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Biochimica et Biophysica Acta 1813 (2011) 1619–1633

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

The MAPK cascades: Signaling components, nuclear roles and mechanisms of nuclear translocation ☆

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ARTICLE INFO

Article history:
Received 22 June 2010
Received in revised form 2 December 2010
Accepted 8 December 2010
Available online 16 December 2010

Keywords:
MAPK
ERK1/2
JNK
p38
ERK5
Nuclear translocation

ABSTRACT

The MAPK cascades are central signaling pathways that regulate a wide variety of stimulated cellular processes, including proliferation, differentiation, apoptosis and stress response. Therefore, dysregulation, or improper functioning of these cascades, is involved in the induction and progression of diseases such as cancer, diabetes, autoimmune diseases, and developmental abnormalities. Many of these physiological, and pathological functions are mediated by MAPK-dependent transcription of various regulatory genes. In order to induce transcription and the consequent functions, the signals transmitted via the cascades need to enter the nucleus, where they may modulate the activity of transcription factors and chromatin remodeling enzymes. In this review, we briefly cover the composition of the MAPK cascades, as well as their physiological and pathological functions. We describe, in more detail, many of the important nuclear activities of the MAPK cascades, and we elaborate on the mechanisms of ERK1/2 translocation into the nucleus, including the identification of their nuclear translocation sequence (NTS) binding to the shuttling protein importin7. Overall, the nuclear translocation of signaling components may emerge as an important regulatory layer in the induction of cellular processes, and therefore, may serve as targets for therapeutic intervention in signaling-related diseases such as cancer and diabetes. This article is part of a Special Issue entitled: Regulation of Signaling and Cellular Fate through Modulation of Nuclear Protein Import.

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1. Introduction

The mitogen-activated protein kinase (MAPK) cascades are evolutionary conserved, intracellular signal transduction pathways that respond to various extracellular stimuli and control a large number of fundamental cellular processes including growth, proliferation, differentiation, motility, stress response, survival and apoptosis [1–3]. Each cascade consists of three core kinases (MAP3K, MAPKK, and MAPK), and often also additional upstream (MAP4K) and downstream (MAPKAPK) components. Within each of the cascades, the signal is propagated by sequential phosphorylation and activations of the sequential kinases, and they eventually lead to the phosphorylation of target regulatory proteins by the MAPK and MAPKAPK components. At present, four different mammalian MAPK cascades have been identified, and named according to their MAPK components: extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38, and ERK5. Other kinases that share sequence similarities with components of the MAPK cascades have been identified as well (e.g. ERK3/4 and ERK7/8). However, the distinct mode of activation of these kinases suggests that they are not components of genuine MAPK cascades [4].

In order to execute their functions, the MAPKs and MAPKAPKs phosphorylate and modulate the activities of hundreds of substrates. The subcellular localization of these substrates may vary depending on the stimulation or regulated functions. Currently, such substrates were identified in the cytoplasm, mitochondria, Golgi, ER, and particularly the nucleus [5–7]. The transmission of signals to the latter compartment is important for the common role of the cascade, namely the induction and regulation of de-novo gene expression [8]. For this purpose, the signals transmitted via the different cascades need to be transported across the nuclear envelope and modulate the activity of a large number of transcription factors, transcription suppressors, and chromatin remodeling proteins, to secure the proper cellular responses [9,10]. The transmission of signals to the nucleus is mediated mostly by a stimulated physical translocation of components of the MAPK cascade into the nucleus. In this review, we will briefly describe the nature of the various cascades, and will mainly concentrate on their nuclear roles and mechanisms of nuclear import, which are important regulatory steps in the function of these cascades.

☆ This article is part of a Special Issue entitled: Regulation of Signaling and Cellular Fate through Modulation of Nuclear Protein Import.

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doi:10.1016/j.bbamcr.2010.12.012

2. The MAPK signaling cascades and their functions

The ERK1/2 cascade was the first MAPK pathway elucidated [11], and is considered as a prototype of these kinase cascades. This cascade plays a central role in the signaling of a wide variety of extracellular agents that operate via various receptors. In most cases, the activation of such receptors is transmitted by several mechanisms to the small GTPase Ras, which is activated mainly at the plasma membranes. In turn, activated Ras recruits the MAP3K tier of the cascade (mostly Raf-1 and B-Raf; Rafs) to the plasma membrane in order to induce their activation (Fig. 1). The mechanism of B-Raf and C-Raf activation is not fully understood yet, but is likely to include homo- or hetero-dimerization as well as phosphorylation [12]. The identity of the kinases that participate in the activating phosphorylation of Rafs, and therefore regarded as MAP4Ks, is still under investigation, but may include under some conditions PKC [13] and MLK3 [14]. Thereafter, the signal is transmitted to the MAPKKs, MEK1 and MEK2 (MEK1/2), and to a lesser extent, the alternative spliced form MEK1b [15], by phosphorylation of two Ser residues in their activation loop. In turn, the activated MEK1/2 transmit their signal to ERK 1 and ERK2 (ERK1/2) [16] and the alternatively spliced ERK1b and ERK1c [17,18] in the MAPK tier, by phosphorylating the regulatory Thr and Tyr residues in the Thr-Glu-Tyr domain within their activation loop [11]. Finally, the signals continue to the MAPKAPK components (RSKs, MNKs and MSKs) and/or to many other substrates, which are localized either in the cytoplasm, the nucleus, or in other cellular organelles [7]. Interestingly, as initially reported for yeast MAPKs [19], ERK1/2 can also bind directly to DNA sequences, and act as transcriptional repressors of several cytokine-induced genes [20].

After stimulation, the ERK1/2, as well as the MAPKAPK components phosphorylate hundreds of substrates in many cellular locations, and these are responsible for the induction of ERK1/2-dependent cellular processes, including mainly proliferation and differentiation, but also morphology determination, neuronal plasticity, survival, and under some conditions stress response and apoptosis [5]. On the other hand, when dysregulated, this cascade plays a major role in various pathologies, inducing neurodegenerative diseases [21], developmental diseases [22], diabetes [23], and particularly notable — cancer [24,25]. The involvement of the ERK1/2 cascade in cancer has been attracting considerable attention in the last decade, as activating mutations of signaling components upstream or within the cascade are responsible for more than half of all cancers [25]. Notably, activated ERK1/2 were found also in cancers where components of the cascade were not mutated, indicating that the ERK1/2 cascade plays a role even in carcinogenesis induced by apparently non-related oncogenes.

The JNK cascade was initially identified as a regulator of the transcription factor c-Jun, and a mediator of intra- or extra-cellular stresses, which gave it its other name, stress-activated protein kinase (SAPKs) cascade [26]. However, like the rest of the MAPKs, it was later shown that this cascade is also stimulated by a large number of stress-independent stimuli and receptors including mitogens [27–29]. Upon activation, the stress and other stimuli transmit their signals to small GTPases such as CDC42 and Rac1, which activate the MAP3K level kinases either directly or via MAP4Ks. Alternatively, the MAP3Ks and MAP4Ks of the cascade can be directly activated also by stimulated interaction with adaptor proteins such as TRAF [30]. A large number of MAP4Ks [31] and MAP3Ks [32] has been identified thus far, and each of these seems to transmit the signal of the cascade by binding to distinct scaffold proteins under distinct conditions (e.g. JIPs [33]). Upon activation, the kinases at the MAP3K tier transmit the signals further by phosphorylating Thr and Ser residues in the activation loop, and thereby activating the kinases at the MAPKK level (MKK4 and MKK7 [34]). In turn, these kinases activate the three components at the MAPK level (JNK1-3; 46 and 54 kDa; JNKs) by direct phosphorylation of Tyr and Thr residues in their activation loop's Thr-Pro-Tyr motif. Recently, MST1 [35] and MAPKAPK3 [36] were reported to be phosphorylated by JNKs, but their function as classical MAPKAPKs is still controversial.

Upon stimulation, the JNKs and possibly their putative MAPKAPKs, phosphorylate a large number of substrates existing mostly in the nucleus, but also in the cytoplasm. These phosphorylated targets further regulate the transcription of many genes, which in turn, mediate cellular processes such as apoptosis [37], immunological effects [38], neuronal activity [39], insulin signaling [39], and more [8]. The exact targets of JNKs that are involved in the regulation of all these processes require further clarification. As would be expected from these central roles, dysregulation of the JNK cascade leads to several diseases. It was shown that JNKs play a role in a list of neurodegenerative diseases, including Alzheimer, Parkinson, amyotrophic lateral sclerosis (ALS, [21]) and lissencephaly [40]. Other pathologies in which JNKs are involved include diabetes [23], inflammation [41], and several cancer types [42]. Therefore, better understanding of the regulation of this cascade is likely to lead to better strategies to combat these serious diseases.

The p38 cascade is yet another SAPK pathway that exhibits a considerable cross-talk and shared components with the other stress-induced cascade of JNKs [43]. Similarly, this cascade seems to be activated primarily by stress-related stimuli, but is also known to respond to signals transmitted by a wide variety of other receptors and cellular processes [44,45]. Upon activation of the receptors, or induction of stress conditions by environmental cues, the signals to the p38 cascade are transmitted via adaptor proteins, small GTPases, MAP4Ks and MAP3K that are usually similar to those functioning in the JNK cascade. The differences in activation of these two cascades, when existing, are mediated by specific scaffold proteins, compartmentalization, and variable substrates [2,46,47]. Upon transmission by one or more components in the MAP3K level of the cascade, the signals induce phosphorylation and activation of the MAPKK components of the p38 cascade, which are mostly MKK3 and MKK6 [48], but under some circumstances, can also include MKK4 [49]. At this stage, the signals are transmitted to the four isoforms (p38 α–δ; p38s) and few functional alternative spliced forms at the MAPK tier of the cascade (32–54 kDa). These components are activated due to phosphorylation of the regulatory Thr and Tyr within the Thr-Gly-Tyr domain in their activation loop. Interestingly, the activation of p38s can also be mediated by autophosphorylation in a MAPKK-independent manner. This type of activation is induced either by stimulated interaction with the adaptor proteins Tab1 [50], by phosphorylation of Tyr323 by ZAP-70 [51], or by interaction with lipidic phosphatidyl inositol analogues [52], which in turn, catalyze an enhanced autophosphorylation of p38s on its activatory residues. This is an important mode of activation that allows additional and unique levels of regulation of this cascade. Unlike JNK, the p38 cascade has several components at its MAPKAPK tier, including MAPKAPK2-5 [53], MNK1/2 [54] and MSK1/2 [55]. MNK1/2 and MSK1 are shared with the ERK1/2 cascade, and therefore, are a point of signal integration between these two MAPK cascades. Finally, the signals are transmitted by the p38s, or the MAPKAPKs to target molecules, which are responsible for the many processes that are induced and regulated by the cascade.

Aside from their role in stress response [45], p38s play a central role in the regulation of immunological effects [56], apoptotic [57], cellular senescence [58], cell cycle checkpoints and even survival [59]. This involvement in important processes predicts that dysregulation of the cascade may have pathological manifestations. Indeed, p38s were found to act as a key component in the induction of inflammation, and are currently considered as important therapeutic targets for inflammation-related diseases [60,61]. This role is also extended to other diseases of the immune system, including autoimmune diseases and others. In addition, p38s were shown to play a role in cardiovascular

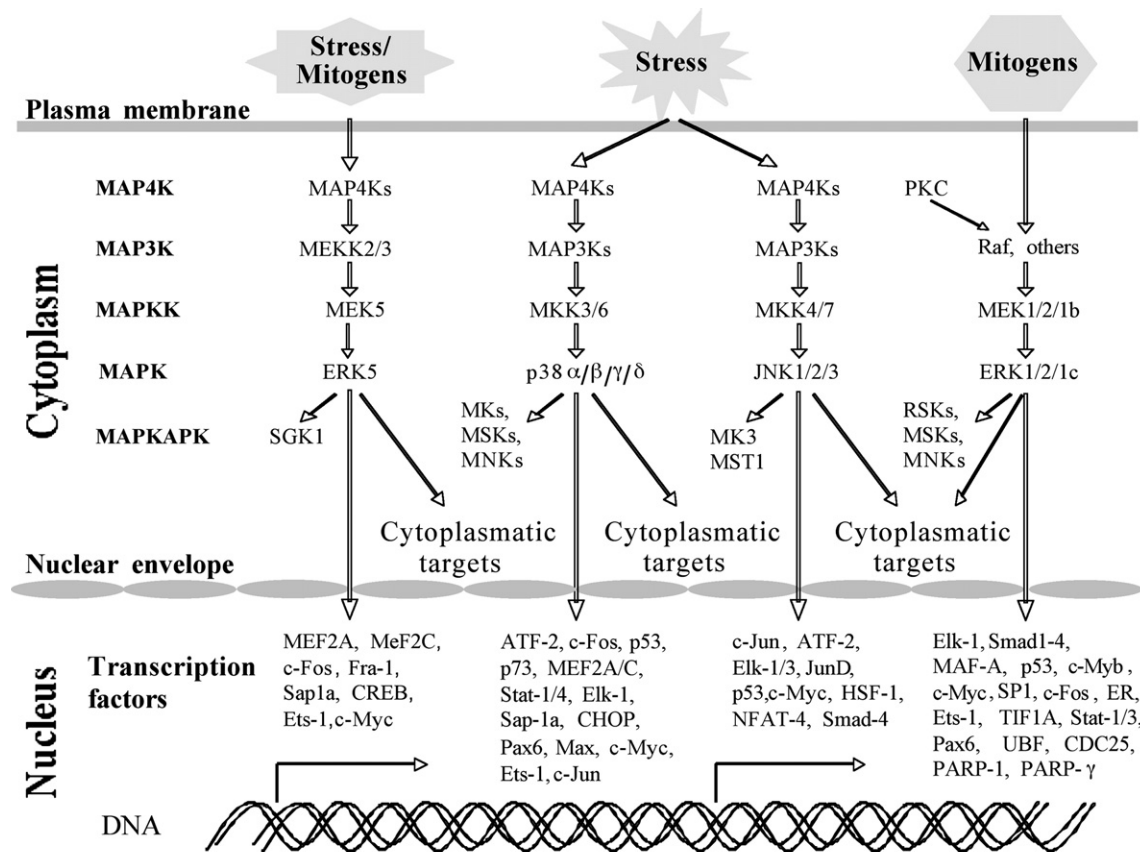

Fig. 1. Schematic representation of the MAPK cascades and their nuclear targets. The main stimulants of the cascades are mentioned, although each cascade can be activated by additional stimulations (see text for details).

diseases, and inhibition of this activity has a cardio-protective effect [45]. It was also implicated in the induction and maintenance of neurodegenerative diseases [21], in diabetes [62], and even in cancer [63]. Unlike the other MAPK cascades, p38s were primarily shown to act as a tumor suppressor, acting through downregulation of Ras-dependent and independent transformation, invasion, and also by inducing apoptosis. However, in fewer cases, p38s were actually shown to induce cancer progression, mainly due to its role in cancer-associated inflammations or in the regulation of cell cycle [42]. The nature of these different roles is not fully understood, but could result from the different function of different p38 isoforms [63].

