## 1. Phylogeny

Mitogen‐activated protein kinase 8 (MAPK8), commonly known as c‑Jun N‐terminal kinase 1 (JNK1), belongs to the stress‐activated subgroup of mitogen‐activated protein kinases (MAPKs) within the CMGC group of protein kinases. JNK1 is encoded by the MAPK8 gene, and together with its paralogues MAPK9 (JNK2) and MAPK10 (JNK3), it forms a conserved family that arose early in eukaryotic evolution through gene duplication events (bogoyevitch2006usesforjnk pages 1-2). JNK1 and JNK2 are ubiquitously expressed across mammalian tissues, whereas JNK3 has a more restricted expression pattern in brain, heart, and testis (bogoyevitch2006usesforjnk pages 2-3, bubici2014jnksignallingin pages 1-2). Phylogenetic analyses indicate that the JNK family is evolutionarily conserved and that the structural features, including the catalytic domain and the docking interface necessary for substrate and regulator binding, are preserved across species from yeast to mammals (orand2023revealingthemechanism pages 41-45). In the context of the larger kinome, JNK1 clusters with other stress‐responsive MAPKs, such as p38 isoforms, and its evolutionary history traces back to early duplication events that expanded the MAPK modules necessary for responding to diverse environmental and cellular stress signals (kyriakis2012mammalianmapksignal pages 3-5, li2011evolutionaryhistoryof pages 11-12).

## 2. Reaction Catalyzed

MAPK8/JNK1 functions as a serine/threonine protein kinase catalyzing the transfer of a phosphate group from ATP to specific serine or threonine residues on substrate proteins. The chemical reaction can be summarized as:  
  ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺  
This phosphorylation reaction is central to the activation or inhibition of substrate functions and is characterized by a proline-directed phosphorylation mechanism whereby a proline residue typically immediately follows the phosphoacceptor site (bogoyevitch2006usesforjnk pages 4-6, chen2011mapk8(mitogenactivatedprotein pages 1-2)). In addition to transcription factors such as c‑Jun, MAPK8/JNK1 phosphorylates a variety of substrates including replication licensing factors, proteins involved in apoptosis (e.g., p53), cytoskeletal regulators like STMN2, and components involved in circadian regulation. The kinase displays a sequential and sometimes random mechanism of phosphorylation on substrates whereby docking interactions serve to enhance specificity and efficiency (orand2023revealingthemechanism pages 25-29).

## 3. Cofactor Requirements

The enzymatic activity of MAPK8/JNK1 is contingent upon several essential cofactors and ions. As with most protein kinases, JNK1 requires ATP as the phosphate donor during the phosphorylation reaction (chen2011mapk8(mitogenactivatedprotein pages 1-2). Additionally, divalent metal ions, particularly Mg²⁺, are required to stabilize the ATP molecule and correctly orient the phosphate group within the catalytic cleft (coffey2014nuclearandcytosolic pages 1-2, kyriakis2012mammalianmapksignal pages 2-3). These cofactors are indispensable for efficient catalysis and for maintaining the proper conformation of the kinase domain during the phosphorylation cycle.

## 4. Substrate Specificity

MAPK8/JNK1 exhibits a broad substrate specificity that is tightly regulated by the peptide sequence surrounding the phosphorylation site and by docking interactions that enhance substrate affinity. Its primary substrate recognition motif is characterized as a proline-directed serine/threonine site, in which the phosphorylatable residue is immediately followed by a proline (bogoyevitch2006usesforjnk pages 4-6, bogoyevitch2006usesforjnk pages 25-26). Physiologically, JNK1 phosphorylates numerous transcription factors, most notably c‑Jun – phosphorylating serines 63 and 73 – which leads to the modulation of activator protein 1 (AP‑1) transcriptional activity (bubici2014jnksignallingin pages 1-2, sehgal2013networkmotifsin pages 3-4). In addition to c‑Jun, JNK1 targets other proteins such as ATF2, JDP2, and p53, thereby integrating stress and apoptotic signals into changes in gene expression. Substrate recognition is further enhanced by specific docking sites (often termed D‑domains or common docking motifs) present both on JNK1 and on its substrates, facilitating transient, yet highly specific interactions (bogoyevitch2006usesforjnk pages 30-31, sehgal2013networkmotifsin pages 4-5). Such docking interactions allow JNK1 to distinguish its physiological targets even amongst substrates with overlapping consensus motifs.

