

## LETTER TO THE EDITOR

# Roles of neddylation against viral infections

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The ubiquitin-dependent proteasome pathway can be hijacked by certain viruses to maintain viral genome amplification. A key class of ubiquitin-E3s involved in this pathway is Cullin-RING ligases (CRLs), which are activated by an additional ubiquitin-like protein NEDD8.<sup>1</sup> The process by which the ubiquitin-like protein NEDD8 is conjugated to its target proteins is officially named ‘neddylation’. Consequently, neddylation inhibition, through the use of the pharmacological inhibitor MLN4924, might prevent viral genome amplification.<sup>2,3</sup> Herpesviruses, adenovirus, and influenza virus are MLN4924 susceptible, whereas vaccinia virus and vesicular stomatitis virus are not.<sup>2,3</sup> Neddylation might also promote viral replication independent of CRLs.<sup>4</sup> For example, hepatitis B virus-encoded X protein can act as an NEDD8 substrate and its neddylation by E3 ligase HDM2 enhances its stability and function in viral replication and hepatocarcinogenesis.<sup>4</sup> On the other hand, blockade of neddylation prevents the degradation of host restriction factors and thereby restores the restriction of human immunodeficiency virus by APOBEC3G and SAMHD1,<sup>5,6</sup>

lentivirus by SAMHD1,<sup>7</sup> or Rift Valley fever virus by protein kinase R.<sup>8</sup> Both CRLs-dependent and CRLs-independent mechanisms have been suggested in this regard.<sup>9</sup>

As for the innate immune response, the involvement of neddylation was suggested as early as the year 2006.<sup>10</sup> The transcription of type I interferon genes is controlled by nuclear factor- $\kappa$ B (NF- $\kappa$ B) and interferon regulatory factor (IRF) family members including IRF3. Many viruses execute immune-evasive activities by targeting the type I interferon signaling pathway. RNA viruses such as Sendi virus (SeV), vesicular stomatitis virus, and rotavirus induce the degradation of IRF3.<sup>10–12</sup> However, a herpesvirus family member, herpes simplex virus type I (HSV-1), a DNA virus, has no such effect.<sup>13</sup> Even though the degradation of IRF3 upon infection with rotavirus has been demonstrated to be independent of CRLs,<sup>11,12</sup> a Cullin-based ubiquitin ligase pathway was reported to be involved in SeV-induced IRF3 degradation in 2006.<sup>10</sup> Therefore, neddylation promotes IRF3 degradation upon SeV infection.<sup>10</sup> Theoretically, neddylation should promote IRF3-mediated transcription of type I interferon genes.

In addition to IRF family members, NF- $\kappa$ B also plays a key role in mediating the transcription of type I interferon genes. It is demonstrated that neddylation contributes to NF- $\kappa$ B activity by promoting the ubiquitination and subsequent degradation of I $\kappa$ B proteins since the ubiquitination of I $\kappa$ B proteins

