

## CROSS TALK OF SIGNALING AND APOPTOTIC CASCADES IN THE CNS: TARGET FOR VIRUS MODULATION

Laure Aurelian

*Virology/Immunology Laboratories, Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore MD 21201*

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Apoptosis and disease pathogenesis
  - 3.1. Apoptosis
  - 3.2. Signaling cascades
  - 3.3. Apoptosis and neurodegeneration
4. Distinct gene families regulate apoptosis
  - 4.1. Bcl-2 and IAP families
  - 4.2. Survival pathways
  - 4.3. Heat shock proteins
5. Cross-talk and the integration of distinct signals
6. Viruses regulate signaling and apoptotic cascades
7. Concluding remarks and perspectives
8. Acknowledgement
9. References

### 1. ABSTRACT

Signaling cascades that regulate proliferation, differentiation and apoptosis determine cell life and death decisions. All are targets of virus modulation. These cascades are briefly described in this review, underscoring cross talk and the integration of distinct signals that contribute to central nervous system (CNS) disease.

### 2. INTRODUCTION

Cell life and death decisions are determined by signaling cascades that regulate proliferation, differentiation and programmed cell death (apoptosis). In order to insure their survival, viruses use various strategies to commandeer these processes. This article is a brief summary of present understanding of apoptotic and signaling cascades. It underscores apoptosis regulation, cross talk and integration of different signals and their role in neuronal pathogenesis.

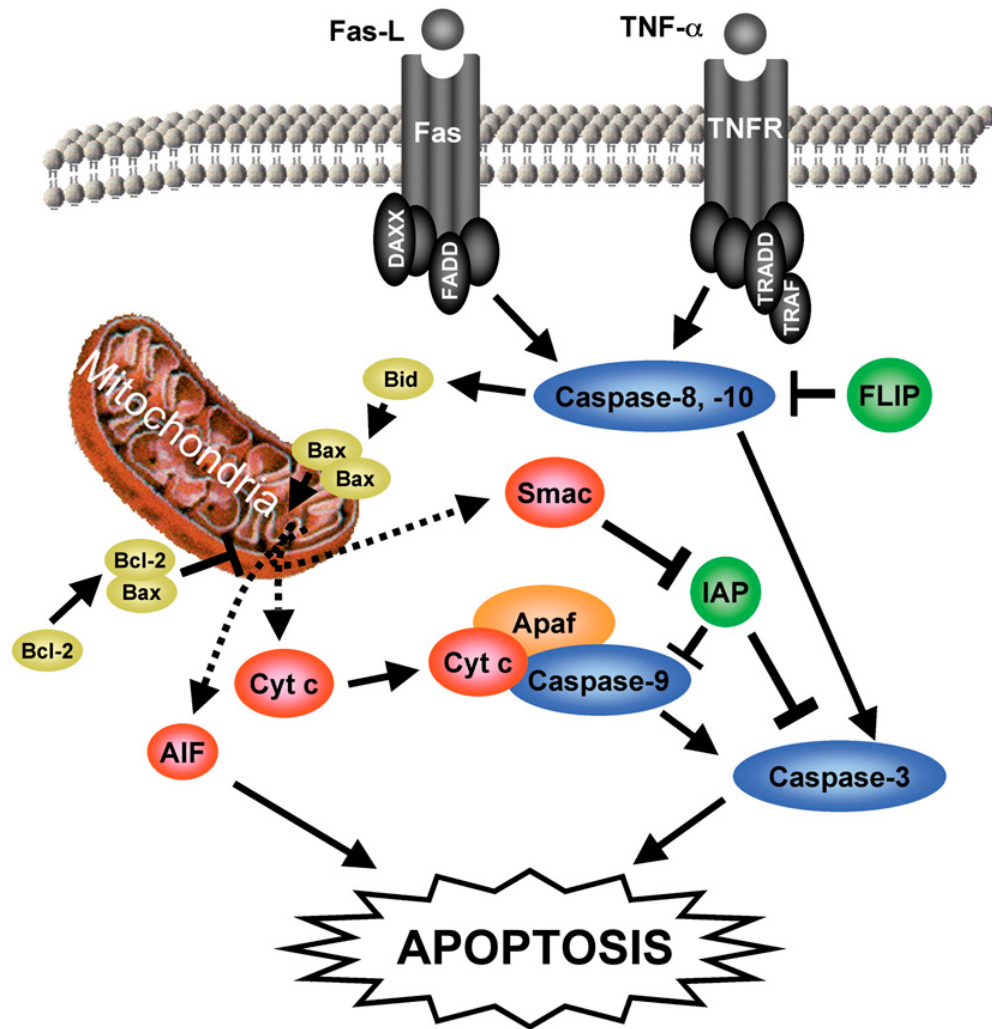
### 3. APOPTOSIS, SIGNALING AND DISEASE PATHOGENESIS

#### 3.1. Apoptosis

Apoptotic pathways and their regulation in neurological disorders were previously reviewed (1,2). Apoptosis is a tightly regulated, irreversible process that results in cell death in the absence of inflammation. It differs from necrosis, an established mechanism of virus-induced cell death, in that it involves active cell participation. As schematically represented in Figure 1, apoptotic stimuli induce mitochondrial release of Cytochrome c (Cyt c), apoptosis-inducing factor (AIF) and Smac/Diablo. Cyt c and AIF are involved in the activation of the caspases, which are cysteine proteases with aspartate

specificity. Caspases are categorized into two major groups: initiators, which include caspases -8, -9, -10, and executioners, which include caspases -3, -6, and -7. Executioner caspases, exemplified by caspase-3, are activated by proteolytic cleavage of precursor zymogens. They are responsible for the morphological and biochemical changes associated with apoptosis, including cleavage of proteins involved in DNA repair and replication, such as poly (ADP-ribose) polymerase (PARP). The intracellular apoptotic pathway is activated by loss of neurotrophic factors [e.g. nerve growth factor (NGF)], excitotoxic injury or virus infection. It initiates with Cyt c release from the mitochondria and its complexation with Apaf-1 and procaspase-9, leading to caspase-9 activation. However, a recent study indicates that apoptosome formation is not required for caspase-9 activation (3). The extra cellular apoptotic pathway is initiated by binding of the ligands Fas or Tumor Necrosis Factor (TNF)-alpha to their respective receptors. These receptors contain cytoplasmic domains that anchor adaptor proteins, which are involved in the recruitment and activation of caspases-8 or -10. Activated initiator caspases, in turn, activate the executioner caspases (4). Apoptosis often results from interference with the strict regulation of these pathways.

Stimuli implicated in apoptosis include oxidative stress, genetic defects, accumulated burden of endogenous or exogenous factors, loss of neurotrophic support, excessive release of neurotransmitters known as excitotoxins, or virus infection. When inappropriate in timing or extent, apoptosis can trigger or account for progression of neurodegeneration in acute and chronic diseases. Excessive amounts of glutamate, or glutamate function for prolonged intervals, trigger neuronal cell death