The ERK5 cascade is less studied and less understood than the other MAPK cascades. As all other MAPK cascades, also this one is activated by a large number of stimuli and receptors, but unlike the others, was implicated equally in both stress and mitogenic signaling [64–66]. The mechanism of activation of this cascade has not been fully elucidated yet, but probably involves either adaptor proteins (e.g. Lad [67]), or MAP4K (e.g. WNK1 [68]). Those components activate the kinases at the MAP3K tier, consisting mainly of MEKK2/3 [69] and probably Cot1 [70]. These kinases activate the sole component in the MAPKK tier, MEK5, by phosphorylating Ser and Thr in its activation loop. ERK5 is the only component in the MAPK level of the cascade, and is also known as a big MAPK (BMK) due to its relatively high molecular weight (110 kDa). As ERK1/2, the activation of ERK5 is mediated by phosphorylation of both Thr and Tyr residues within its Thr-Glu-Tyr motif. However, despite the identical amino acids in the regulatory domain, these MAPKs share very little similarity in their upstream activators or physiological substrates. Upon stimulation, ERK5 can transmit its signals to the one MAPKAPK that was reported thus far (SGK1; [71]), and phosphorylate several cytoplasmic as well as nuclear targets. Interestingly, ERK5 can act as a transcription factor by itself, which is mediated by DNA binding through its non-catalytic region [72]. These downstream activities, allow ERK5 to induce and regulate several physiological processes including proliferation [2], angiogenesis [73], immunological processes [74], stress responses [75] and probably additional processes. The cascade has also been implicated in a few pathologies, including cancer [76,77]. However, due to the small number of studies, the full scope of its functions is not clear, and it is likely that ERK5 will be implicated in the future in additional diseases.

3. Nuclear functions of the ERK1/2 cascade

The MAPK cascades participate in the regulation of essentially all stimulated processes in the cell. In order to execute their functions, the various MAPK components phosphorylate a large number of substrates that are localized either in the cytoplasm, or in various organelles, primarily the nucleus [7,9]. The nuclear functions of the MAPK-dependent signals are important for the activation of transcription factors necessary for the relevant gene expression. Other roles of the nuclear activities, such as chromatin remodeling, direct induction or suppression of transcription and regulation of cell cycle, have been demonstrated as well. Therefore, nuclear translocation of the MAPKs is essential for many of their activities. Indeed, specific abrogation of ERK translocation into the nucleus by irreversible cytoplasmic anchoring [78,79], MKP-2-dependent modulation of nuclear MAPKs [80], or specific inhibition of the nuclear activity of JNKs and p38s [81,82] blocked the progression of many of their regulated cellular processes. In this review, we describe the main nuclear activities of the various MAPK components, and their

involvement in important cellular processes. In this section, we describe some of the nuclear functions of the ERK1/2 cascade, while those of the other cascades are described in the next sections.

ERK1/2 execute their functions by activating a large number of downstream targets that may vary under different conditions and cell types. Currently, the number of identified substrates is as high as 200 (see initial list in [5]), although recent studies indicate that the actual number is likely to be much higher [83]. Interestingly, the substrates are localized in several cellular compartments, including the cytoplasm [84], cytoplasmic organelles such as mitochondria [85], Golgi [86], endosomes/lysosomes [87], various membranes [88], and particularly the nucleus. In fact, about half of the identified ERK1/2 substrates are nuclear proteins, and those participate in the regulation of many stimulated nuclear processes including induction or suppression of transcription, chromatin remodeling and nuclear disintegration in mitosis. Some of the nuclear activities of ERK1/2 are described below.

3.1. Regulation of immediate early genes

The main activity of ERK1/2 in the nucleus seems to be the regulation of transcription factors. Indeed, one of the first substrates identified for ERK1/2 was the transcription factor Elk1 [89,90], which is one of the main regulators of immediate early genes (IEG; [91]). The rapid IEG transcription upon extracellular stimulation requires that their transcription factors will be activated within minutes after stimulation, and this occurs mostly by the ERK1/2 cascade. Indeed, Elk1, which is a nuclear ETS-domain transcription factor, was found to contain several MAPK consensus phosphorylation sites in its activation domain [92]. Stimulation of cells by various agents induces the phosphorylation of Elk1 on no less than 6–9 distinct sites, and most of these phosphorylations were shown to induce the activation of this factor [93]. Importantly, the rapid phosphorylation of Elk1 by ERK1/2 occurs upon a direct binding of the two proteins, which like in many other ERK1/2 substrates is mediated by two docking sites. One site is the D-domain of Elk1, which interacts with the CD/CRS domain in ERK1/2 [94], and the other is the hydrophobic DEF region in Elk1 [95], which interacts with the hydrophobic interaction domain of ERK1/2.

One of the earliest transcriptional events regulated by Elk1 upon stimulation, is the induction of the IEG c-Fos. This induction is important for the proper progression of various cellular processes, including proliferation and differentiation and seems to be dysregulated within oncogenic transformation [96]. Thus, resting cells do not express c-Fos, but this rapidly changes after stimulation when c-Fos is induced for a few minutes up to several hours via a mechanism that involves Elk1 activation. The changes in expression time of the c-Fos protein are regulated by a combined action of protein kinases and proteinases. Without phosphorylation, c-Fos is rapidly degraded, and therefore, is expressed for only several minutes. However, phosphorylation by nuclear ERK1/2 and their downstream RSKs on Ser374 and Ser362 in the C-terminus of c-Fos is the first step in its stabilization [97]. The differences in c-Fos stability upon distinct stimulation make it a good interpreter of differences in ERK1/2 kinetics of activation, and therefore in the determination of ERK1/2-dependent signaling specificity [98]. Moreover, the phosphorylation of c-Fos might partially explain the induction of pathological processes including oncogenic transformation that might be mediated only upon prolonged ERK1/2 activation [97]. Similar phosphorylation by ERK1/2 seems to be important for the stability and activity of additional IEGs such as c-Myc and Fra1 [99], and the ERK1/2 cascade can regulate other transcription factors by modulating ubiquitylation and sumoylation as well [8]. Thus, ERK1/2 serve as key regulators of transcription factors and IEGs in general, and AP-1 in particular, under many conditions and in most cell lines and organisms.

3.2. Involvement of ERK1/2 in transcriptional suppression

Aside from the role of ERK1/2 in the induction of immediate early transcription upon stimulation, they were also shown to play a role in the regulation of many other transcription factors or transcription repressors [5]. One of the main transcription repressors that are regulated by ERK1/2 is the Ets2 repressor factor (Erf1), which suppresses transcription in many resting cells [100]. Thus, in serum-starved cells, Erf1 is dephosphorylated and located in the nucleus where it suppresses transcription. Upon mitogenic and other stimulations, Erf1 is phosphorylated by the translocated active ERK1/2. This phosphorylation, in turn, induces CRM1-dependent nuclear export of Erf1 [101,102], which thereby alleviates its suppression of transcription [103]. The phosphorylation of Erf1 is regulated mainly by two distinct FXF motifs that interact with the hydrophobic DEF-binding domain in ERK1/2 [104]. Prevention of Erf1 phosphorylation was shown to reverse RAS-induced tumorigenicity and to arrest fibroblasts in the G0/G1 phase of the cell cycle. Hence, ERK phosphorylation-dependent Erf1 export affects cell cycle progression, thus providing another link between the ERK1/2 cascade and cellular proliferation [105].

Interestingly, the involvement of ERK1/2 in suppressing gene activity is mediated not only through the regulation of transcription factors/repressors, but also by the direct interaction of ERK1/2 with DNA [20]. This unexpected effect was recently demonstrated by a high-throughput examination of protein–DNA interaction profiles. A comprehensive screen identified not less than 17,718 direct protein–DNA interactions between 460 DNA motifs and 4191 human proteins. Many of these interactions were either known or expected, but some were unanticipated interactions, either for known transcription factors or for additional proteins that had not as yet been known to interact with DNA. One of these proteins was ERK2, which was found to bind to the DNA sequence G/CAAAG/C, independent of its catalytic kinase activity. The authors showed that the interaction causes a direct suppression of various transcriptional elements, including gamma activated transcriptional element (GATE), which is responsible for the activity of the transcription factor C/enhancer binding protein (EBP)-beta. Importantly, ERK2 was shown to mainly repress interferon-γ-induced genes that are normally regulated by GATE, and this suppression is relieved upon interferon-γ treatment, by depleting the ERK2 molecules from the regulated promoters [20]. Thus, aside from its well-known regulation of transcription factors, ERK2 can regulate gene expression by activity-independent binding to promoter regions of some genes.

3.3. Involvement of the ERK1/2 cascade in chromatin remodeling

Within the cell nucleus, the DNA is packaged into chromatin, which is a compact structure composed of protein–DNA complexes [106]. The double stranded DNA is coiled around an octamer of core histones (H2A, H2B, H3 and H4) to form nucleosomes, which are organized in higher order structures that are important for the proper chromatin function. This structure is very condensed, and therefore, not accessible by other proteins, such as transcription factors. Consequently, “decompaction” and a change into active open euchromatin is required in order to allow transcription. Indeed, in response to stimulations, several distinct processes induce chromatin remodeling in order to make the various target genes more accessible. This includes histone acetylation, histone phosphorylation, poly ADP ribosylation, changes in DNA conformation, and binding of other proteins to the DNA. Some of these effects are regulated by the MAPK cascades, including mostly ERK1/2 and p38s, and are required for proper transcription and the induction of the MAPK-dependent processes [107,108]. Three examples for such ERK1/2-mediated effects are mentioned below.

One of the effects of the ERK1/2 cascade on chromatin remodeling is mediated by its regulation of histone deacetylation, which is usually associated with a compact chromatin structure and a repressed gene transcription. Indeed, it was shown that histone deacetylase 4 (HDAC4), which is a member of a family of enzymes that catalyze the removal of acetyl groups from core histones, directly interacts with ERK1/2, and is probably phosphorylated by it [109]. In addition, activation of the ERK1/2 cascade induced translocation of HDAC4 into the nucleus. These results indicate that ERK1/2 participate in a process that leads to chromatin condensation, and thereby, induce a negative feedback loop that is required for its long-term desensitization upon stimulation. Another way by which the ERK1/2 cascade may modulate chromatin arrangements is by MSK-induced phosphorylation of the chromatin-arranging proteins histone H3 and HMG-14 [106,107,110]. RSK2 was implicated in the phosphorylation of histone H3 as well, but at this stage, this observation is still controversial [8]. This phosphorylation then, is able to induce looser structure of the chromatin and accelerate a stimulated transcription.

ERK1/2 were also shown to induce chromatin remodeling by its non-conventional influence on PolyADP-ribose polymerase 1 (PARP1). This protein catalyzes a polyADP-ribosylation of nuclear proteins that affects chromatin structure. It was shown that the catalytic activity of this protein is stimulated not only by DNA strand breaks, but also by the ERK1/2 cascade [111]. Interestingly, the effect of ERK1/2 on PARP1 activity was not mediated by phosphorylation of the protein, but rather was induced by a direct interaction with ERK1/2. In addition to its possible effects on chromatin function, the activated PARP-1 also increased ERK2-catalyzed phosphorylation of Elk1, which may be important for the enhanced transcriptional activity of the latter. Similar non-catalytic activation of nuclear proteins by ERK1/2 was also demonstrated in the case of topoisomerase 2α [112], which is activated by this interaction to induce modulation of the DNA structure downstream of the ERK1/2 cascade. Thus, the ERK1/2 cascade is one of the regulators of chromatin remodeling that plays a role in the induction or suppression of transcription. However, at this stage, it seems that this process is mainly regulated by other signaling pathways, and the ERK1/2 cascade plays only an accessory role or is limited to distinct cell lines and conditions.

3.4. Regulation of nuclear import by the ERK1/2 cascade

Another mechanism by which the ERK1/2 cascade regulates cellular processes is by affecting the general nuclear import machinery. The nucleus is separated from the cytoplasm by a double membrane envelope. Cytoplasmatic/nuclear shuttling of molecules through this envelope occurs via specialized nuclear pore complexes (NPC) that coordinate nucleo-cytoplasmic exchange, ensure high selectivity and support proper cytoplasmatic/nuclear molecular balance ([113]; see also description in Section 5). The NPC is a large macromolecular complex, composed of more than 100 proteins that are termed nucleoporins (NUPs) in eukaryotes [114–116]. Interestingly, many studies over the past decade demonstrated a direct interaction of NUPs with ERK1/2. Initial studies showed binding of the kinases to NUP214 [117] and NUP153 [118], which were first suggested to induce a carrier-independent nuclear import/export of ERK1/2. However, the finding that ERK2 can phosphorylate NUP153 in vitro [119], and the elucidation of a distinct mechanism of nuclear translocation [120], suggests that the interaction with these NUPs may affect nuclear shuttling of additional proteins and not only ERK1/2.

The above NUPs are not the only targets of ERK1/2 in the NPC. Another NUP that is phosphorylated by the ERK1/2 cascade is the NUP “translocated promoter region (TPR)”, which was identified as an ERK substrate by an engineered kinase and ATP analogues methodology [121]. It was later shown that the phosphorylation of TPR is required for its binding to ERK1/2, which therefore allows recruitment or ERK1/2 to the NPCs [122]. Similarly, it was also shown that ERK1/2, as well as other MAPKs, constantly interact with NPCs and phosphorylate an additional NUP, termed NUP30 [123]. Although these studies implicated ERK1/2 in NUP regulation, they did not provide any clue regarding the exact function of all these NUP phosphorylations. However, a more informative study has recently identified NUP50 as a bona fide substrate of ERK1/2 [124]. It was further shown that ERK1/2 phosphorylate the FG repeat of this NUP as well as other NUPs, and this phosphorylation reduces their affinity for importin-β family proteins. Importantly, ERK phosphorylation of NUP50 resulted in impaired translocation of importin-β and transportin, strongly suggesting that the ERK1/2 cascade indeed regulates the general nucleocytoplasmic transport machinery by phosphorylating NUPs.

The effect of the ERK1/2 cascade on protein shuttling is not only mediated by NUP phosphorylation, but also by the regulation of RanGTP distribution [125]. Ran is a small GTPase, which is localized in its active, GTP-bound form predominantly in the nucleus [126]. The inactive form of Ran is localized mainly in the cytoplasm, and the resulting Ran gradient regulates import and export of proteins through NPCs. One of the regulators of RanGTP gradient is Ran-binding protein 3 (RanBP3), which regulates nuclear export via CRM1 [127]. Interestingly, RSKs, acting downstream of ERK1/2, interact with, phosphorylate and regulate the activity of RanBP3, and thereby regulate general nuclear shuttling and cell fate [125]. This phosphorylation can be mediated by the AKT pathway as well, suggesting that the effect of RanBP3 phosphorylation is of general importance upon distinct stimulation [125]. Collectively, these studies present new and exciting ways of regulation of various nuclear shuttling machineries by the ERK1/2 cascade. These mechanisms provide an additional way for determination of signaling specificity of the cascades, which is important for cell fate determination upon various stimulations.

