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MK2 and MK3 – a pair of isoenzymes?

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1. ABSTRACT

The MAPK-activated protein kinases MK2 and MK3 form a pair of structurally and functionally closely related enzymes present in mammals and birds. Both protein kinases can bind to p38α MAPK and are activated by p38α via multiple proline-directed phosphorylations in a stress-dependent manner. Although the expression level and activity of MK2 is always significantly higher than that of MK3, the substrate spectrum of both enzymes is indistinguishable and covers proteins involved in cytokines production, endocytosis, reorganization of the cytoskeleton, cell migration, cell cycle control, chromatin remodeling and transcriptional regulation. Functional differences between MK2 and MK3 could result from the more prominent proline-rich SH3-targeting region in MK2, but are not reported so far. Since MK2 and MK3 are the main downstream targets of p38α responsible for posttranscriptional stimulation of cytokine biosynthesis, both enzymes are promising targets for the development of small molecule inhibitors which can be used in anti-inflammatory therapy. MK2-knockout mice show decreased LPS-induced cytokine biosynthesis and increased protection against collagen-induced arthritis. Recently generated MK2/3 double knockout mice show further reduction of LPS-induced cytokine production.

2. INTRODUCTION

Although stress-dependent phosphorylation of rat small heat shock protein (sHsp) has been originally described a quarter of a century ago (1, 2), the enzymes responsible for stress-dependent modification of mammalian sHsps *in vivo* have remained enigmatic for many years and a clear picture of *in vivo* sHsp-kinases has developed only slowly. First attempts to phosphorylate mouse Hsp25 *in vitro* demonstrated the ability of purified PKA and PKC to act as sHsp-kinases (3), but PKC and PKA inhibitors were not able to block sHsp phosphorylation by the endogenous kinase from Ehrlich ascites tumor cells (4). Partial purification of the stress-induced sHsp-kinase (s) revealed a novel kinase distinct from PKA, PKC and RSK that is regulated by phosphorylation (5).

By chance, some left over recombinant Hsp25 (mouse sHsp) (6), which was originally sent to Graham Hardie for testing it as a substrate for AMPK, was also tested as a substrate for a newly purified kinase from Philip Cohen's lab designated MAPK-activated protein kinase 2 (MAPKAPK2, MK2) (7). Surprisingly, Hsp25 and Hsp27 (human sHsp) were proven to be ideal substrates for MK2 and, furthermore, purification of MK2 from rabbit muscle

directly paralleled the purification of sHsp kinase activity, strongly suggesting that MK2 is the sHsp kinase of mammalian cells (8). The primary structure of MK2 was determined by cloning its cDNA revealing a proline-rich N-terminal domain and a C-terminus with a putative nuclear localization signal (NLS) (9, 10). MK2's catalytic domain was found to be most similar to calcium/calmodulin-dependent protein kinases although it was clear that this enzyme is not activated by calcium, but by phosphorylation. Since MK2 was described to be activated by classical MAPK similar to RSK2 (designated MAPKAPK1 at this time) (7), it was still puzzling how stress-dependence of sHsp phosphorylation was archived. This issue was solved when two groups in parallel demonstrated that MAPKAPK2 is activated by a new stress-activated MAPK, designated p40 MAPK (11) or MAPKAPK2 reactivating kinase (RK) which was similar to the stress-activated kinase HOG1 of *Saccharomyces cerevisiae* (12) and which is now known as p38 MAPK (13).

MK3 was also discovered in parallel by two independent groups and by two different ways. In one approach, the search for binding partners of a catalytically inactive p38 MAPK in a Y2H screen lead to the identification of a cDNA which coded for a protein kinase with about 70 % identity to MK2 at the amino acid level (14). In the second approach, putative tumor suppressor genes were searched by analyzing genes in a specific region of human chromosome 3p which is deleted in small cell lung cancer. One of the genes identified in this region displayed high homology to MK2, was originally designated 3pK (chromosome 3p kinase) and turned out to be identical to MK3 (15).

A decade ago and, again, by two groups in parallel, another protein kinase with partial similarity to MK2 and MK3 was identified and termed PRAK or MAPKAPK5 (16, 17). Since this enzyme does not act as a stress-activated sHsp-kinase *in vivo* (18) and since its activator and activation mechanism are different from MK2 and MK3 (19, 20), this protein kinase is discussed in a separate review of this special issue (Perander *et al.*, 2008).

Homologue kinases of MK2 and MK3 lacking the proline-rich N-terminal domain of MK2 were identified in insects, nematodes and sea urchin (designated MK4 in this organism (21)), while in vertebrates full length homologues of MK2 are present. In worms, insects and lower vertebrates there is only one homologous kinase, while MK2 and MK3 co-exist in birds and mammals indicating a relatively late gene duplication event (22).

3. EXPRESSION AND ISOFORMS

MK2 and MK3 mRNAs are present in most human and mouse tissues analyzed (9, 10, 15). MK2 and MK3 proteins are ubiquitously expressed, but the levels of MK2 expression is significantly higher than MK3 levels (23). Furthermore, two protein isoforms have been described for MK2, which migrate as two distinct bands in SDS–PAGE (mouse: 45 and 55 kDa (24); rabbit: 53 and 60 kDa (7)). Correspondingly, two biochemically distinct forms (p43 and p49) of MK2 have also been detected in rat cardiac myocytes (25). Both isoforms of MK2 show bands of comparable intensity in SDS-PAGE, bind to p38α and show p38α-dependent activation (23) and are absent in MK2 knockout cells (26). So far, it is not clear whether these MK2 isoforms reflect a differential splicing event, post-translational modifications or proteolysis. For human MK2, an alternative cDNA coding for a truncated and modified C-terminus (MK2short, MK2S in Figure 1) has been identified (27). However, since this cDNA is very rare in EST-libraries, it is unlikely to account for the two comparable protein bands in SDS-PAGE.

4. STRUCTURE, ACTIVATION AND SUBCELLULAR TRANSLOCATION

MK2 is highly homologous to MK3 (75% amino acid identity for the human enzymes) suggesting that MK2 and MK3 form a pair of related kinases. Human MK2 features 400 residues with an N-terminal proline-rich region, that is able to bind SH3 domains (28) (residues 10-44), a catalytic kinase domain (residues 51-325), and a C-terminal autoinhibitory region (residues 328-364) (cf. Figure 1) (29). The kinase domains of MK2 and MK3 are most similar (35 to 40% identity) to CaMK and the CTKD of the RSK isoforms. The C-terminal region contains a nuclear export signal (NES) of hydrophobic residues (356-MTSALATMRVDYE-368), while the far C-terminus (residues 365-400) features a bipartite nuclear localization signal (NLS) of basic residues (373-KK-X_{10}-KRRKK-389) (30, 31). The NLS is overlapping with the key p38 binding site: (366-DYEQIKIKKIEDASNPLLLKRRKKA-390). These characteristic features of MK2 could be endorsed by MK3, however the proline-rich region is much shorter or almost non-existing and the NLS is degraded to a monopartite one: 352-KD-X_{10}-KRRKK-368. Several crystal structures were published, resolving non-phosphorylated MK2, determined at 2.8 Å resolution (PDB: 1KWP) (32), complexed with staurosporine at 2.7 Å (PDB: 1NXK), complexed with ADP at 3.2 Å (PDB: 1NY3) (33) and complexed with specific inhibitors at 2.8 Å and 2.0 Å resolution, respectively (PDB: 2P3G and 2OKR) (34, 35).

