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Review

RNA viruses and the mitogenic Raf/MEK/ERK signal transduction cascade

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Abstract

The Raf/MEK/ERK signal transduction cascade belongs to the mitogen-activated protein kinase (MAPK) cascades. Raf/MEK/ERK signaling leads to stimulus-specific changes in gene expression, alterations in cell metabolism or induction of programmed cell death (apoptosis), and thus controls cell differentiation and proliferation. It is induced by extracellular agents, including pathogens such as RNA viruses. Many DNA viruses are known to induce cellular signaling via this pathway. As these pathogens partly use the DNA synthesis machinery for their replication, they aim to drive cells into a proliferative state. In contrast, the consequences of RNA virus-induced Raf/MEK/ERK signaling were less clear for a long time, but since the turn of the century the number of publications on this topic has rapidly increased. Research on this virus/host-interaction will broaden our understanding of its relevance in viral replication. This important control center of cellular responses is differently employed to support the replication of several important human pathogenic RNA viruses including influenza, Ebola, hepatitis C and SARS corona viruses.

Keywords: extracellular signal-regulated kinase; virus replication.

Introduction

This review aims to give an overview of current knowledge regarding the importance of the RNA virus-induced Raf/MEK/ERK signaling pathway for viral replication. Owing to differences in the number of publications dealing with specific viruses, the focus is on the human pathogens influenza viruses, Ebola virus, HIV, hepatitis C virus (HCV), coxsackievirus B3, and coronaviruses. In addition, some representatives of animal pathogens that are being studied as model systems are discussed. Therefore, this review does not claim to give a complete overview. Nevertheless, as every virus depends on its host cell, the understanding of cellular functions such as signaling pathways that are essential for viral replication may be suitable to define targets for anti-viral therapy and pave the way towards effective drugs against essential cellular activities supporting viral replication (see also Ludwig and Planz, 2008; Wolff et al., 2008).

MAPK cascades

Mitogen-activated protein kinase (MAPK) cascades are important signaling pathways that convert extracellular signals into cellular responses (reviewed in Pearson et al., 2001). They regulate proliferation, differentiation, cell activation and immune responses. Four different members organized in separate cascades have been identified so far: (i) ERK (extracellular signal-regulated kinase), (ii) JNK (Jun-N-terminal kinase), (iii) p38 and (iv) ERK5 (Figure 1). For each MAPK, different isoforms are known. All these enzymes are activated by phosphorylation mediated by an upstream MAPK kinase (MAPKK, MEKs or MKKs). The MAPKs ERK1/2 are activated by the MAPKKs MEK-1/2 that are controlled by the MAPKKK Raf. Raf, MEK and ERK form the prototype module of an MAPK pathway. The MAPKs p38 and JNK in turn are activated by the MAPKKs MKK3/6 and MKK4/7, respectively, and are predominantly activated by pro-inflammatory cytokines and certain environmental stress conditions. ERK5 is activated by the MAPKK MEK5. This kinase module is activated by mitogens and certain stress inducers (reviewed in Ludwig et al., 2003, 2006).

Negative-strand RNA viruses

Orthomyxoviridae

Influenza viruses belong to the genus influenza A, B or C virus in the order Orthomyxoviridae. These enveloped viruses possess a segmented single-stranded RNA genome of negative polarity, which assembles with the viral RNA-dependent RNA-polymerase (RDRP) and the nucleocapsid protein to form biologically active replication/transcription complexes: ribonucleoprotein complexes (RNPs). The genome is replicated in the nucleus of infected cells and therefore undergoes complex regulated bi-directional nuclear transport. All four MAPK cascades mentioned above have been shown to be activated upon infection with a variety of avian and human influenza virus strains (Ludwig et al., 2003; Noah et al., 2003; Sarkar et al., 2004; V. Korte and S. Ludwig, unpublished). Recent work has led to a better understanding of the importance of these signaling pathways for influenza virus replication, especially of the Raf/MEK/ERK (MAPK) cascade.