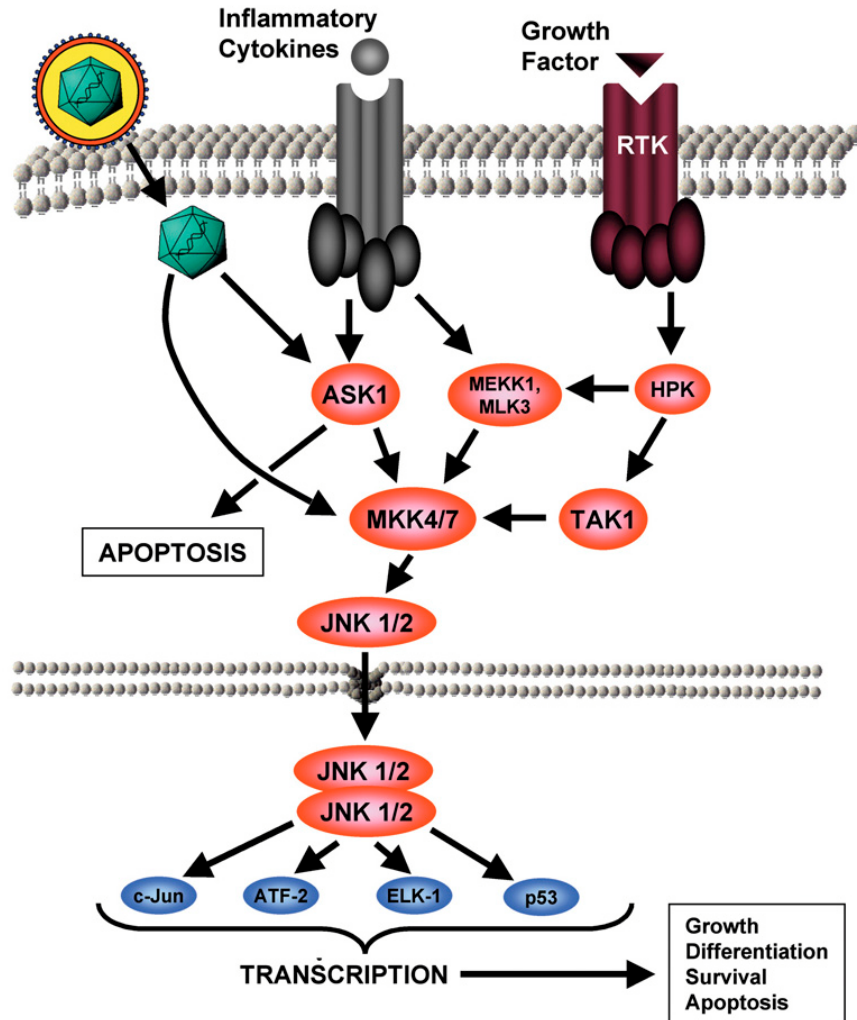
**Figure 1.** Schematic representation of the apoptotic cascade. Apoptosis can be triggered by a variety of stimuli including death receptors on the cell surface (extracellular pathway) and intracellular signals. It is regulated by the interplay of pro- and anti-apoptotic members of the Bcl-2 family. Pro-apoptotic members include Bax, Bad, Bid, Bim and Bik. They contain an alpha-helical BH3 death domain that binds anti-apoptotic family members Bcl-2 and Bcl<sub>XL</sub> forming heterodimers that block their survival activity. The pro-apoptotic Bcl-2 proteins decrease the mitochondrial transmembrane potential causing cytochrome c release. In the cytoplasm and in the presence of dATP, cytochrome c complexes with and activates Apaf-1, that in turn, binds and processes the initiator caspases (viz. procaspase 9) into a proteolytically active form. This begins the caspase cascade, which culminates in the activation of the executioner caspases (viz. caspase 3) and apoptosis. The relative abundance of pro- and anti-apoptotic Bcl-2 family members determines the susceptibility of the cell to apoptosis. IAP family members regulate apoptosis downstream of the executioner caspases.

by over stimulating cognate receptors (4). Excitotoxicity is involved in the pathogenesis of ischemic brain injury, epilepsy and neurodegenerative diseases. Cerebral ischemia was associated with activation of caspases-1, -3, -8, -9 and -11 and the release of Cyt c. Ischemic release of glutamate, IL-1beta, TNF-alpha or reactive oxygen species (ROS), can affect surrounding neurons, a bystander cell-death that is associated with activation of caspase-1 (1).

### 3.2. Signaling cascades

Stress-activated kinases are also implicated in neuronal apoptosis, and they include the c-Jun N-terminal

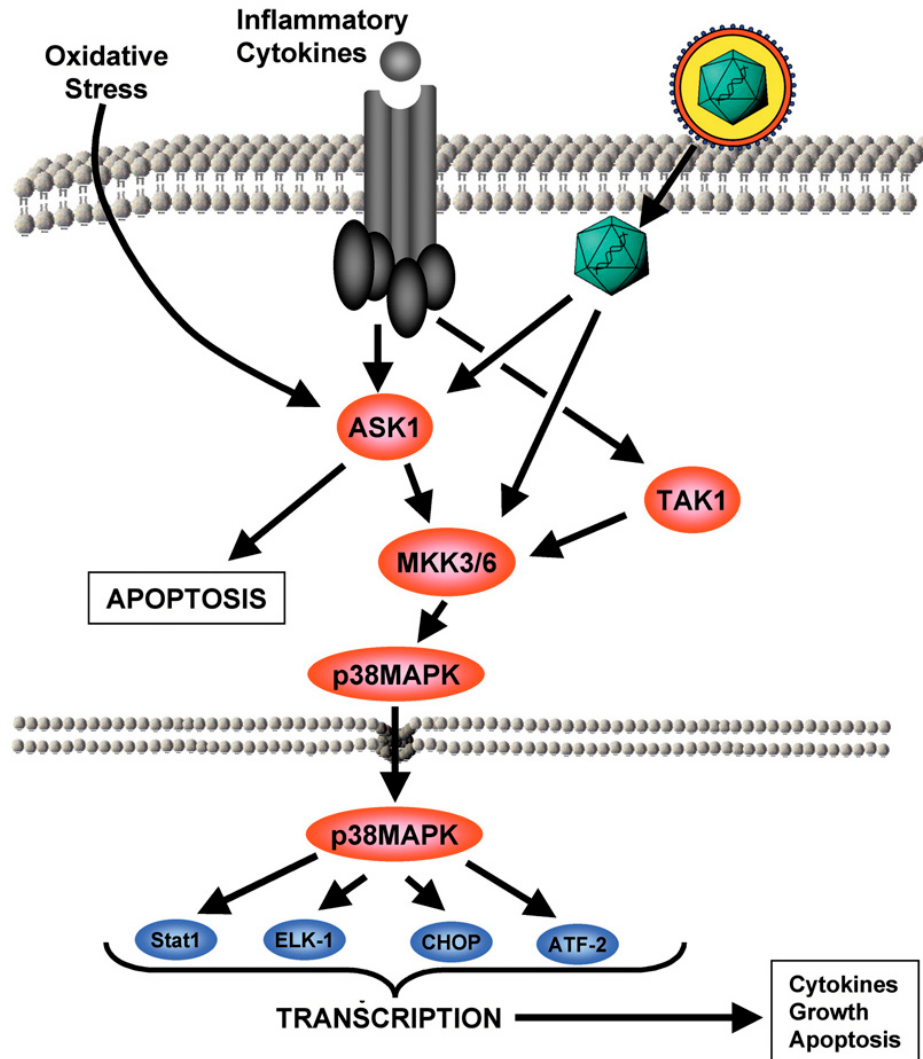
kinase (JNK) (Figure 2) and p38 MAPK (Figure 3) cascades. These pathways initiate with MAP kinase kinase kinases apoptosis signal-regulating kinase 1 (Ask-1), mitogen-activated protein kinase/ERK kinase kinase 1 (MEKK1), and TGF-beta activated kinase 1 (TAK1). These in turn activate MKK4 and/or MKK7 to mediate JNK activation, and MKK3/6 to mediate p38MAPK activation. Rac1, RhoA, and Cdc42 GTPases also modulate JNK activity. The pathways culminate in the activation of transcription factors. JNK induced apoptosis is generally transcription-dependent, involving c-Jun as a target. In PC12 cells, JNK3 can trigger apoptosis or increase NGF-



