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Extracellular-Regulated Kinase—Mitogen-Activated Protein Kinase cascade: Unsolved Issues

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ABSTRACT

This review point out several aspects regarding the mitogen-activated protein kinase (MAPK)/extracellular-regulated kinase (Erk) network, which are still pending issues in the understanding how this pathway integrate information to drive cell fates. Focusing on the role of Erk during cell cycle, it has to be underlined that Erk downstream effectors, which are required for mitosis progression and contribute to aneuploidy during tumorigenesis, remain to be determined. In addition to the identity of the terminal enzymes or effectors of Erk, it has to be stressed that the dynamic nature of the Erk signal is itself a key factor in cell phenotype decisions. Development of biophotonics strategies for monitoring the Erk network at the spatiotemporal level in living cells, as well as computational and hypothesis-driven approaches, are called to unravel the principles by which signaling networks create biochemical and biological specificities. Finally, Erk dynamics might also be impacted by other post-translational modification than phosphorylation, such as *O*-GlcNAcylation. J. Cell. Biochem. 109: 850–857, 2010. © 2010 Wiley-Liss, Inc.

KEY WORDS: MAPK; SPATIOTEMPORAL DYNAMICS; G2/M TRANSITION; POST-TRANSLATIONAL MODIFICATIONS; REGULATION MOTIF

The irreversible decision a cell makes when choosing its fate is based on diverse sources of information. How a cell integrates multiple signals into a decision is a fascinating and challenging subject of study because cell-specific responses are achieved through common effectors of signaling networks. An answer to this question is often addressed by considering the downstream effector substrates (including cytoplasmic targets, transcription factors, and terminal enzymes) that "ultimately" process the activation of transducer proteins into cell-specific phenotypes. Specific cell response to stimuli then arise from two possible mechanisms. First, molecular contributors of signal integration are cell specific and a signaling event drives different cell phenotypes, depending solely on the cell type that is stimulated. Alternatively, the nature of the activated signal (level of activation and combination of recruited effectors) is responsible for the typical or adapted cell response to a stimulus.

Typically, the extracellular-regulated kinase (Erk) cascade is a highly conserved signaling pathway throughout eukaryotic cells, bridging cell surface receptors and diverse executor proteins and integrating signals modulating many aspects of cell life such as cell cycle, survival, differentiation, and cell migration. Serving as a node into a network for signal integration, the Erk cascade consists of three layers, each one being composed of a kinase (Fig. 1). The kinase nearest to the signal source is referred as a MAP kinase kinase kinase (MAPKKK, MEKK, MAP3K), which is activated by an upstream signaling protein (e.g., small GTPases) and phosphorylates a MAP kinase kinase (MAP2K, MEK). The activated MAPKK then phosphorylates the third layer of the cascade, the MAPK. The Erk cascade is not the only signaling cascade that operates in response to receptors stimulation. Among the other cascade operating in parallel are Jnk, p38MAPK and Erk 5 cascades, all being built upon the three tiers model for MAPK [Rubinfeld and Seger, 2005].

The molecular details that link transducers to effectors and responses are still only partially understood. In the particular case of this kinase family, many questions remain unanswered (see below), regarding (1) the involvement of MAPK/Erk during M-phase progression (2), the nature of the pathways becoming engaged that contribute to sustained Erk activity or to turn off rapidly Erk activity, (3) the exact role of protein–phosphatases, scaffold proteins, and new post-translational modifications such as *O*-GlcNAcylation on MAPK dynamics during cell cycle. Indeed, while involvement of Raf-MEK 1&2–Erk cascade has been considered for the control of growth signals, cell survival, tumor progression, and invasion (angiogenesis and cell adhesion), the influence of Erk dysregulation in aneuploidy and its consequences has not received much attention. So far, the significance of Ras/Erk signaling at the ER and the Golgi regulation

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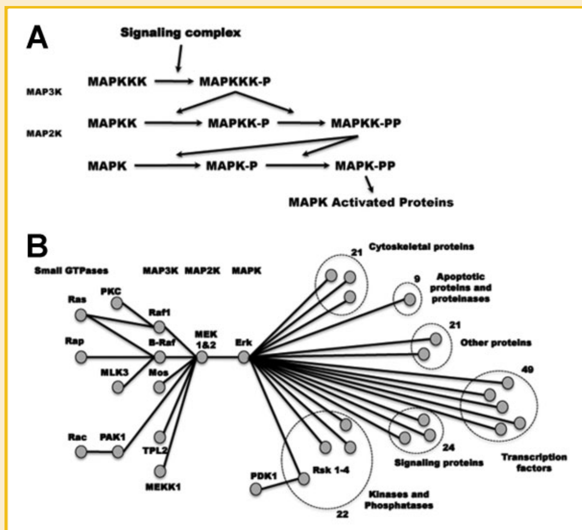

