Chapter

Shaping the Transcriptional Landscape through MAPK Signaling

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Abstract

A change in the transcriptional landscape is an equilibrium-breaking event important for many biological processes. Mitogen-activated protein kinase (MAPK) signaling pathways are dedicated to sensing extracellular cues and are highly conserved across eukaryotes. Modulation of gene expression in response to the extracellular environment is one of the main mechanisms by which MAPK regulates proteome homeostasis to orchestrate adaptive responses that determine cell fate. A massive body of knowledge generated from population and single-cell analyses has led to an understanding of how MAPK pathways operate. MAPKs have thus emerged as fundamental transcriptome regulators that function through a multi-layered control of gene expression, a process often deregulated in disease, which therefore provides an attractive target for therapeutic strategies. Here, we summarize the current understanding of the mechanisms underlying MAPK-mediated gene expression in organisms ranging from yeast to mammals.

Keywords: MAP kinases, signal transduction, transcription, gene expression, chromatin

1. Introduction

The intracellular matrix is physically separated from the dynamic extracellular environment; however, their functions are intimately coordinated in order to ensure cell adaptation and survival. Mitogen-activated protein kinase (MAPK) cascades sense and integrate extracellular cues through sequential activation of protein kinases. These highly conserved transduction pathways are involved in a myriad of fundamental cellular processes and determine cell fate. Misregulation of these signaling cascades has major consequences for numerous diseases such as cancer, diabetes, inflammatory, and immune response diseases.

About 300 genes encode signaling proteins directly involved in signal transduction, including their positive and negative regulators as well [1]. Upon cell stimulation, in order to adapt to an extracellular insult, these seemingly simple linear signaling pathways harbor the potential to target a large number of substrates of which many are involved in gene expression. In fact, MAPKs control every step studied to date of the highly dynamic process of gene expression. The overall

picture of MAPK pathway substrates and interactors is still far from complete; however, the knowledge generated over the last 20 years has allowed a more holistic understanding of the underlying mechanisms of MAPK-regulated transcription. Due to the growing interest in MAPK-biology and the sheer volume of literature available, in this chapter, we not only mainly focus on the main mammalian MAPK cascades in humans (ERK1/2, JNK, p38, and ERK5), but we also discuss the main findings regarding MAPK cascades in the model organism *Saccharomyces cerevisiae*.

2. MAP kinase pathways

MAPKs mediate the transmission of extracellular information through a series of consecutive chemical reactions that lead to the activation of a terminal MAPK to orchestrate the appropriate gene expression pattern. To date, four major MAPK signaling cascades have been characterized in mammals, which are named according to their MAPK components: extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase 1 to 3 (JNK), p38 α/β/γ/δ (p38), and ERK5. Apart from these main MAPKs, several atypical MAPKs have also been described (ERK 3/4, ERK 7/8, and NLK, among others) with less well-defined functions and distinct modes of activation [2].

2.1 MAPK activators

MAPKs can be activated in two different ways: (1) ligand-dependent that requires the physical interaction of a ligand (e.g., growth factors, hormones, or cytokines) with a receptor or (2) ligand-independent that mediates the signaling of physical stressors (e.g., radiation, injury, and osmotic pressure). In general, ERK1/2 responds to proliferative and survival stimuli such as growth factors, serum, and phorbol esters and, to a lesser extent, to ligands of G protein-coupled receptors (GPCRs) or cytokines, or to osmotic stress and microtubule disorganization. ERK5 is activated by growth factors [e.g., EGF, NGF, FGF-2, and brain-derived neurotrophic factor (BDNF)] and cytokines (e.g., Leukemia inhibitory factor—LIF) as well as by some stresses such as osmotic stress and hydrogen peroxide [3]. JNKs and p38 MAPKs are functionally related and are collectively named stress-activated protein kinases (SAPKs). The JNK pathway strongly responds to cytokines, growth factor deprivation, intracellular stimuli (e.g., DNA damage, cytoskeletal changes, oxidative, and ER stress), and extracellular stresses (e.g., UV radiation and osmotic stress) and less efficiently responds to stimulation by some GPCRs, serum, and growth factors [4]. Finally, p38 signaling has been shown to be consistently activated by a wide variety of environmental stresses and inflammation but to be inconsistently activated by insulin and growth factors in certain cell types [1].

2.2 Modular architecture of the MAPK signaling cascades

MAPK signaling is triggered by the stimulation of different membrane receptor families (e.g., receptor tyrosine kinases (RTKs), GPCRs, cytokine receptors, Ser/Thr kinase receptors, and membrane-bound stress sensors) that are coupled to the MAPK signaling cascades. Depending on the stimulus, the signal is transmitted downstream through small G proteins, kinases, or adaptor proteins that are the immediate upstream activators of the conventional MAPK signaling cascades.

A major and highly conserved feature of MAPK pathways is their central three-tiered core signaling module of sequentially activating kinases (**Figure 1**). In the first tier, a Ser/Thr kinase MAPKKK (MAP3K) is activated by the effectors