**Figure 2.** Schematic representation of the JNK pathway. JNKs respond to stress signals including heat shock, osmotic stress and pro-inflammatory cytokines. They are also activated by growth factors and virus infection. Activation is by dual phosphorylation (at Thr-Pro-Tyr motif) by MAPK kinase 4 (MKK4) and MKK7. Upstream of the MKKs are their MAPKKs, which include MEKKs 1-4, a member of the mixed-lineage kinases (MLK3) and ASK-1. In turn, these are activated by GTP-binding proteins of the Rho family. Activated JNKs dimerize and translocate to the nucleus where they activate (phosphorylate) transcription factors including c-Jun, ATF-2, Elk-1 and p53. Activation of the JNK signaling cascade generally results in apoptosis and is involved in inflammation.

induced neurite outgrowth (differentiation) concomitant with target switch from ATF-2 in the apoptotic context, to c-Jun in the differentiation context (6). The cell type, stimulus, and differentiation state affect the way in which cells die and their vulnerability to death inducing signals (7). Cross talk is underscored by the identification of novel regulators, which connect these pathways to the receptor kinases that regulate cell growth and differentiation via Ras-MAPK pathways. For example, Sef is a recently identified negative regulator of fibroblast growth factor (FGF) signaling that inhibits ERK activation and cell proliferation as well as PC12 cell differentiation. When overexpressed, Sef binds TAK1 and activates JNK through a TAK1-MKK4-JNK pathway causing apoptosis, a function distinct from its inhibitory effect on FGF receptor signaling and ERK activation (8).

Neuronal survival is stimulated by trophic factors, which function through specific receptors. The extracellular signal-related kinase (ERKs) and phosphoinositide 3-kinase (PI3-K) pathways (Figure 4) serve as a conduit for signaling from various receptors, including receptors for neurotrophic factors (e.g. NGF), glutamate or other neurotransmitters. These pathways are generally involved in neuronal cell proliferation, differentiation, development, cell cycle, and transmission of survival and mitogenic signals (9). They are linked to G protein-linked cell surface receptors and receptor kinases, including neurotransmitters. They operate through sequential phosphorylation events to activate (phosphorylate) transcription factors, such as c-Fos and the calcium/cAMP response elements binding protein (CREB), and regulate downstream signaling proteins. In response to survival stimuli, the membrane bound G

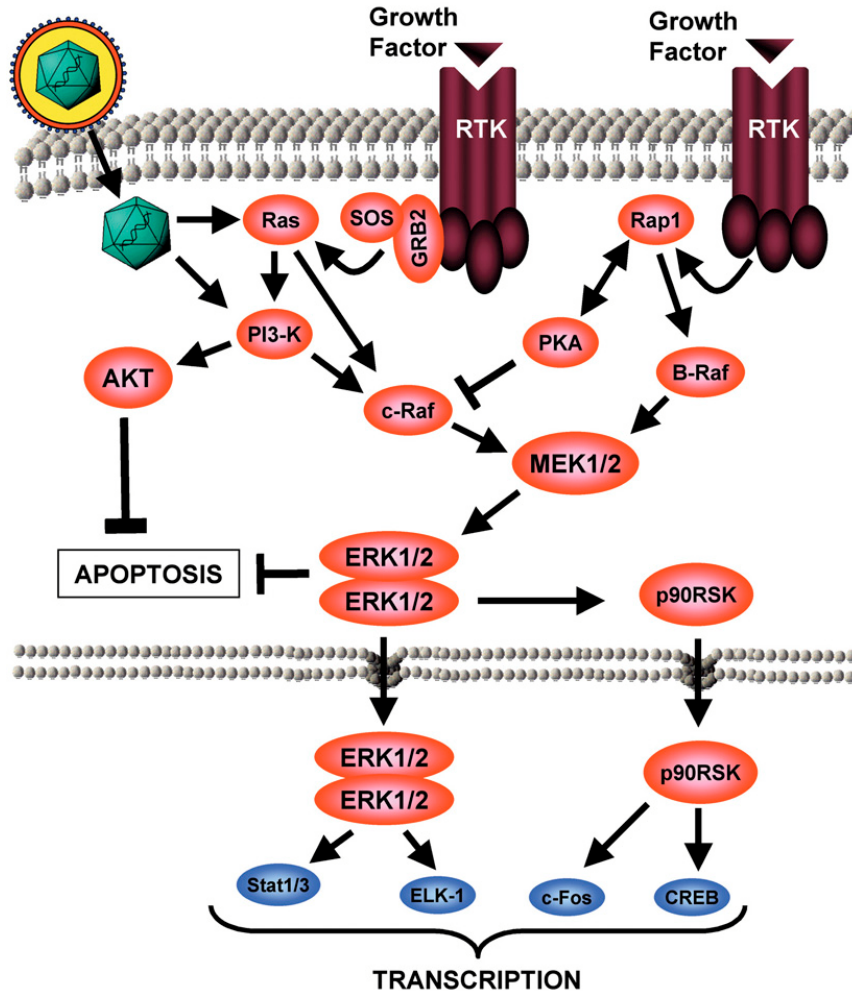


**Figure 3.** Schematic representation of the p38MAPK pathway. p38 MAPKs undergo dual phosphorylation at the Thr-Gly-Tyr motif and are generally activated by environmental stress, including heat, osmotic and oxidative shock. They are also activated by inflammatory cytokines and virus infection. The upstream kinases acting on p38MAPK include MKKs 3/6, which are in turn activated by MEKKs, MLKs and ASK1. Transcription factors affected by the p38 family include Stat1, Elk-1 ATF-2 and CHOP.

protein Ras adopts an active, GTP-bound state and it, in turn, coordinates the activation of a multitude of downstream effectors. The MEK/ERK survival pathway begins with the activation of c-Raf-1 kinase. It is followed by the activation of MAP kinase kinase (MEK) and ERK and culminates in the activation of transcription factors. The PI3-K pathway functions via Akt. The signaling pathway that is activated as a result of survival signals is determined, at least in part, by the specific phosphorylation site on the receptor (10). Different patterns of activation of NMDA-type glutamate receptors can trigger long-term potentiation (LTP) or long-term depression (LTD) of synaptic strength. Long lasting forms of synaptic plasticity are pathways for encoding memories in the brain (11). The convergence of distinct pathways on final effectors may cause signal amplification and provides a fail-safe mechanism in case one pathway becomes non-functional.

### 3.3. Apoptosis and neurodegeneration

Virus-induced neuronal cell death results from a multiplicity of virus-host cell interactions with distinct pathogenesis outcomes, and can be necrotic, apoptotic or both. Apoptosis is a major component of HSV-1 induced encephalitis in humans (12). It is also the predominant form of cell death in chronic neurodegenerative diseases. In some cases it is associated with genetic mutations that activate the caspase cascade. One example is amyotrophic lateral sclerosis (ALS), which is characterized by the progressive loss of motor neurons in the brain, brain stem and spinal cord and is associated with mutations in the gene encoding the free radical scavenging enzyme Cu, Zn superoxide dismutase 1 (SOD1) in 3-4% of ALS cases. The pro-apoptotic activity of the mutant SOD1 is presumably due to an unstable conformation that leads to increased levels of intracellular free radicals. It was



**Figure 4.** Schematic representation of the ERK and PI3-K pathways. A major grouping of the mitogen-activated protein kinase (MAP kinase) pathways activated by stimuli, such as growth factors (viz. NGF) and virus infection. Signals are transduced via small G proteins such as Ras or Rap-1, to multiple tiers of protein kinases that amplify them and/or regulate each other. In the canonical pathway the cascade originates with receptor occupation and dimerization resulting in its activation by tyrosine or serine-threonine phosphorylation. This leads to the activation of Ras, which in turn, activates Raf. Raf activates the Map kinase kinases MEK1/2 and these kinases activate downstream Map kinases ERK1/2. Activated ERK1/2 activate (by phosphorylation) kinases such as p90 RSK, which in turn activate transcription factors such as c-Fos and CREB. Activated ERK1/2 also translocate to the nucleus where they activate transcription factors such as ELK-1 and Stat3. Ras is also involved in the activation of the PI3-K/Akt pathways. The activated ERK pathway can override apoptosis.

associated with activation of caspases-1 -3 and -9, Cyt c release and pro-apoptotic changes in the Bcl-2 protein family (13). Overall, available data underscore the functional versatility of the apoptosis-related molecules in physiological and pathological conditions, stress the complexity and specificity of their cross-talk, and caution against facile conclusions relating to therapeutic interventions.