Fig. 1. Erk network. Typically, Erk cascade is built from three layers activated upon the activation of signaling complex (A) but also serves as a node to integrate cell response (B). More than 160 substrates have been identified for Erk 2 and may be grouped (dashed circle, number indicated are those of proteins included in each group) [Yoon and Seger, 2006]. It is to note that unrelated MAPK cascade elements play important roles such as PDK–PKB, mTOR–S6K, NIK–IKK and others. All these components, together with small GTP binding proteins, act in concert and create a complex network of interacting proteins regulating cell functions.

and cell trafficking has not been fully elucidated and has been discussed elsewhere [Murphy and Blenis, 2006].

Unsolved issues

- What are the identities of the phosphoproteins regulated directly or indirectly by Erk required for M-phase progression?
- How does Erk cascade dysregulation promote aneuploidy?
- How to build reliable kinase activity reporters for Erk network monitoring in living cells?
- What are the causes of rapid activation of Erk cascade? And inversely, as the signal increases, what pathways become engaged that contribute to sustained Erk activity?
- Which are the roles of Erk- and MEK–phosphatases in controlling the spatiotemporal dynamics of Erk cascade?
- How does *O*-GlcNAcylation regulate Erk cascade activity?

ERK-DRIVEN TRANSFORMATION BY MOS OR RAS CREATES GENOMIC INSTABILITY

Small changes in the Erk pathway induced by MAP3K oncogenes such as Raf and Mos profoundly impact the fidelity of the cell division through non-genomic pathways: dysregulation of MAPK/Erk pathway leads to cell division catastrophic events, mainly associated to defective mitosis progression. When transformed by v-Mos, cells exhibit genetic instability [Steffen et al., 1992], and binucleated tumor cells were one of the earliest and historically unexplained histopathological observations found in Mo-MuSV-induced sarcomas in mice. Similarly the injection of a bacterially expressed maltose binding protein (MBP)-*Xenopus* fusion protein into potoroo epithelial cells (PtK1), blocks mitosis by preventing normal metaphase spindle organization. Localization of Mos fusion protein at kinetochores was proposed to result in congression failure and in raised chromosomal instability [Wang et al., 1994]. In the absence of p53, apoptosis which was induced during S phase by Mos was abrogated and cells continued to cycle: non-partitioning chromosomes and multinucleation cells rate were dramatically enhanced by Mos-Erk [Fukasawa and Vande Woude, 1997]. Mos-driven transformation depends mainly upon its ability to activate the Erk cascade. Indeed, MEK overexpression is sufficient to induce transformation [Mansour et al., 1994] and greatly enhances Mos transformation activity whereas co-expression of dominant negative MEK 1 or CL100 MAPK–phosphatase both inhibit Mos-driven transformation [Okazaki and Sagata, 1995].

MAPK is activated in a variety of transformed cells and the MEK-MAPK pathway also mediates, if not totally, transformation induced by Ras, Raf and other oncoproteins. Predominant defects induced by v-Ras are the formation of mitotic bridges, resulting from the acquisition of one or more centromeres, and centrosome amplification, which results in the formation of multiple mitotic spindles and chromosomes missegregation [Saavedra et al., 1999]. It was stressed from these studies that transformed phenotypes associated with high genomic instability induced by Ras (micronuclei formation, abnormal centrosomes amplification, and DNA bridges) were strongly dependent upon Erk activity. Similarly, Erk sustained activity is also involved in genomic instability induced by other carcinogens such as hepatitis B virus X oncoprotein (HBx). Integration of hepatitis B virus has been suggested to facilitate genetic alterations of the host genome at stages of chronic viral hepatitis and cirrhosis, thereby increasing the risk for hepatocarcinoma development. Ectopic expression of HBx in hepatoma cells leads to multinucleated cell population increase, resulting from aberrant mitosis progression, and to chromosomal aberrations like chromosomes rearrangement and micronuclei formation [Arbuthnot et al., 2000]. Similarly, ectopic expression of HBx in Chang liver cell line induced multipolar spindle formation and chromosomal missegregation during mitosis but also increased cell population with multinuclei or micronuclei. These effects, related to centrosome amplication, were shown to be mediated by the Ras-MEK–Erk pathway [Yun et al., 2004]. Nevertheless, exact underlying mechanisms through which sustained activation of Erk promote aneuploidy remain unclear.

