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The Mnks: MAP kinase-interacting kinases (MAP kinase signal-integrating kinases)

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1. ABSTRACT

The human MAP kinase-interacting kinases (or MAP kinase signal-integrating kinases), Mnks, comprise a group of four proteins derived from two genes (Gene symbols: MKNK1 and MKNK2) by alternative splicing. Mnk1a/b differ at their C-termini, as do Mnk2a/2b: in each case, the a-form possesses a longer C-terminal region than the b-form, which lacks the MAP kinase-binding region. The N-termini of all forms contain a polybasic region which binds importin α and the translation factor scaffold protein eukaryotic initiation factor (eIF) 4G. The catalytic domains of Mnk1a/b and Mnk2a/b share three unusual features: two short inserts and a DFD feature where other kinases have DFG. Mnk isoforms differ markedly in their activity and regulation, and in subcellular localization. The best-characterised Mnk substrate is eIF4E. The cellular role of eIF4E phosphorylation remains unclear: it may promote export of certain mRNAs from the nucleus. Other Mnk substrates bind to AU-rich elements that modulate the stability/translation of specific mRNAs. Mnks may also control production of inflammatory mediators and signaling from tyrosine kinase receptors, as well as cell proliferation or survival.

2. THE MNK FAMILY OF PROTEIN KINASES

2.1. Discovery of the Mnks

The Mnks were discovered, independently but almost simultaneously, as a consequence of two new and quite different screens for proteins regulated by the 'classical' MAP kinases, the ERKs. In one approach, ERK substrates were sought by screening bacterial expression libraries; in the other, binding partners for the ERKs were identified using the yeast two-hybrid system (1,2). This study resulted in the identification of human Mnk1 and revealed that it was phosphorylated by ERK and also by p38 MAP kinases α/β, but not by the related JNK enzymes (1). The yeast two-hybrid screen (2) yielded clones for (mouse) Mnk1 and also Mnk2, and showed that Mnk1 interacted with ERK2 and p38 MAP kinase, but not with JNK1. Phosphorylation of ERK decreased its binding to Mnk1. In contrast, Mnk2 only bound stably to ERK2. These studies also demonstrated that Mnk1 was activated by agents that stimulate ERK (such as phorbol esters or serum) or p38 MAP kinase α/β (e.g., stress conditions such as hyperosmolarity and UV-C irradiation or pro-inflammatory cytokines such as tumour necrosis factor α or interleukin 1β).

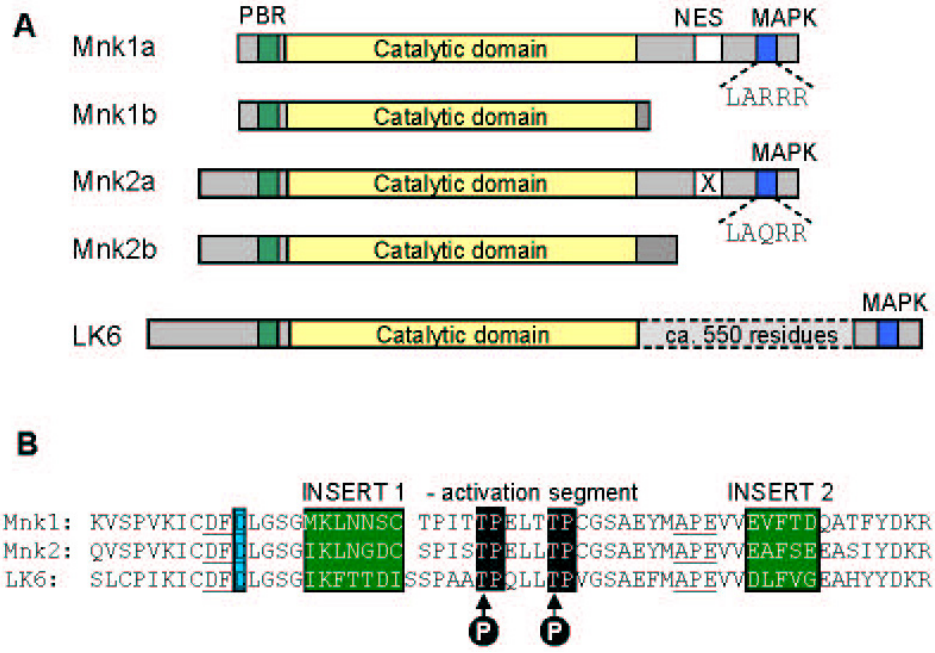

Figure 1. Organization of the Mnks and the related *Drosophila* enzyme, LK6. A: this figure depicts the overall layout on the four known Mnk isoforms and of the closely-related *Drosophila* enzyme LK6. The region demarcated by dashed lines denotes the large region of LK6 that is absent from the mammalian Mnks. The polybasic region (PBR) that binds importin α and eIF4G is shown, as is the MAP kinase binding site, and the core motif of this region. The nuclear-export sequence (NES) in Mnk1a and the related but non-functional motif on Mnk2a are shown. B: sequences around the activation loops of Mnk1 and Mnk2 showing the DFD motif, the two Mnk-specific inserts and the threonine residues which are phosphorylated by the ERKs/p38 MAP kinases αβ.

Importantly, the latter study identified the first substrate for the Mnks – eukaryotic initiation factor (eIF) 4E, the translation initiation factor that binds the 5'-cap structure found on all eukaryotic cytosolic mRNAs (2).

. Its phosphorylation was subsequently shown to be regulated through the ERK and p38 MAP kinase α/β pathways (2-4) and to depend upon the Mnks (5).

2.2. Relationship of the Mnks to other protein kinases

The catalytic domains of Mnk1 and Mnk2 display approximately 70% sequence identity (6). Sequence comparisons revealed that the Mnks were most closely related to other protein kinases that are activated by members of the MAP kinase family, e.g., MK2 (also called MAPKAP-K2), pK3 (also called MAPKAP-K3 or MK3), MK5 (also called PRAK or MAPKAP-K5), and the C-terminal kinase domain of the RSKs (1,2), as well as the MSKs. In common with these other enzymes, the activation loop of the Mnks contains phosphorylatable (threonine) residues followed by prolines which are thus potential substrates for phosphorylation by MAP kinases. In the Mnks, there are actually three threonines each followed by a proline: mutation of the second two yields an inactive enzyme. Recent studies suggest that the first threonine is not required for Mnk activation (Goto and Proud, unpublished data).

Compared to all other protein kinases, the catalytic domains of Mnk1 and Mnk2 show three specific and unusual features. First, a striking difference between Mnks and all other kinases in the main superfamily is the presence of a DFD (Asp-Phe-Asp) motif in subdomain VII, where all other protein kinases have DFG (Figure 1B). In addition the catalytic domains of Mnk1 and Mnk2 contain two 'inserts', i.e., short sequences not found in other protein kinases in the calmodulin-dependent kinase subfamily to which the Mnks are most closely related (Figure 1B). The first insert lies immediately C-terminal to the DFD motif and just N-terminal of the 'activation loop' (or 'activation segment') which contains the two threonines that are phosphorylated by the MAP kinases. The second insert, of five residues, lies immediately C-terminal to the APE motif (subdomain VIII) and therefore just after the activation loop. These features are discussed further below. Both the unusual DFD motif and the two insertions are also present in the fruitfly kinase LK6, the closest relative of the Mnks found in that species.

The initial report for mouse Mnk1 and Mnk2 identified cDNAs that lacked 12 residues present at the N-terminus of human Mnk1 (1,2) and 47 residues at the N-terminus of human Mnk2 (7). A subsequent study (5) identified the 'missing' N-terminus of mouse Mnk2 and showed that it is highly similar to the corresponding region

Table 1. Comparison of Mnk1 and Mnk2

Mnk1
HS1 MVSSQKLEKPIEMGSSEPLPIADGDRRRKKKRRGRATDSLPGKFEDMYKL
MM1 MGSSEPLPIVDSDKRRKKKRKTRATDSLPGKFEDVYQL * *
Mnk2
HS2
MVQKKPAELQGFHRSFKGQNPFELAFSLDQPDHGDSDFGLQCSARPDMPASQPIDIPDAKKRGKKKRGRATDSFSGRFE
MM2
MVQKRTAELQGFHRSFKGQNPFELAFSLDLAQHRDSDFSPQCEARPDMPSSQPIDIPDAKKRGRKKKRCRATDSFSGRFE

N-termini of the Mnks: HS1, human Mnk1; HS2, human Mnk2; MM1, mouse Mnk1, MM2, mouse Mnk2. The polybasic sequence that binds eIF4G and/or importin α is underlined. Residues that differ between human and mouse proteins are shaded grey. The residues in mouse Mnk1 that are phosphorylated by PAK-2 are indicated by asterisks (see text).

of the human protein. This information is summarized in Table 1.

The protein kinase PAK-2, which is generated from a precursor by caspase-3 cleavage, phosphorylates mouse Mnk1 at T22 and S27 (8). This prevents Mnk1 from binding to eIF4G. These residues lie just C-terminal to the polybasic sequence involved in binding eIF4G, but T22 is not conserved in the human protein, being replaced by glycine, which cannot be phosphorylated (Table 1).

2.3. Existence of splice variants

The Mnk proteins that were initially described (the longer forms, Mnk1a and Mnk2a) each contain a C-terminal motif that can bind MAP kinases. The MAP kinase-binding motifs in Mnk1 and Mnk2 differ, the former containing the sequence LARRR, while Mnk2 has LAQRR (Figure 1A). The best-characterised consensus sequence for MAP kinase binding (the 'D-domain'; $(R/K)_{1-2}$- $(X)_{2-6}$-ϕ-X-ϕ- (9) is found in many MAPK substrates including transcription factors, MAPKKs, and MAPK phosphatases (9,10), but does not match the sequences in MAPK-activated protein kinases including the Mnks (10,11). Instead, they appear to fit the KIM consensus sequence (12).