#### 4. DISTINCT GENE FAMILIES REGULATE APOPTOSIS

##### 4.1. Bcl-2 and IAP families

Distinct gene families regulate apoptosis. The Bcl-2 proteins that also function in neurologic disorders consist

of anti-apoptotic (e.g. Bcl-2) and pro-apoptotic (e.g. Bax) members that are differentially mobilized by various stimuli (Figure 1). Pro- and anti-apoptotic proteins heterodimerize and the balance between the two classes determines, at least in part, the susceptibility to apoptosis (14). The pro-apoptotic function of some of these proteins is inactivated by phosphorylation, while that of other members is activated by cleavage or mitochondrial translocation (8). Another class of cell death inhibitors, the inhibitor of apoptosis proteins (IAPs), can bind and inhibit activated caspases-3 and -7, and one member, XIAP, can also associate with Cyt c and inhibit caspase-9. Smac/Diablo, which is also released from mitochondria upon apoptotic stress, can neutralize the function of the IAPs (Figure 1). Smac/Diablo may also potentiate the



caspase cascade by displacing XIAP from mature caspase-9 (4), underscoring the strict cross-regulation of the apoptotic pathways by various 'check and balance' mechanisms. However, an alternative mechanism for apoptosis inhibition by XIAP was also described, and it depends on the selective activation of TAK1-dependent JNK1 (15).

### 4.2. Survival pathways

The ERK survival pathway (Figure 4) overrides the effects of apoptotic signals, apparently by upregulating anti-apoptotic Bcl-2 proteins through transcription-dependent and independent mechanisms. One such transcription factor is CREB. It binds to the cAMP response element (CRE) and activates gene transcription in response to a wide variety of extracellular signals, growth factors, hormones and neurotransmitters. Transcriptional activation of CREB is controlled through phosphorylation at Ser<sup>133</sup> by the ERK1/2 mediated activation of the pp90 ribosome S6 kinase (Rsk). Other transcription factors that are targets of the ERK pathway include c-Fos, which is also regulated by Rsk and was implicated in cell growth, differentiation and development, and Elk-1, which induces gene transcription in response to serum and growth factors (16). Transcription-independent survival mechanisms mediated by activated ERK involve Rsk-2 (one of the three Rsk isoforms), which phosphorylates (and thereby inactivates) the pro-apoptotic protein Bad. In addition to mediating neuronal survival, activated ERK also increases axonal growth, enhances axonal regeneration after axotomy (17), and plays an important role in synaptic plasticity, learning and memory (18). Cell surface receptors can also activate PI3-K, which in turn, activates Akt (19). ERK/Rsk-2 and PI3-K/Akt converge at the level of Bad inactivation to promote cell survival. Ras activation by the NGF receptor TrkA is independent of PI3-K, but PI3-K is required for the activation of Rap-1 (Figure 4), and both pathways converge on MEK (20).

### 4.3. Heat shock proteins

Heat shock proteins (Hsp) are rapidly emerging as a major and versatile apoptosis regulatory family. Most Hsp have anti-apoptotic activity and are overexpressed in tumor tissues, where they contribute to tumor progression and resistance to chemotherapy. Hsp70 proteins prevent Cyt c release from mitochondria, inhibit the JNK and p38MAPK signaling cascades and function downstream of caspase-3. They also bind and sequester activated caspases, Apaf-1 and AIF. Hsp90 is required for the maintenance of the c-Raf-1 function, and Hsp27 sequesters Cyt c (21). However, Hsp27 had only a minimal level of neuroprotection, with loss of CA3 hippocampal neurons reduced from 38% in wild type animals to 17% in Hsp27 transgenics (22). This may reflect the relative importance of other Hsp families in neuroprotection, or the contribution of necrotic cell death in ischemic injury.

An interesting question is whether Hsp modulate apoptosis strictly based on their chaperone activity. Hsp70, for example, complexes with nascent or damaged proteins and chaperones them for refolding and function resumption, or for degradation by the proteasome complex. Its co-chaperone Bag-1 plays a crucial role in the decision whether

client proteins will be refolded for resumed function or targeted to the proteasome for degradation (23). In this context, it is significant to point out that Bag-1 is expressed as distinct isoforms that arise from a common transcript through alternative in-frame translational start sites. One of these, Bag-1M inhibits the refolding reaction of Hsp70, while the other, Bag-1S displays stimulating activity (24). Both isoforms could have anti-apoptotic activity, depending on the function of the Hsp70 client protein. For example, refolding and resumed function of a pro-apoptotic client will favor apoptosis while its proteasomal degradation will favor cell survival. Bag-1 also functions independently of Hsp70, in that it activates the Raf-1 kinase and stabilizes Bcl-2 (25). The ability of Hsp to regulate apoptosis does not always depend on chaperone activity. Hsp70 inhibits JNK and AIF independent of ATPase (chaperone) activity (26) and H11, the first Hsp reported to have pro-apoptotic activity, activates the caspase and p38MAPK pathways, likely involving protein-protein interaction. After single site mutation at residue 51 (H11-W51C), H11 acquires anti-apoptotic activity involving activation of the MEK/ERK pathway (27).

## 5. CROSS-TALK AND THE INTEGRATION OF DISTINCT SIGNALS

How do pathways common to many systems integrate signals from a wide spectrum of activities? In other words, how does a set of similar components control completely different biological responses? The answer appears to lie in the distinct assembly of the pathway components in different cell types. Distinct assembly is likely to alter the duration and intensity of signaling, for example through ERK, thereby dictating its subcellular compartmentalization and/or trafficking. In turn, this dictates whether ERK-expressing cells enter apoptosis, survival or differentiation. In humans, the epidermal growth factor receptor (EGFR) and the fibroblast growth factor receptor are receptor tyrosine kinases that control cell proliferation and death and are implicated in common diseases. They both signal through the ERK pathway, but the components are differently wired together apparently changing the amplitude, duration, and localization of the signals. The dual specificity phosphatases, MKPs, might be the link between the kinetics of ERK activation and its subcellular localization (28). Thus, ERK stimulation by NGF is both rapid and sustained. Sustained activation depends on signaling through the Rap1/B-Raf module (Figure 4) while the Ras/c-Raf-1 module accounts for the immediate response (29). Translational control by ERK signaling was also implicated in long-term synaptic plasticity and memory (30). However, chronic ERK activation was implicated in neurodegeneration (28) and ERK was associated with caspase-independent neuronal injury after ER stress (31).