GETTING THE PICTURE OF ERK AT WORK DURING G2/M: FROM OOCYTES TO SOMATIC CELLS

The Erk cascade is considered as one of the most important hallmarks in cancer models, acting as a transducer activated by numerous growth factors and oncogenes. There has been compelling evidence for the involvement of Erk in pathogenesis progression and oncogenic behavior in many human tumors including breast

carcinoma, glioblastoma, as well as primary tumor cells derived from kidney, colon, and lung tissues [Bodart et al., 2002]. The role of Erk in transcription upregulation at G1/S has been largely involved in tumorigenesis mechanisms. Nevertheless, this cascade is acting at other cell-cycle checkpoints such as the onset of M-phase and the spindle assembly checkpoint. Thus, a translational as well as a non-translational role of the cascade during cell-cycle progression has become an issue for pharmacological therapeutic strategies targeting the Erk-activating network.

The notion that the Erk pathway exerts functions responsible for cell reorganization at M-phase and genetic material segregation came primarily from studies in amphibian egg extracts and oocytes, which have provided numerous insights in spindle morphogenesis and provide a physiological model for chromosome segregation unrelated to transcription regulation. Oocytes offer a unique playground to study cell-cycle regulation because of their large size, their year-around availability, their ease of manipulation, being relative to most mammalian and their cell-cycle synchronization at the onset of M-phase. Erk cascade is activated in response to hormonal stimulation in *Xenopus* oocytes and facilitates meiotic resumption by promoting the activation of MPF (an heterodimer complex made up of two subunits: a cyclin-dependent kinase, Cdc2, and regulatory subunit, cyclin B [Haccard and Jessus, 2006]). G2/M transition is induced by injecting active MEK, Erk or downstream effector Rsk. In vertebrate oocytes, oncoprotein Mos is an oocyte-expressed kinase appeared early during animal evolution and functioned ancestrally in regulating specializations of female meiosis [Amiel et al., 2009]. Once accumulated, Mos is the pivotal enzyme for the activation of the cascade in *Xenopus* oocyte and its injection is sufficient to promote M-phase entry [Ferrell and Machleder, 1998; Dupre et al., 2002; Baert et al., 2003]. The Erk cascade regulates spindle bipolarity through its direct or indirect effects on microtubule dynamics in amphibian oocytes. No bipolar spindle anchored at the plasma membrane is observed when MAPK/Erk activity is inhibited by chemical inhibitors of MEK such as U0126 [Kotani and Yamashita, 2002; Horne and Guadagno, 2003; Bodart et al., 2005]. *Rana japonica* oocytes treated with U0126 also fail to organize a Microtubule Organizing Center (MTOC) at the bottom of the germinal vesicle and chromosomes are only partially condensed [Kotani and Yamashita, 2002]. In vitro, as well as in vivo, MAPK activity inhibition leads to the formation of monopolar spindle or aster-like structures, attesting the failure to establish a bipolar organization [Horne and Guadagno, 2003]. Similarly, when Mos accumulation is prevented in vivo, *Xenopus* oocytes exhibit aster-like structures. Such structures remain to be fully characterized but do not enable oocytes to properly segregate their genomic content [Bodart et al., 2005].