Subsequently, it became clear that each gene also gave rise to an alternative polypeptide, with a different C-terminus that lacks the MAP kinase-binding motif. The original polypeptides are now termed Mnk1a and Mnk2a (see Figure 1A). Mnk2b was initially found as a binding partner for oestrogen receptor β (ERβ) (7). Mnk1b was first described by O'Loghlen *et al.* (13). The 'b'-isoforms have so far only been identified conclusively in human cells. The mouse Mnks so-far studied correspond to the human 'a'-forms which have longer C-termini. Nevertheless, for consistency, these will be referred to as mouse Mnk1a and Mnk2a. Studies involving mice in which the genes for Mnk1 and/or Mnk2 had been knocked out suggested the presence of a shorter Mnk2 species in mouse cells: this may correspond to Mnk2b (5).

Although the four distinct human Mnk polypeptides display distinct characteristics, they do share a number of features in common. Firstly, Mnk1 and Mnk2 contain very closely related catalytic domains, as described above. All four isoforms also contain a polybasic sequence that lies N-terminal to the catalytic domain (Figure 1A). This feature is involved in binding to the translation factor scaffold protein eIF4G to recruit Mnks to phosphorylate eIF4E, its best known substrate, which also interacts with eIF4G (Figure 2). The Mnk/eIF4G interaction is required for the efficient phosphorylation of eIF4E by the Mnks (4,14). The polybasic region also binds to importin α, a karyopherin involved in the import of proteins into the cell nucleus. However, the residues within the polybasic region that are required for binding to importin α and to eIF4G are not identical (15).

Although all Mnk isoforms contain the importin α-binding sequence, not all of them are nuclear. Indeed, Mnk1a is essentially only found in the cytoplasm (15,16). This reflects the presence in its C-terminal region of a nuclear export signal (NES) of the CRM1 type. Mutation of key residues within this feature renders Mnk1a nuclear. Mnk1b has a different (very short) C-terminal region and lacks this NES: accordingly, a high proportion of Mnk1b is nuclear (13). The C-terminal region of Mnk2b lacks an NES and a substantial fraction of Mnk2b is also nuclear (16). The situation for Mnk2a is a little more complicated: its extreme C-terminal domain appears to interfere with binding of its N-terminal polybasic region to eIF4G or to importin α. Consequently, Mnk2a is mainly cytoplasmic, even though it lacks an NES.

The activities of these Mnk isoforms – and their regulation – also differ very markedly. Mnk1a has quite low activity in serum-starved cells, and this is enhanced by stimuli that activate either ERK or p38 MAPK α/β (see, e.g., (2-4)), and inhibited by agents that block these pathways. In contrast, Mnk2a displays high basal activity and this is only slightly enhanced by agents that activate ERK (17). Inhibitors of ERK or p38 MAPK signalling can decrease Mnk2a activity, but only slightly. These properties seem to be related to the ability of Mnk2a to bind phosphorylated, active, ERK, to which Mnk1a does not bind stably. Binding to active ERK means that Mnk2a is physically associated with its activating kinase. The ability of Mnk2a to remain bound to phosphorylated ERK is conferred by features of its C-terminus, as discussed in more detail below.

Mnk2b (which lacks the MAP kinase binding site) shows very low activity under all conditions so far

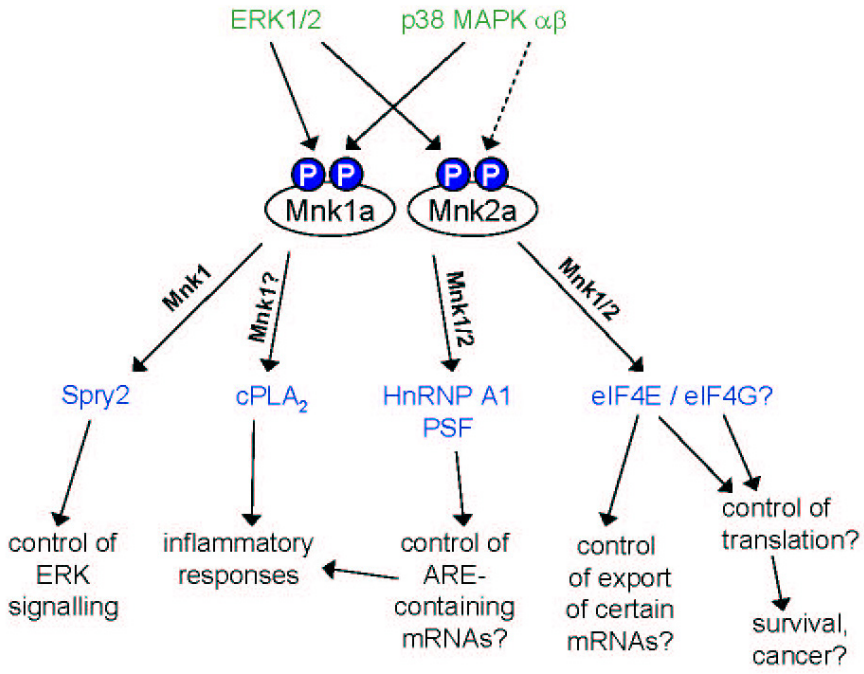

Figure 2. Downstream signaling from the Mnks. The figure shows a schematic depiction of the upstream control of the Mnks by MAP kinase pathways and of known Mnk substrates indicating their possible roles in cellular regulation. The dashed line indicates a probable but weak input from p38 MAP kinases αβ to Mnk2a. The roles of Mnk1b/2b are less clear: this reflects their lack of known regulation and relatively lower levels of activity. The question marks indicate that it is not completely certain that Mnk1 itself is the kinase that phosphorylates $cPLA_2$ or whether Mnk1-mediated phosphorylation of eIF4G affects mRNA translation.

tested. It is not yet clear under which circumstances, if any, it is activated (16). In contrast, Mnk1b shows significant basal activity, indeed higher activity than Mnk1a shows in serum-starved cells ((13,18) Goto, Yao and Proud, unpublished data). However, as expected since Mnk1b does not have a D-motif similar to the one present in Mnk2b, its activity is hardly affected by blocking the ERK or p38 MAP kinase α/β signalling pathways, indicating that its basal activity does not require inputs from ERK or p38 MAP kinase activity. This is consistent with the observation that Mnk1b is not phosphorylated by ERK (13,18). Since the two T-loop phosphorylation sites are each followed by a proline, other proline directed kinases could be responsible. However, treatment of cells with roscovitine, which inhibits several cyclin-dependent (proline-directed) kinases does not inhibit either the activity of Mnk1b or its T-loop phosphorylation, while treatment with pervanadate (which activates a broad spectrum of MAP kinase-family enzymes (see, e.g., (19)), does not enhance these parameters (Goto, Yao and Proud, unpublished data). It therefore remains unclear what accounts for the basal phosphorylation and activity of Mnk1b: O'Loghlen *et al.* (13,18)have suggested that this reflects features of the short (12 residue) C-terminus that is unique to Mnk1b. The properties of the different Mnk isoforms are summarized in Table 2. Our own recent data confirm that the activity of Mnk1b is higher than that of Mnk1a in serum-starved cells, and does not increase upon addition of agents that activate ERK or p38 MAP kinase α/β (Goto *et al.*, unpublished data). However, our data do not support the idea that this is due to specific features of the 12-amino acid C-terminus of Mnk1b, as a truncation of Mnk1a that also has only twelve remaining residues of its C-terminus shows the same characteristics as Mnk1b, as does a truncated protein lacking either C-terminal region.

The reasons for differences in the levels of activity between Mnk1a and Mnk2a involve features of both the C-terminus and the catalytic domain: this is illustrated by the properties of chimaeras that contain the N-terminus and catalytic domain of one isoform and the C-terminus of the other (termed Mnk1-CT2 and Mnk2-CT1). Thus, while Mnk2 with its own C-terminus shows very high 'basal' activity in serum-starved cells, the basal activity of Mnk2-CT1 is far lower (20). While this could suggest that it is the C-terminus of Mnk2 that confers high basal activity, the Mnk1-CT2 chimaera actually shows low basal activity. It is clear, however, that the C-terminus of Mnk2 does contain the features that allow stable binding to phosphorylated, active, ERK, which cannot bind to Mnk1 (20). The interaction with Mnk2a also protects phospho-ERK from dephosphorylation and inactivation (likely explaining the resistance of Mnk2a activation to ERK signalling blockade (20)). The association of active ERK with Mnk2, likely help explain why Mnk2 is active in serum-starved cells – since it is bound to residual phospho-ERK, which phosphorylates and activates it. As noted above, the MAP kinase-binding motifs in Mnk1 and Mnk2 differ (the third residue is Q in Mnk2, R in Mnk1; Figure 1A). However, this difference is not sufficient to explaintheir differing abilities to bind phospho-ERK and other residues in their C-termini are also important for this (20).

Table 2. Characteristics of human Mnk isoforms

Isoform	Activity	Localisation	Comments
Mnk1a	Basally low: increased by ERK or p38 MAPKαβ	Cytoplasmic; perinuclear	Long C-terminus contains NES and MAP kinase-binding site
Mnk1b	Some basal activity; not due to or increased by signaling through ERK or p38 MAPKαβ	Partially nuclear	Very short C-terminus lacks MAP kinase-binding site; may still promote activity
Mnk2a	Basally high; increased a little by signaling through ERK or p38 MAPKαβ	Mainly cytoplasmic	C-terminus contains MAP kinase-binding site, but lacks functional NES
Mnk2b	Low; not known circumstances where it is increased	Partially nuclear	C-terminus lacks NES and MAP kinase-binding site

Features of the catalytic domain are also important in explaining the high activity of Mnk2. One such feature is a residue located immediately after insert 1 in the catalytic domain: this residue is Ser in Mnk1 and Asp in Mnk2 (20). Mutation of this residue to aspartate in Mnk1 does not increase basal activity but greatly enhances its activity after treatment of cells with phorbol ester (which activates ERK). However, making the same mutation in the Mnk1-CT2 chimaera raises its basal activity to the level observed for Mnk2 itself. This indicates that this single residue in the catalytic domain together with features in the C-terminal tail, likely related to phospho-ERK binding, suffice to confer high basal activity on Mnk2a .Nevertheless, determination of the crystal structures of catalytic domains of Mnk1 and Mnk2 also point to additional features that may play a role in this.

