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Review

ERK implication in cell cycle regulation

Jean-Claude Chambard, Renaud Lefloch, Jacques Pouysségur, Philippe Lenormand *

Institute of Signaling Developmental Biology and Cancer, CNRS UMR 6543, Universite de Nice-Sofia Antipolis, Centre A. Lacassagne, 33 Avenue de Valombrose, 06189 Nice, France

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Abstract

The Ras/Raf/MEK/ERK signaling cascade that integrates an extreme variety of extracellular stimuli into key biological responses controlling cell proliferation, differentiation or death is one of the most studied intracellular pathways. Here we present some evidences that have been accumulated over the last 15 years proving the requirement of ERK in the control of cell proliferation. In this review we focus (i) on the spatio-temporal control of ERK signaling, (ii) on the key cellular components linking extracellular signals to the induction and activation of cell cycle events controlling G1 to S-phase transition and (iii) on the role of ERK in the growth factor-independent G2/M phase of the cell cycle. As ERK pathway is often co-activated with the PI3 kinase signaling, we highlight some of the key points of convergence leading to a full activation of mTOR via ERK and AKT synergies. Finally, ERK and AKT targets being constitutively activated in so many human cancers, we briefly touched the cure issue of using more specific drugs in rationally selected cancer patients.
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1. Introduction

Cells receive constantly clues from their environments via activation of surface receptors and extra-cellular matrix. Intracellularly, cells need to integrate the extent and timing of diverse signaling pathways to trigger an appropriate biological response. The Ras/Raf/MEK/ERK signaling cascade is one of the key signaling pathways that integrates extracellular clues. ERK1 and ERK2 isoforms are highly conserved serine/threonine kinases activated *via* phosphorylation on both threonine and tyrosine residues within the TEY sequence in their activation loop by the dual specificity kinase MEK. Activated ERK phosphorylates cytoplasmic, membranous and nuclear substrates, many of which are protein kinases such as RSK whose activity prolong and diversify the signaling cascade. However, in the Raf branch of Ras signaling, ERK is a central and key player for several reasons. Upstream of MEK, Raf can activate very few partners other than MEK, and no other substrates for MEK than ERK have been found so far. Downstream of ERK, signaling cascades continue to flow, but no single downstream kinase can activate the wide variety of ERK substrates. Furthermore, accumulating evidences suggests that subtle differences in the spatio-temporal activation of ERK generate variations in signaling outputs that regulate biological responses. Moreover, crosstalk between ERK and other pathways has been shown to be crucial for determining cell fate decisions.

This review will first recapitulate the array of evidences that led to establish that ERK activation is absolutely indispensable for cell proliferation to occur in mature differentiated eukaryotic cells. Then we will study the cascades of events driven by ERK activation that lead cells to proceed into proliferation, review the necessary interplay between the ERK and the PI3K/AKT pathways for normal proliferation to occur, and finally assess the difficulties encountered when blocking MEK/ERK activation to fight cancer.

2. ERK activation is required for cell proliferation to proceed: array of evidences

2.1. Cell proliferation needs long term but controlled ERK activation

Several years prior to ERK identification in 1991 [1], the close correlation between mitogen action and the increased

* Corresponding author. Tel.: +33 492031227; fax: +33 492031225.
E-mail address: Lenorman@unice.fr (P. Lenormand).

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phosphorylation of two proteins of 41 and 43 kDa on phosphotyrosine, phosphothreonine and/or phosphoserine was revealed by two-dimensional polyacrylamide-gel electrophoresis [2,3]. Because of the sustained phosphorylation during the critical part of G0/G1 phase of the cell cycle, these two proteins known as p41 and p43 were suspected of playing a key role in cell cycle entry. Just after the discovery of ERK it was demonstrated in fibroblasts that ERK activation occurred in two phases, an initial rapid phase lasting about half to 1 h in response to many stimuli, and a late phase lasting several hours that is induced by the persistent presence of mitogenic agonists. For example, the mitogenic agonist alpha-thrombin induces a biphasic activation of ERK in CCL39 cells, addition of a thrombin antagonist after 30 s allowed the first phase of ERK activation while the late phase of activation was abolished and DNA synthesis prevented [4] Furthermore, serotonin, which is not a mitogen by its own, had no effect on late phase ERK activity, but synergized with basic fibroblast growth factor to induce late ERK activation and DNA synthesis. Many observations converged, letting to propose that sustained ERK activation is an obligatory event for growth factor-induced cell cycle progression [4–8] (reviewed by [9]). Recent evidences indicate that ERK activation throughout G1 is required for entry in S phase [10,11].

However experiments in PC12 pheochromocytomas cells indicate that the critical role of prolonged versus transient activation of ERK to trigger biological outcome maybe dictated by cell fate. Stimulation of PC12 cells by nerve growth factor (NGF) leads to growth arrest and neuronal differentiation, whereas insulin or epidermal growth factor (EGF) stimulates cell proliferation. Stimulation with NGF leads to the sustained activation of ERK, whereas insulin or epidermal growth factor induces the transient activation of ERK. Temporal control of ERK is the key modulator for inducing proliferation versus differentiation in PC12 cells, since overexpression of the insulin or the EGF receptor [12,13] led to prolonged ERK activation and differentiation.

Why would late phase ERK activation correlate with proliferation in fibroblasts and cell differentiation in PC12 cells remains to be understood. One hypothesis resides in the possibility that the fate of fibroblasts is to proliferate whereas the fate of PC12 cells is to differentiate, thus the prolonged activation of ERK may be necessary for the cell fate to be accomplished. As will be detailed later, long-term ERK activation can promote accumulation of p21cip1 an inhibitor or cell cycle entry. Cell types may need different threshold of ERK activity to tip the balance between proliferation and growth arrest, in this case, PC12 would be more sensitive to the duration of ERK activation than fibroblasts. In neuronal and kidney cells, complete deregulation of ERK activity leads to ERK dependent cell death [14,15]. Similarly, many cytotoxic drugs are known to induce a prolonged activation of ERK, that is required for promoting apoptosis [16–19]. These findings illustrate the need for a potent control of ERK temporal activation which is achieved by a sharp temporal induction of specific ERK phosphatases [20].

2.2. Blunting mitogen-induced ERK activation blocks cell proliferation

The first indication that ERK activation was required for fibroblast cell proliferation came from the transient expression of the entire antisense mRNA for ERK1 or non-phosphorylatable mutant of ERK1 (T192AY194F) into CCl39 Chinese hamster fibroblasts [21]. Both approaches strongly inhibited endogenous ERK1/ERK2 activation, which led to diminished cell growth. The very elevated transient expression of a full-length antisense mRNA or of the dominant-negative ERK1 were sufficient to block the activation of the other ERK isoform, and thus could also block ERK5 activation (not known at that time), however co-expression of wild-type-ERK1 reversed the inhibition, establishing the requirement of ERK1 for cell cycle entry. Later the discovery of specific MEK inhibitors confirmed the implication of MEK/ERK activation in the control of cell proliferation. First PD98059 inhibited stimulation of cell growth and reversed the phenotype of ras-transformed BALB 3T3 mouse fibroblasts and rat kidney cells [22]. However, it was later discovered that PD98059 was also inhibiting MEK5, the activator of ERK5 [23]. The discovery of inhibitors that discriminate between MEK1/2 and MEK5 [24] led to more conclusive experiments.

Indeed growth factor-stimulated ERK activity was completely inhibited by concentrations of PD184352 (CI-1040) below 1 μM that do not affect ERK5 activation [24]. Furthermore, serum-stimulated cyclin D1 expression and DNA synthesis were inhibited by low doses of PD184352, which abolished ERK activity but had no effect on ERK5 [25]. These results indicate that the antiproliferative effect of PD184352 is due to inhibition of the classical ERK pathway and does not require inhibition of the ERK5 pathway. Other kinases are likely not to be inhibited due to the uncommonly high specificity of this series of MEK inhibitors [26].

Further an independent proof that ERK activation is absolutely required for cell proliferation and differentiation came from gene targeted disruption of both ERK1 and ERK2 in thymocytes. To determine the roles of ERK in thymocytes proliferation and differentiation, conditional mice for erk2 were crossed with mice harboring a germline deficiency in erk1 [27]. In thymocytes development the transition from DN3 stage to the DN4 stage requires six to eight rounds of cell division. After conditional knock-out of erk2 gene, at first sight thymocytes lacking erk1 and erk2 genes did not display any decrease in DN4 cells. However, because erk2 was conditionally deleted, there are cells that escape the recombination event. Indeed, it was found that only the DN4 cells expressing ERK2 continue to divide indicating that in the absence of ERK1, hence there is a selection for the retention of ERK2 for proliferation to proceed [27].

