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The ERK Cascade: Distinct Functions within Various Subcellular Organelles

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Abstract

The extracellular signal-regulated kinase 1/2 (ERK1/2) cascade is a central signaling pathway that regulates a wide variety of stimulated cellular processes, including mainly proliferation, differentiation, and survival, but apoptosis and stress response as well. The ability of this linear cascade to induce so many distinct and even opposing effects after various stimulations raises the question as to how the signaling specificity of the cascade is regulated. Over the past years, several specificity-mediating mechanisms have been elucidated, including temporal regulation, scaffolding interactions, crosstalks with other signaling components, substrate competition, and multiple components in each tier of the cascade. In addition, spatial regulation of various components of the cascade is probably one of the main ways by which signals can be directed to some downstream targets and not to others. In this review, we describe first the components of the ERK1/2 cascade and their mode of regulation by kinases, phosphatases, and scaffold proteins. In the second part, we focus on the role of MEK1/2 and ERK1/2 compartmentalization in the nucleus, mitochondria, endosomes, plasma membrane, cytoskeleton, and Golgi apparatus. We explain that this spatial distribution may direct ERK1/2 signals to regulate the organelles' activities. However, it can also direct the activity of the cascade's components to the outer surface of the organelles in order to bring them to close proximity to specific cytoplasmic targets. We conclude that the dynamic localization of the ERK1/2 cascade components is an important regulatory mechanism in determining the signaling specificity of the cascade, and its understanding should shed a new light on the understanding of many stimulus-dependent processes.

Keywords: MAPK, ERK, MEK, nucleus, mitochondria, Golgi

Introduction

The intracellular communication between membranal receptors and their nuclear or cytoplasmic targets upon stimulation is mediated by a limited number of signaling pathways, including a group of mitogen-activated protein kinase (MAPK) cascades. This group includes 4 distinct cascades, which are named after their MAPK tier component: 1) extracellular signal-regulated kinase 1 and 2 (ERK1/2[1-3]); 2) c-Jun N-terminal kinase 1 to 3 (JNK1-3[4,5]); 3) p38MAPK α, β, γ, and δ (p38α-δ[6-9]); and 4) ERK5 (also known as Big MAPK[10,11]). Additional MAPK-like molecules were termed ERK3/4[2,12] and ERK7/8,[13,14] but at this stage, it is not clear whether these kinases are activated by phosphorylation cascades in a manner similar to the signaling via other MAPKs, and therefore, they are not considered as genuine MAPK cascades.[15] The ERK1/2 cascade transmits mostly mitogenic signals, whereas the p38 and JNK cascades transmit mainly stress signals. ERK5 seems to play a role in both mitogenic and stress-response processes. Each MAPK cascade is activated by either a small GTP-binding protein (smGTP; Rap, Ras), or an adaptor protein, which transmits the signal directly, or through a mediator kinase (MAP4K) to the MAPK kinase kinase (MAP3K) level of the cascade. The signal is then transmitted to the MAPK kinase (MAPKK), MAPK, and eventually to MAPK-activated protein kinase (MAPKAPK). MAP3K, MAPKK, and MAPK are considered the core components, while MAP4K and MAPKAPK are required in specific conditions. While the MAP4K, MAP3K, and MAPKK levels are used mainly for the signal transmission, the MAPK and MAPKAPK components phosphorylate a large number of substrates that eventually regulate most stimulated cellular processes, including proliferation, differentiation, survival, apoptosis, and more.[16-18]

The first MAPK to be discovered is the ERK1/2 cascade that acts downstream of Ras and usually involves sequential phosphorylation and activation of the MAP3Ks Raf-1, B-Raf, and A-Raf (Rafs); MAPK/ERK kinases (MEK) 1/2; ERK1/2; and MAPKAPKs.[3,19-20] In this review, we will describe the components of the cascade as well as the various aspects of their mode of regulation. In this sense, we will concentrate mainly on the subcellular distribution of MEK1/1b/2 and ERK1/1c/2 and their roles in the nucleus, plasma membranes, mitochondria, endosomes, cytoskeleton, and Golgi apparatus. Although Ras and Rafs may be localized in additional organelles, such as endoplasmic reticulum or lysosomes, these organelles do not seem to contain any

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MAPKK and MAPK components of the cascade and therefore will not be discussed here. Understanding the role of the ERK1/2 cascade in the above organelles should provide a wider view on their regulation as well as the determination of signaling specificity by the ERK1/2 cascade.

The Components of the ERK1/2 Cascade

The activation of the ERK1/2 cascade is mostly initiated at membrane receptors, such as receptor Tyr kinases (RTKs[23]), G protein–coupled receptors (GPCRs),[24] ion channels,[25] and others. These receptors transmit the signal by recruiting adaptor proteins (e.g., Grb2) and exchange factors (e.g., SOS) that, in turn, induce the activation of Ras at the plasma membranes, or membranes of other organelles. The activated, GTP-bound Ras then transmits the signal by activating the protein kinases Raf-1, B-Raf, and A-Raf (Rafs) within the MAP3K level of this cascade.[26] This activation occurs by recruiting Rafs to the membranes, where they are then phosphorylated and activated by a mechanism that is not fully understood.[27] Under specific conditions, other MAP3K components may participate in the activation of ERK1/2. Examples that depict this are c-Mos, which acts specifically in the reproductive system,[28] the proto-oncogene TPL2, which seems to act in transformed cells,[29] and MEKK1, which may act as a MAP3K in the ERK1/2 cascade under stress conditions.[30]

Upon activation, the Rafs transmit their signal by phosphorylating the MAPKKs, MEK1/2.[31] The MEK1/2 were first identified as ERK1/2 activators,[32,33] and their study revealed 2 main proteins with molecular masses of 45 kDa (MEK1) and 46 kDa (MEK2), which share a high degree (more than 85%) of homology.[34-36] These proteins are composed of a large regulatory N-terminal domain containing a nuclear export signal (NES), followed by a catalytic kinase domain and a shorter C-terminal region. MEK1/2 are activated through serine phosphorylation at the MAPKK-typical Ser-Xaa-Ala-Xaa-Ser/Thr motif in their activation loop (residues 218-222 in human MEK1[37]). In turn, MEK1/2 activate their only known substrates, native ERK1/2, which function as their sole downstream targets, suggesting that the MEK1/2 serve as the specificity-determining components of the ERK1/2 cascade. The MEK1/2 are the only kinases that can phosphorylate both regulatory Thr and Tyr residues of ERK1/2, and therefore, they belong to the small family of dual-specificity protein kinases.[38]

The next components of the cascade, namely ERK1/2, belong to its MAPK level and are important executers of the upstream signals. They are evolutionary conserved kinases that are gene products of ERK1 (MAPK3) and ERK2 (MAPK1). Each gene encodes one main product, which are the primary 44-kDa (ERK1) and 42-kDa (ERK2) proteins. In addition, these genes encode several splice variants, including ERK1b (46 kDa[39]), ERK1c (42 kDa[40]), and others that will be described below. The MEK1/2-mediated phosphorylation of ERK1/2 on the Thr and Tyr residues in their Thr-Xaa-Tyr signature motif (Thr202 and Tyr204 in human ERK1[41]) is the mechanism that induces their full activation. Because of the high similarity between ERK1 and ERK2, their function and regulation are similar under most circumstances,[42,43] although some differences between their functions have been recently elucidated under distinct conditions.[44-46] The ERK1/2 are "Pro-directed" protein kinases, meaning that they phosphorylate Ser or Thr residues, neighbors to Pro residues. Pro-Xaa-Ser/Thr-Pro is the most common consensus sequence for substrate recognition by ERK1/2, although Ser/Thr-Pro can serve as a substrate as well.[47]