2.4. Structural studies

Jauch and co-workers solved the three-dimensional structures of the catalytic domains of Mnk1 and Mnk2 expressed in bacteria (21,22). The first to be reported was that of a fragment of Mnk2 containing the catalytic domain. The two main features revealed by this structure were, first, that the activation loop showed an unusual open conformation. This is the structure of the non-phosphorylated protein and rearrangements of this region, contributing to kinase activation, presumably occur upon phosphorylation of the two threonine residues in the loop. Second, as mentioned above, both Mnks possess a DFD rather than a DFG motif. The crystal structure revealed that this potentially interferes with ATP binding. This feature favours a so-called DFG/D-OUT conformation which obstructs the usual mode in which ATP is bound to protein kinases. Certain other protein kinases also adopt this conformation. Mutation of the DFD to the canonical DFG sequence had little effect on ATP-binding or kinase activity of the kinase domain fragment, suggesting that the inhibitory conformation may be unstable, at least in the context of this fragment (21,22). Nevertheless, this DFG/D-OUT conformation and unusual features of the Mg^{2+}-binding loop may provide scope for the development of highly specific Mnk inhibitors, along the lines of the p38 MAP kinase inhibitor BIRB, which induces a DFG/D-OUT conformation (reviewed in (23)).

A second study determined the structure of the kinase region of Mnk1 and revealed a number of unusual features of its activation loop (21,22), pointing to a novel mechanism for the activation of the Mnks. In particular, the activation loop appears to function as an "autoinhibitory module". This involves two specific features of the Mnks referred to above, i.e., residues within the second Mnk-specific insertion, I2 at subdomain VIII, and the locking of the DFD motif into the DFG/D-OUT conformation, which presumably impairs ATP binding. This conformation is stabilized by the repositioning of the activation segment. Specifically, the F of the DFD motif projects into the ATP-binding site. In the structure of the Mnk1 kinase domain the activation loop was disordered, so it provides little direct information on how phosphorylation within this loop leads to kinase activation. In this study (22), mutational analysis lent support to the conclusion that insertion 2 and specifically the F residue within the DFD motif operate in an autoinhibitory fashion.

It is interesting to note that certain differences between Mnk1 and Mnk2 may weaken the autoinhibition in the latter: for example, two residues that form interactions that appears to stabilize this conformation in Mnk1 (Y60, T97) are replaced in Mnk2 by residues that seem less able to perform this function (H95, M132). Further mutational and biochemical analyses, most appropriately of full-length Mnk1/2, is required to test these predictions.

3. SUBSTRATES FOR THE Mnks

Mnks have now been reported to phosphorylate a number of different proteins in addition to eIF4E, the first *in vitro* Mnk substrate to be described (2). Like eIF4E, several of these are either components of the translational machinery (e.g., eIF4G (14) or proteins that bind mRNA (e.g., heterogeneous nuclear RNA-binding protein (hnRNP) A1 (24,25) and the polypyrimidine-tract binding protein-associated splicing factor (PSF) (26). Additional substrates include other cytoplasmic proteins (cytoplasmic phospholipase A2 (27) and Sprouty (28)).

3.1. eIF4E

Studies on knock-out mice lacking both Mnk1 and Mnk2 demonstrated very clearly that Mnks are the sole eIF4E kinases in mouse cells, at least for the range of tissues and conditions tested (5). Despite extensive studies over more than two decades, no consensus, or even a clear model, has emerged to explain the possible functional significance of the phosphorylation of eIF4E (Figure 2), which occurs at S209 (Table 3). This topic was last reviewed in detail in 2002, (29), and we will summarize the position and discuss data that have appeared since then.

Table 3. Sequence around known phosphorylation sites for Mnk1 or Mnk2

Substrate	Site (s)	Comments	Reference
eIF4E	S209: TATKSGSTTKNRFVV	Mnk1 and Mnk2	2
cPLA2	S727: QNPSRCSVSLSNVEAR	Reported for Mnk1	27
HnRNP A1	S192: QEMASASSSQRGRSGS	Mnk1 and Mnk2	24
	S310-2: GYGGSSSSSSYGSGRR		
PSF	S8: SRDRFRSRGGGGGGFH	Mainly Mnk2	26
	S283: GFKANLSLLRRPGE	Mnk1 and Mnk2	
Sprouty	S112/121: PLSRSISTVSSGSRSSTRTSTSSSS	Reported for Mnk1	28

The Mnk inhibitor CGP57380 was first identified by Gram and colleagues, who subsequently used it to study the role of eIF4E phosphorylation in cap-dependent and –independent translation (30,31). Their approach involved using bicistronic reporter vectors in transient transfection. In such vectors, the first (more 5') cistron is translated in a cap-dependent manner while the second, placed after an internal ribosome entry site, is translated in a cap-independent manner. They showed that co-expression of active Mnks enhanced eIF4E phosphorylation but impaired cap-dependent relative to IRES-driven translation. Consistent with the inhibitory effect on cap-dependent translation, Mnk expression decreased protein synthesis and clonal growth in human embryonic kidney 293 cells. Conversely, treatment of cells expressing the bicistronic reporter with CGP57380 slightly enhanced cap-dependent translation, but in this case did not affect cap-independent translation.

On the basis of these and other findings, it was suggested that phosphorylation of eIF4E may be part of the normal cycle of events involved in cap-dependent translation. As discussed in (29) and then developed further (32,33), phosphorylation of eIF4E may occur at a certain stage of the cap-dependent initiation process. eIF4E is part of the initiation complex that also contains eIF4G and the Mnks: perhaps conformational changes within this complex favour eIF4E phosphorylation at a certain point in the initiation process. This might explain why a forced increase in eIF4E phosphorylation – due to overexpression of Mnks – impairs cap-dependent translation (32,33). Such a model would be consistent with the findings of Knauf *et al.* (30).

Since the individual steps in cap-dependent translation initiation are not yet clear, further work will be needed to design experiments to test this idea. It is also possible that the effects of the Mnks and CGP57380 are related to other Mnk substrates, perhaps eIF4G itself.

It is now clear that phosphorylation of eIF4E decreases its affinity for the capped mRNA (34). However, the role of eIF4E phosphorylation in the control of translation is still not understood. It has been reported that the recovery of overall protein synthesis rates from osmotic shock is not impaired by inhibition of the Mnks, implying that it does not require phosphorylation of eIF4E (35). However, these data did point to an impairment of polysome assembly in cells treated with the Mnk inhibitor CGP57380, raising the possibility that eIF4E phosphorylation somehow contributes to polysome formation.

Mohr and colleagues have studied the possible role of eIF4E phosphorylation during viral infection/replication. Inhibiting eIF4E phosphorylation using CGP57380 dramatically decreased the replication of herpes simplex virus-1 and the translation of viral mRNAs in primary human cells (36). Interestingly, the effect of the p38 MAP kinase α/β inhibitor SB203580 was even greater, suggesting that additional events dependent on these kinases are involved in virus replication. The same group subsequently reported that CGP57380 also greatly inhibited the replication of human cytomegalovirus (37). As the authors rightly point out, their data do not shed any light on the role of eIF4E phosphorylation in the shut-off of host cell protein synthesis in virus-infected cells: rather, they may reflect roles of eIF4E phosphorylation in ensuring efficient translation of viral transcripts. Given that the Mnks do have other substrates, any effects of CGP57380 may reflect alterations in the phosphorylation of those proteins rather than eIF4E. Furthermore, this compound can inhibit other protein kinases (38) and such effects may also account, at least in part, for some of the reported effects of the Mnks.

Very recent data suggest that signalling through PI 3-kinase can also provide a positive input to eIF4E phosphorylation, independently of the mammalian target of rapamycin, mTOR (39): in fact, mTOR inhibition, likely by inhibiting a negative feedback loop from mTOR (via S6 kinases) to PI 3-kinase, actually promotes eIF4E phosphorylation. This finding was quite unexpected: as noted above, the eIF4E·eIF4G·Mnk complex is critical for efficient phosphorylation of eIF4E (4,14). By causing the dephosphorylation of the 4E-BPs, and thereby increasing their binding to eIF4E, agents such as rapamycin would be expected to inhibit eIF4E phosphorylation – since eIF4E should now be unable to bind eIF4G. This study did not examine the impact of mTOR inhibition on the formation of these complexes. However, the data do indicate that PI 3-kinase can positively regulate the Mnks, and data from cells lacking Mnk1, Mnk2 or both indicate that both Mnk isoforms can be controlled in this way. In combination, rapamycin and CGP57380 exerted a much stronger growth-inhibitory effect on a lung cancer cell line than either agent alone (39). These data are of particular interest in view of other findings that link eIF4E phosphorylation to events that promote cell proliferation (e.g., export of specific mRNAs from the nucleus (see below). See also Section 3.1.

3.2. Observations on the use of CGP57380

It should be noted that two major caveats apply to the interpretation of data obtained using CGP57380. First,

reliance on CGP57380 as the sole indicator of the importance of the Mnks in a biological process may be misleading, since the specificity of this inhibitor is not fully established as recent data indicate that this compound can also inhibit certain other protein kinases (38)). Additional data is therefore essential to support the interpretation that Mnks are involved in a given process. This could be obtained from RNA interference-mediated knock-down of Mnks or from expression of dominant interfering Mnk mutants (see, e.g., (1,2)), although it would be important to rule out that its expression affected other targets of the ERKs/p38 MAP kinase αβ, especially for Mnk2a, which as discussed stably binds to active ERK, for example, and could therefore interfere with other signalling components downstream of ERK. The use of cells from Mnk knockout mice (5) should provide valuable information. Second, it is now clear that the Mnks phosphorylate other proteins in addition to eIF4E, and therefore the effects of this compound may reflect impaired phosphorylation of other Mnk substrates. Nonetheless, CGP57380 can certainly be used to help to rule out a role for eIF4E in a process: if CGP57380 does not affect it, then eIF4E phosphorylation is unlikely to be required for the process under study.

