Regulation of protein trafficking
JNK3 at the Golgi complex

Guang Yang[†] and Max S Cynader

Brain Research Centre; University of British Columbia; Vancouver, British Columbia, Canada

[†]Current affiliation: Hospital for Sick Children; Toronto, Ontario, Canada

In many neurological diseases, pathological stress causes functional deficits of neurons before cell death occurs. These neuronal deficiencies may be caused by compromised Golgi complex functions, leading to impaired sorting of receptors and other key components to the cell surface.[1] Although the dysregulation of protein secretion has been observed in neurological conditions, the molecular links between neuronal stress and the secretion system at the Golgi complex remain elusive.

Members of the c-Jun N-terminal kinase (JNK) family, in particular JNK3, are key players in the stress response in neurons.[2] We hypothesized that JNK3 may be involved in modulating Golgi functions in response to neuronal stress and examined this possibility in our recent work.[3] Several lines of evidence support this idea. First, JNK3 in neurons undergoes isoform-specific palmitoylation, a lipid modification that generally enhances the hydrophobicity of proteins and regulates their association with membrane structures. Palmitoylation of JNK3 impedes axon growth, a process that relies on active Golgi functions.[4] Second, palmitoylation directs JNK3 to the Golgi complex. Fusing a pseudo-constitutive palmitoylation motif to JNK3 or introducing palmitoyl acyltransferases (PATs) to palmitoylate JNK3 promotes enrichment of JNK3 at the Golgi.[3] Third, JNK3 palmitoylation and translocation to the Golgi are enhanced by neuronal stress,[3] and fourth, depletion of JNK3 attenuates the stress-induced suppression of surface delivery of glutamate receptor GluR1

subunits. These observations motivated us to further investigate how JNK3 links neuronal stress to secretory trafficking via its translocation to the Golgi.

By examining the trafficking of the marker protein VSV-G and several neuronal synaptic and membrane proteins, we confirmed that JNK3 represses secretory trafficking at the Golgi in neurons. In a series of experiments with palmitoylation-deficient or kinase-deficient JNK3 mutants, we demonstrated that the stress-induced suppression of GluR1 trafficking is mediated via JNK3 palmitoylation, but is independent of JNK3's kinase activity. Instead of directing phosphorylation, JNK3 may interact with and recruit partners at the Golgi to inhibit protein secretion. One candidate is Sac1, a lipid phosphatase that converts Golgi-resident phosphatidylinositol 4-phosphate (PI4P) to phosphatidylinositol (PI).[5] PI4P is required for post-Golgi secretion.[6] By shuttling between the ER and the Golgi, Sac1 balances the amount of PI4P in the Golgi and controls protein secretion. Indeed, the level and location of Sac1 are critical for maintaining surface delivery of GluR1 in neurons.[3] We discovered that JNK3 interacts directly with Sac1, and that the interaction is strengthened by neuronal stress. Through this interaction, JNK3 enriches Sac1 in the Golgi, which then depletes the local pool of PI4P and represses secretory trafficking. Consistent with these findings, depletion of Sac1 attenuates the stress-induced impairment of GluR1 trafficking in neurons. After identification of the JNK3-binding motifs on Sac1, we were able to disrupt the

endogenous JNK3–Sac1 interaction with synthetic decoy peptides. Application of the decoy peptides efficiently rescued the surface delivery of GluR1, and maintained the integrity of synapses against stress-induced destruction. Our studies have therefore identified a molecular mechanism that links neuronal stress and secretory trafficking at the Golgi complex.[3]

It is worth noting that, in addition to JNK3, a palmitoylation motif is also present on JNK1, but not on the JNK2 isoform.[4] Surprisingly, the palmitoylation of JNK1 is not detectable in resting neurons.[4] However, in the presence of PATs, JNK1 can be palmitoylated and will then become enriched in the Golgi, though to a lesser extent than JNK3. In contrast, JNK2 does not show this behavior.[3] These observations identify palmitoylation as a novel mechanism for isoform-specific regulation of JNK signaling and localization. Our findings suggest that JNK3 is likely to be the major JNK isoform controlling secretory trafficking in neurons, whereas JNK1 may play a similar role in other cell types in which JNK3 is not present.

Among all 23 PATs, zDHHC17 (also known as HIP14) showed the highest efficiency in palmitoylating JNK3. It is thus possible that zDHHC17 is the major PAT in neurons that functions to palmitoylate JNK3 in response to neuronal stress. This is intriguing when considering that zDHHC17 is also able to activate JNK3 to induce neuronal cell death under excitotoxic stress conditions.[7] In this scenario, the ankyrin motifs of

zDHHC17 interact directly with JNK3 and its upstream kinase MKK7 to form a signaling module for JNK3 activation.[7] However, the high efficiency of zDHHC17 in palmitoylating JNK3 seems not to be due simply to its interaction with JNK3, because another PAT, zDHHC13, that binds JNK3 with similar ankyrin motifs only weakly palmitoylates JNK3, whereas zDHHC15, which does not interact with JNK3, also consistently palmitoylates it.[3] Other mechanisms may be used by zDHHC17 to achieve its high selectivity of palmitoylation on JNK3. Our work highlights a potential multilayer regulation of JNK3 signaling by zDHHC17 in neurons. Under different conditions, zDHHC17 may activate and/or palmitoylate JNK3 to induce neuronal cell death and/or secretion repression. We have found that blocking the zDHHC17-JNK3 interaction protects neurons against cell death induced by excitotoxic stress.[3,7] But stress-induced secretion suppression and synaptic loss can only be prevented if the JNK3-Sac1 interaction is blocked at the same time.[3] These observations suggest that combining synaptoprotection by strengthening Golgi functions and neuroprotection by preventing JNK3 activation may represent a potent therapeutic strategy for protection of neuronal functions under adverse conditions.

References

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