MK2 and MK3 are activated upon different stress conditions such as UV-irradiation, heat shock, oxidative state, hyperosmolarity, LPS- or IL1-treatment. Using a p38-inhibitor (36) and a gene targeting approach in mice (37) it was demonstrated, that stress-induced MK2 activation is exclusively dependent on p38α. Activated p38 phosphorylates MK2 in the T-loop (on residue T222 in human enzyme), at S272 within the catalytic domain and at a regulatory phosphorylation site T334 which is located in a hinge region between the catalytic core and the autoinhibitory helix (29, 38). In addition to T222, S272 and T334 some minor (auto)-phosphorylation sites were also described (38). Upon activation, the autoinhibitory helix is assumed to be released from the core of the kinase domain and to interact with nuclear export receptors, ultimately resulting in the export of activated MK2 from the nucleus into the cytoplasm (30). This release simultaneously makes

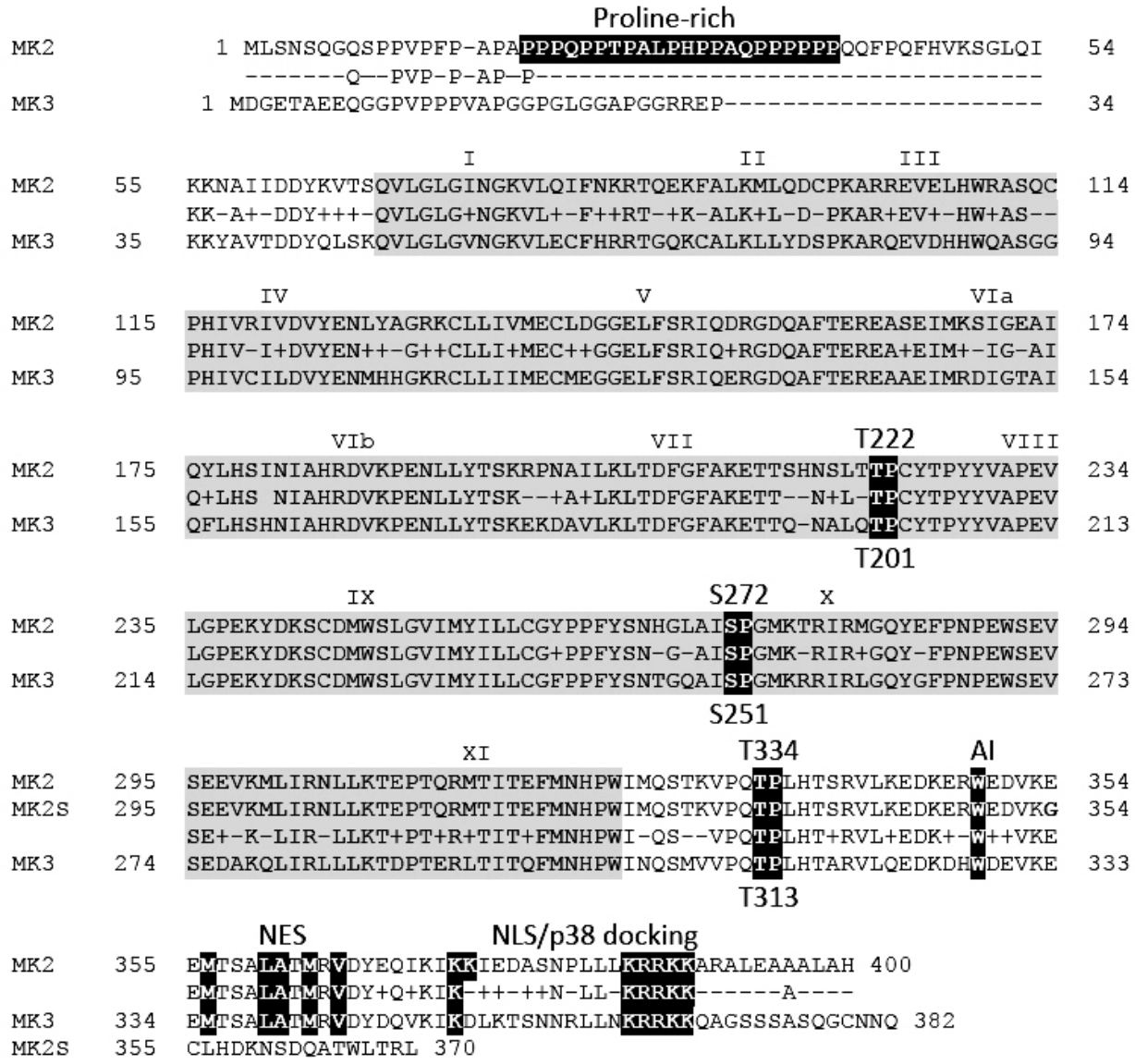

Figure 1. Amino acid sequence alignment of human MK2 (1-400), MK3 (1-382) and MK2S (1-370), an amino acid sequenced with a different C-terminus derived from a 3' altered cDNA. The proline-rich motif of MK2, the proline-directed phosphorylation sites of MK2 (T222, S272, T334) and MK3 (T201, S251, T313), the central tryptophan residue of the autoinhibitory helix (AI), the nuclear export (NES) and import signal (NLS) are indicated. The NLS overlaps with the p38-binding site. Subdomains of the kinase catalytic domain (gray) are indicated by Roman numbers.

accessible the substrate peptide binding site of MK2, therefore representing a so called bifunctional switch (22, 32). In contrast to regulated NES function, the C-terminal bipartite NLS is active independently of the phosphorylation state, enabling this kinase for active shuttling between nucleus and cytoplasm. However, because of partial overlapping of the NLS with the p38 docking site it remains unclear how the p38 binding interferes with re-localisation of endogenous MK2.

It was shown that MK2 and p38α form a stable complex (31, 39). The knockout studies demonstrated decreased levels of p38α in MK2-deficient cells (40), as well as reduction of MK2 expression in p38α knockout cells (41). Since the catalytic activity of MK2 is not required for p38 stabilization, mutual stabilization by complex formation and/or intracellular relocalisation was proposed (22). Characterisation of the p38α-MK2 interaction using different methods show, that the heterodimer is a stable, high affinity complex with a Kd of 2.5 nM (39) or even 0.5 nM (42) and phosphorylation of MK2 attenuates the affinity of the binary MK2:p38α complex by an order of magnitude. Importantly, the 3D structure of the p38:MK2 complex was recently resolved with 4.0A (PDB: 2ONL) (43) and 2.7A (PDB: 2OZA) resolution (44), respectively. Interacting p38α and MK2a

