MAPKAP kinases — MKs — two's company, three's a crowd

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Abstract | Downstream of mitogen-activated protein kinases (MAPKs), three structurally related MAPK-activated protein kinases (MAPKAPKs or MKs) — MK2, MK3 and MK5 — signal to diverse cellular targets. Although there is no known common function for all three MKs, these kinases are involved in important processes: MKs regulate gene expression at the transcriptional and post-transcriptional level, control cytoskeletal architecture and cell-cycle progression, and are implicated in inflammation and cancer.

SAPKs
Stress-activated protein kinases that consist of the JNK and p38 subgroups.

Kinome
The protein kinase complement of the genome, which contains 518 genes and 106 pseudogenes in humans, and 540 genes and 97 pseudogenes in mice.

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Attempts to understand the structure and function of various protein kinases led to the discovery of unexpected regulatory mechanisms and physiological functions for the enzymes that were analysed. Recently, techniques that allow the specific inactivation or downregulation of kinase genes in cells and whole organisms, as well as the availability of specific kinase inhibitors, accelerated the process of assigning new kinase substrates and identifying new signalling pathways. Mitogen-activated protein kinase (MAPK)-activated protein kinases (MAPKAPKs or MKs) benefited from these developments.

Initially, the existence of several intracellular MAPK cascades that have distinct (but structurally related) central MAPKs and stress-activated protein kinases (SAPKs) led to there being a relatively blurred definition for downstream kinases, which are generally known as the MAPKAPKs or MKs. However, from our present knowledge of the phylogenetic relationships of mammalian[1,2] as well as yeast, worm and fruitfly kinomes[3], it has become apparent that different protein-kinase subfamilies exist downstream of MAPKs and SAPKs (FIG. 1a).

Based on the homology of the kinase catalytic domain, all these downstream protein kinases belong to the calcium/calmodulin-dependent protein kinase (CaMK) superfamily. The tandem arrangement of two catalytic domains is characteristic for the ribosomal S6 kinase (RSK) subfamily (the members of which are also known as MAPKAPK1A–D) and for the mitogen- and stress-activated kinase (MSK) subfamily. Although RSKs are exclusively activated by the extracellular signal-regulated kinases (ERKs), MSKs are downstream of both ERKs and p38 MAPK (REF. 4). The two MAPK-interacting kinases, MNK1 and MNK2[5,6], which each contain only one catalytic domain, are also activated by both the ERK and p38 pathways. RSKs and MSKs are mainly involved in the regulation of gene expression at the transcriptional level by phosphorylating substrates such as Fos and cyclic-AMP-responsive-element-binding protein (CREB; RSKs) or histone H3 (MSKs). MNKs, on the other hand, contribute to the general regulation of translation by targeting the eukaryotic translation-initiation factor (eIF)4E. Finally, the three structurally related enzymes, which are designated MAPKAPK2/MK2, MK3/3pK and MK5/PRAK (p38-regulated and -activated kinase) are defined as the real MAPKAPK (MK) subfamily (FIG. 1a).

MK2 turned out to be essential for lipopolysaccharide (LPS)-induced upregulation of cytokine mRNA stability and translation and, therefore, for stimulation of cytokine biosynthesis, which is necessary for the inflammatory response[7,8]. Recently, new insights into the molecular mechanism of post-transcriptional regulation by MK2 were obtained[9–11], and MK2 was also shown to be involved in cell-cycle control at the CDC25- and p53-dependent checkpoints[12–15]. MK3 was found to regulate chromatin remodelling[16] and a novel mechanism of activation of MK5 in development was also shown[17,18]. These findings add substantial new information to the understanding of the physiological role of the MK family, and indicate an unexpected diversity in the regulation and function of these structurally related enzymes.

Starting with a comparison between the structural properties of MKs, this review will discuss the differences in the regulation of MK2, MK3 and MK5. An overview of the substrates and the physiological functions that were revealed by MK gene-deletion and knockdown studies will be then discussed. Finally, and as far as possible, common features and general mechanisms for the action of MKs will be proposed.