Enzymatic activation may not be sufficient for the successful propagation of a signal. Recent studies described scaffold-like molecules that tether components of a specific cascade into oligomeric protein complexes and increase their local concentration or exclude illegitimate cross-interaction. One such molecule is the scaffold

protein Raf kinase inhibitor protein (RKIP), which inhibits the ERK pathway (32). MEK partner 1 (MP1) is another scaffold protein, and it enhances the activation of ERK-1, but not ERK-2 (33), suggesting that it helps to discriminate between the two ERK isoforms that may be functionally distinct. Indeed, distinct functions were described for the JNK isotypes. Recent studies in murine disease models indicated that JNK1/2, which are widely expressed, have different functions. JNK1 regulates insulin resistance and obesity, while JNK2 is required for collagen-induced arthritis and arthrosclerotic plaque formation (34). The overall conclusion is that under various cellular milieus, ERK, for example, may activate different transcription factors or promote cell survival or growth arrest by a transcription-independent mechanism. Negative regulatory signals, such as those provided by protein phosphatases, receptor endocytosis, and proteosomal degradation, provide an additional layer of control that is promoted by cross talk between distinct pathways and is subject to feedback regulation. Mitogenic and pro-apoptotic signaling cascades are often linked, in order to prevent unwanted cell proliferation. In the CNS, the neuron type (cortical, striatal or hippocampal) and its state of differentiation may also contribute to the outcome of ERK activation. Apoptosis, differentiation, survival and proliferation result from the balance of various signaling modules and their targets. In turn, these depend on the combination of neurotrophins and receptors as well as other first messengers available in a specific cellular milieu.

The recent recognition that Hsp are involved in immune responses underscores the cross-talk between various signaling cascades and immune pathways, and is particularly relevant to disease pathogenesis. As part of the putative presentosome, an organized region in which proteins are degraded and peptides are loaded onto the major histocompatibility complex (MHC) class I for presentation to T cells, Hsp bind to antigenic peptides (such as viral antigens) and facilitate their presentation to T cells (35). Antigen presenting cells (APC) can also pick up extracellular Hsp-peptide complexes through the CD91 receptor, resulting in peptide presentation on MHC class I and class II molecules, a process known as cross-presentation (35). Hsp can also stimulate innate immunity. Hsp70 induces APC to release inflammatory cytokines such as TNF- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-6 through its interaction with the CD14/toll-like receptor (TLR) complex and promotes dendritic cell maturation. Interestingly, Hsp70 ATPase activity is not required for induction of inflammatory cytokines (36). By virtue of their ability to influence cell life and death decisions both intracellularly and in the context of the immune response, Hsp are a logical target for virus modulation.

This is particularly relevant within the context of CNS infection, because it is becoming increasingly evident that the outcome of virus infection in the brain is complicated by the contribution of glial cells (astrocytes, oligodendrocytes and microglia). IL-6, TGF- $\beta$  and TNF- $\alpha$  produced by microglial cells, are involved in excitotoxic neuronal damage (37), and it is still not entirely clear whether microglia protect or harm neurons, or

whether TNF- $\alpha$  is beneficial or toxic (38,39). Hsp70 also sensitizes cells to certain apoptotic stimuli like TNF- $\alpha$  (40). The cross talk between signaling and immune cascades modulated by virus infection is further underscored by recent findings that Hsp60 activates innate immunity involving the ubiquitinated TRAF6 protein, a signal transducer that activates JNK in response to stress and inflammatory signals (41,42). The obvious challenge for the future is to decipher how signals vary in the context of site and time, and how the resulting minor differences in connectivity produce robust changes that affect disease pathogenesis. Viruses can target all these components differentially, determined by the site of infection, the route of virus spread in the infected organism, and the modulation of the immune response. Elucidating these interactions is essential in order to manipulate them towards an ultimate beneficial (therapeutic) goal.

## 6. VIRUSES REGULATE SIGNALING AND APOPTOTIC CASCADES

Viruses use various strategies to hijack the cells forcing them to become virus-producing factories. A basic mechanism used by the infected cell to escape virus control is to undergo apoptosis, thereby limiting virus replication and the infection of bystander neighboring cells. To counteract apoptosis, viruses have developed various strategies that modulate regulatory gene families. They include upregulation of various family members, increasing their activity, or encoding homologues of regulatory proteins. Indeed, the balance between cell survival and apoptosis often determines the relative success of virus replication. Modulation of the appropriate signaling cascades favors virus replication and survival and plays a pivotal role in the pathogenesis of virus infections. It, therefore, defines novel targets for therapeutic interventions. CNS pathogenicity probably relates to more than one mechanism and may include cytopathic effects, induction of cytokines, effects on immune function (immune suppressive), and effects on other viruses (e.g. transactivation). However, a common thread in virus-induced pathogenesis of the CNS is neuronal cell apoptosis. Based on present knowledge this may be due to virus replication or as a consequence of an immune reaction triggered by antigen expression.

The articles in this miniseries focus on some of the strategies viruses use to commandeer processes that define cell life and death decisions. They emphasize neurons and the impact of virus-induced modulation of signaling and apoptotic cascades on the pathogenesis of virus infection of the CNS. In her article "*Virus Signaling and Apoptosis in Central Nervous System Infection*", Perkins reviews the strategies used by human pathogens to cause acute disease (encephalitis) or those implicated in neurodegenerative diseases. One strategy that is of increased recent interest is immune evasion, which often results from a balance shift that favors apoptosis through activation of death receptor pathways, such as TNF (Figure 1). Viruses achieve this goal by various mechanisms. One mechanism is to encode homologues of the cellular anti-apoptotic proteins that interfere with the death receptor

apoptotic cascade. Poxviruses encode several genes that are homologues of the TNF receptor and compete with the cellular receptor for TNF (43). Human herpes virus type 8 (HHV-8) encodes a FLIP homologue, which interacts with FADD, and procaspase-8, blocking the latter's activation and subsequent apoptosis (44). Another approach is to encode homologues of signaling proteins that are unrelated to the immune system and/or proteins that regulate apoptosis. For example, baculovirus encodes p35, a homologue of the caspase inhibitor IAP (45) and adenovirus and HHV-8 encode Bcl-2 homologues (46). Viruses also use various strategies to activate the ERK survival pathway, which overrides apoptosis. Vaccinia virus encodes a protein that mimics epidermal growth factor (EGF) in terms of its ability to stimulate cognate receptors (47). ERK is incorporated into HIV virions and is required for the translocation of the reverse transcriptase complex to the nucleus (48). SV40 small T antigen binds protein phosphatase 2A, thereby preventing it from dephosphorylating MEK and MAPK2 and prolonging their activated state (49). A Herpesvirus saimiri protein (STP-C488) associates with Ras and activates it (50) and a coxsackievirus protein (Sam68) binds Ras-GAP and inactivates it, thereby activating Ras (51).

Another strategy used by viruses to regulate apoptosis is to alter the expression and activation of cellular apoptosis modulator proteins. The hepatitis B virus (HBV) protein HBVx and the EBV protein LMP1 upregulate EGFR expression by promoter activation (52), the bovine papilloma virus (BPV) E5 protein binds the EGFR cytoplasmic domain enhancing its kinase activity (53) and the human papilloma virus type 16 (HPV-16) E5 protein binds EGFR and inhibits its downregulation (54). Human cytomegalovirus activates ERK by inhibiting a phosphatase responsible for its dephosphorylation (55) and the HSV-2 protein ICP10PK activates Ras by binding the guanine exchange factor Sos and by inhibiting the activity of the Ras downregulatory protein Ras-GAP through phosphorylation (56).

The second article in this miniseries, entitled "*Herpes Simplex Virus Type 2 Encodes a Heat Shock Protein Homologue with Apoptosis Regulatory Functions*" focuses on the Hsp as targets of virus mediated apoptosis regulation. In this article, Gober et al., review present understanding of the role of Hsp in apoptosis, the mechanism of Hsp-mediated control of cell life and death decisions and the ability of Hsp to cross talk to other signaling cascades as well as the immune system. The authors also introduce an HSV-2 protein (ICP10PK), which is an Hsp homologue and regulates apoptosis. They suggest that ICP10PK may have been co-opted from its cellular homologue in order to improve the efficiency of virus replication. This is particularly relevant within the context of latency, defined as the ability of DNA to persist in sensory neurons indefinitely in a largely untranscribed state with periodic episodes of virus reactivation. The mechanism of latency establishment and reactivation is of major interest in the field, but despite extensive investigation, it is still unresolved. Presumably, ICP10PK responds to stress induced AP-1 expression and enables

resumed transcription of the viral DNA by virtue of its ability to activate the Ras/MEK/ERK pathway. This also results in the inhibition of virus-mediated apoptosis increasing the number of live neurons that can support virus replication. Indeed, Bag-1S, the anti-apoptotic isoform, is also upregulated by the ICP10PK-activated MEK/ERK pathway and it accounts for over 90% of the ICP10PK anti-apoptotic activity. It is likely that Bag-1 functions to stabilize Bcl-2 and provide a feedback amplification loop for Raf-1 kinase (57). However, Gober et al., show that ICP10PK also upregulates Hsp70 and Hsp27 that may contribute to apoptosis inhibition by chaperoning damaged proteins to the proteasome.

