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**REVIEW**

# JNK: A Key Modulator of Intracellular Signaling

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**Abstract**—JNK is a family of stress activated protein kinase enzymes that is under intense study. JNK family members are involved in diverse phenomena, but the focus of research has been until now involvement of JNK in apoptosis. A great number of JNK substrates indeed play major roles in cell death. Conversely, accumulating data support a key role of JNK substrates in cell survival and proliferation. Continuous progress is being made, while several important questions remain unanswered. Does JNK cause cancer or prevent it? This paper attempts to evaluate the role of JNK in cell physiology and describe the effects of intracellular signaling pathways that are mediated by JNK family members.

*Key words*: JNK, apoptosis, inflammation, reactive oxygen species (ROS), gene promoter, cancer

JNK is a protein phosphorylase enzyme (a kinase) that is ubiquitous in animals [1]. The term JNK is derived from "c-Jun N-terminal kinase", which refers to its best-known substrate, the mammalian protooncogene c-jun [2]. A different term for JNK is SAPKα, stress-activated protein kinase α, because JNK is activated in response to several stress conditions. Other stress activated protein kinases are p38 and Erk5. They all belong to the family of MAP ("mitogen activated protein") kinases. MAP kinases transduce signals from a variety of cell surface transmembrane receptors and other extracellular stimulants to any of the intracellular compartments. Signals proceed via cascades of sequential enzyme phosphorylation by their upstream activators (Fig. 1).

JNK enzymes are present in species ranging from nematodes and fruit flies to man [3-6]. In mammals there are three genes which encode JNK isoforms: JNK1, JNK2, and JNK3. The first two are ubiquitous, occurring in all tissues; the third is encountered in the brain, heart, and testes. Each of these genes encodes proteins of 46 to 55 kD through alternative splicing. Substrate preferences vary depending on the spliced isoform [1, 6]. The structure of JNK3 has been analyzed by X-ray diffraction [7]. JNK and its homologs Erk (extracellular signal-regulated kinase) and p38 (protein kinase, protein of apparent molecular weight 38 kD) from other MAP kinase cascades represent the downstream enzymes of the signal cascades in which they participate. While upstream MAPKK and MAPKKK are activated by Ser/Thr phosphorylation, JNK and its homologs are activated by Thr/Tyr phosphorylation [1, 6]. From the crystal structure it can be deduced that Thr phosphorylation promotes domain closure, while Tyr phosphorylation stabilizes the substrate-binding pocket [7].

Depending on the context of its expression, JNK is essential for processes as divergent as cell death, cell survival, cell proliferation, and cell differentiation [1, 6]. In

*Abbreviations*: ASK) apoptosis signal regulating kinase; ATF) activating transcription factor; ATM) ataxia telangiestasia mutated; Bcl-2) B-cell lymphoma-2; Bad) Bcl-2 associated death promoter; Bax) Bcl-2 associated protein X; BH3) Bcl-2 homology domain 3; bZip) basic region-leucine zipper; cdc) cell division control; c-jun) cellular homolog of avian myeloblastosis virus gene 17; c-Fos) cellular homolog of Finkel Biskis Jinkins murine osteosarcoma virus oncogene; c-myc) cellular homolog of myelocytomatosis oncogene; Cot) cancer Osaka thyroid; CREB) cyclic AMP response element-binding protein; Daxx) death domain associated protein xx; DLK2) dual leucine zipper; Elk-1) Ets-like gene 1; ER) endoplasmic reticulum; Erk) extracellular signal-regulated kinase; Ets) E twenty six; FasL) ligand for first autoimmune disease antigen; GCK) germinal center kinase; GnRHR) gonadotropin-releasing hormone (GnRH) receptor; GST) glutathione-S-transferase enzyme; GST-c-jun) recombinant GST fused to c-Jun protein; GSTPi) glutathione-S-transferase enzyme class Pi; ICAM) intercellular adhesion molecule; IFN) interferon; IL) interleukin; IRE1) inositol response 1; MAPKK) MAPK kinase; MMP9) matrix metalloproteinase 9; NFAT) nuclear factor of activated T-cells; NFκB) nuclear factor κB; NCX) sodium calcium exchanger; P38) protein kinase, protein of apparent molecular weight 38 kD; P53) tumor suppressor, protein of apparent molecular weight 53 kD; Ras) oncogene of rat sarcoma; ROS) reactive oxygen species; TAO) thousand and one amino acid protein; TNF) tumor necrosis factor; VEGF) vascular endothelial growth factor.