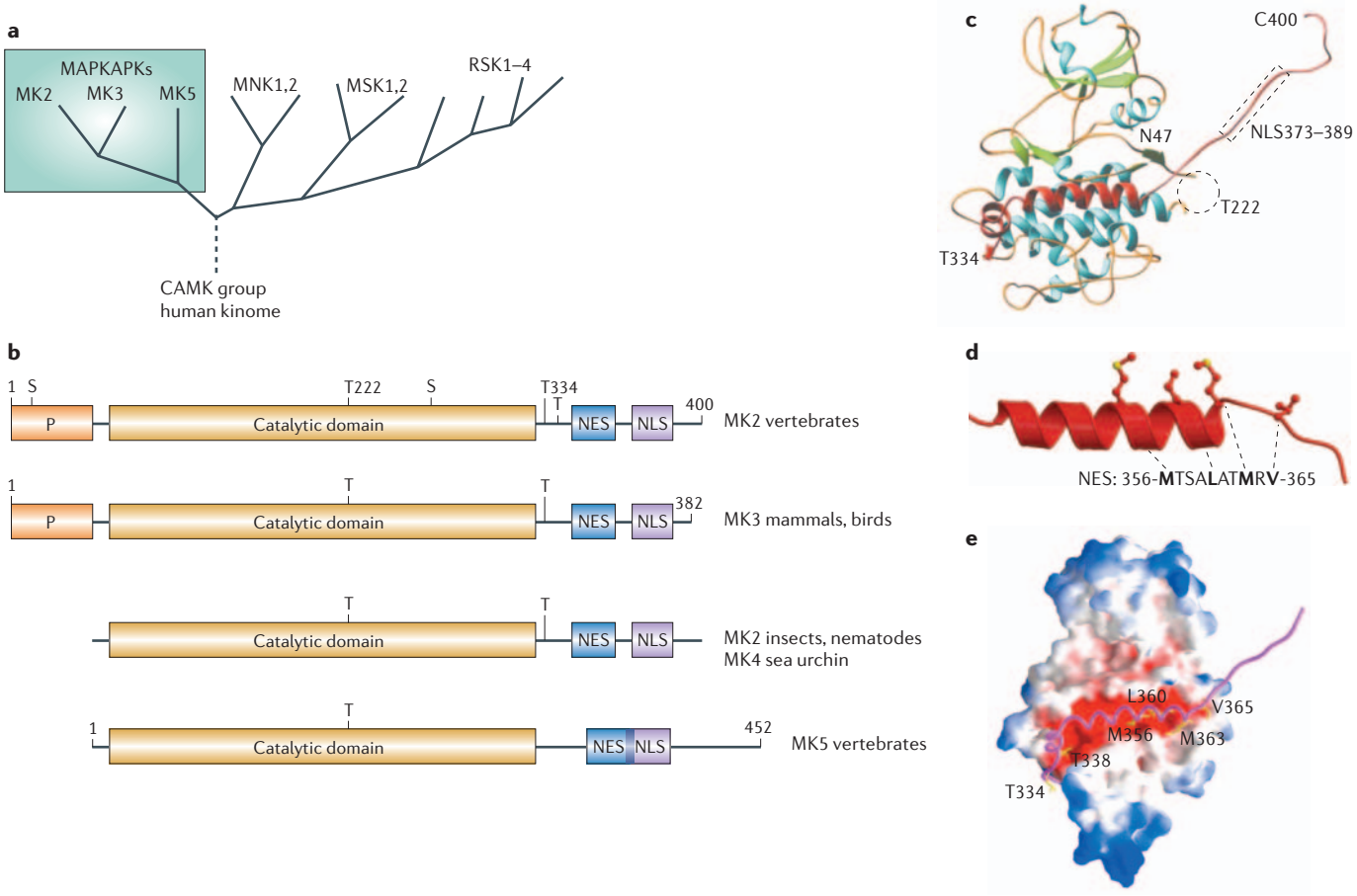

Figure 1 | **Definition and Structure of MKs. a** | Phylogenetic tree of protein kinases of the calcium/calmodulin-dependent protein-kinase (CaMK) supergroup that function downstream of mitogen-activated protein kinases (MAPKs). This family includes the ribosomal-S6-kinase subfamily (RSK1–4), the mitogen- and stress-activated-kinase subfamily (MSK1,2), and MAPK-interacting kinases -1 and -2 (MNK1,2). The MAPK-activated protein kinases (MAPKAPs or MKs) are represented by MK2, MK3 and MK5. **b** | Schematic primary structure of MKs. Sequence identities between the full-length human MK2/MK3 and MK2/MK5 are 75% and 42%, and between the catalytic domains alone are 78% and 48%, respectively. Ser/Thr (S/T)-phosphorylation sites are shown and the numbers represent the amino acids in the human sequence. The nuclear localization sequence (NLS) and the nuclear export sequence (NES; see BOX 1) are shown. **c** | Three-dimensional structure of an inactive MK2 fragment (amino acids 47–400) in the closed conformation and localization of the main phosphorylation sites T222 and T334. T222 is in the flexible activation loop, which is unstructured and missing in the drawing. T334 is in the hinge region between the catalytic core and the C-terminal helix. The C-terminal helix is shown in red[52,53]. **d** | The part of the MK2 C-terminal helix (amino acids 356–365), which contains the NES hydrophobic residues (shown in bold) orientated to the same side of the helix[52]. **e** | Masking of the NES in a hydrophobic pocket of the catalytic core[52]. Part **c** of the figure is reproduced, with permission, from REF. 53 © (2003) Elsevier. Parts **d** and **e** of the figure are reproduced, with permission, from REF. 52 © (2002) the American Society for Biochemistry and Molecular Biology.

Identification and primary structure of MKs

MK2 was the first MK enzyme to be purified and its primary structure was determined by cDNA sequencing almost 15 years ago[19–21]. A subsequent search for its activator led to the identification of the p38 MAPK two years later[22,23]. MK3/3pK was identified and analysed by two different laboratories simultaneously — as a novel substrate for p38α/β[24], and as a new MK that is located on human chromosome 3p21.3 in a small-cell lung cancer tumour-suppressor gene region[25]. Finally, two years later, MK5 was described as PRAK[26], and as a kinase that was activated by ERK and p38, but not by Jun N-terminal kinases (JNKs) *in vitro*[27].

Genome analysis from different model organisms revealed that the catalytic domain and the C-terminal regulatory part of MK2 are conserved in the worm, the fruitfly and mammals, whereas the N-terminal proline-rich region is only present in vertebrates (FIG. 1b). The genes encoding MK3 and MK5 are absent from the worm and the fruitfly. MK5 is expressed in all vertebrates, but the expression of MK3 is restricted to birds and mammals, which indicates that this gene might have arisen from a relatively late gene-duplication event. In addition, the term 'MK4' has been used for a protein from the sea urchin, which might be a structural homologue of MK2 (REF. 28). Yeast lack obvious structural homologues of

Box 1 | **Regulation of nuclear–cytoplasmic transport by phosphorylation**

Many proteins carry mono- or bipartite basic nuclear localization signals (NLSs, see below) and/or nuclear export signals (NESs, see below; conserved hydrophobic residues are shown in bold) with variable hydrophobic residues. Nuclear import of NLS-containing proteins is mediated through binding to the cytosolic importins. This is followed by docking to nuclear pore complexes and subsequent translocation of the importin–cargo complex into the nucleus. Similarly, nuclear export is mediated by the interaction of nuclear NES-containing proteins with the exportins.

Nuclear transport can be regulated through intra- or intermolecular masking of the NES or NLS of a protein as a result of altered phosphorylation. Intramolecular masking of a NLS by phosphorylation has been shown for the nuclear factor of activated T cells (NFAT) — it translocates to the nucleus after dephosphorylation by calcineurin[105]. Intramolecular de-masking of a NES by phosphorylation and nuclear export takes place for mitogen-activated protein kinase (MAPK)-activated protein kinase (MK)2 and MK3. Intermolecular masking can also be regulated through phosphorylation. For example, IκB — the inhibitor of the Rel-homology-domain transcription factor NFκB — masks the NLS of NFκB and prevents its translocation into the nucleus until IκB is released and degraded as a result of phosphorylation. Other mechanisms that are involved in the regulation of nuclear transport are the retention of NLS/NES-containing proteins in the cytoplasm or nucleus (for example, MK5; REFS 17,18), and increased affinity of the proteins for the importins or the exportins as a result of phosphorylation (for example, the fruitfly morphogen Dorsal[106]).

- NLS: R/K-R/K-R/K-R/K-R/K-R/K
- Bipartite NLS: R/K-R/K-$(X)_{10}$-R/K-R/K-R/K-R/K-R/K-R/K
- NES in cAMP-dependent protein-kinase inhibitor-α (PKIα): **L**-A-**L**-K-**L**-A-G-**L**-D-**I**
- NES in MK2: **M**-T-S-A-**L**-A-T-**M**-R-**V**

Importins
Proteins that facilitate the nuclear import of cargo proteins. In many cases, importin-α binds to the NLS of the cargo protein, and then importin-β binds to and translocates the complex through the nuclear pore. In the nucleus, Ran•GTP displaces importin-β and the cargo protein is released.

Exportins
Proteins that facilitate the nuclear export of proteins and mRNA. Exportins bind to their cargo in the nucleus together with Ran•GTP. After translocation through the nuclear pore, GTP is hydrolysed and the cargo is released.

Src-homology-3 (SH3) domain
A protein domain of approximately 60 amino acids that has homology to an N-terminal region of the protein tyrosine kinase Src. There are more than 250 SH3 domains in the human proteome, which can bind to proline-rich protein regions that form a left-handed helix with the minimal consensus motif P-X-X-P (where X is any amino acid).

Nuclear export signal
(NES). An amino-acid sequence that often contains several leucines or other hydrophobic residues and interacts with CRM/exportin-1, thereby functioning as a signal to facilitate export from the nucleus.