With regard to studies performed in gametic cells, which have emphasized the role of MAPK/Erk cascade in spindle formation, contribution of MAPK/Erk to cell-cycle G2/M transition has also been explored in somatic cells. Treatments of synchronized mouse NIH 3T3 cells with U0126 have been reported to lead to spindle defects: chromosome misalignment, abnormal chromosome segregation, and multipolar spindles [Horne and Guadagno, 2003]. Nevertheless, spindle assembly in HeLa and RPE1 cells, was not affected by MEK 1/2 inhibition with U0126 or PD184352 [Roberts et al., 2002]. These results were confirmed by observations in NIH3T3, HeLa, Ptk or RPE1 cells, where inhibition of Erk activity seemed to have no deleterious effects on spindle assembly [Shinohara et al., 2006]. Finally, the formation of centromere-containing micronuclei induced in Chinese hamsters fibroblasts by alkylating agents (methylnitrosurea, MNU) has been suggested to be linked to the inhibition of Rsk [Campagna et al., 2003]. These contradictory observations have shaded the hypothesis that negative dysregulation of MAPK activity drives catastrophic events during M phase progression similarly to what has been observed in gametic cells. Nevertheless, Erk activity remains required for normal M-phase progression by a non-overlapping Cdc2 manner and through a complex and indirect role on a small subset of G2/M phosphoproteins [Roberts et al., 2006].

SENSORS FOR THE ERK NETWORK

The biological outcome of external stimuli might depend on the temporal profile of active, biphosphorylated MAPK in the peri-nuclear area [Murphy et al., 2002]. Spatiotemporal features of Erk dynamics are then critical for establishing a specific and adapted cellular signals and mobilization of downstream effectors. Western blotting and immunostaining for active Erk under its bi-phosphorylated isoforms have provided valuable insights into Erk function but these methods only allow to obtain static snapshots of cellular events. The development of imaging approaches is called to overcome the shortcomings of traditional methods, when providing high temporal and spatial resolution for single living cell analysis [Verveer and Bastiaens, 2008]. Recently, by customizing a generic design for FRET (Förster resonance energy transfer)-based kinase activity reporters, a specific Erk activity sensor was built, namely EKAR (extracellular signal-regulated kinase activity reporter [Harvey et al., 2008]). The latter included a fluorescent donor–acceptor FRET pair (namely EGFP and mRFP1), a substrate phosphorylation peptide from Cdc25 containing an Erk-consensus sequence (PRTP), a proline-directed WW phospho-binding domain and a specific docking site for Erk (FQFP), adjacent to the phosphorylation site, to increase the sensor specificity; upon Erk activation, the phosphorylation of the consensus sequence by Erk activity drives a conformational rearrangement, which triggers a change in the distance between the donor and the acceptor and thus in the measured FRET efficiency. While tested in HEK293 cells and hippocampal pyramidal neurons and providing a promising attempt for Erk signaling pathway analysis, EKAR's observed signals were weaker compared to other similar kinase activity reporters and its innocuousness under biologically relevant conditions remains to be fully established. Similar FRET-based reporters/sensors have been successfully used, such as chameleons, for monitoring spatiotemporal patterns calcium signaling. Challenge is now to perform biosensing using two FRET-based system simultaneously within living cells. To this extent, two approaches have been reported, one aiming at an accurate measurement of caspase-3 activity using dual FRET pairs [Ai et al., 2008], the other one aiming at detecting calcium concentrations and caspase-3 activity in the same cells

using two FRET pairs (Sirius-mseCFP & Sapphire-DsRed) while exciting at a single wavelength [Tomosugi et al., 2009].

Thus, functional fluorescence microscopy is expected to play a crucial role in elucidating the dynamic properties of a whole network, which determining biological function, by isolating and probing protein reactions in living cells.

MAPK/ERK TAKES SPACE

Due to the development of biophotonics methodologies, cells are no longer to be considered as "bags" of enzymes, because being non-homogenous spaces with well-bounded compartments in which dynamical signals are transmitted to their targets. Cells are considered more and more as a mosaic of spatial microdomains, which can change qualitative and quantitative aspects of signaling events. From their studies to model the flow of spatial information from the β-adrenergic receptor to MAPK/Erk 1&2 in hippocampal neurons, Neves et al. [2008] have proposed that the dynamics of the signaling component microdomains arise from the interplay between physical and biochemical cell properties: cell shape, subcellular localization of components, network topology, and kinetic parameters for biochemical reactions. For instance, the dendritic structures, as projection of neurons exhibiting a high surface-to-volume ratio, favor the local activation of signaling components. Nevertheless, it is unlikely that physical constraints on their own permit the transmission of information to downstream component.