2.3. Nuclear translocation of ERK is necessary for cell cycle entry

Interestingly, not only ERK activity needs to be elevated persistently during the G1 phase of the cell cycle to proceed to

S-phase entry, ERK must be able to translocate to the nucleus. Indeed, mitogenic stimulation triggers the translocation of ERK from the cytoplasm to the nucleus, whereas all the upstream activators of the module remain cytosolic. The expression of a catalytically inactive form of cytoplasmic MAP kinase phosphatase (MKP-3/DUSP6) [28] was found to be sufficient to sequester ERK in the cytoplasm. Sequestering ERK in the cytoplasm did not alter its ability to phosphorylate cytoplasmic substrates but strongly inhibited Elk1-dependent gene transcription and the ability of cells to reinitiate DNA replication in response to growth factors. This observation indicated that nuclear substrates, most likely ERK-regulated transcription factors, were required for quiescent cells to enter the cell cycle.

Natural biological regulation of ERK nuclear localization has been demonstrated to regulate cell proliferation. For example, the cytoplasmic PEA15 protein that associates tightly with ERK is actively exported out of the nucleus via a nuclear export sequence [29]. Increased PEA15 expression impedes ERK entry in the nucleus, ERK-dependent transcriptional regulation and cell proliferation. Indeed over-activation of AKT has been shown to prevent the nuclear translocation of ERK by stabilizing endogenous PEA15, resulting in cell proliferation restriction [30]. This is one example of the many cross-talk regulations between the ERK and Akt signaling pathways; here a balanced activation of Akt is required to avoid blocking ERK nuclear translocation, and thus blocking cell proliferation.

3. Targets of ERK implicated in cell proliferation

3.1. ERK regulates the production of building blocks for cell growth

Prior to engage into division, cells must insure that the metabolites required for building new proteins, DNA, ribosomal RNA and membranes are present or that the cell will be able to synthesize them de novo. It appears that the activity of many regulators of the production of metabolites is orchestrated by ERK activation.

3.1.1. Synthesis of pyrimidine

In order to build more DNA and rRNA, ERK activation instructs the cell to produce more pyrimidine nucleotides. The rate-limiting step of the de novo synthesis of pyrimidine nucleotides pathway is catalysed by carbamoyl phosphate synthetase (CPS II), part of the multifunctional enzyme CAD. ERK increases the activity of CAD by phosphorylating threonine 456 of CPS II which induces allosteric modifications [31]. Interestingly, when CAD is actively located in the nucleus, it is more phosphorylated on threonine 456 than when it is trapped in the cytosol [32]. Trapping CAD in the nucleus had a minimal effect on pyrimidine metabolism. In contrast, when CAD was excluded from the nucleus, the rate of pyrimidine biosynthesis, the nucleotide pools, and the growth rate were reduced by 21, 36, and 60%, respectively. ERK is located in the nucleus of fibroblasts only upon long lasting mitogenic stimulation [33], hence the prolonged increase of pyrimidine nucleotide synthesis may occur only during the long lasting second phase of ERK activation, in response to mitogenic signaling.

3.1.2. Chromatin remodeling

Transcriptional activation is not linked solely to increase activation of transcription factors; concomitant regulation of chromatin structure is also required. To constitute chromatin, DNA is wrapped by proteins, including histones and high mobility group proteins (HMG-proteins).

ERK activation leads to increase stimulation of its substrates MSK1 and MSK2 that in turn phosphorylate histone H3 and HMG-14, leading to chromatin remodeling [34]. In mice lacking MSK1 and MSK2, histone H3 and HMG-14 phosphorylation is severely reduced or abolished, leading to reduced induction of immediate early gene transcription.

3.1.3. Ribosome synthesis

Cell proliferation requires more synthesis of ribosomes to meet the increased demand of protein synthesis. Transcription of the ribosomal RNA genes by RNA polymerase I is rapidly activated upon ERK stimulation. This activation is mediated by direct phosphorylation of the HMG box DNA binding domains of the architectural transcription factor UBF. In fact ERK phosphorylation of UBF prevents DNA bending by its first two HMG boxes, leading to a cooperative unfolding of the enhancesome, and increased transcription of ribosomal RNA genes [35,36].

3.1.4. Protein translation

One direct substrate of ERK, the protein kinase MNK1 is responsible for induced phosphorylation of the translation initiation factor 4E (eIF4E) on serine 209 following cell stimulation [37]. This phosphorylation increases the affinity of eIF4E for capped mRNA, leading to increased translation. Analysis of embryonic fibroblasts from single KO mice revealed that MNK1 is responsible for the inducible phosphorylation of eIF4E in response to ERK (and p38MAPK) activation, whereas MNK2 mainly contributes to eIF4E's basal, constitutive phosphorylation [38].

Another pathway to regulated protein translation is mTOR/p70S6K pathway (reviewed by [39]). One important regulator of mTOR is the heterodimer TSC1/TSC2, a tumor suppressor complex which "represses" mTOR activity. Mutations in TSC1 or TSC2 genes induce a tumor syndrome called tuberous sclerosis. The main regulator of TSC function is mediated via activation of the PI3K/AKT pathway that leads to the direct phosphorylation and inactivation of TSC2 by AKT on serine 939 and threonine 1462 (reviewed by [40]). However, it has been shown recently, that ERK phosphorylates TSC2 predominantly on serine 664 in vitro and in vivo, leading to the disruption of the TSC1–TSC2 complex, and ultimately elevated mRNA translation upon unleashing of mTOR activity [41]. Furthermore, the kinase RSK, a direct downstream substrate of ERK, can also phosphorylate TSC2 on serine 1798 to inhibit the function of TSC1/TSC2 complex [42].

This cooperation between the AKT and the ERK pathway to activate TOR is one of the many synergies developed by cells between these two pathways to regulate cell proliferation, in this case to increase protein translation and metabolism.

3.2. ERK role in G1/S phase transition

The key step for quiescent cells to undergo cell cycle entry is the formation of an active cyclinD–CDK4/6 complex. This complex is formed when newly synthesized cyclin D associates with existing CDKs (cyclin dependent kinases). CDK4/6 kinase activity release E2F family of transcription factors from Rb repression inducing expression of a second class of G1 cyclins, cyclin E required for S phase entry. It is worthy to note that the stimulatory effects of cyclins can be counteracted by CDK inhibitors (CDKIs). Repression of CDKIs is also a pre-requisite for G1/S phase transition, elevating the level of cyclin D1 for example is not sufficient to induce cell cycle entry. ERK activation acts at several levels to increase the activity of CDKs in late G1, ERK also plays a role in the inhibition of CDKIs.

3.2.1. ERK regulates cyclin D1 transcriptional induction via Fos family members

ERK activation was first implicated in the induction of cyclin D1 expression, when it was shown that activation of the ERK pathway by stimulating the stably expressed chimera deltaRaf1: ER led to increase cyclin D1 expression; whereas blocking ERK activity by expressing a dominant negative form of MEK led to decreased cyclin D expression [43]. Cyclin D1 is induced several hours after growth factor addition, interestingly it was then shown that only a sustained ERK activity could lead to PDGF-induced cyclin D1 expression [44]. Indeed, blocking ERK activity by adding 10 μM of the MEK inhibitor PD98059 up to 6 h post-stimulation was sufficient to block cyclin D1 expression and cell proliferation.

What are the intermediates between ERK persistent activation and the delayed expression of cyclin D1? Candidates are members of the Fos family of transcription factors since mouse embryo fibroblasts lacking *fos-B* and c-*fos* genes display a profound proliferation defect that can be restored by ectopic expression of cyclin D1 [45].

However, c-Fos must act indirectly to induce cyclin D1 transcription [46] because c-Fos expression strongly repressed cyclin D1 reporter promoter expression [47]. In fact, c-Fos has been shown to interact with the transcriptional repressor p300 [48], whose ectopic expression induces cell cycle arrest in G0/G1 [49]. Contrary to c-Fos, Fra-1 (another member of the Fos family) led to moderate activation of the cyclin D1 promoter [47]. Interestingly, it was shown that only the late phase activity of ERK lead to Fra-1 expression [50] while the initial phase was sufficient to induce Fos mRNA. When looking at chromatin it became evident that Fos family members display a dynamic temporal pattern of binding. For example, it was shown that Fra-1 was expressed and bound to chromatin only when c-Fos expression and binding to chromatin decreased [46]. Taken collectively these data converge to propose a model, in which a well-orchestrated spatio-temporal activation of ERK is required for cyclin D1 expression via Fos family members.

First phase: active ERK phosphorylates pre-existing transcription factors, such as Elk to induce transcription of c-*fos* gene [51]. Transient activation of ERK is not sufficient to express c-Fos protein for extended periods. The reason is that although transient and sustained ERK activations induce as well c-*fos* gene transcription, only sustained ERK activity can phosphorylate C-terminal phosphorylation sites on c-Fos [52,53]. The unmasking of the FXFP site/DEF domain upon c-*fos* C-terminal sites phosphorylation increases c-Fos binding to ERK to ensure phosphorylation of the other sites for full stabilization of the c-Fos protein.

Second phase: persistent activation of ERK leads to persistent expression of c-Fos in the nucleus. c-Fos increases the transcription of many genes, such as Fra-1 and at the same times c-Fos blocks transcription of cyclin D1.