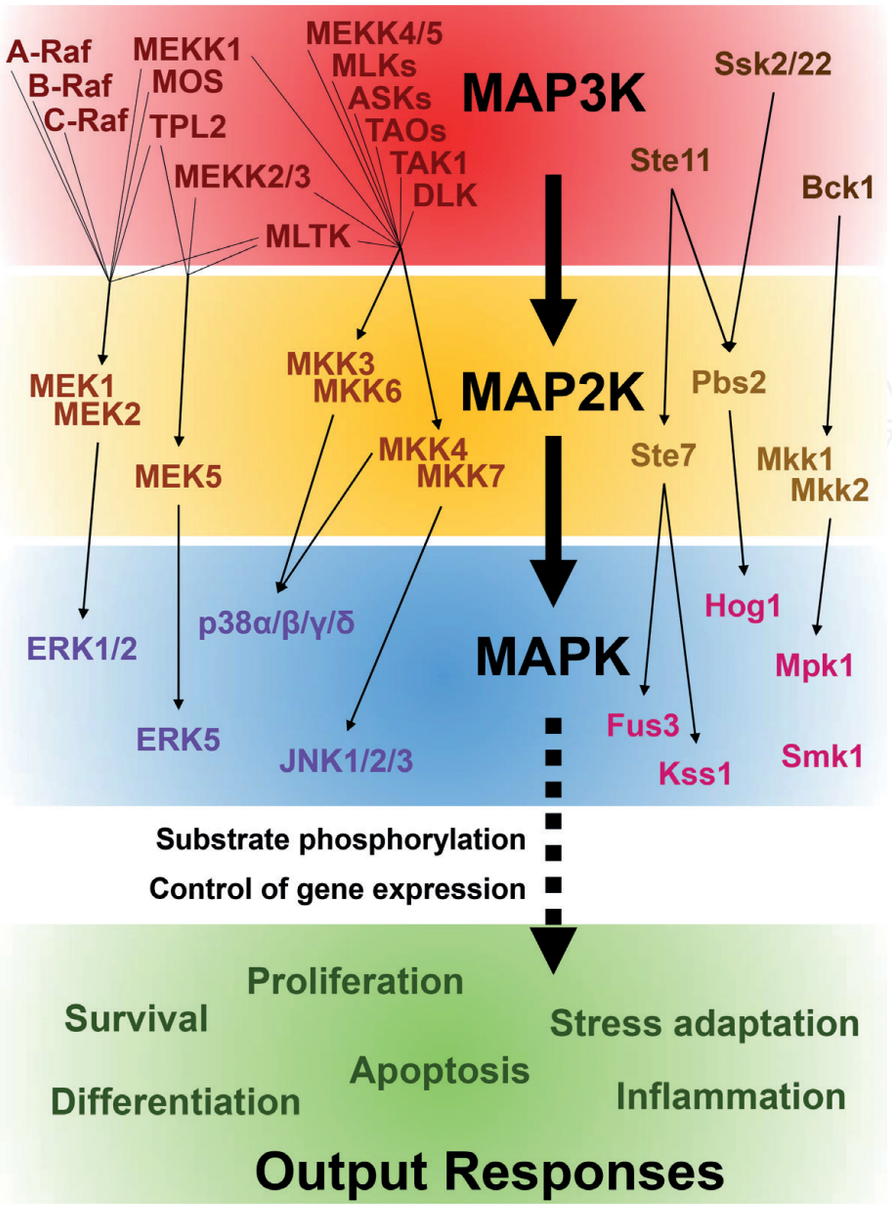

Figure 1.
Conceptual representation of the three core components of a MAPK signaling cascade. A typical MAPK cascade is composed of three consecutively activated tiers of kinases: MAP3Ks, MAP2Ks, and MAPKs. Kinases are grouped by layers according to their position in the signaling cascade. Arrows link components of different layers representing activation pathways. The core modules of mammalian (left) and yeast (right) signaling pathways are shown. Output responses resulting from MAPK activation through substrate phosphorylation and control of gene expression are indicated.

mentioned above. This MAPKKK then phosphorylates and activates a MAPKK (MAP2K) in the second tier; the MAPKK is a dual specificity kinase that phosphorylates both threonine and tyrosine within a conserved Thr-Xaa-Tyr motif in its substrate. Finally, there is a terminal Ser/Thr MAPK in the third tier, which, upon activation, phosphorylates a huge number of cytoplasmic and nuclear substrates on consensus Ser/Thr-Pro sites. Although not always present, other kinases are involved in MAPK signal transduction. One such kinase is the MAP4K that phosphorylates and activates the MAP3K; downstream MAPK-activated protein kinases (MAPKAPKs) contribute to the spread of the signal transduction.

The first MAPK pathway identified was ERK1 whose activation depends on the dimerization and autophosphorylation of the ligand-activated tyrosine kinase receptors (RTKs and GPCRs). These ligand-induced chemical and conformational receptor changes trigger recruitment of the adaptor proteins Shc and Grb2, a guanine exchange factor (SOS), and the small GTPase (Ras) to the plasma membrane.

The interaction of these four elements leads to the homo- and hetero-dimerization of the Raf family of kinases (B- or C-Raf) that activate the MAP3K module. The MAP3K then phosphorylates MEKK1/2 (MAP2K) at two serine within their activation loop (Ser-Met-Ala-Asn-Ser). Activated MEKK1/2 in turn phosphorylates ERK1/2 (MAPK) on the tyrosine and threonine residues of the Thr-Glu-Tyr motif in their activating loop. Additionally, MAPKAPKs have been identified that propagate ERK signaling (RSKs, MSKs, MNKs, and in some cases MK3/5) [1, 5].

The least studied of the four MAPK cascades is ERK5, whose mechanisms of upstream activation may include activation of tyrosine kinase receptors, the protein tyrosine kinase c-Src, the small GTPase Ras, the adaptor protein Lad1, and the protein Ser/Thr kinase WNK1, which acts as a MAP4K [1, 3]. Activation of these signaling molecules leads to activation of the MAP3Ks (not only MEKK2/3 but also TPL2 and MLTK) to phosphorylate the two alternatively spliced MEK5 isoforms (MEK5a and MEK5b, MAP2K) at the Ser-Xaa-Ala-Xaa-Thr activation motif, leading to ERK5 activation at the Thr-Glu-Tyr motif. The ERK5 pathway also involves downstream MAPKAPKs such as the serum and glucocorticoid-activated kinase (SGK) and p90 ribosomal S6 kinases (RSKs) [2].

The signal through the JNK cascade is transmitted through adaptor proteins (TRAFs), small GTPases (Rac1, Cdc42), or Ste20-like kinases that act as MAP4Ks [6]. A large number of MAP3Ks convey the signal to the main MAP2Ks (MKK4/7) by phosphorylating the sequence Ser-Xaa-Ala-Xaa-Ser/Thr in their activation loop [4]. Ultimately, the three components of the MAPK level (JNK1–3) are activated by dual Thr/Tyr phosphorylation at the Thr-Pro-Tyr motif. As for other kinases in the JNK cascade, MAPKAPKs such as MST1 are well-defined JNK substrates that can act as both upstream and downstream of JNK [7].

