleus and phosphorylates two different transcription factors. One of these transcription factors (TF1) activates the expression of gene $D_1$ and the other TF (TF2) represses the expression of gene $D_2$. By solving the set of equations, it was observed that, the threshold stimulus to activate or repress a particular gene expression depends on the translocation constant of active MAPK. Fig. 4 shows the effect of MAPK translocation constant (in absence of feedback) on the activation of transcription factors and the corresponding gene expressions. The simulated results reveal that, as the translocation constant $K_t$ is increased, the threshold input stimulus for activation or repression of gene expression decreases indicating signal amplification as MAPK translocates from cytoplasm to nucleus. Even though, the nuclear concentration of MAPK is very less at low $K_t$ (curve a in Fig. 4A and B), it is capable of activating and repressing gene $D_1$ and $D_2$ expression, respectively (curve c in Fig. 4A and B). As $K_t$ increases, the nuclear concentration of MAPK increases and which in-turn amplifies the performance of TF1 and TF2 (curve b in Fig. 4). Since, the reversible phosphorylation of transcription factors is assumed to operate under zero order and first order conditions, respectively, the response curves of transcription factor activation and the expression of gene $D_1$ and $D_2$ show distinct steepeyness with respect to input stimulus. Thus, at low $K_t$ values even though the MAPK concentration in nucleus
is negligible, it is sufficient for full activation of TF1, whereas TF2 display partial activation (curve b in Fig. 4A and B). At high $K_t$ values both TF1 and TF2 display full activation (curve b in Fig. 4E and F) with Hill coefficient of 10 and 4.5, respectively. It can be noted that as $K_t$ increases the threshold response of transcription factor activation and gene expression display potential signal amplification (curve c in Fig. 4) without affecting the steepness of response.

A separate set of conditions was chosen, to analyze the effect of ultrasensitivity arising out of MAPK cascade on the activation of TF and gene expression (see Appendix A) such that the output response of MAPK cascade (fractional phosphorylation of MAPK to input stimulus) is hyperbolic with a Hill coefficient of one. Fig. 5A and B shows that, when MAPK activation is graded function of the input stimulus, the activation of TF1 and TF2 display characteristic performance of ultrasensitive and graded output response, respectively. TF1 activation is ultrasensitive (with Hill coefficient of 11) due to its operation in the zero order region, whereas, TF2 activation is almost graded (with Hill coefficient of 1) due to its operation in the first order region. The transfer of signal from the active TF1 to expression of genes...
Fig. 5. Effect of hyperbolic output of MAPK cascade on the dose–response curves of fractional concentration nuclear MAPK, activation of transcription factor and fractional gene expression in absence of feedback loop. (A) The performance response in the case of TF-1 and (B) activation of TF-2. Curve a represents fractional concentration of nuclear MAPK, curve b for TF activation and curve c for fractional gene expression. Input concentration refers to the input applied to MAPK cascade. The Hill coefficient of curves a–c in (A) are about 1, 11 and 7, whereas the Hill coefficient of curves b and c in (B) are about 1 and 6. The translocation constant $K_t = 100$ and other parameters used for the simulations are given in Appendix A.

appears to be attenuated since the steepness of fractional gene expression is less ultrasensitive (Hill coefficient of 7) than transcriptional factor activation (Hill coefficient of 11). The fractional expression of gene $D_2$ displays ultrasensitivity to graded output of TF$_2$ activation and this is primarily due to the inherent nonlinearity embedded in dimerization of TF and high association constant of enhancer sites with dimerized TF.

Fig. 6 shows the effect of positive feedback on the nuclear translocation of MAPK, activation of transcription factors and gene expression:

![Fig. 6](image_url)

Fig. 6. Effect of positive feedback loop embedded in MAPK cascade on activation of transcription factor and gene expression: two representative cases are analyzed for showing the effect of translocation constant on the overall response. (A and C) The performance of the TF1 system (for $K_t = 0.01$ and 100, respectively), whereas (B and D) the output response in the case of TF2 (for $K_t = 0.01$ and 100, respectively). Curve a represents fractional concentration of nuclear MAPK, curve b for TF activation and curve c for fractional gene expression. Input concentration refers to the input applied to MAPK cascade. Dashed line indicates the discrete responses and the dots represent the stable states between which the signaling components switch depending upon the threshold input concentration. For simulating the feedback effects following parameters were used: feedback strength of $10^{-3}$, Hill coefficient of 5 and half saturation constant of $10^{-5}/H_9262 M$. 