## 5. Structure

The three-dimensional structure of MAPK8/JNK1 is characterized by a conserved bilobal kinase domain typical of the MAPK family. The N-terminal lobe is rich in β-strands, including a glycine-rich loop (G-loop) that contributes to ATP binding, while the C-terminal lobe is predominantly α-helical and houses the catalytic loop and activation segment (bogoyevitch2006usesforjnk pages 2-3, coffey2014nuclearandcytosolic pages 1-2). A key structural element is the activation loop, which contains the Thr-X-Tyr (TPY) motif; dual phosphorylation on these residues by MKK4 and MKK7 is necessary for full catalytic activation (kyriakis2012mammalianmapksignal pages 10-11). Docking sites on the surface of JNK1––characterized by acidic residues––mediate interactions with basic docking motifs present in substrates and scaffold proteins (bogoyevitch2006usesforjnk pages 28-29, bardwell2015twohydrophobicresidues pages 14-14). Structural studies using crystallography have detailed these interactions and have provided insights into inhibitor binding, with multiple PDB deposits available for JNK complexes. Overall, the architecture of JNK1 includes a conserved kinase fold with essential motifs for ATP binding, substrate docking, and activation loop phosphorylation that are critical for its function and regulation.

## 6. Regulation

MAPK8/JNK1 is regulated by a complex interplay of upstream kinases, docking proteins, and phosphatases. Activation of JNK1 occurs through dual phosphorylation on threonine and tyrosine residues within its activation loop by the dual-specificity kinases MKK4 and MKK7, which themselves are activated in response to various stress stimuli such as pro-inflammatory cytokines, oxidative stress, and UV radiation (bogoyevitch2006usesforjnk pages 1-2, kyriakis2012mammalianmapksignal pages 2-3). Scaffold proteins, particularly members of the JNK-interacting protein (JIP) family, facilitate the assembly of signaling complexes by binding upstream MAP3Ks, MAP2Ks, and JNK1, thereby ensuring efficient signal relay and substrate specificity (kyriakis2012mammalianmapksignal pages 16-17, sehgal2013networkmotifsin pages 3-4). In addition to these kinases and adaptors, dual-specificity phosphatases (DUSPs) serve as negative regulators by dephosphorylating the active JNK1, thereby modulating the duration and amplitude of its signaling (ha2019phosphorylationdynamicsof pages 7-9). Other regulatory mechanisms involve protein–protein interactions that either promote or inhibit JNK1 activity when bound to cofactors or inhibitory proteins. For instance, interactions with regulatory proteins such as β-arrestin or Hsp72 can influence JNK1’s subcellular localization and stability, further fine-tuning its activity (bogoyevitch2006usesforjnk pages 4-6, sehgal2013networkmotifsin pages 5-5).

## 7. Function

MAPK8/JNK1 plays multifaceted roles in cellular signaling, integrating diverse extracellular and intracellular stress signals into appropriate cellular responses. It is involved in the regulation of cell proliferation, differentiation, migration, transformation, and programmed cell death. Upon activation, JNK1 phosphorylates key transcription factors, particularly c‑Jun, which is a core component of the AP‑1 transcription factor complex, thereby modulating the expression of genes associated with inflammatory responses, apoptosis, and cell survival (bubici2014jnksignallingin pages 1-2, bogoyevitch2006usesforjnk pages 1-2). In the context of cell cycle regulation, JNK1 phosphorylates the replication licensing factor CDT1, disrupting its interaction with the histone acetylase HBO1 and thereby influencing replication initiation (Information section, PubMed:21856198). Furthermore, JNK1 mediates stress‐induced apoptosis by targeting factors such as p53 and YAP1, and it is also known to phosphorylate BAD to promote erythroid cell survival upon EPO stimulation (Information section, PubMed:21095239). In neuronal systems, JNK1 regulates microtubule dynamics and neurite elongation through phosphorylation of substrates like STMN2, affecting both cytoskeletal remodeling and neuronal migration (Information section and castrotorres2020involvementofjnk1 pages 15-17). Additional roles include the modulation of autophagy via BCL2 phosphorylation, regulation of circadian rhythms through phosphorylation of the CLOCK-BMAL1 heterodimer, and the control of cellular responses to oxidative stress by targeting proteins such as EIF4ENIF1 and SIRT6 (Information section, PubMed:22441692, PubMed:27568560). JNK1’s diverse substrate profile underpins its involvement in numerous physiological processes and pathologies including neurodegeneration, cancer, inflammatory diseases, and metabolic disorders (wagner2009signalintegrationby pages 1-2, cicenas2015jnkinhibitorsis pages 7-7).