Third phase: after several hours, when persistent ERK activity declines, especially in the nucleus, c-Fos protein is dephosphorylated and degraded. Fra-1 replaces c-Fos in the cyclin D1 promoter, brings-in new transcriptional activators and cyclin D1 is expressed [46]. This sequence stresses the importance of inhibition of ERK in the nucleus during the long lasting phase of ERK activation [54].

3.2.2. ERK also regulates cyclin D1 transcription via myc

Another candidate to mediate ERK transcriptional induction of cyclin D1 gene is the Myc transcription factor. ERK phosphorylates serine 62 of Myc which increases its stability [55], then Myc participates directly in the transcriptional induction of the cyclin D1 gene as indicated by nuclear run-on experiments [56].

3.2.3. ERK regulates the assembly of cyclin/CDK complex

Induction of cyclin E by E2F regulates CDK2 to enforce Rb phosphorylation, creating a positive feedback loop that helps contribute to the irreversibility of the G1/S transition (reviewed by [57]). The formation of the cyclinE/CDK2 complex seems to be indirectly regulated by ERK at two levels.

First of all ERK activity is required for proper nuclear translocation of CDK2 in the nucleus, a compartment where it has to go to be activated by phosphorylation of threonine 160 by CDK activating kinase (CAK) and dephosphorylation of threonine 14 and tyrosine 16 by the CDC25 phosphatase. Blocking ERK activation did not modify the levels of cyclin E/CDK2 complexes, solely the nuclear localization of CDK2 [58].

Second, ERK activity has been shown to regulate phosphorylation of threonine 160 of CDK2, an activating site of CDK2 [59]. The pathways linking ERK activation to the ERK-dependent CDK2 nuclear translocation and activating threonine 160 phosphorylation are not known, further studies are awaited to resolve these questions.

3.2.4. ERK represses the transcription of anti-proliferative genes

Cells cease to enter S phase if ERK is inactivated even in late G1 phase. A means for ERK to mediate this effect was unveiled

recently: ERK is required for the continuous down-regulation of antiproliferative genes throughout G1 phase to allow cell cycle progression [10]. Up to 175 genes have been identified whose expression is diminished during the G1 phase of the cell cycle via an ERK-dependent mechanism. Interestingly, withdrawal of serum or MEK inhibition during the G1 phase was sufficient to re-establish normal expression of these antiproliferative genes, and co-incidentally block S phase entry. The down-regulation of many genes was shown to be dependent on AP1 activity since transfection of dominant negative c-Fos protein blocked the down-regulation of many antiproliferative genes. Forced expression in late G1 of two “antiproliferative proteins” blocked S phase entry (JunD and Gadd45alpha expressed at normal levels). The molecular mechanism of action of these anti-proliferative genes is unknown for most of them. However among them, both Tob1 and JunD have been shown to regulate cyclin D1 expression negatively [60,61], providing another means for ERK to control cell cycle progression.

3.2.5. Conclusion

ERK activation plays a fundamental role for G1/S transition since its activation is required for the induction of the cyclin D1 protein via several mechanisms, and sustained activity of ERK is required for the down-regulation of many antiproliferative genes throughout the whole G1 phase of the cell cycle. Furthermore, it seems that ERK activation induces CDK2 nuclear localization and co-activation by phosphorylating threonine 160. However, as we will study later, ERK activation is not sufficient by itself to promote cell cycle entry.

3.3. Role of ERK at the G2/M transition

The implication of ERK in the meiotic M phase progression is beyond the scope of this review; suffice to say that Mos/ERK activates MPF at the onset of meiosis I and Mos/ERK induces metaphase arrest in meiosis II.

In somatic cells, from the time of ERK discovery it has been shown that ERK was also activated during the G2/M phase of the cell cycle, whereas it was dephosphorylated in metaphase-arrested cells [7]. In synchronized HeLa and NIH 3T3 cells, it has then been shown that phosphorylation and activation of ERK occurred in late S phase and persisted until the end of mitosis. Blocking ERK activation by transfection of dominant negative MEK [62] or MEK inhibitors [63] first retarded the entry into mitosis and then increased the duration of mitosis due to the retarded progression from metaphase to anaphase. When ERK is inhibited during G2/M transition, M phase is nearly twice longer in mammalian cultured cells [62].

Entry into mitosis requires the formation of the cyclin B/CDC2 complex which like cyclinE/CDK2 is activated in the nucleus upon phosphorylation by CAK on threonine 161 and dephosphorylated by CDC25 on threonine 14 and tyrosine 15.

3.3.1. ERK participates in the nuclear translocation of cyclinB1

It has been shown recently that ERK can phosphorylate two of the four phosphorylation sites of the cytoplasmic retention sequence (CRS) of cyclin B1, a third site is phosphorylated by the kinase Plx while the identity of the kinase that phosphorylates the fourth site is not known. Phosphorylation of the four sites of the CRS of cyclin B1 is necessary for nuclear localization of cyclin B1 and mitosis progression, probably by interfering with the nuclear export sequence localized in the CRS [64]. Consequently, when ERK activity is inhibited, cyclin B/Cdc2 complex is retained in the cytosol, leading to a poor activation of Cdc2 and retardation in mitosis entry.

3.3.2. ERK, via RSK, blocks negative phosphorylation of Cdc2 by Myt1

The kinase RSK has been shown to phosphorylate and inactivate Myt1 [65], a dual specificity kinase that regulates negatively Cdc2–cyclin B complexes by phosphorylating Cdc2 on threonine 14 and tyrosine 15 [66]. RSK being a direct substrate of ERK, upon ERK inhibition, RSK activity decreases, Myt1 becomes active and inhibits Cdc2 by double phosphorylation on tyrosine 15 and threonine 14, leading to G2/M phase arrest.

3.3.3. Controlling the strength of ERK activation during G2/M transition is critical: the case of BRCA1 ectopic expression

Germ line mutations in the BRCA1 gene are associated with an increased susceptibility to the development of breast and ovarian cancers. It has been shown that ectopic expression of BRCA1 in human cells trigger an ERK-dependant G2/M cell cycle arrest [67].

BRCA1 transfection increases markedly ERK activity in G2/M which blocks Cyclin B/Cdc2 activity by three mechanisms. First, ERK activity increases activity of the kinase Wee1 that phosphorylates Cdc2 on tyrosine 15 to inactivate the cyclin B/Cdc2 complex. Second, ERK activity increases activity of the kinase Chk1 which induces the cytoplasmic retention and degradation of CDC25. When the phosphatase CDC25 is not in the nucleus, it cannot dephosphorylate Cdc2 on tyrosine 14 and threonine 15 to activate it. Finally, BRCA1-induced ERK activation leads to decreased expression of the CDC25 phosphatase due to its degradation in the cytoplasm. CDC25 expression is reversed upon inhibition of MEK/ERK activity [67].

Altogether, ERK activity is required for mitosis but too much ERK activity at the G2/M transition, for instance as a consequence of reduced VHR/DUSP3 activity [68] blocks entry in mitosis.

3.3.4. Localization of active MEK and active ERK at the G2/M transition

Two reports showed that activated ERK was present in mitosis on kinetochores and mitotic tubulin of proliferating PtK1 and HeLa cells [69,70]. Active MEK localization on kinetochores is likely to be an artefact of the phospho-antibody. Indeed phospho-MEK antibody recognises phosphorylation sites on nucleophosmin/B23, a protein phosphorylated by Cdc2 during mitosis [71,72]. However, the localization of active MEK and active ERK on the spindle and midbody during mitosis was recently confirmed in Swiss 3T3 cells [73].

One of the putative targets of active ERK localized on microtubules in mitosis is CENP-E. CENP-E has been shown to

be phosphorylated in vitro by ERK on sites that are known to regulate its interactions with microtubules and was found to associate in vivo preferentially with active ERK during mitosis [70]. Hence, ERK may play a role to organise interactions between chromosomes and microtubules at mitosis through CENP-E.

In conclusion, ERK is clearly implicated in the regulation of the G2/M transition, furthermore a strict control of the kinetic and strength of ERK activation is required for mitosis to occur normally. However the exact cascades of events occurring in G2/M phase downstream of ERK activation are not yet fully understood. In particular the nature of the signals reactivating ERK at the G2/M transition are not fully resolved.

4. Deregulation by over-activation of ERK pathway

4.1. Persistant over-activation of ERK blocks cell cycle entry

It has been known for long time that expression of the activated form of Ras induced growth arrest in many cells unless a functional collaborating oncogene was present [74,75]. One of the targets of Ras activation is the Raf/MEK/ERK pathway, thus activating ERK could also induce cell cycle arrest. This apparent paradox was solved using cells expressing stably deltaRaf:ER chimeras [76]. Upon addition of estrogen or tamoxifen, the catalytic moiety of the chimeras is unmasked and the Raf/MEK/ERK pathway is persistently activated. Interestingly, only a very intense Raf activity triggered cell cycle arrest while modest activation led to enhanced cell cycle entry. Next it was demonstrated that high-intensity Raf signaling caused cell cycle arrest by inducing massive expression of the cell cycle inhibitor (CDKI) p21cip1 [77,78]. In mouse embryo fibroblasts lacking p21cip1, strong Raf activity led to robust cell-cycle entry as measured by thymidine incorporation [78].