Finally, p38 operates through different receptors from apoptosis-related receptors to physical sensors. The initial signal is transferred using Cdc42, Rac1, and Ste20-like kinases (shared with JNK) and results in phosphorylation of the activation loop (Ser-Xaa-Ala-Xaa-Ser/Thr) of the MAP2Ks MKK3/6 that uniquely target p38. The differences between the p38 and JNK pathways lie within the specific scaffold proteins and substrates. All p38 isoforms, either the major isoforms (p38α,β,γ,δ) or the minor isoforms generated through alternative splicing, are activated through dual phosphorylation at the Thr-Gly-Tyr motif [1]. The main p38 isoform (p38α) is constitutively expressed, while the remaining isoforms are tissue-restricted. Uniquely for a MAP kinase, p38 can be activated through MAP2K-independent mechanisms that involve adaptors that promote p38 autophosphorylation [6]. Finally, the downstream MAPKAPK layer is partially shared with ERK and includes MAPKAPK2,3,5, MNKs, and MSKs [1].

2.2.1 Specificity of signaling cascades

The signaling proteome is composed of a limited number of genes that specifically integrate a virtually endless number of extracellular stimuli. Several strategies have evolved in order to maintain the signaling fidelity. For instance, this is achieved by the interaction of MAPKs with other components of the pathway and with substrates through docking sites composed of specific consensus motifs. Two types of docking motifs have been reported: D-motif and docking site for ERK (FXF)-motif, which ensure fidelity of signaling. D-motifs contain at least two basic residues flanking hydrophobic residues and are located opposite to the catalytic pocket in MAPKs [8]. The FXF-motif is composed of two Phe residues separated by one residue [9]. Another mechanism to gain specificity of signaling is the use of MAPK-scaffold proteins, which were first described in yeast (Ste5 and Pbs2) [10, 11]. Scaffolds are crucial for maintenance of signaling specificity

as they sequester multiprotein interactions to prevent crosstalk by controlling stability and subcellular localization.

2.2.2 Regulators of signaling cascades

The amplitude, frequency, and localization of activated MAPK-activity is tightly controlled, not only through positive and negative feedback mechanisms at the post-translational level mediated by regulatory proteins (e.g., phosphatases and kinases) but also through post-transcriptional control mediated by RNA-binding proteins and microRNAs (miRNAs).

The fastest mechanism of ablating MAPK activity is to remove one of the two activating phosphates through the activity of specific phosphatases. Their role in regulating the terminal MAPK has been extensively studied, but little is known about their effect on upstream signaling components. Phosphatase activity is mainly derived from Ser/Thr phosphatases, Tyr phosphatases, and the dual specificity phosphatases (DUSP) known as MAPK phosphatases (MKP) [1]. Based on sequence homology, substrate specificity, and subcellular localization, DUSPs can be divided into three groups: nuclear inducible (DUSP1/2/4/5), cytoplasmic and ERK-specific (DUSP6/7/9), and DUSPs with no specific cellular localization that targets JNK and p38 SAPKs (DUSP8/10/16) [4, 12]. MAPKs also exert a transcriptional control of regulatory elements such as these phosphatases and thereby generate a negative feedback loop. Another relevant type of negative feedback regulation is driven by the direct phosphorylation of different upstream components of the MAPK cascade by the MAPK itself to modulate basal [13] and stimuli-dependent signaling dynamics [5]. Additionally, scaffold proteins and other enzymatic activities either positively or negatively regulate different levels of MAPK signal transduction such as, for example, the formation of the ligand-receptor signaling complex, the intracellular modular interactions, and the degradation of the components [14]. Post-transcriptional regulation of MAPKs can also be achieved at the RNA level. RNA-binding proteins and miRNA negatively regulate MAPK gene expression by directly cleaving their mRNAs or through complementary pairing [15].

2.3 Yeast MAPK cascades

Five MAPK pathways have been well characterized in the budding yeast, *S. cerevisiae*. In vegetative cells, the four MAPKs Fus3, Kss1, Hog1, and Slt2/Mpk1 are involved in the mating-pheromone response, the filamentous-invasion pathway, the high osmolarity growth, and the cell integrity pathway, respectively. The fifth MAPK, Smk1, is believed to play a role in spore wall assembly [16, 17].

Haploid yeast cells sense the reciprocal mating pheromones (α-factor or a-factor) through Ste2 and Ste3 GPCRs. The signal is then transmitted by GTPases to the p21-activated kinase (PAK)-like kinase Ste20, the MAPK scaffold Ste5, Cdc42, a guanine-nucleotide exchange factor (GEF), and Far1. Ste5 signals and serves as a scaffold that links the MAP4K and the MAPK signalosome (Ste11 → Ste7 → Fus3; the latter is the ERK1 homolog) [18].

The high osmolarity glycerol (Hog1) MAPK, the yeast homolog of p38, is activated in response to osmotic stress as a consequence of signaling elicited from two upstream-independent mechanisms (Sln1/Sho1). The Sln1 sensor is the primary osmosensor and is a complex variation of the well-known bacterial two-component system. Upon osmostress, inactivation of the transmembrane histidine kinase Sln1 leads to the derepression and activation of the MAP3K (Ssk2/22) via Ypd1/Skk1. The Sho1 osmosensing branch is mediated by mucin-like proteins (Hkr1 and Msb2) and ultimately activates the MAP3K Ste11 through the integral transmembrane

protein Opy2, the GTPase Cdc42 and the MAP4K Ste20. These two osmosensing branches converge at the MAP2K (Pbs2) that acts as a scaffold protein for phosphorylation of the MAPK Hog1 [19].