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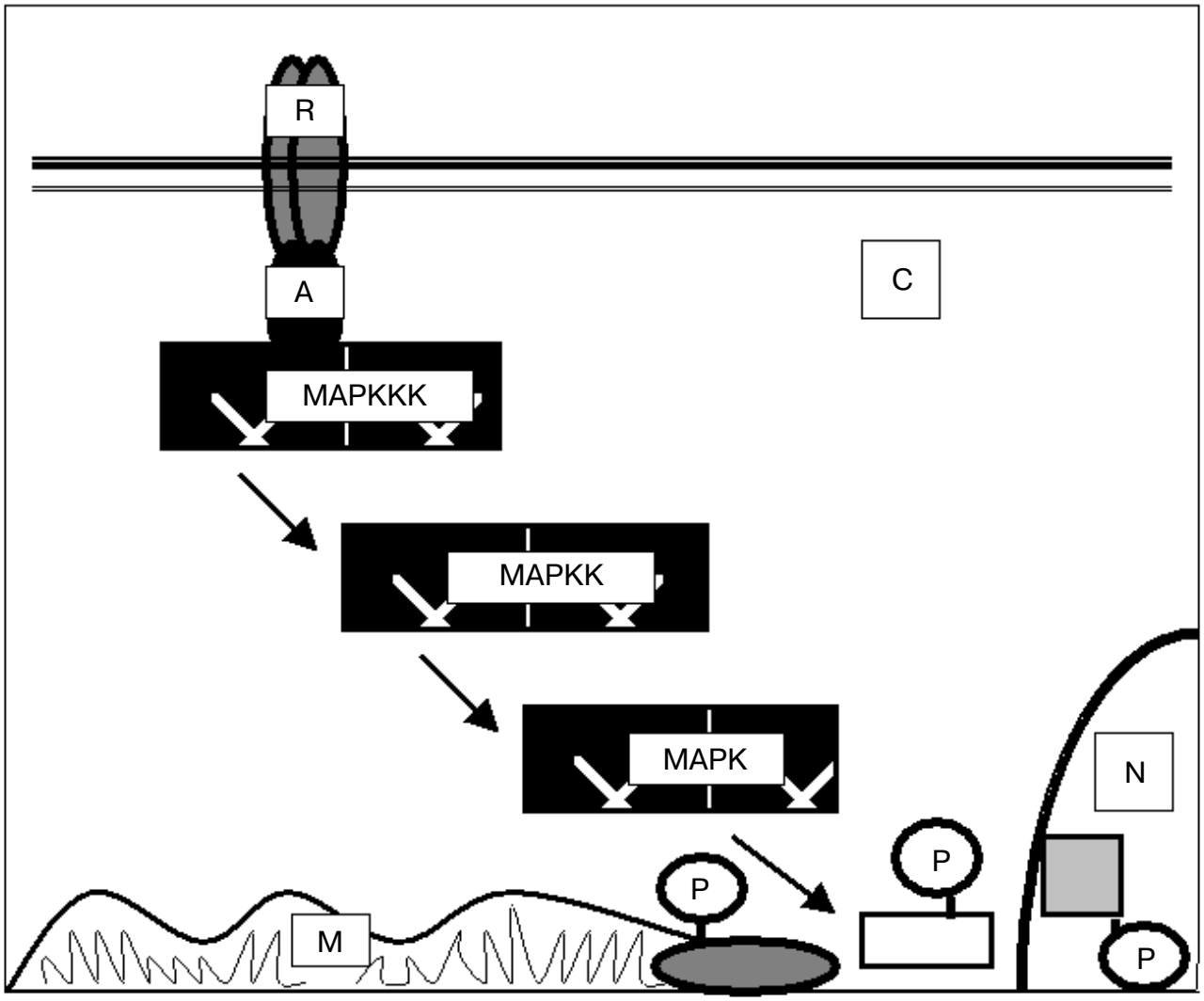


**Fig. 1.** An intracellular MAPK cascade. Activated by a protein A associated with a transmembrane receptor R, MAPKKK activates MAPKK by phosphorylating it on Ser/Thr. Then MAPKK activates MAPK by phosphorylating it on a Thr or a Tyr. MAPK in turn activates transcription factors in the nucleus (N) or other proteins in the cytoplasm (C), on mitochondria (M), or in another compartment.

addition to this, JNK is essential for the induction of the expression of many genes under a variety of conditions. These genes include cytokines (e.g., interleukins IL-2, IL-4, IL-8, IL-18, interferon γ (IFN-γ), tumor necrosis factor α (TNF-α)) [8-12], vascular endothelial growth factor (VEGF) [12], transmembrane receptor CD44 [13], transactivators c-jun and ATF3 [8, 14, 15], enzymes COX-2, collagenase-3, matrix metalloproteinase 9 (MMP-9), heme oxidase (HO)-1, and Aquaporin-1 [8, 16-19], extracellular proteins collagen and fibronectin [20, 21], cell cycle regulator cyclin D1 [22], transmembrane proteins GnRHR (gonadotropin-releasing hormone receptor), ICAM-1 (intercellular adhesion molecule), and NCX1 (sodium calcium exchanger) [23-25]. Under certain conditions, some of these genes are repressed by JNK activity (TGF-β-activated collagen expression was blocked by TNF-α-activated JNK in fibroblasts [26], and flurbiprofen-activated JNK caused cyclin D1 inhibition in colon cancer cells [27]), demonstrating the complexity of signal pathways.

