NSD3 keeps IRF3 active

In this issue of JEM, Wang et al. (https://doi.org/10.1084/jem.20170856) show a novel antiviral innate mechanism by which methyltransferase NSD3 directly monomethylates a transcription factor IRF3 and maintains IRF3 phosphorylation to enhance its transcriptional activity, consequently promoting antiviral innate immune responses.

Insight from Takashi Mino and Osamu Takeuchi

Antiviral innate immune responses are a critical first line of host defense against invading viral pathogens (Akira et al., 2006; Takeuchi and Akira, 2010; Moresco et al., 2011). Viral RNA and DNA is initially recognized by pattern recognition receptors (PRRs) such as TLRs, retinoic acid–inducible gene-I (RIG-I)–like receptors (RLRs), Nod-like receptors (NLRs), and cyclic GMP-AMP (cGAMP) synthase (cGAS). TLRs are transmembrane proteins recognizing microbial components on the cell surface or in the endosomes. Among the TLRs expressed on the endosome, TLR3, TLR7, and TLR9 sense viral double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and DNA with a CpG motif, respectively. In addition to TLRs, cytoplasmic RLRs, RIG-I and melanoma differentiation-associated gene 5 (MDA5) recognize 5′-triphosphate end dsRNA and long dsRNA, respectively (Yoneyama and Fujita, 2009). Cytosolic DNA sensor, cGAS, senses viral DNAs (Barrat et al., 2016). Extensive studies have revealed that the PRR signaling pathways lead to transcription of type I IFNs via transcription factors, including IFN-regulatory factor 3 (IRF3) and IRF7 (Honda et al., 2006). PRR signaling also activates another transcription factor, NF-κB, which contributes to the transactivation of proinflammatory cytokines as well as type I IFNs.

IRF3 and IRF7 are key transcription factors responsible for induction of type I IFNs by viral infection and play a critical role in host antiviral innate immunity (Banchereau and Pascual, 2006; Honda et al., 2006; Sadler and Williams, 2008). IRF3 is constitutively expressed and resides in the cytosol in its latent form. Posttranslational modifications (PTMs), including phosphorylation and polyubiquitination, are key features of signal transduction pathways that allow the modulation of protein function (Deribe et al., 2010; Mowen and David, 2014; Liu et al., 2016). Upon viral infection, PTMs can affect the activation of signaling molecules, as well as their cellular translocation, stabilization, or interaction with other molecules. Indeed, IRF3 undergoes phosphorylation by TBK1 and IKKε after the PRR signaling, which induces IRF3 dimerization and nuclear translocation, resulting in transcription of type I *IFN* mRNA (Fitzgerald et al., 2003; Takeuchi and Akira, 2009). In addition, unconventional PTMs, including methylation, acetylation, sumoylation, and succinylation, have also been implicated in the regulation of innate immune system (Mowen and David, 2014). However, the role of IRF3 methylation in antiviral responses has not been understood.

In this issue of JEM, Wang et al. demonstrate that monomethylation of IRF3 at lysine 366 (K366) is induced by infection with herpes simplex virus (HSV) and vesicular stomatitis virus (VSV). Methylation-defective substitution at K366 (K366A) significantly abolished IRF3-driven *Ifnb* activation and IFN-β production upon VSV infection. These data suggest that viral infection induces monomethylation of IRF3 at K366, which is responsible for promoting IRF3 activation and IFN-β production.

To identify methyltransferases mediating the K366-monomethylation of IRF3, the Wang et al. (2017) performed coimmunoprecipitation and mass spectrometry analysis. They found that a lysine methyltransferase, NSD3, directly binds to IRF3. The K366 methylation of IRF3 was inhibited by VSV infection in infected NSD3-deficient macrophages. Moreover, an in vitro methylation assay showed that NSD3 directly methylates IRF3. NSD3-deficient mice were more susceptible to VSV infection and showed a decreased level of IFN-β production in serum and organs, as well as increased VSV replication and titers in organs compared with control mice. These results show that NSD3 directly methylates IRF3 at K366 upon viral infection, and NSD3 is an essential methyltransferase for the production of type I IFN and antiviral innate responses. Although the authors demonstrated that NSD3 interacts with the IRF3 C-terminal region through its PWWP1 domain and that the NSD3-mediated IRF3 methylation occurs in the nucleus, it is not yet clear how NSD3 specifically methylates IRF3 at K366 upon viral infection. It is interesting to speculate that the activity of NSD3 to methylate IRF3 is also dynamically controlled by viral infection.