The filamentous/invasive growth pathway leads to the activation of Kss1 (an ortholog of mammalian Erk2) under nutrient limiting conditions and, to a much lesser extent, to pheromone stimulation. Remarkably, it relies on proteins involved in the HOG pathway and the pheromone pathway (Mep2, Gpr1, Msb2, Sho1, Ste20, Ste11, and Ste7). In this case, specific activation of Kss1 is achieved by the absence of the Ste5 scaffold that liberates Ste7 allowing its interaction with Kss1 [20].

Cell wall instability is sensed through the cell wall integrity pathway (CWI) (Mpk1 MAPK) and is detected by five mechanosensors (Wsc1–3, Mid2, and Mtl1) that interact with the guanine nucleotide exchange factor (GED) Rom2 to activate Rho1 GTPase leading to protein kinase C (Pkc1) phosphorylation. Yeast Pkc1 serves as a MAP4K that phosphorylates the MAP3K Bck1, which leads to activation of Mpk1 through activation of the redundant MAP2K (Mkk1/2). Despite the absence of a cell wall in higher eukaryotes, mammalian ERK5 has been characterized as a functional ortholog of the CWI pathway [21].

Finally, the meiosis-specific MAPK Smk1 controls the postmeiotic program in diploid cells subjected to nutrient starvation. Activation of Smk1 differs from activation of MAPKs in the classical three-tiered MAPK cascade in which a CDK-activating kinase (CAK1) phosphorylates Smk1 and induces its auto-phosphorylation [22].

2.4 Dynamics of signal transduction

According to the nature of its input signal, MAPK activation can range from minutes (transient) to hours (sustained). The dynamics of MAPK activation results from the interplay between the extracellular environment and a myriad of intracellular feedforward/feedback regulators that give rise to cell fate decisions during cancer progression or development. For example, pulses of or continuous high EGF administration induce transient ERK activation and cell proliferation in rat adrenal cells, whereas repeated pulses of low EGF induce ERK-mediated differentiation into sympathetic-like neurons [23]. Similarly, different dynamics of JNK can generate opposing behaviors as persistent JNK activation has been shown to trigger apoptosis while its transient activation promotes cell survival [24]. Despite different signaling dynamics can determine cell fate, the underlying molecular mechanisms are not well understood.

2.5 Output responses to MAPK activation

The first response to extracellular insults is the immediate arrest of cell growth and hence a blockage of or a delay in cell cycle progression. Once activated, the different MAPKs phosphorylate a large number of substrates that distribute over many cellular compartments. In general terms, the ERK1/2 pathway is mainly associated with the promotion of growth in most cell types and is often linked with differentiation processes, although it can occasionally suppress cell survival [25]. Similarly, ERK5 also promotes proliferation during normal cell growth and differentiation [3]. On the other hand, JNK and p38 pathways have a well-established role in apoptosis, although they have also been shown to contribute to survival, immunity, development, and differentiation [4, 26–29]. One of the main mechanisms by which MAPKs modulate the abovementioned cellular processes is by controlling gene expression, mainly through regulation of the transcriptional machinery, chromatin structure, and post-transcriptional modifications.

3. Nuclear localization and function of MAPKs

In resting cells, MAPK components are usually located in the cytoplasm through their interaction with different anchor proteins, scaffolds, or phosphatases. Upon stimulation, MAPK signaling cascades rapidly transmit information into the nucleus to ensure the appropriate transcriptional response (**Figure 2A**). Across eukaryotes, this process is often initiated by transient accumulation of the MAPKs within the nucleus. The duration and the type of stimuli affect the nuclear localization of MAPK signaling proteins and play an important role in determination of the transcriptional output. Translocation of MAPK molecules requires specialized transport elements to travel through the nuclear pore complex (NPC).