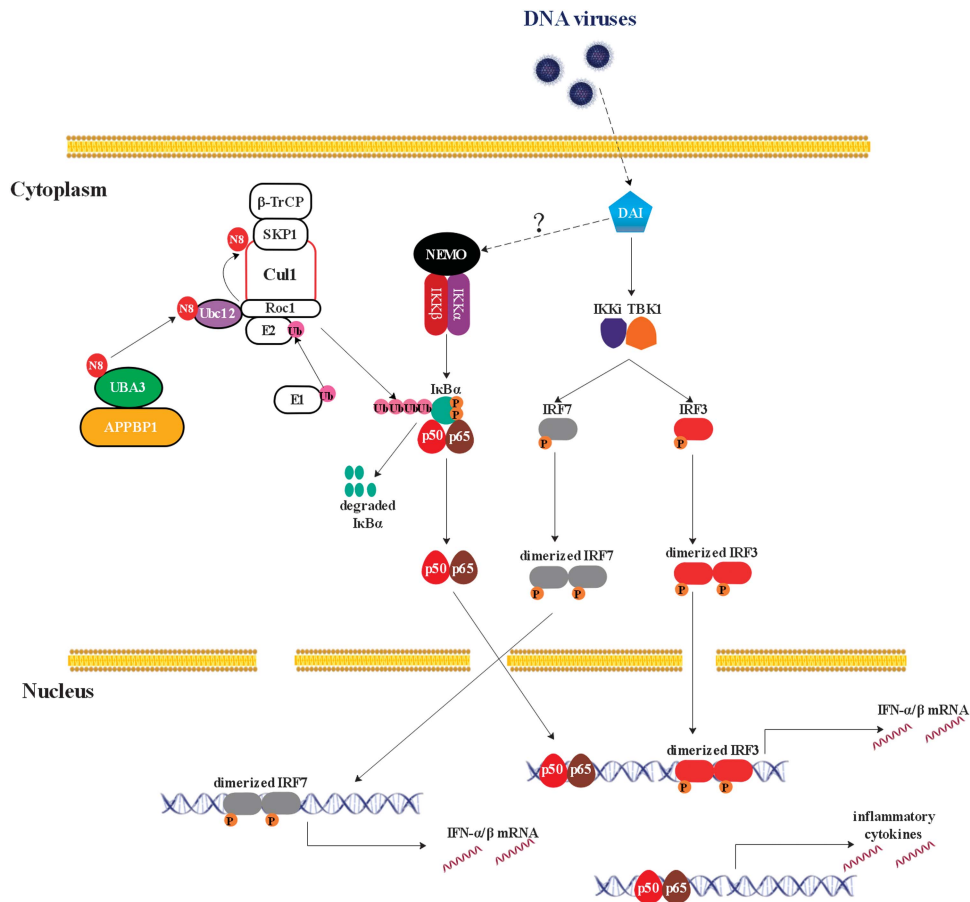
is carried out by certain CRLs members. Therefore, we speculated that neddylation should promote the HSV-1-induced production of type I interferons, and this hypothesis was confirmed in our study, which was recently published in *Cellular & Molecular Immunology* (Figure 1).<sup>14</sup> With primary macrophages deficient of the catalytic subunit of neddylation E1, we have provided the first evidence that neddylation indeed plays a role in type I interferon production. Interestingly, neddylation blockade only delays but does not completely abrogate HSV-1-induced p65 nuclear translocation.<sup>14</sup> Consequently, the early phase type I interferon production in HSV-1 infection is much more impaired than the late phase.<sup>14</sup> Recently, the notion that the host also exploits the neddylation pathway to fight against virus infection has been supported by additional evidence. Zhang *T et al.* reported that the polymerase basic protein 2 of influenza A virus can be covalently modified by NEDD8, which reduces its stability and blocks the replication of influenza A virus.<sup>15</sup>

Another group later reported that NEDD8 knockdown showed no effect on type I interferon production triggered by lipopolysaccharide (LPS), poly (I:C), or SeV.<sup>16</sup> Since poly (I:C) and SeV induce IRF3 degradation, whereas HSV-1 does not,<sup>10</sup> perhaps the interplay between the NF- $\kappa$ B pathway and IRF pathway determines the outcome. Intriguingly, MLN4924 significantly inhibited type I interferon production triggered by LPS, poly (I:C), or SeV,<sup>16</sup> which has been

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**Figure 1** Neddylation contributes to DNA virus-induced production of type I interferons. The transcription of type I interferon genes is controlled by nuclear factor- $\kappa$ B (NF- $\kappa$ B) and interferon regulatory factor (IRF) family members IRF3 and IRF7. DNA virus-induced NF- $\kappa$ B activation depends on the ubiquitination and subsequent degradation of I $\kappa$ B proteins. The ubiquitination of I $\kappa$ B proteins such as I $\kappa$ B $\alpha$  is carried out by a certain member of the Cullin-RING ligases (CRLs), that is, SKP1/Cullin-1/F-box protein  $\beta$ -TrCP/Roc1 complex. As CRLs are activated by the covalent conjugation of an additional ubiquitin-like protein NEDD8 to Cullins, neddylation promotes DNA virus-induced NF- $\kappa$ B activation and thereby promotes DNA virus-induced production of type I interferons.

attributed by the authors to its off-target effects. However, the potential off-target effects of small interfering RNAs and the relatively low efficiency of small RNA interference cannot be excluded. Future studies are required to address this issue.

