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Mini Review

MEK1/2 dual-specificity protein kinases: Structure and regulation

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This paper is dedicated to the memory of Dr. Jack D. Herbert (1940–2011), a founding member of the Board of Directors of the Blue Ridge Institute for Medical Research.

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ABSTRACT

MEK1 and MEK2 are related protein kinases that participate in the RAS–RAF–MEK–ERK signal transduction cascade. This cascade participates in the regulation of a large variety of processes including apoptosis, cell cycle progression, cell migration, differentiation, metabolism, and proliferation. Moreover, oncogenic mutations in RAS or B-RAF are responsible for a large proportion of human cancers. MEK1 is activated by phosphorylation of S218 and S222 in its activation segment as catalyzed by RAF kinases in an intricate process that involves a KSR scaffold. Besides functioning as a scaffold, the kinase activity of KSR is also required for MEK activation. MEK1 regulation is unusual in that S212 phosphorylation in its activation segment is inhibitory. Moreover, active ERK catalyzes a feedback inhibitory phosphorylation of MEK1 T292 that serves to downregulate the pathway.

1. Introduction

Protein kinases play a predominant regulatory role in nearly every aspect of cell biology. The human protein kinase family consists of 518 genes thereby making it one of the largest gene families [1]. These enzymes catalyze the following reaction:

$$MgATP^{-1} + protein{-}OH \rightarrow Protein{-}OPO_3^{2-} + MgADP + H^+$$

Based upon the nature of the phosphorylated –OH group, these proteins are classified as protein-serine/threonine kinases (385 members), protein-tyrosine kinases (90 members), and tyrosine-kinase like proteins (43 members). A small group of dual-specificity kinases including MEK1 and MEK2 catalyze the phosphorylation of both tyrosine and threonine in target proteins; dual-specificity kinases are included within the protein-serine/threonine kinase family. Protein phosphorylation is the most widespread class of post-translational modification used in signal transduction. Families of protein phosphatases catalyze the dephosphorylation of proteins thus making phosphorylation–dephosphorylation an overall reversible process.

MEK1 and MEK2 are ubiquitously expressed hydrophilic non-receptor proteins that participate in the RAS–RAF–MEK–ERK signal transduction cascade, which is sometimes denoted as the mitogen-activated protein kinase (MAPK) cascade [2]. H-RAS, K-RAS, and N-RAS function as molecular switches as an inactive RAS-GDP is converted into an active RAS-GTP [3]. This exchange is promoted by the action of several receptor protein-tyrosine kinases. RAS-GTP has about one dozen downstream effector pathways including the RAF–MEK–ERK signaling module [2]. Active RAS-GTP is converted to inactive RAS-GDP by the intrinsic RAS-GTPase activity.

RAS-GTP leads to the activation of the RAF kinase family (A-, B-, and C-RAF) by an intricate multistage process [4]. The RAF kinases have restricted substrate specificity and catalyze the phosphorylation and activation of MEK1 and MEK2. MEK1/2 are dual-specificity protein kinases that mediate the phosphorylation of tyrosine and then threonine in ERK1 or ERK2, their only known physiological substrates. This phosphorylation activates ERK1/2, which are protein-serine/threonine kinases. Unlike the RAF kinases and MEK1/2, which have narrow substrate specificity, ERK1 and ERK2 have dozens of cytosolic and nuclear substrates.

Abbreviations: C-spine, catalytic spine; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated protein kinase; KD, kinase domain; KSR, kinase suppressor of RAS; MAPK, mitogen-activated protein kinase; MEK, MAP/ERK kinase; PAK, p21-activated kinase; PKA, protein kinase A; R-spine, regulatory spine.

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MEK1/2 catalyze the phosphorylation of threonine and tyrosine residues in the activation segment of ERK1/2, which contains the sequence Thr-Glu-Tyr. MEK1/2 do not phosphorylate denatured ERK1/2 nor do they phosphorylate ERK1/2 peptides [5]. However, MEK1/2 display some activity toward myelin basic protein.