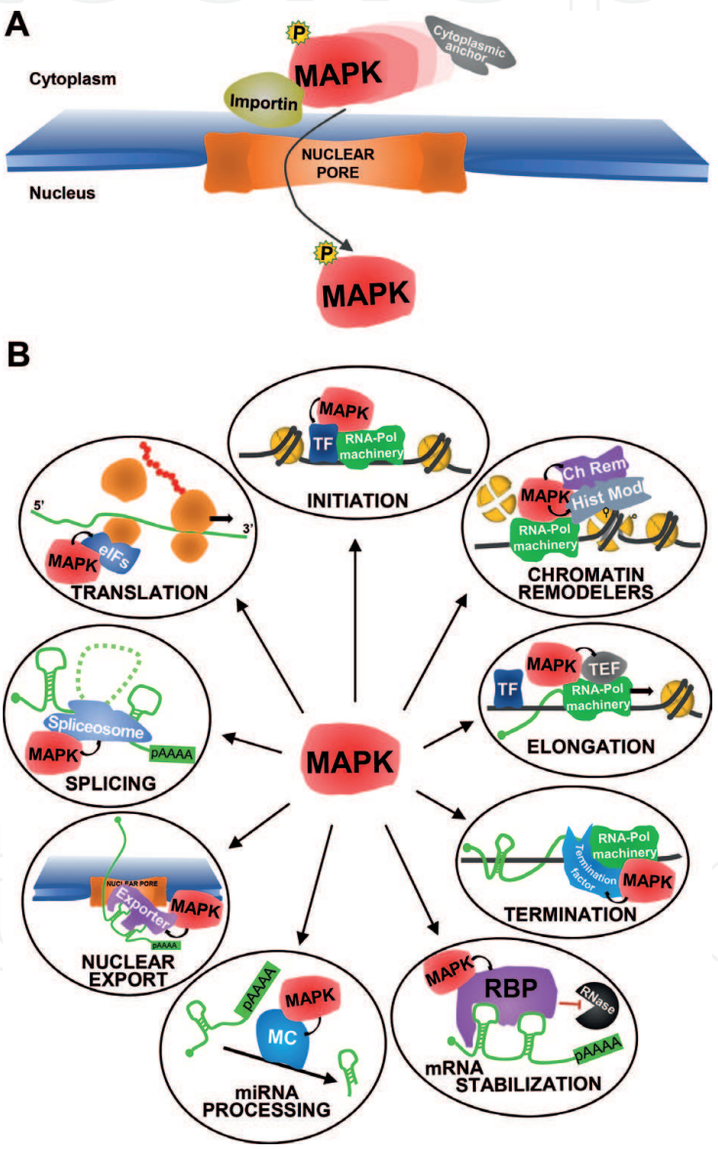

Figure 2.
*MAPK regulatory roles on gene expression: From transcription initiation to translation. (***A***) Activated MAPK is released from its cytoplasmic anchor and translocated to the nucleus. (***B***) From top and clockwise, MAPK regulation on different targets is represented by a black arrow; MAPKs are known to activate transcription factors (TFs) through phosphorylation and to recruit PolII to initiate transcription. Moreover, MAPKs also target several chromatin remodelers (Ch Rem) and histone modifiers (Hist mod) to regulate chromatin structure and histone eviction. MAPK interacts with transcription elongating (TEF) and termination factors to enhance transcription rate. mRNA is shown as a green line with a 5′ cap (green dot) and the polyA at the 3′ end. MAPKs also regulate several stabilizing RNA-binding proteins (RBPs), target miRNA processing through the microprocessor complex (MC), nuclear exporters, and splicing factors. Finally, MAPKs are also known to regulate translation elongation initiation factors (eIFs) to stimulate rapid mRNA translation. Overall, all these mechanisms aim to promote a rapid and efficient response for maximal cell adaptation.*

Nucleoplasmic shuttling of active MAPK can be mediated mainly by three strategies: (1) active regulation of import-export through the NPC; (2) escape from cytoplasmic anchors and/or sequestration by nuclear components; and (3) passive diffusion.

Canonical nuclear localization is an active process during which nuclear α/β importin complexes deliver cargo containing mono- or bi-partite stretches of basic residues (nuclear localization signals—NLSs) to the nucleus. Once in the nucleus, targeted proteins dissociate from importins by interacting with RanGTP. For example, in its inactive state, a nuclear export signal (NES) is exposed in ERK2, which confines it to the cytoplasm. Upon activation of ERK2, a conformational change disrupts its N- and C-terminal interactions, thereby exposing a NLS that sends the kinase into the nucleus. Similarly, activation of ERK1/2 allows their interaction with importin-7 and their nuclear accumulation [30]. Not only ERK1/2 nuclear accumulation is mediated through Ran as direct interaction, but also phosphorylation of nucleoporins (NUP50) facilitates translocation through importin-β [31]. The mechanisms by which p38 and JNK translocate into the nucleus are far less well understood. Recently, the motifs for interaction of both p38 and JNK with importins 3, 7, and 9 have been mapped at their N-terminal region. Ablated interaction of p38/JNK with their importins selectively impairs their nuclear accumulation and phosphorylation of their nuclear but not their cytosolic targets [32]. Nuclear translocation of the budding yeast Hog1 requires both Ran (GSP1) and importin-β (NMD5). Phosphorylation of Hog1 by its MAP2K Pbs2 is essential for its translocation, while MAPK activity is dispensable for its import. Similar to mammalian MAPKs, transcription factors such as Msn2/4, Hot1, Sko1, and the nuclear phosphatase Ptp2 contribute to the nuclear retention of Hog1. Dephosphorylated Hog1 is exported out of the nucleus through an importin-β homolog, XPO1. Blocking its nuclear export traps Hog1 in the nucleus but does not prevent its dephosphorylation [33].

An increasing body of knowledge supports the presence of other upstream kinases: MEK1/2, MEK5, MEKK2/3, and MKK6 in the nucleus [3, 6, 34, 35]. The role of upstream signaling components in transcriptional regulation has not received much attention and requires deeper understanding.

4. MAPK-regulated gene expression

Nuclear localized MAPKs have the capacity to rewire the transcriptional architecture by controlling several layers of mRNA biogenesis (**Figure 2B**). Nuclear localized MAPKs are competent to govern the transcription cycle by acting on several layers of the process. Temporal integration of MAPK signaling into transcription is generally mediated by the phosphorylation of hundreds of transcription-related targets. How this transcriptional control is achieved will be discussed in this section.

4.1 Genes regulated by MAPK activation: global induction/repression patterns

MAPK activation overrides the homeostatic transcriptional program by transiently governing the simultaneous upregulation and downregulation of gene expression. Activation of different MAPK cascades leads to a pathway-specific transcriptional landscape. This stimuli-specific response is required to redefine the demands of each condition and involves the regulation of all RNA species. Unbiased approaches such as tiling arrays and RNA-seq have further extended the type of MAPK-regulated transcripts to noncoding RNA (ncRNA), long noncoding RNA (lncRNA), and, specifically in higher eukaryotes, the expression of miRNA.

The MAPK-induced transcriptional response encompasses not only stimuli-specific genes but also a set of well-defined genes that respond to multiple signals, providing coping mechanisms for adaptation. The transcriptional program induced by MAPK activation is classically described in two stages: A primary response is independent of protein synthesis and triggers the expression of immediate and delayed early genes (IEG and DEG, respectively). Then, the secondary response follows in a protein synthesis-dependent manner to induce the expression of secondary response genes [36]. Here, we will focus our attention on the mechanisms that promote gene induction.

During this early or primary response, cells have to be able to repress cell cycle and growth genes while upregulating several transcription factor genes, which, once translated, will amplify the signal to generate a secondary or late response [37]. Thus, while a selected group of genes are upregulated, the rest of the transcriptome is transiently downregulated. Understanding of the mechanisms of MAPK-mediated gene repression has lagged behind when compared to the activating mechanisms, but some well understood prominent targets of repression are cell cycle- and growth-related genes (cyclins, tRNAs, and rRNAs).

4.2 MAPK as components of the transcriptional machinery

MAPKs localize and interact with all of the regulatory regions of their target genes to control gene expression through similar principles but through distinct molecular mechanisms. These mechanisms include the coordinated control of transcription initiation, elongation, and termination together with modulation of chromatin architecture to ensure proper transcription through its target genes. MAPK phosphorylation of chromatin-related factors alters their activity by regulating their nuclear localization, protein stability, or affinity to DNA [38].