## 8. Other Comments

Owing to its central role in stress signaling and apoptosis, MAPK8/JNK1 is an attractive therapeutic target. Several small molecule inhibitors and peptide-based inhibitors have been developed to modulate JNK1 activity. For example, reversible covalent inhibitors targeting specific cysteine residues in JNK1 have been designed with precision-guided Michael-acceptor warheads to achieve high selectivity (balint2024reversiblecovalentcjun pages 17-18). Peptide inhibitors that interfere with JNK docking interactions have demonstrated promising results in reducing tissue damage in myocardial ischemia-reperfusion injury and in mitigating tau hyperphosphorylation in neurodegenerative disease models (cicenas2015jnkinhibitorsis pages 7-7). Disease associations with aberrant JNK1 activity include cancer, where dysregulation of apoptotic signaling can contribute to tumor progression or suppression depending on context, as well as inflammatory conditions and neurodegenerative disorders such as Alzheimer’s disease (wagner2009signalintegrationby pages 8-9, rehfeldt2020cjunnterminalkinase pages 9-11). Current research is actively focused on identifying more selective JNK inhibitors and elucidating the isoform-specific roles of JNK1 compared to JNK2 and JNK3, particularly through the analysis of differential substrate specificity and regulatory interactions (orand2023revealingthemechanism pages 33-38, bogoyevitch2006usesforjnk pages 25-26). Notable mutations within the MAPK8 gene that impact kinase regulation have been identified in model systems, and further characterization of these mutations may provide insights into pathological mechanisms and potential therapeutic interventions.

## 9. References

1. bogoyevitch2006usesforjnk pages 1-2
2. bogoyevitch2006usesforjnk pages 2-3
3. bogoyevitch2006usesforjnk pages 4-6
4. bogoyevitch2006usesforjnk pages 25-26
5. bogoyevitch2006usesforjnk pages 28-29
6. bubici2014jnksignallingin pages 1-2
7. castrotorres2020involvementofjnk1 pages 15-17
8. chen2011mapk8(mitogenactivatedprotein pages 1-2
9. coffey2014nuclearandcytosolic pages 1-2
10. ha2019phosphorylationdynamicsof pages 7-9
11. kyriakis2012mammalianmapksignal pages 2-3
12. kyriakis2012mammalianmapksignal pages 3-5
13. kyriakis2012mammalianmapksignal pages 7-8
14. kyriakis2012mammalianmapksignal pages 10-11
15. kyriakis2012mammalianmapksignal pages 16-17
16. li2011evolutionaryhistoryof pages 11-12
17. orand2023revealingthemechanism pages 25-29
18. orand2023revealingthemechanism pages 33-38
19. orand2023revealingthemechanisma pages 41-45
20. sehgal2013networkmotifsin pages 3-4
21. sehgal2013networkmotifsin pages 4-5
22. sehgal2013networkmotifsin pages 5-5
23. wagner2009signalintegrationby pages 1-2
24. wagner2009signalintegrationby pages 8-9
25. wagner2009signalintegrationby pages 12-13
26. rehfeldt2020cjunnterminalkinase pages 9-11
27. roux2004erkandp38 pages 1-2
28. balint2024reversiblecovalentcjun pages 17-18
29. bardwell2015twohydrophobicresidues pages 14-14
30. cicenas2015jnkinhibitorsis pages 7-7