2. MEK1/2 structures and catalytic residues

MEK proteins consist of a trifunctional N-terminal sequence of about 70 amino acids, a protein kinase domain of about 290 residues, and a C-terminal sequence of about 30 residues [6]. The N-terminal sequence contains an inhibitory segment, a nuclear export sequence, and a segment that aids in binding its ERK substrates (Fig. 1). MEK1/2, like all protein kinases, have a small N-terminal lobe and large C-terminal lobe that contain several conserved α-helices and β-strands, first described in PKA [7].

The small lobe is dominated by a five-stranded antiparallel β-sheet (β1–β5) [8]. It also contains an important and conserved αC-helix that occurs in active or inactive orientations. The small lobe contains a conserved glycine-rich (GxGxxG) ATP-phosphate-binding loop, sometimes called the P-loop, which occurs between the β1- and β2-strands (Fig. 2). The glycine-rich loop, which is the most flexible part of the N-lobe, positions the γ-phosphate of ATP for catalysis. The β1- and β2-strands harbor the adenine ring of ATP. The glycine-rich loop is followed by a conserved valine (V81/85 in MEK1/2) that makes a hydrophobic contact with the adenine of ATP. The β3-strand typically contains an AxK sequence, the lysine of which (K97/101 of MEK1/2) couples the phosphates of ATP to the αC-helix. A conserved glutamate occurs near the center of the αC helix (E114/118 in MEK1/2) in protein kinases. The presence of a salt-bridge between the β3-lysine and the αC-glutamate is a hallmark of the activated state. The absence of this salt-bridge indicates that the kinase is inactive.

The large lobe is mainly α-helical (Fig. 2) with six conserved segments (αD–αI) [8]. It also contains four short conserved β-strands (β6–β9) that contain most of the catalytic residues associated with the phosphoryl transfer from ATP to the ERK substrates. The primary structure of the β-strands occurs between those of the αE- and αF-helices.

Hanks and colleagues identified 12 subdomains with conserved amino acid residue signatures that constitute the catalytic core of protein kinases [9]. Of these, the following three amino acids, which define a K/D/D (Lys/Asp/Asp) motif, illustrate the catalytic properties of MEK1/2. An invariant β3-strand lysine (K97/101 in MEK1/2) forms salt bridges with the α- and β-phosphates of ATP (Fig. 3). Asp190/194, which is a base occurring within the catalytic loop, abstracts the proton from the –OH group of tyrosine or threonine thereby facilitating the nucleophilic attack of oxygen on the γ-phosphorus atom of MgATP. The second aspartate of the K/D/D signature, Asp208/212, is the first residue of the activation segment. Asp208/212 binds two Mg^{2+} ions, which in turn coordinate the α-, β- and γ-phosphates of ATP. The activation segments of nearly all protein kinases including MEK1 begins with DFG and ends with APE. Although the activation segment of MEK1 begins with DFG, it ends with SPE. The primary structure of the catalytic loop of MEK1, which occurs between those of the β6- and β7-strands, contains His188, Arg189, Asp190, and Lys192 (Fig. 3). The primary structure of the activation segment occurs after that of the catalytic loop and before that of the αF-helix. Functionally important MEK1/2 residues are listed in Table 1. The large lobe characteristically binds the protein substrates, in this case ERK1 or ERK2. The N-terminus of MEK1/2 participates in this interaction (Fig. 1).

