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The JNK signal transduction pathway

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The c-Jun NH_2-terminal kinases (JNKs) are an evolutionarily conserved sub-group of mitogen-activated protein (MAP) kinases. Recent studies have improved our understanding of the physiological function of the JNK pathway. Roles of novel molecules that participate in the JNK pathway have been defined and new insight into the role of JNK in survival signaling, cell death, cancer and diabetes has been achieved.

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Introduction

The response of a cell to changes in its environment is induced, in part, by a diverse array of intracellular signaling pathways. These pathways serve to relay, amplify and integrate signals from extracellular stimuli, ultimately resulting in a genomic and physiological response. In mammalian systems, these responses include cellular proliferation, differentiation, development, the inflammatory response and apoptosis. Mitogen-activated protein (MAP) kinases are one such family of signaling proteins.

The c-Jun NH_2-terminal kinase (JNK) pathway represents one sub-group of MAP kinases that is activated primarily by cytokines and exposure to environmental stress [1,2]. Specific stimuli trigger the activation of MAP3Ks, which then phosphorylate and activate the MAP2K isoforms MKK4 and MKK7, which in turn phosphorylate and activate JNK [1,2]. Components of the JNK pathway can be organized into signaling complexes, mediated by one of the protein kinases (e.g. a MAP3K or a MAP2K) or by a scaffold protein, for example a member of the JNK-interacting protein (JIP) family [3]. A major target of the JNK signaling pathway is the activator protein-1 (AP-1) transcription factor, which is activated, in part, by the phosphorylation of c-Jun and related molecules [1,2]. This review aims to highlight recent progress in JNK-related research; we refer the reader to earlier reviews [1–3] for references and discussion of previous work.

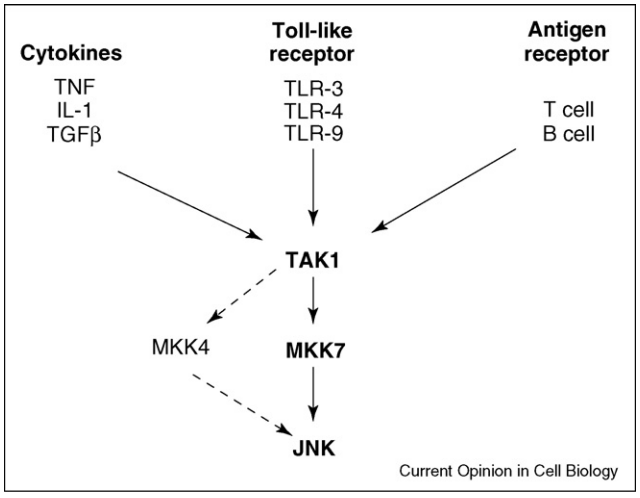
Novel components of the JNK signaling pathway

Recent research has provided new insights into regulatory components of the JNK signaling pathway. These fall broadly into three categories: upstream regulators (e.g. MAP3Ks); down-stream inhibitors (e.g. phosphatases); and scaffold proteins (e.g. JIPs).

The canonical JNK signaling cascade has been well characterized; however, the specific role of MAP3K in the response to various stimuli remains largely unresolved. Recent studies of the MAP3K isoform transforming growth factor-β activated kinase -1 (TAK1) demonstrate that it is critical for JNK activation in response to inflammatory cytokines (e.g. interleukin-1, lymphotoxin-β, tumor necrosis factor, and transforming growth factor-β), and also for activation of Toll-like receptors (TLR-3, TLR-4, and TLR-9), the B cell receptor and the T cell receptor [4••–6••]. This role of TAK1 is illustrated in Figure 1. The MAP3K MEKK3 appears to be critical for JNK activation by TLR-8 [7]. In addition, the MAP3K isoforms TPL2 and MLK3 have been reported to contribute to tumor necrosis factor-stimulated JNK activation in embryonic fibroblasts [8,9]. Further studies are required to define the role of additional members of the MAP3K group in the activation of JNK [1,2].