ERK activation upon productive influenza virus infection (Noah et al., 2003) contributes to virus-induced cyto-

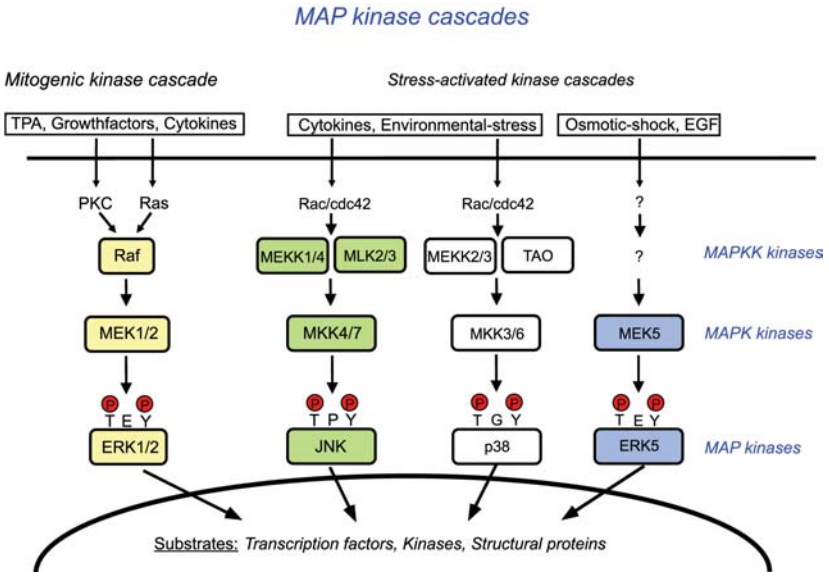

Figure 1 MAP kinase cascades.
The four MAPK cascades belong to either mitogen- or stress-activated MAPK cascades. They can be activated by many factors. Among others, these include phorbol esters (TPA), growth factors and cytokines, stress, osmotic shock and epidermal growth factor (EGF). How these signals are registered and passed on to the downstream kinases is only partially known. Protein kinases of the MAPKKK class may be activated by small regulatory GTPases such as Rac and cdc42 or other protein kinases. These again may receive their signal from growth and cytokine receptors. The cascades have a stepwise activation in common, including a MAP kinase (ERK, JNK; p38, ERK5) that is activated by dual phosphorylation through a MAPK kinase (MEK1/2, MKK4/7, MKK3/6, MEK5), which in turn has to be activated by a MAPKK kinase (Raf, MEKK1/4, MLK2/3). These again are activated via specific factors (PKC, Ras, Rac) responding to different stimuli. MLK, mixed lineage kinase; TAO, thousand-and-one-amino-acid protein kinase; ERK, extracellular signal-regulated kinase; JNK, Jun-N-terminal kinase; MEK, MAP/ERK kinase; MEKK, MEK kinase.

kine production and airway inflammation (Mizumura et al., 2003), although at the same time appears to support viral replication (Pleschka et al., 2001). Strikingly, specific blockade of the pathway strongly impaired growth of avian and human influenza A and human B-type viruses (Pleschka et al., 2001; Ludwig et al., 2004). Conversely, virus titers are enhanced in cells with an activated MAPK pathway (Ludwig et al., 2004; Olschlager et al., 2004; Marjuki et al., 2007). This has been demonstrated in cell culture and *in vivo* in infected mice expressing a constitutively active Raf kinase in the alveolar epithelial cells of the lung, which led to enhanced virus replication in cells expressing the transgene (Olschlager et al., 2004). Thus, activation of the MAPK pathway is required for efficient virus growth. With respect to the underlying molecular mechanisms, it was shown that cascade inhibition led to nuclear retention of the viral RNP complexes in late stages of the replication cycle. This suggests that the pathway controls the active nuclear export of RNPs, most likely due to interference with the activity of the viral nuclear export protein NEP (Pleschka et al., 2001). This finding is reminiscent of the impaired Rev function found under MAPK inhibition in Visna virus-infected cells (see below). So far the detailed mechanism of how ERK regulates RNP export is unknown, but there are two likely scenarios: it occurs via direct phosphorylation of a viral protein involved in RNP transport or by control of a cellular export factor. Although no change in the overall phosphorylation status of NP and NEP proteins was observed in initial studies (Pleschka et al., 2001), there are now first indications that certain phosphorylation sites of NP are indeed affected by MEK inhibition (S. Pleschka, unpublished data). With regard to a cellular factor that might be involved, there is first evidence that the MAPK cascade specifically regulates nuclear export of certain cellular RNA-protein complexes (Dumitru et al., 2000). Thus, the ERK pathway may regulate phosphorylation of both viral NP and an as yet unknown cellular factor to specifically mediate RNP export of influenza A and B viruses.