## SUBSTRATES OF JNK

The most intensely studied substrates of JNK are inducible transcription factors, of which c-Jun is the best known [2]. c-Jun is an inducible transactivator that belongs to the bZip (basic region leucine zipper) class of proteins, named so because the molecules dimerize via formation of a coiled coil, the Leucine Zipper. The dimerizing element is preceded by a basic region, which is mainly responsible for specific DNA binding. Farther towards the N-terminal direction is the activation domain, which is phosphorylated by JNK on two amino

acid residues. Phosphorylation of c-Jun at serine residues 63 and 73 results in enhanced transcriptional activity. c-Jun protein can homodimerize, or form heterodimers with other proteins that belong to the families of c-Fos (cellular homolog of Finkel Biskis Jinkins murine osteosarcoma virus oncogene) or ATF2 (activating transcription factor) [27, 28]. The dimers are called AP-1 (activator protein-1) because they recognize and bind, via complex macromolecular interactions (between amino acid residues of the protein, and the major groove plus backbone of DNA), to specific DNA sequences on certain gene promoters, which contain AP-1 sites (the pseudopalindrome ATGACTCAT or variants of this). The bZip transactivator ATF2 is also activated by JNK on a Ser/Thr-Pro motif on its activation domain [1, 6].

Another transcriptional activator and substrate of JNK is the tumor suppressor protein P53, which is phosphorylated at threonine 81 [29]. Upon phosphorylation by JNK, P53 causes apoptosis, which is in accordance with its anti-oncogenic role. In contrast, when JNK is inactive it binds to P53 between residues 97 and 116 and targets P53 for proteasomal degradation. The transcriptional regulator c-Myc (cellular homolog of myelocytomatosis oncogene) is also directly phosphorylated by JNK, in a manner that controls c-Myc-dependent apoptosis and c-Myc-induced telomerase gene expression [30, 31].

Other transcription factors phosphorylated by JNK include Ets (E twenty six), Elk-1 (Ets-like gene 1), NFAT4 (nuclear factor of activated T-cells), and the glucocorticoid receptor [1, 6, 32-34]. The latter two upon phosphorylation by JNK exit the nucleus of the cell and sequester in the cytoplasm. To regulate transcription factors, JNK must also be present in the nucleus [32, 35].

JNK can also translocate to mitochondria where it phosphorylates proteins of the Bcl-2 family [36-40] that regulate cell survival.

## ACTIVATORS OF JNK

MAP kinases are activated by dual phosphorylation by upstream kinases on the motif Thr-x-Tyr, where x is a given amino acid. JNK kinases are activated on Thr-Pro-Tyr [1]. JNK can be activated by two enzymes, MKK4 and MKK7. Those enzymes are activated by further upstream MKKK enzymes through phosphorylation at Ser or Thr residues. One such example is mixed lineage protein kinase MLK3 (mixed lineage protein kinase). Scaffold protein JIP (JNK interacting protein) binds MLK3, MKK7, and JNK [41]. MLK3 activates MKK7 by phosphorylation, and MKK7 activates JNK in turn. JIP has been described as being an inhibitor of JNK because when overexpressed it binds JNK and causes it to be retained in the cytoplasm, blocking its activation by stress stimulants such as UV light or the protein synthesis inhibitor anisomycin. When, however, JIP is coexpressed with MLK3 or MKK7, it potentiates their effect on JNK induction. Other scaffold proteins are JLP [42] and JSAP1 [43]. MKKK enzymes include MEKK1, -2, -3, and -4, MLK2, DLK2 (dual leucine zipper), TAK1, Cot (cancer Osaka thyroid), and TAO1 and -2 (thousand and one amino acid protein) [1]. Further upstream are enzymes such as the GCK (germinal center kinase) family proteins and proteins of the TRAF (TNF-receptor associated factor) type. These can activate MKKK following extracellular stimuli such as the binding of cytokines to external receptors [1].

Activators of JNK include cytokines, reactive oxygen species (ROS), UV light, protein synthesis inhibitors, and other stress stimulants [1, 44-47]. Most of these act by inducing MAPKKK (such as ASK protein) activity, which initiates the signal cascade. Many reports have appeared that indicate JNK involvement in a variety of stress responses; however, a single pathway for JNK activation does not appear to exist. Many stimulants cause JNK activation through more than one mechanisms. A notable case is ROS, which can modify JNK activity via several different reactions, both chemical [48] and enzymatic [49-51]. Increased levels of intracellular ROS very often correlate with increased JNK phosphorylation and activation. In the last two chapters, we discuss JNK induction by ROS in some detail, as it has importance for inflammatory and cytotoxic signal cascades.

## TOOLS TO STUDY JNK-DEPENDENT SIGNALS

A plethora of scientific data has resulted from the recent discovery of the chemical SP600125, a selective inhibitor of JNK [8, 49]. Definitive measurement of JNK activity can be made with assays that measure phosphorylation of GST-c-jun (recombinant glutathione-S-transferase fused to c-Jun protein), using [$\gamma$-$^{32}$P]ATP as the substrate [49]. JNK activation can also be assessed using antibodies that recognize phosphorylated (activated) JNK [52, 53]. The involvement of JNK in a pathway can be confirmed by the introduction of dominant negative (inhibitory) JNK [54]. Another specific inhibitor of JNK is the JNK-binding-domain of the scaffold protein JIP [41, 55]. Antisense oligonucleotides have also been used to ablate JNK gene expression in cultured cells [56].