(A) $G_1$ (B) $G_2$

(C) $G_1$ (D) $G_2$
transcription factors and regulation of gene expression at two different translocation constants. From our earlier simulations (Fig. 2B) it is clear that the final output response of MAPK cascade displays binary characteristics. Fig. 6 shows that nuclear translocation, activation of transcription factor and gene expression can also exhibit binary on-off characteristics in presence of the MAPK cascade embedded with positive feedback loop. The threshold input stimulus required for the activation displays amplification of the signal due to presence of positive feedback loop, even though the activation levels of both transcription factors depended on their cascade parameters.

3. Discussion

Signaling pathways are made up of intricate network of enzyme cascades, allosteric interactions and multiple feedback/feedforward loops. The molecular connectivity within and across these signaling networks are responsible for mounting appropriate input-output dose-response relationships. In the present work, we demonstrate through steady state modeling that, the system-level performance of a signaling pathway is dependent on the numerous operational conditions and on the multiple levels of regulatory mechanisms in the pathway. Our results indicate that, in the case of signaling pathways with common signaling modules like the MAPK cascade, the nuclear translocation, nuclear retention of active signaling molecules, modulation and operatibility of transcription factors control the differential regulation of gene expression. The steady state simulation further shows that, in the presence of positive feedback loop with appropriate feedback strength and translocation constant of communicating species across the compartments modify the threshold level of output response. The translocation constant primarily regulates the signal amplification, while the properties of reversible modification cycles influence the response sensitivity. The positive feedback loop embedded in MAPK cascade system imparts binary or digital characteristic to the cascade output and other downstream signaling components to an applied input stimulus. The binary output response in this case is characterized by the discontinuous dose-response curves and not by the hysteresis feature commonly observed in bistable responses. Since, the fractional modified pathway species were estimated by numerically solving a set of algebraic equations and not by solving the ordinary differential equations at steady states, we obtained only the switching-on property of the response curves. The results indicate that, the modification of terminal kinase MAPK is highly sensitive to the applied feedback strength than that of upstream kinases MAPK and MAPKKK.

Our analysis show that, the regulation of gene expression can demonstrate switch-like outputs even in absence of ultrasensitivity at the level of upstream MAPK cascade. That is, even though the dose-response curve of activation of MAPK may still be hyperbolic, the downstream components may show distinct response sensitivity. This emergence of ultrasensitivity at the level of gene expression to graded input stimuli can be attributed to the nuclear translocation, activation and oligomerization of transcription factors and other regulatory mechanisms of gene expression. Our results indicate that, this regulatory machinery can also cause attenuation of terminal response sensitivity. This can be clearly seen in Fig. 5A in which the fractional phosphorylation of TF1 is ultrasensitive with apparent Hill coefficient of 11, whereas the fractional gene expression shows an apparent Hill coefficient of 7. This sensitivity attenuation appears to be due to the diminization of active transcription factor and its affinity with the enhancer cites. Thus, these results indicate that, the upstream ultrasensitivity in TF activation is not entirely transmitted down to the gene expression level and may represent an important feature of the cellular regulation.

Our results are in agreement with recent experimental analysis, which demonstrated that graded MAPK activity is preceded with switch-like induction of immediate early genes and cell cycle progression in mammalian cells (MacKeigan et al., 2005). Similar analysis in primary human cells demonstrated that, the activation of MAPK is graded in mammalian cells (Whitehurst et al., 2004) whereas it is reported to be bistable in progesterone-induced Xenopus oocytes (Ferrell and Machleder, 1998). Then the question arises as to what may be the significance of such variability in response sensitivity to signaling modules, which share a common regulatory architecture. This may indicate the context or cell type specific responses displayed by the signaling modules underpinning the importance of regulatory design principles behind the system-level output responses. Such variability in response sensitivity may represent the advantage for a specific case in terms of flexibility and adaptability to different environmental cues. Our analysis suggests that, the differential sensitivity of signaling modules can facilitate in regulating numerous other target proteins across compartments, thus avoiding excessive signal amplification or higher response sensitivity.
Most often, the biological responses are highly nonlinear with respect to input signal displaying sigmoidal dose-response characteristics (Koshland et al., 1982; Koshland, 1987, 1998). In an extreme nonlinearity, the dose-response curves approach a step function, in which a very small increment in the input concentration (signal) above the threshold level, the system switches from off-state to on-state. The biological reaction networks, which demonstrate such sigmoidal dependence on an input signal, are known as molecular/regulatory switches (Koshland, 1987; Ferrell, 1996, 2002). These molecular switches typically exhibit a threshold phenomenon in which the concentration of input stimulus just below the threshold are unable to exhibit output response, while concentration just above the threshold can induce stronger responses (Mutalik et al., 2003, 2004, Tyson et al., 2003; Wolf and Arkin, 2003).