Nuclear localization signal (NLS)
An amino-acid sequence that often consists of a bipartite arrangement of basic amino acids and interacts with importin-α, thereby functioning as a signal to facilitate import into the nucleus.

MKs, but functionally homologous kinases with high structural similarity to the catalytic domain of the CaMK superfamily do exist[1] — these are Rck1 and Rck2 (REF. 29) in budding yeast and Srk1 (also known as Mkp1) and Mkp2 (REF. 30) in fission yeast, and they are activated by the MAPK homologues Hog1 or Sty1, respectively.

Primary structure. In the primary structure, MKs share high homology within the catalytic domain (FIG. 1b). A conserved regulatory phosphorylation site within the activation loop (T222 in human MK2) exists in all the MKs. Another important regulatory phosphorylation site is located in the hinge region between the catalytic domain and the C-terminal regulatory domain (T334 in human MK2), but this site is not conserved in MK5. Vertebrate MK2 and MK3 contain an N-terminal proline-rich region that interacts with Src-homology-3 (SH3) domains *in vitro*[31]. This region is not conserved in fruitfly or worm MK2 and is also not present in vertebrate MK5.

The C termini of MKs contain sequences that mediate subcellular targeting to the nucleus and to the cytoplasm (known as the nuclear export signal (NES) and the nuclear localization signal (NLS); see BOX 1). The NLS and NES are located in distinct C-terminal regions of MK2 and MK3, whereas, in MK5, the two signals overlap (FIG. 1b). Interestingly, the C termini of MK2 and MK3 can also have an auto-inhibitory function for the catalytic domain[32,33]. The basic residues of the C-terminal NLS of all the MKs form the core docking domain for p38 MAPKs, which bind to this region through their common docking motif, and modulate the function of the NLS[34,35].

Do isoforms matter? MK mRNAs are expressed at detectable levels in most tissues and cells that have been analysed[21,26]. Furthermore, different protein isoforms have been described for MK2 and MK5. SDS-PAGE[19,36] and chromatography[37] revealed two distinct isoforms for MK2, which migrate with comparable intensity and might arise due to differential splicing, or, more likely, due to post-translational modifications or limited proteolysis of MK2. Both isoforms bind to p38α and show p38α-dependent activation (A. Kotlyarov and M.G. unpublished observations). A rare human *MK2* cDNA has also been cloned, which encodes an alternative C terminus that lacks the NES, the NLS and, therefore, the p38α/β-docking domain[38]. Although the alternative coding mRNA region is located in the *MK2* gene, this sequence is not adequately represented in expressed sequence tag (EST) libraries, so this cDNA is probably the result of a rare alternative-splicing event of the *MK2* pre-mRNA.

Two different isoforms of human MK5 have been described that differ in two amino acids in the C-terminal extension, which is unique to MK5. In the mouse, five MK5 isoforms have been detected; they result from a combination of the lack of the two amino acids in the C-terminal region and the deletion of an N-terminal part of the catalytic domain (B. Allen, unpublished observations).

Regulation of the different MKs

Activation of MKs by phosphorylation is paralleled by significant conformational changes, specific protein interactions and subcellular translocations, which proceed, depending on the MK considered, in a simultaneous or sequential manner. Our knowledge of the regulatory phosphorylation sites and the three-dimensional structure of MK2 can explain at least some of the molecular mechanisms that are involved. MKs are activated by one (MK5; REF. 26) or two to three (MK2 and MK3; REFS 21,39,40) important regulatory phosphorylations. MKs show binding to and activation by p38, but activation by the ERKs or even the JNKs has also been observed *in vitro* and in overexpression studies[27,39]. These discrepancies were only resolved when MK-specific antibodies

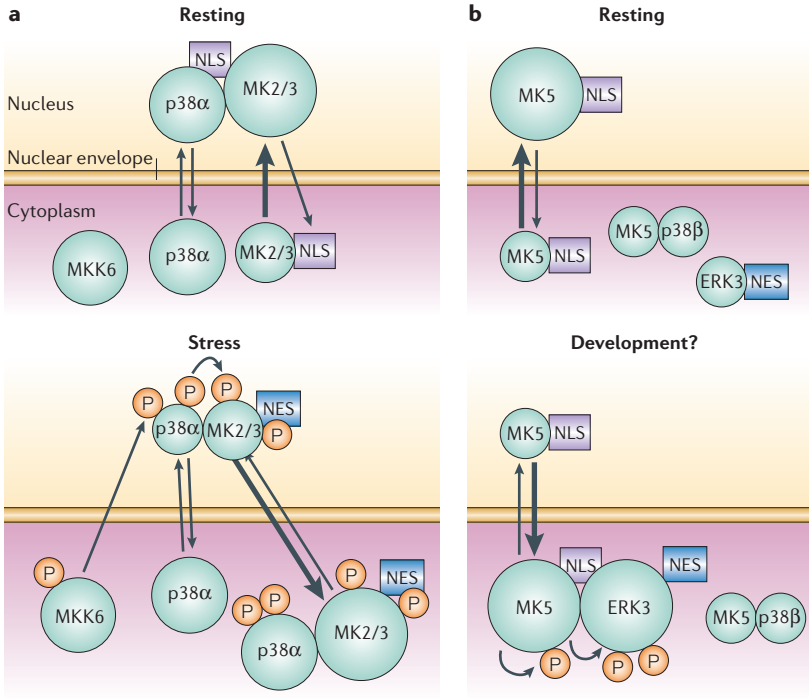

Figure 2 | **Models for activation and translocation of MKs.** The size of the circles represents the subcellular steady-state concentrations of mitogen-activated protein kinase (MAPK)-activated protein kinases (MAPKAPs or MKs), and other proteins that they interact with, before and after their activation. **a** | Simultaneous p38α-dependent activation and translocation of MK2/3. In resting cells, a MK2/3–p38α complex exists in the nucleus, owing to a specific interaction between p38α and MK2/3 — the MK2/3 nuclear localization signal (NLS) is functional whereas the nuclear export signal (NES) is masked. After stress stimulation, MAPK kinase-6 (MKK6) phosphorylates and activates p38α, which, in turn, phosphorylates and activates MK2/3 in the nuclear complex. During the activation of MK2/3, the NES is exposed and the complex of active p38α and MK2/3 is actively exported from the nucleus. A steady state is then reached, in which active MK2/3–p38α is mainly in the cytoplasm of the cell. **b** | Sequential translocation and activation of MK5 by the cytoplasmic extracellular-regulated kinase-3 (ERK3). In resting cells, MK5 is mainly localized in the nucleus owing to a functional NLS, whereas ERK3, which has a NES, is instable and hardly detectable in the cytoplasm. In specific stages of development, ERK3 is stabilized and accumulates in the cytoplasm, where it interacts with MK5, anchors it in the cytoplasm and changes the steady-state distribution of MK5 to be more cytoplasmic. Through autophosphorylation or other as-yet-unknown mechanisms, cytoplasmic MK5 is activated and, in turn, phosphorylates its binding partner ERK3. MK5 also interacts with p38β *in vitro*, mainly in the cytoplasm.

and enzyme inhibitors as well as MK-knockout cells became available, as these reagents allowed the study of the mechanism of activation of endogenous MKs in non-transfected cells *in vivo*.