In the last article in this miniseries entitled "*The Herpes Simplex Virus Type 2 Protein ICP10PK: a Master of Versatility*", Smith uses ICP10PK as a model to exemplify the cell specificity of the viral modulatory effects. Thus, ICP10PK activates the Ras/MEK/ERK signaling pathway in all the cells studied so far. However, the outcome of this stimulation is cell type specific and depends on the mitotic or post-mitotic state of the cell. At the molecular level, the distinct biological outcome probably reflects the activation of a range of transcription factors, which is determined by the cell type and its position along the cell cycle. This results in increased expression of different target genes. Thus, activation of the Ras/Raf-1/MEK/ERK pathway in immortalized cells leads to upregulation of the AP1 transcription factor c-Fos, which is responsible for increased expression of positive cell cycle regulators and downregulation of tumor suppressor genes that inhibit cell cycle progression. However, in post mitotic neurons, the activated pathway increases the expression of anti-apoptotic transcription factors, such as CREB. It will be interesting to see whether ICP10 PK induces distinct survival pathways in response to apoptotic stimuli other than virus infection, and to better elucidate the contribution of specific cellular genes that are upregulated /activated in neurons as compared to mitotic cells in which ICP10PK induces proliferation.

## 7. CONCLUDING REMARKS AND PERSPECTIVE

Factors involved in the CNS pathogenesis of virus infection include lytic effects due to virus replication and/or inflammatory responses that result, at least in part, from cytokine production by microglial cells, and neuronal cell apoptosis. Apoptosis is a determining aspect of virus-induced CNS pathogenesis. Apoptotic cascades are affected by signaling pathways, and none of these function in isolation. The ranges of connections, which allow integration between the various signaling and apoptotic pathways, and their modulation by virus infection, are emerging as a topic of major relevance to disease pathogenesis and the identification of therapeutic targets. The ERK, PI3-K/Akt and cAMP/PKA pathways are ubiquitous and pivotal to cellular growth, survival and function. The JNK and p38MAPK stress-induced pathways and the caspase cascades are generally associated with cell death. However, ERK-associated cell death and JNK-mediated cell survival have also been reported, and viruses can hijack any one of these pathways, altering this



sensitive balance. For example, HSV-1 triggers apoptosis in CNS neurons, involving JNK/c-Jun and caspase activation and culminating in the induction of the pro-apoptotic protein Bad. In humans, HSV-1 causes a non-epidemic, sporadic, acute focal encephalitis (HSE) that accounts for 10-20% of viral encephalitis cases among adults and older children in the US and has a 50-60 % mortality rate in the presence of antiviral therapy. JNK-dependent apoptosis appears to be a significant component of HSE pathogenesis. By contrast, HSV-2 does not trigger apoptosis in CNS neurons nor cause encephalitis. This is likely due to the anti-apoptotic activity of the ICP10PK protein, which is poorly conserved in HSV-1 (12). HSV-1 has also been associated with Alzheimer's disease, a chronic neurodegenerative disorder characterized by intracellular accumulation of neurofibrillary tangles, abnormal deposition of the beta-amyloid (A $\beta$ ) protein into focal plaques and extensive neuronal death also related to apoptosis. The hypothesis is that people who harbor latent HSV-1 in the brain suffer from mild encephalitis (and apoptosis) related to periodic virus reactivation, and this problem is augmented by the age-related decline in the immune system. Individuals homozygous or heterozygous for the type epsilon4 allele of the apolipoprotein E gene (ApoE4) may have a higher frequency of virus reactivation (58).

It is becoming increasingly evident that the outcome of virus infection in the brain is complicated by the contribution of glial cells (astrocytes, oligodendrocytes and microglia). IL-6, TGF- $\beta$  and TNF- $\alpha$  produced by microglial cells are involved in excitotoxic neuronal damage (36). However, microglia can also produce neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), NGF and GDNF that are neuroprotective (59). It is still not entirely clear whether microglia protect or harm neurons or whether TNF- $\alpha$  is beneficial or toxic. Some studies suggest that TNF- $\alpha$  enhances injury (37), while others indicate that it induces anti-apoptotic factors and is protective (38). Still other studies suggest that TNF- $\alpha$  has a deleterious effect during the acute response that occurs in the traumatized brain, but it plays a key role in the long-term behavioral recovery and tissue repair (60). Although TNF- $\alpha$  may not be sufficient to trigger apoptosis on its own, TNF- $\alpha$  triggered apoptosis was initiated or made worse when Hsp70 expression increased to high levels that disrupt NF- $\kappa$ B signaling (39). This is particularly relevant since HSV-1/2 upregulate Hsp70 that has both anti-apoptotic activity and stimulates the apoptotic activity of other stimuli, such as TNF- $\alpha$ . In HSV-2 infected cells, ICP10PK upregulates Hsp70 together with its anti-apoptotic co-chaperone Bag-1, suggesting that under these conditions, Hsp70 contributes to anti-apoptotic activity. By contrast, in HSV-1 infected cells, in which Bag-1 is not induced and the activated JNK pathway is pro-apoptotic, Hsp70 may contribute to apoptosis development.

The cross talk between signaling pathways and immune responses that are modulated by virus infection is underscored by recent findings that Hsp60 can activate innate immunity involving the ubiquitinated TRAF6 protein,

a signal transducer that activates JNK in response to stress and inflammatory signals (40,41). Hsp60 triggers production of TNF- $\alpha$ , IL-6 or IL-12 and activates macrophages and dendritic cells *via* the Toll-like/IL-1 receptor. TRAF6 is involved and ultimately leads to the activation of NF- $\kappa$ B, the stress-activated kinases JNK and p38MAPK and the mitogen-activated kinases ERK1/2 (61). Hsp70 also stimulates the Toll-like/IL-1 receptor signal pathway (62).

Astrocytes reduce neuronal cell death following a variety of cellular stresses, such as excitotoxicity and oxidative stress. They protect neurons from apoptosis caused by serum deprivation, at least in part, by releasing TGF- $\beta$ , which activates the JNK/c-Jun pathway *via* the TGF- $\beta$  type II receptors. In this system, pathway activation leads to increased transcription of neuroprotective genes (63), presumably because the pro-apoptotic function of JNK/c-Jun is counteracted by neurotrophic factors in the CNS milieu. This underscores the importance of the cell type towards functional definition of pathway activation.