3. The protein kinase hydrophobic skeleton

Taylor and Kornev [8] and Kornev and colleagues [10] analyzed the structures of the active and inactive conformations of some two dozen protein kinases and determined functionally important residues by a local spatial alignment (LSP) algorithm. This analysis reveals a skeleton of four non-consecutive hydrophobic residues that constitute a regulatory, or R-, spine and eight hydrophobic residues that constitute a catalytic, or C-, spine. The MEK1/2 regulatory spine consists of a residue from the beginning of the β4-strand (F129/134) and one from the C-terminal end of the αC-helix (L118/122) and a hydrophobic residue after the DFG of the activation loop (F209/213), and the HRD-histidine (H188/192) of the catalytic loop. L118/122 is four residues C-terminal to the conserved αC-glutamate. The backbone of H188/192 is anchored to the F-helix by a hydrogen bond to a conserved aspartate residue (D245/249). Table 2 lists the residues of the spines in human MEK1/2 and the catalytic subunit of murine PKA, and Fig. 2 shows the location of the catalytic and regulatory spines of MEK1.

The catalytic spine of protein kinases consists of residues from the N-terminal and C-terminal lobes that is completed by the adenine ring of ATP [8,10]. This structure mediates catalysis via ATP localization thereby accounting for the term catalytic. The two residues of the N-terminal lobe of MEK1/2 that bind to the adenine ring of the nucleotide substrate include V81/85 from the beginning of the β2-strand and A95/99 from the conserved AxK of the β3-strand. Moreover, L197/201 from the middle of the β7-strand is predicted to bind to the adenine ring in the active enzyme. I196/

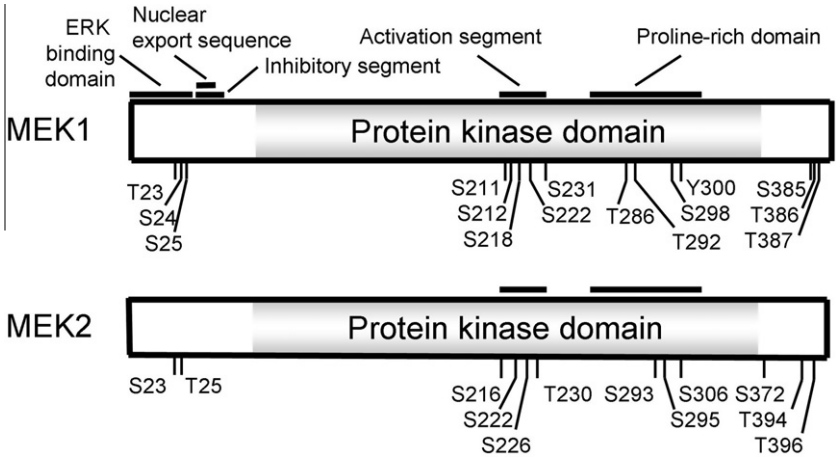

Fig. 1. Organization of MEK1/2. The experimentally confirmed phosphorylation sites are from www.phosphonet.ca.

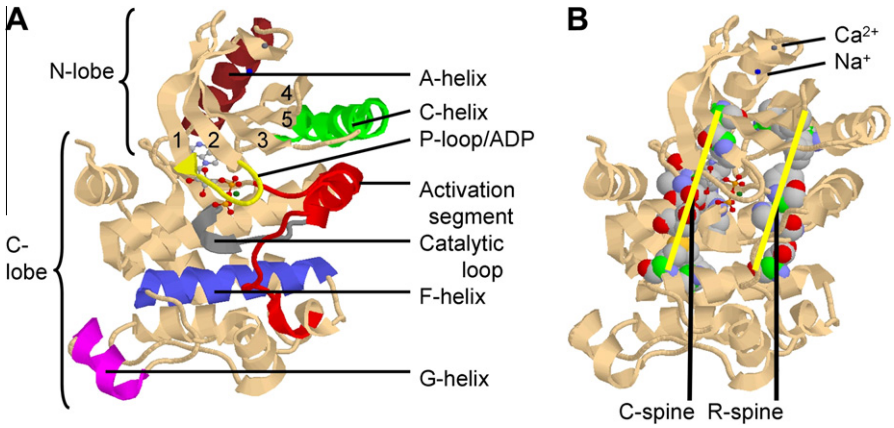

Fig. 2. (A) Ribbon diagram of human MEK1 bound to MgADP. The P-loop, or glycine-rich loop, resides over MgADP (ball-and-stick format), which occurs in the active site cleft between the N- and C-lobes. The numbers in the N-lobe label the β-strands (1–5). (B) The yellow lines denote the residues (space-filling models) that constitute the regulatory and catalytic spines. This structure corresponds to an inactive enzyme based upon the lack of contact between Lys97 and Glu114 (not shown). The view is the same as that of (A). Prepared from protein data bank file 3EQI.