One important approach that has been used to demonstrate that a particular enzyme acts downstream of a certain step in a pathway is to introduce a constitutively activated form of the enzyme. If the introduction of the constitutively active enzyme makes inhibition of the step irrelevant to the activation of the signal cascade, then it can be concluded that the enzyme does, in fact, act downstream in the same cascade [57]. Constitutively activated enzymes are made by replacing phosphorylatable residues with a negatively charged amino acid. The generation of constitutively active JNK has not been possible, however, because JNK is normally activated by phospho-

rylation on threonine and tyrosine [1]. While Ser/Thr phosphorylation is mimicked by Asp or Glu in a recombinant constitutively active enzyme, phosphorylated Tyr cannot be mimicked by any other amino acid. Some groups have bypassed this problem, using a fusion protein of JNK with its upstream activator MKK7 [58, 59]. This construct has truly constitutive activity due to substrate proximity, but it can still be subject to inhibition by any protein that can reverse this phosphorylation. This fusion protein, however, has not been surpassed to date and represents an irreplaceable tool for JNK analysis.

Transgenic mice and cells deficient in JNK isoforms and upstream activators have been generated [60-63]. These have provided a wealth of information on the precise role of JNK1, -2, and -3 in tissue physiology. Each JNK appears to have distinct roles in cell function, but there is also a very significant degree of redundancy, especially between JNK1 and JNK2.

A classical readout of JNK effects is an AP-1 dependent reporter gene, as AP-1 is the major JNK-dependent transcription factor [2, 64]. The signals from many other protein kinases converge at this point, but valuable information can be gleaned when this approach is combined with inhibition studies. Measurements of AP-1 activation indicate a completed signal cascade in a great number of cases of JNK activation.

A potential alternative to JNK-deletion in cells or animals is the use of small interfering RNA (siRNA) to silence JNK gene expression via the degradation of JNK-encoding mRNA [65]. It remains to be proven in how many systems this approach will be effective.

## OXIDANT STRESS AND INFLAMMATION: JNK AS A LINK

The field that currently has the greatest potential for pharmaceutical intervention is JNK involvement in inflammatory and autoimmune disorders [66]. An enormous amount of data has emerged during the past two decades associating oxidant stress and inflammatory conditions [67-69]. Aging and atherosclerosis have also been associated with changes in the regulation of inflammatory mediators [66, 70]. At the same time, aging and atherosclerosis have been also associated with oxidant stress. JNK was very soon after its discovery recognized as an being induced by ROS, intermediates of which propagate oxidant stress [71]. There are many ways, in which ROS can change JNK activity. While ROS-induced JNK activity often has an important role in the determination of cell fate, the expression of proinflammatory mediators is a separate, important field of application for JNK inhibitors and warrants a great deal of further research [66].

The definition of an event as ROS-dependent is often linked to its inhibition by antioxidants. A great deal of caution should, however, be used in the interpretation of such experimental results, because the spectrum of antioxidant-sensitive mechanisms in any given cell is large. In addition to this, several antioxidants can sometimes have prooxidant effects, due to autooxidation or the presence of electron-accepting compounds [72]. Also, the prooxidant concentrations used to assay for the ROS-inducibility of a signal can deviate from physiological or even pathological levels. The JNK signal cascade, however, can be safely regarded as an ROS-inducible pathway in many cell types. Very extensive studies have demonstrated the significance of ROS for JNK-mediated effects under many conditions, as will be elaborated in the chapter on cell fate.

JNK activity is necessary for the expression of inflammatory mediators, such as cytokines, metalloproteinases, and adhesion molecules [73-75]. It has also been proved that the expression of several inflammatory mediators requires ROS [76, 77]. Inhibition of JNK inhibition and/or ROS can block the expression of several cytokines and adhesion molecules, showing JNK to be a promising candidate target for interventions aiming to relieve chronic inflammation [66, 78].

There are several different ways that JNK can be activated by ROS. ROS, for example, cause ASK activation by inducing its dissociation from thioredoxin [63]. ASK phosphorylates MKK7, which in turn phosphorylates JNK. Another mechanism by which JNK is activated by ROS results from the fact that JNK can be held in an inactive form by GSTPi (glutathione-S-transferase enzyme class Pi) [49-51]. Upon increase in oxidative stress or UV irradiation JNK dissociates from GSTPi, and can then enter the nucleus, or translocate to the mitochondria and activate substrates. An increase in oxidant stress can occur in many different ways. JNK is activated in response to both exogenous and endogenous ROS [79-81]. Endogenous ROS can become a factor following cell stimulation with the cytokine TNF. TNF causes glutathione (GSH) efflux ([82] and Boldogh, Vlahopoulos, and Brasier, unpublished results), increasing the longevity of endogenous reactive oxygen species. ROS are byproducts of normal cellular metabolism, e.g., from mitochondria, but also from cell-specific enzyme complexes, such as macrophage NADPH oxidase [79]. In some systems, activation of JNK by ROS was dependent on intracellular calcium [83-85]. One potential effect of calcium might be to activate the kinase Rac1, an enzyme that acts upstream of JNK in some cell types [86, 87]. Another effect of calcium is to prolong oxidant stress, by activating channels that allow GSH efflux [88]. There exist also systems where JNK activation is independent of calcium [89]. The interplay between calcium and ROS is very complex, probably due to the great variety of signal transducers the two agents activate and the ways the transducers interact with each other (reviewed in [90]).