4.2.1 Transcription initiation: transcription factor modification

Transcription initiation is the first step in governing gene expression and can be either directly or indirectly regulated by MAPKs. The most common regulatory mechanism involves the control of promoters by the regulation of an intricate network of transcription factors usually through direct phosphorylation and/or by induction of their expression [39]. Transcription factors serve as anchoring platforms for the recruitment of MAPKs to chromatin. Chromatin-tethered MAPK nucleates the key signaling components to promoters and other regulatory elements to form a competent pre-initiation complex (PIC). Examples of "hubs" in the transcription factor network that facilitate the recruitment of active MAPK to chromatin are Elk-1, c-Jun and c-Fos for p38, ERKs, and JNKs. ERK5 is a rare MAPK that contains a transcriptional coactivator domain and has the capability of stimulating transcription through transcription factors or by direct binding to DNA through its noncatalytic region [3].

One of the best characterized transcription factors is c-Jun upon which stimulation is phosphorylated by JNK in its transactivator domain, which is required for induction of its maximal transcriptional activity and increased protein stability [39]. A single-transcription factor can serve to integrate signals from different MAPKs, or several MAPKs can cooperate in regulation of the same target. In response to UV light, both p38 and ERK contribute to the activation of c-Fos. On the other hand, efficient Elk1 phosphorylation is achieved by its differential interaction with ERK1/2, p38, and JNK. Activated Elk1 induces the expression of c-Fos and c-Jun transcription factors that will subsequently regulate a second transcriptional wave that includes other transcription factors and phosphatases [38]. Alternatively,

a more indirect method to promote transcription is to activate downstream kinases that will themselves activate other transcription factors. For example, p38 activates two downstream kinases, mitogen- and stress-activated kinase 1/2 (MSK1–2), that activate another set of transcription factors STAT1/3, CREB, ATF1, and NF-κB [40].

In yeast, Fus3 and Kss1 MAPKs activate the transcription factor Ste12 that induces the expression of over 200 genes, including its own gene [41]. For example, in yeast, the combination of deletions of transcription factors and genome-wide analyses has been especially useful in providing a detailed view of the circuitry activated by the Hog1 or Fus3 MAPKs [42, 43].

The interrelationship between transcription factors and MAPKs is conserved throughout evolution, although the number of players and their functions has increased over time. MEF2 family transcription factors are substrates for several ERKs and in particular for p38 [44]. In yeast, it has been widely reported that the different transcription factors relevant for osmoresponsive gene expression are phosphorylated and recruited to target genes in a Hog1-dependent manner [45, 46]. Targeted recruitment of the MAPK activation machinery can also include recruitment of upstream MAPK-regulatory kinases. Examples of such in mammals are the recruitment of MEK1/2 to ERK-dependent genes [47] and the recruitment of MKK6 to p38 targeted regions in a MAPK-dependent manner [35]. Yeast upstream MAPK components have received far less attention than those of mammals, although Ste5 also associates with chromatin upon pheromone stimulation [48].

Besides controlling transcription factors, MAPKs control several other enzymatic activities, protein complexes, and targets that contribute to the formation of a transcriptionally competent Pre-Initiation Complex (PIC) (SAGA, Mediator, Ubp3) [49, 50]. A critical downstream node for MEK1/2 and ERK1/2 signaling upon the induction of EGF responsive genes is the integrator complex, a transcriptional coactivator. The binding of integrator to chromatin depends on catalytically active ERK1/2. Indeed, inhibition of the MAPK resulted in diminished association of integrator and RNA Pol II to chromatin [51].

4.2.2 Transcription elongation

Our knowledge of MAPK-regulated transcriptional control extends far beyond its control of transcription initiation and mainly originates from analysis of yeast MAPKs. The detection of MAPKs at the coding regions of their target genes suggested a far more extensive role for MAPKs as crucial components of the transcription regulatory complex. Seminal work regarding this phenomenon has been done in *S. cerevisiae* in which the association of Hog1, Fus3, and Mpk1 MAPKs with the coding regions of their target genes has been reported. Mpk1 elicits elongation of stress-responsive genes in a catalytic-independent manner by its interaction with the Paf1 elongation complex. Mpk1 is tethered to its target genes through binding to Paf1 that serves as a scaffold to escort Mpk1 into the elongating RNA Pol II. This binding requires the presence of the cell cycle transcriptional regulator SBF. The loss of this interaction restricts Mpk1 to the promoter region, which impairs both transcription and cell viability upon stress [52]. In response to osmotic stress, Hog1 and Paf1 interact through an unknown region, but the function and outcome of the Paf1 complex are kinase-specific.

The majority of genes targeted by Hog1 display an enrichment of the MAPK throughout the coding region [53, 54] that is mediated by the 3'UTR and is independent of promoter association. ORF-bound Hog1 behaves as a selective elongation factor by traveling and interacting with phosphorylated RNA Pol II (Rpb1). As RNA Pol II moves across the gene, it regulates chromatin structure through the recruitment of chromatin remodelers and chromatin-modifying enzymes (Section 4.3).

Moreover, Hog1 phosphorylates the Spt4 elongation factor to regulate RNA Pol II processivity to stimulate elongation efficiency at stress-responsive genes [55]. As happens during initiation, Hog1 recruits other protein complexes with specific enzymatic activities such as deubiquitinase (Ubp3) to ensure the proper production of stress-responsive genes [50]. Further studies in mammalian cells also corroborated p38 binding to coding regions of genes not only in response to osmotic stress but also during skeletal muscle differentiation, suggesting that the mechanism and purposes of Hog1/p38 transcriptional regulation are conserved throughout evolution.