About 200 distinct substrates of ERK1/2 have been identified to date, which seem to be responsible for the induction and regulation of various ERK1/2-dependent processes.[19,48] Among the first identified substrates/interactors of the ERK1/2 cascades are the cytoplasmic PLA2,[26] cytoskeletal elements,[49] and the intercellular domains of membranal receptors.[50] However, not less important are the large number of nuclear ERK1/2 targets identified, including transcription factors such as Elk1,[51] c-Fos,[52] and c-Jun.[53] These latter substrates require stimulation-dependent nuclear translocation of ERK1/2 for their phosphorylation, and this is regulated by the novel nuclear translocation signal (NTS) on ERK1/2, recently identified in our laboratory.[54] Finally, among the large number of substrates, several MAPKAPKs may further extend the ERK1/2 cascade. The first identified MAPKAPK was RSK in which its phosphorylation by ERK1/2 was one of the main building blocks for the elucidation of the whole signaling cascade.[1] MAPK-interacting protein kinase (MNK[55,56]) and mitogen- and stress-activated protein kinase (MSK[57]) were also identified as MAPKAPKs of the cascade. However, the activation of these kinases is not restricted to the ERK1/2 cascade, as they are also activated by the p38 cascade, mainly under stress conditions. Thus, the signals of the ERK1/2 cascade are well-disseminated, and it is estimated that a few minutes after extracellular stimulation, the cascade is responsible for no less than 500 phosphorylations throughout the cells.

Alternative Splicing within the ERK1/2 Cascade

As mentioned above, aside from the main components, each tier of the ERK1/2 cascade contains one or more alternatively spliced isoforms. These variants are much less abundant than the main isoforms described above, and their expression and activity are usually cell type or condition specific. However, the spliced forms have been reported to manifest unique important functions that are not shared by the main isoforms, and therefore, they may be considered as individual signaling components. At the MAP3K level of the cascade, B-Raf was reported to express up to 10 spliced variants in the mouse and human.[58,59] Interestingly, some of the spliced isoforms demonstrate higher

basal B-Raf activity, while in others, the activity is compromised. An example of such cases is the alternative splicing of exons 8b and 10 that are located just before the kinase domain.[60] Thus, the insertion of the exon 10 sequence increases the affinity of B-Raf for MEK1/2, its basal kinase activity, and transforming properties. On the other hand, insertion of the exon 8b sequence seems to have the opposite, inhibitory effects. More recently, it was shown that aberrant B-Raf splicing can serve as an alternative mechanism for oncogenic B-Raf activation *in vivo*.[61] Thus, novel B-Raf splice variants that lack the N-terminal autoinhibitory domain were detected in patients with thyroid carcinoma. These variants were constitutively active and significantly associated with the oncogenic V600E mutation and an advanced stage of the disease. All these studies indicate that splice variants of B-Raf may function differently from the most abundant isoform, either by activating or inhibiting downstream activities of the ERK1/2 cascade.

The MAP3K tier of the ERK1/2 cascade is not the only one to contain alternatively spliced isoforms. Thus, the MAPKK tier contains one alternatively spliced isoform, MEK1b, which lacks a 26–amino acid stretch in the kinase domain, as compared to the main transcribed form. This kinase was initially identified as an inactive form of MEK1, as it failed to phosphorylate ERK1/2,[35,36] but was later shown to phosphorylate an alternative spliced isoform of ERK1 (ERK1c) and thereby to form an independent signaling cascade that differs from the main MEK1/2-ERK1/2 cascade.[62] Thus, the elimination of 26 amino acids does not cause inactivation but rather modifies the substrate specificity of the kinase without modifying the remarkable selectivity of MEK family members to their respective ERKs.[3] In the next tier of the cascade, the alternatively spliced isoform ERK1b was identified mainly in rodents as a 46-kDa protein that contains a 26–amino acids insert, just C-terminal to the kinase domain of ERK1.[39] This kinase seems to be activated similar to the main ERK1/2, although under some circumstances, it is regulated differently because of distinct regulation of its altered CRS/CD domain.[63] Interestingly, a similar splicing event also occurs in primates; in this case, however, the insert consists of 103 base pairs that are followed by a stop codon, which leads to the translation of a 42-kDa protein termed ERK1c.[40] It was shown that ERK1c is expressed in most human cells, is activated primarily by MEK1b, and is unique in regulating mitotic Golgi fragmentation.[62,64] Other ERK1/2 splice variants, such as ERK1d (unpublished) and ERK2b,[12] seem to be generated as well, and extend the specificity of the ERK1/2 cascade, as described below.

Regulation of ERK1/2 by Kinases and Phosphatases

The activation of ERK1/2 is induced by MEK1/2 phosphorylation of both Thr and Tyr residues in the ERK1/2's activation loop. This phosphorylation causes dramatic conformational changes, which enable full activation and interaction of ERK1/2 with their substrates. These global conformational changes elevate the catalytic rate of ERK1/2 to approximately 5 mM/min/mg, which is 5 to 6 orders of magnitude higher than the basal activity.[65] Interestingly, aside from this activation loop phosphorylation, ERK1/2 were demonstrated to undergo regulatory phosphorylation on additional residues. For example, it was shown that 2 Ser residues (Ser244 and Ser246 of ERK2) in their kinase insert domain (KID) are phosphorylated upon stimulation.[54] These phosphorylations, which are probably mediated by more than one kinase, are important for binding of ERK1/2 to importin7 and their nuclear translocation, as described below. In addition, autophosphorylation of a residue in the activation loop (Thr188 in ERK2) was shown to affect the subcellular localization of ERK1/2 as well.[66] This phosphorylation seems to exhibit a unique role of ERK1/2 in integrating G protein–coupled receptor–initiated signals in order to induce cardiac hypertrophy. Importantly, other residues in close proximity to the activation loop of ERK1/2 are phosphorylated as well[67] and thereby may affect this important region of ERK1/2.[68] Notably, these Thr-Glu-Tyr–independent phosphorylations are usually not important merely for the activation mechanisms of ERK1/2 but rather play a role in the regulation of ERK1/2 localization or downregulation and thereby in the determination of their signaling specificity, as described below.