The role of protein phosphatases in the regulation of the JNK pathway is poorly understood. However, recent studies have led to new insight into the function of phosphatases in JNK signaling. Specifically, studies of knockout mice have shown that members of the MAPK phosphatase (MKP) family act as negative regulators of JNK signaling. Thus, *Mkp1*$^{-/-}$ mice [10•–12•] and *Mkp5*$^{-/-}$ mice [13•] exhibit increased JNK activity. Physiological inhibition of MKP activity by reactive oxygen species may cause prolonged JNK activation [14]. Indeed, MKP inhibition may be sufficient for JNK activation following some stimuli [15]. These studies suggest that MKP1 and MKP5 contribute to the resolution of immune responses by suppressing signal transduction [11•–13•]. The recent determination of the atomic structure of MKP5 provides the foundation for further mechanistic studies of JNK inactivation by phosphatases [16]. MKP isoforms may be directly targeted to JNK, but these interactions may also be mediated by scaffold proteins. For instance, the JNK phosphatase MKP7 has been found

Figure 1



The MAP3K isoform. TAK1 plays a central role in JNK activation mediated by inflammatory cytokines, Toll-like receptors and ligation of antigen receptors. The role of TAK1 in the JNK signaling pathway is illustrated schematically.

to form complexes [17,18] with the JNK scaffold proteins β-arrestin 2 and JIP1 [3].

Kinesins are molecular motor proteins that use the energy from ATP hydrolysis to move cargo along microtubule tracks. Several JNK scaffold proteins interact with motor proteins such as kinesin-I [3], and recent additions to this group include APLIP1 [19], JLP [20], UNC-16 [21] and JIP4 [22]. The JNK scaffold/kinesin interaction raises questions concerning the relationship between JNK signaling and vesicular transport, particularly in neurons, where JIP-mediated signals are transmitted between the cell body and the distantly located nerve terminals [23]. Indeed, recent research has shown that the JNK scaffold protein JIP3 (also known as Sunday Driver), together with JNK3, is present on vesicular structures in axons, and interacts with kinesin-1 and the dynactin complex. Nerve injury induces local activation of JNK and transport of JNK and JIP3, suggesting that a mobile JNK–JIP3 complex may generate a transport-dependent surveillance system to detect axonal damage [24•]. Similar retrograde transport of the JIP1 scaffold protein with JNK following exposure of cultured hippocampal neurons to anoxic stress has been described [23].

It is established that JIP1 acts as a scaffold protein for the JNK signaling pathway because *Jip1*$^{-/-}$ mice exhibit defects in JNK activation in response to anoxic stress *in vivo* and *in vitro* [23,25], glucose deprivation *in vitro* [26] or a high fat diet *in vivo* [27]. The mechanism that mediates the effects of JIP1 on JNK activation has not yet been fully established. Nevertheless, it is known that the protein kinase components of the JNK pathway dynamically associate with JIP1 [28–30]. It is also established that JIP-mediated JNK activation is regulated by the Notch [26] and Akt [31,32] signaling pathways. Recent studies have provided insight into the structure of JIP complexes, with reports describing the atomic structure of the JIP/JNK interaction [33] and the SH3/SH3 dimerization interface between JIP1 molecules [34]. These atomic structures provide some initial understanding of the biochemical basis of JIP protein function. Progress that has been achieved towards understanding the function of other JNK scaffold proteins has recently been reviewed [3].

JNK in cell death

A role for JNK in apoptosis is well established [1,2]; however, the mechanism by which this occurs is controversial and appears to be stimulus- and tissue-specific [35]. One explanation for some of the differences observed could be the temporal aspect of JNK activation, and two recent studies have addressed this issue [36]. First, Chang and colleagues have described how sustained, but not transient, JNK activation promotes TNF-α killing via the E3 ubiquitin ligase Itch-mediated degradation of the caspase-8 inhibitor $cFLIP_L$ [37•]. JNK1-dependent phosphorylation and activation of Itch is necessary for the ubiquitination of $cFLIP_L$ and its subsequent degradation by the proteosome. Second, Ventura and colleagues used a chemical genetic approach to generate a JNK mutant that can be selectively inhibited by the addition of a drug [38•]. These authors demonstrate that both the transient and sustained phases of JNK activation contribute to the induction of gene expression; however, early transient JNK activation promotes cell survival, whereas prolonged JNK activation can mediate apoptosis [38•]. These results significantly increase our understanding of how the temporal regulation of JNK activation is critical to the cellular response.