3.3. eIF4G

eIF4G is a scaffold protein that binds a range of components of the translational machinery including the Mnks themselves (4,14) and eIF4E. eIF4G comprises a number of distinct 'domains'. Mnks bind to the C-terminal part of eIF4G: this involves the polybasic motif in the N-terminus of the Mnks (4,14,15). eIF4E binds near the centre of the eIF4G molecule while eIF4G's N-terminus interacts with the poly- (A)-binding protein (PABP). These interactions with proteins that engage the 5'-cap and the 3'-poly (A) tail mean that eIF4G in effect facilitates the circularization of the mRNA, a feature that is believed to be important for efficient translation of the message (40). eIF4G also binds to eIF4A, an RNA helicase that is thought to facilitate the translation of those mRNAs whose 5'-untranslated regions (UTRs) contain substantial secondary structure. By unwinding such structures, eIF4A likely facilitates the scanning process by which the 40S ribosomal subunit locates the start codon (41).

Although it was suggested some time ago that eIF4G is a substrate for phosphorylation by the Mnks (14), no further information on this has become available about the location of the sites or the functional significance of these modifications (Figure 2). Further work is clearly needed to clarify both points.

3.4. Proteins that binds AU-rich elements

3.4.1. hnRNP A1

Many mRNAs that correspond to 'immediate early' genes, or encode cytokines or other immuno-modulatory proteins contain, within their 3'-UTRs, elements that are rich in A and U residues (hence 'AU-rich elements, AREs) (42). Expression of proteins encoded by ARE-containing messages is subject to tight regulation and at the post-transcriptional level this mainly involves the ERK and p38 MAP kinase α/β pathways, which positively control their production (43). The AREs regulate the stability and/or translation of the mRNAs through their interaction with ARE-binding proteins (ARE-BPs). Several ARE-binding proteins involved in mRNA translation and turnover have been identified, although much less is known about the upstream signalling pathways that regulate them. There is extensive evidence that MK2, a protein kinase that is activated by p38 MAP kinases α/β , controls the fate of some ARE-containing mRNAs and phosphorylates ARE-BPs such as TTP, HuR, hnRNP A0 or PABP. In contrast, the link to ERK signalling is much less well-characterized. The ERK pathway has been implicated in the translation of neuronal mRNAs during synaptic activity (44), the nuclear export of mRNAs such as TNFα (45) or Hdm2 (46) and the translation of DICE (differentiation-control element) containing mRNAs through the phosphorylation-dependent cellular localization of hnRNPK (47). However, evidence supporting the involvement of the ERK pathway in ARE-dependent translational control of specific mRNAs is limited. A recent paper established that not only p38 MAPK α/β but also ERK is required for TNFα stabilization through the inhibition of TTP (48). However, downstream of the ERK and the p38 MAPK pathways, the Mnks have been implicated in the control of ARE-containing mRNAs in T lymphocytes (24) as well as macrophages (49,50). In T-cells, this may involve the newly identified Mnk-substrate, the ARE-binding protein hnRNP A1.

hnRNP A1, a major component of eukaryotic heterogeneous nuclear RNA-ribonucleoprotein complexes, is a very abundant nuclear protein that has an important role in mRNA metabolism. Although it is primarily nuclear, hnRNP A1 shuttles continuously between the nucleus and the cytoplasm, and this involves the M9 motif, which mediates the bidirectional transport of hnRNP A1 (51,52). hnRNP A1 is a sequence-specific RNA binding protein (53) that can regulate splice-site selection (54), nuclear export of mature mRNAs (52) and internal ribosome entry site-mediated translation (55,56), among others. hnRNP A1 is also an ARE-binding protein (57) and has been implicated in controlling mRNA stability (58,59).

hnRNP A1 undergoes several modifications including phosphorylation. Osmotic stress via p38 MAPK signalling results in the hyperphosphorylation and cytoplasmic accumulation of hnRNP A1 (60) to stress granules (25). Recruitment of hnRNP A1 to stress granules probably involves Mnk1/2-dependent phosphorylation of mRNA-bound hnRNP A1, as dominant negative mutants of Mnk1/2 block the stress-induced hyperphosphorylation and cytoplasmic accumulation of hnRNP A1. The stress-induced phosphorylation sites in hnRNP A1 lie in a stretch of serines located adjacent to the M9 motif, the sequence termed the F peptide (51).

The Mnks not only phosphorylate hnRNP A1 upon osmotic stress but also in response to T-cell activation (24). In this paper, the authors identified hnRNP A1 as an ARE-binding protein that is also an Mnk substrate. Mnks phosphorylate hnRNP A1 at two sites *in vitro*, S192 and S310-312 (which fall within the stress-induced phosphorylation region). T-cell activation

results in phosphorylation of hnRNP A1 at the Mnk sites and this decreases the ability of hnRNP A1 to bind the TNFα mRNA *in vivo*, perhaps thereby derepressing its translation While Mnk-phosphorylation of hnRNP A1 upon osmotic stress causes its relocalisation to the cytoplasm and an increase binding to poly (A) mRNA, upon T-cell activation, Mnk-mediated hnRNP A1 phosphorylation does not promote hnRNP A1 accumulation into the cytoplasm but causes a decreased binding of hnRNP A1 to the TNFα mRNA. It is possible that other modifications, including additional phosphorylation sites, account for the differential behaviour of hnRNP A1 in response to osmotic stress or T cell activation.

Overall, Buxade *et al.* (24) provided several lines of evidence that the Mnks play a key role in the regulation of the translation of specific messages such as TNFα by phosphorylating proteins that bind the regulatory AREs in its 3'UTR such as hnRNP A1 (Figure 2).

3.4.2. PSF

PSF (polypyrimidine tract-binding protein (PTB)-associated splicing factor) was first identified as a 100kDa protein that forms a large complex with PTB necessary for pre-mRNA splicing (61). Since then, multiple functions have been attributed to PSF, a nuclear protein involved in transcription and RNA processing.

Together with $p54^{nrb}$, a nuclear DNA/RNA-binding protein with and extensive homology to PSF, PSF forms an heterodimeric transcription-splicing factor implicated in nuclear processes such as transcription, nuclear RNA processing, nuclear retention of edited RNA, DNA relaxation, tumorigenesis, DNA double-strand break rejoining, transcription termination and pre-mRNA processing. Also involved in RNA metabolism, PSF/$p54^{nrb}$ positively regulate translation of the Myc family of onco-proteins, being a novel IRES-transacting factor (ITAF) specific for the myc IRES (62), inhibit HIV-1 mRNA expression through binding to the HIV-1 *cis*-acting instability elements (63) and are involved in the posttranscriptional regulation of the COX2 mRNA, regulating its alternative polyadenylation (64).

PSF and $p54^{nrb}$ are both phosphoproteins. PSF is N-terminally hyperphosphorylated on serine/threonine during apoptosis, and this alters its protein-protein interactions and relocalises it to alternative nuclear structures. However, neither the kinase (s) nor the mechanism involved has yet been identified (65). It has also been reported that PSF and $p54^{nrb}$ can be phosphorylated *in vitro* by protein kinase C (PKC) and that this inhibits their binding to RNA (66,67). Moreover, PSF can be phosphorylated *in vitro* by SR kinases within the N-terminus and SR phosphorylation of PSF inhibits its binding to the polypyrimidine tract of pre-mRNA introns (68). In addition, $p54^{nrb}$ is multiply phosphorylated by Cdk1 and possibly by other kinases during mitosis thereby regulating its binding properties (69).

More recently PSF has been identified as a novel intracellular Mnk substrate and together with its partner $p54^{nrb}$, as a new binding complex for mRNAs that possess AREs, such as those encoding for cytokines, immediate early genes or other proteins involved in inflammation (26).

The Mnks phosphorylate PSF at two sites *in vitro*, S8 and S283, and S8 is known to be phosphorylated within cells in a Mnk-dependent manner. Interestingly, Ser8 is preferentially phosphorylated by Mnk2, providing the first evidence for differences between the specificities of Mnk1 and Mnk2. Mnk-mediated phosphorylation of PSF increases the *in vivo* binding of PSF to the TNFα mRNA, suggesting that the Mnks may regulate the fate of specific mRNAs by modulating their binding to PSF/$p54^{nrb}$ (26).

PSF is thought mainly to participate in nuclear events (reviewed in (70)) and the Mnk2b isoform is nuclear (16). It is therefore attractive to speculate that its Mnk-regulated ability to bind the TNFα mRNA (and likely other ARE-containing mRNAs) plays a role in modulating the nuclear processing of such mRNAs or their transport into the cytoplasm. However, regulation at the level of cytoplasmic mRNAs, similar to that reported for the c-Myc mRNA (62), cannot be excluded.

3.5. $cPLA_2$

Cytoplasmic phospholipase A2 plays a key role in the production of eicosanoids, which play a number of important roles especially in immunity and inflammation. $cPLA_2$ releases arachidonate from glycerophospholipids thereby providing the precursor for eicosanoid synthesis $cPLA_2$ can be activated by Ca^{2+}-ions or by phosphorylation. Phosphorylation occurs at least at two main sites (S505 and S727) and is induced via signalling through p38 MAP kinase α/β (27). Since these sites are not followed by prolines (which are required for direct phosphorylation by p38 MAP kinases), they were likely to be targets for kinases that are activated by p38 MAP kinase α/β. To test the role of Mnk1 in this, a mutant of Mnk1 in which both T-loop threonines were mutated to alanine was used. This mutant appears to act as a dominant negative (4) and inhibited – albeit incompletely - arachidonate release induced by interleukin-1 (27). Mnk1 was also shown to phosphorylate $cPLA_2$ at S727 *in vitro (*see Table 3). As noted, this site is regulated in a p38 MAP kinase α/β-dependent manner in living cells. These and other data suggest that signalling through p38 MAP kinase α/β leads via Mnk1 to the activation of $cPLA_2$. However, since MSK1 and MK5 (which are also downstream of p38 MAP kinase α/β) can also phosphorylate $cPLA_2$, and since S505 is not a target for Mnk1, other links between p38 MAP kinase and $cPLA_2$ appear to operate.

These data are of particular interest in the context of the evidence that Mnk1 also regulates the production of the pro-inflammatory cytokine TNFα (24,50). They suggest that Mnk1 may control both the synthesis of cytokines and the activity of enzymes that generate other modulators of inflammation, such as eicosanoids (Figure 2).