The activation of the ERK1/2 cascade upon extracellular stimulation lasts between 20 minutes (transient activation) up to 2 to 3 hours (sustained activation). Because the duration of the signal is necessary for proper ERK1/2 signaling (see below), the downregulation phase of the ERK1/2 cascade is not less important than its activation. Although degradation[69] or distorting scaffolding interactions[70] have been reported to participate in the regulation of ERK1/2, the main mechanism by which ERK1/2 are inactivated involves the removal of 1 or 2 of the phosphates from its activation loop.[71] Since the phosphorylation of both Tyr and Thr residues is required to induce the full ERK1/2 activity, removal of phosphate from just one of them is sufficient for full inactivation. Thus, protein Ser/Thr phosphatases, protein Tyr phosphatases, and dual-specificity phosphatases (MKPs) all act to inactivate ERK1/2 under various conditions. MKPs are mostly products of inducible genes that are expressed only 30 to 60 minutes after stimulation, and therefore, the short-term inactivation of ERK1/2 is mediated mainly by protein Ser/Thr phosphatases or protein Tyr phosphatases that are responsible for ERK1/2's transient activation.[72] Escape from these 2 phosphatases often results in a prolonged ERK1/2 activation, which is finally downregulated by the induced, newly expressed MKPs.[73] In summary, it is clear that ERK1/2 activity is heavily regulated by a set of MEK1/2-independent kinases and phosphatases that play an important role in regulating the substrate specificity of the ERK1/2 cascade.

Determination of Signaling Specificity

The ERK1/2 cascade is a linear signal transduction pathway that induces distinct and even opposing physiological processes. The above regulatory processes are not sufficient to allow the plethora of ERK1/2-induced effects, which raises the question as to the full scope of the mechanisms that are involved in the determination of the signaling specificity of this cascade. The current described mechanisms can be categorized into at least 5 distinct types,[20] and these are as follows: 1) differences in the duration and strength of the signal; 2) interaction with scaffold proteins; 3) crosstalk of the MAPK components with other signaling pathways that are activated or inhibited simultaneously; 4) presence of multiple components in each level of the cascade, including substrate competition; and 5) compartmentalization of the MAPK cascade components and their targets in certain organelles or other cellular regions. The last mechanism is the main issue of this review and therefore will be covered in detail. A brief description of the first 4 mechanisms follows.

1. Differences in the duration and strength of the signal: This was the first mechanism elucidated for signaling specificity determination. In early experiments, it was shown that in PC12 cells, both EGF and NGF induce strong activation of ERK1/2, albeit with distinct outcomes. EGF stimulation caused a transient activation of ERK1/2 (peaking at 15 minutes and reduced back to basal levels after 40 minutes) and proliferation of PC12, while NGF stimulation led to a sustained activation (after 15-180 minutes), which resulted in differentiation of the cells.[74,75] It was later demonstrated that these effects are mainly interpreted by immediate early genes that induce distinct cellular processes dependent on the signal length.[76] As mentioned above, the duration of the signal is regulated mainly by protein phosphatases,

which are therefore key regulators of ERK1/2 signaling outcomes.[77]

2. Scaffold proteins: The term "scaffold protein" is given to a protein that interacts with more than one protein in a specific cascade.[78] In this way, scaffold proteins allow the formation of multicomponent complexes that may be important for the regulation of all MAPK cascades. For example, scaffold proteins may bring various components of the same cascade together and thereby facilitate the kinetics and duration of MAPK activation. Scaffold proteins may also direct the cascade to either specific upstream receptors or to unique downstream targets contributing to proper signal distribution. In addition, they may contribute to the stabilization of some components, determination of signaling thresholds, direction of the localization of the cascade components, or protecting signaling components from phosphatases. All these effects of scaffold proteins make them important regulators of the specificity of the ERK1/2 cascade.[20] The regulation of ERK1/2 by scaffold proteins in different compartments is described below.

3. Crosstalk with other signaling cascades: Although the ERK1/2 cascade is a central signal transduction pathway in the cell, the same stimuli can also activate other cascades, such as PI3K-AKT, NF-κB, and others. These cascades may interact with each other and thus modulate the signaling output by cross-phosphorylation between the cascades, by combinatorial effects on downstream targets, or by modulation of activity.[20] For example, several components of the PI3K-AKT pathway interact with and regulate the ERK1/2 cascade.[79,80] Thus, MEK1/2 were suggested to be a focal point for cross-cascade regulation because they are affected by Rho family proteins and PAK1 downstream of PI3K.[81] In addition, PI3K is thought to affect the ERK1/2 cascade via a direct interaction of its p110 catalytic subunit with Ras.[82,83]

Importantly, several improper interactions with other cascades are thought to induce pathologies, such as cancer.[84]

4. Multiple components in each level of the cascade: The presence of various components with distinct functions or regulation in each level of the cascade is, as yet, another important mechanism for the determination and extension of signaling specificity. Thus, different proteins in the MAP3K tier, including Rafs, MEKK1, Cot, and Mos, may be involved in the activation of the cascade under varying conditions. Although the activity of MEK1 and MEK2 in the MAPKK tier of the cascade is similar under most circumstances, the multiple phosphorylation sites in the Pro-rich domain of MEK1, but not MEK2, suggested different functions between these isoforms under some conditions. Indeed, it has been demonstrated that MEK1 and MEK2 have distinct functions during cell cycle progression[85] and other processes.[86] In addition, the alternative spliced isoform MEK1b is specifically instrumental in regulating mitotic Golgi fragmentation. In the next tier of the cascade, ERK1/2, which also share a high degree of similarity, were initially considered to be functionally redundant. However, several studies have demonstrated that differences between ERK1 and ERK2 do exist. For example, different outcomes were noticed with ERK1 or ERK2 depletion,[85] whereas ERK2, and not ERK1, was shown to play a role in the induction of epithelial-to-mesenchymal transformation.[46] Finally, the alternatively spliced isoforms described above, as well as substrate competition,[87] might participate in the determination of signaling specificity as well.

5. Localization of Components of the ERK1/2 Cascade

Restriction of components of the ERK1/2 cascade to specific cellular compartments as well as the dynamic

changes in their localization after stimulation is an important way of signaling specificity determination. In resting cells, the components of the ERK1/2 cascade are localized to the cytoplasm mainly because of interaction with specialized anchor/scaffold proteins.[78] Upon stimulation, Rafs are recruited to the plasma membrane, and to membranes in other compartments, because of their interaction with activated Ras. Upon activation, MEK1/2, ERK1/2, and RSKs are usually released from their cytoplasmic anchors, and this allows translocation of a large portion of their molecules into the nucleus and other cellular organelles.[88] It should be noted, however, that not all the molecules of the signaling kinases are released from their anchors, and it was shown that a significant portion of ERK1/2 molecules remain attached to specific cytoplasmic anchoring proteins (i.e., PEA15[70]), preventing their nuclear translocation while directing them to particular cytoplasmic targets. The ability of ERK1/2 molecules to be released from the anchoring proteins upon stimulation is dependent upon their mode of interaction. Thus, the interaction of ERK1/2 with many of their anchoring proteins is mediated through their CRS/CD domain.[89,90] Activating phosphorylation of the ERK1/2's Thr-Glu-Tyr domain induces a large conformational change, which forces a release of ERK1/2 from the CRS/CD-dependent interactions.[91] Other docking sites of ERK1/2 that may mediate their cytoplasmic interaction are the DEJL as well as loop 6 of the kinase. The binding of ERK1/2 through these domains does not seem to be reversed upon stimulation, and thereby, it may fix the ERK1/2 molecules to a certain region in the cytoplasm and prevents their stimulated translocations.[72] The hydrophobic DEJL domain is usually necessary for proper ERK1/2 substrate phosphorylation,[92] and its irreversible binding may modulate ERK1/2 translocations and activity.[93] The binding through loop 6 seems to mediate interaction of ERK1/2 with cytoskeletal elements.[94,95] As for DEJL,

this interaction is probably not affected by Thr-Glu-Tyr phosphorylation and seems to direct ERK1/2 to cytoskeletal elements and the proper site of action.