Understanding the cross-talk processes that interlink signaling systems in defined cell types is crucial for the development of appropriate therapies. The therapeutic approach dictated by the complex multifactorial nature of neurodegenerative disorders includes the targeting of both the apoptotic cell death and the decline in cognitive processes. Gene therapy of neurological disorders that involve apoptosis, using HSV vectors for delivery of anti-apoptotic genes, has attracted much recent attention. Interest in the use of HSV vectors was stimulated by the ability of HSV to infect post-mitotic neurons and its large foreign gene insertion capacity (up to 50 kb). However, the development of effective HSV-1 vectors for the CNS is hampered by their cytotoxicity and apoptotic activity. Therefore, recent of attention has shifted towards maintaining neuronal function in addition to promoting survival (64,65). HSV-2-based vectors may have an additional advantage when compared to HSV-1 vectors, in that the ICP10 PK confers broad anti-apoptotic activity in neurons. This includes inhibition of apoptosis triggered by virus infection, neurotrophic factor deprivation (a known etiologic factor of neurodegenerative disorders) genetic defects (including ALS) and excitotoxicity (models of stroke and epilepsy). In addition, ICP10 PK activates the ERK/CREB LTP pathway, providing an improvement in cognitive functions, a benefit sought after, but never achieved by the current therapeutic strategies of neurodegenerative disorders.

## 8. ACKNOWLEDGEMENT

The studies in my laboratory were supported by NINDS, National Institutes of Health (NIH) public health service grant NS45169

## 9. REFERENCES

1. Friedlander RM Apoptosis and caspases in neurodegenerative diseases. *N Engl J Med.* 348, 1365-1375 (2003)

2. Aurelian L. HSV induced apoptosis in herpes encephalitis. In . Role of apoptosis in infection. *Current Topics in Microbiology and immunology* 289, Griffin, D. Ed., (2005)
3. Chang DW, Ditsworth D, Liu H, Srinivasula SM, Alnemri ES, and Yang X. Oligomerization is a general mechanism for the activation of apoptosis initiator and inflammatory procaspases. *J Biol Chem* 278, 16466-16469 (2003)
4. Hill MM, Adrain C, and Martin SJ. Portrait of a killer: the mitochondrial apoptosome emerges from the shadows. *Mol Interv.* 3, 19-26 (2003)
5. Arundine M, and Tymianski M. Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. *Cell Mol Life Sci.* 61, 657-668 (2004)
6. Waetzig V, and Herdegen T. A single c-Jun N-terminal kinase isoform (JNK3-p54) is an effector in both neuronal differentiation and cell death. *J Biol Chem.* 278, 567-572 (2003)
7. Lambeng N, Willaime-Morawek S, Mariani J, Ruberg M and Brugg B. Activation of mitogen-activated protein kinase pathways during the death of PC12 cells is dependent on the state of differentiation. *Mol Brain Res.* 111, 52-60 (2003)
8. Yang X, Kovalenko D, Nadeau RJ, Harkins LK, Mitchell J, Zubanova O, Chen PY, and Friesel R. Sef interacts with TAK1 and mediates JNK activation and apoptosis. *J Biol Chem.* 279, 38099-38102 (2004)
9. Lowes VL, Ip NY, and Wong YH. Integration of signals from receptor tyrosine kinases and G protein-coupled receptors. *Neurosignals* 11, 5-19 (2002)
10. Chao MV Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci.* 4, 299-309 (2003)
11. Sheng M and Kim MJ (2002) Postsynaptic signaling and plasticity mechanisms. *Science* 298,776-780 (2002)
12. Perkins D, Gyure KA, Pereira EFR and Aurelian L. Herpes simplex virus type 1 induced encephalitis has an apoptotic component associated with activation of c-Jun N-terminal kinase. *J. Neurovirol.* 9, 101-111 (2003)
13. Pasinelli P, Houseweart MK, Brown RH Jr, and Cleveland DW. Caspase-1 and -3 are sequentially activated in motor neuron death in Cu,Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A.* 97, 13901-13906 (2000).
14. Chittenden T. Mammalian Bcl-2 family genes. In Wilson J.W., Booth C., Potten C.S., eds., Apoptosis genes, Kluwer Acad. Publishers pp. 37-83 (1998)
15. Sanna MG, da Silva Correia J, Ducrey O, Lee J, Nomoto K, Schrantz N, Deveraux QL, Ulevitch RJ. IAP suppression of apoptosis involves distinct mechanisms: the TAK1/JNK1 signaling cascade and caspase inhibition. *Mol Cell Biol.* 22, 1754-1766 (2002)
16. Nebreda AR and Gavin A-C. Cell Survival Demands Some Rsk. *Science* 286,1309-1310 (1999)
17. Atwal JK, Massie B, Miller FD, and Kaplan DR. The TrkB-Shc site signals neuronal survival and local axon growth via MEK and P13-kinase. *Neuron.* 27, 265-277 (2000)
18. Adams JP, and Sweatt JD. Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Annu Rev Pharmacol Toxicol.* 42, 135-163 (2002)
19. Kandel ES, and Hay N. The regulation and activities of the multifunctional serine/ threonine kinase Akt/PKB. *Exp. Cell Res.* 253, 210-229 (1999)
20. York RD, Molliver DC, Grewal SS, Stenberg PE, McCleskey EW, and Stork PJ (2000) Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via Ras and Rap1. *Mol Cell Biol.* 20, 8069-8083 (2000)
21. Beere, H.M. Stressed to death: regulation of apoptotic signaling pathways by the heat shock proteins. *Sci. STKE.* 93, 1-6 (2001)
22. Latchman DS. Protective effect of heat shock proteins in the nervous system. *Current Neurovascular Res* 1, 21-27 (2004)
23. Luders J, Demand J, and Hohfeld J. The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. *J Biol Chem* 275, 4613-4617 (2000)
24. Luders J, Demand J, Papp O, Hohfeld J. Distinct isoforms of the cofactor BAG-1 differentially affect Hsc70 chaperone function. *J Biol Chem.* 275,14817-14823 (2000)
25. Wang HG, Takayama S, Rapp UR, Reed JC. Bcl-2 interacting protein, Bag-1, binds to and activates the kinase Raf-1. *Proc Natl Acad Sci U S A.* 93, 7063-7068 (1996)
26. Ravagnan L, Gurbuxani S, Susin SA, Maise C, Daugas E, Zamzami N, Mak T, Jaattela M, Penninger JM, Garrido C, and Kroemer G. Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nat Cell Biol* 3, 839-843 (2001)
27. Gober MD, Smith CC, Ueda K, Toretzky JA, and Aurelian L. Forced expression of the H11 heat shock protein can be regulated by DNA methylation and trigger apoptosis in human cells. *J Biol Chem* 278, 37600-37609 (2003)