MK2/3 as a bifunctional switch of activation and translocation. By using small-molecule pyrimidyl imidazol compounds of the SB203580 family of cytokine-suppressive anti-inflammatory drugs[41] — in concentrations where they specifically inhibit p38α/β — it was shown that stress and cytokine-dependent activation of MK2 and MK3 was exclusively dependent on p38α/β[42]. Furthermore, MK2 activation by the antibiotic anisomycin and the oxidative-stress stimulant arsenite was almost completely impaired in p38α-deficient embryonic stem cells[43]. The tight binding between MK2 and p38α enabled biochemical identification of a stable p38α–MK2 complex[35,44]. The expression levels of p38α are significantly reduced in MK2-deficient tissues and cells, and MK2 expression is reduced in *p38α*-knockout-mouse embryonic fibroblasts, which indicates that the proteins are mutually stabilized by forming a complex[45,46]. So far, it is unclear whether the residual p38α protein in MK2-deficient cells is stabilized through its interaction with MK3.

All MKs are able to shuttle between the nucleus and the cytoplasm due to their C-terminal NES and NLS[44,47–51]. The co-existence of a regulatory phosphorylation site, an autoinhibitory domain and a NLS-overlapping MAPK-binding site within the C terminus of MKs indicates a complex interdependence of activity and subcellular localization. Accordingly, their catalytic activity and their interaction with MAPKs might influence their subcellular localization. Indeed, MK2 and MK3 are clearly bifunctional switches that couple their activation by phosphorylation with the nuclear export of themselves and of their activator to the cytoplasm. The inactive conformation of MK2 (REFS 52,53) (FIG. 1c), shows that the C-terminal autoinhibitory helix (in red) tightly binds to the catalytic core of MK2. The regulatory phosphorylation site T334 is located in a hinge region between the catalytic core and the autoinhibitory helix. Furthermore, it becomes obvious that the NES in this helix is masked by binding to and hiding its core residues (M356, L360, M363 and V365) in a hydrophobic pocket of the catalytic domain[52] (FIG. 1d,e). The more C-terminal part interferes with the activation loop and substrate-binding site and contains a NLS and a p38α/β-docking site, which are still accessible (FIG. 1c).

MK2 and p38α exist as a preformed inactive complex in the nucleus, which indicates that the complex has a functional NLS[44] or is too large for permeation of the nuclear pore by diffusion. After activation by an upstream kinase — for example, MAPK kinase-6 (MKK6) — p38α phosphorylates MK2 at the regulatory sites. In particular, phosphorylation of T334 leads to the weakening of the interaction between the C-terminal helix and the catalytic core and, subsequently, the unmasking of the NES[48,54]. As a result, the activated MK2–p38α complex translocates to the cytoplasm and a new steady state is reached — the phospho-MK2–phospho-p38α complex is mainly localized in the cytoplasm (FIG. 2a). This process probably represents a mechanism of increasing the specificity of signalling whereby a cytoplasmic substrate is separated from the basal activity of a nuclear enzyme and can only be reached by the enzyme after complete activation and translocation. Alternatively, this could be a mechanism for rapid downregulation of a short pulse of kinase activity in the nucleus. As the C termini of MK2 and MK3 share high similarity, it can be postulated that this mechanism is also operating for MK3. Regulation of MK2/3 clearly involves catalytic stimulation by phosphorylation within the activation loop (on residue T222 in humans) and some other minor (auto)-phosphorylations[33,40].

Anisomycin
An antibiotic that inhibits protein synthesis and, at lower concentrations, stimulates stress signalling.

Box 2 | **Gene regulation by the polycomb group (PcG)**

Polycomb group (PcG) proteins participate in the repression of homeotic genes that contain PcG-response elements. These proteins form large dynamic protein complexes, in which most individual components do not display DNA-binding activity[107,108]. Two cooperating PcG complexes that are involved in chromatin remodelling have been identified; the polycomb intitiation complex (also known as the polycomb repressive complex (PRC)2) and the polycomb maintenance complex (also known as PRC1). PRC2 has histone-modifying enzymatic activity, whereas it has been postulated that PRC1 specifically interacts with modified histones to maintain gene repression. The localization and activity of PcG proteins, such as the oncoprotein BMI1 (REF. 109), is regulated by phosphorylation. *Bmi1* and *Mel18* mouse knockouts show defects in the self-renewal of haematopoietic stem cells (HSCs), whereas in RAE28-deficient mice enhanced HSC self-renewal is observed (*Rae28*, is the mouse homologue of human polyhomoetic-1 (*HPH1*); reviewed in REF. 110). This demonstrates the complex role of PcG proteins in mammalian systems.

Activation of MK5: cytoplasmic anchoring. Understanding the regulation of the activity and the localization of MK5 was more puzzling than for the other MKs for several reasons. The formation of the complex between MK5 and p38α does not proceed with the same efficiency as for MK2, and there is no reduction in the levels of p38α in MK5-deficient cells[55]. Furthermore, the NLS and NES directly overlap, making separate regulation unlikely and there is no regulatory phosphorylation site present in the hinge region between the catalytic core and the C terminus. Finally, it is still controversial whether endogenous MK5 is present in the nucleus or the cytoplasm of resting cells, whereas overexpressed tagged versions of MK5 clearly accumulate in the nucleus[49,51].

The first indication that MK5 behaves differently from MK2/3 derived from its preference for p38β during *in vitro* activation[26]. Interestingly, p38β is mainly localized to the cytoplasm, and, when co-expressed with green fluorescent protein (GFP)-tagged MK5, it leads to the translocation of this fusion protein to the cytoplasm by docking to and masking the NLS of MK5 (REFS 49,51). In parallel, MK5 phosphorylation in the activation loop of the catalytic domain at T182 might allosterically activate its NES and contribute to MK5 cytoplasmic localization[49]. However, formation of the p38β–MK5 complex seems to be dominant for the translocation, because a MK5 mutant, which still carries a NES and a NLS, but does not bind to p38β with high affinity, does not accumulate in the cytoplasm even after T182 phosphorylation[51]. Cytoplasmic anchoring of MK5 by p38β is independent of tumour necrosis factor (TNF)-stimulated p38α/β activity[49], so, it is unlikely that MK5 functions as a bifunctional switch of simultaneous stress-dependent activation and translocation as MK2 and MK3 do.

Recently, the atypical MAPK ERK3 was identified as a specific interaction partner for MK5 (REFS 17,180). Although the activation of ERK3 by phosphorylation remains enigmatic, ERK3 is mainly cytoplasmic and is regulated by the ubiquitin–proteasome pathway[56,57]. Expression of ERK3 leads to the cytoplasmic anchoring and subsequent activation of MK5 (FIG. 2b) — MK5 activation by ERK3 is more efficient than activation by p38β. In contrast to p38β, ERK3 does not require the basic residues of the docking motif in MK5 and binds to a more C-terminal region of MK5 (REF. 17). So far, it is not clear whether the catalytic activity of ERK3 is necessary for the activation of MK5 or whether MK5 first autophosphorylates within the cytoplasmic ERK3 complex and subsequently also phosphorylates ERK3 at several sites[17,18].