While most of the ERK1/2 molecules migrate to the nucleus, a smaller portion of these, as well as MEK1/2 and Raf molecules, migrates into distinct subcellular compartments/organelles, where they are conducting specific functions[88] (Fig. 1). The various translocation events and distinct localizations, which have important physiological functions, can be categorized into 2 main functional groups. The first is the ability of the translocated ERK1/2 to regulate specific activities within certain organelles, such as the regulation of transcription in the nucleus and mitochondria[22,96] or the regulation of mitotic Golgi fragmentation.[64] The second function is to bring components of the ERK1/2 cascade into proper localization in the outer surface of the organelles, where the signaling components phosphorylate specialized substrates without significant nuclear translocation. These unique localizations often result in a distinct signaling fate under varying conditions, and thereby, it is an additional important method for the determination of signaling specificity.[97,98] As described above, scaffold and other interacting proteins often regulate the targeting to the organelles. Examples of such directing proteins are MP1 that directs ERK1 to endosomes,[99] VDAC to the mitochondria,[96] and Sef1 to the Golgi.[100] Cytoskeletal elements, as well as cytoskeleton-related proteins, may also participate in this effect.[78] The distinct functions of ERK1/2 in various organelles are described below.

ERK1/2 in the Nucleus

As mentioned above, many ERK1/2 molecules detach from the anchor proteins upon stimulation and translocate to various organelles and other cellular compartments.[88] Most notably, a large portion of the ERK1/2 molecules (50%-70%) can be found in the nucleus within 10 to 20 minutes after cellular stimulation.[101] It

was shown that specific abrogation of ERK1/2 translocation into the nucleus blocks growth factor–induced gene expression and other induced processes.[70,102] Therefore, it is likely that understanding nuclear activities should shed light on the regulation of many growth factors or oncogene-dependent cellular processes including proliferation, differentiation, and oncogenic transformation. The nuclear effects of ERK1/2 are executed via a large number of substrates, interacting proteins, and direct DNA interactions in this location. Significantly, about half of the currently identified ERK1/2 substrates are nuclear proteins, and those participate in the regulation of many stimulated nuclear processes.[19] These effects include mainly transcription (i.e., Elk1 activation[103]) as well as modulation of transcription suppression (i.e., Erf-1 suppression[104]), which are described below. Other mechanisms by which ERK1/2 affect the nuclear process include chromatin remodeling (i.e., PARP-1 regulation[105]) and nuclear translocation (i.e., phosphorylation of NUP50[106]). The direct effect of MEK1/2[107] and RSKs[108] translocation to the nucleus is less studied at this stage and therefore will not be covered here.

The main group of effectors identified is that of transcription factors, most notably those that regulate immediate early genes (IEG[109]). The rapid IEG transcription after stimulation requires activation of their transcription factors within minutes after stimulation, and this occurs mainly by the rapidly translocated ERK1/2. One of the best-studied ERK1/2-activated transcription factors is a nuclear ETS domain–containing Elk1.[51,103] The rapid phosphorylation of Elk1 by ERK1/2 occurs on 6 to 9 sites[110] and requires a direct docking interaction between the 2 proteins.[111] One of the earliest transcriptional events regulated by Elk1, upon stimulation, is the induction of the IEG c-Fos, which is important for the proper progression of proliferation and differentiation.[112] Thus, the expression of c-Fos, which is minimal in quiescent cells, is dramatically induced upon stimulation for
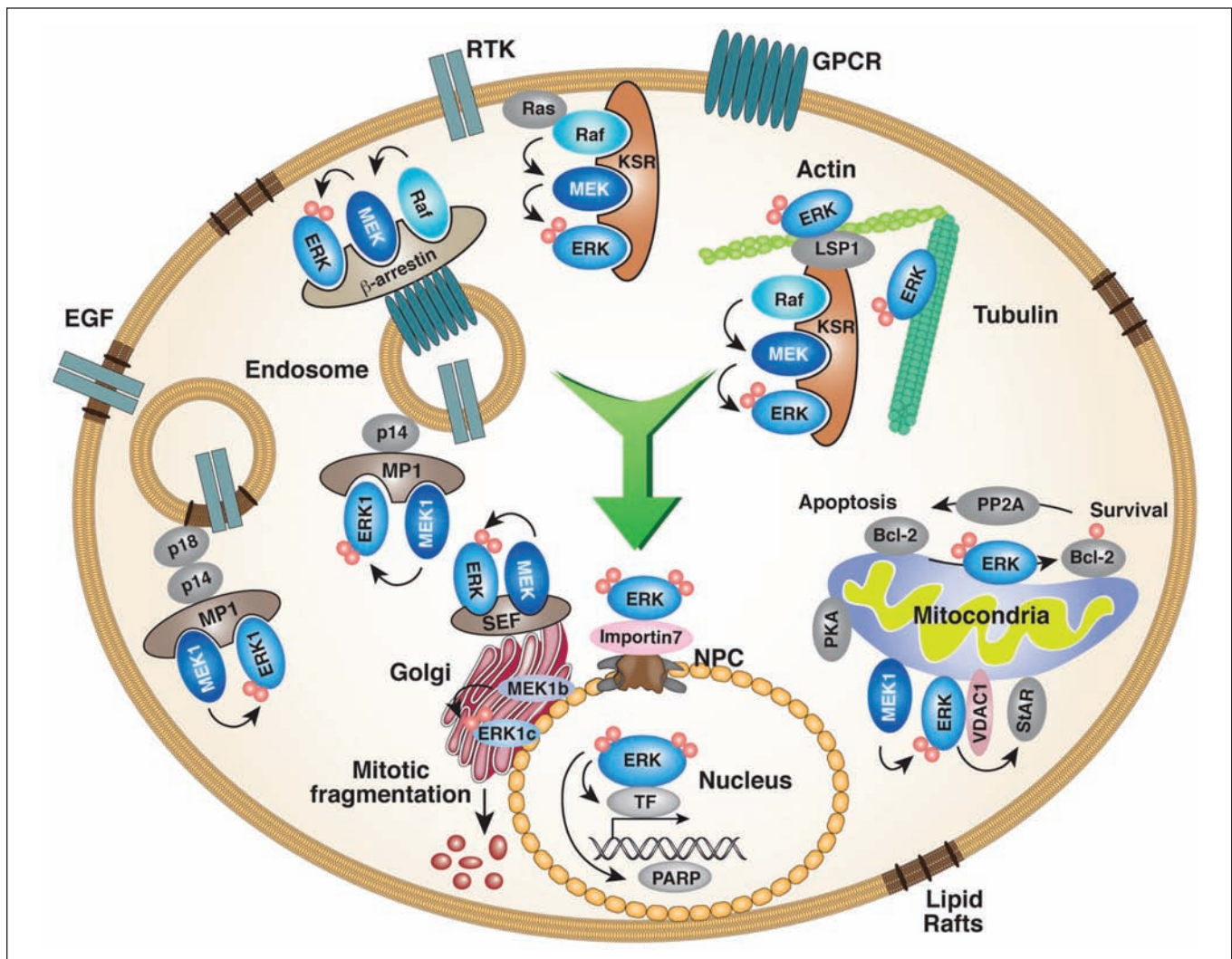
Figure 1. ERK1/2 distribution within the various compartments of the cell. The activation of the ERK1/2 cascade results in a significant translocation of the ERK1/2 molecules to the nucleus, which is mediated by interaction with importin7 to induce mainly proliferation and differentiation. In addition, ERK1/2 translocate into various cellular organelles usually because of interaction with specific scaffold proteins. In each of these organelles, ERK1/2 can either regulate intrinsic activities or direct ERK1/2 signals to nearby cytoplasmic substrates (for details, see text).