A new JNK substrate has been identified that is phosphorylated in apoptotic cells: H2AX, a histone H2A variant [39]. The site of phosphorylation on H2AX corresponds to a non-canonical site for MAPK phosphorylation [40]. H2A functions as a core component of the histone octamer that forms the nucleosome. In response to DNA damage, phosphorylated H2AX accumulates at the site of double strand breaks, where it is thought to restructure chromatin and assist in the recruitment of DNA repair and signaling factors. Previous studies have suggested that H2AX phosphorylation by members of the PIKK group of kinases is a consequence of apoptosis [41,42]; however, the recent study by Lu and colleagues indicates that JNK-mediated H2AX phosphorylation may be essential for DNA fragmentation in UV-stimulated cells [39]. *In vitro* studies indicate that H2AX phosphorylation is required for caspase-activated DNase (CAD)-mediated nucleosomal fragmentation of chromosomal DNA [39]. JNK-mediated phosphorylation of H2AX may therefore contribute to apoptosis.

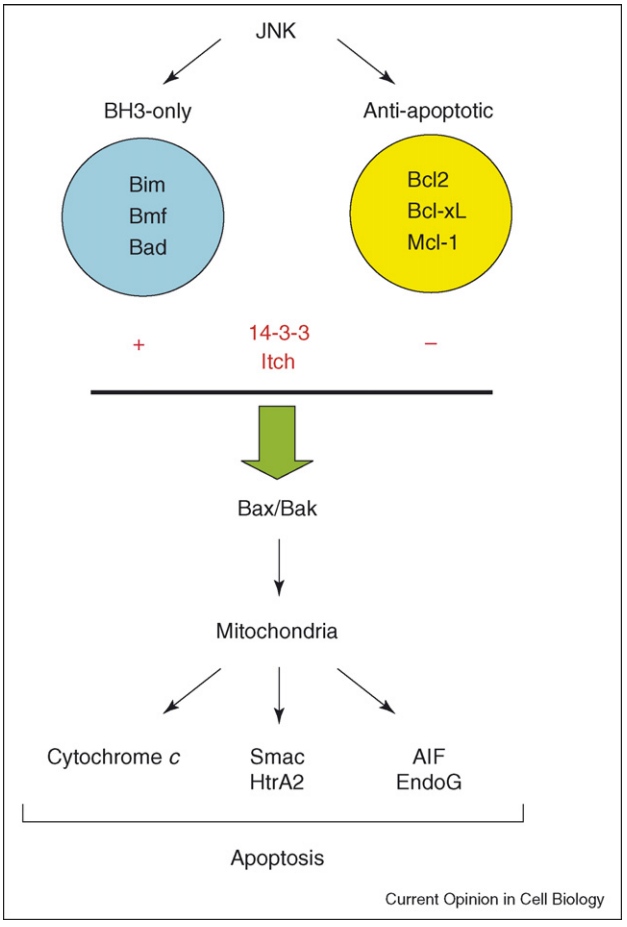
Many previous studies have focused on the role of JNK in apoptotic death. However, recent studies have demonstrated that JNK may also play important roles in other forms of cell death, including necrosis and autophagy. Thus, Ventura and colleagues have demonstrated that JNK contributes to TNF-stimulated necrosis in cells when the NF-κB pathway is inhibited by promoting the production of cytotoxic reactive oxygen species [43•]. A second example comes from Adhami and colleagues [44•]. Targeted deletion of *Jnk3* has previously been shown to protect mice from brain injury after cerebral ischemia-hypoxia [45]; however, Adhami and colleagues have demonstrated that during cerebral ischemia-hypoxia, very few cells complete the apoptotic process, but instead many damaged neurons exhibited features of autophagic/lysosomal cell death [44•]. Together, these reports indicate that JNK may have a more complex role in cell death than previously anticipated and that JNK may contribute to multiple forms of cell death.