In addition to GSTPi, other mechanisms also account for JNK induction by oxidant stress. Hydroxynonenal, an end product of lipid peroxidation, forms

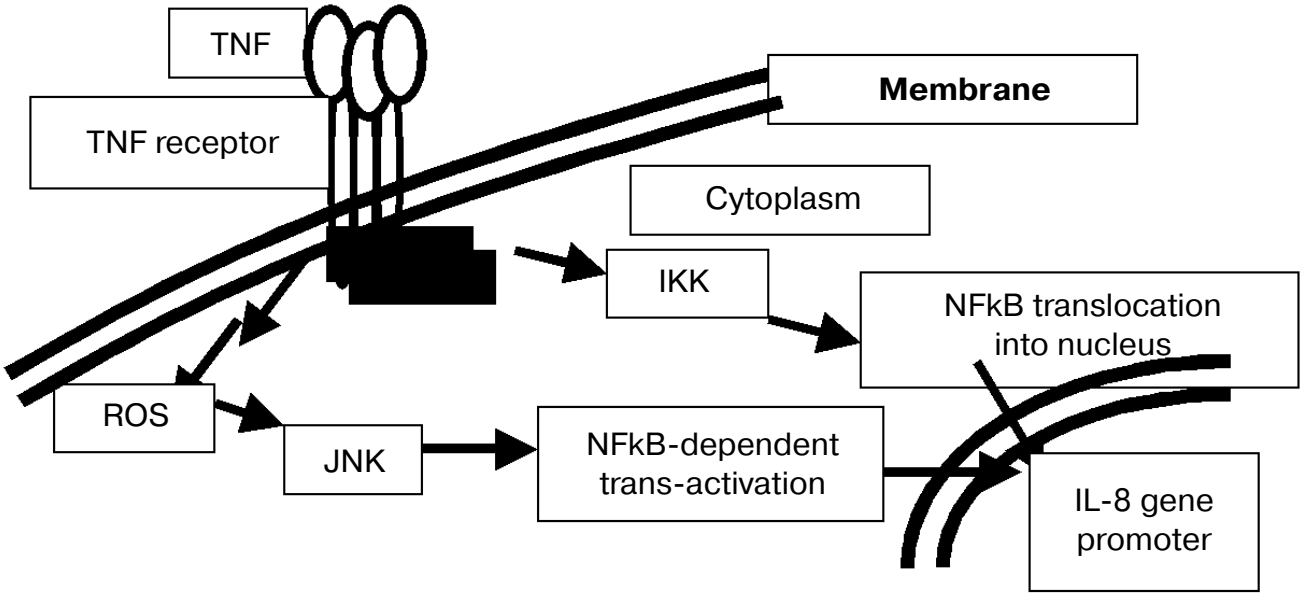


**Fig. 2.** NFκB-dependent induction of IL-8 gene promoter requires JNK1.

adducts with JNK and causes its activation [48]. Oxidant stress also inactivates protein phosphatases, which would otherwise remove phosphate groups from specific enzymes, causing their inactivation [91]. Inactivation of a JNK-specific phosphatase by hydrogen peroxide would permit prolonged activation of JNK if no other antagonistic factors were present. This would contrast with the normally transient activation of JNK by cytokine stimulation [92] and could have significant effects on cell fate.

JNK mediation of an ROS-transmitted signal can be illustrated in the case of interleukin-8 (IL-8) gene expression [93]. IL-8 is a proinflammatory cytokine that is inducibly expressed by a variety of cell types. Upon exposure of a monocyte to TNF, glutathione efflux occurs. The resulting increase in ROS levels, in combination with TNF-induced NFκB (nuclear factor κB) translocation, activates the IL-8 gene promoter [93]. This IL-8 promoter induction is JNK1-dependent [94]. ROS increase causes JNK activation. Upon exposure to IL-8, circulating neutrophils cross the vascular endothelium and release a respiratory burst, increasing ROS levels in the surrounding tissue. As JNK can be activated by endogenous, as well as exogenous ROS [79-81], it becomes clear how in this case JNK1 has the potential to mediate the propagation of inflammation, connecting a systemic endocrine signal (TNF) to a localized, paracrine response (IL-8) (Fig. 2).

## DETERMINATION OF CELL FATE AFTER JNK ACTIVATION

JNK is involved in a great variety of phenomena. Depending on the cell type and state, JNK can be involved in proliferation [22, 56], differentiation [9, 61], or apoptosis [1, 7, 55]. Even though the major pharmaceutical promise of JNK inhibitors has so far been as anti-inflammatory agents (due to JNK involvement in expression of proinflammatory molecules [66]), most research on JNK has unambiguously been directed toward involvement of the stress kinase in determination of cell fate. There are at least three major reasons for this. 1) Death of cancer cell lines often involves JNK activation, and therefore activation of stress kinases or selected downstream targets can help to eliminate cancer cells. 2) Proliferation and the metastatic phenotype of some cancer cell lines is associated with and requires JNK activity [95-97]. Blocking JNK or downstream targets offers therefore a way to treat some forms of cancer. 3) Neurodegenerative diseases have been associated with undesirable JNK-mediated, cell death [57, 88, 98, 99]. Therefore, the study of JNK upstream activation may offer opportunities to find a cure, while selective JNK inhibition may be a way to stop disease progression.