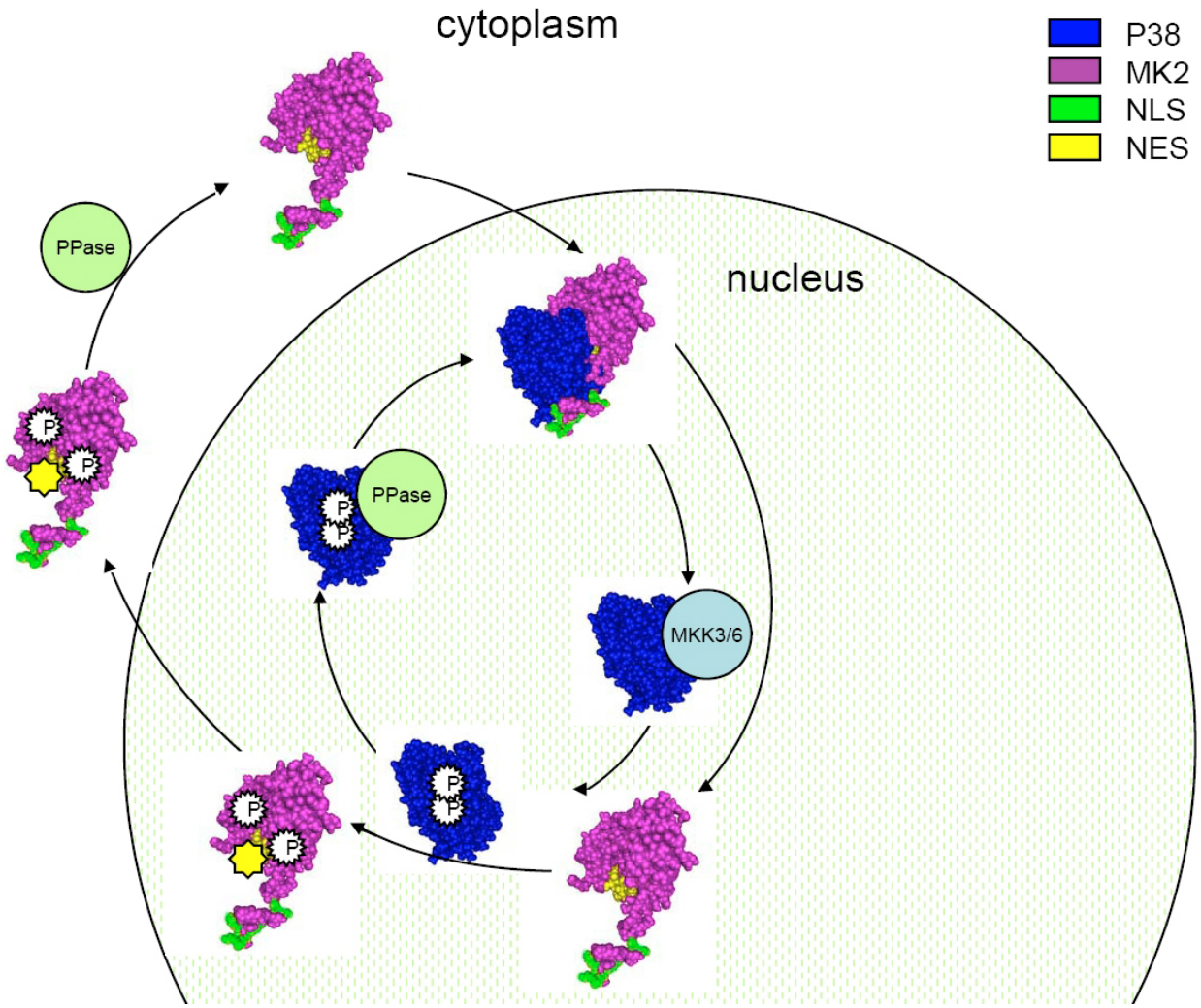

Figure 2. The MK2 activation and nuclear-cytoplasmic translocation cycle based on the recent p38/MK2 heterodimer structure where p38 masks the NES (17). Inactive MK2 forms a stable complex with p38 in the nucleus. Activated MKK3/6 displaces MK2 from p38 and phoshorylates the latter on the TGY motif. Activated p38 transiently interacts with MK2, leading to the phosphorylation of MK2 on T222 and T334 (and, may be, also at S272). Phosphorylation of T334 induces conformational changes in hinge region, resulting in the exposure/activation of previously masked NES. Strong NES defies constitutively active NLS causing MK2 translocation to the cytoplasm, which is reversible as soon as MK2 becomes dephosphorylated. In the nucleus, inactive MK2 can again undergo association with dephosphorylated p38. Structure of MK2:p38 complex is based on PDB: 2OZA (17).

molecules are positioned "face to face" so that the ATP-binding sites of both kinases are at the heterodimer interface. The C-terminal segment of MK2 (residues 368–400), wraps around p38α and inserts into the docking groove. The C-terminal end of MK2 binds in the docking groove of p38α and makes key interactions with the ED and CD regions. These interactions are a combination of hydrophobic and polar contacts. Resolving of heterodimer structure has some important consequences. Since the same regions are employed in binding of p38 activating kinases, inactivating phosphatases, and substrates, MK2 clearly competes with p38-binding of these other partners. Moreover, the tight complex with p38 sterically precludes MK2 phosphorylation at T222 and T334 and a second, transient interaction is required for MK2 phosphorylation. Most importantly, p38 binding shields the NES of MK2 from solvent preventing the co-export of the complex to the cytoplasm as it was originally postulated by Ben-Levy *et al.* (31) (Figure 2). Since intracellular staining of endogenous p38 with pan p38 antibodies as well as with phosphospecific p38 antibodies revealed no p38 redistribution in MK2/MK3 deficient MEFs, but equable reduction of signal intensity both in nucleus and cytoplasm (Ronkina and Gaestel, unpublished data), a role of MK2/MK3 in subcellular redistribution of p38 is further unlikely. Interaction of MK2 and MK3 with components of polycomb silencing complex EDR1 and EDR2 was shown (45, 46) which could lead to the recruitment of at least a subpopulation of MK2 to polycomb. To our knowledge a

specific subcellular staining of endogenous MK2 and MK3 was not reported in the literature until now.

5. SUBSTRATES AND FUNCTIONS

MK2/3 are activated *in vivo* by stimulation of the p38 MAPK cascade and participate in diverse cellular processes such as cytokines production (23, 26), endocytosis (47), reorganization of the cytoskeleton (48), cell migration (49-51), cell cycle control (52), chromatin remodeling (45, 46) and gene expression (Ronkina and Gaestel, unpublished data). The optimal phosphorylation site motif for MK2/3 has been defined as ϕ-X-R-X- (L,N)-(pS/pT)- (I,V,F,L)-X, where ϕ represents a hydrophobic amino acid (15, 52, 53). MK2 and MK3 have similar kinetic constants for the phosphorylation of peptide substrates and phosphorylate the same serine residues in Hsp27 and Hsp25 (also known as Hspb1) with the same relative rates (53). However, the level of *in vivo* activity of MK2 is always markedly higher than that of MK3. Accordingly, it was shown that MK3 could have significant effects only in an MK2-free background. Consistently, MK2/MK3 double-knockout mice showed further reduction of TNF production and expression of p38 and TTP compared to MK2 single knockout mice. This finding, together with the observation that ectopically expressed MK3 can rescue MK2 deficiency similarly to ectopic MK2, indicates that both kinases share physiological functions *in vivo* but are expressed to different levels (23).

Interestingly, the dramatic reduction of p38 protein levels in MK2/3 double knockout cells resulted also in attenuation of p38 functions besides the MK2/3 signaling pathway. For example, stress-induced, but not mitogen-induced, phosphorylation and activation of another direct p38 downstream substrate, MSK (Mitogen- and Stress-activated protein Kinase, see review of J.S.C. Arthur within this special issue), is significantly reduced in MK2/3 double knockout macrophages and MEF cells, indicating that residual p38 is not sufficient to fulfill its function completely (Ronkina, Arthur and Gaestel, unpublished data). p38 protein levels and catalytic function could be rescued by expression of exogenous MK2, MK3 or by catalytic dead mutant of MK2 (23, 54). Restoration of p38 protein levels by ectopically expressed MKs or by catalytic dead mutant of MK2 recovers stress-induced MSK and CREB phosphorylation (Ronkina and Gaestel, unpublished data). Taking in account that MK2 and MK3 strongly interact with p38, we conclude that this interaction stabilizes p38 protein and thereby influences p38 functions.

A number of substrates of MK2/3 have been identified. Some of them are described below; the others are summarized in a recent review (22).

5.1 Small mammalian heat shock protein

One of the major *in vivo* substrates of MK2/3 is the small heat shock protein Hsp25/27. Mammalian Hsp25/27 are rapidly phosphorylated by MAPKAP kinase 2/3 at two or three serine residues in response to various extracellular stresses (7, 53). Upon phosphorylation large sHsp oligomers dissociate to tetramers and lose their chaperone activities and ability to protect against oxidative stress effectively (55, 56). On the other hands it was shown that MK2/3 dependent Hsp25/27 phosphorylation increases the stability of the actin microfilaments providing homeostatic functions at the actin cytoskeleton level during stress (48). With regard to the functional versatility ascribed to Hsp25, it is rather surprising that studies with Hsp25 knockout mice do not show any notable effects of Hsp25 deficiency on ontogenesis and tissue physiology under normal conditions. Nevertheless the same studies demonstrate a critical role of Hsp25 in thermotolerance development and protection of cells from apoptosis under thermal stress (57).

5.2 ARE-mRNA binding proteins

MK2 knockout mice show decreased production of inflammatory cytokines such as TNF and IL-6 upon LPS-stimulation (26). Impaired cytokine production make MK2 deficient mice less sensitive to LPS-induced endotoxic shock, but more susceptible to bacterial infection (58). Lack of MK3 alone does not lead to any notable changes in inflammatory response, but absence of both, MK2 and MK3, results in further reduction of TNF production compared to MK2 single knockout. This finding suggests that MK2 and MK3 function additively in stress-induced cytokine production (23). MK2 was shown to regulate TNF and IL-6 production posttranscriptionally by increasing the stability and translation of their mRNAs. Such regulation depends on AU-rich elements (AREs) within the 3′-untranslated regions of these mRNAs (59, 60). A similar mechanism of MK2/3-dependent regulation was shown also for other inflammatory mediators such as COX-2 (cyclooxigenase 2), MIP-2 (macrophage inflammatory protein-2), GM-CSF (granulocyte-macrophage colony-stimulating factor), CXCL-1 (C-X-C motif ligand 1) (23, 61, 62).