The regulatory switches are known to control a wide range of biological processes, from stress/starvation responses and chemotaxis to more complex cellular development and cell fate decisions (Ferrell, 1996, 2002; Anderson et al., 2002). In such systems the nonlinearity resides either at the level of signal transmitting receptor and enzyme cascades or at the response executing gene expression level. Depending upon the regulatory design, these switches can elicit monostable ultrasensitive responses or bistable discrete all-or-none responses.

The system-level approaches in experimental and theoretical studies have shown that, bistability is one of the recurring themes in regulatory circuits across all living systems (Ferrell, 2002). At the level of individual cell, the gene expression appears to be regulated essentially by positive feedback loops or double negative regulatory circuits. Positive feedback loops or double negative feedback loops with embedded nonlinearity are known to be essential to display binary responses, which switch between two stable on-off states (Ferrell and Xiong, 2001; Becskei et al., 2001; Louis and Becskei, 2002). The digital output response of transcriptional process is maximally expressed or is not expressed at all (Smolen et al., 1998, Rossi et al., 2000; Biggar and Crabtree, 2001; Ferrell, 1996, 2002). These switches (Koshland, 1987; Ferrell, 1996, 2002). These regulatory design, these switches can elicit monostable switching gene expression level. Depending upon the regulatory design principles present at the modulatory design, these switches can elicit monostable cutting gene expression level. Depending upon the regulatory design principles present at the modulatory design, these switches can elicit monostable

### Appendix A

#### A.1. Steady state model used for analyzing MAPK cascade system (Fig. A.1)

For convenience, following nomenclature is used: A, MAPKK, AP, MAPKKK, B, MAPKK, BP, MAPKKP, C, MAPK, CP, MAPKP, CPP, MAPKPP.

<table>
<thead>
<tr>
<th>Mass balances</th>
<th>Rate expressions</th>
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| \[ \begin{align*} 
  \dot{A} &= A + 4P \\
  \dot{B} &= B + BP + BPP \\
  \dot{C} &= C + CP + CPP \\
  \dot{A}_t &= 4E_1A \\
  \dot{B}_t &= 4E_1B \\
  \dot{C}_t &= 4E_1C \\
  \dot{E}_t &= \dot{E}_1 = E_1 + 2E_2 + E_3 |
| \[ k_1AE_1 + k_2AE_2 \\
  k_1BP + k_2BPP \\
  k_1CP + k_2CPP \\
  k_1CBPP + k_2CPP \\
  k_1CBPP + k_2CPP |

Complexes

| \[ \begin{align*} 
  A_E &= \frac{E_1}{E_2} \\
  B_P &= \frac{E_2}{E_1} \\
  B_PP &= \frac{E_2}{E_1} \\
  B_PP &= \frac{E_2}{E_1} \\
  C_PP &= \frac{E_2}{E_1} \\
  B_PP &= \frac{E_2}{E_1} |

The parameters used for the analysis were:

- Analysis of MAPKK cascade was performed using Goldbeter and Koshland equation and was connected to MAPKK and MAPK analysis, which in turn was done following parameters on isofluor program of Matlab (The MathWorks Inc.) software. The solution strategy is shown in Fig. A.1. Parameter set referred from Huang and Ferrell (1996). See figure legends in the text for nomenclature. The total enzyme concentrations: \( A_t \), \( B_t \), \( C_t \), (total MAPKK) = 0.003 \( \mu \) M, \( B_t \), (total MAPK) = 1.2 \( \mu \) M; phosphate concentration: \( E_0 = E_0 = 0.0803 \mu \) M; \( E_0 = 0.12 \mu \) M; reaction rates: \( k_1-4k_6 = 150 \) min⁻¹, Michaelis constants:
To obtain a hyperbolic MAPK response, following parameter set was used:

The total enzyme concentrations: $A_t$ (total MPKKK) = 0.2 μM, $B_t$ (total MPKK) = 0.18 μM, $C_t$ (total MPK) = 0.036 μM; phosphatase concentration: $E_{2t} = E_{3t} = 0.224$ μM, $E_{4t} = 0.0032$ μM; reaction rates (min⁻¹): $k_1 = 24, k_2 = 6, k_3 = 60, k_4 = 60, k_5 = 9, k_6 = 60$; Michaelis constants (μM): $K_{m1} = 66.6, K_{m2} = 15.65, K_{m3} = 0.159, K_{m4} = 65, K_{m5} = 0.46, K_{m6} = 0.75$.

Positive feedback effects:

To incorporate the positive feedback of active MAPK on upstream kinase, following modified input relationship was used:

$$E_{in} = E_{in} + \frac{C_{PP, in}}{K_{in, 0.5} + C_{PP, in}}$$

$E_{in}$ is the total input kinase, $v$ the feedback strength, $n_H$ the Hill coefficient and $K_{in, 0.5}$ is the half saturation constant. The feedback parameters are given in the figure legends.
Fig. A.2: Solution strategy for analyzing MAPK cascade system, nuclear translocation of active MAPK, transcription factor activation, dimerization and gene expression at steady state (results shown in Figs. 4–6 in text).
A.2. Steady state model used for analyzing MAPK activation, nuclear translocation, activation of transcription factors and regulation of gene expression (Fig. A.2)

The mass balance equations and equilibrium relationships for MAPK cascade components are same as given in Section A1. The following equations are for gene expression module.

<table>
<thead>
<tr>
<th>Mass balances</th>
<th>Complexes</th>
<th>Equilibrium relationships</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF1 activation</td>
<td>TF(CPP) = ( \frac{(\text{CPP})}{(\text{E})} )</td>
<td>TF1(CPP) = ( k_1 \text{TF1} \cdot \text{E} )</td>
</tr>
<tr>
<td>TF2 activation</td>
<td>TF(CPP) = ( \frac{(\text{CPP})}{(\text{E})} )</td>
<td>TF2(CPP) = ( k_2 \text{TF2} \cdot \text{E} )</td>
</tr>
</tbody>
</table>

Translocation constant: \( k_t = \frac{\text{CPP}}{\text{E}} \)

A.3. The parameters used for the analysis were

MAPK cascade parameters are same as above. The nuclear translocated MAPK, \( \text{CPP} \) (calculated in presence or absence of feedback) was taken as an input to the gene expression module. The modification of transcription factors (TF1, TF2, and TF2) was calculated using Goldbeter and Koshland equation. The regulation of gene expression module was solved by using solve program of Matlab (see solution strategy in Fig. A.2). The parameters used for the simulation are: total concentration of TF’s (\( \mu M \))—TF1 = 2, TF2 = 0.002; phosphatase concentration (\( \mu M \))—\( E_0 = 0.003 \), \( E_0 = 0.02 \); reaction rates (\( \text{min}^{-1} \))—\( k_1 = 150 \), \( k_2 = 40 \), \( k_3 = 360 \); Michaelis constants (\( \mu M \))—\( K_{m1} = 0.03 \), \( K_{m2} = 0.3 \), \( K_{m3} = 20 \), \( K_{m4} = 150 \); total enhancer sites (\( \mu M \))—\( D_0 = D_1 = D_2 = \text{Id}-6 \); dissociation constants (\( \mu M \))—\( K_{d1} = 0.1 \), \( K_{d2} = 0.0001 \), \( K_{d3} = 1 \), \( K_{d4} = 0.001 \). The translocation constant values are given in Figs. 4 and 6. The final output response is fractional expression of gene \( D_1 \) and \( D_2 \) and is defined as \( f_{\text{gene1}} = \left( \frac{D_1 (\text{TF1})}{D_1 (\text{TF1}) + D_1 (\text{TF2})} \right) ; f_{\text{gene2}} = \left( \frac{D_2 (\text{TF2})}{D_2 (\text{TF2}) + D_2 (\text{TF2})} \right) \).

References