Transcription elongation rates for many genes depend on the entangled interplay of factors and complexes that regulate RNA Pol II. During elongation, a number of positive and negative elongation factors (P-TEFs and N-TEFs, respectively) have been shown to accelerate or attenuate Pol II, and, not surprisingly, these factors are targeted by MAPKs at stress-responsive genes. In response to hormone stimulation, MEK1 and ERK1/2 promote elongation and abolish pausing of RNA Pol II [56].

4.2.3 Termination

Unlike initiation and elongation, transcription termination can be carried out through different pathways depending on the coding or noncoding nature of the transcript. The two best defined termination pathways that are also highly conserved are the polyA-dependent pathway for protein coding and the Sen1-dependent pathway for noncoding transcripts.

One of the best studied examples of the involvement of MAPKs in the control of transcription termination is that of the role of Mpk1 in transcription termination during heat stress in yeast. As mentioned before, Paf1 and Mpk1 interact at heat responsive genes; this association prevents the recruitment of the Sen1-Nrd1-Nab3 termination machinery (NNS). Interestingly, the same study showed that human ERK5 and human Paf1 complex expressed in yeast also regulated termination in response to cell wall stress [52]. Mpk1 has recently been shown to directly phosphorylate Tyr1 in the RNA Pol II CTD as it traverses the coding region with the elongating machinery. This phosphorylation occurs in a stress-dependent manner and prevents early termination through the NNS pathway [57]. Deep sequencing of osmotically stressed neuronal cell lines identified a new set of transcripts termed downstream of gene-containing transcripts (DoGs). These noncoding transcripts span large region downstream of annotated gene features (>45 Kb) and are actively regulated through IP3 signaling [58].

4.3 MAP kinases and their effects on chromatin

MAPKs facilitate the abovementioned transcription activity by also regulating several chromatin remodelers to generate the proper chromatin environment for the transcription machinery. For induction of gene expression, chromatin must be accessible to allow the assembly of transcription factors, RNA Pol II and other factors, during initiation, elongation, and termination. These chromatin remodelers have been studied in both yeast and mammalian models as has been extensively reviewed in [38].

There are numerous examples of MAPKs interacting with chromatin remodelers. For instance, both Hog1 and p38 govern the recruitment of the remodeling complex SWI/SNF to target genes [38]. On the other hand, MAPK regulation goes beyond the substrate phosphorylation. As described in previous sections, MAPKs can also regulate chromatin remodeling through direct protein-protein interactions. This is the case with ERK2, which contacts PolyADP-ribose polymerase (PARP1), thereby

increasing its activating activity on chromatin remodelers [59]. Apart from chromatin remodelers, MAPKs govern a cohort of histone modifiers that not only destabilize nucleosomes but also, in a more complex manner, generate selective marks that dictate nucleosome dynamics. An example of this type of regulation is the Hog1-dependent gene recruitment of Rpd3, a histone deacetylase, that induces gene expression by promoting the eviction of histones at osmoresponsive genes [60] and the regulation of H3K4 monomethylation to dictate specificity of chromatin remodelers [61]. During elongation, as Hog1 travels with the elongating RNA polymerase, it recruits the RSC remodeling complex, thereby facilitating transcription along the gene body [62]. In mammals, ERKs, p38, and JNK promote the phosphorylation of H3S10 either directly or through their downstream kinases [38, 63]. p38 also phosphorylates the transcription factor MEF2D, which, in turn, leads to recruitment of the Ash2L-containing methyltransferase complex that generates an increase in the activating mark H3K4me3 [64]. These examples highlight the relevance of MAPK-mediated histone modification to generate an efficient chromatin remodeling robustly achieved through different mechanisms.

MAPKs also regulate gene silencing through chromatin remodelers. ERK1/2 directly interacts with the histone deacetylase 4 (HDAC4) that removes acetyl groups leading to chromatin condensation [6]. Similarly, Hog1 promotes the transcription of *PNC1*, which encodes an activator of Sir2, a histone acetyltransferase that protects sensible rRNA-coding regions from DNA damage [65]. In these two cases, MAPKs act as repressing elements of chromatin remodeling.

5. Role of MAPK signaling in post-transcriptional regulation

The ultimate goal of MAPK-mediated transcriptional reprogramming is to change the proteome composition. This change becomes especially important upon extracellular challenge when a massive pool of previously low-abundant RNAs needs to be expressed. Activated MAPKs target mRNA-binding proteins to downregulate unnecessary mRNAs and favor expression of the required genes in order to adapt to the new conditions [31, 45].

5.1 Transcript/RNA stability

Genomic run-on (GRO) experiments that have revealed global changes of gene expression in response to stress are also achieved through the regulation of mRNA stability and decay [66]. In yeast, upon osmotic stress, there is a broad mRNA destabilization, while Hog1 plays a role on the stabilization of osmo-induced mRNAs [67]. The p38 MAPK pathway is also a key regulator of the mRNA stability of both TTP (tristetraprolin), a protein that shortens the half-lives of adenine-uracil rich element (ARE)-containing mRNA, and HuR (human antigen R), a protein that stabilizes such mRNA. The role of p38 turns out to be opposed depending on the cell type [68]. Like p38, ERK and possibly JNK are thought to target HuR, changing its localization to the cytosol, where it stabilizes ARE-containing mRNA [69]. As a further example of the role of p38 in regulating mRNA stability, p38-mediated phosphorylation of ADAR1p110, another mRNA-binding protein, suppresses apoptosis in stressed cells by protecting many antiapoptotic gene transcripts from mRNA decay [70].

Another layer of transcriptional regulation coordinated by MAPKs, which has gained importance over the years, is the regulation of miRNA biogenesis. A relevant example of the coordination of the regulation of miRNA by different MAPK cascades is the regulation that takes place in the early stages of the inflammatory response. JNK and p38 trigger transcription of the miRNA let-7f, which downregulates the

expression of Blimp1 and PRDM1, two transcriptional repressors of inflammatory genes. Since a sustained expression of inflammatory genes is detrimental, later activation of ERK promotes the transcription of Lin28, an inhibitor of let-7f biogenesis, thereby increasing the expression of the Blimp1 and PRDM1 repressors [71]. Here, the same stimuli generate a time-dependent regulated activation of multiple signaling pathways to achieve a finely tuned transcriptional response.