These findings led to the hypothesis that active RNP export is an induced rather than a constitutive event. Whereas RNPs have to reside in the nucleus for sufficient replication and transcription of the viral genome in the early stages, they have to be exported from the nucleus late in the replication cycle to be enwrapped into budding progeny virions at the cell membrane. This coincides well with late activation of ERK in the viral life cycle. Thus, a regulatory mechanism can be predicted, raising the question as to how timely coordination of this regulatory pathway can be achieved. A major step forward in solving this puzzle was the demonstration that membrane accumulation of viral hemagglutinin (HA) protein and its tight association with lipid-raft domains triggers protein kinase Cα (PKCα)-dependent activation of the ERK cascade late in the infection cycle, and thus induces RNP export (Marjuki et al., 2006). These findings are strongly supported by results indicating that clustering of raft-associated HA in the external membrane leaflet modulates diffusion and signaling of H-Ras at the internal leaflet, which, besides PKCα, is also a major upstream activator of Raf (Eisenberg et al., 2006). As HA together with other cell membrane-integrated viral proteins (NA, M2 and M1) forms electron-dense patches at the site of

virus membrane budding, signaling components at the inner membrane layer could be affected, leading to activation of the MAPK pathway. ERK activation by membrane-accumulated HA may therefore represent an auto-regulatory mechanism that coordinates timing of RNP export to a point at which all viral components are ready for virus budding.

Filoviridae

This order comprises the genus Filovirus with its three representatives, Ebola virus, Marburg virus and Reston virus. These BSL-4 pathogens are enveloped viruses with a single-stranded non-segmented RNA genome of negative polarity. Ebola virus is a highly lethal pathogen that causes hemorrhagic fever in humans and non-human primates. Among the seven known viral gene products, the envelope glycoprotein (GP) by itself induces cell death. GP-induced cell death follows down-modulation of cell-surface molecules involved in signaling pathways. Investigation of GP-induced cytotoxicity revealed that ERK activation was reduced in cells transiently expressing GP. Impaired ERK activity enhanced the decrease in αV integrin expression associated with toxicity. Consequently, activation of the pathway counteracted this effect. These results indicate that the MAPK cascade is involved in GP-mediated cytotoxicity. This GP-induced effect seems to be dependent on the heavily glycosylated mucin domain of GP (Zampieri et al., 2007a). In contrast to this inhibiting effect on MAPK signaling of GP expressed in cells, treatment of dendritic cells (DCs), which are important early targets of Ebola virus infection *in vivo*, with Ebola virus-like particles (VLPs) containing the viral matrix protein (VP40) and the viral GP activated DCs. This response included the production of several pro-inflammatory cytokines and activation of ERK. Again the heavily glycosylated mucin domain was essential for this GP activity (Martinez et al., 2007; reviewed in Zampieri et al., 2007b). Transient expression of the VP40 matrix protein of Ebola virus results in VLP budding from mammalian cells. Intracellular levels of calcium are known to affect the localization and function of host proteins involved in virus budding. BAPTA/AM, a calcium ion chelator, and calmodulin antagonists reduced budding of VP40 VLPs, indicating that this process depends on intracellular free calcium. VP40 VLP budding was also reduced by the MEK inhibitor U0126, indicating that the mechanism of calcium/calmodulin-mediated Ebola VLP budding may involve the MAPK signaling pathway, which can be activated in a calcium-dependent manner (Han and Harty, 2007). These data indicate substantial differences between transient GP expression, VLP treatment and VLP generation in cells that require further investigation.

Positive-strand RNA viruses

Retroviridae

A great wealth of knowledge, mostly concerning the genus Lentivirus in the order Retroviridae, especially HIV type I (HIV-I)-induced ERK signaling, has been accumulated. Since these viruses are not 'pure' RNA viruses, they are only mentioned briefly here.

Lentiviruses have many common morphological and biological properties. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period. Lentiviruses are transmitted as enveloped viruses with a single-stranded, positive-sense RNA genome. Upon entry into the target cell, the viral RNA genome is converted to double-stranded DNA by a virus-encoded reverse transcriptase that is present in the virus particle. This viral DNA is then integrated into the cellular DNA by a virus-encoded integrase so that the genome can be transcribed by cellular polymerases. Once the virus has infected the cell, two pathways are possible: either the virus becomes latent and the infected cell continues to function, or the virus becomes active and replicates and a large number of virions are produced to infect other cells.