durations that may vary from minutes to hours. These changes in duration are regulated by ERK1/2-dependent stabilization of c-Fos, which is achieved only when ERK1/2 activation is sustained, namely strong enough when the c-Fos is significantly expressed.[113] Therefore, the differences in stability make c-Fos a good interpreter of differences in ERK1/2 kinetics of activation, which was shown to play a role in the determination of ERK1/2-dependent signaling specificity.[75] Similar phosphorylation by ERK1/2 seems to be important for the stability and activity of additional IEGs, such as c-Myc and Fra1,[76] which indicates that ERK1/2 serve as key regulators of transcription factors and IEGs upon various conditions.

Aside from the role of ERK1/2 in regulating IEGs, they can phosphorylate and modulate the activity of additional transcription factors that participate in the induction of intermediate or late genes. Notably, ERK1/2 were demonstrated to regulate several members in the subgroup of nuclear receptors, including estrogen receptor (ER), which under most conditions can be activated by this phosphorylation.[114] In addition, components of the ERK1/2 cascade also affect the genomic activity of peroxisome proliferator–activated receptor gamma (PPARγ). However, unlike the activation of ER, the phosphorylation of this nuclear receptor by ERK1/2 actually inhibits the nuclear functions of this nuclear receptor.[115] Attenuation of activity by ERK1/2-mediated phosphorylation was also reported for retinoic X

receptor alpha (RXRα[116]), and some studies suggest that this might be the case for the glucocorticoid receptor (GR[117]) as well. Thus, the main function of ERK1/2-dependent phosphorylation of nuclear receptors is probably suppression of their activity or not activating them, as is the case for the IEG-related transcription factors. Interestingly, suppression of transcription by the ERK1/2 cascade was reported in the case of other transcription or repression factors as well. One of them is the Ets2 repressor factor (Erf1), which suppresses transcription in many resting cells.[118] The phosphorylation by ERK1/2 induces CRM1-dependent nuclear export of Erf1, which thereby alleviates its suppression of transcription.[104] Finally, the cascade was also implicated in suppressing gene activity by the direct interaction of ERK2 with DNA.[119] This suggests that aside from its well-known regulation of transcription factors, ERK2 can regulate gene expression by activity-independent binding to promoter regions.

One of the questions that attracted much attention in the last few years is the mechanism by which ERK1/2 translocate into the nucleus.[88,120] The nuclear envelope separates the nucleus from the rest of the cell and guarantees a selective transport into the nucleus. Proteins and other molecules shuttle into the nucleus via specialized nuclear pores (NPCs) that coordinate nucleocytoplasmic exchange.[121] These NPCs allow free diffusion of small molecules and proteins (up to 40 kDa), whereby bigger proteins penetrate through the pore by using energy-dependent transport machinery. In many cases, this machinery includes the binding of a basic region in the transported protein (cargo), which serves as a nuclear localization signal (NLS) to the trafficking proteins, importins α and β. These importins then serve as shuttle for the NLS-containing cargo proteins and facilitate their transport through the NPCs.[122] Other less-frequent and less-understood mechanisms of translocation seem to involve either

nonconventional NLS,[123] binding of the cargo to the NPC,[124] or binding to non-α/β importins.[125] Interestingly, ERK1/2 translocation seems to be mediated by the latter mechanism, namely by binding to importin7.[126] Our recent findings[54] suggest that upon stimulation, the activatory Thr and Tyr residues of ERK1/2 are phosphorylated by MEK1/2 to induce their activation and detachment from cytoplasmic anchoring proteins. This dissociation then allows the phosphorylation of 2 Ser residues within the kinase insert domain of ERK1/2 (Ser244 and Ser 246 in human ERK2, termed nuclear translocation signal [NTS] domain), which further induces interaction with importin7. NTS-like sequences were also found in other proteins like MEK1/2 (TPT) and SMAD3. In the nucleus, ERK1/2 are downregulated and are eventually exported from the nucleus, probably by coupling to MEK1/2 that induce the export by their binding to exportins.[120] However, more studies are required in order to better understand the full mechanism by which ERK1/2 shuttling is regulated in various cell lines and processes.

ERK1/2 in the Mitochondria

The mitochondrion is a point of integration of signaling cascades because of its pivotal role in cellular metabolism, redox biochemistry, and survival/death decisions.[127] Regulation of the number of the mitochondrial organelles in relation to environmental/cellular conditions would require coordinated transcription of nuclear and mitochondrial genes and the genesis of, or mitochondria trafficking to, appropriate regions with high-energy utilization. Various signaling components and cascades are involved in this type of cell fate processing via the mitochondria, including ERK1/2, PKA, PKC, PI3K-Akt, JNK, and p38 MAPK.[128] In the following section, we describe the role of the ERK1/2 cascade in mitochondria regulation and activity. Unlike other organelles (see below), at this stage, there is no convincing

evidence for ERK1/2 signaling from the outer surface of the mitochondria towards cytosolic targets.

Many studies, using pharmacological inhibitors of MEK1/2, have indicated that ERK1/2 can modulate mitochondrial functions, particularly those associated with cell death. While some of these studies demonstrated an antiapoptotic effect of ERK1/2,[129,130] others showed that ERK1/2 have either proapoptotic effects or even induce nonapoptotic cell death.[131,132] Although it is clear from these studies that ERK1/2 are important regulators of mitochondria activities, the reason for the different effects in specific cell types and conditions is not fully understood. These studies have also raised the question as to how the ERK1/2 signals are transmitted into the mitochondrial proteins and activities. Although it is conceivable that ERK1/2 may affect the mitochondrial activities by regulating the expression of mitochondrial proteins in the nucleus,[133] it appears that these kinases may have intrinsic mitochondrial activities as well. One of the first evidence supporting mitochondrial localization and activity of ERK1/2 was derived from biochemical studies in renal tubular cells. In that study, it was found that upon cisplatin treatment, activated ERK1/2 as well as PKCα are present in enriched mitochondrial fractions. This localization was suggested to contribute to increased mitochondrial membrane potential, decreased oxidative phosphorylation, and increased caspase-3 activation and apoptosis in these cisplatin-treated cells.[134] In addition, it was shown that ERK1/2 colocalize with Bcl2 in the mitochondria, where the latter is phosphorylated by ERK1/2 on Ser87 to exert its antiapoptotic effect. This effect of ERK1/2 on Bcl2 is reversed by mitochondrial PP2A.[135,136] Additional cells in which ERK1/2 were identified in the mitochondria include murine cardiac myocytes, where the kinases are associated with enhanced Bad phosphorylation and cardioprotection,[137] and human alveolar macrophages, where ERK1/2 maintain mitochondrial membrane potential and

ATP production.[138] Finally, detailed immunoelectron microscopy studies have established the presence of phosphorylated ERK1/2 in the outer membrane and intermembrane space of brain mitochondria,[139,140] suggesting their role in multiple mitochondria functions.

