Delicate coordination of TRIM21's dual activity in virus neutralization and signaling

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TRIM21 belongs to the tripartite motif (TRIM) family of ubiquitin E3 ligases. Approximately 100 TRIM proteins exist in humans, and they all share a common domain structure composed of a RING domain that confers the E3 ligase enzymatic activity, one or two B-boxes, and a coiled-coil domain (1). Many TRIM proteins play crucial roles in antiviral immune responses, often by targeting specific viral components. For example, TRIM5 α inhibits the infection of many retroviruses, including HIV-1, by binding to incoming viral capsids, leading to capsid dissociation and inhibition of reverse transcription of the viral genome. TRIM19, also known as promyelocytic leukemia protein, is a key component of subnuclear structures that restrict a variety of RNA and DNA viruses. Furthermore, TRIM proteins modulate signal transduction pathways that lead to antiviral cytokine production, ultimately contributing to the establishment of an antiviral state in both the infected and uninfected cell (1). For example, TRIM25 activates the innate immune sensor RIG-I, potentiating the production of antiviral cytokines, such as type-I interferons (IFN- α/β) (2). Recent studies

demonstrated that several TRIM proteins, including TRIM5 α and TRIM19, play dual roles in antiviral immunity by acting both as “effectors” that neutralize viral infection, and as “sensors” that induce innate immune signaling. It has also been shown that the ability of TRIM proteins to catalyze different types of ubiquitination is important for their effector and sensor functions. However, the molecular details of how the dual activity of TRIM proteins is temporally and mechanistically coordinated are not well understood. In PNAS, Fletcher et al. uncover a mechanism of stepwise ubiquitination and deubiquitination in synchronizing the sensor and effector functions of TRIM21, providing molecular-level insights into the antiviral activity of TRIM proteins (3).

TRIM21 is a cytosolic receptor for antibody-opsonized virus particles that mediates proteasomal degradation of the virus (4). In addition, upon detection of virus, TRIM21 initiates a signaling cascade that results in NF- κ B activation and subsequent production of proinflammatory cytokines (5). It remains unclear whether TRIM21's sensor and effector functions are mechanistically connected

and whether there is a molecular “switch” that coordinates both activities.

Ubiquitination is a posttranslational protein modification that is involved in a number of cellular processes in eukaryotes. Ubiquitin is a small protein of 76 amino acids that is usually attached to lysine residues of proteins by a cascade of enzymes. Ubiquitin molecules are first activated by E1 enzymes, then conjugated by E2 enzymes, and are finally ligated to the substrate by E3 ligases (6). Ubiquitination may result in monoubiquitinated or polyubiquitinated proteins. For polyubiquitination, either the N-terminal methionine or one of the seven internal lysine residues of ubiquitin (K6, K11, K27, K29, K33, K48, and K63) can be modified in consecutive conjugation cycles, leading to a chain of ubiquitins. Polyubiquitin chains can be either attached covalently to the substrate protein or bound noncovalently as unanchored “free” polyubiquitin chains. Whereas the E3 ligase determines the substrate specificity, it is the E2 enzyme that determines the type of polyubiquitin linkage that is made. Given the large number of ubiquitinated proteins in the cell, it is not surprising that more than 600 E3 ligases exist in humans that, together with ~ 40 different E2 enzymes (and two E1 enzymes), are responsible for the human “ubiquitinome.” To reverse the concerted action of E1, E2, and E3 enzymes, the human genome also encodes ~ 100 deubiquitinating enzymes (DUB), which remove mono- or polyubiquitin from substrate proteins.

Over the past several years, significant progress has been made on the roles of different polyubiquitin linkage types in determining the fate of the modified protein. K48-linked polyubiquitin chains are well known to be recognized by the proteasome, triggering the degradation of the modified substrate. In contrast, K63-linked ubiquitin chains—both the covalently attached and unanchored forms—are generally believed to not trigger the proteasomal degradation of the substrate

Fig. 1. Regulation of TRIM21's sensor and effector functions through stepwise ubiquitination and deubiquitination. Binding of TRIM21 to virus-immunoglobulin (Ig) complexes results in TRIM21 auto-monoubiquitination mediated by the E2 enzyme Ube2W. Monoubiquitination of TRIM21 serves as a substrate for K63-linked polyubiquitination mediated by Ube2N and Ube2V2. Translocation of TRIM21 to the proteasome leads to the degradation of virus-antibody complexes, as well as release of the K63-linked polyubiquitin chain from TRIM21 by the proteasome-associated enzyme Poh1. The released unanchored K63-linked polyubiquitin chains then trigger NF- κ B activation.