3.5. Direct involvement of MEK1/2 in nuclear processes

MEK1/2 are unique 45/46 kDa dual specificity kinases that appear to phosphorylate only ERK1/2, and therefore, are the specificity determinants of their cascade [128,129]. Initial studies described MEK1 as a cytoplasm-resident protein, both in resting cells and after stimulation, in contrast to its substrates ERK1/2 that were found in the nucleus upon stimulation [130,131]. This subcellular distribution was attributed to an association with cytoplasmic anchoring proteins such as KSR [132], as well as to a leucine-rich sequence in the N-terminus of the protein that acts as a CRM1-dependent nuclear export signal (NES; [133]). This sequence was initially suggested to act as a safety mechanism to secure the cytoplasmic localization of MEK1, which is important for its function, as well as that of MEK2, as anchoring proteins of ERK1/2 in the cytoplasm [134,135]. In support of that, when the NES is interrupted by replacing the sequence's Leu with Ala residues, MEK1 is found both in the nucleus and in the cytoplasm, in contrast to the wild-type MEK1, which is found exclusively in the cytoplasm [136]. However, it was later shown that MEK1 and probably MEK2 could rapidly translocate into the nucleus upon cellular stimulation [136–138]. Thus, initiation of signaling via the cascade causes MEK1/2 to detach from ERK1/2 and from other anchoring molecules, and translocate separately from ERK1/2 into the nucleus. Unlike ERK1/2 that can be retained in the nucleus for minutes to hours after stimulation, most MEK1/2 molecules are rapidly exported back to the cytoplasm shortly after translocation, giving rise to the apparent constant cytoplasmic distribution. This rapid, CRM1-dependent export is important for mediating the functions of ERK1/2 cascade-dependent processes. Therefore, it became important to study the role and

mechanism of this rapid shuttle in and out of the nucleus, both in resting and stimulated cells.

Although the role of this rapid MEK1/2 shuttle is not fully understood yet, some studies may have shed light on this issue. Since MEK1/2 were shown to be very specific towards ERK1/2, it was thought that the translocation might affect nuclear ERK1/2 molecules. Indeed, constant nuclear localization was reported for alternatively spliced isoforms of ERK1, namely ERK1b [17], and to some extent ERK1c in G1/S phase of the cell cycle [18]. The phosphorylation of these isoforms is MEK1/2-dependent, and therefore is likely to involve the translocated active MEK1/2 molecules. In addition, it was shown that translocated MEK1, and probably MEK2 may be involved in the export of ERK1/2 out of the nucleus at late stages after stimulation [139]. Thus, nuclear inactive MEK1/2, mostly generated by dephosphorylation of the translocated molecules, are able to re-bind to inactive ERK1/2 molecules in this location. This is followed by interaction of the NES of MEK1/2 with CRM1 and a rapid export of the complex out of the nucleus, resulting in the resetting of the ERK1/2 cascade. Interestingly, it was later shown that MEK1 could induce export of other nuclear proteins beside ERK1/2. These include the nuclear receptor PPARγ [140], whose nuclear export upon mitogenic stimulation participates in its functional inactivation. Finally, one study reported that MEK1/2 can be identified within the nucleus of muscle cells where they bind the nuclear MyoD transcriptional complex and thereby repress its activity [141]. These findings suggest that the nuclear translocation of MEK1/2 has an important physiological role, but additional studies are required to elucidate the full scope of this effect in various systems.

4. Nuclear functions of the other MAPK cascades

Although less studied than the “prototypic ERK1/2 cascade” [48], the nuclear activities of the JNK, p38 and ERK5 cascades have been implicated in various important cellular processes as well. As was shown for ERK1/2, also the other MAPKs regulate transcription, chromatin remodeling, stability of nuclear proteins and other nuclear processes [6,8]. These processes are regulated by a set of nuclear substrates, which are either targeted only by one of the cascades, or more frequently, by two and even three of the MAPKs. Examples for the latter case are Elk1, which is regulated by ERK1/2, JNKs and p38s [142]; c-Fos, by ERK1/2 and ERK5 [143]; and c-Jun, which is regulated by JNKs and ERK1/2 [144]. The ability of the various cascades to regulate the activity of similar substrates is not very surprising, since the different components in the MAPK and most MAPKAPKs share the same consensus phosphorylation site (Pro-Xaa-Ser/Thr-Pro for MAPKs [145,146], and basic amino acid N-terminal of Ser/Thr for MAPKAPKs [147,148]). However, it is not very clear how the activation of these nuclear targets by distinct MAPK cascades still results in distinct outcomes. These distinct specificities may be mediated by spatio-temporal differences, interaction with distinct scaffold proteins, distinct interaction affinities with various substrates, and regulation by additional nuclear components [1]. Overall, the various phosphorylations on similar, as well as distinct targets lead to integration of the MAPK and other signals, and allow progression of nuclear processes such as transcription [44], chromatin remodeling [149], protein stability [150], subcellular localization [81], and even cell cycle progression [151]. In this section, we cover some of the nuclear processes that seem to be preferentially induced by the stress-activated MAPK cascades.

4.1. Regulation of transcription factors by stress-activated cascades

Over the past years, it was reported that JNKs, p38s and their responsive MAPKAPKs directly regulate a wide range of transcription factors. Some of the main transcription factors regulated by JNKs are: c-Jun, JunB, JunD, ATF2, p53, JDP2, and c-Myc [152], and by p38s: ATF-1/2/6, Sap1, CHOP, p53, C/EBP, MEF2C, MEF2A, and HBP1 [44]. The activation of all these transcription factors is likely to regulate different aspects of the response of cells to various stresses and other stimuli. Here we focus on the phosphorylation and activation of three well-studied transcription factors, c-Jun, MEF2A, and p53. These may be considered as prototype transcription factors, and therefore, their activation probably exemplifies the mechanism of activation of other members of the group as well.

The first identified, and one of the best-known transcription factors regulated by JNKs, is c-Jun, which gave JNKs their name [153]. c-Jun is constantly expressed in resting, as well as stimulated cells. In order to exert its activity upon stimulation, c-Jun interacts with other transcription factors such as c-Fos, and ATF to form a complex known as “activator protein 1” (AP-1) [154]. In addition, the activation of c-Jun requires phosphorylation of its transactivation domain, mainly on Ser63 and Ser73 [155], but also on Thr91 and Thr93 [144]. This phosphorylation, which is mediated by all three JNK isoforms and to some extent by other MAPKs, induces the full transcriptional activity within the AP-1 complex, independent of DNA binding [156]. Aside from the induction of transactivation, the phosphorylation was also shown to increase the half-life of c-Jun protein [157], which is important for the timing of the transcriptional response. Therefore, the phosphorylation of c-Jun is a central mechanism by which JNKs and other MAPKs induce their responses upon various extracellular stimulations.

The induction of c-Jun phosphorylation is important for the regulation of many distinct cellular processes, ranging from proliferation to apoptosis. However, MAPKs often modulate the activity of specific transcription factors that regulate the activity of more limited number of cellular processes. An example of such an effect is MEF2A, which is phosphorylated by two isoforms of p38, p38α and p38β2, and to some extent by ERK5 [158,159]. This unique specificity can be mediated, in principle, by scaffold proteins or by specific docking interactions to the substrates [160,161]. In the case of MEF2A, it was shown that the second possibility prevails, and this occurs due to variations in the docking (D) domains of the transcription factor, which secured interactions exclusively with relevant MAPKs. In similarity to the phosphorylation of c-Jun described above, the phosphorylation by MAPKs facilitates the activity and stability of MEF2A, thereby promoting its function in several cells including cardiac muscles, neurons and T cells [162].

An additional transcription factor that plays a central role in the cellular response to stress and is phosphorylated by MAPKs, is p53. In unstressed cells, p53 is maintained at low expression levels through targeted degradation. However, genotoxic agents and other stresses that stabilize the p53 protein induce its phosphorylation on various residues, and activate its transcriptional activity. This activation leads either to growth arrest, mostly at the G1/S phase, or often to apoptosis, which is required for the elimination of hyper-mutagenized cells [163,164]. The phosphorylation of p53 occurs on multiple sites and is mediated by several protein kinases, including JNKs [165], p38s [166], and even ERK1/2 [167]. Interestingly, the distinct MAPKs phosphorylate several sites on p53, as JNKs were reported to phosphorylate Ser6 and Ser36, which are both correlated with elevated p53 activity. Interestingly, this phosphorylation seems to be mediated mainly by the interacting JNK2, while JNK1 does not phosphorylate these residues. Rather, activation of JNK1 in this system seems to accelerate the activation of JNK2, which in turn is able to phosphorylate p53 and induce apoptosis [168]. ERK1/2, phosphorylate Thr73 and Thr83, the functions of which are not fully understood, and Ser15, which upon phosphorylation by JNKs and also by p38s induces the activation of p53. More studies are required to better characterize p53 phosphorylation by MAPKs and the specific outcomes of these phosphorylations.

4.2. Regulation of nuclear receptors by stress-activated cascades

Another subgroup of transcription factors that is regulated by MAPK cascades is the family of nuclear receptors, which include estrogen receptor (ER), glucocorticoid receptor (GR), peroxisome proliferator-activated receptor (PPAR), androgen receptor (AR) and retinoic acid receptor (RAR). These nuclear receptors are usually activated by specific ligands, and the phosphorylation by MAPKs does not always induce their activity, but rather modulate it. This difference from the activation of the above transcription factors prompted their description under this separate subsection. Although the ERK1/2 cascade seems to be a major regulator of these nuclear receptors [169,170], over the past years it became clear that other MAPKs are centrally involved in the regulation of some of the receptors as well. One way by which these MAPKs influence the nuclear receptor is through modulating the transcriptional activity of the latter. For example, it was shown that PPARα is phosphorylated by p38s upon ischemia, hypoxia, and other stresses of cardiac myocytes [171,172]. This phosphorylation leads to a significantly enhanced ligand-dependent transactivation, and therefore, enhanced expression of energy metabolic genes [171]. In addition, PPARα phosphorylation by p38s was shown to mediate adiponectin signaling, which is important for the regulation of fatty acid oxidation in muscle cells [172].

Another nuclear receptor that is regulated by the p38 cascade is the retinoic acid receptor gamma (RARγ). A well-studied effect of p38 is the direct phosphorylation of RARγ, which facilitates the recruitment of the coactivator SUG1, and consequently the transactivation of the nuclear receptor [173]. However, it was shown that the same phosphorylation of RARγ is important also for the late degradation and downregulation of RARγ. Therefore, this phosphorylation by p38s may be important not only for the activation, but also for the downregulation and the transactivation-linked turnover of RARγ. Moreover, RAR signaling may be modulated by p38-mediated phosphorylation of the interacting protein SRC-3, which was shown to affect the fate of RAR signaling in embryonic cortical neurons [174]. In view of the above, p38s seem to be central, multifaceted regulators of the nuclear receptors PPAR and RAR in various systems and conditions. Importantly, the unexpected ability of MAPKs to regulate nuclear receptors by phosphorylation of their interacting regulators is not limited to RAR. Thus, it was shown that p38s stimulate estrogen-dependent transcription through phosphorylation and potentiation of the p160 coactivator GR-interacting protein 1. This elevation in transcription seems to enhance estrogen-mediated proliferation and maybe important for oncogenic transformation as well [175]. More such indirect effects on other nuclear receptors such as ER and AR still await clarification.

As mentioned regarding RARγ, phosphorylation by stress-activated MAPKs does not only activate the nuclear receptors, but may also participate in their inactivation and downregulation. One example of the downregulation of nuclear receptors is the AR, which is phosphorylated on Ser650 upon stress stimulation by both JNKs and p38s [176]. This phosphorylation induces nuclear export of AR, and therefore, reduces its transcriptional activity, indicating that JNKs and p38s are both negative regulators of AR activity. However, in most cases, the effects by the p38 and JNK cascade are distinct from each other, as the p38 cascade often enhances the activity of the nuclear receptors (as above), whereas the JNK cascade downregulates them. Example for inhibitory effects of the JNK cascade is the regulation of the glucocorticoid receptor. It was shown that upon UV exposure, GR is phosphorylated by JNKs on Ser226, and this induces nuclear export of the nuclear receptor in the absence of the ligand for GR, dexamethasone [177]. Therefore, this phosphorylation is important for the termination of GR-mediated transcription after stress response. Aside from the inhibitory effects on subcellular localization of the nuclear receptors, JNKs may negatively regulate the nuclear receptors by a direct phosphorylation. Such an effect was reported for PPARγ that is phosphorylated on its inhibitory Ser82 by JNKs, similarly to the negative regulation by ERK1/2 [178]. In addition, JNKs are also involved in the inhibition of RXRα and RARα activities. In the case of RXRα, JNKs phosphorylation inhibits the activity of the nuclear receptor upon treatment with arsenic trioxide [179]. In the case of RAR, JNK phosphorylation induces proteasomal degradation of RARα, thereby promoting aberrant RAR signaling in lung cancer cells [180]. These examples indicate that nuclear receptors are important MAPK targets, and their phosphorylation modulates the activity of these transcription factors independently of their corresponding ligand-induced activation.

4.3. Regulation of the subcellular localization of transcription factors by MAPKs

One way for the regulation of transcription factors by MAPKs is via the modulation of their subcellular localization [181]. Such a regulation was described above for the nuclear export of the transcription factors PPARγ and GR, which are regulated by MEK1/2, and JNKs respectively. In the past years, it was shown that this type of regulation, mainly by the stress activated MAPKs, plays an important role in the regulation of additional transcription factors as well. One of the best examples for such a regulation is the transcription factor NFAT. The activity of this factor is usually induced by elevated cellular calcium concentrations, which induce nuclear translocation of the otherwise cytoplasmic protein [182]. In most cases, the nuclear translocation is mediated by interaction with the phosphatase PP2B, which translocates to the nucleus along with NFAT. At later stages after stimulation when the signals are reduced, NFAT is phosphorylated again, and this allows its export back to the cytoplasm. A group of kinases that mediate the later phosphorylation is that of p38s, which inhibit NFAT action upon stress stimulation [182]. Interestingly, the subcellular localization of the different isoforms of NFAT is not regulated exclusively by the p38 cascade, but by other signaling cascades including ERK5 and mTORC1 [183] as well. It was shown that each of these kinases can phosphorylate NFATc4 on Ser168 and Ser170, and enhance nuclear export of this transcription factor. In addition, NFATc4 is phosphorylated by ERK5, which primes NFATc4 to phosphorylation by CK1. These ERK5 and CK1 phosphorylations then cooperate to stabilize the transcription factor, and are probably dispensable for the determination of its localization.