There are some ARE-binding proteins involved in posttranscriptional regulation of ARE-containing mRNAs which were described to be MK2/3 substrates. Among them: tristetraprolin (TTP) (63, 64), heterogeneous nuclear ribonucleoprotein (hnRNP) A0 (62), polyA-binding protein 1 (PABP1) (61) and human R-antigen (HuR) (65). Phosphorylation of ARE-binding proteins by MK2/3 could influence their stability, and/or ARE-binding affinities, and/or their interaction with other proteins. TTP, a zinc-finger protein that binds to ARE containing mRNAs and causes their destabilization by targeting them to exosomes (66) or proteasomes (67), was shown to be major target of MK2 involved in the posttranscriptional regulation of TNF. Phosphorylation of TTP by MK2 increases its stability and binding to 14-3-3 proteins and reduces its ARE affinity leading to inhibition of TTP-dependent degradation of ARE-containing transcripts (64, 68, 69).

5.3 RSK

MK2 and MK3 additively contribute to Toll-like receptor (TLR) signalling response in dendritic cells (DC) trough phosphorylation of RSK (p90 ribosomal S6 kinase, see review of Carrière *et al.*, this special issue) at S386 which is pivotal for RSK activation (47). Whereas lack of only MK2 or MK3 alone does not lead to remarkable

endocytic defects, dual deficiency of both MK2 and MK3 significantly attenuates TLR4-induced edocytosis in DC. The involvement of MK2/3 in TLR-driven endocytosis is cell type specific and was observed only in DC. In other cell types, RSK autophosphorylation at S386 is catalysed by Erk1/2-activated C-terminal kinase domain (CTKD) of RSK. Thus, direct phosphorylation of S386 by MK2/3 in DC bypasses the necessity of the CTKD phosphorylation by Erk1/2. Taking into account the critical importance of DC for effective triggering of innate immune response, it could be supposed that even light stimuli which weakly activate Erk1/2 or p38 should still be able to induce endocytosis in DC. This could be a possible explanation for unordinary signalling mechanism evolved by DC and not by other cell types.

5.4 TAB3

IL-1 induced phosphorylation of TAB3 (TAK1-binding subunit 3) at S506 is mediated by MK2/3 and does not occurs in MK2/3 double knockout MEFs (70) TAB3 is structurally related to TAB2 and demonstrates functional redundancy to TAB2 in mediation of IL-1 induced activation of TAK1 (71). IL-1 stimulates binding of TAB2/TAB3 to K63-linked polyubiqitinated TRAF6 and facilitates the formation of TRAF6-TAB2/TAB3-TAK1 complex, leading to the activation of TAK1 by auto-phosphorylation (72). How MK2/3-dependent phosphorylation of TAB3 influences its function is not clear so far.

6. USE OF MK2/3 KNOCKOUT MICE IN ANIMAL MODELS FOR HUMAN DISEASES

Stress-induced p38-MK2/3 activation has been implicated in various pathological conditions (73). The usage of specific p38 inhibitors helped to determine whether this signalling pathway contributes to designated pathologies, but side effects of p38 MAPK inhibitors, such as for example hepatotoxicity (74), limit their application in experimental disease models. Therefore, MK2/3 knockout mice, which are viable in contrast to the embryonic lethal p38 alpha knockout, become a very useful, if not indispensable, tool for the analysis of the functional role of MK2/3 in different mouse models for human diseases. The reaction to bacterial LPS/galactosamine is a well characterized stress response in mice and results in endotoxic shock mediated by inflammatory cytokines, especially the toxic TNF response in liver (75). In contrast to WT littermates, MK2-/- mice were partially protected and more than 50% survived endotoxic shock (26). Production of cytokines such as TNFα and interleukin (IL)-6 upon LPS-stimulation is severely affected. It is now clear that MK2 is the main target of p38α in the signalling pathway which regulates the stability and translation of TNFα and IL-6 mRNAs through a process that involves the AU-rich elements in the 3′ non-coding region of these mRNAs (59).

Interestingly, MK2 knockout mice demonstrate increased resistance to collagen-induced arthritis which is the murine model of rheumatoid arthritis (76). MK2 mediates joint inflammation by upregulation of inflammatory cytokines such as TNF-α and IL-6. Significantly reduced incidence and severity of collagen-induced arthritis in MK2 deficient mice disclose MK2 as a key player in the pathogenesis of this systemic autoimmune inflammatory disease. Atherosclerosis is another chronic inflammatory disease, where MK2 was shown to play a functional role (77). MK2 promote recruitment of monocytes into the vessel wall, by increasing VCAM-1 (vascular cell adhesion molecule) and MCP-1 (monocyte chemoattractant protein) expression in endothelial cells upon feeding an atherogenic diet of hypercholesterolemic mice (ldlr-/-). Consequently, MK2-deficiency of hypercholesterolemic mice (ldlr-/-/mk2-/-) decreases the diet-induced accumulation of lipids, macrophages and foam cells in the aorta and thus ameliorates atherosclerosis.

In the model of experimental asthma, the endothelial permeability as well as expression of particular chemokines (IL-4, IL-5, IL-10, IL-13, MCP-1) and adhesion molecules (VCAM-1) were reduced in the lung of allergen treated MK2 knockout mice indicating that MK2 is essential for the development of the local lung inflammation (78). In the model of pulmonary fibrosis, lack of MK2 prevents myofibroblast formation and in this way exacerbates fibrosis, suggesting that MK2 mediates myofibroblast differentiation in fibrotic injury (79, 80). The increased Hsp25 phosphorylation in early diabetic nephropathy was almost completely inhibited in MK2 knockout mice (81). The suppression of MK2-induced Hsp25 phosphorylation, however, did not prevent MK2-deficient mice from renal hypertrophy, glomerular hyperfiltration, renal fibrosis and albuminuria in the early diabetic state. This observation challenges the finding that phosphorylation of Hsp25 is responsible for abnormalities in glomerular hemodynamics in the early stages of diabetes mellitus (82). The increased Hsp25 phosphorylation in the diabetic state was markedly reduced, but not completely abolished in the MK2 knockout mice suggesting that other kinases, most probably MK3, might compensate for the loss of MK2.

MK2 was shown to be responsible for generating ischemia-induced cardiomyocyte apoptosis and in accordance with this observation, the hearts of the MK2 knockout mice were resistant to myocardial ischemic reperfusion injury (83). While the hearts of the MK2 knockout mice were protected form ischemia-induced myocardial injury, the cardioprotective effects of ischemic preconditioning was markedly reduced in the absence of MK2 (84). Lack of MK2 in mice subjected to focal cerebral ischemia protects brain from neurological deficits and results in prominent reduction of infarct expanses (85). Furthermore, the crucial role of MK2 in microglial neuroinflammation and neurodenegation was demonstrated in microglia – neuron coculture with possible relevance to Alzheimer disease (AD) (86). Upon LPS + IFN-γ or -β stimulation, MK2 deficient microglia produces decreased amount of neurotoxic pro-inflammatory mediators such as TNF, CXCL-1 and MIP-1. Consistently, MK2 deficient microglia is not able to induce neuronal cell injury. In agreement with that, lack of MK2 in microglia protects neurons from inflammation-induced neurotoxicity. In the

cortical tissue of TASTPM mice, a transgenic model of Alzheimers disease (AD), enhanced production of the same cytokine set as well as up-regulation of MK2 expression and activity has also been demonstrated. However, direct toxicity of one or combined chemokine/cytokine on cortical neurons was not demonstrated in this study. Crossing TASTPM mice on MK2-deficient background could be useful to validate MK2 role in this AD model.

Thus MK2 and MK3, as key drivers of inflammatory response, represent an attractive therapeutic target for the inhibitors that could ameliorate numerous inflammatory diseases, including rheumatoid arthritis, atherosclerosis and asthma. However, intact cytokine response is also essential for efficient host defence against invading pathogens. For example, global suppression of cytokine production via anti-inflammatory glucocorticoid therapy strongly increases susceptibility to microbial infection. Consistent with the affected cytokine synthesis, MK2 ablation may result in increased susceptibility to infections as well. Indeed, MK2-/- mice displayed significantly increased mortality when infected with a facultative intracellular bacterium *Listeria monocytogenes* reflected by significantly higher bacterial loading in spleen and lung (58). Additionally to impaired TNFα and IFNγ production, MK2-deficient macrophages displayed affected phagocytosis.