It is possible that the regulation of MK5 is similar to the regulation of LKB1 — the Ser/Thr protein kinase that is mutated in Peutz–Jeghers cancer syndrome — which autophosphorylates within a cytoplasmic complex with the pseudokinase STRAD (sterile-20-related adaptor). STRAD lacks amino acids in the kinase catalytic domain that are indispensable for enzyme activity. However, it activates LKB1 by complex formation and nucleo–cytoplasmic translocation[58]. Both the STRAD–LKB1 and ERK3–MK5 signalling molecules could provide examples for a general mechanism through which sustained protein-kinase activation can be achieved by a cytoplasmic binding partner, which is regulated through protein stability rather than phosphorylation. The levels of ERK3 are strongly reduced in MK5-deficient cells[17] and MK5 activity is impaired after ERK3 depletion[18], which highlights the *in vivo* relevance of the ERK3–MK5 complex.

Phosphorylation motif and substrates

Although MK2 was purified using a peptide from glycogen synthase as a substrate[19], the first protein substrates that were identified for MKs were the human and mouse small heat-shock proteins (Hsps) HSP27 and HSP25 — these proteins were phosphorylated by MK2 at three and two serine residues, respectively[59]. The sites are located in an optimal substrate-recognition consensus sequence L-L-X-R-X-X-pS[20], which has been recently extended to (L,F,I,V)-(Q,A,M)-R-(Q,M)-(L,N)-S(p)-(I,V,F,L) using a peptide library that contains a fixed R in the –3 position (X-X-X-X-R-X-X-S-X-X-X-X)[13]. However, in this library, X was not S, T, Y or C and, therefore, these amino acids are not represented in this extended motif. The combination of library results, kinetic analysis and known substrate sequences led to the motif being refined to (L/F/I)-X-R-(Q,S,T)-L-pS/pT-hydrophobic.

MK3 and MK5 seem to use a recognition sequence that is similar[39], or identical[26,42], to that of MK2, and are also able to phosphorylate the same sites within HSP27 *in vitro*[24,26]. However, phosphorylation of HSP25 is strongly reduced in stress-stimulated MK2-deficient cells[8], whereas it is not affected in MK5-deficient cells[55]. This observation indicates that MK2 is the main HSP25-kinase *in vivo*.

Several other substrates for MKs have been identified, which illustrates the ability of MKs to couple the p38 MAPK cascade to different targets, depending on the cell type (see online supplementary information S1 (table)). The MK substrates include enzymes[60,61], proteins that interact with the cytoskeleton[62,63], mRNA-binding proteins[64,65,10,11], transcription factors[47,66,67], and regulators of the cell cycle and apoptosis[13,14,68]. So far, the extent of the physiological relevance of most of these substrates remains to be analyzed *in vivo*.

Homeotic genes
Genes that specify how structures develop in different segments of the body during embryogenesis. Homeotic genes are often organized in clusters and encode DNA-binding proteins.

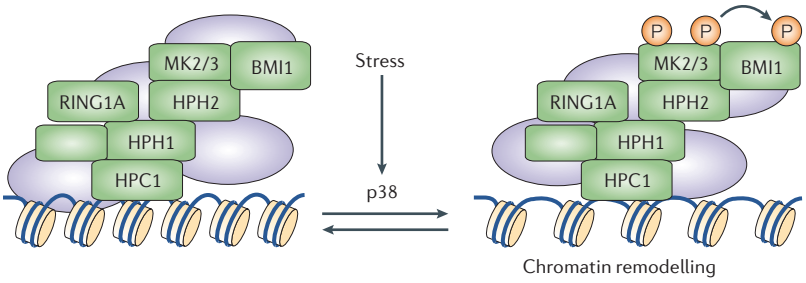

Figure 3 | **MK2 and MK3 interact with components of the polycomb repressive complex.** MK2 and MK3 bind to the human polyhomeotic protein-2 (HPH2), which is a component of the large and dynamic polycomb repressive complex-1 (PRC1). PRC1 contains several components, including human polyhomeotic protein-1 (HPH1), human polycomb-1 (HPC1), and the RING-finger-domain protein RING1A. After stress stimulation, MK2 and MK3 are activated by p38 MAPK and phosphorylate proteins of the complex, such as the oncoprotein BMI1. These interactions result in changes in structure and composition of PRC1 and induce chromatin remodelling, and possibly transcriptional derepression.

Biological functions of the MKs

The substrates of MKs (see online supplementary information S1 (table)) indicate a role for these enzymes in diverse cellular processes, such as reorganization of the cytoskeleton, cell migration, cell-cycle regulation and chromatin remodelling. The physiological evidence for these roles and their underlying mechanisms are discussed below.

MKs in actin remodelling. Stress-dependent phosphorylation of small Hsps by MKs has been associated with modification of their oligomerization and chaperone properties[69], as well as with regulation of the actin cytoskeleton[70]. HSP27 oligomers are ATP-independent chaperones[71], which keep unfolded proteins in a folding-competent state before these proteins can be refolded by the ATP-dependent chaperone HSP70. As a result of phosphorylation, large HSP27 oligomers decay to dimers or tetramers that have a lower binding capacity for unfolded proteins. This might provide a mechanism for facilitating the transfer of unfolded proteins to HSP70 or for clearance of HSP27 from otherwise irreversibly bound proteins[72]. Whether the chaperone properties of HSP27 are also responsible for its function in stabilizing the actin filaments is unclear.

As for HSP25, it has been postulated that non-phosphorylated HSP25 binds to, or chaperones, the barbed, rapidly growing ends of actin filaments, stabilizes them, and inhibits further actin polymerization. After phosphorylation, HSP25 is released from the barbed ends and actin polymerization and remodelling might take place concomitantly with the binding of many other proteins to actin[73]. An alternative mechanism of HSP25 action in actin remodelling could involve its binding to 14-3-3 proteins (see below). Some substrates of MKs — such as lymphocyte-specific protein-1 (LSP1; REF. 74), F-actin-capping protein Z-interacting protein (Cap-ZIP)[75] and the p16 subunit (p16-Arc) of the seven-member actin-related protein-2/3 complex (Arp2/3)[76]— are involved in actin remodelling. Other substrates — such as vimentin[63] and αB-crystallin[62]— could interact with intermediate filaments and microtubules. This opens the possibility for complex regulation of cytoskeletal architecture by the MKs. Whether there is a specific role for a certain MK in actin remodelling remains unclear. MK2 is the main HSP25 kinase, therefore it might be the main component in this regulation.