Mechanism of JNK-induced activation of the mitochondrial apoptotic pathway

Primary fibroblasts prepared from $Jnk1^{-/-}Jnk2^{-/-}$ embryos and from $Mkk4^{-/-}Mkk7^{-/-}$ embryos exhibit marked defects in stress-induced apoptosis [46,47]. Detailed analysis demonstrated that the apoptosis defect was associated with failure to release mitochondrial pro-apoptotic proteins, including cytochrome *c*. Indeed, micro-injection experiments demonstrated that the mutant cells did not exhibit defects in apoptosis if cytochrome *c* was directly injected into the cytoplasm [47]. These studies establish that mitochondria are a primary target of pro-apoptotic signaling by JNK [1]. A critical role for the pro-apoptotic proteins Bax and Bak was demonstrated by the observation that in $Bax^{-/-}Bak^{-/-}$ compound mutant fibroblasts, activated JNK was not able to cause the release of mitochondrial cytochrome *c* and apoptosis [48]. Indeed, the conformational changes in Bax/Bak and the mitochondrial redistribution of Bax/Bak observed in wild-type cells was not found in JNK-deficient cells [48]. Together, these data indicate that Bax/Bak are targets of the JNK-induced apoptotic signaling pathway. How can JNK regulate Bax and Bak? One plausible mechanism is that JNK regulates members of the Bcl2 protein family. Indeed, several Bcl2-like proteins have been proposed to mediate the effects of JNK on cell death (Figure 2).

It has been reported that Bax is a JNK substrate, although the site of phosphorylation has not been identified. The JNK-mediated Bax phosphorylation may cause Bax activation [49]. Bax has also been reported to be sequestered by 14-3-3 proteins via an interaction mediated by the COOH-terminal region of 14-3-3, which is separate from the previously characterized phospho-serine binding site [50]. Recently, it has been reported that four of the seven

Figure 2



JNK can activate the mitochondrial apoptotic pathway, can phosphorylate and activate several pro-apoptotic members of the Bcl2-related protein family, and can also phosphorylate and inhibit several anti-apoptotic members of the Bcl2-related protein family. The balance of these pro-apoptotic and anti-apoptotic signals can cause activation of the mitochondrial apoptotic pathway by engaging the pro-apoptotic proteins Bax and Bak.

isoforms of 14-3-3 are phosphorylated by JNK and that this phosphorylation causes the dissociation of Bax from inactive 14-3-3 complexes [51•]. Thus, 14-3-3 protein phosphorylation represents one mechanism that may contribute to JNK-mediated regulation of the pro-apoptotic activity of Bax. JNK-mediated phosphorylation of 14-3-3 proteins may also lead to the release of other pro-apoptotic proteins, including FOXO transcription factors [52•].

The anti-apoptotic proteins Bcl2, Bcl-xL and Mcl-1 are phosphorylated by JNK *in vitro* and transfection studies indicate that this phosphorylation may suppress the anti-apoptotic functions of these proteins [35]. However, questions have been raised concerning the role of JNK, compared to other protein kinases (e.g. Cdk isoforms), in the regulation of this phosphorylation *in vivo*, since JNK

activation does not necessarily lead to phosphorylation *in vivo* and maximal phosphorylation *in vivo* can be observed at times when JNK is not activated [46].

The BH3-only protein Bid can be proteolytically processed in a caspase-independent but JNK-dependent manner in cells exposed to UV radiation [47]. Recent studies indicate that TNF can cause the JNK-dependent processing of Bid to a novel form (jBid) that is required for TNF-stimulated apoptosis in cells with inhibited NF-κB signaling [53]. However, the mechanism employed by JNK to regulate Bid processing is unknown and the structure of jBid has not been defined.

The BH3-only protein Bad is phosphorylated by JNK *in vitro*. This phosphorylation has been reported to lead to either increased [54] or decreased [55] Bad-mediated apoptotic activity in transfection assays, although the significance of the pro-apoptotic phosphorylation of Bad by JNK has been questioned [56].