After noting in the previous paragraph that exquisite temporal regulation of ERK activation is required for cell cycle entry, here regulation of the strength of ERK signaling also proves to be essential. Intense ERK activation throughout G1 leads to the accumulation of p21cip1 that inhibits cyclin E/CDK2 complexes to block S-phase entry. Controlled ERK activation leads to a modest induction of p21cip1 that is progressively titrated by nascent cyclinD/CDK4 complexes during G1 phase progression [79]. When p21cip1 bound to cyclin E-CDK2 lowers under a threshold level, cyclin E-CDK2 activity is released from inhibition rendering S-phase progression irremediable. The level of p21cip1 expression can be considered a sensor of ERK signal strength.

4.2. What are the mechanisms linking ERK hyper-activation to massive induction of the CDK inhibitor p21cip1?

(1) ERK activation increased the phosphorylation of the transcription factors Ets2, C/EBPalpha, and C/EBPbeta, and rapidly increased transcription from the p21 promoter via multiple Ets- and C/EBP-elements within the enhancer region [80].

(2) As seen previously, ERK increases cyclin D1 protein expression, and hyper-activation of the ERK pathway leads to a massive accumulation of cyclin D1 protein [77,78]. p21 is a short-lived protein that is degraded through association with the C8alpha subunit of the 20S proteasome catalytic complex [81] Upon high ERK activation, cyclin D1 protein accumulates massively, associates with p21cip1 to inhibit its degradation by masking the binding site of p21cip1 to C8alpha [82]. This process is somewhat autocatalytic because p21 binding to cyclin D1 impedes cyclin D1 export out of the nucleus, and its subsequent degradation in the cytoplasm [83].

(3) Hence, a combination of ERK driven transcriptional induction of p21cip1 and cyclin D1 (by different transcription factors), and auto-stabilization of the p21cip1/cyclin D1 complex, triggers a massive accumulation of both cyclin D1 and p21cip1 during abnormal hyper-activation of ERK, leading to cell cycle arrest by p21cip1.

4.3. How Ras transformation bypass the p21cip1-mediated growth arrest mediated by hyper-activation of the ERK pathway?

In melanomas, an active form of NRas is associated with wild type PTEN, on the contrary a constitutive active form of BRaf is frequently associated to a defect in PTEN [84]. The phosphatase PTEN is a negative regulator of AKT, thus inactivation of PTEN leads to constitutive activation of the PI3K/AKT pathway. Considering the fact that NRas can activate both the ERK and the PI3K/AKT pathway, whereas BRaf can activate only the ERK pathway, a simple explanation for the frequent association of PTEN and B-Raf mutations considers that both the PI3K/AKT and the ERK pathways must be constitutively activated to induce tumorigenic growth.

Indeed, it has been shown that constitutive activation of AKT can override the G1 cell cycle arrest caused by over-activation of the ERK pathway. This has been demonstrated in cells harboring conditionally active, steroid hormone-regulated forms of Raf and AKT in which activation of Raf/MEK/ERK and AKT could be performed independently or in combination [85]. Under conditions where activation of neither Raf nor AKT alone promoted S-phase progression, co-activation of both kinases elicited a robust cell proliferation. In this setting, while ERK activation induced p21cip1, AKT activation promoted nuclear export of p21Cip1 into the cytoplasm where it is degraded by the proteasome. As a consequence, p21Cip1 levels diminished and the cyclinE/cdk2 complex ceased to be inhibited, cell cycle progression goes on [85].

Nuclear export of p21Cip1 upon AKT activation is not the sole convergent aspect of the Raf/MEK/ERK and the PI3K/AKT pathways to promote cell proliferation. Previously we have already seen that over-activation of PI3K/AKT pathway prevents ERK nuclear translocation in some cells by stabilization of the PEA-15 protein, hence blocking cell proliferation. In addition we have seen that AKT, ERK and RSK can phosphorylate TSC2 on distinct sites to cooperate in the disruption of the TSC1/TSC2 complex and thus diminishing repression of mTOR to increase protein synthesis. Hereafter are

other examples of cooperations between these two signaling pathways to induce cell proliferation.

4.4. Cooperation between ERK and AKT to induce Cyclin D1 expression

We have seen previously, that ERK activation leads to cyclin D1 transcriptional induction and protein stabilization. Nonetheless, full induction of cyclin D1 by mitogens requires activation of the PI3K/AKT pathway, to negatively regulate GSK3beta (reviewed by [57]). Indeed, GSK3beta phosphorylates threonine 286 of cyclin D1, which enhances its nuclear export, and accelerates its ubiquitin-dependent proteasomal degradation in the cytoplasm, shortening the half-life of cyclin D1 to as little as 10 min. Direct phosphorylation of GSK3beta by AKT can inhibit its activity, thus impeding GSK3beta-mediated cyclin D1 degradation.

4.5. Cooperation between ERK and AKT to activate Myc and repress p27kip1

One of the consequences of ERK activation in early G1 phase is the induction of the myc protein, an absolute requirement for cell cycle entry. It has been known for many years that ERK phosphorylates directly Myc on serine 62 which increases its stability [55]. Phosphorylated serine 62 constitutes a priming site for GSK3-mediated threonine58 phosphorylation leading to the rapid degradation of Myc via the proteasome [86]. AKT activation intervenes again by phosphorylating and inhibiting GSK3beta, thus impeding phosphorylation of threonine 58. This regulation is essential for the orderly expression of Myc protein during cell cycle progression. In fact early into G1, the activation of the ERK and AKT pathways synergize to increase myc expression, however late in G1 the decrease of AKT activity unlashes GSK3beta to phosphorylate myc and induce its degradation. Persistent AKT activation would lead to persistent inhibition of GSK3beta and persistent myc expression which could lead to induction of apoptosis.

One of the many consequences of Myc stabilization is p27kip1 degradation. p27kip1 is another cyclin dependent inhibitor (CDKI) that must be degraded for cell progression to proceed. Myc affects both p27kip1 gene transcription [87] and p27kip1 protein degradation [88].

Another way for AKT to regulate p27kip1 occurs via FOXO family of transcription factors. FOXO enhance transcription of p27kip1 leading to increased protein expression. However, upon phosphorylation of FOXO by AKT, FOXO is exported out of the nucleus and degraded; leading to p27kip1 reduced transcription [89,90].

Overall, the molecular cooperations between ERK and PI3K/AKT activation provide explanations for the strong synergy observed between many growth factors (potent ERK activators) and insulin/IGF-I (potent AKT activator) to promote cell cycle entry in many cell types. Furthermore, the main target of the cooperation between ERK and AKT pathways in early G1 is the regulation of the transcription, complex-assembly and nuclear transport of G1 cyclins (cyclin D and E). This cooperation renders G1-cyclins an essential growth factor sensor, most likely the molecular culprit of Pardee's cell cycle restriction point.

5. Blocking ERK pathway to fight cancer

5.1. ERK is constitutively activated in many cancer and cancer derived cell lines

It has been shown that many primary human tumors and derived cell lines display constitutive activation of ERK. For example, among 138 tumor cell lines and 102 primary tumors derived from various human organs, 36% display constitutive activation of ERK [91]. In fact it is known for long time that about 15% of human cancer harbor mutations of the Ras protein that renders it constitutively active. More recently it has been shown that B-Raf was mutated in many human cancers, to the point of being constitutively active in about 60% of all melanomas [92]. Considering that B-Raf is the most potent kinase isoform for ERK activation, this array of evidences highlights the Ras/Raf/MEK/ERK pathway as therapeutic target for cancer treatment.

5.2. Inhibitors to block ERK activation in vivo

To block activation of this pathway, the focus has been put on MEK inhibitors due to their very unusual specificity among kinase inhibitors, since they are not ATP competitors. Potent Raf inhibitors such as BAY43-9006 are presently tested in clinical trials. ERK inhibitors have not yet received as much attention due to the fact that the ATP binding pocket of ERK is similar to that of cyclin dependent kinases, rendering specificity of inhibition difficult.