Another transcription factor, of which the cellular localization is regulated mainly by MAPKs, is Foxo3a [184]. This regulation was demonstrated in the processes of muscle atrophy, which involves loss of muscle mass due to activation of ubiquitin-dependent proteasomal protein degradation. Nuclear Foxo3a seems to participate in this process by activation of the ubiquitin ligase Atrogin-1, further allowing progression of atrophy [184]. JNKs, or p38s participate in the regulation of Foxo3a during these processes by inducing its CRM1-mediated nuclear export, and this effect is not related to its nuclear translocation that is mediated independently by the PI3K/AKT activity. Stress-activated MAPKs control Foxo3a activity also by modulation of its ubiquitylation in muscle cells, which demonstrate a multifaceted regulation of atrophy and other muscle-specific processes. Another important example for the effect of JNKs on subcellular localization of regulators of transcription is the phosphorylation of β-catenin, which prevents its nuclear accumulation [185]. This prevention of nuclear translocation inhibits the interaction of β-catenin with its nuclear effector, the transcription factor TCF. Thus, the effects of MAPKs on the subcellular localization of transcription factors and regulators consist an important layer of regulation of various cellular processes.

As described for ERK1/2 above, another mechanism by which MAPKs can regulate the nuclear shuttling of transcription factors upon stimulation is via phosphorylation of NUPs. In this sense, it was shown that similarly to ERK1/2, JNKs and p38s are constitutively associated with NPCs, and therefore, may regulate nuclear pore functions [123]. Indeed it was shown that JNKs phosphorylate NUP214, which may participate in the induction of neuronal apoptosis [186], and p38s (and ERK1/2) participate in the regulation of nuclear import upon mechanical stretching [187]. Moreover, p38s may regulate nuclear import by disrupting RanGTP gradient upon hyperosmotic stress [188]. This change in the RanGTP gradient was prevented by p38 inhibitors, supporting the role of this MAPK in the general regulatory process of protein accumulation in the nucleus. Thus, the localization of transcription factors is heavily regulated by MAPKs, but the full scope of this regulation requires further clarification.

4.4. ERK5 is a potent transcription factor

As described above, components of the MAPK cascades usually phosphorylate and modulate the activity of a large number of transcription factors. However, some MAPKs can also act as either transcription factors (ERK5; [72]) or transcriptional repressors by themselves (ERK1/2; [20]). In this sense, the action of ERK5 as a transcription factor is a unique example of a dual activity enzyme that not only regulates the action of many transcription factors (e.g. Sap1a, c-Fos, Fra1, and MEF2), but also acts as one by itself [65]. ERK5 has a large, unique C-terminal-half, which is not found in other MAPKs, and contains two proline-rich domains and a bipartite NLS. This C-terminal part demonstrates a very little similarity with other proteins, and was shown to exhibit potent intrinsic stimulus-dependent transcriptional activity [72]. This transcriptional activation domain of ERK5 is required for maximal MEF2 activity in response to calcium flux in T cells, and for the activation of the endogenous Nur77 gene, when recruited to the Nur77 promoter via MEF2 sites. Later it was also shown that the transactivation activity of ERK5 is enhanced upon phosphorylation and activation of the ERK5 kinase activity [189]. Furthermore, active ERK5 can autophosphorylate itself at the C-terminal-half, and this was found to be necessary for the enhancement of the transactivation activity of the protein. Moreover, this activity is enhanced by the constant nuclear localization of ERK5 in most cells [190], and is likely to exert many stimulated activities of ERK5 and possibly of other protein kinases. Thus, ERK5 is a dual activity protein that is activated upon stimulation, autophosphorylates its C-terminus to enhance its transcriptional activity, and thereby transcribes genes that are necessary for various cellular processes.

5. Mechanisms of ERK5 and ERK1/2 translocation into the nucleus

In resting cells, the components of the ERK1/2 cascade are localized primarily in the cytoplasm, where they are retained mainly due to interaction with various anchoring proteins [7]. Upon stimulation, these components are rapidly activated and change their location in order to execute their functions. Thus, Rafs move mainly to the plasma membranes, but also to other cellular membranes, where they are activated by Ras GTPases. On the other hand, MEK1/2, and ERK1/2 translocate to the nucleus shortly after stimulation. MEK1/2 are rapidly exported out of this compartment by their N-terminal NES, while ERK1/2 are retained in that location for many minutes or even hours. The translocation of these components into the nucleus is essential for their proper activity and especially for the induction of proliferation, but the mechanism that allows this translocation is not fully understood. As for the ERK5 cascade, the subcellular localization of its components seems to vary in different cell lines. In most cell lines examined, ERK5 and MEK5 are constantly localized in the nucleus, and the activating signals reach them through the cytoplasmic MEKK2 that translocates into the nucleus upon stimulation [190,191]. However, in other cells, the distribution of these components seems to resemble that of ERK1/2, whereby ERK5 is cytoplasmic in resting cells, and translocates to the nucleus upon stimulation [192,193]. Although it is likely that similarly to ERK1/2, JNKs and p38s are cytoplasmic in resting cells and translocate to the nucleus upon stimulation, the current knowledge about their localization is very limited. Several recent studies presented new information on the mechanism of nuclear translocation of ERK1/2 and ERK5, which seems to be distinct from the canonical NLS-dependent shuttling of housekeeping proteins. In this section we describe the canonical NLS-dependent, as well as the ERK1/2 and ERK5 mechanisms of translocation; explain the differences between them; and discuss their role as an unexpected layer of regulation in stimulated cellular processes.

5.1. The canonical, NLS/importin-α/β-dependent, mechanism of nuclear shuttling

As mentioned above, shuttling of molecules through the nuclear envelope occurs via specialized nuclear pore complexes (NPC) that coordinate nucleo-cytoplasmic exchange [113]. Each NPC complex allows a relatively free transport in and out of the nucleus of ions, metabolites, and small macromolecules (up to 40 kDa). These small molecules usually shuttle via the NUPs by passive diffusion, whereas big proteins and RNA must utilize specific and active transportation mechanisms for this purpose. The first step of active transport of macromolecules from the cytoplasm to the nucleus through NPCs is their interaction with special shuttling transport factors — karyopherins (importins and transportins). Proteins that need to be transported (cargo proteins) include newly translated nuclear proteins upon their release from the ribosomes, or signaling proteins, which undergo acute nuclear translocation upon cellular stimulation. The best-known mechanism of translocation of many of these proteins is based on their interaction with importin-α/β that rapidly escort them into the nucleus. This interaction is usually mediated by a specific nuclear localization signal (NLS) within the sequence of the cargo proteins [194].

NLS is usually composed of a cluster of 4 or more basic amino acids (e.g. PKKKRKV in SV40 large T-antigen [195]), but in many cases contains even an additional separated basic cluster (e.g. KR [PAATKKAGQA]KKKK in nucleoplasmin [196]), which turn it to a "bipartite basic NLS". Other non-canonical NLSs were identified as well [197], but their mechanism of action is still unclear. The various NLSs bind to a specific sequence in importin-α, which in turn recruits importin-β to the complex. The role of importin-β is to interact with NUPs within the NPC and allow the proper sliding of the transporting complex through the pores [198]. Subsequently, the complex is dissociated at the nuclear face by the small GTPase (Ran) that is localized at this site in its GTP bound form, which allows its action on the transported complex. The dissociated importins are then exported from the nucleus by the CRM1 export machinery [101], while the dissociated cargo is freed to execute its nuclear functions. Thus, this classical mechanism that shuttles many proteins into the nucleus is important for the proper localization of most house-keeping proteins, but is responsible for the stimulated nuclear shuttling of only few signaling proteins including NFkB [199] and ERK5 [65] (see below). Most signaling molecules do not contain the canonical NLS, and their mechanism of translocation is currently under investigation. Export of proteins out of the nucleus is mediated by a unique sequence termed nuclear export signal (NES [200]) that interacts with exportins such as CRM1 [101,102]. This induces an efficient nuclear export of the interacting proteins to the cytoplasm.

5.2. Mechanism of ERK5 translocation into the nucleus

Most of the MAPKs do not contain the canonical NLS in their sequence, and therefore, are likely to use a specialized mechanism for their stimulus-dependent translocation. However, ERK5 does contain two NLSs in its C-terminal non-kinase domain, which might be used for the canonical mode of nuclear shuttling. As mentioned above, although ERK5 is localized in the nucleus of many cells, there are systems in which it is localized in the cytoplasm and translocates into the nucleus upon stimulation. This translocation requires phosphorylation by the upstream MEK5, which is essential not only for the activation of ERK5, but also for conformational changes that allow the nuclear accumulation [193]. Interestingly, aside from its NLSs, ERK5 contains one NES in its C-terminal domain, which seems to play an important role in the subcellular localization of the kinase. In resting cells, ERK5 is folded in such a way that its N-terminal domain is tightly bound to the C-terminal part. This folding exposes both the NES and the NLSs of the ERK5, but since the CRM1 system that interacts with the NES is more potent than NLS/importin α/β activity, the net result of this folding is a predominant cytoplasmic localization. After stimulation, phosphorylation of the TEY motif in the kinase domain of the ERK5 disrupts the intramolecular interaction and hinders the NES domain, thus preventing ERK5 interaction with the nuclear export machinery. As a result, ERK5 translocates into the nucleus due to its NLSs, which continue to be exposed [193]. Thus, cytoplasmic ERK5 utilizes the canonical NLS/importin-α/β for its translocation, whereas in cells where MEK5 and ERK5 are constitutively nuclear, their activation is induced by translocation of active MEKK2/3 [190]. These MAP3Ks' sequence does not contain a canonical NLS, and therefore, its mechanism of translocation is not clear. Mechanisms of NLS/importin-α/β-independent translocation are described below, and it is possible that one or two of them are used by MEKK2/3 for their stimulated translocation.

5.3. NLS-independent translocation mechanisms

Unlike the NLS-containing components, many signaling proteins translocate to the nucleus shortly after stimulation using distinct, importin-α/β-independent mechanisms. Due to the rapid nuclear translocation and the size of most proteins (>40 kDa), it is unlikely that the translocation of these proteins is mediated by a passive diffusion. Previous studies on nuclear translocation of signaling proteins suggested various possible NLS-independent mechanisms, which were later suggested to be also important for the translocation of MAPKs under various conditions. One such signaling protein is PKA, which transmits signals of many hormones, and thereby, regulates metabolism, differentiation and other cellular processes [201]. The catalytic subunit of PKA was shown to accumulate in the nucleus shortly after stimulation, and such a rapid translocation suggests that PKA uses different mechanism in addition to the passive diffusion that had been previously suggested [202]. Another type of signaling molecules that translocate to the nucleus upon stimulation is signal transducers and activators of transcription (STATs), which are localized in the cytoplasm of resting cells. Upon stimulation, STATs undergo phosphorylation by the protein tyrosine kinase JAK, allowing them to translocate to the nucleus, where they regulate gene expression [203]. Since STATs do not contain active canonical NLS motif, the mechanism of their nuclear translocation is not clear. One suggested mechanism is that STAT1 and STAT2 dimerize upon stimulation, and this dimerization creates a structural NLS motif by bringing together residues from both proteins [204]. It has also been suggested that NLS-containing signaling proteins other than importin-α/β escort STATs to the nucleus upon stimulation [205,206]. A third option is that STATs interact by themselves with NUPs, and therefore, do not require the assistance of importins for the translocation (as suggested for SMADs [207], β-catenin [208] and possibly other signaling components [209]). However, none of the mechanisms, for any of these proteins, was properly verified, and other mechanisms are likely to exist as well.

5.4. Studies on the translocation of ERK1/2 into the nucleus

In addition to the above-mentioned signaling molecules, ERK1/2, as well as MEK1/2 translocate into the nucleus in an NLS- and importin-α/β-independent manner [7,9]. The onset of translocation of these molecules is relatively fast (5–15 min after stimulation [130,136,137]), and always transient. However, although ERK1/2 molecules stay in the nucleus from several minutes to hours, MEK1/2 are exported from this location much faster, due to their N-terminal NES [133]. As mentioned above, the translocation of both ERK1/2 and MEK1/2 into the nucleus was shown to be essential for proliferation and probably other physiological functions of the ERK1/2 cascade [79,138]. This importance of nuclear ERK1/2 in proliferation prompted many studies on the molecular mechanism of their nuclear shuttling. In these studies it was shown that ERK1/2 are localized in the cytoplasm of resting cells due to interaction with anchoring proteins, such as microtubules [210], MEK1, [134,135], phosphatases [211], and others [160]. Upon stimulation, most ERK1/2 molecules are detached from their cytoplasmic anchors [212] and rapidly translocate into the nucleus, either by passive diffusion or by active transport [213]. The active transport was initially suggested to involve homo-dimerization of the ERK1/2 molecules [213,214]. However, these findings were later disputed [212,215], and currently the dimerization of ERK1/2 is thought to be essential for cytoplasmic signaling upon scaffold interactions [216].

Further studies claimed that despite the lack of NLS, ERK1/2 translocation might be aided in mammalian cells by components of the canonical nuclear translocation machinery [213], and in *Drosophila*, by D-Importin-7 (DIM-7) plus importin-β [217]. It was also shown that the translocation might be calcium dependent, which suggests that the mechanism of translocation might be dependent, at least in part, on calcium immobilization [218]. Another, more recent, suggestion for a possible translocation mechanism was that, similar to the possible translocation of β-catenin mentioned above, ERK1/2 may translocate to the nucleus by a direct interaction with nuclear pore proteins [117,118]. However, some of these suggestions were not rigorously verified, and others turned out to be specific to only a few cell lines. These uncertainties, as well as mutational analysis, prompted the suggestions that ERK1/2 translocate into the nucleus by multiple mechanisms, each of which might act in different cell lines or upon various stimulations [219,220].