From the use of biosensors and the observation of cell-signaling components, parts of a non-homogenous space, the concept has emerged that spatiotemporal dynamics of signal molecule activities and connectivities create a code that confers signaling specificity. Key designs in connectivities are positive and negative motifs, including feedback and feedforward loops (see definitions). Short positive regulatory circuits, when in place, provide a robust mechanism of signal transmission in neurons. Rapid propagation of survival signals over centimeters of axon length cannot depend upon the retrograde transport of endosomes or the translocation of phosphorylated kinases by molecular motors moving with rates of few μm/s but can depend upon phosphoprotein waves, traveling from the distal axon to the neuron body. In this context, positive feedback loops from MAPK to cytoplasmic MAP2K enable this phosphoprotein wave to propagate with high velocity and constant amplitude over distance exceeding hundreds, if even thousands of millimeters [Markevich et al., 2006].

Definitions

Bistable: a network with two steady states, or two distinguishable phenotypes. This network is able to exist stably in one of two alternative steady states, but cannot rest in an intermediate state.

Feedback: process whereby an action induces the same action, and thus some proportion of the network output will feed back to the input.

Feed-Forward Loop (FFL): The feed-forward loop is a network motif or pattern including three nodes. Classically, it is made of two cascaded transcription factors that jointly regulate a gene. Two FFL types are more common than others. These are termed the coherent FFL, which contains three positive interactions (i.e., X and Y are both transcriptional activators), and the incoherent type in which X activates Y and Z while Y represses Z.

Ultrasensitivity: property of a system, which displays a sigmoidal dose–response curve. Low levels of stimulus generate a poor if no response while higher levels create an abrupt increase in the response.

DIFFERENTIAL RESPONSE OF PC12 CELLS TO EGF AND NGF

Rat adrenal pheochromocytoma (PC12) cells have provided a cell system to address how specific functionalities may arise from the Erk cascade. In these cells, the distinct dynamics of Erk activation between epidermal growth factor (EGF) and nerve growth factor (NGF) through their respective receptors, EGF-R and TrkA, are believed to be the underlying cause for the different cellular responses, differentiation and proliferation [Marshall, 1995]. Several models have been proposed to explain the processes at the origin of these two specific dynamics; they include (1) modulation of strength and duration by immediate early gene products, (2) receptor downregulation or recycling [Murphy and Blenis, 2006], (3) distinct kinetics of small GTPases regulators [Sasagawa et al., 2005], and (4) regulatory feedback loops embedding the cascade [Santos et al., 2007]. Different topological features are clearly involved and determines the distinct Erk dynamics. Two sensitivity analysis approaches have recently been applied to this case. To decipher the architecture of Erk pathway in the context of NGF and EGF stimulation in PC12, Santos et al. [2007] used a sensitivity analysis, the modular response analysis (MRA) developed by Kholodenko [2007], coupled with a systematic perturbation of the three levels of the cascade (Raf, MEK 1&2, Erk 1&2) by downregulation through RNA interference strategies. In their conditions, the network exhibited solely a negative feedback on EGF stimulation whereas it exhibited a positive feedback in case of NGF stimulation, emphasizing the rewiring of the network depending on the growth factor context. In the case of NGF, the positive feedback observed from Erk to Raf is proposed to occur through the PKC-mediated phosphorylation of the Raf kinase inhibitory protein (RKIP), enabling direct Raf-1 phosphorylation by Erk. More recently, Yoon and Deisboeck [2009] applied a systems-level, multiparametric perturbation using Monte Carlo simulations, based on data previously compiled [Brightman and Fell, 2000]. Their analysis in silico revealed a crucial role for intermediate module, Ras and Raf, rather than top-module or MAPK-modules. In addition, the initial concentrations of Raf, RasGTP, and GAP in controlling the distinct dynamics of Erk activation, was underlined. A mechanistic interpretation from their work is summarized in Figure 2: controlling the equilibrium between activation/inactivation of Ras and the stability of Raf are central for shaping the Erk dynamics. It is also to note that while the MRA method is limited to a