Two species of HIV infect humans: HIV-1 and HIV-2. HIV-1 is known to induce the MAPK cascade, which seems to be dependent on integration/receptor binding of the viral glycoprotein gp120 and expression of the viral Tat and Nef proteins, affecting T-cell activity, viral replication, and viral infectivity (Jabado et al., 1997; Lannuzel et al., 1997; Popik et al., 1998; Gibellini et al., 1999; Mischiati et al., 1999; Rusnati et al., 2001; Schrager et al., 2002; Fiala et al., 2004; Gee et al., 2006; Tomkowicz et al., 2006; Toschi et al., 2006; Shan et al., 2007; Wu et al., 2007). Signaling seems to be induced by gp120 through CXCR4 and CD4, leading to ERK activation. Interestingly, gp120 caused rapid cytoskeleton rearrangements resulting in a chemotactic response. Thus, HIV-1 may locally affect the cytoskeleton of target cells to facilitate viral transmission (Balabanian et al., 2004; Haller and Fackler, 2008). Interestingly, MAPK stimulation by phorbol esters induced activation of latent HIV-1 that was suppressed by the MEK inhibitor PD098059 (Yang and Gabuzda, 1999; Osiecki et al., 2005; Marquez et al., 2008). On the other hand, it was also found that viral Vpr protein-dependent cell-cycle arrest involved downregulation of genes in the MAPK pathway and decreased phosphorylation of ERK. Exogenous provision of excess MEK reversed the Vpr-associated cell-cycle arrest, confirming involvement of the MAPK cascade (Yoshizuka et al., 2005). One advantage of MAPK activation by HIV-1 might be seen be that activated ERK is incorporated into HIV-1 particles (Cartier et al., 1997), which seems to play an important role in regulating viral assembly and release by phosphorylating the p6gag protein of HIV-1 (Hemonnot et al., 2004). Moreover, exogenously activated ERK appears to phosphorylate the HIV-1 pre-integration complex, a step necessary for nuclear translocation and successful integration (Marozsan et al., 2001). Infection by HIV-1 results in the formation of a reverse transcription complex (RTC). Efficient RTC release from the cell membrane and subsequent nuclear import requires RTC phosphorylation by virion-associated ERK. Expression of a constitutively active MEK was able to activate virion-associated ERK *in trans*, and stimulation of the Raf/MEK/ERK cascade *in trans* by phorbol ester increased viral infectivity, whereas suppression of virion-associated ERK

by PD098059 markedly impaired viral infectivity (Jacque et al., 1998). This suggests that ERK activation by HIV-1 upon entry promotes HIV-1 integration. Furthermore, ERK activity seems to link cytokine signals to activation of latent HIV-1 infection by stimulating a cooperative action of the transcription factors AP-1 (activating protein 1) and NF-κB, activating the HIV-1 LTR (Briant et al., 1998; Flory et al., 1998). This indicates a signal-dependent activation of HIV-1 replication in latently infected cells and suggests potential therapeutic strategies for unmasking latent reservoirs of HIV-1 (Yang et al., 1999), as well as blocking HIV-1 activation by MEK inhibitors.

Among the non-human Lentiviruses, Visna virus can cause arthritis, encephalitis, pneumonia, wasting and depression in sheep. It was found that susceptible primary cells show induced and sustained MAPK pathway activation. Treatment with the MEK inhibitor PD098059 abolishes Visna virus replication. Early events of viral replication (i.e., reverse transcription, integration, and transcription) seemed to be largely unaffected by PD098059. Interestingly, MEK inhibition results in decreased cytoplasmic expression of gag and env, but not rev mRNA, highly suggestive of an MAPK-dependent defect in Rev function. *In vivo* analysis of ERK activation in brains derived from Visna virus-infected sheep demonstrates a strong correlation between ERK activation and virus-associated encephalitis, suggesting that activation of the MAPK pathway may contribute to viral neurodegenerative pathogenicity (Barber et al., 2002).

Flaviviridae

Among this order of RNA viruses, the genus Hepacivirus with the representative HCV has been investigated for its potential to induce the MAPK cascade and its importance in viral replication and pathology.

HCV has a positive-sense single-stranded RNA with one open reading frame of approximately 9600 nucleotides. At the 5′- and 3′-ends of the RNA there are untranslated regions (UTRs) that are important for translation and replication of the viral RNA. The 5′-UTR has an internal ribosome entry site (IRES) that starts the translation of an approximately 3000-aa polyprotein that is later cut by cellular and viral proteases into 10 active structural and non-structural proteins. HCV mainly replicates within hepatocytes in the liver. Circulating HCV particles bind to receptors on the surfaces of hepatocytes and subsequently enter the cells and initiate the lytic cycle. RNA replication depends on the RDRP, which produces a negative-strand RNA intermediate. The negative-strand RNA then serves as a template for the production of new positive-strand viral genomes. Nascent genomes can then be translated, further replicated, or packaged within new virus particles. New virus particles presumably bud into the secretory pathway and are released at the cell surface.