5.5. The NTS/importin7 mechanism of ERK1/2 translocation

In order to elucidate the mechanism(s) of ERK1/2 translocation into the nucleus, our group mutated residues in the kinase insert domain (KID) of ERK2, which had previously been implicated in the regulation of its subcellular localization [220]. This study identified a critical sequence for the stimulated, as well as non-stimulated, ERK1/2 translocation [9,120]. The sequence contained two Ser residues separated by a Pro (Ser-Pro-Ser; SPS; 244–246 of ERK2) that, when mutated to Ala or deleted, prevented the translocation. The identification of phosphorylatable residues within an area that may serve as a consensus phosphorylation site for several kinases, suggested that the region might be phosphorylated in order to execute its role in nuclear translocation. Mass spectroscopic analysis, as well as anti p-SPS antibody that was raised, confirmed that this site is indeed phosphorylated upon stimulation. As expected, phosphomimetic mutations of the two Ser residues resulted in accelerated nuclear translocation, and thereby verified the importance of the Ser-Pro-Ser phosphorylation for the translocation process. It should be noted that the phosphorylation of Ser246

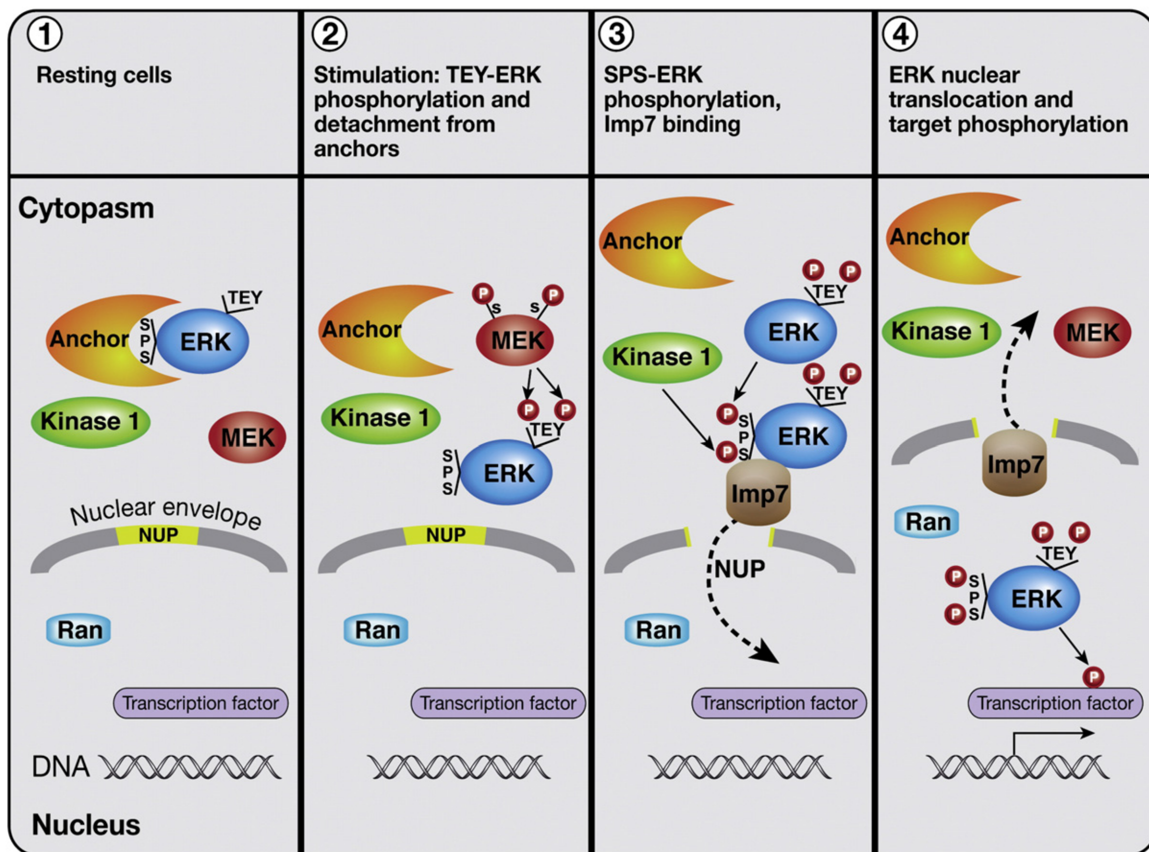

Fig. 2. The mechanism of ERK1/2 translocation into the nucleus. 1) In resting cells, ERK1/2 are retained in the cytoplasm by specific anchors. 2) Cellular stimulation results in phosphorylation of the TEY motif of ERK1/2 by active MEK1/2. This leads to detachment of ERK1/2 from the cytoplasmic anchors and exposure of SPS motif. 3) The two Ser residues of the ERK1/2's NTS are phosphorylated, by an unknown kinase (kinase 1) and probably also by independent, activated ERK1/2. This phosphorylation of the NTS allows the binding of ERK1/2 to importin7 and transportation of the complex into the nucleus. 4) Importin7 is detached from ERK1/2 by Ran-GTP, followed by its export back to the cytoplasm. The free ERK1/2 are then able to interact with their nuclear substrates to activate various processes.

alone drove the translocation, albeit at a slower rate. Phosphorylation of Ser244 alone had only a minor influence, but the phosphorylation of both residues together induced the full rate of translocation upon stimulation. The KID region was shown to act autonomously in mediating nuclear translocation of ERK1/2, as well as other proteins. This was shown by ligating the 19 amino acids of ERK1/2's KID to β-galactodidase-GFP construct (~145 kDa), or to a chimera of 2GFP (~55 kDa). Only a small portion of this large protein was detected in the nuclei of transfected cells, which was probably mediated by endogenous basal phosphorylation, and this shuttle was facilitated by stimulation. The attachment of non-phosphorylatible 19 amino-acid stretch prevented the translocation, whereas phosphomimetic mutants accelerated it without any further effect upon cellular stimulation. These findings led to the conclusion that the KID-ERK1/2 domain is indeed a nuclear translocation signal (NTS), and is likely to have a general role in the translocation of other proteins as well. Indeed, we found that the translocated MEK1 and SMAD3, which do not contain an NLS, do have a similar NTS, and as expected, this sequence was found to be important for their nuclear translocation. However, it should be noted that not all translocated proteins contain this sequence, indicating the existence of additional NTSs.

In order to investigate the involvement of importins in the process of ERK nuclear translocation, we used Si-RNA of most of these nuclear shuttlers. We found that only the Si-RNA of importin7 reduced both non-stimulated and stimulated nuclear translocation of ERK1/2. Moreover, we found that importin7 directly interacts with ERK1/2, and this interaction is enhanced upon mitogenic stimulation dependent on SPS phosphorylation. We also found that ERK1/2 can interact with certain NUPs, and that this interaction is actually prevented by SPS phosphorylation. In view of the above, we proposed a model (Fig. 2) in which cellular stimulation induces first the phosphorylation of the regulatory TEY domain to allow ERK1/2 activation and detachment from anchor proteins. The free active ERK1/2 are then phosphorylated on their SPS domain by several kinases, which may include autophosphorylation by activated ERK1/2. This phosphorylation allows interaction between ERK1/2 and importin7, and this complex then translocates into the nucleus via the nuclear pores, where the phosphorylation is important for proper sliding. Importin7 then detaches from ERK1/2 and is exported back to the cytoplasm, while the ERK1/2 themselves remain free to execute their nuclear functions.

As mentioned above, one of the unique components that participate in ERK1/2's translocation is importin7, which is a member of a group of karyopherins that were recently identified as possible regulators of shuttling processes [221]. The role of different importins in the nuclear translocation of NLS-deficient signaling proteins seems to be very important, but poorly investigated. It has been recently reported that both TGFβ-induced and basal state import of Smad4 require importins 7 and 8 [222]. Other authors demonstrated that in digitonin-permeabilized cells, transportin1, importin7, and importin9 promote efficient import of c-Jun into the nucleus [223]. These results raise the possibility that the role of different importins other than importin-α/β, is to mediate nuclear translocation of various NLS-lacking signaling proteins, including the MAPK cascade components. This may involve a network of signaling protein/importin interactions that may add yet another facet to the complex mechanisms that govern nuclear processes, mainly after cellular stimulations.

6. Summary

The translocation of components of the MAPK cascades into the nucleus is an important step in the regulation of the large number of cellular processes governed by these central signaling pathways. The main components that undergo nuclear translocation are the MAPK, ERK1/2, JNK, p38 and ERK5, although other components including MEK1, MEK2, MEK5, MEKK2/3 and several MAPKAPKs were reported to localize in the nucleus as well. The translocation of the variety of components into the nucleus plays a role in the regulation of a variety of nuclear processes that are essential for the induction of many stimulated cellular functions. The best-studied nuclear process is the regulation of transcription, which is mainly mediated by the phosphorylation and activation of many transcription factors. Interestingly, ERK1/2 and ERK5 were reported to bind DNA, and thereby act as transcriptional repressors or transcription factors of some genes. In the nucleus, the MAPKs also participate in the regulation of chromatin remodeling, which is essential for transcription factors' binding to proper promoters. Finally, MAPKs were reported to regulate the nuclear localization of regulatory proteins, to induce nuclear-dependent restriction points of the cell cycle, and to modulate the activity of nuclear receptors. These processes cooperate in order to maintain the delicate regulatory machinery essential for the induction of most stimulated cellular functions.

Although much information is currently available on the MAPK-regulated nuclear processes, not much is known on the mechanisms of nuclear shuttling of the MAPK components. Aside from ERK5, these components do not contain a canonical NLS, and therefore, their stimulated nuclear translocation is likely to be regulated by unique translocation mechanisms. Recently, our group identified an NTS, localized in the KID of ERK1/2, and showed that phosphorylation of two Ser residues in this location is essential for the translocation. We further demonstrated that this phosphorylation allows the binding of ERK1/2 with importin7, facilitating the penetration through nuclear pores and further into the nucleus. This mode of regulation seems to be common to some other translocating proteins, such as SMAD3 and MEK1. However, many other proteins that translocate into the nucleus upon stimulation do not contain the same sequences, and therefore, are likely to utilize other mechanisms for their translocation. In this regard, it is likely that other regulatory proteins, including importin3-13 and RanBPs play an important role in the regulations of these shuttling processes. The mode of translocation of signaling proteins after stimulation deserves further investigation, as it may emerge as an important regulatory step in various cellular processes. As such, this translocation may serve as a target for therapeutic intervention in signaling-related diseases, such as cancer, inflammation and diabetes.

Acknowledgements

This work was supported by grants from the Israeli Science Foundation, and MINERVA. R.S. is an incumbent of the Yale Lewine and Ella Miller Lewine — professorial chair for cancer research.

References

[1] Y.D. Shaul, R. Seger, The MEK/ERK cascade: from signaling specificity to diverse functions, Biochim. Biophys. Acta 1773 (2007) 1213–1226.
[2] M. Raman, W. Chen, M.H. Cobb, Differential regulation and properties of MAPKs, Oncogene 26 (2007) 3100–3112.
[3] G. Pimienta, J. Pascual, Canonical and alternative MAPK signaling, Cell Cycle 6 (2007) 2628–2632.
[4] P. Coulombe, S. Meloche, Atypical mitogen-activated protein kinases: structure, regulation and functions, Biochim. Biophys. Acta 1773 (2007) 1376–1387.
[5] S. Yoon, R. Seger, The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions, Growth Factors 24 (2006) 21–44.
[6] A.G. Turjanski, J.P. Vaque, J.S. Gutkind, MAP kinases and the control of nuclear events, Oncogene 26 (2007) 3240–3253.
[7] Z. Yao, R. Seger, The ERK signaling cascade — views from different subcellular compartments, Biofactors 35 (2009) 407–416.
[8] A.J. Whitmarsh, Regulation of gene transcription by mitogen-activated protein kinase signaling pathways, Biochim. Biophys. Acta 1773 (2007) 1285–1298.
[9] E. Zehorai, Z. Yao, A. Plotnikov, R. Seger, The subcellular localization of MEK and ERK-A novel nuclear translocation signal (NTS) paves a way to the nucleus, Mol. Cell. Endocrinol. 314 (2010) 213–220.
[10] A. Clark, J. Dean, C. Tudor, J. Saklatvala, Post-transcriptional gene regulation by MAP kinases via AU-rich elements, Front. Biosci. 14 (2009) 847–871.
[11] R. Seger, E.G. Krebs, The MAPK signaling cascade, FASEB J. 9 (1995) 726–735.
[12] T. Niault, M. Baccarini, Targets of Raf in tumorigenesis, Carcinogenesis 7 (2010) 1165–1174.
[13] W. Kolch, G. Heidecker, G. Kochs, R. Hummel, H. Vahidi, H. Mischak, G. Finkenzeller, D. Marme, U.R. Rapp, Protein kinase C alpha activates RAF-1 by direct phosphorylation, Nature 364 (1993) 249–252.
[14] D.N. Chadee, J.M. Kyriakis, MLK3 is required for mitogen activation of B-Raf, ERK and cell proliferation, Nat. Cell Biol. 6 (2004) 770–776, Epub 2004 Jul 2018.
[15] Y.D. Shaul, G. Gibor, A. Plotnikov, R. Seger, Specific phosphorylation and activation of ERK1c by MEK1b: a unique route in the ERK cascade, Genes Dev. 23 (2009) 1779–1790.
[16] R. Seger, Y. Biener, R. Feinstein, T. Hanoch, A. Gazit, Y. Zick, Differential activation of mitogen-activated protein kinase and S6 kinase signaling pathways by 12-O-tetradecanoylphorbol-13-acetate (TPA) and insulin. Evidence for involvement of a TPA-stimulated protein-tyrosine kinase, J. Biol. Chem. 270 (1995) 28325–28330.
[17] Y. Yung, Z. Yao, T. Hanoch, R. Seger, ERK1b, a 46-kDa ERK isoform that is differentially regulated by MEK, J. Biol. Chem. 275 (2000) 15799–15808.
[18] D.M. Aebersold, Y.D. Shaul, Y. Yung, N. Yarom, Z. Yao, T. Hanoch, R. Seger, Extracellular signal-regulated kinase 1c (ERK1c), a novel 42-kilodalton ERK, demonstrates unique modes of regulation, localization, and function, Mol. Cell. Biol. 24 (2004) 10000–10015.
[19] D.K. Pokholok, J. Zeitlinger, N.M. Hannett, D.B. Reynolds, R.A. Young, Activated signal transduction kinases frequently occupy target genes, Science 313 (2006) 533–536.
[20] S. Hu, Z. Xie, A. Onishi, X. Yu, L. Jiang, J. Lin, H.S. Rho, C. Woodard, H. Wang, J.S. Jeong, S. Long, X. He, H. Wade, S. Blackshaw, J. Qian, H. Zhu, Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling, Cell 139 (2009) 610–622.
[21] E.K. Kim, E.J. Choi, Pathological roles of MAPK signaling pathways in human diseases, Biochim. Biophys. Acta 1802 (2010) 396–405.
[22] W.E. Tidyman, K.A. Rauen, The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation, Curr. Opin. Genet. Dev. 19 (2009) 230–236.
[23] J.F. Tanti, J. Jager, Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation, Curr. Opin. Pharmacol. 9 (2009) 753–762.
[24] A.S. Dhillon, S. Hagan, O. Rath, W. Kolch, MAP kinase signalling pathways in cancer, Oncogene 26 (2007) 3279–3290.
[25] C. Montagut, J. Settleman, Targeting the RAF-MEK-ERK pathway in cancer therapy, Cancer Lett. 283 (2009) 125–134.
[26] R.J. Davis, MAPKs: new JNK expands the group, Trends Biochem. Sci. 19 (1994) 470–473.
[27] G.L. Johnson, K. Nakamura, The c-jun kinase/stress-activated pathway: regulation, function and role in human disease, Biochim. Biophys. Acta 1773 (2007) 1341–1348.
[28] C.R. Weston, R.J. Davis, The JNK signal transduction pathway, Curr. Opin. Cell Biol. 19 (2007) 142–149.
[29] M.A. Bogoyevitch, K.R. Ngoei, T.T. Zhao, Y.Y. Yeap, D.C. Ng, c-Jun N-terminal kinase (JNK) signaling: recent advances and challenges, Biochim. Biophys. Acta 1804 (2010) 463–475.
[30] J.R. Bradley, J.S. Pober, Tumor necrosis factor receptor-associated factors (TRAFs), Oncogene 20 (2001) 6482–6491.
[31] I. Dan, N.M. Watanabe, A. Kusumi, The Ste20 group kinases as regulators of MAP kinase cascades, Trends Cell Biol. 11 (2001) 220–230.
[32] E.A. Craig, M.V. Stevens, R.R. Vaillancourt, T.D. Camenisch, MAP3Ks as central regulators of cell fate during development, Dev. Dyn. 237 (2008) 3102–3114.
[33] A.J. Whitmarsh, The JIP family of MAPK scaffold proteins, Biochem. Soc. Trans. 34 (2006) 828–832.
[34] X. Wang, A. Destrument, C. Tournier, Physiological roles of MKK4 and MKK7: insights from animal models, Biochim. Biophys. Acta 1773 (2007) 1349–1357.
[35] W. Bi, L. Xiao, Y. Jia, J. Wu, Q. Xie, J. Ren, G. Ji, Z. Yuan, N-terminal kinase enhances MST1-mediated pro-apoptotic signaling through phosphorylation at serine 82, J. Biol. Chem. 285 (2010) 6259–6264.
[36] S. Ludwig, K. Engel, A. Hoffmeyer, G. Sithanandam, B. Neufeld, D. Palm, M. Gaestel, U.R. Rapp, 3pK, a novel mitogen-activated protein (MAP) kinase-activated protein kinase, is targeted by three MAP kinase pathways, Mol. Cell. Biol. 16 (1996) 6687–6697.
[37] D.N. Dhanasekaran, E.P. Reddy, JNK signaling in apoptosis, Oncogene 27 (2008) 6245–6251.
[38] M. Rincon, R.J. Davis, Regulation of the immune response by stress-activated protein kinases, Immunol. Rev. 228 (2009) 212–224.
[39] W. Haeusgen, R. Boehm, Y. Zhao, T. Herdegen, V. Waetzig, Specific activities of individual c-Jun N-terminal kinases in the brain, Neuroscience 161 (2009) 951–959.
[40] A. Gdalyahu, I. Ghosh, T. Levy, T. Sapir, S. Sapoznik, Y. Fishler, D. Azoulai, O. Reiner, DCX, a new mediator of the JNK pathway, EMBO J. 23 (2004) 823–832.
[41] K. Zhang, R.J. Kaufman, From endoplasmic-reticulum stress to the inflammatory response, Nature 454 (2008) 455–462.