References

1. (bogoyevitch2006usesforjnk pages 1-2): Marie A. Bogoyevitch and Bostjan Kobe. Uses for jnk: the many and varied substrates of the c-jun n-terminal kinases. Microbiology and Molecular Biology Reviews, 70:1061-1095, Dec 2006. URL: https://doi.org/10.1128/mmbr.00025-06, doi:10.1128/mmbr.00025-06. This article has 788 citations and is from a domain leading peer-reviewed journal.
2. (bogoyevitch2006usesforjnk pages 2-3): Marie A. Bogoyevitch and Bostjan Kobe. Uses for jnk: the many and varied substrates of the c-jun n-terminal kinases. Microbiology and Molecular Biology Reviews, 70:1061-1095, Dec 2006. URL: https://doi.org/10.1128/mmbr.00025-06, doi:10.1128/mmbr.00025-06. This article has 788 citations and is from a domain leading peer-reviewed journal.
3. (bogoyevitch2006usesforjnk pages 30-31): Marie A. Bogoyevitch and Bostjan Kobe. Uses for jnk: the many and varied substrates of the c-jun n-terminal kinases. Microbiology and Molecular Biology Reviews, 70:1061-1095, Dec 2006. URL: https://doi.org/10.1128/mmbr.00025-06, doi:10.1128/mmbr.00025-06. This article has 788 citations and is from a domain leading peer-reviewed journal.
4. (bogoyevitch2006usesforjnk pages 4-6): Marie A. Bogoyevitch and Bostjan Kobe. Uses for jnk: the many and varied substrates of the c-jun n-terminal kinases. Microbiology and Molecular Biology Reviews, 70:1061-1095, Dec 2006. URL: https://doi.org/10.1128/mmbr.00025-06, doi:10.1128/mmbr.00025-06. This article has 788 citations and is from a domain leading peer-reviewed journal.
5. (bubici2014jnksignallingin pages 1-2): Concetta Bubici and Salvatore Papa. Jnk signalling in cancer: in need of new, smarter therapeutic targets. British Journal of Pharmacology, Jan 2014. URL: https://doi.org/10.1111/bph.12432, doi:10.1111/bph.12432. This article has 434 citations and is from a highest quality peer-reviewed journal.
6. (castrotorres2020involvementofjnk1 pages 15-17): Rubén Castro-Torres, Oriol Busquets, Antoni Parcerisas, Ester Verdaguer, Jordi Olloquequi, Miren Ettcheto, Carlos Beas-Zarate, Jaume Folch, Antoni Camins, and Carme Auladell. Involvement of jnk1 in neuronal polarization during brain development. Cells, 9:1897, Aug 2020. URL: https://doi.org/10.3390/cells9081897, doi:10.3390/cells9081897. This article has 16 citations and is from a peer-reviewed journal.
7. (chen2011mapk8(mitogenactivatedprotein pages 1-2): F Chen. Mapk8 (mitogen-activated protein kinase 8). Atlas of Genetics and Cytogenetics in Oncology and Haematology, Feb 2011. URL: https://doi.org/10.4267/2042/37949, doi:10.4267/2042/37949. This article has 7 citations and is from a peer-reviewed journal.
8. (coffey2014nuclearandcytosolic pages 1-2): Eleanor T. Coffey. Nuclear and cytosolic jnk signalling in neurons. Nature Reviews Neuroscience, 15:285-299, Apr 2014. URL: https://doi.org/10.1038/nrn3729, doi:10.1038/nrn3729. This article has 379 citations and is from a highest quality peer-reviewed journal.
9. (ha2019phosphorylationdynamicsof pages 7-9): Jain Ha, Eunjeong Kang, Jihye Seo, and Sayeon Cho. Phosphorylation dynamics of jnk signaling: effects of dual-specificity phosphatases (dusps) on the jnk pathway. International Journal of Molecular Sciences, 20:6157, Dec 2019. URL: https://doi.org/10.3390/ijms20246157, doi:10.3390/ijms20246157. This article has 74 citations and is from a peer-reviewed journal.
10. (kyriakis2012mammalianmapksignal pages 2-3): John M. Kyriakis and Joseph Avruch. Mammalian mapk signal transduction pathways activated by stress and inflammation: a 10-year update. Physiological Reviews, 92:689-737, Apr 2012. URL: https://doi.org/10.1152/physrev.00028.2011, doi:10.1152/physrev.00028.2011. This article has 1590 citations and is from a highest quality peer-reviewed journal.