Together, these 10 years of studies suggest that neddylation might exert distinct roles in the infection of different viruses. Targeting the neddylation pathway against virus infection, though hopeful under certain circumstances, should be cast with caution and requires extensive studies to clarify how neddylation works in each step of virus infection.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

- Enchev RI, Schulman BA, Peter M. Protein neddylation: beyond cullin-RING ligases. *Nat Rev Mol Cell Biol* 2015; **16**: 30–44.
- Hughes DJ, Wood JJ, Jackson BR, Baquero-Perez B, Whitehouse A. NEDDylation is essential for Kaposi's sarcoma-associated herpesvirus latency and lytic reactivation and represents a novel anti-KSHV target. *Plos Pathog* 2015; **11**: e1004771.
- Le-Trilling VT, Megger DA, Katschinski B, Landsberg CD, Rückborn MU, Tao S *et al*. Broad and potent antiviral activity of the NAE inhibitor MLN4924. *Sci Rep* 2016; **6**: 19977.
- Liu N, Zhang J, Yang X, Jiao T, Zhao X, Li W *et al*. HDM2 promotes NEDDylation of HBV HBx to enhance its stability and function. *J Virol* 2017; **91**: e00340–17.
- Stanley DJ, Bartholomeeusen K, Crosby DC, Kim DY, Kwon E, Yen L *et al*. Inhibition of a NEDD8 cascade restores restriction of HIV by APOBEC3G. *Plos Pathog* 2012; **8**: e1003085.
- Hofmann H, Norton TD, Schultz ML, Polsky SB, Sunseri N, Landau NR. Inhibition of CUL4A Neddylation causes a reversible block to SAMHD1-mediated restriction of HIV-1. *J Virol* 2013; **87**: 11741–11750.
- Wei W, Guo H, Liu X, Zhang H, Qian L, Luo K *et al*. A first-in-class NAE inhibitor, MLN4924, blocks lentiviral infection in myeloid cells by disrupting neddylation-dependent Vpx-mediated SAMHD1 degradation. *J Virol* 2014; **88**: 745–751.
- Mudhasani R, Tran JP, Retterer C, Kota KP, Whitehouse CA, Bavari S. Protein kinase R degradation is essential for rift valley fever virus infection and is regulated by SKP1-CUL1-F-box (SCF)FBXW11-NSs E3 ligase. *Plos Pathog* 2016; **12**: e1005437.
- Ramirez PW, DePaula-Silva AB, Szaniawski M, Barker E, Bosque A, Planelles V. HIV-1 Vpu utilizes both cullin-RING ligase (CRL) dependent and independent mechanisms to downmodulate host proteins. *Retrovirology* 2015; **12**: 65.
- Bibeau-Poirier A, Gravel SP, Clément JF, Rolland S, Rodier G, Coulombe P *et al*. Involvement of the I $\kappa$ B kinase (IKK)-related kinases tank-binding kinase 1/IKKi and cullin-based ubiquitin ligases in IFN regulatory factor-3 degradation. *J Immunol* 2006; **177**: 5059–5067.

- 11 Ding S, Mooney N, Li B, Kelly MR, Feng N, Loktev AV *et al.* Comparative proteomics reveals strain-specific beta-TrCP degradation via rotavirus NSP1 hijacking a host Cullin-3-Rbx1 complex. *Plos Pathog* 2016; **12**: e1005929.
- 12 Lutz LM, Pace CR, Arnold MM. Rotavirus NSP1 associates with components of the Cullin RING ligase family of E3 ubiquitin ligases. *J Virol* 2016; **90**: 6036–6048.
- 13 Collins SE, Noyce RS, Mossman KL. Innate cellular response to virus particle entry requires IRF3 but not virus replication. *J Virol* 2004; **78**: 1706–1717.
- 14 Zhang X, Ye Z, Pei Y, Qiu G, Wang Q, Xu Y *et al.* Neddylation is required for herpes simplex virus type 1 (HSV-1)-induced early phase interferon-beta production. *Cell Mol Immunol* 2016; **13**: 578–583.
- 15 Zhang T, Ye Z, Yang X, Qin Y, Hu Y, Tong X *et al.* NEDDylation of PB2 reduces its stability and blocks the replication of influenza A virus. *Sci Rep* 2017; **7**: 43691.
- 16 Song H, Huai W, Yu Z, Wang W, Zhao J, Zhang L *et al.* MLN4924, a first-in-class NEDD8-activating enzyme inhibitor, attenuates IFN-beta production. *J Immunol* 2016; **196**: 3117–3123.