The accumulation of ERK1/2 in the mitochondria can vary in different cellular conditions, suggesting that these kinases may translocate into this compartment upon stimulations. Thus, it was shown that in the brain of developing mice, the levels of mitochondrial ERK1/2 peak at stages E19 to P2, decreasing from P3 to adulthood. The decrease in mitochondrial ERK1/2 correlates with their increased nuclear translocation, providing information about mitochondrial energetic and redox status to the proliferating/differentiating cells.[140] In addition, phosphorylated ERK1/2 were found within a subset of mitochondria in degenerating neurons from patients with Parkinson disease and Lewy body dementia.[139] In the transformed Leydig-derived MA-10 cell line, presence of ERK1/2 in the mitochondria and their activation are obligatory for PKA-mediated steroidogenesis and thereby are probably associated with their oncogenic potential.[141,142] Despite the clear accumulation and activation of ERK1/2 in the mitochondria, little is known about the mechanisms that allow these processes, especially because none of the components of the ERK1/2 cascade seems to contain a mitochondrial localization signal. In this regard, it was shown that ERK1/2, as well as p38, JNK, and their respective MAPKKs, are present in the mitochondria of a murine tumor cell line, and the traffic of these MAPKs in and out of the organelle is regulated by hydrogen peroxide.[143] Another important step in understanding the mitochondrial localization, regulation, and role in mitochondrial activities was the recent proteomic study on ERK1/2-interacting proteins in the mitochondria.[96] In this study, it was demonstrated that ERK1 physically associates with structural,

signaling, transport, and metabolic proteins in the mitochondria of HeLa cells. Among the new interactors identified were the voltage-dependent anion channel 1 (VDAC1) that may facilitate the mitochondrial transport of ERK1/2. These interactions suggest that aside from their role in mitochondria-dependent survival/apoptosis, ERK1/2 participate in several unexpected intrinsic mitochondrial processes. These functions include the regulation of various proteins that govern the ATP source in the outer mitochondrial membrane, as well as the activation of mitochondrial transcription factors and transcriptional machinery. However, the exact mechanisms by which ERK1/2 are translocated and regulated in the mitochondria, the mechanism of MAPKKs activation, and the full scope of ERK1/2 functions in this location are still obscure.

ERK1/2 in the Endosomes

Formation of endosomes is crucial not only for signal termination of receptors but also for the activation of various cellular functions, such as nutrient intake/digestion, membrane protein cycling, cell migration, and intracellular signaling.[144] Early endosomes, which are usually formed as clathrin-coated vesicles, are initiated by the internalization of either GPCRs or RTKs. Upon their detachment from the plasma membrane, the early endosomes either recycle back to the plasma membrane or mature into late endosomes, which often migrate into lysosomes, where the receptors are degraded.[145,146] In many cases, the endocytosis of activated receptors is necessary not only for their downregulation but also for the induction of additional signaling outputs. Indeed, several early studies showed that endocytosis and endosomes are required for proper receptor signaling through the ERK1/2 cascade. For example, it was shown that inhibition of internalization attenuates ERK1/2 activation by lysophosphatidic acid (LPA), thrombin, and bombesin receptors in Rat-1 fibroblasts.[147]

Moreover, endocytosis of the GPCR β2 adrenergic receptor was shown necessary for the activation of the ERK1/2 cascade,[148] and the internalization of PAR2 is required for the interaction of the receptor with its downstream effectors, Rafs, and ERK1/2 in the endocytic compartment.[99]

Since these initial findings, much information was accumulated on the role of endosomes in ERK1/2 signaling.[149] The mechanism that allows the dissemination of the signals to downstream signaling components is primarily related to specialized scaffold proteins that bring these components to close proximity to each other and facilitate their activation. One such scaffold protein is β-arrestin, which was initially shown to enhance angiotensin II–induced Raf and MEK-dependent activation and endosomal targeting of ERK2.[150,151] This scaffold directs ERK1/2 mainly to clathrin-coated endosomes that are formed together with receptor internalization, indicating a relatively rapid effect upon stimulation. Interestingly, the interaction of β-arrestin with activated ERK1/2 is irreversible and commits the ERK1/2 to phosphorylate cytoplasmic substrates without many effects on nuclear targets.[152] Another scaffold protein that mainly binds ERK1 and directs it to endosomes is MEK1 partner 1 (MP1). MP1 was first identified in a yeast 2-hybrid screen as a binding partner of MEK1.[153] MP1 binds MEK1 and ERK1 but not MEK2 and ERK2, and unlike β-arrestin that directs ERK1/2 to early endosomes, the recruitment of MP1 seems to be confined to late endosomes. Interestingly, MP1 seems to operate in conjunction with another binding protein, termed p14,[99] which associates with the cytoplasmic face of the endosomes in a variety of cell types. ERK1 activation on endosomes regulates late endosomal traffic and cellular proliferation. While the MP1-p14 complex is required for ERK1 endosomal activation, this complex is irrelevant for ERK1 activation at the plasma membrane.[154]

Another type of ERK1/2 interaction with endosomes is through the adaptor protein p18 that directs the cascade components to lipid rafts on late endosomes and, consequently, to RTKs' recycling.[155] Lipid rafts are dynamic cholesterol-enriched microdomains in plasma and endomembranes and are proposed to serve as signaling platforms by facilitating protein-protein interactions.[156] Importantly, the internalization of RTKs, like EGFR by clathrin-dependent endocytosis, usually results in a rapid recycling of the RTKs back to the plasma membranes. On the other hand, RTKs, but probably not GPCRs, that are internalized together with lipid rafts/caveolae are sorted from early endosomes to late endosomes, leading to lysosomal degradation.[157,158] It was shown that p18 is anchored to lipid rafts of late endosomes and serves as a lipid raft anchor for p14-MP1-MEK1 signaling components.[155] This complex can also recruit ERK1, while the p18-MEK1-ERK1 complex takes part in controlling intracellular membrane dynamics potentially by regulating organelle interactions and/or transports along cytoskeletal elements. Taken together, ERK1/2 do not seem to participate in the regulation of trafficking or other intrinsic endosomal activities. Rather, the endosomes seem to facilitate the activation of the ERK1/2 cascade downstream of internalizing RTKs and GPCRs and direct them to their proper targets in the cytoplasm. In these processes, RTKs and GPCRs are using a different set of scaffold/anchor proteins to execute their functions.