Many apparently contradictory observations on JNK effects on cell growth have been reported: for example, research indicates that in human prostate cancer cells JNK facilitates proliferation [56, 65, 100], while in breast cancer cell lines JNK is not essential for cell growth [56]. In some studies, Ras (oncogene of rat sarcoma)-dependent cell transformation requires JNK-activated c-Jun [101-105], while in other studies JNK and c-Jun blocked Ras-stimulated transformation [106]. A further contradiction: mice lacking JNK2 were resistant to skin tumors [107], while those lacking JNK1 had increased skin tumorigenesis [108] in spite of the substantial redundancy between JNK1 and JNK2. The reason that JNK effects

on cell fate are variable most probably reflects the great heterogeneity of JNK substrates. However, JNK appears to be often at the core of cell life-death decisions. The combined effects of the enzymes and regulatory molecules that control JNK activity make it a sensor that alters the course of cell fate.

To cause cancer, among other events, a cell has to overcome restrictions on the cell cycle, to divide indefinitely, to become immortal, and to invade a number of tissues. The involvement of JNK in cell proliferation is not difficult to understand. JNK activated c-Jun transactivates the promoter of cyclin D1 [22] and probably many other genes. Proliferating cell nuclear antigen (PCNA), cyclin-dependent protein kinases (cdk) 2 and 4, and several other genes are then activated [22, 56, 65]; the cell passes growth cycle checkpoints, DNA is replicated, and cell division proceeds. JNK contributes to immortalization probably by activating c-myc [30, 31], and thereby inducing telomerase activity [109]. The metastatic phenotype is another aspect of JNK-facilitated carcinogenesis. JNK-activated AP-1 transactivator, among other genes, induces the expression of matrix metalloproteinase 9, which facilitates metastasis [17]. The fact that the oncogene v-jun can autonomously (without JNK) transform cells, while delta motif phosphorylation of c-jun by JNK is needed to transform cells [104-106], illustrates perfectly the potential of JNK to cause cancer. Examination of a series of murine cell lines that model the progression of skin cancer has shown increased JNK and AP-1 activities are associated with advancing stages of the model [27, 97].

JNK activation, on the other hand, is the hallmark for certain types of cell death, most notably caused by stress conditions and TNF family proteins. JNK can induce cell apoptosis by phosphorylation and activation of the transcription factor P53 [29, 52], or, after translocation to mitochondria, by phosphorylation and activation of proapoptotic proteins like Bax (Bcl-2-associated protein X) [54] and BAD (Bcl-2-associated death promoter) [40], or by phosphorylation (and inactivation) of antiapoptotic proteins of the Bcl-2 family [35-37]. JNK substrate c-Myc can also become part of the apoptotic mechanism [30, 110]. BAD activation by JNK is antagonized by the phosphorylation of BAD at serine 155 by protein kinase A [111]. Protein kinase A antagonism to JNK may also rely on activation of the transcription factor CREB (cAMP response element binding protein) [112].

The cytokine TNF activates one antiapoptotic pathway via the receptor associated factor TRAF2 (TNF receptor-associated factor 2) which signals to NFκB [113], and a proapoptotic pathway via JNK activation, which may disrupt the antiapoptotic TRAF2-mediated signal via translocation of BH3 (Bcl-2 homology domain 3)-interacting domain death agonist (BID) to mitochondria and subsequent release of second mitochondria-derived activator of caspases (Smac/DIABLO). Alteration of the balance between those two pathways in favor of JNK leads to cell death [114].

A main role of JNK appears to be mediation of stress-induced apoptosis. A variety of forms of stress cause JNK activation [45-47, 99, 100], including heat shock, protein synthesis inhibition, misfolding of proteins, UV light, DNA damage, and ROS. One of the most sensitive switches of JNK activity by stress can be JNK-inactivating phosphatases [115-118], which are both sensitive to protein damaging conditions and highly selective. There are several other potential JNK inhibitors that must be neutralized for JNK signals to commence.

Protein folding is normally assisted by chaperones such as Hsp72. Hsp72 synthesis is induced when cells are subject to heat shock [115-118], and it inhibits JNK through a region different from the chaperone domain [118]. Hsp72 can also protect a JNK-specific phosphatase [113-115], which by dephosphorylation turns off JNK activity. This phosphatase is inactivated by heat shock, thereby allowing prolonged JNK activation unless Hsp72 restores the function of the phosphatase. Hsp72 can inhibit both apoptosis and necrosis [116-118], and reports indicate that it can inhibit cell death via JNK inhibition, but also independently of JNK or ROS [119, 120]. Hsp72 might also have the potential to block cell death at a step subsequent to JNK induction, as inhibition of cell death by Hsp72 can happen also by substantial shortening of JNK activity duration, while there is no inhibition of the initial activation of JNK by heat shock [118]. Initial JNK activation by heat shock can be attributed to misfolded proteins [47]. Misfolded proteins can activate JNK via endoplasmic reticulum stress, using TRAF2, which subsequently activates the JNK pathway via ASK-1, probably via formation of a TRAF2–ASK-1 complex with the transmembrane protein of endoplasmic reticulum IRE1 (inositol response 1) [121]. From the potential of Hsp72 to block cell death by shortening the duration of JNK activation it can be concluded that at least some pathway to cell death requires persistent activation of JNK. We address this issue below where we discuss ROS-induced apoptosis.