The structurally related BH3-only proteins Bmf and Bim are also phosphorylated by JNK [57–59]. This phosphorylation of Bmf and Bim was reported to cause increased apoptosis [57–59]. In addition, the expression of pro-apoptotic Bim can also be transcriptionally induced by JNK-dependent AP-1 activity, leading to JNK-dependent apoptosis [60–62]. JNK-stimulated activation of FOXO transcription factors [63•,64] may also contribute to the increased Bim expression [65].

Further studies are required to define whether any of these proposed mechanisms are relevant to JNK-stimulated apoptosis *in vivo*.

JNK in cancer

JNK is implicated in oncogenic transformation; however, its role in tumor development remains controversial [66]. A role for the JNK pathway in tumorigenesis is supported by the high levels of JNK activity found in several cancer cell lines [66]. Indeed, in a recent study using a *Drosophila* model of tumor formation, oncogenic Raf and JNK were shown to cooperate to induce massive hyperplasia [67]. Studies of JNK signaling in mammals also support a role for JNK in tumor development. Thus, Nateri *et al.* used a mouse model of intestinal cancer to shown that ablation of the *cJun* gene or mutation of the JNK phosphorylation sites on cJun reduced tumor number and size, and prolonged lifespan [68]. These authors propose that a phosphorylation-dependent interaction between cJun and TCF4 regulates intestinal tumorigenesis by integrating JNK and APC/β-catenin [68], two distinct pathways activated by WNT signaling [2]. A further example of a role for JNK in tumorigenesis has been reported in liver, where JNK was shown to promote chemically induced hepatocarcinogenesis [69]. This pro-oncogenic role of JNK may be related to its known ability to promote proliferation.

In contrast to studies that demonstrate a pro-oncogenic role for JNK, other studies have linked JNK to tumor suppression [66]. One mechanism of tumor suppression is mediated by a role for JNK in tumor surveillance by the immune system involving $CD8^+$ T cells [70]. Tumor suppression may also be mediated by the pro-apoptotic effects of JNK activation [66]. Together, these data suggest that JNK may play a context-dependent role in tumorigenesis.

Studies of cancer genetics have implicated the MAP2K isoform MKK4, which activates both JNK and p38 MAPK *in vivo* [71], in human cancer [72•]. Published studies link MKK4 to both tumorigenesis and tumor suppression [72•]. Indeed, a recent study demonstrated that MKK4 co-operated with the PI3K pathway to promote the survival and proliferation of tumor xenografts [73]. A second example is represented by the demonstration that constitutively active MKK4 in human bronchial epithelial cell lines causes increased proliferation and invasion [74]. By contrast, loss-of-function mutations in the *MKK4* gene are found in ~5% of human tumors from a variety of tissues [72•], indicating a role for MKK4 as a tumor suppressor. Interestingly, MKK4 has also been implicated in the suppression of metastases. For example, a recent study by Stark and colleagues described reduced MKK4 expression in breast cancer to brain metastases [75]. Another study showed that mice injected with cells expressing MKK4 exhibited reduced lung metastases and increased survival compared to controls [76]. It therefore appears that, like JNK, the MAP2K isoform MKK4 may play a context-dependent role in tumorigenesis.

Despite an increasing body of evidence implicating the JNK pathway in cancer, the genetic and mechanistic basis for these findings remains largely unresolved [72•]. JNK may influence proliferation, survival, death, angiogenesis and migration. Further studies will be required to identify molecular mechanisms that account for the role of JNK in cancer.

JNK in diabetes and metabolism

Biochemical studies have established that JNK phosphorylates the insulin receptor substrate-1 (IRS-1) at the inhibitory site Ser-307 [77,78]. JNK activation can therefore suppress signal transduction by the insulin receptor. These observations implicate the JNK signaling pathway in insulin resistance, metabolic syndrome and type 2 diabetes. Indeed, JNK is activated in obese mice [79], in part because of lipotoxic stress [80] that may be mediated by a mechanism involving the ataxia telangiectasia mutated (ATM) protein kinase [81]. This activation of JNK causes insulin resistance [82]. Whether IRS-1 phosphorylation represents the only target of JNK that mediates insulin resistance or whether other targets make a contribution requires further study.