3.6. Sprouty (hSpry2)

Sprouty (Spry) proteins belong to a group of membrane-associated proteins that suppress ERK activation and/or signalling (71). In fact, Spry proteins also appear to modulate additional signalling pathways but show specificity in terms of the upstream activators whose effects they modulate: for example, they block the activation of ERK by fibroblast growth factor but not epidermal growth factor (reviewed in (71)). Furthermore, they exert both positive and negative effects on receptor tyrosine kinase signalling. Knock-outs of individual Spry proteins in mice reveal that Spry1 and Spry2 serve quite distinct functions.

Spry proteins are subject to phosphorylation on serine and tyrosine. Recent work shows that Mnk1 phosphorylates Spry2 at two sites, S112 and S121 (Table 3) (28). Conversion of these residues to alanines caused the destabilization of Spry2, as did expression of the inactive 'T2A2' mutant of Mnk1 which appears to act as a dominant negative. The Mnk inhibitor CGP57380 also led to degradation of Spry2, which is mediated by the proteasome. Conversely, expressing a Mnk1 mutant which exhibits increased activity led to the stabilization of Spry2. Spry2 phosphorylation on serine also impairs its phosphorylation at Y55, the docking site for c-Cbl, a ubiquitin C3 ligase, providing a potential mechanism for the stabilising effect of Mnk1-mediated phosphorylation.

The Mnk1-mediated phosphorylation of Spry2 thus provides a mechanism for prolonging the half-life of Spry2 and thus its inhibitory effects on ERK signalling. Interestingly, the closest relative of the Mnks in fruitflies, LK6, was identified in a genetic screen as a modulator of Ras/ERK signalling (72). Overexpression of dSpry2 has a similar effect, which might be explained if LK6 stabilized dSpry2 in a similar way to the effect of Mnk1 on human Spry2. The amino acid sequences of the N-termini of dSpry and Spry2 are not highly homologous, but the former does contain serine and threonine residues in roughly similar positions to S112/121 in Spry2, and might therefore be a substrate for LK6.

In summary, the phosphorylation of Spry2 by Mnk1 would serve to allow the extended operation of a feedback mechanism that impairs or limits the activation of ERK, at least in response to certain stimuli (Figure 2).

4. OTHER CELLULAR FUNCTIONS OF MNKS

4.1. Roles for the Mnks in cell survival and cancer?

The availability of cells (immortalized MEFs) lacking the Mnks (5) has facilitated studies of their cellular roles. For example, double knock-out MEFs (DKO; lacking Mnk1 and Mnk2) more readily undergo apoptosis in response to serum withdrawal, as indicated by caspase 3 cleavage and annexin V binding, than either WT or single Mnk knockout cells (i.e., lacking Mnk1 or Mnk2) (73). This is consistent with the observation that treatment of WT MEFs with the Mnk inhibitor CGP57380 makes them more sensitive to apoptosis upon serum-starvation. Reintroducing Mnk1 or Mnk2 to the DKO MEFs enhances eIF4E phosphorylation to similar levels, but only Mnk1 was found to decrease sensitivity to apoptosis. This is puzzling as the Mnk1 KO cells did not show altered sensitivity to apoptosis but also suggests that the effects on susceptibility to apoptosis are not related to the prevailing levels of eIF4E phosphorylation. These data clearly suggest a role for the Mnks, and likely for a specific Mnk1 substrate, in anti-apoptotic signalling. Earlier data (8) pointed to a link between caspase-3 and Mnk1, in that the active form of the protein kinase PAK-2, generated by caspase-3 cleavage of a precursor, phosphorylates Mnk1 and this prevents Mnk1 from binding to eIF4G and phosphorylating it. However, these effects are downstream of caspase-3, while the data of Chrestensen *et al.* (73) suggest Mnk1 actually functions upstream of caspase-3 activation. Further work is clearly needed to study the links between Mnk1 and cell death/survival.

Other recent data from the same group indicate a possible role for the Mnks in breast cancer (74). For example, the activities of Mnk1 and Mnk2 are enhanced in certain breast cancer cell lines that overexpress HER2. HER2 (and EGFR) are involved in the development of different epithelial and mesenchymal tumours such as breast cancer. At present, HER receptors are targets for different anti-tumour therapeutic strategies. These include monoclonal antibodies against the extracellular domains of HER2 and EGFR or compounds that inhibit their tyrosine kinase activity (75). The search for new targets and more specific inhibitors are two of the most promising strategies to improve these therapies.

Interestingly, treatment of AU565 cells breast cancer cells that over-expresses HER2 with CGP57380, inhibited colony formation (74), while expression of an inactive, and reportedly dominant-negative, Mnk1 mutant inhibited the growth of leukemia cells. As Chrestensen *et al.* (74) point out, their data suggest a role for the Mnks in cell proliferation or invasiveness.

Very recent data support a role for eIF4E phosphorylation in tumorigenesis, apparently by suppressing apoptosis (76). Earlier work from the same group had established a role of eIF4E in lymphomagenesis (77). In the recent study, haematopoietic stem cells were infected with retroviruses encoding different mutants of eIF4E were injected into irradiated mice, and mice were examined for tumours. Consistent with earlier studies, wildtype eIF4E was tumorigenic and a S209D mutant of eIF4E displayed accelerated tumour formation, while the non-phosphorylatable S209A mutant was less effective than wildtype eIF4E. Although it should be noted that the S209A mutant only weakly mimics the effect of S209 phosphorylation on the binding properties of eIF4E *in vitro (*34), expression of an activated mutant of Mnk1 (T332A) also accelerated tumorigenesis, while a non-activatable variant (with alanine mutations at the T-loop phosphorylation sites) did not (76). These data are consistent with eIF4E phosphorylation promoting tumour formation: further analysis revealed that eIF4E (S209D) and activated Mnk1 both suppressed c-Myc induced apoptosis. Conversely, cells from Mnk1/2 double knockout

mice showed an enhanced sensitivity to c-Myc's pro-apoptotic effects. The data further suggest that the effects observed here may be related to increased expression of the anti-apoptotic protein Mcl-1, apparently due to increased translation of its mRNA. Thus, compounds that inhibit the Mnks may have potential for anti-tumour drug development.

4.2. Roles of Mnks and eIF4E phosphorylation in the nucleus

How might the Mnks be working to promote tumour formation, survival or growth? To date most work has focused on their best known substrate, eIF4E, and its potential roles in gene expression. As noted above, Mnk2b is found within the nucleus and appears to associate with the promyelocytic leukemia protein (PML), with which eIF4E also associates (78).

Of particular interest is the idea that eIF4E may play a role in the export of certain mRNAs from the nucleus to the cytoplasm (79,80). Overexpression of eIF4E is observed in many kinds of cancers, and this seems to correlate positively with 'tumour aggression' (81). Artificial overexpression of eIF4E has been known for some time to help transform cells (82,83) and to increase the cytoplasmic levels of certain mRNAs (84), including the mRNA for cyclin D1. Extensive work from the Borden laboratory has demonstrated that overexpression of eIF4E does indeed promote transport of the cyclin D1 mRNA into the cytoplasm. Their recent data indicate that eIF4E co-ordinates the export of a set of mRNAs many of which are involved in cell cycle control (79,85). The ability of eIF4E to regulate mRNA export is dependent of both its phosphorylation state and a 4E-sensitivity element present in the 3'-UTR of these mRNAs (84). It also involves the nuclear shuttling protein CRM1, although details of the mechanism involved remain to be established. In addition, Phillips and Blaydes (86) demonstrated the importance of the eIF4E phosphorylation for the export of the mRNA for Hdm2 and the expression of Hdm2 which was previously identified as one of the transcripts regulated by eIF4E .

To date, most attention has however been devoted to the export of the cyclin D1 mRNA. Analysis of point mutants of eIF4E indicates that it does not need to be able to interact with eIF4G, the partner with which it binds to promote cap-dependent mRNA translation, but does require an intact phosphorylation site at Ser209 (87). In cells expressing a S209A/T210A mutant of eIF4E, relatively less cyclin D1 mRNA was cytoplasmic (87).This mutation did not however alter the nucleocytoplasmic distribution of eIF4E itself. The finding that CGP57380 decreased the cytoplasmic levels of both cyclin D1 mRNA and cyclin D1 protein levels is consistent with the idea that phosphorylation of eIF4E is important for its ability to export at least the cyclin D1 mRNA to the cytoplasm.

These data are potentially very important for understanding the cellular roles of the Mnks and of eIF4E phosphorylation, and add further support to the idea that targeting the Mnks may be of some value in cancer therapy. However, extensive work is needed to understand the role of eIF4E phosphorylation in mRNA export, and indeed other roles that the Mnks play in the control of cell proliferation and survival.

5. COMMENTS ON THE SUBSTRATE SPECIFICITY OF THE MNKS

Although several substrates for the Mnks have now been identified (Table 3), comparison of the sequences around the sites of phosphorylation reveals no obvious consensus of the kind observed, e.g., for members of the AGC family of kinases. However, many of the sequences targetted by Mnks contain numerous serine (or threonine) residues and most contain some basic residues. However, unlike the situation for AGC kinases such as PKB, the RSKs, and the S6 kinases, there is no pattern to their distance from the residue (s) acted on by the Mnks, and they occur on either the N- or C-terminal side of the substrate residue. Since denatured eIF4E is a very poor substrate for phosphorylation by the Mnks (Scheper, Parra-Palau and Proud, unpublished data), it seems likely that higher order structure plays a key role in substrate recognition (at least for eIF4E).

6. Mnks IN NON-MAMMALIAN SYSTEMS

6.1. Drosophila LK6 and eIF4E phosphorylation

The protein kinase LK6 was first identified in a screen using antisera raised against microtubule-associated proteins from *Drosophila melanogaster*, antisera that also recognises centrosomes (88). The antiserum was used to screen expression libraries to identify microtubule-associated centrosomal proteins. One of the cDNAs thus identified encodes LK6. This protein contains, in addition to a canonical protein kinase catalytic domain, an N-terminal extension and a long C-terminal region, and has a total molecular mass of around 200 kDa (88) (Figure 1A). The C-terminal region contains a PEST sequence which likely accounts for the rapid degradation of LK6. LK6 was found to localise (at least in part) in centrosomes and to bind to microtubules. In addition, overexpression of LK6 led to defects in microtubule organisation. Among human protein kinases, the catalytic domain of LK6 is most closely related (61% identical, 77% similar residues) to those of the MAP kinase signal-interacting kinases Mnk1 and Mnk2 (see also Figure 1A). LK6 is the closest homologue of the Mnks in *Drosophila.*

eIF4E from *Drosophila* contains a serine (Ser251) in the position corresponding to Ser209 in mammalian eIF4E and mutation of this residue to alanine completely prevents phosphorylation *in vivo*. Phosphorylation of this site appears to play an important role in normal development and growth in *Drosophila* (89), based on the phenotypes observed when this residue is mutated.