Apart from inflammation and infection, a strong activation of p38/MK2/3 axis accompanies gentoxic stress and treatment with cytostatika, such as cisplatin or doxorubicin, raising the question whether this activation modulates apoptosis or cell cycle checkpoint control. MK2-deficient mouse cells show increased apoptosis after UV irradiation and elevated levels of p53 protein (87). In an *in vitro* kinase assay, MK2 is able to phosphorylate HDM2 on S157 and S166. The consequence of these modifications is enhanced ability of MDM2 (HDM2) to degrade p53 and, hence, lack of this modification in MK2-deficient cells causes increased stability of p53. However, stress-dependent reduction of p53 level is not completely abrogated in MK2-deficient cells, indicating existence of additional mechanism responsible for the downregulation of p53 at the end of the stress response (87). Since several years, it was known that inhibition of p38 pathway in UV treated cells resulted in loss of G2/M checkpoint control (88). Interestingly, MK2 is able to phosphorylate CDC25B, creating 14-3-3 binding sites and inhibiting G2/M progression similar to CHK1 and CHK2, which are activated by ATM/ATR in γ-irradiated cells (52). Moreover, in the absence of p53, MK2 activation was reported to induce Cdc25A-mediated S phase checkpoint in cisplatin treated cells and Cdc25B-mediated G2/M checkpoint after doxorubicin treatment (89). Consequently, depletion of MK2 in p53-/- cells using RNAi resulted in an increase in the sensitivity to both chemotherapeutic agents in cell culture as well as in a tumor model *in vivo*, probably due to increased mitotic catastrophy (89). The two latter evidences are only indicative and the role of MK2 as a tumor promoter or checkpoint kinase should be validated using MK2-deficient animals and established disease models.

Summing up, MK2 knockout mice - and in future also MK2/3 double knockout mice - represent a profitable tool for evaluation of the impact of MK2/3 on the development of different pathologies. However, for the interpretation of the results obtained in these models, attenuated p38 activity due to ablation of MK2/3 should also be considered.

7. MK2/3 INHIBITORS FOR ANTI-INFLAMMATORY THERAPY

In the Christmas issue of Nature in 1994, a bicyclic imidazole, which could act as an efficient cytokine-suppressive anti-inflammatory drug in monocyte culture, was identified as ATP-competitive inhibitor of p38 MAPK (90). This finding initiates the development of different generations of p38 MAPK inhibitors starting with the well known compound SB203580 and leading to more potent and specific inhibitors such as VX-745 and BIRB-796 (91). However, due to liver toxicity and other unexpected side effects, until now none of these p38 MAPK-inhibitors had made it to the clinics (92). Meanwhile, the central role of MK2/3 in posttranscriptional control of inflammatory cytokine production and the viability of the MK2/3 double knockout mice (23), compared to the embryonic lethality of the p38α knockout mice (93), qualifies MK2/3 as a promising target for the development of small molecule inhibitors which can be used as anti-inflammatory therapeutics. Accordingly, screens for small molecule inhibitors for MK2 and MK3 were carried out by various pharmaceutical companies, but the first outcome was rather scanty. Obviously, the ATP-binding pocket of MK2 is shallow and the structural change of the catalytic domain itself from its inactive to its active conformation is small and not easy to be locked by an inhibitor. MK2 activation is probably more regulated by the C-terminal domain and its interaction with the catalytic domain (29, 94) and by protein-protein-interactions within the stable MK2/3/p38 MAPK complex (44). However, some effective small molecule inhibitors for MK2/3 were identified (reviewed in (91)). Recently, pyrrolopyridins and carboline analogs were described as potent and rather selective inhibitors with an IC50 in the low nanomolar range and the 3D structures of the inhibitors bound to MK2 fragments were determined (34, 35). Both classes of inhibitors bind near the P-loop and within the hinge region of MK2 which is a larger pocket than in most other kinases (34, 35). The inhibitors are active in blocking LPS-stimulated TNF production in U937 (35, 95) and THP-1 cells (34) at least in the low micromolar range. Moreover, the pyrrolopyridin “compound 23” when given orally to rats 2 h prior to LPS challenge can suppress more than 80% of TNF production *in vivo* (35).

In the present time the declared targets for developing of small molecule inhibitors for MK2 and MK3 are inflammatory diseases. However, since the disruption of the MK2 signalling pathway enhances sensitivity to chemotherapeutic agents (89), MK2 inhibitors could also be of use in combination with conventional cancer chemotherapy.

8. PERSPECTIVE

So far, MK2 and MK3 appear as structurally and functionally closely related isoenzymes with a seemingly identical manner of p38-dependent activation and an indistinguishable substrate spectrum. In addition, a common physiological function in posttranscriptional regulation of cytokine biosynthesis via TTP and stabilization of p38 has been demonstrated (23) making both kinases promising targets for anti-inflammatory therapy. However, the more prominent proline-rich region in MK2 compared to MK3, which could be of importance for subcellular targeting (28) and seems to be necessary for regulation of cell migration (40), might be the basis for some functional differences between both protein kinases. For analysis of these differences in targeting and for a further understanding of activation-dependent subcellular translocations of MK2/3 in concert with p38, antibodies which allow the subcellular detection of the endogenous kinases will be essential. Finally, the role of MK2 and MK3 in tumorigenesis and cell cycle control will also require further analysis.

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10. REFERENCES

1. Kim, Y. J., J. Shuman, M. Sette & A. Przybyla: Phosphorylation pattern of a 25 Kdalton stress protein from rat myoblasts. *Biochem Biophys Res Commun*, 117, 682-7 (1983)
2. Welch, W. J.: Phorbol ester, calcium ionophore, or serum added to quiescent rat embryo fibroblast cells all result in the elevated phosphorylation of two 28,000-dalton mammalian stress proteins. *J Biol Chem*, 260, 3058-62 (1985)
3. Gaestel, M., W. Schroder, R. Benndorf, C. Lippmann, K. Buchner, F. Hucho, V. A. Erdmann & H. Bielka: Identification of the phosphorylation sites of the murine small heat shock protein hsp25. *J Biol Chem*, 266, 14721-4. (1991)
4. Benndorf, R., K. Hayess, J. Stahl & H. Bielka: Cell-free phosphorylation of the murine small heat-shock protein hsp25 by an endogenous kinase from Ehrlich ascites tumor cells. *Biochim Biophys Acta*, 1136, 203-7 (1992)
5. Zhou, M., H. Lambert & J. Landry: Transient activation of a distinct serine protein kinase is responsible for 27-kDa heat shock protein phosphorylation in mitogen-stimulated and heat-shocked cells. *J Biol Chem*, 268, 35-43 (1993)
6. Gaestel, M., B. Gross, R. Benndorf, M. Strauss, W. H. Schunk, R. Kraft, A. Otto, H. Bohm, J. Stahl, H. Drabsch & *et al.*: Molecular cloning, sequencing and expression in Escherichia coli of the 25-kDa growth-related protein of Ehrlich ascites tumor and its homology to mammalian stress proteins. *Eur J Biochem*, 179, 209-13. (1989)
7. Stokoe, D., D. G. Campbell, S. Nakielny, H. Hidaka, S. J. Leevers, C. Marshall & P. Cohen: MAPKAP kinase-2; a novel protein kinase activated by mitogen-activated protein kinase. *Embo J*, 11, 3985-94. (1992)
8. Stokoe, D., K. Engel, D. G. Campbell, P. Cohen & M. Gaestel: Identification of MAPKAP kinase 2 as a major enzyme responsible for the phosphorylation of the small mammalian heat shock proteins. *FEBS Lett*, 313, 307-13. (1992)
9. Engel, K., K. Plath & M. Gaestel: The MAP kinase-activated protein kinase 2 contains a proline-rich SH3-binding domain. *FEBS Lett*, 336, 143-7. (1993)
10. Stokoe, D., B. Caudwell, P. T. Cohen & P. Cohen: The substrate specificity and structure of mitogen-activated protein (MAP) kinase-activated protein kinase-2. *Biochem J*, 296, 843-9. (1993)
11. Freshney, N. W., L. Rawlinson, F. Guesdon, E. Jones, S. Cowley, J. Hsuan & J. Saklatvala: Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. *Cell*, 78, 1039-49. (1994)
12. Rouse, J., P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt & A. R. Nebreda: A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell*, 78, 1027-37. (1994)
13. Han, J., J. D. Lee, L. Bibbs & R. J. Ulevitch: A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science*, 265, 808-11. (1994)
14. McLaughlin, M. M., S. Kumar, P. C. McDonnell, S. Van Horn, J. C. Lee, G. P. Livi & P. R. Young: Identification of mitogen-activated protein (MAP) kinase-activated protein kinase-3, a novel substrate of CSBP p38 MAP kinase. *J Biol Chem*, 271, 8488-92. (1996)
15. Sithanandam, G., F. Latif, F. M. Duh, R. Bernal, U. Smola, H. Li, I. Kuzmin, V. Wixler, L. Geil & S. Shrestha: 3pK, a new mitogen-activated protein kinase-activated protein kinase located in the small cell lung cancer tumor suppressor gene region. *Mol Cell Biol*, 16, 868-76. (1996)
16. New, L., Y. Jiang, M. Zhao, K. Liu, W. Zhu, L. J. Flood, Y. Kato, G. C. Parry & J. Han: PRAK, a novel protein kinase regulated by the p38 MAP kinase. *Embo J*, 17, 3372-84. (1998)
17. Ni, H., X. S. Wang, K. Diener & Z. Yao: MAPKAPK5, a novel mitogen-activated protein kinase (MAPK)-activated protein kinase, is a substrate of the extracellular-regulated kinase (ERK) and p38 kinase. *Biochem Biophys Res Commun*, 243, 492-6. (1998)
18. Shi, Y., A. Kotlyarov, K. Laaß, A. D. Gruber, E. Butt, K. Marcus, H. E. Meyer, A. Friedrich, H. D. Volk & M. Gaestel: Elimination of protein kinase MK5/PRAK activity by targeted homologous recombination. *Mol Cell Biol*, 23, 7732-41. (2003)
19. Schumacher, S., K. Laass, S. Kant, Y. Shi, A. Visel, A. D. Gruber, A. Kotlyarov & M. Gaestel: Scaffolding by ERK3 regulates MK5 in development. *Embo J*, 23, 4770-4779. Epub 2004 Nov 11. (2004)
20. Seternes, O. M., T. Mikalsen, B. Johansen, E. Michaelsen, C. G. Armstrong, N. A. Morrice, B. Turgeon, S. Meloche, U. Moens & S. M. Keyse: Activation of MK5/PRAK by the atypical MAP kinase ERK3 defines a novel signal transduction pathway. *Embo J*, 23, 4780-91. Epub 2004 Dec 2. (2004)
21. Komatsu, S., N. Murai, G. Totsukawa, M. Abe, K. Akasaka, H. Shimada & H. Hosoya: Identification of MAPKAPK homolog (MAPKAPK-4) as a myosin II