5.2 mRNA export

There are numerous examples of interactions between MAPK pathways and different components of the mRNA exporting machinery in biological systems ranging from yeast to mammalian cells. In yeast, it has been reported that, in response to osmotic or heat stress, Hog1 and Mpk1, respectively, phosphorylate components of the nuclear pore complex to increase the export efficiency of stress-responsive mRNAs [72, 73]. Similarly, in mammals, both p38 and ERK pathways regulate RNA-binding proteins such as eIF4E or hDl1 that facilitate mRNA export [74, 75]. In the event of stress, the export of the newly transcribed mRNAs is prioritized to maximize the transcriptional response.

5.3 mRNA splicing

The transcriptional response to external stimuli generates an outburst of mRNAs that have to be spliced. The associations between splicing events that modulate MAPK genes are becoming increasingly relevant in human disease [76]. One strategy to regulate splicing under stress is phosphorylation of the splicing factor TDP-43 by MEK1/2, which prevents TDP-43 aggregation [77]. Another mechanism of regulating splicing is by interfering with the localization of splicing factors such as RNM4, hn-RNPA1, or hSlut7 [78–80].

5.4 Translation

Translation plays a pivotal role in the control of gene expression and is tightly regulated by MAPK pathways that modulate the activity of several components within the translational machinery [81]. In yeast, Hog1 promotes Rck2-mediated attenuation of protein synthesis in response to osmotic stress by phosphorylation of the translation elongation factor 2 (EF-2) [82]. ERK- and p38-activated MNKs phosphorylate the elongation initiation factor eIF4E to enhance translation initiation [83]. Another example is the activation of RSK, a downstream kinase of ERK. RSK phosphorylates S6, a component of the 40S ribosomal subunit, as well as the elongation initiation factor eIF4B, which facilitates their binding to eIF3 to promote mRNA translation [84, 85]. Besides the targeting of newly transcribed mRNAs, translation regulation can also target mRNAs that have not been transcriptionally induced, a type of regulation found in yeast and mammals [86].

6. Future perspectives and challenges

Due to their master regulatory role, MAPKs have sparked a lot of interest and have been the main focus of multiple researchers worldwide over the last 30 years. As MAPK knowledge advances, it has become obvious that the external control of MAPK activity has the potential to modulate cellular behavior and survival. Moreover, MAPK signaling has been found to be altered or defective in many human diseases such as cancer; therefore, achievement of the control of

MAPK activity could provide an attractive intervention point for new therapeutic approaches. However, despite the tremendous amount of knowledge generated, there are fundamental questions that remain to be elucidated in order to transform the biomedical potential of MAPKs into a reality.

While the central core of MAPK signaling cascades has been extensively described, branches of the networks have not yet been completely identified. This is especially true in terms of the upstream sensors, where the picture is not well defined, especially in higher eukaryotes. In the immediate future, we therefore foresee that more sophisticated approaches using CRISPR/Cas9 and RNAi-based libraries will provide a means to perform systematic genome-wide genetic screens to reveal missing components of these pathways.

Additionally, there are other features of MAPK signaling that might have been overlooked. An example of such a feature is the peptide-mediated blockage of p38/JNK interaction with importins, which reduces their nuclear export. The presence of this peptide impaired MAPK nuclear localization and decreased cell proliferation and tumor growth to a larger extent than the presence of commercial p38 inhibitors. These data open up a new perspective on MAPK regulation and need to be further examined as they could provide a new therapeutic intervention strategy to regulate MAPK activity [32]. It is clear that nuclear localization stimulates the encounter of MAPKs with defined chromatin loci, where their targets are located to provide specificity for gene induction; however, how the kinases are directed to these regions is not clear. Similarly, the molecular mechanisms of transcriptional termination have only recently been uncovered, and there are few reports regarding the targets and the control of MAPKs in termination, as many noncoding RNAs have been shown to be regulated by MAPKs such as Hog1/p38 and ERK2 [87, 88].

Remarkably, upstream MAPK pathway components as transcriptional regulators are also unclear, although the recruitment of several MAP2Ks (MEK1/2, MKK6) to chromatin has been detected. Furthermore, an extreme case has been reported in which, upon insulin stimulation, the entire signaling pathway from the insulin receptor to the ERK signaling cascades is recruited to insulin inducible loci [89]. The functions and consequences of such recruitment require further investigation. Due to their master regulatory role, MAPKs have generated a lot of interest. It is clear that controlling MAPK activity could provide a means of controlling cell behavior. Additionally, understanding the consequences of heterogeneity for MAPK-regulated events will be crucial for understanding differential responses to extracellular stimuli and therapeutic treatments. In conclusion, it is of utmost importance that MAPK-mediated mechanisms of controlling gene expression are fully characterized in order to further identify druggable targets/processes that are relevant to human diseases.

Acknowledgements

MNR is a recipient of a Maria de Maeztu Postdoctoral Fellowship (Doctores Banco de Santander-María de Maeztu at Universitat Pompeu Fabra). GMC is a recipient of an FPI Predoctoral Fellowship (Spanish Ministry of Economyv Industry and Competitiveness (MINECO). The FP and EdN's laboratory is supported by grants from the Spanish Ministry of Economy and Competitiveness (BFU2015-64437-P to FP and BFU2017-85152-P and FEDER to EN), the Catalan Government (2017 SGR 799), the Fundación Botín, and the Banco Santander through its Santander Universities Global Division to FP and the Unidad de Excelencia Maria de Maeztu, MDM-2014-0370. FP is a recipient of an ICREA Acadèmia (Generalitat de Catalunya). We gratefully acknowledge funding from the Spanish Ministry

of Economy, Industry, and Competitiveness (MINECO) through the Centers of Excellence Severo Ochoa award and from the CERCA Program of the Catalan Government.

Conflict of interest

The authors declare no conflict of interest.

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