Arquier and collaborators showed that eIF4E phosphorylation is controlled by LK6 and that phosphorylation appears to control cell size and number (90,91). Work by Reiling *et al.* showed that LK6 is dispensable for normal growth and development. However,

under conditions of reduced dietary protein and oxidative stress loss of LK6 affects cell number and size (92). Work in this laboratory showed that LK6 phosphorylates mammalian eIF4E *in vivo* and *Drosophila* eIF4E *in vitro*. LK6 is activated by ERK signalling but not by the stress-activated MAPK α/β pathway. Like the Mnks, LK6 also binds to the scaffolding protein eIF4G which is crucial for the phosphorylation of eIF4E. However, although LK6 contains a polybasic motif and a NLS consensus at the N and C-terminus similar to Mnk1 it failed to relocalise to the nucleus after leptomycin B treatment suggesting that the polybasic motif does not act as a NLS or other export mechanisms may be involved in its cytoplasmic localization (90,91).

6.2. eIF4E phosphorylation in *Aplysia*

The Sossin group has focused on the phosphorylation of eIF4E in the nervous system of the mollusk, *Aplysia californica*. Translational control is of interest in this context as mRNA translation is needed for sensory neuron connections, but transcription is not. The neurotransmitter serotonin (also termed 5-hydroxytryptamine) increases protein synthesis rates in sensory neurons in *Aplysia*: it is therefore important to establish both how translation is activated and how it contributes to neuronal function. Of particular relevance to the present discussion are that serotonin decreases phosphorylation of eIF4E (93). In *Aplysia* neurons, eIF4E phosphorylation seems to be mediated via p38 MAP kinase (rather than ERK). Dephosphorylation of eIF4E in this system appears to be linked to a switch to cap-independent translation (i.e., enhanced translation of the mRNA for egg-laying hormone, which contains an internal ribosome-entry site (IRES; (94)), as assessed using mutants of eIF4E which are mutated at the conserved phosphorylation site and an adjacent phosphorylatable residue. The enhanced expression of *Aplysia* Mnk, which enhances the phosphorylation of eIF4E, also inhibits cap-dependent translation (32,33). The sequence of *Aplysia* Mnk shows its MAP kinase binding site is more similar to that of Mnk1 than Mnk2, but some other features may be shared with Mnk2, so it is not possibly easy to say which one it more closely resembles.

Interestingly, 5-HT did not affect the T-loop phosphorylation of Aplysia Mnk, suggesting that it reduces eIF4E phosphorylation through other mechanisms. Most importantly, overexpression of Mnk inhibited cap-dependent translation more strongly than cap-independent (IRES-driven) translation (32,33). These data are reminiscent of those of Knauf *et al.* (30) which also indicated that Mnks impair cap-dependent translation.

7. PERSPECTIVE

In the ten years since the first Mnks were identified we have learned a good deal about their fundamental properties, such as the control of their activities and their subcellular localization. However, although eIF4E was identified as an Mnk substrate right from the start, the physiological significance of this modification remains unclear. Several Mnk substrates have now been identified including proteins that bind to specific mRNAs, which play roles in immune and inflammatory responses; including $cPLA_2$, an enzyme involved in generating inflammatory signals; and modulators of tyrosine kinase signalling (Spry2). However, the real physiological roles of the Mnks – including any specific functions of individual isoforms, remain unclear. There are growing indications that Mnks may have function in inflammatory responses, and in cell survival or proliferation, leading to tumorigenesis. Features of the structures of their catalytic domains may aid in the development of specific Mnk inhibitors which may prove to be useful in treating inflammatory conditions or as anti-cancer agents. Substantial further work is first necessary to define more fully the cellular roles played by the Mnks, including the functions of specific isoforms.

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9. REFERENCES

1. Fukunaga R, and T. Hunter: Mnk1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. *EMBO J* 16**,** 1921-1933 (1997)

2. Waskiewicz A. J, A. Flynn, C. G. Proud and J. A. Cooper: Mitogen-activated kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J* 16**,** 1909-1920 (1997)

3. Wang X, A. Flynn, A. J. Waskiewicz, B. L. J. Webb, R. G. Vries, I. A. Baines, J. Cooper and C. G. Proud: The phosphorylation of eukaryotic initiation factor eIF4E in response to phorbol esters, cell stresses and cytokines is mediated by distinct MAP kinase pathways. *J Biol Chem* 273**,** 9373-9377 (1998)

4. Waskiewicz A. J, J. C. Johnson, B. Penn, M. Mahalingam, S. R. Kimball and J. A. Cooper: Phosphorylation of the cap-binding protein eukaryotic translation factor 4E by protein kinase Mnk1 *in vivo*. *Mol Cell Biol* 19**,** 1871-1880 (1999)

5. Ueda T, R. Watanabe-Fukunaga, H. Fukuyama, S. Nagata and R. Fukunaga: Mnk2 and Mnk1 are essential for constitutive and inducible phosphorylation of eukaryotic initiation factor 4E but not for cell growth or development. *Mol Cell Biol* 24**,** 6539-6549 (2004)

6. Roux P. P. and J. Blenis: ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 68**,** 320-344 (2004)

7. Slentz-Kesler K, J. T. Moore, M. Lombard, J. Zhang, R. Hollingsworth and M. P. Weiner: Identification of the human Mnk2 gene (MKNK2) through protein interaction with estrogen receptor β. *Genomics* 69**,** 63-71 (2000)

8. Orton K. C, J. Ling, A. J. Waskiewicz, J. A. Cooper, W. C. Merrick, N. L. Korneeva, R. E. Rhoads, N. Sonenberg

and J. A. Traugh: Phosphorylation of Mnk1 by caspase-activated Pak2/gamma-PAK inhibits phosphorylation and interaction of eIF4G with Mnk. *J Biol Chem* 279**,** 38649-38657 (2004)
9. Bardwell A. J, M. Abdollahi and L. Bardwell: Docking sites on mitogen-activated protein kinase (MAPK) kinases, MAPK phosphatases and the Elk-1 transcription factor compete for MAPK binding and are crucial for enzymic activity. *Biochem J* 370**,** 1077-1085 (2003)
10. Sharrocks A. D, S. H. Yang and A. Galanis: Docking domains and substrate-specificity determination for MAP kinases. *Trends Biochem Sci.* 25**,** 448-453 (2000)
11. Tanoue T, M. Adachi, T. Moriguchi and E. Nishida: A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nat Cell Biol* 2**,** 110-116 (2000)
12. MacKenzie S. J, G. S. Baillie, I. McPhee, G. B. Bolger and M. D. Houslay: ERK2 mitogen-activated protein kinase binding, phosphorylation, and regulation of the PDE4D cAMP-specific phosphodiesterases. The involvement of COOH-terminal docking sites and NH2-terminal UCR regions. *J Biol Chem* 275**,** 16609-16617 (2000)
13. O'Loghlen A, V. M. Gonzalez, D. Pineiro, M. I. Perez-Morgado, M. Salinas and M. E. Martin: Identification and molecular characterization of Mnk1b, a splice variant of human MAP kinase-interacting kinase Mnk1. *Exp Cell Res.* 299**,** 343-355 (2004)
14. Pyronnet S, H. Imataka, A. C. Gingras, R. Fukunaga, T. Hunter and N. Sonenberg: Human eukaryotic translation initiation factor 4G (eIF4G) recruits Mnk1 to phosphorylate eIF4E. *EMBO J* 18**,** 270-279 (1999)
15. Parra-Palau J. L, G. C. Scheper, M. L. Wilson and C. G. Proud: Features in the N and C termini of the MAPK-interacting kinase Mnk1 mediate its nucleocytoplasmic shuttling. *J Biol Chem* 278**,** 44197-44204 (2003)
16. Scheper G. C, J.-L. Parra, M. L. Wilson, B. van Kollenburg, A. C. O. Vertegaal, Z.-G. Han and C. G. Proud: The N and C termini of the splice variants of the human mitogen-activated protein kinase-interacting kinase Mnk2 determine activity and localization. *Mol Cell Biol* 23**,** 5692-5705 (2003)
17. Scheper G. C, N. A. Morrice, M. Kleijn and C. G. Proud: The MAP kinase signal-integrating kinase Mnk2 is an eIF4E kinase with high basal activity in mammalian cells. *Mol Cell Biol* 21**,** 743-754 (2001)
18. O'Loghlen A, V. M. Gonzalez, T. Jurado, M. Salinas and M. E. Martin: Characterization of the activity of human MAP kinase-interacting kinase Mnk1b. *Biochim Biophys Acta* 2007)
19. Zhao Z, Z. Tan, C. D. Diltz, M. You and E. H. Fischer: Activation of mitogen-activated protein (MAP) kinase pathway by pervanadate, a potent inhibitor of tyrosine phosphatases. *J Biol Chem* 271**,** 22251-22255 (1996)
20. Parra J. L, M. Buxade and C. G. Proud: Features of the catalytic domains and C termini of the MAPK signal-integrating kinases Mnk1 and Mnk2 determine their differing activities and regulatory properties. *J Biol Chem* 280**,** 37623-37633 (2005)
21. Jauch R, S. Jakel, C. Netter, K. Schreiter, B. Aicher, H. Jackle and M. C. Wahl: Crystal structures of the Mnk2 kinase domain reveal an inhibitory conformation and a zinc binding site. *Structure* 13**,** 1559-1568 (2005)
22. Jauch R, M. K. Cho, S. Jakel, C. Netter, K. Schreiter, B. Aicher, M. Zweckstetter, H. Jackle and M. C. Wahl: Mitogen-activated protein kinases interacting kinases are autoinhibited by a reprogrammed activation segment. *EMBO J* 25**,** 4020-4032 (2006)
23. Fabian M. A, W. H. Biggs, III, D. K. Treiber, C. E. Atteridge, M. D. Azimioara, M. G. Benedetti, T. A. Carter, P. Ciceri, P. T. Edeen, M. Floyd, J. M. Ford, M. Galvin, J. L. Gerlach, R. M. Grotzfeld, S. Herrgard, D. E. Insko, M. A. Insko, A. G. Lai, J. M. Lelias, S. A. Mehta, Z. V. Milanov, A. M. Velasco, L. M. Wodicka, H. K. Patel, P. P. Zarrinkar and D. J. Lockhart: A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotechnol* 23**,** 329-336 (2005)
24. Buxade M, J. L. Parra, S. Rousseau, N. Shpiro, R. Marquez, N. Morrice, J. Bain, E. Espel and C. G. Proud: The Mnks Are Novel Components in the Control of TNFalpha Biosynthesis and Phosphorylate and Regulate hnRNP A1. *Immunity* 23**,** 177-189 (2005)
25. Guil S, J. C. Long and J. F. Caceres: hnRNP A1 relocalization to the stress granules reflects a role in the stress response. *Mol Cell Biol* 26**,** 5744-5758 (2006)
26. Buxade M, N. Morrice, D. L. Krebs and C. G. Proud: The PSF/p54nrb complex is a novel Mnk substrate that binds the mRNA for TNFalpha. *J Biol Chem* 283, 57-65 (2007)
27. Hefner Y, A. G. Borsch-Haubold, M. Murakami, J. I. Wilde, S. Pasquet, D. Schieltz, F. Ghomashchi, J. R. Yates, III, C. G. Armstrong, A. Paterson, P. Cohen, R. Fukunaga, T. Hunter, I. Kudo, S. P. Watson and M. H. Gelb: Serine 727 phosphorylation and activation of cytosolic phospholipase A2 by MNK1-related protein kinases. *J Biol Chem* 275**,** 37542-37551 (2000)
28. Dasilva J, L. Xu, H. J. Kim, W. T. Miller and D. Bar-Sagi: Regulation of sprouty stability by mnk1-dependent phosphorylation. *Mol Cell Biol* 26**,** 1898-1907 (2006)
29. Scheper G. C. and C. G. Proud: Does phosphorylation of the cap-binding protein eIF4E play a role in translation initiation? *Eur J Biochem* 269**,** 5350-5359 (2002)
30. Knauf U, C. Tschopp and H. Gram: Negative regulation of protein translation by mitogen-activated protein kinase-interacting kinases 1 and 2. *Mol Cell Biol* 21**,** 5500-5511 (2001)
31. Tschopp C, U. Knauf, M. Brauchle, M. Zurini, P. Ramage, D. Glueck, L. New, J. Han and H. Gram: Phosphorylation of eIF-4E on Ser 209 in response to mitogenic and inflammatory stimuli is faithfully detected by specific antibodies. *Mol Cell Biol Res Commun* 3**,** 205-211 (2000)
32. Carroll M, O. Warren, X. Fan and W. S. Sossin: 5-HT stimulates eEF2 dephosphorylation in a rapamycin-sensitive manner in Aplysia neurites. *J Neurochem* 90**,** 1464-1476 (2004)
33. Ross G, J. R. Dyer, V. F. Castellucci and W. S. Sossin: Mnk is a negative regulator of cap-dependent translation in Aplysia neurons. *J Neurochem* 97**,** 79-91 (2006)
34. Scheper G. C, B. van Kollenburg, J. Hu, Y. Luo, D. J. Goss and C. G. Proud: Phosphorylation of eukaryotic initiation factor 4E markedly reduces its affinity for capped mRNA. *J Biol Chem* 277**,** 3303-3309 (2002)