UV radiation may activate JNK signal cascades via clustering and the resultant activation of receptors for the TNF family of cytokines [46]. Subsequent to receptor clustering, the signal to JNK might proceed via TRAF2 and ASK-1 [122], utilizing also Daxx (death domain associated protein xx). Daxx overexpression or ablation results in apoptosis [123-125]. Daxx can monitor the progress of cell growth, facilitating apoptosis whenever the Daxx concentration changes, or an associated protein is dissociated from it. The fact that there have to be certain common aspects between events of cell death caused by different stimuli is obvious by the observation that mild heat shock can block UV-induced apoptosis probably through induction of Hsp72 [119]. UV-induced cell death

under certain conditions requires activation of ceramide production [126]. A different signal transducer, breast cancer tumor suppressor protein BRCA1, is cleaved by protease caspase-3 in response to cell treatment with UV radiation, causing JNK-mediated apoptosis [127].

DNA damaging agents such γ-radiation or alkylating agents, and several conditions that result in DNA strand breaks, may use c-Abl, p53, ATM (Ataxia telangiestasia mutated), and other proteins to initiate JNK signal pathways [128-131]. In response to DNA damage, JNK translocates to mitochondria to phosphorylate and inactivate Bcl-2 family members and cause apoptosis [36-39]. This type of death can also take place in the absence of protein synthesis [132], which means that regulation of transcription factors becomes irrelevant in the case that protein synthesis inhibition is persistent (because no gene expression takes place). As would be expected, this type of death can also be mediated by signal cascades that do not utilize JNK [133].

In *Drosophila*, where oxidant stress inhibition is associated with an increased lifespan of short-lived flies [134], JNK has been proved to condition cells against ROS-associated toxicity [135]. In mammalian systems, conditioning of cells against oxidant stress [136] is associated with activation of the PI3-Akt-erk pathway (activated by energy restriction, which increases mammalian lifespans), inhibition of calcium signaling [81, 83], and induction of antioxidant enzymes [72, 137]. The last effect can also be traced to JNK [137]. Antiapoptotic factor Bcl-2 was reported to block cell death by altering the balance between the activities of MAP kinases Erk and JNK in favor of Erk [82]. In many studies, the activity of Erk or its upstream inducers had to be blocked or overcome by a rise in JNK activity for cell death to occur [138, 139]. Using histone deacetylase inhibitors, Yu et al. caused cell death that could be prevented by constitutively active MEK1 (upstream activator of Erk) or by antioxidant treatment coincident with JNK inhibition [140].

The Erk–JNK antagonism is a field where ROS also play an important role [136-144]. Initially MAP kinases were considered as ROS-responsive, but the methods used to determine ROS involvement were not uniform. High concentrations of various compounds were used, and the effects at those concentrations include enzyme inhibition, and sometimes even prooxidant side effects [72]. Insight into the induction of MAP kinases by ROS was given by teams [141-143] who demonstrated that in a variety of cell types, electron spin resonance measured certain low ROS levels that correlated with cell proliferation. Enzymes such as catalase and superoxide dismutase used at levels sufficient to quench those ROS levels inhibited Erk activity and cell cycle progression. Thus, it was possible to differentiate this phenomenon from TNF-induced ROS, which clearly were associated with JNK activation. This observation was also corroborated by observations that low concentrations of ROS, in this case resulting from the application of exogenous micromolar hydrogen peroxide ($H_2O_2$), caused Erk activation in cardiac myocytes. Higher concentrations of $H_2O_2$ further increased Erk activity and began to cause JNK activation, and still higher concentrations further augmented JNK activity and induced cell death, which could be inhibited by dominant negative JNK [144]. In renal epithelial cells, blocking $H_2O_2$-induced cell death by ER stress preconditioning required Erk activation and was inversely correlated to JNK activity [47]. In mouse liver, glutathione peroxidase (Gpx) was shown to protect hepatocytes against oxidant stress-mediated cell death, which was associated with p53 phosphorylation by JNK [145]. Gpx null mice had higher concentrations of activated JNK and p53.

The fact that JNK requires higher levels of ROS than Erk to become activated is in full accordance with its role as a sensor of stress and inflammatory conditions. If, for example, JNK was turned on by the lower ROS levels that are required for normal progress of the cell cycle [141-143], then its downstream targets designed to react to stress conditions would be activated without purpose. The high concentration of ROS that is expected at the site of inflammation will turn on JNK, which will in turn facilitate activation of inflammatory mediators and cause the death of cells that have lost certain regulators of the cell cycle. Such cells would arise from exposure to genotoxic levels of stress [146-149], and uncontrolled growth of those cells would otherwise give rise to tumors.

While NFκB can block JNK-dependent apoptosis [150], NFκB can also cause apoptosis, overcoming a JNK-dependent, antiapoptotic signal [151]. When does JNK lead to cell death? According to one model, apoptosis can be caused by prolonged JNK activation [152]. A temporal restriction on the length of JNK activation might explain the permanent cell growth arrest observed on prolonged exposure of cells to ROS [153], where prolonged exposure of the cell to ROS correlated with a permanent growth arrest. This growth arrest may facilitate apoptosis, when combined with signals that under other conditions would cause cell proliferation. Prolonged activation of JNK would allow P53 to be stabilized [29], and to block the cell cycle, while a shorter activation might not protect P53 from proteolysis for long enough for it to cause cell cycle arrest. Short activation of JNK, therefore, may indeed be consistent with cell cycle progression.