Persistent HCV infection can lead to the development of human hepatocellular carcinoma (HCC), but the exact mechanism of HCV-induced hepatocarcinogenesis remains unclear (see also Bode et al., 2008). Nevertheless, many forms of cancer involve deregulation of the MAPK cascade. Therefore, a functional role of the MAPK cascade in HCV-induced HCC can be suspected. It was demonstrated that the viral core protein can activate the MAPK cascade and its downstream transcription factor Elk 1 in stably transfected cell lines, and that this activation could be specifically be blocked by PD098059, demonstrating induction of the cascade by the viral protein (Hayashi et al., 2000; Fukuda et al., 2001; Erhardt et al., 2002). A model providing new insight into the mechanism of hepatocarcinogenesis by HCV infection based on HCV core-induced MAPK activation was recently proposed. Transient expressed HCV core induced an increase in NF-κB activity leading to TGFα transcription. The growth-promoting activity and activation of ERK were reduced by treatment with anti-TGFα antibodies. These results suggest that the HCV core protein activates NF-κB-dependent TGFα expression, which in turn promotes proliferation of human hepatoma cells by TGFα activation of the MAPK pathway (Sato et al., 2006). Moreover, ERK and Elk-1 activation was found to be significantly prolonged in response to mitogenic stimulus by EGF. A sustained response to EGF in cells expressing HCV core protein occurred despite normal induction of the MAPK phosphatases (MKP) regulatory feedback, which might therefore contribute to the transformation of HCV-infected liver cells (Giambartolomei et al., 2001). The finding that ERK activity was increased in core-transgenic mice fed ethanol suggests that HCV core protein cooperates with ethanol in MAPK activation, contributing to the pathogenesis of liver disease in HCV-infected patients with high ethanol consumption (Tsutsumi et al., 2003). Interestingly the HCV core protein is able to impair T-cell proliferation by inhibition of ERK activation, resulting in the inhibition of IL-2 and IL-2Rα gene expression. These results imply that HCV core-induced blockage of intracellular events in T-cell activation may play a critical role in the establishment of HCV persistence during the acute phase of viral infection (Yao et al., 2001).

Besides MAPK activation via the HCV core protein, interaction between the HCV envelope glycoprotein E2 and CD81 is considered a molecular mechanism contributing to HCV infection and pathogenicity. ERK phosphorylation was related to the concentration of HCV E2 proteins and to the length of stimulation. This suggests that HCV E2-CD81 interaction might be involved in intracellular signal transduction, as well as cell proliferation, and might play an active role in HCV pathogenicity (Zhao et al., 2001, 2005, 2006, 2007). A potential pathologic result of this interaction is upregulation of matrix metalloproteinase-2, a major enzyme involved in the degradation of normal hepatic extracellular matrix, following the interaction between E2 and CD81 (Mazzocca et al., 2005).

Another viral interaction with the MAPK pathway seem to be transmitted via the viral non-structural NS5A protein, which was shown to be able to inhibit the transcription factor AP-1 function by perturbing ERK signaling (Macdonald et al., 2003), possibly mediated by HCV NS5A activation of phosphatase 2A activity (Georgopoulou et al., 2006).

It is not clear whether MEK inhibition might therefore open a therapeutic opportunity, as it was also reported that HCV IRES-dependent protein synthesis is enhanced by the MEK inhibitor PD098059. Although the finding is

based on a reporter replicon-based system and the underlying molecular mechanisms were not explained, the authors concluded that PD098059 is a potent accelerator of HCV RNA replication (Murata et al., 2005). In contrast, activation of ERK in HCC indicates aggressive tumor behavior, confirming that HCV infection activates the Raf/MEK/ERK pathway and thus might contribute to HCC carcinogenesis. Determination of ERK activity might thus be used for identification of high-risk patients who may benefit from new anti-cancer drugs targeting the ERK pathway (Schmitz et al., 2008).