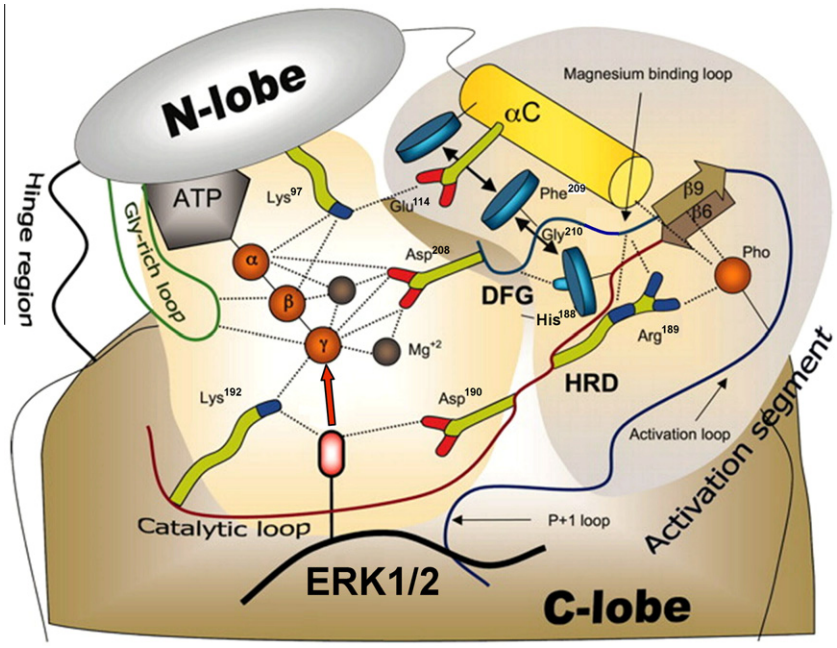

Fig. 3. Diagram of the inferred interactions between human MEK1 kinase catalytic core residues, ATP, and ERK1/2 (the protein substrates). Catalytically important residues that are in contact with ATP and ERK occur within the light khaki background. Secondary structures and residues that are involved in regulation of catalytic activity occur within the gray background. Hydrophobic interactions between the HRD motif, the DFG motif, and the αC-helix are shown by black arrows while polar contacts are shown by dashed lines. Pho, phosphate. This figure is adapted from Ref. [8] copyright *Proceedings of the National Academy of Sciences USA*.

200 and V198/202, hydrophobic residues that flank L197/201, bind to L151/155 at the beginning of the D-helix. The D-helix L151/155 residues bind to L253/257 and M256/260 in the F-helix. Besides the hydrophobic interactions with the adenine ring, the exocyclic nitrogen of ATP characteristically forms hydrogen bonds with backbone residues in the hinge region that connects the N- and C-lobes. The X-ray structure of human MEK1 (pdb file 3EQI) shows that the exocyclic 6-amino nitrogen of ADP binds to the peptide carbonyl oxygen of Glu144 while the N-5 nitrogen of the adenine ring binds to the backbone nitrogen of Met146. The R-spine binds to the F-helix by hydrophobic bonds as do the catalytic loop, the P + 1 loop, the activation segment, and the αH–αI loop [8].

The P + 1 loop binds the amino acid residue immediately following the residue that is phosphorylated. MEK1/2 first mediate the phosphorylation of the tyrosine residue in the ERK activation segment. Tyrosine-phosphorylated ERK dissociates from MEK and then reassociates with the same or another active MEK that then catalyzes the phosphorylation of the activation-segment threonine, which is two residues upstream from the ERK phosphotyrosine [11]. The phosphorylation of a single residue in ERK fails to activate the enzyme; activation requires the phosphorylation of both activation segment residues.