28. Colucci-D'Amato L, Perrone-Capano C, and di Porzio U. Chronic activation of ERK and neurodegenerative diseases. *Bioessays* 25, 1085-1095 (2003).
29. Vaudry D, Stork PJ, Lazarovici P, and Eiden LE. Signaling pathways for PC12 cell differentiation: making the right connections. *Science* 296, 1648-1649 (2002)
30. Kelleher RJ 3rd, Govindarajan A, Jung HY, Kang H, and Tonegawa S. Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* 116, 467-479 (2004)
31. Arai K, Lee SR, van Leyen K, Kurose H, and Lo EH. Involvement of ERK MAP kinase in endoplasmic reticulum stress in SH-SY5Y human neuroblastoma cells. *J Neurochem* 89, 232-239 (2004)
32. Chong H, Vikis HG, and Guan KL. Mechanisms of regulating the Raf kinase family. *Cell Signal* 5, 463-469 (2003)
33. Schaeffer HJ, Catling AD, Eblen ST, Collier LS, Krauss A, and Weber MJ. MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. *Science* 281, 1668-1671 (1998)
34. Ricci R, Sumara G, Sumara I, Rozenberg I, Kurrer M, Akhmedov A, Hersberger M, Eriksson U, Eberli FR, Becher B, Boren J, Chen M, Cybulsky MI, Moore KJ, Freeman MW, Wagner EF, Matter CM, Luscher TF. Requirement of JNK2 for scavenger receptor A-mediated foam cell formation in atherosclerosis. *Science* 306, 1558-1561 (2004)
35. Srivastava P. Roles of heat-shock proteins in innate and adaptive immunity. *Nat Rev Immunol* 2, 185-194 (2002)
36. Wang Y, Kelly CG, Singh M, McGowan EG, Carrara AS, Bergmeier LA, and Lehner T. Stimulation of Th1-polarizing cytokines, C-C chemokines, maturation of dendritic cells, and adjuvant function by the peptide binding fragment of heat shock protein 70. *J Immunol* 169, 2422-2429 (2002)
37. Acarin L, Gonzalez B, and Castellano B. Neuronal, astroglial and microglial cytokine expression after an excitotoxic lesion in the immature rat brain. *Eur J Neurosci* 12, 3505-3520 (2000)
38. Barone FC, Arvin B, White RF, Miller A, Webb CL, Willette RN, Lysko PG, and Feuerstein GZ. Tumor necrosis factor- $\alpha$ . A mediator of focal ischemic brain injury. *Stroke* 28, 1233-1244 (1997)
39. Suzuki T, Hide I, Ido K, Kohsaka S, Inoue K, and Nakata Y. Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia. *J Neurosci* 24, 1-7 (2004)
40. Ran R, Lu A, Zhang L, Tang Y, Zhu H, Xu H, Feng Y, Han C, Zhou G, Rigby AC, and Sharp FR. Hsp70 promotes TNF-mediated apoptosis by binding IKK gamma and impairing NF-kappa B survival signaling. *Genes Dev* 18, 01466-1481 (2004)
41. Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, and Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 412, 346-351 (2001)
42. Wang W, Zhou G, Hu MC, Yao Z, and Tan TH. Activation of the hematopoietic progenitor kinase-1 (HPK1)-dependent, stress-activated c-Jun N-terminal kinase (JNK) pathway by transforming growth factor beta (TGF-beta)-activated kinase (TAK1), a kinase mediator of TGF beta signal transduction. *J Biol Chem* 272, 22771-22775 (1997)
43. Everett H and McFadden G. Poxviruses and apoptosis: a time to die. *Curr Opin Microbiol* 5, 395-402 (2002)
44. Djerbi M, Screpanti V, Catrina AI, Bogen B, Biberfeld P, and Grandien A. The inhibitor of death receptor signaling, FLICE-inhibitory protein defines a new class of tumor progression factors. *J Exp Med* 190, 1025-1032 (1999)
45. Seshagiri S and Miller LK. Baculovirus inhibitors of apoptosis (IAPs) block activation of Sf-caspase-1. *Proc Natl Acad Sci U S A* 94, 13606-13611 (1997)
46. Cheng EH, Nicholas J, Bellows DS, Hayward GS, Guo HG, Reitz MS and Hardwick JM. A Bcl-2 homolog encoded by Kaposi sarcoma-associated virus, human herpesvirus 8, inhibits apoptosis but does not heterodimerize with Bax or Bak. *Proc Natl Acad Sci U S A* 94, 690-694 (1997)
47. King CS, Cooper JA, Moss B, and Twardzik DR. Vaccinia virus growth factor stimulates tyrosine protein kinase activity of A431 cell epidermal growth factor receptors. *Mol Cell Biol* 6, 332-336 (1986)
48. Jacque JM, Mann A, Enslen H, Sharuva N, Brichacek B, Davis RJ, and Stevenson M. Modulation of HIV-1 infectivity by MAPK, a virion associated kinase. *EMBO J* 17, 2607-2618 (1998)
49. Sontag E, Fedorov S, Kamibayashi C, Robbins D, Cobb M, and Mumby M. The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates MAP kinase pathway and induces cell proliferation. *Cell* 75, 887-897 (1993)
50. Jung JU and Desrosiers RC. Association of viral oncoprotein, STP-C488 with cellular ras. *Mol Cell Biol* 15, 6506-6512 (1995)
51. Huber M, Watson KA, Selinka HC, Carthy CM, Klingel K, McManus BM, and Kandolf R. Cleavage of RasGAP and phosphorylation of mitogen-activated protein kinase in the course of coxsackievirus B3 replication. *J Virol* 73, 3587-3594 (1999)
52. Menzo S, Clementi M, Alfani E, Bagnarelli P, Iacovacci S, Manzin A, Dandri M, Natoli G, Levrero M

and Carloni G. Trans-activation of epidermal growth factor receptor gene by the hepatitis B virus X-gene product. *Virology* 196, 878-882 (1993)

53. Cohen BD, Goldstein DJ, Rutledge L, Vass WC, Lowy DR, Schlegel R, and Schiller JT. Transformation-specific interaction of the bovine papillomavirus E5 oncoprotein with the platelet-derived growth factor receptor transmembrane domain and the epidermal growth factor receptor cytoplasmic domain. *J Virol* 67, 5303-5311 (1993)

54. Miller WE and Raab-Traub N. The EGFR as a target for viral oncoproteins. *Trends Microbiol* 7, 453-458 (1999)

55. Rodems SM and Spector DH. Extracellular signal-regulated kinase activity is sustained early during human cytomegalovirus infection. *J Virol* 72, 9173-9180 (1998)

56. Smith CC, Peng T, Kulka M, and Aurelian L. The PK domain of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10) is required for immediate-early gene expression and virus growth. *J Virol* 72, 9131-9141 (1998)

57. Perkins D, Pereira EF and Aurelian L. The herpes simplex virus type 2 R1 protein kinase (ICP10 PK) functions as a dominant regulator of apoptosis in hippocampal neurons involving activation of the ERK survival pathway and upregulation of the antiapoptotic protein Bag-1. *J Virol* 77, 1292-1305 (2003)

58. Dobson CB, Wozniak MA, and Itzhaki RF. Do infectious agents play a role in dementia? *Trends in Microbiol.* 11, 312-317 (2003)

59. Suzuki H, Imai F, Kanno T, and Sawada M. Preservation of neurotrophin expression in microglia that migrate into the gerbil's brain across the blood-brain barrier. *Neurosci Lett.* 312, 95-98 (2001)

60. Scherbel U, Raghupathi R, Nakamura M, Saatman KE, Trojanowski JQ, Neugebauer E, Marino MW, and McIntosh TK. Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. *Proc Natl Acad Sci U S A.* 96, 8721-8726 (1999)

61. Vabulas RM, Ahmad-Nejad P, da Costa C, Miethke T, Kirschning CJ, Hacker H, and Wagner H. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J Biol Chem* 276, 31332-31339 (2001)

62. Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, and Wagner H. HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. *J Biol Chem.* 277, 15107-15112 (2002)

63. Dhandapani KM, and Brann DW. Transforming growth factor-beta: a neuroprotective factor in cerebral ischemia. *Cell Biochem Biophys.* 39, 13-22 (2003).

64. Dumas T., McLaughlin J., Ho D., Meier T., and Sapolsky R. Delivery of herpes simplex virus amplicon-based vectors to the dentate gyrus does not alter hippocampal synaptic transmission in vivo. *Gene Ther.* 6, 1679-1684 (1999)

65. Mc Laughlin J, Roozendaal B, Dumas T, Gupta A, Ajilore O, Hsieh J, Ho D, Lawrence M, McGaugh JL, and Sapolsky R. Sparing of neuronal function post-seizure with gene therapy. *Proc. Natl. Acad. Sci. USA* 97, 12804-12809 (2000)

**Key Words:** Signaling Pathways, Signal Integration, Apoptosis Cascades, ERK, JNK, and p38MAPK, Review

**Send correspondence to:** Laure Aurelian, Ph.D, University of Maryland School of Medicine, Department of Pharmacology and Experimental Therapeutics, Virology/Immunology Laboratories, 655 W. Baltimore Str., Room 4-023, Baltimore, MD 21201, Tel 410-706-3895, Fax 410-706-2513, E-mail: laurelia@umaryland.edu

<http://www.bioscience.org/current/vol10.htm>