In melanoma cell lines where B-Raf is constitutively active, knock-down of B-Raf, but not A-Raf nor C-Raf was sufficient to reduce markedly cell cycle entry and increase apoptosis, an effect that was mimicked by a specific Raf inhibitor (BAY43-9006). Furthermore, in vivo, colon carcinoma tumor growth was inhibited as much as 80% in mice with the MEK inhibitor PD184352 (also named CI-1040) [93], furthermore the Raf inhibitor BAY43-9006 led to a substantial growth delay in melanoma tumor xenografts [94]. In fact it has just been demonstrated that B-Raf mutations confer a preferential sensitivity to MEK inhibition in human cancer cells. In cells that display B-Raf mutations, doses of MEK inhibitor PD184352 that block ERK activation were sufficient to block cyclin D1 expression which was not the case with cells that did not display B-Raf mutations. In tumor cells where B-Raf is normal, higher doses of MEK inhibitor were necessary to lower cyclin D1 expression than to decrease ERK activity. Furthermore, the concentration of inhibitor that led to 50% inhibition of cell proliferation was much lower in cells harboring B-Raf mutations (24-111 nM) compared to cells harboring wild-type B-Raf (100 to 500 nM) [95].

These encouraging results in mice prompted clinical trials to treat human cancers. For example, phase II clinical study was undertaken to assess the anti-tumor activity and safety of

the MEK inhibitor, PD184352 (CI-1040) in breast cancer, colon cancer, non-small-cell lung cancer, and pancreatic cancer. PD184352 was generally well tolerated but clinical trials were not continued due to insufficient anti-tumor activity in the four types of tumors tested [96]. In the 2005 ASCO meeting it was reported that a more potent MEK inhibitor (PD0325901) was able to strongly suppressed ERK activity in tumors, thus PD 0325901 is being tested in phase II clinical trials at present. A recent review details all the strategies engaged to target Raf/MEK/ERK pathway to fight cancer [97].

5.3. Pitfalls of ERK inhibition to fight cancer

The failure of the MEK inhibitor PD184352 to cure several types of human tumors was disappointing, but xenograft experiments had already indicated the difficulty to get tumors regression by using MEK inhibitors. For example, anti-tumor effectiveness of a new MEK inhibitor (PD184161) has been assessed after implanting human hepatocellular carcinoma (HCC) cells in nude mice [98]. PD184161 significantly suppressed tumor engraftment and initial tumor growth however, established tumors were not significantly affected. After the first exposure of the cells and tumors to PD184161, MEK activity was reduced markedly; however MEK activity became normal in HCC xenografts during long-term treatment [98]. The systemic efficacy of PD184161 is unlikely to be responsible for the lack of drug effectiveness because in the lung, high MEK/ERK activity was effectively suppressed after repeated PD184161 treatments. This observation may simply indicate that the cells that survive the first dose of treatment adapted rapidly and lost the high sensitivity to MEK inhibition that was described in many human tumors cells after the first exposure to the MEK inhibitor [95].

In line with these results we have shown in the laboratory that reducing the level of both MEK1 and MEK2 expression by up to 95% with siRNA had no effects on the kinetic of ERK activity following serum stimulation (J. Shama J. Pouyssegur and E. Vial; personal communication). Furthermore, siRNA-mediated ablation of ERK1 and ERK2 levels (more than 95%), diminished ERK activity and cell proliferation only 40 to 50% (R. Lefloch, J. Pouysségur and P. Lenormand; personal communication). These results imply that the MEK/ERK pathway is resilient to diminution of its components, and can adapt rapidly to maintain ERK activation nearly normal. A likely biochemical explanation for the lack of impact of MEK1 and MEK2 knock-down is provided by stoichiometric analysis of this pathway. It was shown recently that activating only 5% of the MEK kinase pool was sufficient to activate up to 60% of the total ERK pool. Indeed, there is a large stoichiometric excess of MEK over Raf, and about twice as much MEK than ERK [99]. If inhibitors spare a small percentage of MEK, the downstream impact on ERK activity may be negligible, not taking into account negative feed-back loops that would certainly restore ERK activation to near normal levels. For example, many downstream regulatory phosphatases are induced upon ERK activation, thus decreasing ERK activity will lead to diminished expression of the phosphatases and more sustained ERK activation.

Another explanation for the decreased efficiency of MEK inhibitors during long-term treatment lies in the fact that the tumor environment is altered during tumorigenesis, consequently the cells may require less activity of a particular signaling pathway for tumor growth. Initially, tumor cells are surrounded by normal tissue and later mostly by tumor tissue. As a consequence, tumor cells may encounter autocrine factors, a different cell matrix and neo-vascularization. One experimental setting has demonstrated that oncogenic Ras needed full downstream activities only to initiate tumor growth in vivo (RalGEF, Raf and PI3K pathways) [100]. Once tumors have been established, during what is called tumor maintenance, only the PI3K activity downstream of oncogenic Ras was necessary for tumor progression. This was demonstrated by injecting tumor cells co-expressing oncogenic Ras mutated to avoid activating Raf and deltaRaf:ER. These cells engage in tumor formation only if deltaRaf:ER is active from the beginning. Once the tumors are formed, removal of tamoxifen led to reduced Raf activity, which did not induce tumor regression. On the contrary, in tumors co-expressing oncogenic Ras mutated to avoid PI3K activation and ER:AKT, removal of tamoxifen led to reduced AKT activity and tumor regression [100].

Recent evidences indicate that many tumors encompass cancer stem cells (reviewed by [101]). Although cancer stem cells represent a very small portion of the tumor mass (0,1 to 2%), they may grow independently of strong MEK/ERK activity. Indeed, it is known that embryonic stem cells do not require ERK activity for proliferation [102]. On the contrary, strong ERK activation appears to impair self-renewal of embryonic stem cells by promoting their differentiation [103]. If cancer stem cells behave similarly to embryonic stem cells, then treatment with MEK inhibitors will not eradicate this population, which then has the potential to promote recurrence of the tumor.

In conclusion, targeting the Raf/MEK/ERK pathway to control tumorigenicity is tempting, considering the frequent occurrence of constitutive activation of this pathway in human cancers. Restricting treatment to patients that display tumors with constitutive activation of ERK will certainly be beneficial. However, combinatorial therapies will certainly be needed to induce regression of pre-existing tumors, especially considering the cytostatic effect of MEK inhibition in most tumor cells. For example, in cancer cells where ERK is constitutively activated, MEK inhibitors induce p27kip1 and cell cycle arrest [104]. Furthermore, in pancreatic cancer cells, it was demonstrated that the cytostatic effect of MEK inhibitors was mainly due to increased p27kip1 expression, since inhibition of p27Kip1 expression restored the activity of cyclin/cdk2 and partially relieved the effects of the MEK inhibitor U0126 on pancreatic cancer cell cycle arrest [105]. However, MEK inhibitors may prove to be highly beneficial to slow tumor-metastasis. In fact when human melanoma cells were injected in the lateral vein of the tail of mice, most cells died within 1 day, only the ones that could implant in the lung formed tumors. In this model of

metastatic melanoma cells, administration of PD184352 inhibited formation of pulmonary metastases [106].

More specific drugs are being developed and we must be ready to offer by a set of functional markers a simple profile of active signaling pathways to stratify in a rational way each cancer patient for the best appropriate cure.

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References

[1] T.G. Boulton, S.H. Nye, D.J. Robbins, N.Y. Ip, E. Radziejewska, S.D. Morgenbesser, R.A. DePinho, N. Panayotatos, M.H. Cobb, G.D. Yancopoulos, ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF, Cell 65 (1991) 663–675.

[2] J.A. Cooper, B.M. Sefton, T. Hunter, Diverse mitogenic agents induce the phosphorylation of two related 42,000-dalton proteins on tyrosine in quiescent chick cells, Mol. Cell Biol. 4 (1984) 30–37.

[3] M. Kohno, J. Pouyssegur, Alpha-thrombin-induced tyrosine phosphorylation of 43,000- and 41,000-Mr proteins is independent of cytoplasmic alkalinization in quiescent fibroblasts, Biochem. J. 238 (1986) 451–457.

[4] S. Meloche, K. Seuwen, G. Pages, J. Pouyssegur, Biphasic and synergistic activation of p44mapk (ERK1) by growth factors: correlation between late phase activation and mitogenicity, Mol. Endocrinol. 6 (1992) 845–854.

[5] S.J. Cook, F. McCormick, Kinetic and biochemical correlation between sustained p44ERK1 (44 kDa extracellular signal-regulated kinase 1) activation and lysophosphatidic acid-stimulated DNA synthesis in Rat-1 cells, Biochem. J. 320 (Pt 1) (1996) 237–245.

[6] C. Kahan, K. Seuwen, S. Meloche, J. Pouyssegur, Coordinate, biphasic activation of p44 mitogen-activated protein kinase and S6 kinase by growth factors in hamster fibroblasts. Evidence for thrombin-induced signals different from phosphoinositide turnover and adenylylcyclase inhibition, J. Biol. Chem. 267 (1992) 13369–13375.

[7] H. Tamemoto, T. Kadowaki, K. Tobe, K. Ueki, T. Izumi, Y. Chatani, M. Kohno, M. Kasuga, Y. Yazaki, Y. Akanuma, Biphasic activation of two mitogen-activated protein kinases during the cell cycle in mammalian cells, J. Biol. Chem. 267 (1992) 20293–20297.