4. Active and inactive MEK1/2

The catalytic site lies in the cleft between the small and large lobes. In Fig. 2, this site contains MgADP. The two lobes of protein

Table 1
Important residues in human MEK1/2 and KSR1/2.

	MEK1	MEK2	KSR1	KSR2
Protein kinase domain	68–361	72–367	611–881	666–931
Glycine-rich P-loop	74–82	78–86	618–623	673–678
β3-lysine (K) or arginine (R)	K97	K101	R637	R692
αC-glutamate	114	118	650	710
Hinge residues between the N- and C-lobes	144–149	148–153	684–689	739–744
Gatekeeper residue	M143	M147	T684	T739
Beginning of the catalytic loop	HRD 188–190	HRD 192–194	HKD 729–731	HKD 784–786
Catalytic loop lysine	192	196	733	788
DFG (Asp-Phe-Gly)	208–210	212–214	748–750	803–805
Activation loop phosphorylation sites	S218, S222	S222, S226	S755[a], S770[a]	S808[a], S810[a]
End of the activation loop	SPE 231–233	APE 235–237	APE 778–780	APE 833–835
No. of residues	392	400	921	950
Proline-rich insert	262–326	266–334	Absent	Absent
Molecular wt. (kDa)	43.4	44.4	102	108
UniProtKB accession no.	Q02750	P36507	Q6VAB6	Q81VT5

[a] Not confirmed experimentally.

Table 2
Human MEK1/2 and murine PKA residues that form the R-spine and the C-spine.

	MEK1/2	PKA[a]
Regulatory spine		
β4-strand (N-lobe)	F129/134	L106
C-helix (N-lobe)	L118/122	L95
Activation loop (C-lobe)	F209/213	F185
Catalytic loop (C-lobe)	H188/192	Y164
F-helix (C-lobe)	D245/249	D220
Catalytic spine		
β2-strand (N-lobe)	V81/85	V57
β3-AxK motif (N-lobe)	A95/99	A70
β7-strand (C-lobe)	L197/201	L173
β7-strand (C-lobe)	I196/200	L172
β7-strand (C-lobe)	V198/202	I174
D-helix (C-lobe)	L151/155	M128
F-helix (C-lobe)	L253/257	L227
F-helix (C-lobe)	M256/260	M231

[a] From Ref. [10].

kinases move relative to each other during the catalytic cycle and can open and close the cleft. The open form allows access of ATP and release of ADP from the active site. The closed form brings residues into the catalytically active state during which the phosphoryl transfer from ATP to the protein substrate occurs.

Within each lobe is a polypeptide segment that has active and inactive conformations [12]. In the small lobe, the regulatory segment is the αC-helix. This helix rotates and translates with respect to the rest of the lobe, making or breaking part of the active site. In the active state, a conserved lysine residue from the β3-strand (MEK1/2 K97/101) forms a salt bridge with the conserved glutamate from the αC-helix (MEK1/2 E114/118). An inactive state occurs when the αC-helix moves and the salt bridge is broken, and the inhibitory αA-helix (Fig. 2) stabilizes the inactive conformation by shifting the αC-helix from its active-state orientation [6].

The activation segment of the large lobe exhibits active and inactive orientations. In the inactive conformation, the aspartate side chain (MEK1/2 E208/212) of the conserved DFG sequence faces away from the active site. This is called the DFG-aspartate out conformation. In the active state, the aspartate side chain faces into the ATP-binding pocket and coordinates Mg^{2+} (Fig. 3). This is called the DFG-aspartate in conformation. This terminology is better than "DFG-in" and "DFG-out" because, in the inactive state, the DFG-phenylalanine may move into the active site while the DFG-aspartate moves out; it is the aspartate binding (aspartate in) or not binding (aspartate out) to Mg^{2+} in the active site that is the key.