11. (orand2023revealingthemechanism pages 41-45): T Orand. Revealing the mechanism of action of intrinsically disordered proteins in mapk cell signalling. Unknown journal, 2023.
12. (sehgal2013networkmotifsin pages 3-4): Vasudha Sehgal and Prahlad T. Ram. Network motifs in jnk signaling. Genes & Cancer, 4:409-413, Sep 2013. URL: https://doi.org/10.1177/1947601913507577, doi:10.1177/1947601913507577. This article has 82 citations.
13. (sehgal2013networkmotifsin pages 4-5): Vasudha Sehgal and Prahlad T. Ram. Network motifs in jnk signaling. Genes & Cancer, 4:409-413, Sep 2013. URL: https://doi.org/10.1177/1947601913507577, doi:10.1177/1947601913507577. This article has 82 citations.
14. (sehgal2013networkmotifsin pages 5-5): Vasudha Sehgal and Prahlad T. Ram. Network motifs in jnk signaling. Genes & Cancer, 4:409-413, Sep 2013. URL: https://doi.org/10.1177/1947601913507577, doi:10.1177/1947601913507577. This article has 82 citations.
15. (bogoyevitch2006usesforjnk pages 28-29): Marie A. Bogoyevitch and Bostjan Kobe. Uses for jnk: the many and varied substrates of the c-jun n-terminal kinases. Microbiology and Molecular Biology Reviews, 70:1061-1095, Dec 2006. URL: https://doi.org/10.1128/mmbr.00025-06, doi:10.1128/mmbr.00025-06. This article has 788 citations and is from a domain leading peer-reviewed journal.
16. (kyriakis2012mammalianmapksignal pages 10-11): John M. Kyriakis and Joseph Avruch. Mammalian mapk signal transduction pathways activated by stress and inflammation: a 10-year update. Physiological Reviews, 92:689-737, Apr 2012. URL: https://doi.org/10.1152/physrev.00028.2011, doi:10.1152/physrev.00028.2011. This article has 1590 citations and is from a highest quality peer-reviewed journal.
17. (kyriakis2012mammalianmapksignal pages 16-17): John M. Kyriakis and Joseph Avruch. Mammalian mapk signal transduction pathways activated by stress and inflammation: a 10-year update. Physiological Reviews, 92:689-737, Apr 2012. URL: https://doi.org/10.1152/physrev.00028.2011, doi:10.1152/physrev.00028.2011. This article has 1590 citations and is from a highest quality peer-reviewed journal.
18. (kyriakis2012mammalianmapksignal pages 3-5): John M. Kyriakis and Joseph Avruch. Mammalian mapk signal transduction pathways activated by stress and inflammation: a 10-year update. Physiological Reviews, 92:689-737, Apr 2012. URL: https://doi.org/10.1152/physrev.00028.2011, doi:10.1152/physrev.00028.2011. This article has 1590 citations and is from a highest quality peer-reviewed journal.
19. (kyriakis2012mammalianmapksignal pages 7-8): John M. Kyriakis and Joseph Avruch. Mammalian mapk signal transduction pathways activated by stress and inflammation: a 10-year update. Physiological Reviews, 92:689-737, Apr 2012. URL: https://doi.org/10.1152/physrev.00028.2011, doi:10.1152/physrev.00028.2011. This article has 1590 citations and is from a highest quality peer-reviewed journal.
20. (li2011evolutionaryhistoryof pages 11-12): Meng Li, Jun Liu, and Chiyu Zhang. Evolutionary history of the vertebrate mitogen activated protein kinases family. PLoS ONE, 6:e26999, Oct 2011. URL: https://doi.org/10.1371/journal.pone.0026999, doi:10.1371/journal.pone.0026999. This article has 96 citations and is from a peer-reviewed journal.
21. (orand2023revealingthemechanism pages 25-29): T Orand. Revealing the mechanism of action of intrinsically disordered proteins in mapk cell signalling. Unknown journal, 2023.
22. (orand2023revealingthemechanism pages 33-38): T Orand. Revealing the mechanism of action of intrinsically disordered proteins in mapk cell signalling. Unknown journal, 2023.