[8] V. Vouret-Craviari, E. Van Obberghen-Schilling, J.C. Scimeca, E. Van Obberghen, J. Pouyssegur, Differential activation of p44mapk (ERK1) by alpha-thrombin and thrombin-receptor peptide agonist, Biochem. J. 289 (Pt 1) (1993) 209–214.

[9] K. Roovers, R.K. Assoian, Integrating the MAP kinase signal into the G1 phase cell cycle machinery, BioEssays 22 (2000) 818–826.

[10] T. Yamamoto, M. Ebisuya, F. Ashida, K. Okamoto, S. Yonehara, E. Nishida, Continuous ERK activation downregulates antiproliferative genes throughout G1 phase to allow cell-cycle progression, Curr. Biol. 16 (2006) 1171–1182.

[11] S.M. Jones, A. Kazlauskas, Growth-factor-dependent mitogenesis requires two distinct phases of signalling, Nat. Cell Biol. 3 (2001) 165–172.

[12] I. Dikic, J. Schlessinger, I. Lax, PC12 cells overexpressing the insulin receptor undergo insulin-dependent neuronal differentiation, Curr. Biol. 4 (1994) 702–708.

[13] S. Traverse, K. Seedorf, H. Paterson, C.J. Marshall, P. Cohen, A. Ullrich, EGF triggers neuronal differentiation of PC12 cells that overexpress the EGF receptor, Curr. Biol. 4 (1994) 694–701.

[14] S. Cagnol, E. Van Obberghen-Schilling, J.C. Chambard, Prolonged activation of ERK1,2 induces FADD-independent caspase 8 activation and cell death, Apoptosis 11 (2006) 337–346.

[15] M. Stanciu, D.B. DeFranco, Prolonged nuclear retention of activated extracellular signal-regulated protein kinase promotes cell death generated by oxidative toxicity or proteasome inhibition in a neuronal cell line, J. Biol. Chem. 277 (2002) 4010–4017.

[16] D. Xiao, S.V. Singh, Phenethyl isothiocyanate-induced apoptosis in p53-deficient PC-3 human prostate cancer cell line is mediated by extracellular signal-regulated kinases, Cancer Res. 62 (2002) 3615–3619.

[17] D. Tang, D. Wu, A. Hirao, J.M. Lahti, L. Liu, B. Mazza, V.J. Kidd, T.W. Mak, A.J. Ingram, ERK activation mediates cell cycle arrest and apoptosis after DNA damage independently of p53, J. Biol. Chem. 277 (2002) 12710–12717.

[18] Y.L. Hsu, P.L. Kuo, L.T. Lin, C.C. Lin, Asiatic acid, a triterpene, induces apoptosis and cell cycle arrest through activation of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways in human breast cancer cells, J. Pharmacol. Exp. Ther. 313 (2005) 333–344.

[19] A. Calcabrini, J.M. Garcia-Martinez, L. Gonzalez, M.J. Tendero, M.T. Ortuno, P. Crateri, A. Lopez-Rivas, G. Arancia, P. Gonzalez-Porque, J. Martin-Perez, Inhibition of proliferation and induction of apoptosis in human breast cancer cells by lauryl gallate, Carcinogenesis 27 (2006) 1699–1712.

[20] S.M. Keyse, Protein phosphatases and the regulation of MAP kinase activity, Semin. Cell Dev. Biol. 9 (1998) 143–152.

[21] G. Pages, P. Lenormand, G. L'Allemain, J.C. Chambard, S. Meloche, J. Pouyssegur, Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 8319–8323.

[22] D.T. Dudley, L. Pang, S.J. Decker, A.J. Bridges, A.R. Saltiel, A synthetic inhibitor of the mitogen-activated protein kinase cascade, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 7686–7689.

[23] S. Kamakura, T. Moriguchi, E. Nishida, Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus, J. Biol. Chem. 274 (1999) 26563–26571.

[24] N. Mody, J. Leitch, C. Armstrong, J. Dixon, P. Cohen, Effects of MAP kinase cascade inhibitors on the MKK5/ERK5 pathway, FEBS Lett. 502 (2001) 21–24.

[25] M.S. Squires, P.M. Nixon, S.J. Cook, Cell-cycle arrest by PD184352 requires inhibition of extracellular signal-regulated kinases (ERK) 1/2 but not ERK5/BMK1, Biochem. J. 366 (2002) 673–680.

[26] S.P. Davies, H. Reddy, M. Caivano, P. Cohen, Specificity and mechanism of action of some commonly used protein kinase inhibitors, Biochem. J. 351 (2000) 95–105.

[27] A.M. Fischer, C.D. Katayama, G. Pages, J. Pouyssegur, S.M. Hedrick, The role of erk1 and erk2 in multiple stages of T cell development, Immunity 23 (2005) 431–443.

[28] A. Brunet, D. Roux, P. Lenormand, S. Dowd, S. Keyse, J. Pouyssegur, Nuclear translocation of p42/p44 mitogen-activated protein kinase is required for growth factor-induced gene expression and cell cycle entry, EMBO J. 18 (1999) 664–674.

[29] E. Formstecher, J.W. Ramos, M. Fauquet, D.A. Calderwood, J.C. Hsieh, B. Canton, X.T. Nguyen, J.V. Barnier, J. Camonis, M.H. Ginsberg, H. Chneiweiss, PEA-15 mediates cytoplasmic sequestration of ERK MAP kinase, Dev. Cell 1 (2001) 239–250.

[30] M. Gervais, C. Dugourd, L. Muller, C. Ardidie, B. Canton, L. Loviconi, P. Corvol, H. Chneiweiss, C. Monnot, Akt down-regulates ERK1/2 nuclear localization and angiotensin II-induced cell proliferation through PEA-15, Mol. Biol. Cell 17 (9) (2006) 3940–3951.

[31] L.M. Graves, H.I. Guy, P. Kozlowski, M. Huang, E. Lazarowski, R.M. Pope, M.A. Collins, E.N. Dahlstrand, H.S. Earp III, D.R. Evans, Regulation of carbamoyl phosphate synthetase by MAP kinase, Nature 403 (2000) 328–332.