regulatory light-chain kinase in sea urchin egg extracts. *Arch Biochem Biophys*, 343, 55-62. (1997)
22. Gaestel, M.: MAPKAP kinases - MKs - two's company, three's a crowd. *Nat Rev Mol Cell Biol.*, 7, 120-30. (2006)
23. Ronkina, N., A. Kotlyarov, O. Dittrich-Breiholz, M. Kracht, E. Hitti, K. Milarski, R. Askew, S. Marusic, L. L. Lin, M. Gaestel & J. B. Telliez: The mitogen-activated protein kinase (MAPK)-activated protein kinases MK2 and MK3 cooperate in stimulation of tumor necrosis factor biosynthesis and stabilization of p38 MAPK. *Mol Cell Biol*, 27, 170-81 (2007)
24. Cano, E., Y. N. Doza, R. Ben-Levy, P. Cohen & L. C. Mahadevan: Identification of anisomycin-activated kinases p45 and p55 in murine cells as MAPKAP kinase-2. *Oncogene*, 12, 805-12. (1996)
25. Chevalier, D. & B. G. Allen: Two distinct forms of MAPKAP kinase-2 in adult cardiac ventricular myocytes. *Biochemistry*, 39, 6145-56. (2000)
26. Kotlyarov, A., A. Neininger, C. Schubert, R. Eckert, C. Birchmeier, H. D. Volk & M. Gaestel: MAPKAP kinase 2 is essential for LPS-induced TNF-alpha biosynthesis. *Nat Cell Biol*, 1, 94-7. (1999)
27. Zu, Y. L., F. Wu, A. Gilchrist, Y. Ai, M. E. Labadia & C. K. Huang: The primary structure of a human MAP kinase activated protein kinase 2. *Biochem Biophys Res Commun*, 200, 1118-24. (1994)
28. Plath, K., K. Engel, G. Schwedersky & M. Gaestel: Characterization of the proline-rich region of mouse MAPKAP kinase 2: influence on catalytic properties and binding to the c-abl SH3 domain *in vitro*. *Biochem Biophys Res Commun*, 203, 1188-94. (1994)
29. Engel, K., H. Schultz, F. Martin, A. Kotlyarov, K. Plath, M. Hahn, U. Heinemann & M. Gaestel: Constitutive activation of mitogen-activated protein kinase-activated protein kinase 2 by mutation of phosphorylation sites and an A-helix motif. *J Biol Chem*, 270, 27213-21. (1995)
30. Engel, K., A. Kotlyarov & M. Gaestel: Leptomycin B-sensitive nuclear export of MAPKAP kinase 2 is regulated by phosphorylation. *Embo J*, 17, 3363-71 (1998)
31. Ben-Levy, R., S. Hooper, R. Wilson, H. F. Paterson & C. J. Marshall: Nuclear export of the stress-activated protein kinase p38 mediated by its substrate MAPKAP kinase-2. *Curr Biol*, 8, 1049-57 (1998)
32. Meng, W., L. L. Swenson, M. J. Fitzgibbon, K. Hayakawa, E. Ter Haar, A. E. Behrens, J. R. Fulghum & J. A. Lippke: Structure of mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 suggests a bifunctional switch that couples kinase activation with nuclear export. *J Biol Chem*, 277, 37401-5. (2002)
33. Underwood, K. W., K. D. Parris, E. Federico, L. Mosyak, R. M. Czerwinski, T. Shane, M. Taylor, K. Svenson, Y. Liu, C. L. Hsiao, S. Wolfrom, M. Maguire, K. Malakian, J. B. Telliez, L. L. Lin, R. W. Kriz, J. Seehra, W. S. Somers & M. L. Stahl: Catalytically active MAP KAP kinase 2 structures in complex with staurosporine and ADP reveal differences with the autoinhibited enzyme. *Structure*, 11, 627-36 (2003)
34. Wu, J. P., J. Wang, A. Abeywardane, D. Andersen, M. Emmanuel, E. Gautschi, D. R. Goldberg, M. A. Kashem, S. Lukas, W. Mao, L. Martin, T. Morwick, N. Moss, C. Pargellis, U. R. Patel, L. Patnaude, G. W. Peet, D. Skow, R. J. Snow, Y. Ward, B. Werneburg & A. White: The discovery of carboline analogs as potent MAPKAP-K2 inhibitors. *Bioorg Med Chem Lett*, 17, 4664-9 (2007)
35. Anderson, D. R., M. J. Meyers, W. F. Vernier, M. W. Mahoney, R. G. Kurumbail, N. Caspers, G. I. Poda, J. F. Schindler, D. B. Reitz & R. J. Mourey: Pyrrolopyridine Inhibitors of Mitogen-Activated Protein Kinase-Activated Protein Kinase 2 (MK-2). *J Med Chem.*, 50, 2647-54. Epub 2007 May 5. (2007)
36. Clifton, A. D., P. R. Young & P. Cohen: A comparison of the substrate specificity of MAPKAP kinase-2 and MAPKAP kinase-3 and their activation by cytokines and cellular stress. *FEBS Lett*, 392, 209-14 (1996)
37. Adams, R. H., A. Porras, G. Alonso, M. Jones, K. Vintersten, S. Panelli, A. Valladares, L. Perez, R. Klein & A. R. Nebreda: Essential role of p38alpha MAP kinase in placental but not embryonic cardiovascular development. *Mol Cell*, 6, 109-16 (2000)
38. Ben-Levy, R., I. A. Leighton, Y. N. Doza, P. Attwood, N. Morrice, C. J. Marshall & P. Cohen: Identification of novel phosphorylation sites required for activation of MAPKAP kinase-2. *Embo J*, 14, 5920-30. (1995)
39. Lukas, S. M., R. R. Kroe, J. Wildeson, G. W. Peet, L. Frego, W. Davidson, R. H. Ingraham, C. A. Pargellis, M. E. Labadia & B. G. Werneburg: Catalysis and function of the p38 alpha.MK2a signaling complex. *Biochemistry*, 43, 9950-60 (2004)
40. Kotlyarov, A., Y. Yannoni, S. Fritz, K. Laass, J. B. Telliez, D. Pitman, L. L. Lin & M. Gaestel: Distinct cellular functions of MK2. *Mol Cell Biol*, 22, 4827-35. (2002)
41. Sudo, T., K. Kawai, H. Matsuzaki & H. Osada: p38 mitogen-activated protein kinase plays a key role in regulating MAPKAPK2 expression. *Biochem Biophys Res Commun*, 337, 415-21 (2005)
42. Zhang, W. X., R. Wang, D. Wisniewski, A. I. Marcy, P. LoGrasso, J. M. Lisnock, R. T. Cummings & J. E. Thompson: Time-resolved Forster resonance energy transfer assays for the binding of nucleotide and protein substrates to p38alpha protein kinase. *Anal Biochem*, 343, 76-83 (2005)
43. ter Haar, E., P. Prabhakar, X. Liu & C. Lepre: Crystal structure of the p38 alpha-MAPKAP kinase 2 heterodimer. *J Biol Chem*, 282, 9733-9 (2007)
44. White, A., C. A. Pargellis, J. M. Studts, B. G. Werneburg & B. T. Farmer, 2nd: Molecular basis of MAPK-activated protein kinase 2:p38 assembly. *Proc Natl Acad Sci U S A*, 104, 6353-8 (2007)
45. Yannoni, Y. M., M. Gaestel & L. L. Lin: P66 (ShcA) interacts with MAPKAP kinase 2 and regulates its activity. *FEBS Lett*, 564, 205-11 (2004)
46. Voncken, J. W., H. Niessen, B. Neufeld, U. Rennefahrt, V. Dahlmans, N. Kubben, B. Holzer, S. Ludwig & U. R. Rapp: MAPKAP kinase 3pK phosphorylates and regulates chromatin association of the polycomb group protein Bmi1. *J Biol Chem*, 280, 5178-87 (2005)
47. Zaru, R., N. Ronkina, M. Gaestel, J. S. Arthur & C. Watts: The MAPK-activated kinase Rsk controls an acute Toll-like receptor signaling response in dendritic cells and is activated through two distinct pathways. *Nat Immunol*, 8, 1227-35 (2007)
48. Guay, J., H. Lambert, G. Gingras-Breton, J. N. Lavoie, J. Huot & J. Landry: Regulation of actin filament dynamics