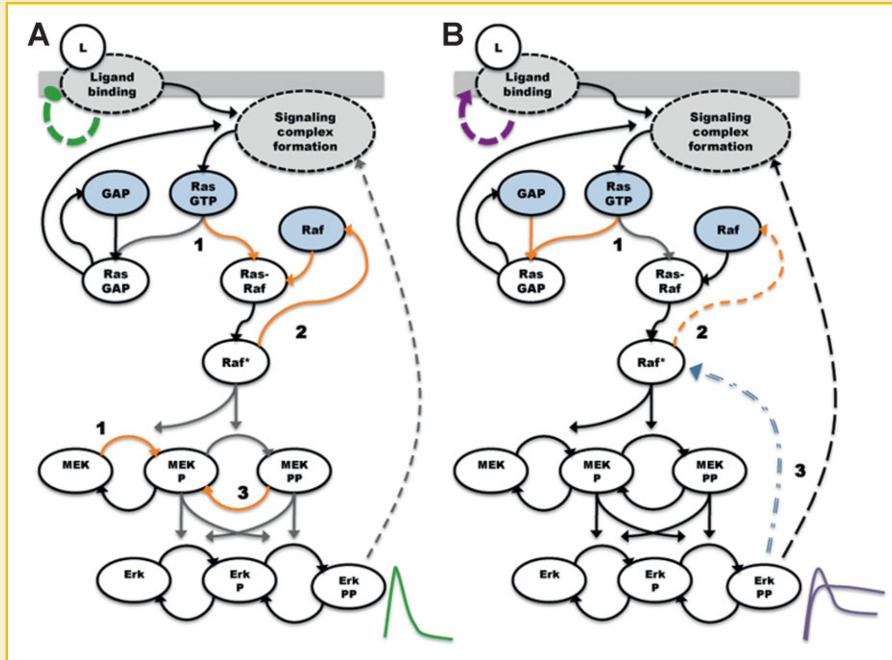

Fig. 2. Schematic overview of molecular network associated with transient (A) and sustained (B) Erk activation. Differences within Erk dynamics may be attributed to receptor modulation, such as regeneration and recycling (purple dashed circle) or downregulation driving a decrease in receptor density and incoming input (green dashed circle). Based upon a multiparametric global sensitivity analysis, the following mechanistical interpretation has been proposed: (A) Raf is quickly activated, due to the binding of Ras–GTP to Raf, occurring faster than the binding to GAP. In addition, Raf inactivation kinetics occurs rapidly (2) and MEK is rapidly dephosphorylated and inactivated (3). The inhibitory feedback is not depicted in case A, and is embedded in the signaling complex formation module. Initial concentrations of Ras–GTP, Raf, and GAP were found to be crucial (blue circle). In case B, binding of GAP to RasGTP occurs faster, increasing the availability of Ras–GDP, so the inactive Ras can be reactivated (1). Raf inactivation is slowed in these conditions (2). Positive feedback proposed by different authors have been depicted, blue broken line: positive feedback proposed by Santos et al. [2007]. Raf*, active Raf. Faster reactions are in orange, slower kinetics are in gray.

small number of interacting proteins, the in silico analysis performed by Yoon and Deisboeck failed to capture interactive effects between ‘‘distant’’ proteins in the proposed architecture of the network, like the feedback effect of Erk to SOS (within the signaling complex). Further experimental validation will be required as well as an extension of future models to include other effectors such as adaptor proteins (Grb2) or PI3kinase–Akt pathway.

CASE STUDY OF FEMALE GAMETES: ALL-OR-NONE ERK RESPONSE

A specific all-or-none response for Erk activation is observed in *Xenopus* oocytes [Ferrell and Machleder, 1998], which contrasts with the gradual answer of the Erk cascade to external stimuli in mammalian somatic cells. As mentioned above, the paradigm of the amphibian oocyte has been advantageously used to analyze the role of the Erk pathway in the morphological events of M-phase. Physical properties of Erk activation in amphibian oocytes are ultrasensitivity, bistability, and irreversibility (see definitions). Abruptness of Erk response to progesterone stimulation (Fig. 3) is traditionally attributed to a strong feedback loop, lacking in mammalian cells. This lack of feedback loop may explain why Erk activity is graded in these cells and why mammalian cells do not exhibit such a switch-like behavior as seen in oocytes. Nevertheless ultrasensitivity, as defined by Goldbeter and Koshland [1981], may not only result from the feedback loop in which the cascade is embedded in [Ferrell and Machleder, 1998] but also from the existence of a feed-forward loop (see definitions), involving Erk or MEK–phosphatases down-regulation [Russo et al., 2009b, Beaujois and Bodart, unpublished work]. Indeed, Mos promotes MEK phosphorylation in mice oocytes while at the same time, inhibiting a MEK–phosphatase [Verlhac et al., 2000]. This double-punch action reminds of feed-forward motif and may contribute to build a switch-like response of MAPK. Consistent with this hypothesis, it was reported that at low levels of MAPK–phosphatase, a system stimulated by PDGF exhibits bistable states and that increasing the levels of MAPK–phosphatase drives the system into a monostable regime [Bhalla et al., 2002]. One has also to note that MPF play a crucial role in the dynamics of Erk activity, potentially enabling the tuning of the cascade [Russo et al., 2009a] while fine tuning of the threshold system itself could be achieved by a nested feedback loop, engaging the inhibition of the glycogen synthase kinase 3-β (Gsk3-β) [Justman et al., 2009].