5. Phosphorylation of MEK1/2

The activation segment of most protein kinases contains one or more phosphorylation sites. These sites can be phosphorylated by reactions catalyzed in *trans* by the same kinase family member (e.g., EGFR-1 by EGFR-1) or by a different kinase family member (MEK1 by B-RAF). Zheng and Guan reported that human MEK1 activation requires the phosphorylation of two serine residues (S218 and S222) in the activation segment [13]. Expression of either S218A or S222A mutant in Swiss 3T3 cells abolishes EGF-stimulated MEK1 activation suggesting that both residues must be phosphorylated to attain activation. Alessi and co-workers demonstrated that human C-RAF is able to catalyze the phosphorylation of the equivalent serine residues in rabbit MEK1 [14]. After the first phosphorylation *in vitro*, the second occurs rapidly without the accumulation of any mono-phosphorylated species. However, removal of one phosphate from di-phosphorylated MEK1 as catalyzed by protein phosphatase 2A leaves the enzyme fully activated. Perhaps di-phosphorylation is required for activation and the subsequent removal of a single phosphate leaves MEK1 in an activated state. The corresponding residues on MEK2 are S222 and S226.

Gopalbhai et al. demonstrated that phosphorylation of S212 in the activation loop of MEK1 decreases kinase activity [15]. They reported that this residue, which is conserved among all MEK family members, is phosphorylated *in vivo*. Mutation of S212 to alanine enhances the basal activity of MEK1, whereas the phosphomimetic aspartate mutation completely suppresses the activation of both wild-type MEK1 and a constitutively activated MEK1 (S218D/S222D) mutant. Phosphorylation of S212 does not interfere with the phosphorylation of MEK1 at S218 or S222 or with binding to the ERK2 substrate. MEK1 S212 phosphorylation is thus quite unusual because phosphorylation of activation loop residues is almost always associated with activation, not inhibition.

Eblen and colleagues showed that the association of MEK with ERK is enhanced by the phosphorylation of T298 of MEK1, which is mediated by the p21-activated kinase-1 (PAK1), downstream of the small G-protein RAC [16]. When this phosphorylation accompanies the phosphorylation of S218 and S222, it facilitates the activation of ERK in response to various stimuli. Moreover, the association of MEK1 or MEK2 with RAF kinases facilitates the rate of MEK1/2 activation. The association of MEK1 and MEK2 with their upstream or downstream components is mediated by scaffold proteins such as KSR1/2; thus, the scaffolds are supportive activators of the RAF–MEK–ERK cascade [2].

MEK1 can be inhibited by phosphorylation. Thus, phosphorylation of several residues on MEK1 results in reduced MEK activity.