[42] E.F. Wagner, A.R. Nebreda, Signal integration by JNK and p38 MAPK pathways in cancer development, Nat. Rev. Cancer 9 (2009) 537–549.
[43] J.M. Kyriakis, J. Avruch, Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation, Physiol. Rev. 81 (2001) 807–869.
[44] T. Zarubin, J. Han, Activation and signaling of the p38 MAP kinase pathway, Cell Res. 15 (2005) 11–18.
[45] L.R. Coulthard, D.E. White, D.L. Jones, M.F. McDermott, S.A. Burchill, p38(MAPK): stress responses from molecular mechanisms to therapeutics, Trends Mol. Med. 15 (2009) 369–379.
[46] D.K. Morrison, R.J. Davis, Regulation of MAP kinase signaling modules by scaffold proteins in mammals, Annu. Rev. Cell Dev. Biol. 19 (2003) 91–118.
[47] B.D. Cuevas, A.N. Abell, G.L. Johnson, Role of mitogen-activated protein kinase kinase kinases in signal integration, Oncogene 26 (2007) 3159–3171.
[48] H. Rubinfeld, R. Seger, The ERK cascade: a prototype of MAPK signaling, Mol. Biotechnol. 31 (2005) 151–174.
[49] A.J. Whitmarsh, R.J. Davis, Role of mitogen-activated protein kinase kinase 4 in cancer, Oncogene 26 (2007) 3172–3184.
[50] B. Ge, H. Gram, F. Di Padova, B. Huang, L. New, R.J. Ulevitch, Y. Luo, J. Han, MAPKK-independent activation of p38alpha mediated by TAB1-dependent autophosphorylation of p38alpha, Science 295 (2002) 1291–1294.
[51] J.M. Salvador, P.R. Mittelstadt, T. Guszczynski, T.D. Copeland, H. Yamaguchi, E. Appella, A.J. Fornace Jr., J.D. Ashwell, Alternative p38 activation pathway mediated by T cell receptor-proximal tyrosine kinases, Nat. Immunol. 6 (2005) 390–395.
[52] J.J. Gills, S.S. Castillo, C. Zhang, P.A. Petukhov, R.M. Memmott, M. Hollingshead, N. Warfel, J. Han, A.P. Kozikowski, P.A. Dennis, Phosphatidylinositol ether lipid analogues that inhibit AKT also independently activate the stress kinase, p38alpha, through MKK3/6-independent and -dependent mechanisms, J. Biol. Chem. 282 (2007) 27020–27029.
[53] A. Shiryaev, U. Moens, Mitogen-activated protein kinase p38 and MK2, MK3 and MK5: menage a trois or menage a quatre? Cell. Signal. 22 (2010) 1185–1192.
[54] M. Buxade, J.L. Parra-Palau, C.G. Proud, The Mnks: MAP kinase-interacting kinases (MAP kinase signal-integrating kinases), Front. Biosci. 13 (2008) 5359–5373.
[55] L. Vermeulen, W.V. Berghe, I.M. Beck, K. De Bosscher, G. Haegeman, The versatile role of MSKs in transcriptional regulation, Trends Biochem. Sci. 34 (2009) 311–318.
[56] G. Huang, L.Z. Shi, H. Chi, Regulation of JNK and p38 MAPK in the immune system: signal integration, propagation and termination, Cytokine 48 (2009) 161–169.
[57] S.J. Sohn, J. Thompson, A. Winoto, Apoptosis during negative selection of autoreactive thymocytes, Curr. Opin. Immunol. 19 (2007) 510–515.
[58] J. Maruyama, I. Naguro, K. Takeda, H. Ichijo, Stress-activated MAP kinase cascades in cellular senescence, Curr. Med. Chem. 16 (2009) 1229–1235.
[59] T.M. Thornton, M. Rincon, Non-classical p38 map kinase functions: cell cycle checkpoints and survival, Int. J. Biol. Sci. 5 (2009) 44–51.
[60] A. Cuenda, S. Rousseau, p38 MAP-kinases pathway regulation, function and role in human diseases, Biochim. Biophys. Acta 1773 (2007) 1358–1375.
[61] G.L. Schieven, The p38alpha kinase plays a central role in inflammation, Curr. Top. Med. Chem. 9 (2009) 1038–1048.
[62] Z. Liu, W. Cao, p38 mitogen-activated protein kinase: a critical node linking insulin resistance and cardiovascular diseases in type 2 diabetes mellitus, Endocr. Metab. Immune Disord. Drug Targets 9 (2009) 38–46.
[63] M. Loesch, G. Chen, The p38 MAPK stress pathway as a tumor suppressor or more? Front. Biosci. 13 (2008) 3581–3593.
[64] J. Moscat, M.T. Diaz-Meco, A. Albert, S. Campuzano, Cell signaling and function organized by PB1 domain interactions, Mol. Cell 23 (2006) 631–640.
[65] S. Nishimoto, E. Nishida, MAPK signalling: ERK5 versus ERK1/2, EMBO Rep. 7 (2006) 782–786.
[66] X. Wang, C. Tournier, Regulation of cellular functions by the ERK5 signalling pathway, Cell. Signal. 18 (2006) 753–760.
[67] W. Sun, K. Kesavan, B.C. Schaefer, T.P. Garrington, M. Ware, N.L. Johnson, E.W. Gelfand, G.L. Johnson, MEKK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway, J. Biol. Chem. 276 (2001) 5093–5100.
[68] B.E. Xu, S. Stippec, L. Lenertz, B.H. Lee, W. Zhang, Y.K. Lee, M.H. Cobb, WNK1 activates ERK5 by an MEKK2/3-dependent mechanism, J. Biol. Chem. 279 (2004) 7826–7831.
[69] T.H. Chao, M. Hayashi, R.I. Tapping, Y. Kato, J.D. Lee, MEKK3 directly regulates MEK5 activity as part of the big mitogen-activated protein kinase 1 (BMK1) signaling pathway, J. Biol. Chem. 274 (1999) 36035–36038.
[70] M. Chiariello, M.J. Marinissen, J.S. Gutkind, Multiple mitogen-activated protein kinase signaling pathways connect the cot oncoprotein to the c-jun promoter and to cellular transformation, Mol. Cell. Biol. 20 (2000) 1747–1758.
[71] M. Hayashi, R.I. Tapping, T.H. Chao, J.F. Lo, C.C. King, Y. Yang, J.D. Lee, BMK1 mediates growth factor-induced cell proliferation through direct cellular activation of serum and glucocorticoid-inducible kinase, J. Biol. Chem. 276 (2001) 8631–8634.
[72] H.G. Kasler, J. Victoria, O. Duramad, A. Winoto, ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain, Mol. Cell. Biol. 20 (2000) 8382–8389.
[73] O.L. Roberts, K. Holmes, J. Muller, D.A. Cross, M.J. Cross, ERK5 and the regulation of endothelial cell function, Biochem. Soc. Trans. 37 (2009) 1254–1259.
[74] S.J. Sohn, G.M. Lewis, A. Winoto, Non-redundant function of the MEK5-ERK5 pathway in thymocyte apoptosis, EMBO J. 27 (2008) 1896–1906.
[75] C. Yan, M. Takahashi, M. Okuda, J.D. Lee, B.C. Berk, Fluid shear stress stimulates big mitogen-activated protein kinase 1 (BMK1) activity in endothelial cells. Dependence on tyrosine kinases and intracellular calcium, J. Biol. Chem. 274 (1999) 143–150.
[76] J. Whyte, O. Bergin, A. Bianchi, S. McNally, F. Martin, Key signalling nodes in mammary gland development and cancer. Mitogen-activated protein kinase signalling in experimental models of breast cancer progression and in mammary gland development, Breast Cancer Res. 11 (2009) 209.
[77] Q. Yang, X. Deng, B. Lu, M. Cameron, C. Fearns, M.P. Patricelli, J.R. Yates 3rd, N.S. Gray, J.D. Lee, Pharmacological inhibition of BMK1 suppresses tumor growth through promyelocytic leukemia protein, Cancer Cell 18 (2010) 258–267.
[78] A. Brunet, D. Roux, P. Lenormand, S. Dowd, S. Keyse, J. Pouyssegur, Nuclear translocation of p42/p44 mitogen-activated protein kinase is required for growth factor-induced gene expression and cell cycle entry, EMBO J. 18 (1999) 664–674.
[79] E. Formstecher, J.W. Ramos, M. Fauquet, D.A. Calderwood, J.C. Hsieh, B. Canton, X.T. Nguyen, J.V. Barnier, J. Camonis, M.H. Ginsberg, H. Chneiweiss, PEA-15 mediates cytoplasmic sequestration of ERK MAP kinase, Dev. Cell 1 (2001) 239–250.
[80] M. Tresini, A. Lorenzini, C. Torres, V.J. Cristofalo, Modulation of replicative senescence of diploid human cells by nuclear ERK signaling, J. Biol. Chem. 282 (2007) 4136–4151.
[81] B. Bjorkblom, J.C. Vainio, V. Hongisto, T. Herdegen, M.J. Courtney, E.T. Coffey, All JNKs can kill, but nuclear localization is critical for neuronal death, J. Biol. Chem. 283 (2008) 19704–19713.
[82] C.A. Hazzalin, E. Cano, A. Cuenda, M.J. Barratt, P. Cohen, L.C. Mahadevan, p38/RK is essential for stress-induced nuclear responses: JNK/SAPKs and c-Jun/ATF-2 phosphorylation are insufficient, Curr. Biol. 6 (1996) 1028–1031.
[83] H. Daub, J.V. Olsen, M. Bairlein, F. Gnad, F.S. Oppermann, R. Korner, Z. Greff, G. Keri, O. Stemmann, M. Mann, Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle, Mol. Cell 31 (2008) 438–448.
[84] B. Casar, A. Pinto, P. Crespo, ERK dimers and scaffold proteins: unexpected partners for a forgotten (cytoplasmic) task, Cell Cycle 8 (2009) 1007–1013.
[85] S. Galli, O. Jahn, R. Hitt, D. Hesse, L. Opitz, U. Plessmann, H. Urlaub, J.J. Poderoso, E.A. Jares-Erijman, T.M. Jovin, A new paradigm for MAPK: structural interactions of hERK1 with mitochondria in HeLa cells, PLoS ONE 4 (2009) e7541.
[86] S. Torii, M. Kusakabe, T. Yamamoto, M. Maekawa, E. Nishida, Sef is a spatial regulator for Ras/MAP kinase signaling, Dev. Cell 7 (2004) 33–44.
[87] D. Teis, N. Taub, R. Kurzbauer, D. Hilber, M.E. de Araujo, M. Erlacher, M. Offterdinger, A. Villunger, S. Geley, G. Bohn, C. Klein, M.W. Hess, L.A. Huber, p14-MP1-MEK1 signaling regulates endosomal traffic and cellular proliferation during tissue homeostasis, J. Cell Biol. 175 (2006) 861–868.
[88] N. Fehrenbacher, D. Bar-Sagi, M. Philips, Ras/MAPK signaling from endomembranes, Mol. Oncol. 3 (2009) 297–307.
[89] H. Gille, A.D. Sharrocks, P.E. Shaw, Phosphorylation of transcription factor p62TCF by MAP kinase stimulates ternary complex formation at c-fos promoter, Nature 358 (1992) 414–417.
[90] R. Marais, J. Wynne, R. Treisman, The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain, Cell 73 (1993) 381–393.
[91] R. Treisman, Regulation of transcription by MAP kinase cascades, Curr. Opin. Cell Biol. 8 (1996) 205–215.
[92] G. Buchwalter, C. Gross, B. Wasylyk, Ets ternary complex transcription factors, Gene 324 (2004) 1–14.
[93] F.H. Cruzalegui, E. Cano, R. Treisman, ERK activation induces phosphorylation of Elk-1 at multiple S/T-P motifs to high stoichiometry, Oncogene 18 (1999) 7948–7957.
[94] A.D. Sharrocks, S.H. Yang, A. Galanis, Docking domains and substrate-specificity determination for MAP kinases, Trends Biochem. Sci. 25 (2000) 448–453.
[95] D. Jacobs, D. Glossip, H. Xing, A.J. Muslin, K. Kornfeld, Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase, Genes Dev. 13 (1999) 163–175.
[96] R. Eferl, E.F. Wagner, AP-1: a double-edged sword in tumorigenesis, Nat. Rev. Cancer 3 (2003) 859–868.
[97] L.O. Murphy, S. Smith, R.H. Chen, D.C. Fingar, J. Blenis, Molecular interpretation of ERK signal duration by immediate early gene products, Nat. Cell Biol. 4 (2002) 556–564.
[98] C.J. Marshall, Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation, Cell 80 (1995) 179–185.
[99] L.O. Murphy, J.P. MacKeigan, J. Blenis, A network of immediate early gene products propagates subtle differences in mitogen-activated protein kinase signal amplitude and duration, Mol. Cell. Biol. 24 (2004) 144–153.
[100] G. Mavrothalassitis, J. Ghysdael, Proteins of the ETS family with transcriptional repressor activity, Oncogene 19 (2000) 6524–6532.
[101] M. Fornerod, M. Ohno, M. Yoshida, I.W. Mattaj, CRM1 is an export receptor for leucine-rich nuclear export signals, Cell 90 (1997) 1051–1060.
[102] K. Stade, C.S. Ford, C. Guthrie, K. Weis, Exportin 1 (Crm1p) is an essential nuclear export factor, Cell 90 (1997) 1041–1050.
[103] D.N. Sgouras, M.A. Athanasiou, G.J. Beal Jr., R.J. Fisher, D.G. Blair, G.J. Mavrothalassitis, ERF: an ETS domain protein with strong transcriptional repressor activity, can suppress ETS-associated tumorigenesis and is regulated by phosphorylation during cell cycle and mitogenic stimulation, EMBO J. 14 (1995) 4781–4793.
[104] S. Polychronopoulos, M. Verykokakis, M.N. Yazicioglu, M. Sakarellos-Daitsiotis, M.H. Cobb, G. Mavrothalassitis, The transcriptional ETS2 repressor factor associates with active and inactive Erks through distinct FXF motifs, J. Biol. Chem. 281 (2006) 25601–25611.