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protein, but rather have important roles in activating signal transduction pathways (6). Mechanistically, K63-linked polyubiquitin can activate signaling pathways by either stabilizing the substrate protein or inducing its multimeric active form, or by facilitating the recruitment of other signaling proteins. In particular, TRIM proteins have been implicated in the regulation of immune-defense pathways by modifying key signaling proteins with K63-linked polyubiquitin. Upon viral infection, TRIM25 mediates the K63-linked ubiquitination of the intracellular viral RNA sensor RIG-I, thereby inducing RIG-I tetramerization, which is necessary for RIG-I translocation to mitochondria where signaling is perpetuated (2, 7). In the case of TRIM21, *in vitro* experiments indicated that TRIM21, together with the E2 enzymes Ube2N/Ube2V1, can induce the formation of unanchored K63-polyubiquitin chains (5), which are known to play a role in TAK1-dependent NF- κ B activation (8). The mechanistic details, however, of how TRIM21 induces innate signaling in a virus-infected cell have not been well characterized. In particular, it remains unclear how the dual activity of TRIM21 in virus neutralization and proinflammatory signal initiation is coordinated.

Fletcher et al. (3) first tested whether NF- κ B activation by TRIM21 is a prerequisite for its ability to neutralize virus particles. Using various inhibitors of the proteasome and of NF- κ B activation, the authors show that the two functions of TRIM21 are indeed tightly linked. Furthermore, upon depletion of Ube2N, the virus could no longer be neutralized by TRIM21, indicating that the synthesis of K63-linked polyubiquitin precedes viral neutralization. In a subsequent series of experiments the authors depleted Ube2W, an E2 enzyme that has been reported to facilitate covalent K63-linked polyubiquitination mediated by Ube2N/Ube2V2. Depletion of Ube2W abolished both the TRIM21-mediated degradation of virus-Ig complexes as well as NF- κ B activation. Collectively, these results suggest that both Ube2W and Ube2N/Ube2V2 play roles in regulating TRIM21's dual antiviral activity.

In a series of elegant *in vitro* experiments, Fletcher et al. (3) demonstrate that Ube2W first facilitates TRIM21 auto-monoubiquitination, and that TRIM21 monoubiquitination then serves as a substrate for the covalent attachment of K63-polyubiquitin chains to TRIM21 catalyzed by Ube2N/Ube2V2. Without monoubiquitination of TRIM21 by Ube2W, TRIM21 was still capable of catalyzing

unanchored K63-linked ubiquitin chains; however, unexpectedly, these unanchored K63-linked chains did not trigger NF- κ B activation in the cell. This finding suggested that regulation of TRIM21-mediated proinflammatory signal activation is governed by an additional mechanism.

How does TRIM21 induce innate immune signaling? Fletcher et al. (3) hypothesized that a DUB enzyme associated with the proteasome

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cleaves off anchored K63-polyubiquitin from TRIM21, releasing it to generate unanchored K63-polyubiquitin, which then induces NF- κ B activation. The authors focused on Poh1, a proteasome-associated DUB that is known to remove ubiquitin chains *en bloc*, thereby generating unanchored ubiquitin chains (9). Depletion of endogenous Poh1 increased the levels of K63-polyubiquitin covalently attached to TRIM21, indicating that Poh1 indeed removes this modification from TRIM21. Silencing of Poh1 also resulted in a dramatic reduction in NF- κ B activation, suggesting that the Poh1-mediated release of K63-linked polyubiquitin induces activation of NF- κ B signaling. Fletcher et al. (3) further show that production of proinflammatory cytokines triggered by virus-Ig complexes is dependent on Poh1. The authors thus propose a model in which covalently bound K63-linked polyubiquitin chains on TRIM21 are cleaved off by Poh1, generating "free" K63-linked polyubiquitin,

which subsequently induces proinflammatory signal-transduction (Fig. 1).

The work of Fletcher et al. (3) indicates that monoubiquitination of TRIM21 is a prerequisite for subsequent K63-polyubiquitination, and that two different E2 enzymes—Ube2W and Ube2N/Ube2V2—are needed for the stepwise activation of TRIM21. This unveils a complex regulatory mechanism for TRIM21 activation, which could be critical for controlling aberrant signaling to prevent unwanted inflammatory responses in the absence of infection. Although their study has considerably advanced our understanding of TRIM21 activity, the findings of Fletcher et al. also raise several important questions. It remains unclear how the proteasome is recruited to TRIM21, and in particular how K48-linked ubiquitin chains, which are also conjugated to TRIM21 (4, 10), contribute to TRIM21-mediated degradation of antibody-pathogen complexes. Furthermore, Fletcher et al. (3) do not address which downstream targets are activated by K63-polyubiquitin chains released by Poh1 and their contributions to proinflammatory cytokine induction.

In conclusion, the work of Fletcher et al. (3) proposes an intriguing model of TRIM21 activation through stepwise autoubiquitination and Poh1-mediated deubiquitination. Interestingly, a similar activation mechanism has been recently reported for TRIM5 α (11). It has been shown that TRIM5 α monoubiquitination mediated by Ube2W and subsequent K63-polyubiquitination by Ube2N/Ube2V2 is required for TRIM5 α 's ability to inhibit HIV reverse transcription. A comprehensive view of how ubiquitination/deubiquitination controls the antiviral and immunomodulatory functions of TRIM proteins will greatly enhance our understanding of antiviral restriction mechanisms and may lead to novel antiviral therapies.

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