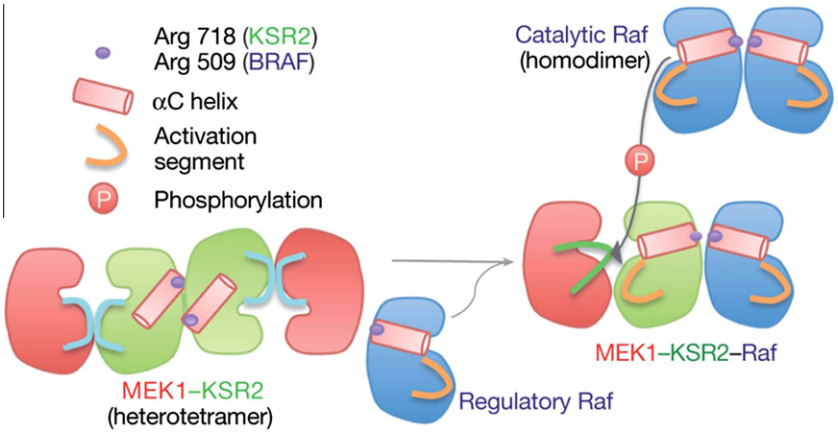

Fig. 4. Catalytic B-RAF–mediated activation of MEK1 phosphorylation is facilitated by regulatory B-RAF as part of a MEK1–KSR2–B-RAF ternary complex. In the KSR2–MEK1 heterotetramer (left), the inaccessible activation segment of MEK1 is released through the interaction of KSR2 with regulatory B-RAF, which is induced by a conformational change of the αC-helix, thereby allowing catalytic B-RAF to mediate the phosphorylation of MEK1 (right). B-RAF bound to KSR2 through a side-to-side heterodimer is sterically unable to phosphorylate MEK1 in *cis*, an activity that must therefore be performed by a different B-RAF molecule. This figure is adapted from Ref. [22] copyright *Nature*.

These include: (1) S212, which is in the activation segment as noted above and is the substrate of an unknown protein kinase [15]; (2) T286, which is in the proline-rich segment of the kinase domain and is a substrate of Cdk5 [17]; (3) T292, which is also in the proline-rich segment and is an ERK substrate, phosphorylation of which decreases T298 phosphorylation as catalyzed by PAK1 [16,17]; and (4) T386, which is a Cdk5 [17] and ERK [18,19] substrate. Although several other phosphorylation sites have been reported for MEK1/2 based upon large-scale proteomics analysis by mass spectrometry (Fig. 1), the physiological effects resulting from their phosphorylation is unknown.

6. MEK1–MEK2 heterodimers and duration of the ERK signal

Catalanotti and colleagues confirmed that MEK1-deficiency in mice is embryonic lethal as a result of placental defects [20]. Moreover, they reported that *Mek1* ablation decreases fibroblast migration toward extracellular matrix components (haptotaxis), but it unexpectedly enhances growth-factor induced fibroblast migration (chemotaxis). This phenotype is due to increased ERK activation in MEK1-deficient cells. These workers showed that MEK1 and MEK2 form heterodimers subject to negative feedback by ERK catalyzed phosphorylation of MEK1 on T292, facilitating the dephosphorylation of S218 and S222 in the MEK1 activation loop. An equivalent T292 residue is lacking in the proline-rich region of MEK2. If MEK1 is absent or unable to bind to MEK2, the negative feedback inhibition is disabled and MEK2 phosphorylation and ERK activation are prolonged. These investigators showed that ablation of *Mek1* upregulates MEK and ERK signaling in mouse fibroblasts, embryos, epidermis, and brain.

7. Role of KSR protein kinase activity in MEK activation

Besides the four classical components in the RAS–RAF–MEK–ERK signaling pathway, scaffolding proteins such as kinase suppressor of RAS (KSR) play an important role in signaling. KSR functions as an essential scaffolding protein to coordinate the assembly of RAF–MEK–ERK complexes. KSR was discovered in *Drosophila* and *C. elegans* as a positive effector of MAP kinase signaling [21]. The designation as suppressor is somewhat of a misnomer because KSR facilitates RAS signaling (the initial experiments showed that mutants of KSR abrogated RAS signaling). Humans possess one gene that encodes KSR1 and another that encodes KSR2. Sequence analysis indicates that KSR1/2 belong to the protein-serine/threonine kinase family. However, it was first thought that these proteins were catalytically inactive owing to the absence of critical conserved amino acid residues. Whereas most active kinases possess His-Arg-Asp (or Tyr-Arg-Asp) at the beginning of the catalytic loop, KSR1/2 have His-Lys-Asp in its place in humans (Table 1), *Drosophila* (UniProtKB Q24171), and *C. elegans* (UniProtKB Q19380). Moreover, the essential lysine residue in the β3-strand of most kinases, including the KSR of *Drosophila* and *C. elegans*, is replaced by an arginine in mammalian KSR1 and KSR2 (human KSR1/2 with R637/R692). Recent work, however, indicates that KSR1 [21] and KSR2 [22] possess catalytic activity.