ERK1/2 in the Plasma Membrane and Cytoskeletal Elements

The plasma membrane is the origin of most intracellular signaling, as most of them are initiated by the membranal receptors. In addition, many signaling components are associated with the plasma membrane either by direct or indirect interactions. One of the main regulators of the ERK1/2 cascade in the plasma membrane is kinase suppressor of Ras (KSR), first identified by genetic screens in *Drosophila* and *Caenorhabditis elegans* as a positive regulator of the ERK1/2 cascade.[159] Studies on the nature of KSR action led to the conclusion that it acts as a scaffold protein by facilitating ERK1/2 signaling, and as such, KSR was the first scaffold protein identified for the cascade. Interestingly, mammalian KSR1 (and probably also KSR2) is a central component of complex signaling machinery that initiates ERK1/2 signals from a close vicinity to the plasma membranes. Thus, in resting cells, KSR1 interacts with inactive MEK1/2[160] but not with ERK1/2 or Rafs. For its regulation, KSR1 also interacts with c-Tak1, which constitutively phosphorylates its Ser392,[161] as well as with the adaptor protein 14-3-3,[162] inactive PP2A,[163] and the inhibitory E3 ubiquitin ligase, IMP1.[164] These components form a big protein complex that is localized primarily in the cytoplasm. Upon stimulation, IMP1 is recruited by Ras-GTP, which further induces its polyubiquitination and degradation. This induces a big change in the structure of the complex, allowing the associated PP2A to dephosphorylate Ser392 in KSR1, leading to dissociation of the KSR1 from the 14-3-3 protein and translocation to the plasma membrane. In this location, active Raf1 joins the complex and activates the pre-existing MEK1/2 that further recruit and activate ERK1/2 molecules.[160] Finally, the activated ERK1/2 detach from the complex and shuttle to various cellular compartments, mainly the nucleus, to induce most ERK1/2-dependent cellular functions.[20,159] Another membranal protein that may participate in the regulation of the ERK1/2 cascade is caveolin,[165] localized mainly in caveolae. However, this interaction seems to mostly inhibit ERK1/2 activation and is probably specific to particular cell lines and conditions.

Yet another way to secure explicit localization of components of the ERK1/2 cascade and their proper regulation is achieved by interactions with cytoskeletal components.[88,166] Several cytoskeletal elements have been reported to directly interact with ERK1/2 and other components of the cascade. Thus, it was initially shown that ERK1/2 associate with the microtubule and actin filaments both before and after cellular stimulation.[49,167] This interaction may be induced by either a direct binding to microtubules or actin, or indirectly by adaptor proteins like calponin, which is an actin-binding protein. One of the main purposes of this interaction is to direct the ERK1/2 to their right localization and as such to restrict nuclear entry of activated ERK1/2. One example for the latter effect was demonstrated for retinoic acid–induced differentiation, which is accompanied by a reduction in cell proliferation. This reduced proliferation is mediated by restricting nuclear entry of ERK1/2, which requires intact actin and microtubule cytoskeleton.[168] The association of ERK1/2 with the cytoskeleton was also suggested to be involved in the transport of phosphorylated ERK1/2 over long distances within the cell, using the cytoskeletal motors. For example, in lesioned nerves, the binding of vimentin to phosphorylated ERK1/2 enables spatial translocation of the kinases by importins and dynein.[169] Aside from the direct interaction, and the interaction through cytoskeletal adaptors that recruit only ERK1/2 molecules, it was shown that scaffold proteins that recruit additional components of the ERK cascade might direct cytoskeletal localization of ERK1/2 as well. Thus, it was shown that IQGAP1 interacts with B-Raf, MEK1/2, and ERK1/2; recruits them to actin filaments; and thereby influences mitogenic, morphological, and migratory cell behavior.[170,171] Another ERK1/2 scaffold protein that is also an actin-binding protein is LSP1, which associates with MEK1, ERK2, and KSR and targets them to peripheral actin filaments and thereby regulates signals to proliferation.[172,173] Finally, not only are ERK1/2 regulated by cytoskeletal elements, they can also regulate cytoskeletal reorganization and

thereby downstream cellular processes. An example of such an effect is the influence of ERK1/2 on cell motility, which is mediated in part by phosphorylation of myosin light chain kinase (MLCK) to enhance its activity and facilitate cell motility.[174]

ERK1/2 and ERK1c in the Golgi Apparatus

The Golgi apparatus of mammalian cells is organized into stacks of cisternae, which are anchored in the perinuclear region.[175] Most of the processes within the Golgi, including glycosylation and trafficking, are probably not regulated by phosphorylation. However, the process of Golgi fragmentation may be different. Thus, once the cell enters mitosis, the perinuclear stacks of Golgi cisternae undergo extensive fragmentation, and the fragments are dispersed throughout the cytoplasm, later dividing between the separating cells. Golgi fragmentation is considered to be a "mitotic check point," as once it starts, mitosis must be completed.[15] Interestingly, it was previously shown that the fragmentation is inhibited by MEK1/2 inhibitors, indicating that the ERK1/2 cascade might be involved in its regulation.[176] Moreover, it was shown that the fragmentation is associated with the accumulation of monophosphorylated ERK1/2 in the Golgi.[177] However, although activated, no ERK1/2, and probably no MEK1/2, could be detected in the Golgi, either in the G2 or M phases of the cell cycle. Therefore, it became important to elucidate the mechanism by which MEK1/2 or ERK1/2 operate in order to induce the Golgi fragmentation. In this regard, our group has shown that the ERK1 splice variant, ERK1c, but not ERK1/2, accumulates in the Golgi in the relevant cell cycle stages.[40,64] In addition, it was shown that ERK1c activity is elevated towards mitosis because of phosphorylation by MEK1b,[62] and knockdown of MEK1b or ERK1c reduces mitotic Golgi fragmentation and attenuates mitotic progression. These results indicate that in late G2, Golgi-resident
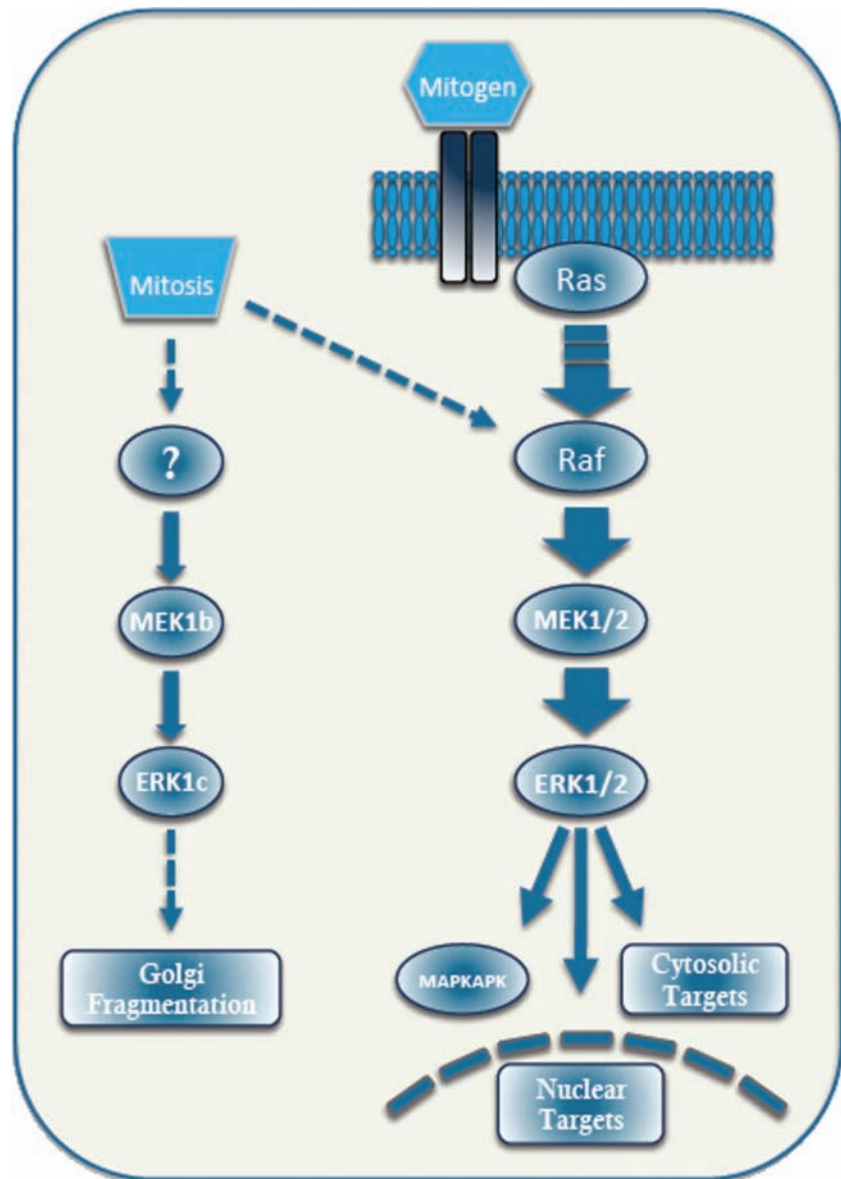