Wang et al. (2017) subsequently investigated mechanisms of how the NSD3-mediated IRF3 methylation regulates IRF3 activity. Interestingly, VSV-induced IRF3 phosphorylation at Ser388 requires NSD3, and NSD3-mediated IRF3 methylation suppressed the interaction of IRF3 with protein phosphatase 1 (PP1), which is involved in the regulation of IRF3 activity via dephosphorylation (Gu et al., 2014). These data demonstrate that NSD3 decreases the binding of PP1 to IRF3, preventing

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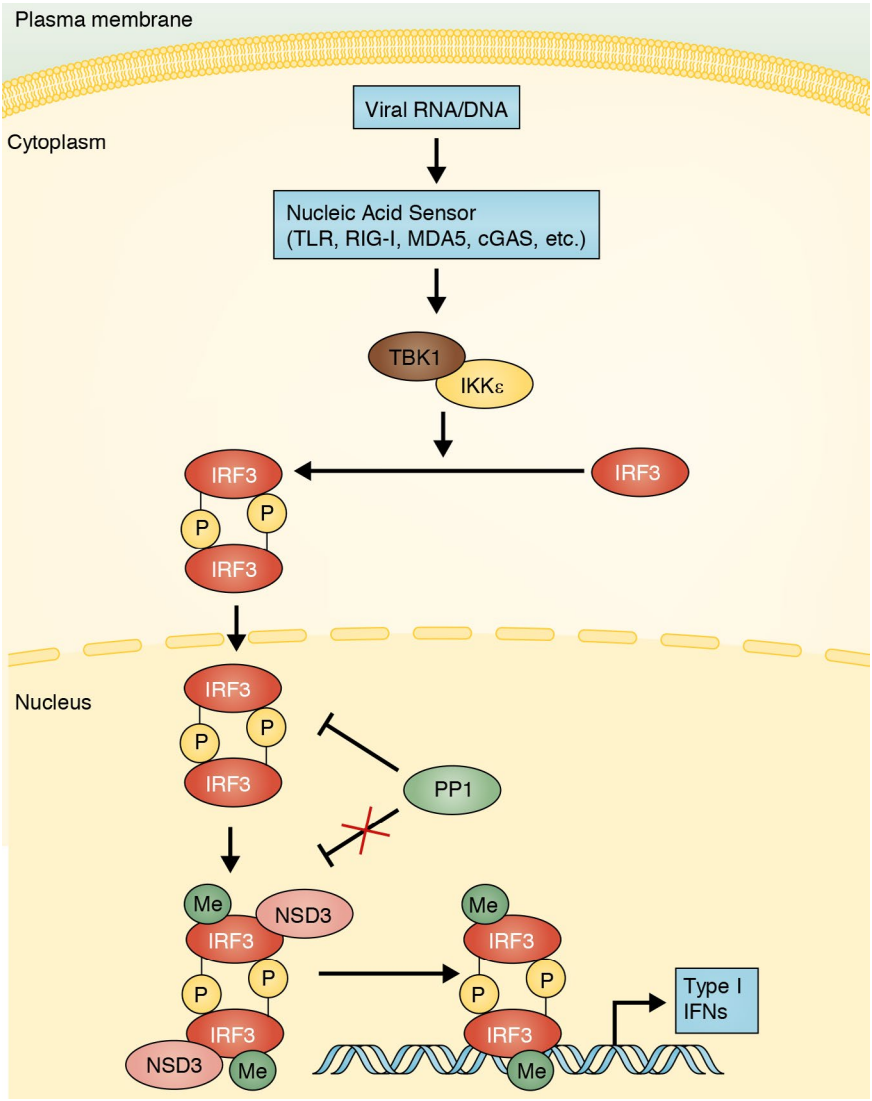
dephosphorylation of IRF3 by PP1 and consequently resulting in maintenance of IRF3 phosphorylation and IFN-β production (see figure). However, it is unclear how the NSD3-mediated IRF3 methylation blocks the binding of PP1 to IRF3. Because the PP1-binding domain of IRF3 does not overlap with the K366 site and the IRF3 methylation does not have a direct effect on the binding of PP1 to IRF3, it is interesting to speculate that potential proteins associating with methylated IRF3 block the binding of PP1 to IRF3. It will be interesting to investigate whether IRF7 methylation is also important for antiviral innate responses because IRF7 is also key transcription factor responsible for induction of type I IFNs by viral infection.

In summary, this study provides a novel layer of IRF3 regulation via NSD3-mediated regulation controlling IRF3 activity in response to viral infection. PP1-mediated dephosphorylation functions as a fail-safe system of the IRF3 activation, and NSD3-induced methylation seems to remove the safety device of type I IFN production. Given that dysregulated production of type IFNs is the cause of type I interferonopathy, NSD3 in addition to TBK1 and IKKε appear to be required for tight control of innate immunity (Crow and Manel, 2015). Antiviral immunoreactivity might potently be manipulated by controlling NSD3 activity by using an activator or inhibitor of NSD3 via IRF3-mediated IFN production. Future studies may further characterize the mechanisms and importance of PTMs in antiviral immune responses.



NSD3 maintains IRF3 phosphorylation to enhance its transcriptional activity, promoting antiviral innate immune response. Transcription factor IRF3 is phosphorylated upon viral infection, and the phosphorylated IRF3 undergoes dimerization and nuclear translocation. In the nucleus, methyltransferase NSD3 directly methylates IRF3 at K366, and the NSD3–mediated IRF3 methylation maintains phosphorylation of IRF3 by preventing IRF3 dephosphorylation via disrupting the association of PP1 with IRF3.

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