MKs in cell migration and development. Remodelling of the cytoskeleton is a prerequisite for changes in cell shape and for cell migration. The phosphorylation of HSP27 contributes to cell motility[77], therefore, it was not surprising that MK2-deficient neutrophils showed defects in chemotaxis and altered chemokinesis[78], and that migration of different MK2-deficient cell types was compromised in a Boyden-chamber assay. Interestingly, rescue of the migratory phenotype requires the proline-rich N-terminal region of MK2, whereas rescue of other functions of MK2 (see below) does not require this region[45]. Directed cell migration is important for embryonic development, but migratory defects were not detected in MK2-deficient animals, which indicates that MK3, which also contains a proline-rich domain, might be able to compensate for the loss of MK2 during mouse development.

However, MK5-deficient animals show developmental defects that, depending on the genetic background, lead to embryonic lethality with incomplete penetrance between embryonic day (E)11.5 (REF. 17) and birth (A. Kotlyarov and M. Gaestel, unpublished observations). The spatio-temporal co-expression of MK5 and ERK3 during mouse development[17,79], and the ability of ERK3 to cause sustained activation of MK5, further supports the idea of the functional importance of this complex during embryonic development.

MKs in the regulation of the cell cycle. The budding yeast functional MK homologues (Rck1 and Rck2) suppress cell-cycle-checkpoint mutations when expressed in fission yeast by prolonging the G2/M transition[29]. But can MKs also contribute to cell-cycle regulation in multicellular organisms? This idea was supported by the finding that the p38 MAPK cascade is involved in cell-cycle regulation in mammalian cells, especially in ultraviolet light (UV)-induced phosphorylation of the CDC25B phosphatase, which is responsible for the G2/M checkpoint[80]. In addition, a genome-wide small interfering (si)RNA survey of protein kinases that are involved in cell-cycle progression in fruitflies[15], revealed that of the 228 protein kinases that were analysed, downregulation of only 80 members, including the fruitfly homologue of MK2, led to cell-cycle defects. MK2 knockdown was associated with chromosomal-alignment defects and spindle abnormalities during mitosis in fruitfly S2 cells. Whether these defects are also observed in MK2-deficient flies, which are viable but show increased sensitivity to high-salt feeding (G. Seisenbacher, H. Stocker and E. Hafen, unpublished observations), remains to be determined.

It has been recently shown that MK2 directly phosphorylates CDC25B and CDC25C at relevant sites in

Chaperone (molecular chaperone)
Proteins that assist the folding of nascent proteins, or the re-folding of partially misfolded proteins, without being part of the final folded protein structure.

Barbed end
The rapidly growing end of the polar actin microfilaments at which globular actin polymerizes.

14-3-3 proteins
A family of ~30-kDa proteins that form cup-like structures that bind to discrete phosphoserine-containing motifs. They are present in fungi, plants and mammals and, in mammals, nine structurally related family members have been identified.

Boyden chamber
An experimental setting that was developed by Stephen Boyden in 1961 to test the chemotactic activity of motile cells *in vitro*. Two compartments are separated by a membrane of such pore size that only actively migrating cells can pass through it; a gradient of the chemotactic substance is applied, and the number of cells that pass through the membrane is determined.

Box 3 | **AU-rich elements in mRNA**

AU-rich elements (AREs) were identified in the 3′ untranslated region (3′ UTR) of unstable mRNAs and are also able to destabilize reporter mRNAs when transferred to their 3′ UTR [111]. AREs represent the most common *cis*-acting element of mRNA stability in mammalian cells and can be regulated in response to extracellular signals such as phorbol esters, calcium ionophores, cytokines and inhibitors of transcription[112]. According to the number of overlapping AUUUA motifs, AREs are clustered in five groups (I–V) that contain five, four, three, two or one pentameric repeats. mRNAs of group I have 5 or more overlapping motifs and members of this group are cytokines such as tumour-necrosis factor, interleukin (IL)-1β, IL-11, granulocyte/macrophage colony-stimulating factor (GM-CSF) and the oncoprotein PIM1. The recently constructed human AU-rich-element-containing mRNA database includes more than 1,000 human transcripts that contain AREs[113].

Different ARE-binding proteins have been identified that mainly stabilize, destabilize or influence the translation of ARE-containing mRNAs. Stabilizing proteins include RNA-binding protein homologous to human A–D or R antigen (HuA–D/R; which are also known as embryonic-lethal normal vision (ELAV)1–4); destabilizing proteins include heterogeneous nuclear ribonucleoprotein (hnRNP)A1, -A2, -C and -D (which is also known as AU-rich element binding factor (AUF)1), tristetraprolin (TTP), K homology-type splicing regulatory protein (KSRP) and butyrate-response factor (BRF1); and influencing proteins include T-cell-restricted intracellular antigen-1 (TIA1) and TIA1-related protein (TIAR). The p38 mitogen-activated protein kinase (MAPK) cascade has also been shown to regulate the stability of approximately 40 AU-rich mRNAs after stimulation of the human monocytic cell line THP-1 with bacterial lipopolysaccharide (LPS)[114]. An attractive hypothesis for the action of the p38 MAPK cascade is that it stimulates the modification of ARE-binding proteins by phosphorylation.

UV-treated osteosarcoma cells, and that knockdown of MK2 leads to loss of the G2/M checkpoint[13]. Therefore, in these cells, MK2 can be considered as the third member of the DNA-damage-checkpoint-kinase family that functions in parallel with CHK1 and CHK2. This idea is supported by the finding that, in fission yeast, a functional homologue of MKs, the kinase Srk1/Mkp1 phosphorylates Cdc25 at the same sites as Chk1 and Chk2 (REF. 81).

The tumour-suppressor protein p53, which is essential for cell-cycle regulation at G1/S and entry into apoptosis, is also known to be a target of the p38 MAPK cascade[82]. Interestingly, p53 is activated through the p38 pathway and is a direct substrate of p38, whereas the p53-interacting ubiquitin ligase HDM2 (which is involved in the degradation of p53) was recently identified as a target of MK2 (REF. 14). Phosphorylation of HDM2 by MK2 leads to HDM2 activation and increased degradation of p53, which resembles the activation of HDM2 by the survival kinase AKT/protein kinase B (PKB). It was therefore proposed that MK2 dampens the activity of p53 after short stimulation by p38-phosphorylation and contributes to the fine tuning of the DNA-damage response. Whether MK3 and MK5 also contribute to the regulation of the cell cycle remains to be determined.

MKs in chromatin remodelling via polycomb. The human polyhomeotic protein-2 (HPH2) was identified as a specific interaction partner for both MK2 and MK3 in yeast two-hybrid screens[16,83]. HPH2 is a component of the polycomb repressive complex-1 (PRC1), which contributes to the stable silencing of specific genes through maintenance of chromatin modifications after initiation of silencing by PRC2 (REF. 84) (BOX 2). Together with the human polycomb proteins (HPC1–3) and the proteins RING1A and BMI1, HPH2 forms the core functional group of a highly dynamic repressive complex with different combinations of associated components. BMI1 can be phosphorylated by MK3 *in vitro*[16], therefore, it can be postulated that MK3 binds to the complex through HPH2, and directly phosphorylates BMI1 *in vivo*; this leads to dynamic changes in PRC1 and modulation of transcriptional repression (FIG. 3). The phosphorylation-dependent nuclear export of MK2 and MK3 does not contradict this scenario, as it changes the steady state of nuclear/cytoplasmic distribution but still allows the existence of a remaining subpopulation of activated enzyme in the nucleus[85]. Interestingly, BMI1-deficient mice show defects in the self-renewal of haematopoietic stem cells[86], but whether MKs are also involved in the regulation of stem-cell renewal remains to be investigated.