Figure 2. Schematic representation of the different components of the ERK1/2 cascade in resting and mitotic cells. In stimulated cells, most of the ERK cascade outcomes are facilitated by MEK1/2 and ERK1/2. During G2/M phases, some signals are transmitted by MEK1/2 and ERK1/2. In addition, the expression of MEK1 and ERK1 splice variants, MEK1b and ERK1c, is increased. At this stage, the activation of MEK1b and ERK1c is essential for mitotic Golgi fragmentation, while MEK1/2 and ERK1/2 activation induces other mitotic processes.

MEK1b activates ERK1c, which consequently regulates the Golgi fragmentation during mitosis (Fig. 2). One of the important questions that remained open in this study is what could be the downstream substrate of ERK1c that mediates its fragmentation effect? Interestingly, the Golgi protein GRASP55, which may be the protein that converts Golgi stacks into small fragments, was shown to be phosphorylated during early mitosis in a MEK-dependent manner.[178] This and other Golgi proteins containing ERK1/2 consensus phosphorylation sites are likely candidates to be the MEK/ERK-dependent effectors during Golgi

fragmentation, but the exact mechanism of action involving such proteins needs further clarification. A better understanding of the molecular mechanism by which ERK1c acts in the Golgi will shed light on a crucial step in mitosis and will expand our understanding of the signal specificity of the ERK1/2 cascade.

Aside from the role of MEK1b/ERK1c in the regulation of Golgi fragmentation, the outer surface of this organelle was suggested to serve as an anchoring platform for MEK1/2-ERK1/2–generated processes that lead to Golgi-independent cellular effects. This anchoring is mediated by at least one Golgi-specific scaffold protein, termed Sef1.[100] This is a transmembranal Golgi protein that, in resting cells, seems to bind inactive ERK1/2 on the outer part of the Golgi apparatus. Upon stimulation, Sef1 binds irreversibly to activated MEK1/2 and thereby facilitates the activation of the prebound ERK1/2 molecules. At this stage, most of the Sef1-MEK1/2-ERK1/2 complexes remain bound to the Golgi, where they can initiate particular cytoplasmic signals. The irreversible nature of the ERK1/2-MEK1/2 interactions, induced by their interaction with Sef1, prevents the translocation of active ERK1/2 to the nucleus and thereby exerts an inhibitory effect on ERK1/2-dependent transcription. Indeed, knockdown of Sef1 results in enhanced ERK1/2 translocation to the nucleus and upregulation of ERK1/2-dependent genes, such as c-FOS, Egr1, and JunB.[100] In addition, a small amount of the complexes translocate after stimulation to the plasma membranes and activate a particular set of targets at this vicinity as well. Another Golgi protein that may influence the ERK1/2 cascade is RKTG, which binds only to Raf1 and not MEK1/2 and ERK1/2.[179] This protein seems to inhibit the ERK1/2 cascade by sequestering Raf1 from the other downstream signaling components. Thus, both Golgi scaffolds may inhibit rather than facilitate the rate of proliferation upon certain types of stimulations. In summary, it appears that although components of the ERK1/1c/2

cascade may influence Golgi function, the outer surface of this organelle may have a general inhibitory effect on part of the signal propagation downstream of the ERK1/2 cascade.

Summary

The ERK1/2 cascade is a central signaling pathway that participates in the regulation of many distinct and even opposing cellular processes. In order to execute all the diverse functions, the ERK1/2 cascade utilizes several mechanisms that determine its signaling specificity. These include differential temporal activation, scaffold proteins, interaction with different cascades, multiple components in each tier of the cascade, and differential spatial regulation. Interestingly, the relatively large number of ERK1/2 molecules in each cell ($\sim 10^7$ molecules per cell) suggests that distinct pools of molecules mediate distinct functions. Such distinct subgroups of molecules can be maintained by the compartmentalization of the ERK1/2 molecules in different organelles or by interaction with specialized scaffold/anchoring proteins that prevent the translocation of the activated molecules to undesired compartments and bring them to close vicinity to their proper signaling partners under distinct conditions. These allow proper and specific signaling circuits that eventually result in the proper activation of the wanted cellular processes upon distinct activations.

Dysregulation of the ERK1/2 cascade is known to result in various pathologies, inducing neurodegenerative diseases,[180] developmental diseases,[181] diabetes,[182] and cancer.[183] The involvement of the ERK1/2 cascade in cancer is of particular interest, as it was shown that activating mutations of upstream components of ERK1/2 are responsible for more than half of all cancers.[184] Notably, activated ERK1/2 were also found in cancers in which components of the cascade were not mutated, indicating that the ERK1/2 cascade plays a role even in carcinogenesis

induced by apparently nonrelated oncogenes. It is also clear that the translocation of ERK1/2 into the nucleus, and probably other organelles, is essential for the proliferation of cancer cells as well as metastasis. Therefore, interference with the localization of certain components, and in particular with the translocation of ERK1/2 to the nucleus, may serve as potential therapeutic targets for some diseases. Further studies of the compartmentalization of the ERK1/2 cascade will broaden our knowledge of signal specificity determination and ERK1/2 involvement in diseases and may eventually lead to the development of new therapeutic strategies in combating diabetes, developmental disorders, and cancer.

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