35. Morley S. J. and S. Naegele: Phosphorylation of eukaryotic initiation factor (eIF) 4E is not required for de novo protein synthesis following recovery from hypertonic stress in human kidney cells. *J Biol Chem* 277**,** 32855-32859 (2002)
36. Walsh D. and I. Mohr: Phosphorylation of eIF4E by Mnk-1 enhances HSV-1 translation and replication in quiescent cells. *Genes Dev* 18**,** 660-672 (2004)
37. Walsh D, C. Perez, J. Notary and I. Mohr: Regulation of the translation initiation factor eIF4F by multiple mechanisms in human cytomegalovirus-infected cells. *J Virol* 79**,** 8057-8064 (2005)
38. Bain J, L. Plater, M. Elliott, N. Shpiro, C. J. Hastie, H. McLauchlan, I. Klevernic, J. S. Arthur, D. R. Alessi and P. Cohen: The selectivity of protein kinase inhibitors: a further update. *Biochem J* 408**,** 297-315 (2007)
39. Wang X, P. Yue, C. B. Chan, K. Ye, T. Ueda, R. Watanabe-Fukunaga, R. Fukunaga, H. Fu, F. R. Khuri and S. Y. Sun: Inhibition of mammalian target of rapamycin induces phosphatidylinositol 3-kinase-dependent and Mnk-mediated eukaryotic translation initiation factor 4E phosphorylation. *Mol Cell Biol* 27**,** 7405-7413 (2007)
40. Sachs A. (2000) Physical and functional interactions between the mRNA cap structure and the poly (A) tail. In Translational control of gene expression (Sonenberg,N, Hershey,J.W.B. and Mathews,M.B. Eds.), pp. 447-465. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
41. PestovaT.V, Lorsch,J.R. and Hellen,C.U.T. (2006) The mechanism of translation initiation in eukaryotes. In Translational Control in Biology and Medicine (Mathews,M.B, Sonenberg,N. and Hershey,J.W.B. Eds.), pp. 87-128. Cold Spring Harbor Laboratory Press.
42. Barreau C, L. Paillard and H. B. Osborne: AU-rich elements and associated factors: are there unifying principles? *Nucleic Acids Res* 33**,** 7138-7150 (2005)
43. Espel E.: The role of the AU-rich elements of mRNAs in controlling translation. *Semin Cell Dev Biol* . 16**,** 59-67 (2005)
44. Kelleher R. J, III, A. Govindarajan, H. Y. Jung, H. Kang and S. Tonegawa: Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* 116**,** 467-479 (2004)
45. Dumitru C. D, J. D. Ceci, C. Tsatsanis, D. Kontoyiannis, K. Stamatakis, J.-H. Lin, C. Patriotis, N. A. Jenkins, N. G. Copeland, G. Kollias and P. N. Tsichlis: TNF-α induction by LPS is regulated posttranscriptionally via a Tpl2/ERK-dependent pathway. *Cell* 103**,** 1071-1083 (2000)
46. Phelps M, A. Phillips, M. Darley and J. P. Blaydes: MEK-ERK signaling controls Hdm2 oncoprotein expression by regulating hdm2 mRNA export to the cytoplasm. *J Biol Chem* 280**,** 16651-16658 (2005)
47. Habelhah H, K. Shah, L. Huang, A. Ostareck-Lederer, A. L. Burlingame, K. M. Shokat, M. W. Hentze and Z. Ronai: ERK phosphorylation drives cytoplasmic accumulation of hnRNP-K and inhibition of mRNA translation. *Nat Cell Biol* 3**,** 325-330 (2001)
48. Deleault K. M, S. J. Skinner and 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)
49. Rowlett R. M, C. A. Chrestensen, M. Nyce, M. G. Harp, J. W. Pelo, F. Cominelli, P. B. Ernst, T. T. Pizarro, T. W. Sturgill and M. T. Worthington: MNK kinases regulate multiple TLR pathways and innate proinflammatory cytokines in macrophages. *Am J Physiol Gastrointest Liver Physiol* epub ahead of print. (2007)
50. Andersson K. and R. Sundler: Posttranscriptional regulation of TNFalpha expression via eukaryotic initiation factor 4E (eIF4E) phosphorylation in mouse macrophages. *Cytokine* 33, 52-7 (2006)
51. Allemand E, S. Guil, M. Myers, J. Moscat, J. F. Caceres and A. R. Krainer: Regulation of heterogenous nuclear ribonucleoprotein A1 transport by phosphorylation in cells stressed by osmotic shock. *Proc Natl Acad Sci USA* 102**,** 3605-3610 (2005)
52. Izaurralde E, A. Jarmolowski, C. Beisel, I. W. Mattaj, G. Dreyfuss and U. Fischer: A role for the M9 transport signal of hnRNP A1 in mRNA nuclear export. *J Cell Biol* 137**,** 27-35 (1997)
53. Burd C. G. and G. Dreyfuss: RNA binding specificity of hnRNP A1: significance of hnRNP A1 high-affinity binding sites in pre-mRNA splicing. *EMBO J* 13**,** 1197-1204 (1994)
54. Yang X, M. R. Bani, S. J. Lu, S. Rowan, Y. Ben-David and B. Chabot: The A1 and A1B proteins of heterogeneous nuclear ribonucleoparticles modulate 5' splice site selection in vivo. *Proc Natl Acad Sci USA* 91**,** 6924-6928 (1994)
55. Bonnal S, F. Pileur, C. Orsini, F. Parker, F. Pujol, A. C. Prats and S. Vagner: Heterogeneous nuclear ribonucleoprotein A1 is a novel internal ribosome entry site trans-acting factor that modulates alternative initiation of translation of the fibroblast growth factor 2 mRNA. *J Biol Chem* 280**,** 4144-4153 (2005)
56. Cammas A, F. Pileur, S. Bonnal, S. M. Lewis, N. Leveque, M. Holcik and S. Vagner: Cytoplasmic Relocalization of Heterogeneous Nuclear Ribonucleoprotein A1 Controls Translation Initiation of Specific mRNAs. *Mol Biol Cell* 18**,** 5048-5059 (2007)
57. Hamilton B. J, E. Nagy, J. S. Malter, B. A. Arrick and W. F. Rigby: Association of heterogeneous nuclear ribonucleoprotein A1 and C proteins with reiterated AUUUA sequences. *J Biol Chem* 268**,** 8881-8887 (1993)
58. Hamilton B. J, C. M. Burns, R. C. Nichols and W. F. Rigby: Modulation of AUUUA response element binding by heterogeneous nuclear ribonucleoprotein A1 in human T lymphocytes. The roles of cytoplasmic location, transcription, and phosphorylation. *J Biol Chem* 272**,** 28732-28741 (1997)
59. Henics T, A. Sanfridson, B. J. Hamilton, E. Nagy and W. F. Rigby: Enhanced stability of interleukin-2 mRNA in MLA 144 cells. Possible role of cytoplasmic AU-rich sequence-binding proteins. *J Biol Chem* 269**,** 5377-5383 (1994)
60. van der Houven van Oordt, M. T. az-Meco, J. Lozano, A. R. Krainer, J. Moscat and J. F. Caceres: The MKK (3/6)-p38-signaling cascade alters the subcellular distribution of hnRNP A1 and modulates alternative splicing regulation. *J Cell Biol* 149**,** 307-316 (2000)
61. Patton J. G, E. B. Porro, J. Galceran, P. Tempst and B. Nadal-Ginard: Cloning and characterization of PSF, a novel pre-mRNA splicing factor. *Genes Dev* 7**,** 393-406 (1993)