23. (orand2023revealingthemechanisma pages 41-45): T Orand. Revealing the mechanism of action of intrinsically disordered proteins in mapk cell signalling. Unknown journal, 2023.
24. (rehfeldt2020cjunnterminalkinase pages 9-11): Stephanie Cristine Hepp Rehfeldt, Fernanda Majolo, Márcia Inês Goettert, and Stefan Laufer. C-jun n-terminal kinase inhibitors as potential leads for new therapeutics for alzheimer’s diseases. International Journal of Molecular Sciences, 21:9677, Dec 2020. URL: https://doi.org/10.3390/ijms21249677, doi:10.3390/ijms21249677. This article has 45 citations and is from a peer-reviewed journal.
25. (roux2004erkandp38 pages 1-2): Philippe P. Roux and John Blenis. Erk and p38 mapk-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiology and Molecular Biology Reviews, 68:320-344, Jun 2004. URL: https://doi.org/10.1128/mmbr.68.2.320-344.2004, doi:10.1128/mmbr.68.2.320-344.2004. This article has 3345 citations and is from a domain leading peer-reviewed journal.
26. (wagner2009signalintegrationby pages 1-2): Erwin F. Wagner and Ángel R. Nebreda. Signal integration by jnk and p38 mapk pathways in cancer development. Nature Reviews Cancer, 9:537-549, Aug 2009. URL: https://doi.org/10.1038/nrc2694, doi:10.1038/nrc2694. This article has 3021 citations and is from a domain leading peer-reviewed journal.
27. (wagner2009signalintegrationby pages 12-13): Erwin F. Wagner and Ángel R. Nebreda. Signal integration by jnk and p38 mapk pathways in cancer development. Nature Reviews Cancer, 9:537-549, Aug 2009. URL: https://doi.org/10.1038/nrc2694, doi:10.1038/nrc2694. This article has 3021 citations and is from a domain leading peer-reviewed journal.
28. (wagner2009signalintegrationby pages 8-9): Erwin F. Wagner and Ángel R. Nebreda. Signal integration by jnk and p38 mapk pathways in cancer development. Nature Reviews Cancer, 9:537-549, Aug 2009. URL: https://doi.org/10.1038/nrc2694, doi:10.1038/nrc2694. This article has 3021 citations and is from a domain leading peer-reviewed journal.
29. (balint2024reversiblecovalentcjun pages 17-18): Dániel Bálint, Ádám Levente Póti, Anita Alexa, Péter Sok, Krisztián Albert, Lili Torda, Dóra Földesi-Nagy, Dániel Csókás, Gábor Turczel, Tímea Imre, Eszter Szarka, Ferenc Fekete, Isabel Bento, Márton Bojtár, Roberta Palkó, Pál Szabó, Katalin Monostory, Imre Pápai, Tibor Soós, and Attila Reményi. Reversible covalent c-jun n-terminal kinase inhibitors targeting a specific cysteine by precision-guided michael-acceptor warheads. Nature Communications, Oct 2024. URL: https://doi.org/10.1038/s41467-024-52573-2, doi:10.1038/s41467-024-52573-2. This article has 4 citations and is from a highest quality peer-reviewed journal.
30. (bardwell2015twohydrophobicresidues pages 14-14): A. Jane Bardwell and Lee Bardwell. Two hydrophobic residues can determine the specificity of mitogen-activated protein kinase docking interactions. Journal of Biological Chemistry, 290:26661-26674, Oct 2015. URL: https://doi.org/10.1074/jbc.m115.691436, doi:10.1074/jbc.m115.691436. This article has 33 citations and is from a domain leading peer-reviewed journal.
31. (bogoyevitch2006usesforjnk pages 25-26): Marie A. Bogoyevitch and Bostjan Kobe. Uses for jnk: the many and varied substrates of the c-jun n-terminal kinases. Microbiology and Molecular Biology Reviews, 70:1061-1095, Dec 2006. URL: https://doi.org/10.1128/mmbr.00025-06, doi:10.1128/mmbr.00025-06. This article has 788 citations and is from a domain leading peer-reviewed journal.
32. (cicenas2015jnkinhibitorsis pages 7-7): Jonas Cicenas. Jnk inhibitors: is there a future? MAP Kinase, Dec 2015. URL: https://doi.org/10.4081/mk.2015.5700, doi:10.4081/mk.2015.5700. This article has 20 citations.