Brennan and colleagues performed structural and biochemical studies to elucidate the mechanism of KSR2-stimulated MEK1 phosphorylation as catalyzed by B-RAF [22]. They determined the crystal structure of human MEK1 with rabbit KSR2 and found that their catalytic sites face each other and the heterodimer interface involves their activation segments and αG-helices. The KSR2 scaffold assembles MEK1–KSR2–B-RAF ternary complexes responsible for promoting MEK1 phosphorylation by B-RAF. KSR2(KD)–MEK1 heterodimers assemble into tetramers through a KSR2(KD) homodimer interface centered on Arg718 (Fig. 4). A KSR2 Arg718His mutation abolishes the activation of MEK1 by B-RAF indicating the importance of KSR2 dimer interface in the overall process of MEK1 activation.

Brennan et al. showed that the addition of a kinase-impaired B-RAF(K483S) mutant to KSR2(KD)–MEK1 increases MEK1 phosphorylation 15-fold [22]. The KSR2 inhibitor ASC24 blocks 70% of total MEK1 phosphorylation but less than 10% of S218/S222 activation segment phosphorylation. Sorafinib, a RAF kinase inhibitor, blocks 30% of total MEK1 phosphorylation but over 90% of S218/S222-specific phosphorylation. They conclude that KSR2 is the major protein kinase responsible for increased MEK1 phosphorylation, and this results from a B-RAF(K483S)-induced increase in KSR2 catalytic activity. KSR2-catalyzed phosphorylation of MEK1 on non-activation segment residues may facilitate B-RAF-mediated phosphorylation of the MEK1 activation segment serines (218 and 222). These experiments indicate that KSR2 possesses catalytic activity despite lacking canonical residues as noted above. B-RAF forms active side-to-side dimers [4], and B-RAF presumably allosterically stimulates KSR2 activity by formation of similar heterodimers. KSR2 leads to a release of the MEK1 activation segment for phosphorylation. These

investigators hypothesize that regulatory B-RAF interacts with KSR2 in *cis* to induce a conformational change in KSR2 thereby facilitating phosphorylation of MEK1 by an independent catalytic B-RAF molecule in *trans* (Fig. 4).

Hu and co-workers generated a mouse KSR1 mutant that cannot bind ATP but stabilizes the C- and R-spines critical for the formation of its closed active conformation, and they used this mutant to assess its scaffold versus kinase functions with other mouse proteins [21]. The catalytically inactive KSR1 mutant binds to C-RAF and to MEK1 but does not result in MEK1 activation, unlike wild-type KSR1. They reported that wild-type KSR1 alone lacks kinase activity. However, co-expression and binding of KSR1 with C-RAF results in KSR1 kinase activity. These experiments suggest that KSR1 is a bona fide kinase whose activity, in cooperation with C-RAF, is required for MEK1 activation. Thus, both the scaffolding function and catalytic activity of KSR1 is required for MEK1 phosphorylation and activation by C-RAF.

8. Epilogue

Seger et al. reported in 1992 that human MAP kinase (ERK) activators, now known as MEK, catalyze the phosphorylation of Tyr190 and Thr188 in *Xenopus* MAP kinase [5]. The human gene family of dual-specificity kinases consists of seven members (MAP2K1–MAP2K7). Later studies lead to the elucidation of the RAS–RAF–MEK–ERK signaling pathway. This signaling module is upregulated in perhaps one-third of all human cancers owing to activation of upstream receptors and mutations in RAS and B-RAF [23]. This pathway has attracted considerable attention as a target for anticancer therapy. Inhibition of RAS function has proven intractable. Several inhibitors of the RAF kinase family, MEK1/2, and ERK1/2 have been developed, but none have achieved FDA approval for patient treatment [23]. Sorafinib, an FDA-approved drug for the treatment of renal carcinoma that was initially developed as a RAF inhibitor, most likely exerts its therapeutic effects by inhibiting vascular endothelial growth factor receptors and angiogenesis. Deciphering the mechanisms of RAS–RAF–MEK–ERK signaling continues to be an important and challenging task.

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