62. Cobbold L. C, K. A. Spriggs, S. J. Haines, H. C. Dobbyn, C. Hayes, C. H. de Moor, K. S. Lilley, M. Bushell and A. E. Willis: Identification of IRES-trans-acting factors for the Myc family of IRESs. *Mol Cell Biol* 2007)
63. Zolotukhin A. S, D. Michalowski, J. Bear, S. V. Smulevitch, A. M. Traish, R. Peng, J. Patton, I. N. Shatsky and B. K. Felber: PSF acts through the human immunodeficiency virus type 1 mRNA instability elements to regulate virus expression. *Mol Cell Biol* 23**,** 6618-6630 (2003)
64. Hall-Pogar T, S. Liang, L. K. Hague and C. S. Lutz: Specific trans-acting proteins interact with auxiliary RNA polyadenylation elements in the COX-2 3'-UTR. *RNA* 13**,** 1103-1115 (2007)
65. Shav-Tal Y, M. Cohen, S. Lapter, B. Dye, J. G. Patton, J. Vandekerckhove and D. Zipori: Nuclear relocalization of the pre-mRNA splicing factor PSF during apoptosis involves hyperphosphorylation, masking of antigenic epitopes, and changes in protein interactions. *Mol Biol Cell* 12**,** 2328-2340 (2001)
66. Akhmedov A. T. and B. S. Lopez: Human 100-kDa homologous DNA-pairing protein is the splicing factor PSF and promotes DNA strand invasion. *Nucleic Acids Res* 28**,** 3022-3030 (2000)
67. Deloulme J. C, L. Prichard, O. Delattre and D. R. Storm: The prooncoprotein EWS binds calmodulin and is phosphorylated by protein kinase C through an IQ domain. *J Biol Chem* 272**,** 27369-27377 (1997)
68. Huang C. J, Z. Tang, R. J. Lin and P. W. Tucker: Phosphorylation by SR kinases regulates the binding of PTB-associated splicing factor (PSF) to the pre-mRNA polypyrimidine tract. *FEBS Lett* 581**,** 223-232 (2007)
69. Proteau A, S. Blier, A. L. Albert, S. B. Lavoie, A. M. Traish and M. Vincent: The multifunctional nuclear protein p54nrb is multiphosphorylated in mitosis and interacts with the mitotic regulator Pin1. *J Mol Biol* 346**,** 1163-1172 (2005)
70. Shav-Tal Y. and D. Zipori: PSF and p54 (nrb)/NonO--multi-functional nuclear proteins. *FEBS Lett* 531**,** 109-114 (2002)
71. Bundschu K, U. Walter and K. Schuh: The VASP-Spred-Sprouty domain puzzle. *J Biol Chem* 281**,** 36477-36481 (2006)
72. Huang A. M. and G. M. Rubin: A misexpression sreen identifies genes that can modulate RAS1 pathway signaling in *Drosophila melanogaster*. *Genetics* 156**,** 1219-1230 (2000)
73. Chrestensen C. A, A. Eschenroeder, W. G. Ross, T. Ueda, R. Watanabe-Fukunaga, R. Fukunaga and T. W. Sturgill: Loss of MNK function sensitizes fibroblasts to serum-withdrawal induced apoptosis. *Genes Cells* 12**,** 1133-1140 (2007)
74. Chrestensen C. A, J. K. Shuman, A. Eschenroeder, M. Worthington, H. Gram and T. W. Sturgill: MNK1 and MNK2 regulation in HER2-overexpressing breast cancer lines. *J Biol Chem* 282**,** 4243-4252 (2007)
75. Baselga J.: Targeting tyrosine kinases in cancer: the second wave. *Science* 312**,** 1175-1178 (2006)
76. Wendel H. G, R. L. Silva, A. Malina, J. R. Mills, H. Zhu, T. Ueda, R. Watanabe-Fukunaga, R. Fukunaga, J. Teruya-Feldstein, J. Pelletier and S. W. Lowe: Dissecting eIF4E action in tumorigenesis. *Genes Dev* 21**,** 3232-3237 (2007)
77. Wendel H. G, E. De Stanchina, J. S. Fridman, A. Malina, S. Ray, S. Kogan, C. Cordon-Cardo, J. Pelletier and S. W. Lowe: Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* 428**,** 332-337 (2004)
78. Cohen N, M. Sharma, A. Kentsis, J. M. Perez, S. Strudwick and K. L. B. Borden: PML RING suppresses oncogenic transformation by reducing the affinity of eIF4E for mRNA. *EMBO J* 20**,** 4547-4559 (2001)
79. Culjkovic B, I. Topisirovic, L. Skrabanek, M. Ruiz-Gutierrez and K. L. Borden: eIF4E is a central node of an RNA regulon that governs cellular proliferation. *J Cell Biol* 175**,** 415-426 (2006)
80. Strudwick S. and K. L. Borden: The emerging roles for translation factor eIF4E in the nucleus. *Differentiation* 70**,** 10-22 (2002)
81. De Benedetti A. and J. R. Graff: eIF-4E expression and its role in malignancies and metastases. *Oncogene* 23**,** 3189-3199 (2004)
82. Lazaris-Karatzas A, K. S. Montine and N. Sonenberg: Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5' cap. *Nature* 345**,** 544-547 (1990)
83. Smith M. R, M. Jaramillo, Y. L. Liu, T. E. Dever, W. C. Merrick and N. Sonenberg: Translation initiation factors induce DNA synthesis and transform NIH 3T3 cells. *New Biol* 2**,** 648-654 (1990)
84. Rousseau D, R. Kaspar, I. Rosenwald, L. Gehrke and N. Sonenberg: Translation initiation of ornithine decarboxylase and nucleocytoplasmic transport of cyclin D1 messenger-RNA are increased in cells overexpressing eukaryotic initiation factor 4E. *Proc Natl Acad Sci USA* 93**,** 1065-1070 (1996)
85. Culjkovic B, I. Topisirovic, L. Skrabanek, M. Ruiz-Gutierrez and K. L. Borden: eIF4E promotes nuclear export of cyclin D1 mRNAs via an element in the 3'UTR. *J Cell Biol.* 169**,** 245-256 (2005)
86. Phillips A. and J. P. Blaydes: MNK1 and EIF4E are downstream effectors of MEKs in the regulation of the nuclear export of HDM2 mRNA. *Oncogene* 2007)
87. Topisirovic I, M. Ruiz-Gutierrez and K. L. Borden: Phosphorylation of the eukaryotic translation initiation factor eIF4E contributes to its transformation and mRNA transport activities. *Cancer Res* 64**,** 8639-8642 (2004)
88. Kidd D. and J. W. Raff: LK6, a short-lived protein kinase in Drosophila that can associate with microtubules and centrosomes. *J Cell Sci* 110**,** 209-219 (1997)
89. Lachance P. E. D, M. Miron, B. Raught, N. Sonenberg and P. Lasko: Phosphorylation of eukaryotic translation initiation factor 4E is critical for growth. *Mol Cell Biol* 22**,** 1656-1663 (2002)
90. Parra-Palau J. L, G. C. Scheper, D. E. Harper and C. G. Proud: The Drosophila protein kinase LK6 is regulated by ERK and phosphorylates the eukaryotic initiation factor eIF4E in vivo. *Biochem J* 385**,** 695-702 (2005)
91. Arquier N, M. Bourouis, J. Colombani and P. Leopold: Drosophila Lk6 kinase controls phosphorylation of eukaryotic translation initiation factor 4E and promotes normal growth and development. *Curr Biol* 15**,** 19-23 (2005)

92. Reiling J. H, K. T. Doepfner, E. Hafen and H. Stocker: Diet-dependent effects of the Drosophila Mnk1/Mnk2 homolog Lk6 on growth via eIF4E. *Curr Biol* 15**,** 24-30 (2005)
94. Dyer J. R. and W. S. Sossin: Regulation of eukaryotic initiation factor 4E phosphorylation in the nervous system of Aplysia californica. *J Neurochem* 75**,** 872-881 (2000)
94. Dyer J. R, S. Michel, W. Lee, V. F. Castellucci, N. L. Wayne and W. S. Sossin: An activity-dependent switch to cap-independent translation triggered by eIF4E dephosphorylation. *Nat Neurosci* 6**,** 219-220 (2003)

Abbreviations: ARE: AU-rich element; $cPLA_2$: cytoplasmic phospholipase A_2; eIF: eukaryotic initiation factor; ERK: extracellular ligand-regulated kinase; Hdm2: human homologue of mouse mdm2; HnRNP: heterogeneous nuclear ribonucleoprotein; hSpry: human sprouty; JNK: c-Jun N-terminal kinase; mTOR: mammalian target of rapamycin; MAP kinase: mitogen-activated protein kinase; MAPKAP-K: MAP kinase-activated protein kinase; Mnk, MAP kinase-interacting kinase or MAP kinase signal-integrating kinase; MEF: mouse embryonic fibroblast; NES: nuclear export signal; PABP: poly- (A)-binding protein; PSF: polypyrimidine-tract binding protein-associated splicing factor; RSK: ribosomal protein S6 kinase; TTP: tristetraprolin; UTR: untranslated region.

Key Words: Mnk; MAP Kinase, Initiation Factor, mRNA Translation,; Cytokine, Apoptosis, Review

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