Polycomb repressive complex-1 (PRC1). One of the two subcomplexes (PRC1 and PRC2) that cooperate to form the nuclear polycomb repressive complex (PRC), which has a role in gene silencing during development.

Post-transcriptional regulation in inflammation

MK2-deficient mice show an increased resistance to endotoxic shock, owing to an impaired inflammatory response, as well as a decreased production of cytokines such as TNF and interleukin (IL)-6 upon LPS-stimulation[8]. But, on the other hand, they also show increased susceptibility to bacterial infection[87] and exacerbation of pathology in a genetic model of inflammatory bowl disease[88]. It is now clear that MK2 is the main target of p38α/β, and this signalling pathway regulates the stability and translation of *TNF* and *IL-6* mRNAs[8] through a process that involves the AU-rich elements (AREs; see BOX 3) in the 3′ non-coding region of these mRNAs[89]. Tristetraprolin (TTP), which is a protein that controls the stability and translation of *TNF* mRNA[90], has been recently identified as a direct substrate for MK2 (REFS 10,11). Phosphorylation of TTP by MK2 increases its stability[115,116] and its binding to 14-3-3 proteins (see below), and thereby stimulates TNF expression. Other targets of MK2 that might contribute to increased *TNF*-mRNA translation are the heterogeneous nuclear ribonucleoprotein A0 (hnRNP A0)[64] and the polyA-binding protein-1 (PABP1)[65], which both could be involved in the assembly of the ring-like structure of actively translated mRNA.

MK2 also regulates the stability of other mRNAs, such as those encoding urokinase plasminogen activator (uPA)[91], cyclooxygenase-2 (REF. 92), IL-6 (REF. 89) and IL-8 (REF. 7). Depending on the specific mRNA,

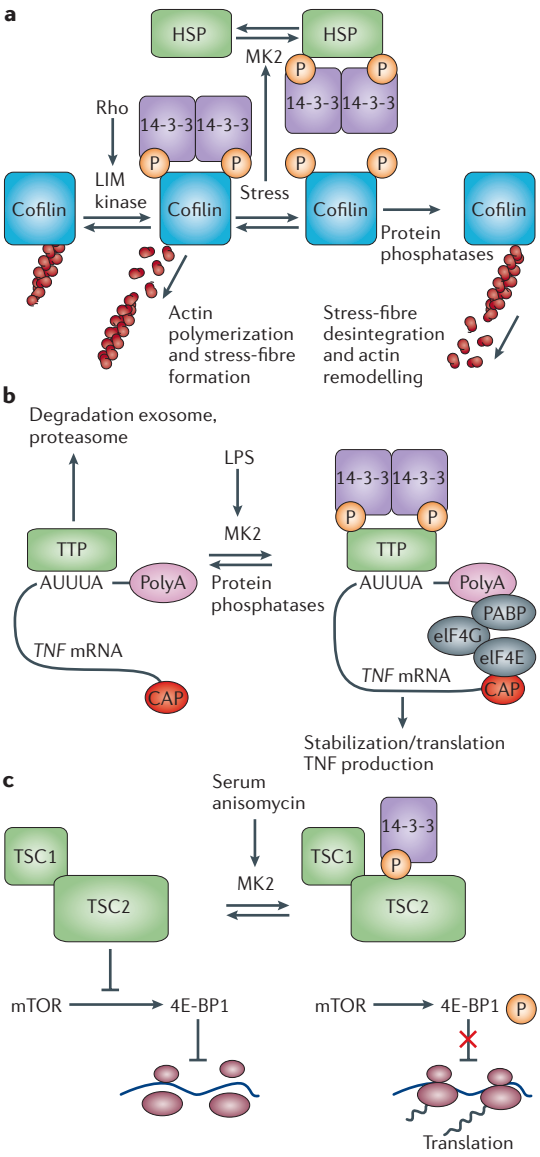

Figure 4 | **14-3-3-protein-dependent mechanisms of MK action. a** | Regulation of actin depolymerization through competitive interactions between 14-3-3 proteins, small heat-shock proteins (Hsps) and cofilin. Activation of the small G protein Rho results in phosphorylation of cofilin by a LIM kinase (which contains a LIM protein-protein-interaction domain) and its release from the barbed ends of the actin filaments, which can now rapidly grow resulting in stress-fibre formation. Cofilin, in its inactive phosphorylated conformation, is bound to 14-3-3 proteins and its dephosphorylation by protein phosphatases is inhibited. After stress stimulation, small Hsps are phosphorylated and compete with phosphorylated cofilin for 14-3-3-protein binding. This results in the dephosphorylation of cofilin and its binding to the barbed ends of actin, which thereby blocks actin polymerization. **b** | Bacterial lipopolysaccharide (LPS)-induced stability and translation of AU-rich element (ARE)-containing mRNAs is mediated through the interaction of tristetraprolin (TTP) with 14-3-3 proteins (AREs are shown as AUUUA in the figure). TTP targets ARE-containing mRNAs to mediate their degradation. After stimulation with LPS, TTP is phosphorylated, binds to 14-3-3 proteins and is excluded from the sites of mRNA degradation. This results in the pre-formation of a translatable mRNA — binding of CAP-binding protein (CAP), eukaryotic translation-initiation factor 4E (eIF4E) and polyA-binding protein (PABP) occurs, and further stabilization by eIF4G, and probably also 14-3-3 proteins, might take place. **c** | The hamartin (TSC1)–tuberin (TSC2) tumour-suppressor complex inhibits translation by inhibiting the phosphorylation of the eIF4E-binding protein-1 (4E-BP1) by the mammalian target of rapamycin (mTOR). TSC2 is phosphorylated by the upstream kinase MK2. This stimulates its binding to 14-3-3 proteins and neutralizes the inhibitory function of the TSC1–TSC2 complex on 4E-BP1 phosphorylation, which results in derepression of protein translation.

Kaposi's sarcoma
Cancer of the connective tissue that was named after the person who described it — the Hungarian dermatologist Moritz Kaposi (1837–1902) — and is caused by infection with the herpes virus, especially in patients with severe immunodeficiencies.

23-nucleotide GC-rich direct repeats
The kaposin locus of herpes virus contains a small coding region (open reading frame (orf) k12) preceded by two families of 23-nucleotide GC-rich direct repeats (termed DR1 and DR2). It is transcribed as a single mRNA and a complex translational programme generates various proteins from this mRNA. Kaposin B results from translation of the repeats alone and consists of a series of tandemly repeated copies of 23-amino-acid peptides that are derived from translation of the DR2 (HPRNPARRTPGTRRG APQEPGAA) and DR1 (PGTWC PPPREPGALLPGNLVPSS) repeats.