Under certain conditions, activation of c-Jun by JNK is essential for apoptosis [154]. Whether JNK activation will rescue cells from apoptosis, or rather causes apoptosis itself, depends on the context in which JNK activation takes place. And indeed, in mouse fibroblasts, c-jun antagonizes p53 binding on the promoter of p21 [155]. Constitutive c-Jun expression resulted in apoptosis in response to UV, while cells lacking c-Jun underwent prolonged cell cycle arrest. Consistent with this observation is a report that chemical inhibition of JNK caused G2/M arrest of three breast cancer cell lines. It did not,

however, have uniform effects on DNA replication; and it imposed a block to apoptosis that was stimulus-dependent and cell line dependent [156].

The above reports indicate that the availability of substrates for JNK plays a role in determining the outcome of JNK activation. P53 state is a factor, but certainly not the only one [149], as JNK can cause cell death also in absence of P53. While from the presented data it is clear that neither JNK1 nor JNK2 would appear to have a purely anti-oncogenic role, JNK3 might, as it has been found to be mutated in cell lines from human brain tumors [157].

Remarkably, embryonic stem cells deficient in stress-induced JNK activation, proceeded normally to apoptosis, and were only impaired in cytokine gene expression [158]. This indicates that embryonic stem cells possess stress sensors that are turned off in subsequent cell lineages. During gastrulation, however, the capacity of JNK to mediate apoptosis has been established, as transmembrane receptor-triggered apoptotic cell death in gastrulating *Xenopus* embryos was blocked by a dominant inhibitor of JNK [159, 160]. In JNK2 null mouse embryonic fibroblasts, TNF induction of the proteases cathepsin (cytosolic, implicated in lysosomal pathway for cell death) and caspase (implicated in mitochondrial cell death) was impaired compared to wild type cells. The JNK2 null cells were also resistant to TNF-induced apoptosis, as could be expected [161].

JNK involvement, under certain conditions, in differentiation of T helper cells has also been established [9, 61], demonstrating the versatility of JNK in the control of cell phenotype. Additionally, experiments in JNK minus mice have indicated that JNKs are essential for embryonic development [162, 163]. Both JNK1 and -2 are required for neural tube formation and normal brain development (Fig. 3).

The effects of JNK on gene expression can be complex. Some genes can be either activated or repressed by JNK. The outcome depends on signal integration on the promoter of such genes. Signals from JNK, but also from many other transducers, converge there [10, 12, 93, 164, 165]. Signal integration on gene promoters, furthermore, depends not only on the transcription factor binding sites, but also on their relative position [9]. In addition to that, the recruitment of regulatory proteins onto a promoter can also happen indirectly, without any specific DNA target sites for these proteins within the promoter. Protein–protein interaction with DNA-bound transactivators that have specific target sites in the promoter enables regulators that do not recognize any specific DNA site within the promoter with high enough affinity to be included in the set of transcription factors that regulate the given gene [166]. Thus, the expression of a particular gene might respond to a great number of different conditions through a relatively simple arrangement of regulatory elements.

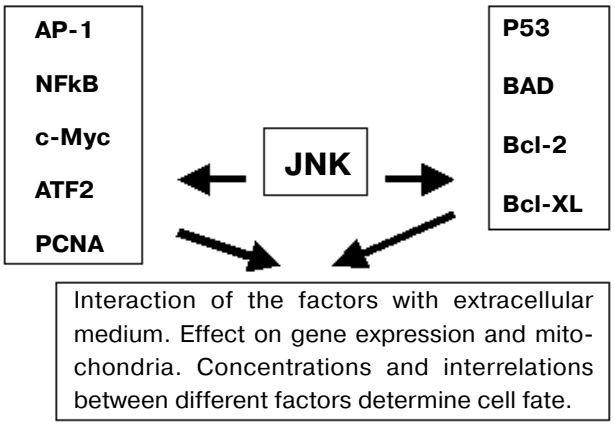


**Fig. 3.** Cell fate depends on factors controlled by JNK, some of which are depicted, and also on other factors that are not regulated by JNK. Combinatorial interaction of all these factors has potential to affect processes such as expression of PCNA, cyclins, antiapoptotic factors, or proapoptotic factors (FasL (Ligand for first autoimmune disease antigen), release of mitochondrial cytochrome *c*, caspase activation). The sum of the effects of all of these factors determines ultimate outcome as cell survival or death, or cell proliferation or differentiation.

While it is evident that interactions of JNK with mitochondrial proteins such as Bcl-2 or Bax play a major role in control of apoptosis, cell fate, when it requires new protein synthesis, can be also monitored at the level of the gene promoter, where multiple signals may be integrated. Due to the fact, that most endpoints of JNK-mediated signal cascades are well characterized with respect to their influence on cell fate, assaying regulation of those endpoints should allow a detailed dissection of the effects of JNK on cell fate. This should have a profound impact on our understanding of cancer cell physiology and other phenomena involving JNK responses to internal and environmental factors, because it is evident that JNK is at the core of a mechanism that integrates the feedback from multiple pathways to determine cell fate.

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