Because different network architectures can produce identical dynamical responses, the physical properties of the Erk dynamics in oocytes may result from different regulation motifs, or even a complex combination of them. The respective roles of these regulation motifs have to be clearly defined. MAPK/Erk- and MEK–phosphatases are also key components in the flexibility of the Erk

Fig. 3. Schematic overview of molecular network associated with all-or-none Erk response in xenopus oocytes. Ligand (L) is progesterone in this cellular context, where the Erk pathway is activated through the stimulation of the protein synthesis machinery and upregulation of polyadenylation processes (oocyte M-phase initiation machinery, 1). Mos is the pivotal enzyme of the Erk cascade (2), while Raf is believed to play little role in the activation of Erk. This cascade has been traditionally depicted as embedded with feedbacks enabling to activate the polyadenylation processes and promoting the stability of the active form of Mos. These feedbacks (3) are exerted directly by Erk or indirectly through other key component like MPF (M-phase promoting factor). A nested feedback (4) has been proposed [Justman et al., 2009], and impact of MEK/Erk phosphatases (5) is this context may be underestimated and has to be re-evaluated. Raf*, active Raf; Mos*, active Mos.

network and the changes of connectivities within this network remain to be fully elucidated. Also, roles of scaffolds and anchoring proteins (AKAPs), which can deeply change reaction kinetics and limit diffusions, will have to be addressed. Indeed, different parameters can lead identical networks to produce different phenotypical responses.

O-GlcNAcylation: A NEW PLAYER IN THE GAME?

The *O*-*N*-acetylglucosaminylation (*O*-GlcNAc) is a highly dynamic process in response to environment, rapidly cycling on and off proteins on a time scale comparable to that for phosphorylation/dephosphorylation. This post-translational modification is governed by a unique couple of enzymes, the *O*-GlcNAc transferase, which promotes the addition of the *O*-GlcNAc residue, and the *O*-GlcNAcase, which removes *O*-GlcNAc residues. *O*-GlcNAc may compete with phosphorylation on the same amino acid residues and it has been shown to be crucial in many aspects of cellular life such as cell division and differentiation [Zachara and Hart, 2006]. Studies on the regulation of M-phase entry in amphibian oocytes showed that M-phase entry was accompanied by an increased in the level of *O*-GlcNAc but the latter was not sufficient to promote both Erk and MPF activations though OGT injection potentiates meiotic resumption induced by hormonal stimulation. Nevertheless, *O*-GlcNAc was observed to be necessary for G2/M transition [Dehennaut et al., 2007, 2008a].

Interplay between the MAPK pathway and the enzymes governing *O*-GlcNAcylation has been suggested because Erk was observed to be *O*-GlcNacylated in G2-phase blocked amphibian oocytes whereas it was phosphorylated at M-phase entry [Dehennaut et al., 2008b]. Sites of *O*-GlcNAcylation and physiological significance have yet to be determined but one might hypothesize that a "passive" occupation of phosphorylation of a site by *O*-GlcNAcylation may create a negative feedback loop and damped potential Erk oscillation, as well as propagate perturbations both forward and backward in this signaling cascade, as for the property unraveled by Sepulchre and coworkers in their phenomenological model [Ventura et al., 2008].

CONCLUDING REMARKS

An interpretation of computational, data and hypothesis-driven approaches are called to help in the efforts that will lead to the discovery of how the dynamics of cell signaling factors impact cell fates and modulate them. Such strategies are also waiting for the development of new imaging strategies within living cells. Space offers also a new and highly attractive dimension in an attempt to unravel the principles by which signaling networks generate biochemical and biological specificity. Combined approaches to create in silico models of protein interaction networks, validated at the experimental level and involved in cell phenotypic decision, will facilitate target discovery and drug development of compounds leading to the extinction and/or modulation of the Erk pathway.

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