Besides the genus Hepacivirus, the genus Flavivirus has another representative that is implicated in RNA virus-induced MAPK signaling, the Japanese encephalitis virus (JEV). JEV is a neurotropic virus that generates a rapid cerebral inflammatory response stimulating inflammatory cytokine expression, peripheral neutrophil leucocytosis and neutrophil infiltration into extra-neural tissue connected to the disease (JE) in humans. The level of inflammation correlates with clinical outcome in JE patients. RANTES expression required viral replication and ERK activation, as well as transcription factors, including NF-κB and nuclear factor IL-6. However, JEV replication was not dependent on ERK activation (Chen et al., 2004). It was recently demonstrated that inhibitors of the Src protein tyrosine kinase (PTK) and Ras attenuated JEV-induced ERK activation. Further results revealed that PTK, Ras, and ERK inhibitors effectively suppressed JEV-induced pro-inflammatory cytokine expression and neurotoxicity. These results suggest that the Src/Ras/Raf/MEK/ERK signaling cascade is involved in JEV-induced pro-inflammatory cytokine expression and neurotoxicity (Raung et al., 2007).

Picornaviridae

Among the order Picornaviridae, coxsackievirus B3 (CVB3), a member of the genus Enterovirus, has been implicated in RNA virus-induced MAPK signaling. Coxsackieviruses (CV) are important human pathogens, causing a remarkable variety of diseases, from minor common colds to fatal myocarditis and neurological disorders. The CVB3 genome is encoded in a single-stranded RNA molecule of positive polarity of approximately 7500 nucleotides in length. Infection of permissive host cells is initiated by virus attachment to the specific cell-surface receptor molecule coxsackie and adenovirus receptor (CAR). Following entry into the cell, the genomic viral plus-strand RNA serves as a template for transcription by viral RDRP to yield the minus-strand RNA replication intermediate, which is subsequently transcribed by the RDRP into large amounts of genomic plus-strand RNA. Translation of genomic viral RNA occurs by a Cap-independent mechanism, yielding the viral precursor polyprotein of 243 kDa. The virus-encoded proteinases cleave the polyprotein co- and post-translationally into mature proteins that exhibit multiple functions.

It was shown that the negative regulator of Ras activity, RasGAP, is cleaved during infections with different CVB3 strains. This cleavage event leading to inactivated RasGAP allows Ras to be active for a longer time (Sprang, 1997) and may therefore promote activation of the Ras/Raf/MEK/ERK pathway, as shown by ERK activation in the late phase of CVB3 infection (Huber et al., 1999). The idea that activation or inhibition of MAPK signaling may play a general role in regulating effective enterovirus replication and pathogenesis was supported by the observation that CVB3 infection induced sustained ERK activation late in the replication cycle. Treatment of cells with the selective MEK inhibitor U0126 significantly inhibited CVB3 progeny release and decreased virus protein production (Luo et al., 2002; Opavsky et al., 2002). It was also shown that caspase 3 is induced before lysis of CVB3-infected cells occurs, leading to the release of progeny virions, and that this caspase 3 induction depends on viral activation of MAPK signaling (Cunningham et al., 2003). These data suggest that ERK activation is important for CVB3 replication and point to a therapeutic approach to stop virus spread and preserve myocardial integrity. When the correlation between ERK signaling and virus replication in the presence of CAR was investigated in CHO cells that do not express CAR and for infection of CAR-expressing cells with replication-defective CVB3, specific ERK activation was not detected and progeny virus was not produced. In contrast, in CAR-expressing cells, specific early and late ERK activation at 0.5 and 8 h post infection (p.i.) was induced, and progeny viruses were produced. In addition, inhibition of late ERK activation by the MEK inhibitor PD098059 4 h p.i. significantly decreased virus replication. The findings suggest that early ERK activation is a response to virus binding to CAR via the viral VP1, -2 and -3 proteins, whereas late ERK activation is related to viral replication (Lim et al., 2005). A possible role for CV-induced ERK activity in virus replication is also suggested by work showing that inhibition of ERK signaling contributes, at least in part, to proteasome inhibitor-mediated reduction of CV replication, demonstrating a converging function of major intracellular signaling and protein degradation pathways in the regulation of CV replication. The ubiquitin/proteasome system (UPS), a major intracellular protein degradation pathway, plays a critical role in CV replication. It was shown that UPS inhibition reduced CV-induced ERK phosphorylation, which was correlated with induction of MKP-1. Blockade of MKP induction attenuated the loss of ERK phosphorylation, and subsequently restored viral replication (Wong et al., 2007).