MK2-dependent stabilization can also involve other proteins and mechanisms. For example, MK2-dependent cytoplasmic accumulation of the mRNA-binding protein homologous to human R-antigen (HuR) contributes to an ARE-dependent stabilization of *uPA* mRNA in the cytoplasm[93].

The impaired inflammatory response in MK2-deficient animals, and the fact that the catalytic activity of MK2, but not the stabilization of p38α by a catalytically inactive MK2, can rescue this phenotype[45] indicate MK2 as a pharmacological target for the treatment of chronic inflammation. MK2 is probably a more specific target than p38α, which has many more substrates besides MKs. Different screenings for small-molecule, ATP-competitive, MK2–p38-interaction- or MK2-export-inhibitors were carried out[94–97], and a promising ATP-competitive MK2 inhibitor, which even inhibits *Streptococcus aureus* cell-wall-induced arthritis in rats, has been recently described[98].

Different lines of evidence indicate that the function of MK2 in the inflammatory response is not shared by the other MKs. Firstly, a dominant-negative mutant of MK2, but not of MK3, is able to block p38-dependent *uPA*-mRNA stabilization[91]. Secondly, and more importantly, no significant reduction of LPS-induced cytokine production could be detected, neither in MK3- (J.B. Telliez and colleagues, unpublished observations) nor in MK5-deficient mouse macrophages[55].

MK2 and its substrate TTP are unique targets, not only for anti-inflammatory therapy, but also for viral strategies that modulate inflammation. The Kaposin B protein from the herpes virus that is associated with Kaposi's sarcoma directly binds, through the sequence region that is encoded by the second 23-nucleotide GC-rich direct repeat (DR2), to a region within the catalytic domain of MK2, which is also a target for the autoinhibitory C-terminal helix. As a result of binding, and through an as-yet-unknown mechanism, Kaposin B facilitates MK2 activation by p38, stabilizes

P-bodies
Cytoplasmic aggregates of translationally inactive ribonucleoproteins (mRNPs), which are sites of mRNA degradation.

cytokine mRNA, and increases cytokine production in infected cells and, therefore, might contribute to the oncogenesis of the virus[9]. By contrast, the host-shut-off protein of herpes simplex virus-1 leads to stimulated degradation of host mRNAs by increasing cytoplasmic TTP levels in virally infected cells[99].

14-3-3-binding regulation: a common mechanism?

The substrate-recognition motif of MKs matches at least the minimal requirements of a binding site for 14-3-3 proteins. It has the optimal consensus sequence R-[S/aromatic]-[basic]-pS-[L/E/A/M]-P, in which the phosphoserine and the arginine in the –3 position are crucial. This reveals the attractive possibility that an important function of MKs is the generation of 14-3-3-binding sites at their substrates.

The function of HSP27 as an inhibitor of Rho-dependent actin-stress-fibre formation can be explained by competition for 14-3-3-protein binding between the phospho-HSP27 and phospho-cofilin. This competition with the abundant phospho-HSP27 leads to an increased release of cofilin from the 14-3-3 protein. This is followed by cofilin dephosphorylation and subsequent binding to actin, which results in actin remodelling[100] (FIG. 4a).

Generation of 14-3-3-protein-binding sites seems important for the function of MKs in the inflammatory response. Phosphorylated TTP binds to 14-3-3 proteins (FIG. 4b), which leads to exclusion of the ternary 14-3-3-protein–TTP–ARE-mRNA complex from stress granules and/or P-bodies, and inhibits TTP-dependent degradation of ARE-containing transcripts[11]. In endothelial cells, p38-independent signalling pathways induce the phosphorylation of a complex that contains 14-3-3β and TTP, as well as the release of TTP from an ARE-containing mRNA[101], which results in the stabilization of the transcript.

Furthermore, in cell-cycle-checkpoint control, phosphorylation of CDC25B and CDC25C by CHK1, CHK2 or MK2 stimulates binding to 14-3-3 proteins[13]. This is responsible for cytoplasmic retention of these phosphatases, which otherwise facilitate cell-cycle progression by dephosphorylating nuclear targets such as CDC2 (REF. 13). In cell-size regulation, a complex of the tumour-suppressor proteins hamartin (also known as tuberous sclerosis (TSC)1) and tuberin (also known as TSC2) inhibits protein translation. MK2 phosphorylates TSC2 (REF. 102), which binds to 14-3-3 proteins and can no longer inhibit phosphorylation of eIF4E-binding protein-1, therefore protein synthesis proceeds (FIG. 4c).

Finally, the 14-3-3ζ protein itself was identified as a substrate of MKs and its phosphorylation regulates dimerization and binding, revealing a mechanism through which MKs might regulate 14-3-3-protein docking at the level of both partner proteins[103]. Furthermore, competition between the relatively abundant phospho-HSP27 and other phosphoproteins, including MK substrates, for 14-3-3-protein binding is an elegant way to explain the multifunctionality of small Hsps.

Conclusions

Although the three MKs share similar structural properties and an overlapping substrate spectrum, there is no known common function between all three MKs. MK2 and MK3 can bind to and, upon stress, are activated by p38α, whereas MK5 is unique in its interaction with and long-term activation by ERK3, but physiological stimuli for its short-term activation are not known. The existence of different subcellular populations of MKs in different cellular compartments is regulated by conformational changes of the MKs themselves, as well as by nuclear export for MK2 and MK3 and by protein–protein interactions and cytoplasmic anchoring for MK5. For each of the various roles of MKs — including the inflammatory response, cell-cycle-checkpoint control, chromatin remodelling, and development — different MKs seem important, whereas others do not. This raises questions about the differences in MK-substrate specificity *in vivo*, as well as the cell-type-specific activation and subcellular localization of these kinases.

In future research, identification of the MK-dependent subset of biological functions of p38α/β should be reached by comparing the effects of specific p38α/β kinase inhibitors and the newly developed MK inhibitors. Although it has been recently shown that MK2 might have important functions in cell-cycle control, and MK3 might be involved in chromatin remodelling, further analysis is required in cells and organisms that lack MKs to elucidate their role in these processes. Further insights into molecular mechanisms are required to investigate the translational regulation of specific mRNAs by MK2 and whether the generation of 14-3-3-protein-binding sites is a general function of MKs.

The functional overlap between MK2, MK3 and MK5 should be further analysed using systems that are depleted in two, or all three, enzymes; using such an approach, we will able to investigate whether MKs can compensate for each other *in vivo*. Furthermore, it will be necessary to identify specific functions for MK3 and MK5 and to understand how these functions are achieved at the molecular level through different subcellular localizations, regulatory protein complexes and specific substrates. Finally, these results, together with the recent findings that indicate that other protein kinases downstream of MAPKs, such as MNKs[104], might also contribute to post-transcriptional regulation of cytokines, could be used to functionally re-define the MK family.

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