Ablation of the JNK pathway in mice can influence susceptibility to obesity and diabetes [82]. For example, knockout mice that lack expression of JNK1 or the JNK scaffold JIP1 are resistant to the effects of a high-fat diet on obesity and insulin resistance [27,79]. A primary role for JNK1 is implicated by these studies of knockout mice. However, the redundant functions of JNK1 and JNK2 together with gain-of-function phenotypes observed in JNK isoform-deficient mice have led to a re- interpretation of the phenotypes of $Jnk1^{-/-}$ and $Jnk2^{-/-}$ mice [83••]. It now appears that both JNK1 and JNK2 contribute to insulin resistance [84].

Ozcan and colleagues have shown that obesity causes endoplasmic reticulum (ER) stress, which in turn can lead to suppression of insulin receptor signaling through hyperactivation of JNK [85••]. These investigators demonstrated that mice deficient in X-box-binding protein-1 (XBP-1), a transcription factor that modulates the ER stress response, develop insulin resistance. These findings demonstrate that ER stress is a central feature of peripheral insulin resistance and type 2 diabetes at the molecular, cellular and organismal levels. Indeed, this link can be exploited for therapeutic purposes using orally active chemical chaperones. Treatment of obese and diabetic mice with 4-phenyl butyric acid and taurine-conjugated ursodeoxycholic acid was shown to alleviate ER stress, normalize hyperglycemia and restore systemic insulin sensitivity in cells and whole animals [86••]. This is an interesting approach with potential for the treatment of type 2 diabetes.

Although most studies have focused on the roles of JNK in type 2 diabetes, a recent study has established a role for JNK in type 1 (insulin-dependent) diabetes that is caused by the autoimmune destruction of β cells [87]. In this study, disruption of the *Jnk2* gene in non-obese diabetic (NOD) mice decreased destructive insulitis and reduced disease progression to diabetes. The authors show that JNK2 is important for controlling the Th1/Th2 balance of the immune response, thereby protecting against the autoimmune disease. Similarly, JNK1 is implicated in a different autoimmune disease (encephalomyelitis), where it plays an important role in regulating expression of the anti-inflammatory cytokine IL-10 [88••].

JNK in lifespan

Aging of a eukaryotic organism is affected by its nutritional state and by its ability to repair oxidative damage. Consequently, signal transduction systems that control metabolism and oxidative stress responses influence lifespan. Two recent studies have shown that JNK can control lifespan in *Drosophila* and *C. elegans* by promoting phosphorylation of the forkhead protein FOXO [63•,89•]. Oh and colleagues show that JNK promotes *daf-16* (FOXO) activity, which regulates life span and stress resistance in *C. elegans* [63•]. Wang and colleagues also show that JNK-dependent life span extension in *Drosophila* also requires *dfoxo* [89•]. The discovery that JNK can exert some of its effects via FOXO introduces a new potential explanation for the ability of JNK to cause insulin resistance. This hypothesis will have to be tested in the appropriate model organisms.

Box 1 The JNK signal transduction pathway in disease

The JNK signal transduction pathway is implicated in multiple disease processes:

Insulin resistance
Metabolic syndrome
Type 2 diabetes

Autoimmune disease
Type 1 diabetes
Encephalomyelitis

Ischemic tissue injury
Stroke
Cardiac infarction

Cancer
Pro-oncogenic
Tumor suppression

Conclusions

Significant progress towards understanding the function of the JNK signaling pathway has been achieved during the past few years. The determination of atomic structures for components of the JNK signaling pathway and also some complexes formed by these components represents a critical step towards a more complete understanding. Recent studies using the chemical genetic approach [90] to define the function of JNK *in vivo* using mice with a germ-line point mutation that confers sensitivity to a small molecule drug [38•, 83••] expands the available methods that can be applied to study the function of JNK *in vivo*. Furthermore, the finding that drugs targeting JNK have demonstrated therapeutic efficacy for protecting mice against type 2 diabetes [91] and tissue injury caused by ischemic disease (e.g. stroke [92–95] and cardiac infarction [96]) provide hope that JNK may be useful for the development of novel therapies for human disease (Box 1). The research progress during the next few years should prove to be very exciting.

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