[32] F.D. Sigoillot, D.H. Kotsis, V. Serre, S.M. Sigoillot, D.R. Evans, H.I. Guy, Nuclear localization and mitogen-activated protein kinase phosphorylation of the multifunctional protein CAD, J. Biol. Chem. 280 (2005) 25611–25620.
[33] P. Lenormand, J.M. Brondello, A. Brunet, J. Pouyssegur, Growth factor-induced p42/p44 MAPK nuclear translocation and retention requires both MAPK activation and neosynthesis of nuclear anchoring proteins, J. Cell Biol. 142 (1998) 625–633.
[34] A. Soloaga, S. Thomson, G.R. Wiggin, N. Rampersaud, M.H. Dyson, C.A. Hazzalin, L.C. Mahadevan, J.S. Arthur, MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14, EMBO J. 22 (2003) 2788–2797.
[35] V. Stefanovsky, F. Langlois, T. Gagnon-Kugler, L.I. Rothblum, T. Moss, Growth factor signaling regulates elongation of RNA polymerase I transcription in mammals via UBF phosphorylation and r-chromatin remodeling, Mol. Cell 21 (2006) 629–639.
[36] V.Y. Stefanovsky, F. Langlois, D. Bazett-Jones, G. Pelletier, T. Moss, ERK modulates DNA bending and enhancesome structure by phosphorylating HMG1-boxes 1 and 2 of the RNA polymerase I transcription factor UBF, Biochemistry 45 (2006) 3626–3634.
[37] A.J. Waskiewicz, J.C. Johnson, B. Penn, M. Mahalingam, S.R. Kimball, J.A. Cooper, Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo, Mol. Cell Biol. 19 (1999) 1871–1880.
[38] T. Ueda, R. Watanabe-Fukunaga, H. Fukuyama, S. Nagata, 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 (2004) 6539–6549.
[39] S. Wullschleger, R. Loewith, M.N. Hall, TOR signaling in growth and metabolism, Cell 124 (2006) 471–484.
[40] K. Inoki, H. Ouyang, Y. Li, K.L. Guan, Signaling by target of rapamycin proteins in cell growth control, Microbiol. Mol. Biol. Rev. 69 (2005) 79–100.
[41] L. Ma, Z. Chen, H. Erdjument-Bromage, P. Tempst, P.P. Pandolfi, Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis, Cell 121 (2005) 179–193.
[42] P.P. Roux, B.A. Ballif, R. Anjum, S.P. Gygi, J. Blenis, Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 13489–13494.
[43] J.N. Lavoie, G. L'Allemain, A. Brunet, R. Muller, J. Pouyssegur, Cyclin D1 expression is regulated positively by the p42/p44MAPK and negatively by the p38/HOGMAPK pathway, J. Biol. Chem. 271 (1996) 20608–20616.
[44] J.D. Weber, D.M. Raben, P.J. Phillips, J.J. Baldassare, Sustained activation of extracellular-signal-regulated kinase 1 (ERK1) is required for the continued expression of cyclin D1 in G1 phase, Biochem. J. 326 (Pt 1) (1997) 61–68.
[45] J.R. Brown, E. Nigh, R.J. Lee, H. Ye, M.A. Thompson, F. Saudou, R.G. Pestell, M.E. Greenberg, Fos family members induce cell cycle entry by activating cyclin D1, Mol. Cell Biol. 18 (1998) 5609–5619.
[46] P.M. Burch, Z. Yuan, A. Loonen, N.H. Heintz, An extracellular signal-regulated kinase 1- and 2-dependent program of chromatin trafficking of c-Fos and Fra-1 is required for cyclin D1 expression during cell cycle reentry, Mol. Cell Biol. 24 (2004) 4696–4709.
[47] C. Albanese, J. Johnson, G. Watanabe, N. Eklund, D. Vu, A. Arnold, R.G. Pestell, Transforming p21ras mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions, J. Biol. Chem. 270 (1995) 23589–23597.
[48] G.A. Preston, D. Srinivasan, J.C. Barrett, Apoptotic response to growth factor deprivation involves cooperative interactions between c-Fos and p300, Cell Death Differ. 7 (2000) 215–226.
[49] S. Baluchamy, H.N. Rajabi, R. Thimmapaya, A. Navaraj, B. Thimmapaya, Repression of c-Myc and inhibition of G1 exit in cells conditionally overexpressing p300 that is not dependent on its histone acetyltransferase activity, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9524–9529.
[50] K. Balmanno, S.J. Cook, Sustained MAP kinase activation is required for the expression of cyclin D1, p21Cip1 and a subset of AP-1 proteins in CCL39 cells, Oncogene 18 (1999) 3085–3097.
[51] H. Gille, M. Kortenjann, O. Thomae, C. Moomaw, C. Slaughter, M.H. Cobb, P.E. Shaw, ERK phosphorylation potentiates Elk-1-mediated ternary complex formation and transactivation, EMBO J. 14 (1995) 951–962.
[52] L.O. Murphy, J.P. MacKeigan, J. Blenis, A network of immediate early gene products propagates subtle differences in mitogen-activated protein kinase signal amplitude and duration, Mol. Cell. Biol. 24 (2004) 144–153.
[53] L.O. Murphy, S. Smith, R.H. Chen, D.C. Fingar, J. Blenis, Molecular interpretation of ERK signal duration by immediate early gene products, Nat. Cell Biol. 4 (2002) 556–564.
[54] V. Volmat, M. Camps, S. Arkinstall, J. Pouyssegur, P. Lenormand, The nucleus, a site for signal termination by sequestration and inactivation of p42/p44 MAP kinases, J. Cell Sci. 114 (2001) 3433–3443.
[55] A. Seth, E. Alvarez, S. Gupta, R.J. Davis, A phosphorylation site located in the NH2-terminal domain of c-Myc increases transactivation of gene expression, J. Biol. Chem. 266 (1991) 23521–23524.
[56] J.I. Daksis, R.Y. Lu, L.M. Facchini, W.W. Marhin, L.J. Penn, Myc induces cyclin D1 expression in the absence of de novo protein synthesis and links mitogen-stimulated signal transduction to the cell cycle, Oncogene 9 (1994) 3635–3645.
[57] C.J. Sherr, J.M. Roberts, CDK inhibitors: positive and negative regulators of G1-phase progression, Genes Dev. 13 (1999) 1501–1512.
[58] S.M. Keenan, C. Bellone, J.J. Baldassare, Cyclin-dependent kinase 2 nucleocytoplasmic translocation is regulated by extracellular regulated kinase, J. Biol. Chem. 276 (2001) 22404–22409.
[59] N.H. Lents, S.M. Keenan, C. Bellone, J.J. Baldassare, Stimulation of the Raf/MEK/ERK cascade is necessary and sufficient for activation and Thr-160 phosphorylation of a nuclear-targeted CDK2, J. Biol. Chem. 277 (2002) 47469–47475.
[60] Y. Yoshida, T. Nakamura, M. Komoda, H. Satoh, T. Suzuki, J.K. Tsuzuku, T. Miyasaka, E.H. Yoshida, H. Umemori, R.K. Kunisaki, K. Tani, S. Ishii, S. Mori, M. Suganuma, T. Noda, T. Yamamoto, Mice lacking a transcriptional corepressor Tob are predisposed to cancer, Genes Dev. 17 (2003) 1201–1206.
[61] J.B. Weitzman, L. Fiette, K. Matsuo, M. Yaniv, JunD protects cells from p53-dependent senescence and apoptosis, Mol. Cell 6 (2000) 1109–1119.
[62] J.H. Wright, E. Munar, D.R. Jameson, P.R. Andreassen, R.L. Margolis, R. Seger, E.G. Krebs, Mitogen-activated protein kinase kinase activity is required for the G(2)/M transition of the cell cycle in mammalian fibroblasts, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 11335–11340.
[63] E.C. Roberts, P.S. Shapiro, T.S. Nahreini, G. Pages, J. Pouyssegur, N.G. Ahn, Distinct cell cycle timing requirements for extracellular signal-regulated kinase and phosphoinositide 3-kinase signaling pathways in somatic cell mitosis, Mol. Cell Biol. 22 (2002) 7226–7241.
[64] S. Walsh, S.S. Margolis, S. Kornbluth, Phosphorylation of the cyclin b1 cytoplasmic retention sequence by mitogen-activated protein kinase and Plx, Mol. Cancer Res. 1 (2003) 280–289.
[65] A. Palmer, A.C. Gavin, A.R. Nebreda, A link between MAP kinase and p34(cdc2)/cyclin B during oocyte maturation: p90(rsk) phosphorylates and inactivates the p34(cdc2) inhibitory kinase Myt1, EMBO J. 17 (1998) 5037–5047.
[66] F. Liu, C. Rothblum-Oviatt, C.E. Ryan, H. Piwnica-Worms, Overproduction of human Myt1 kinase induces a G2 cell cycle delay by interfering with the intracellular trafficking of Cdc2–cyclin B1 complexes, Mol. Cell. Biol. 19 (1999) 5113–5123.
[67] Y. Yan, R.S. Spieker, M. Kim, S.M. Stoeger, K.H. Cowan, BRCA1-mediated G2/M cell cycle arrest requires ERK1/2 kinase activation, Oncogene 24 (2005) 3285–3296.
[68] S. Rahmouni, F. Cerignoli, A. Alonso, T. Tsutji, R. Henkens, C. Zhu, C. Louis-dit-Sully, M. Moutschen, W. Jiang, T. Mustelin, Loss of the VHR dual-specific phosphatase causes cell-cycle arrest and senescence, Nat. Cell Biol. 8 (2006) 524–531.