by p38 map kinase-mediated phosphorylation of heat shock protein 27. *J Cell Sci*, 110, 357-68. (1997)
49. Hannigan, M. O., L. Zhan, Y. Ai, A. Kotlyarov, M. Gaestel & C. K. Huang: Abnormal migration phenotype of mitogen-activated protein kinase-activated protein kinase 2-/- neutrophils in Zigmond chambers containing formyl-methionyl-leucyl-phenylalanine gradients. *J Immunol*, 167, 3953-61 (2001)
50. Wu, Y., M. O. Hannigan, A. Kotlyarov, M. Gaestel, D. Wu & C. K. Huang: A requirement of MAPKAPK2 in the uropod localization of PTEN during FMLP-induced neutrophil chemotaxis. *Biochem Biophys Res Commun*, 316, 666-72 (2004)
51. Rousseau, S., I. Dolado, V. Beardmore, N. Shpiro, R. Marquez, A. R. Nebreda, J. S. Arthur, L. M. Case, M. Tessier-Lavigne, M. Gaestel, A. Cuenda & P. Cohen: CXCL12 and C5a trigger cell migration via a PAK1/2-p38alpha MAPK-MAPKAP-K2-HSP27 pathway. *Cell Signal*, 18, 1897-905 (2006)
52. Manke, I. A., A. Nguyen, D. Lim, M. Q. Stewart, A. E. Elia & M. B. Yaffe: MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation. *Mol Cell.*, 17, 37-48. (2005)
53. Clifton, A. D., P. R. Young & P. Cohen: A comparison of the substrate specificity of MAPKAP kinase-2 and MAPKAP kinase-3 and their activation by cytokines and cellular stress. *FEBS Lett*, 392, 209-14. (1996)
54. Kotlyarov, A., Y. Yannoni, S. Fritz, K. Laass, J. B. Telliez, D. Pitman, L. L. Lin & M. Gaestel: Distinct cellular functions of MK2. *Mol Cell Biol*, 22, 4827-35 (2002)
55. Rogalla, T., M. Ehrnsperger, X. Preville, A. Kotlyarov, G. Lutsch, C. Ducasse, C. Paul, M. Wieske, A. P. Arrigo, J. Buchner & M. Gaestel: Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor alpha by phosphorylation. *J Biol Chem*, 274, 18947-56 (1999)
56. Jakob, U., M. Gaestel, K. Engel & J. Buchner: Small heat shock proteins are molecular chaperones. *J Biol Chem*, 268, 1517-20. (1993)
57. Huang, L., J. N. Min, S. Masters, N. F. Mivechi & D. Moskophidis: Insights into function and regulation of small heat shock protein 25 (HSPB1) in a mouse model with targeted gene disruption. *Genesis*, 45, 487-501 (2007)
58. Lehner, M. D., F. Schwoebel, A. Kotlyarov, M. Leist, M. Gaestel & T. Hartung: Mitogen-activated protein kinase-activated protein kinase 2-deficient mice show increased susceptibility to Listeria monocytogenes infection. *J Immunol*, 168, 4667-73. (2002)
59. Neininger, A., D. Kontoyiannis, A. Kotlyarov, R. Winzen, R. Eckert, H. D. Volk, H. Holtmann, G. Kollias & M. Gaestel: MK2 targets AU-rich elements and regulates biosynthesis of tumor necrosis factor and interleukin-6 independently at different post-transcriptional levels. *J Biol Chem*, 277, 3065-8. (2002)
60. Winzen, R., M. Kracht, B. Ritter, A. Wilhelm, C. Y. Chen, A. B. Shyu, M. Muller, M. Gaestel, K. Resch & H. Holtmann: The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. *Embo J*, 18, 4969-80 (1999)
61. Bollig, F., R. Winzen, M. Gaestel, S. Kostka, K. Resch & H. Holtmann: Affinity purification of ARE-binding proteins identifies polyA-binding protein 1 as a potential substrate in MK2-induced mRNA stabilization. *Biochem Biophys Res Commun*, 301, 665-70. (2003)
62. Rousseau, S., N. Morrice, M. Peggie, D. G. Campbell, M. Gaestel & P. Cohen: Inhibition of SAPK2a/p38 prevents hnRNP A0 phosphorylation by MAPKAP-K2 and its interaction with cytokine mRNAs. *Embo J*, 21, 6505-14. (2002)
63. Chrestensen, C. A., M. J. Schroeder, J. Shabanowitz, D. F. Hunt, J. W. Pelo, M. T. Worthington & T. W. Sturgill: MAPKAP kinase 2 phosphorylates tristetraprolin on *in vivo* sites including Ser178, a site required for 14-3-3 binding. *J Biol Chem*, 279, 10176-84. Epub 2003 Dec 19. (2004)
64. Stoecklin, G., T. Stubbs, N. Kedersha, S. Wax, W. F. Rigby, T. K. Blackwell & P. Anderson: MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. *Embo J*, 23, 1313-24. Epub 2004 Mar 11. (2004)
65. Tran, H., F. Maurer & Y. Nagamine: Stabilization of urokinase and urokinase receptor mRNAs by HuR is linked to its cytoplasmic accumulation induced by activated mitogen-activated protein kinase-activated protein kinase 2. *Mol Cell Biol*, 23, 7177-88 (2003)
66. Chen, C. Y., R. Gherzi, S. E. Ong, E. L. Chan, R. Raijmakers, G. J. Pruijn, G. Stoecklin, C. Moroni, M. Mann & M. Karin: AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell*, 107, 451-64 (2001)
67. Deleault, K. M., S. J. Skinner & S. A. Brooks: Tristetraprolin regulates TNF TNF-alpha mRNA stability via a proteasome dependent mechanism involving the combined action of the ERK and p38 pathways. *Mol Immunol*, 45, 13-24 (2008)
68. Brook, M., C. R. Tchen, T. Santalucia, J. McIlrath, J. S. Arthur, J. Saklatvala & A. R. Clark: Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. *Mol Cell Biol*, 26, 2408-18 (2006)
69. Hitti, E., T. Iakovleva, M. Brook, S. Deppenmeier, A. D. Gruber, D. Radzioch, A. R. Clark, P. J. Blackshear, A. Kotlyarov & M. Gaestel: Mitogen-activated protein kinase-activated protein kinase 2 regulates tumor necrosis factor mRNA stability and translation mainly by altering tristetraprolin expression, stability, and binding to adenine/uridine-rich element. *Mol Cell Biol*, 26, 2399-407 (2006)
70. Mendoza, H., D. G. Campbell, K. Burness, J. Hastie, N. Ronkina, J. H. Shim, J. S. Arthur, R. J. Davis, M. Gaestel, G. L. Johnson, S. Ghosh & P. Cohen: Roles for TAB1 in regulating the IL-1-dependent phosphorylation of the TAB3 regulatory subunit and activity of the TAK1 complex. *Biochem J*, 409, 711-22 (2008)
71. Ishitani, T., G. Takaesu, J. Ninomiya-Tsuji, H. Shibuya, R. B. Gaynor & K. Matsumoto: Role of the TAB2-related protein TAB3 in IL-1 and TNF signaling. *Embo J*, 22, 6277-88 (2003)
72. Kanayama, A., R. B. Seth, L. Sun, C. K. Ea, M. Hong, A. Shaito, Y. H. Chiu, L. Deng & Z. J. Chen: TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Mol Cell*, 15, 535-48 (2004)