[105] L. Le Gallic, L. Virgilio, P. Cohen, B. Biteau, G. Mavrothalassitis, ERF nuclear shuttling, a continuous monitor of Erk activity that links it to cell cycle progression, Mol. Cell. Biol. 24 (2004) 1206–1218.
[106] K. Brami-Cherrier, E. Roze, J.A. Girault, S. Betuing, J. Caboche, Role of the ERK/MSK1 signalling pathway in chromatin remodelling and brain responses to drugs of abuse, J. Neurochem. 108 (2009) 1323–1335.
[107] S.P. Zhong, W.Y. Ma, Z. Dong, ERKs and p38 kinases mediate ultraviolet B-induced phosphorylation of histone H3 at serine 10, J. Biol. Chem. 275 (2000) 20980–20984.
[108] P.S. Rao, A. Satelli, S. Zhang, S.K. Srivastava, K.S. Srivenugopal, U.S. Rao, RNF2 is the target for phosphorylation by the p38 MAPK and ERK signaling pathways, Proteomics 9 (2009) 2776–2787.
[109] X. Zhou, V. Richon, A. Wang, X. Yang, R. Rifkind, P. Marks, Histone deacetylase 4 associates with extracellular signal-regulated, Proc. Natl Acad. Sci. USA 97 (2000) 14329–14333.
[110] A. Soloaga, S. Thomson, G.R. Wiggin, N. Rampersaud, M.H. Dyson, C.A. Hazzalin, L.C. Mahadevan, J.S. Arthur, MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14, EMBO J. 22 (2003) 2788–2797.
[111] M. Cohen-Armon, L. Visochek, D. Rozensal, A. Kalal, I. Geistrikh, R. Klein, S. Bendetz-Nezer, Z. Yao, R. Seger, DNA-independent PARP-1 activation by phosphorylated ERK2 increases Elk1 activity: a link to histone acetylation, Mol. Cell 25 (2007) 297–308.
[112] P.S. Shapiro, A.M. Whalen, N.S. Tolwinski, J. Wilsbacher, S.J. Froelich-Ammon, M. Garcia, N. Osheroff, N.G. Ahn, Extracellular signal-regulated kinase activates topoisomerase IIalpha through a mechanism independent of phosphorylation, Mol. Cell. Biol. 19 (1999) 3551–3560.
[113] E.A. Nigg, Nucleocytoplasmic transport: signals, mechanisms and regulation, Nature 386 (1997) 779–787.
[114] M. Suntharalingam, S.R. Wente, Peering through the pore: nuclear pore complex structure, assembly, and function, Dev. Cell 4 (2003) 775–789.
[115] L.J. Terry, S.R. Wente, Flexible gates: dynamic topologies and functions for FG nucleoporins in nucleocytoplasmic transport, Eukaryot. Cell 8 (2009) 1814–1827.
[116] M. Capelson, M.W. Hetzer, The role of nuclear pores in gene regulation, development and disease, EMBO Rep. 10 (2009) 697–705.
[117] Y. Matsubayashi, M. Fukuda, E. Nishida, Evidence for existence of a nuclear pore complex-mediated, cytosol-independent pathway of nuclear translocation of ERK MAP kinase in permeabilized cells, J. Biol. Chem. 276 (2001) 41755–41760.
[118] A.W. Whitehurst, J.L. Wilsbacher, Y. You, K. Luby-Phelps, M.S. Moore, M.H. Cobb, ERK2 enters the nucleus by a carrier-independent mechanism, Proc. Natl Acad. Sci. USA 99 (2002) 7496–7501.
[119] T. Lee, A.N. Hoofnagle, Y. Kabuyama, J. Stroud, X. Min, E.J. Goldsmith, L. Chen, K.A. Resing, N.G. Ahn, Docking motif interactions in MAP kinases revealed by hydrogen exchange mass spectrometry, Mol. Cell 14 (2004) 43–55.
[120] D. Chuderland, A. Konson, R. Seger, Identification and characterization of a general nuclear translocation signal in signaling proteins, Mol. Cell 31 (2008) 850–861.
[121] S.T. Eblen, N.V. Kumar, K. Shah, M.J. Henderson, C.K. Watts, K.M. Shokat, M.J. Weber, Identification of novel ERK2 substrates through use of an engineered kinase and ATP analogs, J. Biol. Chem. 278 (2003) 14926–14935.
[122] T. Vomastek, M.P. Iwanicki, W.R. Burack, D. Tiwari, D. Kumar, J.T. Parsons, M.J. Weber, V.K. Nandicoori, Extracellular signal-regulated kinase 2 (ERK2) phosphorylation sites and docking domain on the nuclear pore complex protein Tpr cooperatively regulate ERK2-Tpr interaction, Mol. Cell. Biol. 28 (2008) 6954–6966.
[123] R.S. Faustino, T.G. Maddaford, G.N. Pierce, Mitogen activated protein kinase at the nuclear pore complex, J. Cell. Mol. Med. 15 (4) (2010) 928–937.
[124] H. Kosako, N. Yamaguchi, C. Aranami, M. Ushiyama, S. Kose, N. Imamoto, H. Taniguchi, E. Nishida, S. Hattori, Phosphoproteomics reveals new ERK MAP kinase targets and links ERK to nucleoporin-mediated nuclear transport, Nat. Struct. Mol. Biol. 16 (2009) 1026–1035.
[125] S.O. Yoon, S. Shin, Y. Liu, B.A. Ballif, M.S. Woo, S.P. Gygi, J. Blenis, Ran-binding protein 3 phosphorylation links the Ras and PI3-kinase pathways to nucleocytoplasmic transport, Mol. Cell 29 (2008) 362–375.
[126] S. Kuersten, M. Ohno, I.W. Mattaj, Nucleocytoplasmic transport: Ran, beta and beyond, Trends Cell Biol. 11 (2001) 497–503.
[127] I.G. Macara, Transport into and out of the nucleus, Microbiol. Mol. Biol. Rev. 65 (2001) 570–594, table of contents.
[128] S. Bendetz-Nezer, R. Seger, Full molecular page of MEK1, AfCS/Nature Signaling Gateway (2005), doi:10.1038/mp.a001505.01.
[129] S. Bendetz-Nezer, R. Seger, Full molecular page of MEK2, AfCS/Nature Signaling Gateway (2005), doi:10.1038/mp.a001506.01.
[130] P. Lenormand, C. Sardet, G. Pages, G. L'Allemain, A. Brunet, J. Pouyssegur, Growth factors induce nuclear translocation of MAP kinases (p42mapk and p44mapk) but not of their activator MAP kinase kinase (p45mapkk) in fibroblasts, J. Cell Biol. 122 (1993) 1079–1088.
[131] C.F. Zheng, K.L. Guan, Cytoplasmic localization of the mitogen-activated protein kinase activator MEK, J. Biol. Chem. 269 (1994) 19947–19952.
[132] S. Stewart, M. Sundaram, Y. Zhang, J. Lee, M. Han, K.L. Guan, Kinase suppressor of Ras forms a multiprotein signaling complex and modulates MEK localization, Mol. Cell. Biol. 19 (1999) 5523–5534.
[133] M. Fukuda, I. Gotoh, Y. Gotoh, E. Nishida, Cytoplasmic localization of mitogen-activated protein kinase kinase directed by its NH2-terminal, leucine-rich short amino acid sequence, which acts as a nuclear export signal, J. Biol. Chem. 271 (1996) 20024–20028.
[134] M. Fukuda, I. Gotoh, M. Adachi, Y. Gotoh, E. Nishida, A novel regulatory mechanism in the mitogen-activated protein (MAP) kinase cascade. Role nuclear export signal MAP kinase kinase, J. Biol. Chem. 272 (1997) 32642–32648.
[135] H. Rubinfeld, T. Hanoch, R. Seger, Identification of a cytoplasmic-retention sequence in ERK2, J. Biol. Chem. 274 (1999) 30349–30352.
[136] H. Jaaro, H. Rubinfeld, T. Hanoch, R. Seger, Nuclear translocation of mitogen-activated protein kinase kinase (MEK1) in response to mitogenic stimulation, Proc. Natl Acad. Sci. USA 94 (1997) 3742–3747.
[137] N.S. Tolwinski, P.S. Shapiro, S. Goueli, N.G. Ahn, Nuclear localization of mitogen-activated protein kinase kinase 1 (MKK1) is promoted by serum stimulation and G2-M progression. Requirement phosphorylation activation lip signaling downstream MKK, J. Biol. Chem. 274 (1999) 6168–6174.
[138] Z. Yao, I. Flash, Z. Raviv, Y. Yung, Y. Asscher, S. Pleban, R. Seger, Non-regulated and stimulated mechanisms cooperate in the nuclear accumulation of MEK1, Oncogene 20 (2001) 7588–7596.
[139] M. Adachi, M. Fukuda, E. Nishida, Nuclear export of MAP kinase (ERK) involves a MAP kinase kinase (MEK)-dependent active transport mechanism, J. Cell Biol. 148 (2000) 849–856.
[140] E. Burgermeister, D. Chuderland, T. Hanoch, M. Meyer, M. Liscovitch, R. Seger, Interaction with MEK causes nuclear export and downregulation of peroxisome proliferator-activated receptor gamma, Mol. Cell. Biol. 27 (2007) 803–817.
[141] R.L. Perry, M.H. Parker, M.A. Rudnicki, Activated MEK1 binds the nuclear MyoD transcriptional complex to repress transactivation, Mol. Cell 8 (2001) 291–301.
[142] S.H. Yang, A.J. Whitmarsh, R.J. Davis, A.D. Sharrocks, Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1, EMBO J. 17 (1998) 1740–1749.
[143] S. Kamakura, T. Moriguchi, E. Nishida, Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus, J. Biol. Chem. 274 (1999) 26563–26571.
[144] S. Morton, R.J. Davis, A. McLaren, P. Cohen, A reinvestigation of the multisite phosphorylation of the transcription factor c-Jun, EMBO J. 22 (2003) 3876–3886.
[145] E. Alvarez, I.C. Northwood, F.A. Gonzalez, D.A. Latour, A. Seth, C. Abate, T. Curran, R.J. Davis, Pro-Leu-Ser/Thr-Pro is a consensus primary sequence for substrate protein phosphorylation. Characterization of the phosphorylation of c-myc and c-jun proteins by an epidermal growth factor receptor threonine 669 protein kinase, J. Biol. Chem. 266 (1991) 15277–15285.
[146] D. Edbauer, D. Cheng, M.N. Batterton, C.F. Wang, D.M. Duong, M.B. Yaffe, J. Peng, M. Sheng, Identification and characterization of neuronal mitogen-activated protein kinase substrates using a specific phosphomotif antibody, Mol. Cell. Proteomics 8 (2009) 681–695.
[147] D. Stokoe, B. Caudwell, P.T. Cohen, P. Cohen, The substrate specificity and structure of mitogen-activated protein (MAP) kinase-activated protein kinase-2, Biochem. J. 296 (Pt 3) (1993) 843–849.
[148] A. Donella-Deana, A. Lavoinne, O. Marin, L.A. Pinna, P. Cohen, An analysis of the substrate specificity of insulin-stimulated protein kinase-1, a mammalian homologue of S6 kinase-II, Biochim. Biophys. Acta 1178 (1993) 189–193.
[149] B. Drobic, B. Perez-Cadahia, J. Yu, S.K. Kung, J.R. Davie, Promoter chromatin remodeling of immediate-early genes is mediated through H3 phosphorylation at either serine 28 or 10 by the MSK1 multi-protein complex, Nucleic Acids Res. 38 (2010) 3196–3208.
[150] S.Y. Fuchs, V.A. Fried, Z. Ronai, Stress-activated kinases regulate protein stability, Oncogene 17 (1998) 1483–1490.
[151] I.A. Manke, A. Nguyen, D. Lim, M.Q. Stewart, A.E. Elia, M.B. Yaffe, MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation, Mol. Cell 17 (2005) 37–48.
[152] M.A. Bogoyevitch, B. Kobe, Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases, Microbiol. Mol. Biol. Rev. 70 (2006) 1061–1095.
[153] M. Hibi, A. Lin, T. Smeal, A. Minden, M. Karin, Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain, Genes Dev. 7 (1993) 2135–2148.
[154] E. Shaulian, AP-1 — The Jun proteins: oncogenes or tumor suppressors in disguise? Cell. Signal. 22 (2010) 894–899.
[155] B. Derijard, M. Hibi, I.H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, R.J. Davis, JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain, Cell 76 (1994) 1025–1027.
[156] E. Shaulian, M. Karin, AP-1 as a regulator of cell life and death, Nat. Cell Biol. 4 (2002) E131–E136.
[157] A.M. Musti, M. Treier, D. Bohmann, Reduced ubiquitin-dependent degradation of c-Jun after phosphorylation by MAP kinases, Science 275 (1997) 400–402.
[158] D. Barsyte-Lovejoy, A. Galanis, A.D. Sharrocks, Specificity determinants in MAPK signaling to transcription factors, J. Biol. Chem. 277 (2002) 9896–9903.
[159] D. Barsyte-Lovejoy, A. Galanis, A. Clancy, A.D. Sharrocks, ERK5 is targeted to myocyte enhancer factor 2A (MEF2A) through a MAPK docking motif, Biochem. J. 381 (2004) 693–699.
[160] D. Chuderland, R. Seger, Protein–protein interactions in the regulation of the extracellular signal-regulated kinase, Mol. Biotechnol. 29 (2005) 57–74.
[161] A.J. Bardwell, E. Frankson, L. Bardwell, Selectivity of docking sites in MAPK kinases, J. Biol. Chem. 284 (2009) 13165–13173.
[162] D.M. Cox, M. Du, M. Marback, E.C. Yang, J. Chan, K.W. Siu, J.C. McDermott, Phosphorylation motifs regulating the stability and function of myocyte enhancer factor 2A, J. Biol. Chem. 278 (2003) 15297–15303.
[163] E. Appella, C.W. Anderson, Post-translational modifications and activation of p53 by genotoxic stresses, Eur. J. Biochem. 268 (2001) 2764–2772.