[69] P.S. Shapiro, E. Vaisberg, A.J. Hunt, N.S. Tolwinski, A.M. Whalen, J.R. McIntosh, N.G. Ahn, Activation of the MKK/ERK pathway during somatic cell mitosis: direct interactions of active ERK with kinetochores and regulation of the mitotic 3F3/2 phosphoantigen, J. Cell Biol. 142 (1998) 1533–1545.
[70] M. Zecevic, A.D. Catling, S.T. Eblen, L. Renzi, J.C. Hittle, T.J. Yen, G.J. Gorbsky, M.J. Weber, Active MAP kinase in mitosis: localization at kinetochores and association with the motor protein CENP-E, J. Cell Biol. 142 (1998) 1547–1558.
[71] H. Cha, C. Hancock, S. Dangi, D. Maiguel, F. Carrier, P. Shapiro, Phosphorylation regulates nucleophosmin targeting to the centrosome during mitosis as detected by cross-reactive phosphorylation-specific MKK1/MKK2 antibodies, Biochem. J. 378 (2004) 857–865.
[72] C. Hayne, X. Xiang, Z. Luo, MEK inhibition and phosphorylation of serine 4 on B23 are two coincident events in mitosis, Biochem. Biophys. Res. Commun. 321 (2004) 675–680.
[73] F.S. Willard, M.F. Crouch, MEK, ERK, and p90RSK are present on mitotic tubulin in Swiss 3T3 cells: a role for the MAP kinase pathway in regulating mitotic exit, Cell. Signal. 13 (2001) 653–664.
[74] B.R. Franza Jr., K. Maruyama, J.I. Garrels, H.E. Ruley, In vitro establishment is not a sufficient prerequisite for transformation by activated ras oncogenes, Cell 44 (1986) 409–418.
[75] T. Hirakawa, H.E. Ruley, Rescue of cells from ras oncogene-induced growth arrest by a second, complementing, oncogene, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 1519–1523.
[76] M.L. Samuels, M.J. Weber, J.M. Bishop, M. McMahon, Conditional transformation of cells and rapid activation of the mitogen-activated protein kinase cascade by an estradiol-dependent human raf-1 protein kinase, Mol. Cell. Biol. 13 (1993) 6241–6252.
[77] A. Sewing, B. Wiseman, A.C. Lloyd, H. Land, High-intensity Raf signal causes cell cycle arrest mediated by p21Cip1, Mol. Cell. Biol. 17 (1997) 5588–5597.
[78] D. Woods, D. Parry, H. Cherwinski, E. Bosch, E. Lees, M. McMahon, Raf-induced proliferation or cell cycle arrest is determined by the level of Raf activity with arrest mediated by p21Cip1, Mol. Cell. Biol. 17 (1997) 5598–5611.
[79] J. LaBaer, M.D. Garrett, L.F. Stevenson, J.M. Slingerland, C. Sandhu, H.S. Chou, A. Fattaey, E. Harlow, New functional activities for the p21 family of CDK inhibitors, Genes Dev. 11 (1997) 847–862.
[80] J.S. Park, L. Qiao, D. Gilfor, M.Y. Yang, P.B. Hylemon, C. Benz, G. Darlington, G. Firestone, P.B. Fisher, P. Dent, A role for both Ets and C/EBP transcription factors and mRNA stabilization in the MAPK-dependent increase in p21 (Cip-1/WAF1/mda6) protein levels in primary hepatocytes, Mol. Biol. Cell 11 (2000) 2915–2932.
[81] R. Touitou, J. Richardson, S. Bose, M. Nakanishi, J. Rivett, M.J. Allday, A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8 alpha-subunit of the 20S proteasome, EMBO J. 20 (2001) 2367–2375.
[82] M.L. Coleman, C.J. Marshall, M.F. Olson, Ras promotes p21(Waf1/Cip1) protein stability via a cyclin D1-imposed block in proteasome-mediated degradation, EMBO J. 22 (2003) 2036–2046.
[83] J.R. Alt, A.B. Gladden, J.A. Diehl, p21(Cip1) Promotes cyclin D1 nuclear accumulation via direct inhibition of nuclear export, J. Biol. Chem. 277 (2002) 8517–8523.
[84] F.G. Haluska, H. Tsao, H. Wu, F.S. Haluska, A. Lazar, V. Goel, Genetic alterations in signaling pathways in melanoma, Clin. Cancer Res. 12 (2006) 2301s–2307s.
[85] A.M. Mirza, S. Gysin, N. Malek, K. Nakayama, J.M. Roberts, M. McMahon, Cooperative regulation of the cell division cycle by the protein kinases RAF and AKT, Mol. Cell. Biol. 24 (2004) 10868–10881.
[86] E. Yeh, M. Cunningham, H. Arnold, D. Chasse, T. Monteith, G. Ivaldi, W.C. Hahn, P.T. Stukenberg, S. Shenolikar, T. Uchida, C.M. Counter, J.R. Nevins, A.R. Means, R. Sears, A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells, Nat. Cell Biol. 6 (2004) 308–318.
[87] W. Yang, J. Shen, M. Wu, M. Arsura, M. FitzGerald, Z. Suldan, D.W. Kim, C.S. Hofmann, S. Pianetti, R. Romieu-Mourez, L.P. Freedman, G.E. Sonenshein, Repression of transcription of the p27(Kip1) cyclin-dependent kinase inhibitor gene by c-Myc, Oncogene 20 (2001) 1688–1702.
[88] R.C. O'Hagan, M. Ohh, G. David, I.M. de Alboran, F.W. Alt, W.G. Kaelin Jr., R.A. DePinho, Myc-enhanced expression of Cul1 promotes ubiquitin-dependent proteolysis and cell cycle progression, Genes Dev. 14 (2000) 2185–2191.
[89] R.H. Medema, G.J. Kops, J.L. Bos, B.M. Burgering, AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1, Nature 404 (2000) 782–787.
[90] M. Stahl, P.F. Dijkers, G.J. Kops, S.M. Lens, P.J. Coffer, B.M. Burgering, R.H. Medema, The forkhead transcription factor FoxO regulates transcription of p27Kip1 and Bim in response to IL-2, J. Immunol. 168 (2002) 5024–5031.
[91] R. Hoshino, Y. Chatani, T. Yamori, T. Tsuruo, H. Oka, O. Yoshida, Y. Shimada, S. Ari-i, H. Wada, J. Fujimoto, M. Kohno, Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors, Oncogene 18 (1999) 813–822.
[92] H. Davies, G.R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M.J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing, Y. Floyd, K. Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, C. Mould, A. Parker, C. Stevens, S. Watt, S. Hooper, R. Wilson, H. Jayatilake, B.A. Gusterson, C. Cooper, J. Shipley, D. Hargrave, K. Pritchard-Jones, N. Maitland, G. Chenevix-Trench, G.J. Riggins, D.D. Bigner, G. Palmieri, A. Cossu, A. Flanagan, A. Nicholson, J.W. Ho, S.Y. Leung, S.T. Yuen, B.L. Weber, H.F. Seigler, T.L. Darrow, H. Paterson, R. Marais, C.J. Marshall, R. Wooster, M.R. Stratton, P.A. Futreal, Mutations of the BRAF gene in human cancer, Nature 417 (2002) 949–954.
[93] J.S. Sebolt-Leopold, D.T. Dudley, R. Herrera, K. Van Becelaere, A. Wiland, R.C. Gowan, H. Tecle, S.D. Barrett, A. Bridges, S. Przybranowski, W.R. Leopold, A.R. Saltiel, Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo, Nat. Med. 5 (1999) 810–816.
[94] M. Karasarides, A. Chiloeches, R. Hayward, D. Niculescu-Duvaz, I. Scanlon, F. Friedlos, L. Ogilvie, D. Hedley, J. Martin, C.J. Marshall, C.J. Springer, R. Marais, B-RAF is a therapeutic target in melanoma, Oncogene 23 (2004) 6292–6298.
[95] D.B. Solit, L.A. Garraway, C.A. Pratilas, A. Sawai, G. Getz, A. Basso, Q. Ye, J.M. Lobo, Y. She, I. Osman, T.R. Golub, J. Sebolt-Leopold, W.R. Sellers, N. Rosen, BRAF mutation predicts sensitivity to MEK inhibition, Nature 439 (2006) 358–362.
[96] J. Rinehart, A.A. Adjei, P.M. Lorusso, D. Waterhouse, J.R. Hecht, R.B. Natale, O. Hamid, M. Varterasian, P. Asbury, E.P. Kaldjian, S. Gulyas, D.Y. Mitchell, R. Herrera, J.S. Sebolt-Leopold, M.B. Meyer, Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer, J. Clin. Oncol. 22 (2004) 4456–4462.
[97] M. Kohno, J. Pouyssegur, Targeting the ERK signaling pathway in cancer therapy, Ann. Med. 38 (2006) 200–211.
[98] P.J. Klein, C.M. Schmidt, C.A. Wiesenauer, J.N. Choi, E.A. Gage, M.T. Yip-Schneider, E.A. Wiebke, Y. Wang, C. Omer, J.S. Sebolt-Leopold, The Effects of a Novel MEK Inhibitor PD184161 on MEK-ERK Signaling and Growth in Human Liver Cancer, Neoplasia 8 (2006) 1–8.
[99] A. Fujioka, K. Terai, R.E. Itoh, K. Aoki, T. Nakamura, S. Kuroda, E. Nishida, M. Matsuda, Dynamics of the Ras/ERK MAPK cascade as monitored by fluorescent probes, J. Biol. Chem. 281 (2006) 8917–8926.
[100] K.H. Lim, C.M. Counter, Reduction in the requirement of oncogenic Ras signaling to activation of PI3K/AKT pathway during tumor maintenance, Cancer Cell 8 (2005) 381–392.
[101] C.T. Jordan, M.L. Guzman, M. Noble, Cancer stem cells, N. Engl. J. Med. 355 (2006) 1253–1261.
[102] T. Burdon, C. Stracey, I. Chambers, J. Nichols, A. Smith, Suppression of SHP-2 and ERK signalling promotes self-renewal of mouse embryonic stem cells, Dev. Biol. 210 (1999) 30–43.
[103] T. Burdon, A. Smith, P. Savatier, Signalling, cell cycle and pluripotency in embryonic stem cells, Trends Cell Biol. 12 (2002) 432–438.
[104] R. Hoshino, S. Tanimura, K. Watanabe, T. Kataoka, M. Kohno, Blockade of the extracellular signal-regulated kinase pathway induces marked G1

cell cycle arrest and apoptosis in tumor cells in which the pathway is constitutively activated: up-regulation of p27(Kip1), J. Biol. Chem. 276 (2001) 2686–2692.

[105] S. Gysin, S.H. Lee, N.M. Dean, M. McMahon, Pharmacologic inhibition of RAF→MEK→ERK signaling elicits pancreatic cancer cell cycle arrest through induced expression of p27Kip1, Cancer Res. 65 (2005) 4870–4880.

[106] E.A. Collisson, A. De, H. Suzuki, S.S. Gambhir, M.S. Kolodney, Treatment of metastatic melanoma with an orally available inhibitor of the Ras-Raf-MAPK cascade, Cancer Res. 63 (2003) 5669–5673.