73. Lee, J. C., S. Kumar, D. E. Griswold, D. C. Underwood, B. J. Votta & J. L. Adams: Inhibition of p38 MAP kinase as a therapeutic strategy. *Immunopharmacology*, 47, 185-201 (2000)
74. Wang, Y., R. Singh, J. H. Lefkowitch, R. M. Rigoli & M. J. Czaja: Tumor necrosis factor-induced toxic liver injury results from JNK2-dependent activation of caspase-8 and the mitochondrial death pathway. *J Biol Chem*, 281, 15258-67 (2006)
75. Galanos, C., M. A. Freudenberg & W. Reutter: Galactosamine-induced sensitization to the lethal effects of endotoxin. *Proc Natl Acad Sci U S A*, 76, 5939-43 (1979)
76. Hegen, M., M. Gaestel, C. L. Nickerson-Nutter, L. L. Lin & J. B. Telliez: MAPKAP kinase 2-deficient mice are resistant to collagen-induced arthritis. *J Immunol*, 177, 1913-7 (2006)
77. Jagavelu, K., U. J. Tietge, M. Gaestel, H. Drexler, B. Schieffer & U. Bavendiek: Systemic deficiency of the MAP kinase-activated protein kinase 2 reduces atherosclerosis in hypercholesterolemic mice. *Circ Res*, 101, 1104-12 (2007)
78. Gorska, M. M., Q. Liang, S. J. Stafford, N. Goplen, N. Dharajiya, L. Guo, S. Sur, M. Gaestel & R. Alam: MK2 controls the level of negative feedback in the NF-kappaB pathway and is essential for vascular permeability and airway inflammation. *J Exp Med*, 204, 1637-52 (2007)
79. Liu, T., R. R. Warburton, O. E. Guevara, N. S. Hill, B. L. Fanburg, M. Gaestel & U. S. Kayyali: Lack of MK2 inhibits myofibroblast formation and exacerbates pulmonary fibrosis. *Am J Respir Cell Mol Biol*, 37, 507-17 (2007)
80. Sousa, A. M., T. Liu, O. Guevara, J. Stevens, B. L. Fanburg, M. Gaestel, D. Toksoz & U. S. Kayyali: Smooth muscle alpha-actin expression and myofibroblast differentiation by TGFbeta are dependent upon MK2. *J Cell Biochem*, 100, 1581-92 (2007)
81. Park, J. K., N. Ronkina, A. Hoft, C. Prohl, J. Menne, M. Gaestel, H. Haller & M. Meier: Deletion of MK2 signalling *in vivo* inhibits small Hsp phosphorylation but not diabetic nephropathy. *Nephrol Dial Transplant* (2008)
82. Dunlop, M. E. & E. E. Muggli: Small heat shock protein alteration provides a mechanism to reduce mesangial cell contractility in diabetes and oxidative stress. *Kidney Int*, 57, 464-75 (2000)
83. Shiroto, K., H. Otani, F. Yamamoto, C. K. Huang, N. Maulik & D. K. Das: MK2-/- gene knockout mouse hearts carry anti-apoptotic signal and are resistant to ischemia reperfusion injury. *J Mol Cell Cardiol*, 38, 93-7 (2005)
84. Nagy, N., K. Shiroto, G. Malik, C. K. Huang, M. Gaestel, M. Abdellatif, A. Tosaki, N. Maulik & D. K. Das: Ischemic preconditioning involves dual cardio-protective axes with p38MAPK as upstream target. *J Mol Cell Cardiol*, 42, 981-90 (2007)
85. Wang, X., L. Xu, H. Wang, P. R. Young, M. Gaestel & G. Z. Feuerstein: Mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 deficiency protects brain from ischemic injury in mice. *J Biol Chem*, 277, 43968-72 (2002)
86. Culbert, A. A., S. D. Skaper, D. R. Howlett, N. A. Evans, L. Facci, P. E. Soden, Z. M. Seymour, F. Guillot, M. Gaestel & J. C. Richardson: MAPK-activated protein kinase 2 deficiency in microglia inhibits pro-inflammatory mediator release and resultant neurotoxicity. Relevance to neuroinflammation in a transgenic mouse model of Alzheimer disease. *J Biol Chem*, 281, 23658-67 (2006)
87. Weber, H. O., R. L. Ludwig, D. Morrison, A. Kotlyarov, M. Gaestel & K. H. Vousden: HDM2 phosphorylation by MAPKAP kinase 2. *Oncogene*, 24, 1965-72. (2005)
88. Bulavin, D. V., Y. Higashimoto, I. J. Popoff, W. A. Gaarde, V. Basrur, O. Potapova, E. Appella & A. J. Fornace, Jr.: Initiation of a G2/M checkpoint after ultraviolet radiation requires p38 kinase. *Nature*, 411, 102-7. (2001)
89. Reinhardt, H. C., A. S. Aslanian, J. A. Lees & M. B. Yaffe: p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage. *Cancer Cell*, 11, 175-89 (2007)
90. Lee, J. C., J. T. Laydon, P. C. McDonnell, T. F. Gallagher, S. Kumar, D. Green, D. McNulty, M. J. Blumenthal, J. R. Heys, S. W. Landvatter & *et al.*: A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature*, 372, 739-46. (1994)
91. Gaestel, M., A. Mengel, U. Bothe & K. Asadullah: Protein kinases as small molecule inhibitor targets in inflammation. *Curr Med Chem*, 14, 2214-34 (2007)
92. Dominguez, C., D. A. Powers & N. Tamayo: p38 MAP kinase inhibitors: many are made, but few are chosen. *Curr Opin Drug Discov Devel*, 8, 421-30 (2005)
93. Allen, M., L. Svensson, M. Roach, J. Hambor, J. McNeish & C. A. Gabel: Deficiency of the stress kinase p38alpha results in embryonic lethality: characterization of the kinase dependence of stress responses of enzyme-deficient embryonic stem cells. *J Exp Med*, 191, 859-70. (2000)
94. Zu, Y. L., Y. Ai & C. K. Huang: Characterization of an autoinhibitory domain in human mitogen-activated protein kinase-activated protein kinase 2. *J Biol Chem*, 270, 202-6. (1995)
95. Anderson, D. R., S. Hegde, E. Reinhard, L. Gomez, W. F. Vernier, L. Lee, S. Liu, A. Sambandam, P. A. Snider & L. Masih: Aminocyanopyridine inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2). *Bioorg Med Chem Lett*, 15, 1587-90 (2005)

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