Coronaviridae

The genus Coronavirus in the order Coronaviridae includes human pathogenic members causing infections of the upper respiratory tract that can lead to severe clinical conditions, including bronchitis and pneumonia in small children. In this group of patients, infections of the intestinal tract can also lead to gastroenteritis. Animal pathogens are also being widely studied as model systems or because of their impact on domestic animals.

Coronaviruses (CoV) are enveloped viruses with a positive-sense single-stranded RNA genome. The genome size can be up to 31 kb, which is extraordinarily large for RNA viruses. Viral replication takes place in the cytoplasm. The CoV genome has a 5′-methylated cap and a 3′-polyadenylated tail. This allows the viral genomic RNA to be directly translated by cellular ribosomes, leading to a large polyprotein that includes the viral RDRP. The poly-

protein is auto-proteolytically processed and the newly generated RDRP replicates the plus-strand genome into a negative-strand intermediate, which serves as a template for the generation of viral mRNAs for structural viral proteins.

In March 2003, a novel CoV was isolated from patients exhibiting atypical pneumonia. This was subsequently identified as the causative agent of the disease now referred to as severe acute respiratory syndrome (SARS). By analyzing the effects of transiently expressed viral-spike protein (S) of SARS-CoV, it was revealed that the S protein plays an important role in virus-stimulated cyclooxygenase-2 (COX-2) expression (Liu et al., 2007). COX-2 is a prostaglandin synthetase involved in inflammation (Wymann and Schneiter, 2008) that is highly regulated by different factors including cytokines (Funk and FitzGerald, 2007). The upstream calcium-dependent PKCα that modulates the downstream Raf/MEK/ERK pathway is induced by the SARS-CoV S protein. It was revealed that ERK is involved in S protein-induced activation of the COX-2 promoter and the production of COX-2 protein in HEK293T cells. This result helps to explain the function of SARS-CoV S protein in SARS pathogenesis (Liu et al., 2007). More information on relevant MAPK signaling induced by CoV was obtained for mouse hepatitis virus (MHV, murine CoV). MHV infection of cultured cells resulted in activation of the Raf/MEK/ERK signal cascade (Cai et al., 2006), and inhibition of the MAPK signaling pathway by U0126 or knockdown of MEK and ERK by small interfering RNAs significantly impaired MHV progeny production. The inhibitory effect of U0126 on MHV production appeared to be a general phenomenon observed in all six different MHV strains and in three different cell types tested. The treatment did not affect virus entry or cellular and viral mRNA production. However, synthesis of viral genomic and subgenomic RNAs was severely suppressed by U0126 treatment. These findings indicate that the MAPK signaling pathway is involved in MHV RNA synthesis (Cai et al., 2007).

Other RNA viruses

Besides the RNA viruses mentioned above, several others have been shown to interact with the MAPK cascade. The information published only allows a preliminary insight into the relevance of virus-induced MAPK signaling. Among these is the Borna disease virus (BDV, Bornaviridae). This virus replicates its single-stranded RNA genome of negative polarity in the nucleus of infected cells. It was shown that MEK inhibition impairs virus spread in cell culture. Conversely, persistent infection of primary neurons by BDV interfered with ERK signaling (Planz et al., 2001a; Hans et al., 2004). These differences are not yet explained, but might be due to the different cells systems used.

Infection by the respiratory syncytial virus (RSV, Paramyxoviridae, Pneumovirus; RNA viruses with a single-stranded genome of negative polarity that replicate in the cytoplasm) caused an increase ERK activity compared to non-infected cells. This virus-induced ERK activation could be inhibited by PD098059 (Chen et al., 2000). It was also shown that MAPK activation is required for efficient RSV infection (Kong et al., 2004). RSV activates ERK both early in infection, which is connected to virus binding, and late in infection, which is related to viral replication. MAPK activation was dependent on RSV-activated PKC isoforms (Monick et al., 2001). Induction of the MAPK signaling cascade required a replication-competent virus. Inhibition of ERK activation significantly reduced RSV-induced RANTES mRNA and protein secretion without affecting RANTES gene transcription, indicating that the MAPK signaling cascade regulates RANTES production in alveolar epithelial cells at a post-transcriptional level (Pazdrak et al., 2002).