[164] M. Oren, A. Damalas, T. Gottlieb, D. Michael, J. Taplick, J.F. Leal, R. Maya, M. Moas, R. Seger, Y. Taya, A. Ben-Ze'ev, Regulation of p53: intricate loops and delicate balances, Biochem. Pharmacol. 64 (2002) 865–871.

[165] D.M. Milne, L.E. Campbell, D.G. Campbell, D.W. Meek, p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation-induced protein kinase characteristic of the c-Jun kinase, JNK1, J. Biol. Chem. 270 (1995) 5511–5518.

[166] T. Lin, N.K. Mak, M.S. Yang, MAPK regulate p53-dependent cell death induced by benzo[a]pyrene: involvement of p53 phosphorylation and acetylation, Toxicology 247 (2008) 145–153.

[167] D.M. Milne, D.G. Campbell, F.B. Caudwell, D.W. Meek, Phosphorylation of the tumor suppressor protein p53 by mitogen-activated protein kinases, J. Biol. Chem. 269 (1994) 9253–9260.

[168] N.V. Oleinik, N.I. Krupenko, S.A. Krupenko, Cooperation between JNK1 and JNK2 in activation of p53 apoptotic pathway, Oncogene 26 (2007) 7222–7230.

[169] T.S. Eisinger-Mathason, J. Andrade, D.A. Lannigan, RSK in tumorigenesis: connections to steroid signaling, Steroids 75 (2010) 191–202.

[170] E. Burgermeister, R. Seger, MAPK kinases as nucleo-cytoplasmic shuttles for PPARgamma, Cell Cycle 6 (2007) 1539–1548.

[171] P.M. Barger, A.C. Browning, A.N. Garner, D.P. Kelly, p38 mitogen-activated protein kinase activates peroxisome proliferator-activated receptor alpha: a potential role in the cardiac metabolic stress response, J. Biol. Chem. 276 (2001) 44495–44501.

[172] M.J. Yoon, G.Y. Lee, J.J. Chung, Y.H. Ahn, S.H. Hong, J.B. Kim, Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha, Diabetes 55 (2006) 2562–2570.

[173] M. Gianni, A. Bauer, E. Garattini, P. Chambon, C. Rochette-Egly, Phosphorylation by p38MAPK and recruitment of SUG-1 are required for RA-induced RAR gamma degradation and transactivation, EMBO J. 21 (2002) 3760–3769.

[174] Z. Chai, L. Yang, B. Yu, Q. He, W.I. Li, R. Zhou, T. Zhang, X. Zheng, J. Xie, p38 mitogen-activated protein kinase-dependent regulation of SRC-3 and involvement in retinoic acid receptor alpha signaling in embryonic cortical neurons, IUBMB Life 61 (2009) 670–678.

[175] D.E. Frigo, A. Basu, E.N. Nierth-Simpson, C.B. Weldon, C.M. Dugan, S. Elliott, B.M. Collins-Burow, V.A. Salvo, Y. Zhu, L.I. Melnik, G.N. Lopez, P.J. Kushner, T.J. Curiel, B.G. Rowan, J.A. McLachlan, M.E. Burow, p38 mitogen-activated protein kinase stimulates estrogen-mediated transcription and proliferation through the phosphorylation and potentiation of the p160 coactivator glucocorticoid receptor-interacting protein 1, Mol. Endocrinol. 20 (2006) 971–983.

[176] D. Gioeli, B.E. Black, V. Gordon, A. Spencer, C.T. Kesler, S.T. Eblen, B.M. Paschal, M. J. Weber, Stress kinase signaling regulates androgen receptor phosphorylation, transcription, and localization, Mol. Endocrinol. 20 (2006) 503–515.

[177] M. Itoh, M. Adachi, H. Yasui, M. Takekawa, H. Tanaka, K. Imai, Nuclear export of glucocorticoid receptor is enhanced by c-Jun N-terminal kinase-mediated phosphorylation, Mol. Endocrinol. 16 (2002) 2382–2392.

[178] H.S. Camp, S.R. Tafuri, T. Leff, c-Jun N-terminal kinase phosphorylates peroxisome proliferator-activated receptor-gamma1 and negatively regulates its transcriptional activity, Endocrinology 140 (1999) 392–397.

[179] K.K. Mann, A.M. Padovani, Q. Guo, A.L. Colosimo, H.Y. Lee, J.M. Kurie, W.H. Miller Jr., Arsenic trioxide inhibits nuclear receptor function via SEK1/JNK-mediated RXRalpha phosphorylation, J. Clin. Invest. 115 (2005) 2924–2933.

[180] H. Srinivas, D.M. Juroske, S. Kalyankrishna, D.D. Cody, R.E. Price, X.C. Xu, R. Narayanan, N.L. Weigel, J.M. Kurie, c-Jun N-terminal kinase contributes to aberrant retinoid signaling in lung cancer cells by phosphorylating and inducing proteasomal degradation of retinoic acid receptor alpha, Mol. Cell. Biol. 25 (2005) 1054–1069.

[181] Y. Mebratu, Y. Tesfaigzi, How ERK1/2 activation controls cell proliferation and cell death is subcellular localization the answer? Cell Cycle 8 (2009) 1168–1175.

[182] P. Gomez del Arco, S. Martinez-Martinez, J.L. Maldonado, I. Ortega-Perez, J.M. Redondo, A role for the p38 MAP kinase pathway in the nuclear shuttling of NFATp, J. Biol. Chem. 275 (2000) 13872–13878.

[183] T.T. Yang, R.Y. Yu, A. Agadir, G.J. Gao, R. Campos-Gonzalez, C. Tournier, C.W. Chow, Integration of protein kinases mTOR and extracellular signal-regulated kinase 5 in regulating nucleocytoplasmic localization of NFATc4, Mol. Cell. Biol. 28 (2008) 3489–3501.

[184] S. Clavel, S. Siffroi-Fernandez, A.S. Coldefy, K. Boulukos, D.F. Pisani, B. Derijard, Regulation of the intracellular localization of Foxo3a by stress-activated protein kinase signaling pathways in skeletal muscle cells, Mol. Cell. Biol. 30 (2010) 470–480.

[185] G. Liao, Q. Tao, M. Kofron, J.S. Chen, A. Schloemer, R.J. Davis, J.C. Hsieh, C. Wylie, J. Heasman, C.Y. Kuan, Jun NH2-terminal kinase (JNK) prevents nuclear beta-catenin accumulation and regulates axis formation in *Xenopus* embryos, Proc. Natl Acad. Sci. USA 103 (2006) 16313–16318.

[186] C.G. Besirli, E.F. Wagner, E.M. Johnson Jr., The limited role of NH2-terminal c-Jun phosphorylation in neuronal apoptosis: identification of the nuclear pore complex as a potential target of the JNK pathway, J. Cell Biol. 170 (2005) 401–411.

[187] M.N. Richard, J.F. Deniset, A.L. Kneesh, D. Blackwood, G.N. Pierce, Mechanical stretching stimulates smooth muscle cell growth, nuclear protein import, and nuclear pore expression through mitogen-activated protein kinase activation, J. Biol. Chem. 282 (2007) 23081–23088.

[188] J.B. Kelley, B.M. Paschal, Hyperosmotic stress signaling to the nucleus disrupts the Ran gradient and the production of RanGTP, Mol. Biol. Cell 18 (2007) 4365–4376.

[189] H. Morimoto, K. Kondoh, S. Nishimoto, K. Terasawa, E. Nishida, Activation of a C-terminal transcriptional activation domain of ERK5 by autophosphorylation, J. Biol. Chem. (2007).

[190] Z. Raviv, E. Kalie, R. Seger, MEK5 and ERK5 are localized in the nuclei of resting as well as stimulated cells, while MEKK2 translocates from the cytosol to the nucleus upon stimulation, J. Cell Sci. 117 (2004) 1773–1784.

[191] Z. Yao, S. Yoon, E. Kalie, Z. Raviv, R. Seger, Calcium regulation of EGF-induced ERK5 activation: role of Lad1-MEKK2 interaction, PLoS ONE 5 (2010).

[192] M. Buschbeck, A. Ullrich, The unique C-terminal tail of the mitogen-activated protein kinase ERK5 regulates its activation and nuclear shuttling, J. Biol. Chem. 280 (2005) 2659–2667.

[193] K. Kondoh, K. Terasawa, H. Morimoto, E. Nishida, Regulation of nuclear translocation of extracellular signal-regulated kinase 5 by active nuclear import and export mechanisms, Mol. Cell. Biol. 26 (2006) 1679–1690.

[194] A.V. Sorokin, E.R. Kim, L.P. Ovchinnikov, Nucleocytoplasmic transport of proteins, Biochemistry (Mosc) 72 (2007) 1439–1457.

[195] D. Kalderon, B.L. Roberts, W.D. Richardson, A.E. Smith, A short amino acid sequence able to specify nuclear location, Cell 39 (1984) 499–509.

[196] C. Dingwall, J. Robbins, S.M. Dilworth, B. Roberts, W.D. Richardson, The nucleoplasmin nuclear location sequence is larger and more complex than that of SV-40 large T antigen, J. Cell Biol. 107 (1988) 841–849.

[197] D. Christophe, C. Christophe-Hobertus, B. Pichon, Nuclear targeting of proteins: how many different signals? Cell. Signal. 12 (2000) 337–341.

[198] E.J. Tran, S.R. Wente, Dynamic nuclear pore complexes: life on the edge, Cell 125 (2006) 1041–1053.

[199] B. Sun, M. Karin, NF-kappaB signaling, liver disease and hepatoprotective agents, Oncogene 27 (2008) 6228–6244.

[200] J.G. Turner, D.M. Sullivan, CRM1-mediated nuclear export of proteins and drug resistance in cancer, Curr. Med. Chem. 15 (2008) 2648–2655.

[201] P. Sassone-Corsi, Coupling gene expression to cAMP signalling: role of CREB and CREM, Int. J. Biochem. Cell Biol. 30 (1998) 27–38.

[202] A.T. Harootunian, S.R. Adams, W. Wen, J.L. Meinkoth, S.S. Taylor, R.Y. Tsien, Movement of the free catalytic subunit of cAMP-dependent protein kinase into and out of the nucleus can be explained by diffusion, Mol. Biol. Cell 4 (1993) 993–1002.

[203] J.E.J. Darnell, I.M. Kerr, G.R. Stark, Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins, Science 264 (1994) 1415–1421.

[204] K. Melen, L. Kinnunen, I. Julkunen, Arginine/lysine-rich structural element is involved in interferon-induced nuclear import of STATs, J. Biol. Chem. 276 (2001) 16447–16455.

[205] T. Kawashima, Y.C. Bao, Y. Nomura, Y. Moon, Y. Tonozuka, Y. Minoshima, T. Hatori, A. Tsuchiya, M. Kiyono, T. Nosaka, H. Nakajima, D.A. Williams, T. Kitamura, Rac1 and a GTPase-activating protein. MgcRacGAP are required nuclear translocation STAT transcription factors, J. Cell Biol. 175 (2006) 937–946.

[206] C.C. Williams, J.G. Allison, G.A. Vidal, M.E. Burow, B.S. Beckman, L. Marrero, F.E. Jones, The ERBB4/HER4 receptor tyrosine kinase regulates gene expression by functioning as a STAT5A nuclear chaperone, J. Cell Biol. 167 (2004) 469–478.

[207] L. Xu, C. Alarcon, S. Col, J. Massague, Distinct domain utilization by Smad3 and Smad4 for nucleoporin interaction and nuclear import, J. Biol. Chem. 13 (2003) 13.

[208] F. Fagotto, U. Gluck, B.M. Gumbiner, Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of beta-catenin, Curr. Biol. 8 (1998) 181–190.

[209] L. Xu, J. Massague, Nucleocytoplasmic shuttling of signal transducers, Nat. Rev. Mol. Cell Biol. 5 (2004) 209–219.

[210] A.A. Reszka, R. Seger, C.D. Diltz, E.G. Krebs, E.H. Fischer, Association of mitogen-activated protein kinase with the microtubule cytoskeleton, Proc. Natl Acad. Sci. USA 92 (1995) 8881–8885.

[211] A. Zuniga, J. Torres, J. Ubeda, R. Pulido, Interaction of mitogen-activated protein kinases with the kinase interaction motif of the tyrosine phosphatase PTP-SL provides substrate specificity and retains ERK2 in the cytoplasm, J. Biol. Chem. 274 (1999) 21900–21907.

[212] I. Wolf, H. Rubinfeld, S. Yoon, G. Marmor, T. Hanoch, R. Seger, Involvement of the activation loop of ERK in the detachment from cytosolic anchoring, J. Biol. Chem. 276 (2001) 24490–24497.

[213] M. Adachi, M. Fukuda, E. Nishida, Two co-existing mechanisms for nuclear import of MAP kinase: passive diffusion of a monomer and active transport of a dimer, EMBO J. 18 (1999) 5347–5358.

[214] A.V. Khokhlatchev, B. Canagarajah, J. Wilsbacher, M. Robinson, M. Atkinson, E. Goldsmith, M.H. Cobb, Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation, Cell 93 (1998) 605–615.

[215] D.S. Lidke, F. Huang, J.N. Post, B. Rieger, J. Wilsbacher, J.L. Thomas, J. Pouyssegur, T.M. Jovin, P. Lenormand, ERK nuclear translocation is dimerization-independent but controlled by the rate of phosphorylation, J. Biol. Chem. 285 (2010) 3092–3102.

[216] B. Casar, A. Pinto, P. Crespo, Essential role of ERK dimers in the activation of cytoplasmic but not nuclear substrates by ERK-scaffold complexes, Mol. Cell 31 (2008) 708–721.

[217] J.A. Lorenzen, S.E. Baker, F. Denhez, M.B. Melnick, D.L. Brower, L.A. Perkins, Nuclear import of activated D-ERK by DIM-7, an importin family member encoded by the gene moleskin, Development 128 (2001) 1403–1414.

[218] D. Chuderland, R. Seger, Calcium regulates ERK signaling by modulating its protein–protein interactions, Commun. Integr. Biol. 1 (2008) 4–5.

[219] A. Ranganathan, M.N. Yazicioglu, M.H. Cobb, The nuclear localization of ERK2 occurs by mechanisms both independent of and dependent on energy, J. Biol. Chem. 281 (2006) 15645–15652.

[220] M.N. Yazicioglu, D.L. Goad, A. Ranganathan, A.W. Whitehurst, E.J. Goldsmith, M.H. Cobb, Mutations in ERK2 binding sites affect nuclear entry, J. Biol. Chem. 282 (2007) 28759–28767.
[221] K.M. Wagstaff, D.A. Jans, Importins and beyond: non-conventional nuclear transport mechanisms, Traffic 10 (2009) 1188–1198.
[222] X. Yao, X. Chen, C. Cottonham, L. Xu, Preferential utilization of Imp7/8 in nuclear import of Smads, J. Biol. Chem. 283 (2008) 22867–22874.
[223] I. Waldmann, S. Walde, R.H. Kehlenbach, Nuclear import of c-Jun is mediated by multiple transport receptors, J. Biol. Chem. 282 (2007) 27685–27692.