Concluding remarks

An increasing amount of evidence indicating the importance of RNA virus-induced Raf/MEK/ERK (MAPK) sig-

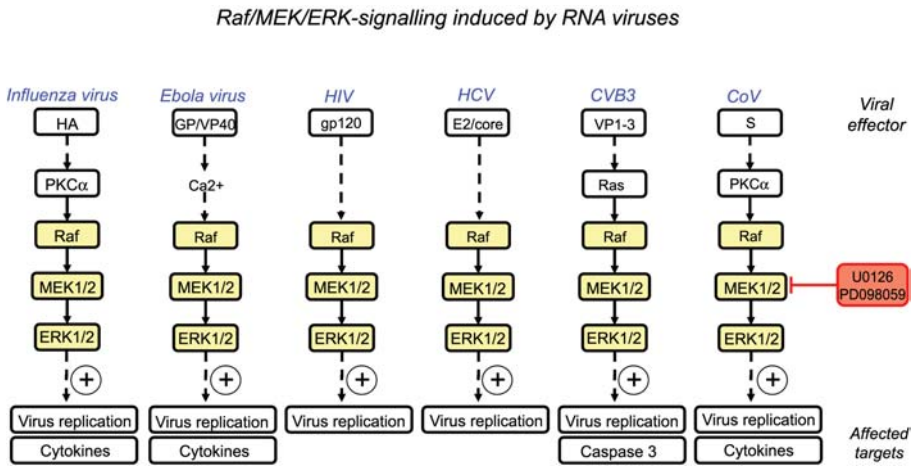

Figure 2 Raf/MEK/ERK signaling induced by RNA viruses.
The different viral effectors that have been connected to the Raf/MEK/ERK cascade activation by the RNA viruses reviewed are shown, as well as the factors that transmit the stimulus to Raf as far as they are known. The MEK inhibitors of ERK activation and major targets/responses to ERK activity are indicated. The broken line indicates that the exact mechanism still has to be determined.

naling for viral replication is emerging. This is connected to intensive investigation of virus/host interactions and provides new insights in virus pathology. It can therefore be expected that our knowledge of RNA virus-induced MAPK signaling will steadily increase over time. A general reason for viruses to upregulate the MAPK pathway is that this activity seems to be involved in downregulation of IFN production and thus impairs the cellular innate immune response, which would be beneficial for virus replication (Battcock et al., 2006; Noser et al., 2007).

Interestingly, there is evidence that RNA viruses induce MAPK signaling via viral surface structures, which then can activate PKCs or Ras, leading to Raf activation (Figure 2). It remains to be determined what specific interactions between the viral factor and host components trigger MAPK signaling. Nevertheless, it can be envisaged that the viral protein either interacts with receptor kinases on the cell surface or recruits intracellular signaling factors. Genomic replication and/or transcription seem to be regulated by the activity of the pathway. This indicates that MAPK activation might be required for efficient virus replication and suggests that this pathway can be considered an interesting cellular target for potent anti-viral approaches. For example, significant anti-viral action against influenza A- and B-type viruses in cell culture (Ludwig et al., 2004) and infected mice (Ludwig et al., 2003; Klumpp, 2004; O. Planz, S. Pleschka, S. Ludwig, unpublished) could be demonstrated for commercially available MEK inhibitors such as U0126, PD098059 and others. Furthermore, these compounds showed surprisingly little toxicity in both cell culture (Planz et al., 2001b; Pleschka et al., 2001; Ludwig et al., 2004) and mice (Sebolt-Leopold et al., 1999) Several promising MEK inhibitors such as CI-1040 are also being tested in clinical trials as anti-cancer agents to be used over longer periods of time, supporting the idea that these compounds might be very useful in anti-viral therapy (Cohen, 2002; Thompson and Lyons, 2005; Roberts and Der, 2007; Wang et al., 2007a,b; Friday and Adjei, 2008). Furthermore, inhibitors that interfere with MAPK signaling are being tested in clinical trials in the treatment of HCV-caused HCC based on: (i) MAPK signaling is an important molecular mechanism involved in HCC; (ii) it is HCV-activated; and (iii) it supports HCV replication. Therefore, blockade of the MAPK pathway would help to fight not only cancer cells, but also the causative agent, HCV. In addition, MAPK inhibition blocks stimulation of the pathway by extracellular signals, such as EGF and TGFα (Pang and Poon, 2007). Moreover, MEK inhibitors did not exhibit any tendency to induce generation of resistant virus variants (Ludwig et al., 2004). Nevertheless, the details of the mechanisms involved in RNA virus-induced MAPK signaling mostly remain elusive and await further investigation, which will foster better understanding of this important virus/host interaction.

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