Virology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Ubiquitination in the antiviral immune response

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ARTICLE INFO

Article history:
Received 2 December 2014
Returned to author for revisions
12 February 2015
Accepted 17 February 2015

Keywords:
Antiviral immunity
Type-I interferon
RIG-I-like receptors
Toll-like receptors
cGAS
STING
Ubiquitin
E3 ligases
TRIM proteins
TRIM25

ABSTRACT

Ubiquitination has long been known to regulate fundamental cellular processes through the induction of proteasomal degradation of target proteins. More recently, 'atypical' non-degradative types of poly-ubiquitin chains have been appreciated as important regulatory moieties by modulating the activity or subcellular localization of key signaling proteins. Intriguingly, many of these non-degradative types of ubiquitination regulate the innate sensing pathways initiated by pattern recognition receptors (PRRs), ultimately coordinating an effective antiviral immune response. Here we discuss recent advances in understanding the functional roles of degradative and atypical types of ubiquitination in innate immunity to viral infections, with a specific focus on the signaling pathways triggered by RIG-I-like receptors, Toll-like receptors, and the intracellular viral DNA sensor cGAS.

Contents

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http://dx.doi.org/10.1016/j.virol.2015.02.033
0042-6822/

Introduction

Infection with viral pathogens triggers an immediate antiviral response in the host cell, commonly termed 'innate immune response'. This response is characterized by rapid gene expression of a variety of antiviral and inflammation-inducing molecules, including type-I interferons (IFN-α/β), type-III IFNs (IFN-λ or IL-28/29), proinflammatory cytokines and chemokines. Upon secretion and subsequent binding to their respective receptors on the surface of surrounding cells, IFNs lead to the upregulation of more than one hundred different interferon-stimulated genes (ISGs) (Ivashkiv and Donlin, 2014; Liu et al., 2011). ISGs encode for either signaling molecules, including transcription factors, that amplify the innate immune response, or for antiviral effector proteins to block virus replication through multiple mechanisms, such as cleavage of viral RNA or shutdown of host cell translation. Furthermore, secreted proinflammatory cytokines and chemokines produced during the innate immune response are critical for priming and fine-tuning the adaptive immune response (Sadler and Williams, 2008; Sen and Sarkar, 2007).

One class of important molecules in the activation of the innate antiviral response are pattern recognition receptors (PRRs), which recognize viral proteins or specific features in the viral nucleic acid, and then trigger immune signaling that results in IFN production (Creagh, 2006; Takeuchi and Akira, 2010). At least three major classes of PRRs recognizing viral nucleic acids have been identified: (1) the cytosolic RIG-I-like receptors (RLRs) sensing viral RNA species produced during both RNA and DNA virus infections; (2) the membrane-bound Toll-like receptors (TLRs) detecting viral RNA or DNA in endolysosomes immediately after virus entry; and (3) a group of structurally-unrelated viral DNA sensors, with cGAS (cyclic GMP-AMP synthase) representing a key sensor of various DNA virus infections. Upon sensing of viral nucleic acid, these sensors activate several kinases belonging to the IKK (inhibitor of nuclear factor kappa-B [IκB] kinase) family, namely the canonical IKKα and IKKβ together with their essential regulatory subunit IKKγ/NEMO, as well as the non-canonical IKKε and TANK-binding kinase-1 (TBK1). IKKα/β/γ and TBK1/IKKε then activate the transcription factors NF-κB and IFN-regulatory factors 3 and 7 (IRF3/7), respectively. In addition, PRRs activate several mitogen-activated protein kinases (MAPK), leading to the activation of AP-1 (activator protein-1). IRF-3/7, NF-κB and AP-1, upon their translocation into the nucleus, transcriptionally induce IFNs and other cytokines, ultimately establishing an antiviral program in the infected host cell or uninfected surrounding cells (Loo and Gale, 2011; Goubau and Deddouche, 2013).

Aberrant PRR activation and signaling can lead to chronic inflammation and tissue damage, and potentially cause autoimmune disorders. Indeed, recent findings indicated that some autoimmune diseases, *e.g.* systemic lupus erythematosus and Aicardi-Goutières Syndrome, are linked to single nucleotide polymorphisms (SNPs) in PRRs that lead to their constitutive activation (reviewed in (Kato and Fujita, 2014; Smith and Jefferies, 2014)). To prevent premature or excessive activation of PRR-induced antiviral signaling, an elegant system of regulation is in place. A key host mechanism for modulating the stability and signaling activity of PRRs and their downstream signaling molecules is reversible posttranslational modification (PTM), with phosphorylation and ubiquitination being the most well studied PTMs. Here we focus on the role of ubiquitination and the reversal of this process, deubiquitination, in the regulation of three major innate sensing pathways of viral infections: the RLR, TLR and cGAS-STING pathways.

Ubiquitin conjugation and deubiquitination of proteins

Ubiquitin is a small, 76 amino acid protein that is conserved across eukaryotic organisms and can be covalently attached to lysines or other residues in target proteins to modify their stabilities or activities. Ubiquitin conjugation is completed through step-wise catalysis using three distinct classes of enzymes, termed E1, E2 and E3 (Pickart and Eddins, 2004; Chernorudskiy and Gainullin, 2013). First, E1 activates the ubiquitin molecule in an ATP-dependent manner by forming an intermediate thioester bond between an active cysteine group in the E1 enzyme itself and the ubiquitin C-terminus. The E1 ubiquitin-activating enzyme next binds to the E2 enzyme, also called ubiquitin-conjugating enzyme, which accepts the ubiquitin at a catalytic cysteine residue. Finally, the E3 ubiquitin ligase, in complex with E2, facilitates the transfer of the ubiquitin moiety to the substrate protein by forming an isopeptide bond, usually between the ε-amino group of a lysine in the substrate and the C-terminal glycine residue of the ubiquitin molecule. Given that the E3 ligase determines the substrate specificity and that there are many different substrate proteins for ubiquitination in human cells, it is not surprising that a large number (more than 700) of E3 ligases exists. Furthermore, in humans, there are two E1 enzymes, which usually do not have any specificity for the E2 or E3 enzyme, and ~40 different E2 enzymes, whose primary function is to determine which types of polyubiquitin chains are catalyzed by the E3.

The E3 ubiquitin ligase superfamily can be classified into four major families: Really Interesting New Gene (RING), homologous to E6-associated protein C-terminus (HECT), UFD2 homology (U-box), and RING-in-between-RING (RBR) E3 ligases (Berndsen and Wolberger, 2014; Mattiroli and Sixma, 2014; Nagy and Dikic, 2010). Members of each E3 ligase family facilitate ubiquitin conjugation to the target protein through different mechanisms. RING E3 ligases, the most prevalent, never directly bind to the ubiquitin moiety. Instead, they serve as mediators for direct transfer of the ubiquitin molecule from the E2 enzyme to the substrate. In contrast, in the case of HECT E3 ligases, an intermediate bond between ubiquitin and a catalytic cysteine of the E3 ligase is formed before transfer of ubiquitin to the target protein. U-box E3 ligases, also dubbed E4 ubiquitin ligases, primarily elongate polyubiquitin chains that have already been begun by another E3 ligase (Koegl et al., 1999). The recently identified family of RBR E3 ligases (further reviewed in (Spratt et al., 2014)) are structurally characterized by two domains which are bioinformatically similar to RING domains, separated by an intervening sequence called IBR (in-between-RING). RBR E3 ligases catalyze ubiquitin conjugation through a hybrid mechanism in which the first RING domain acts as a canonical RING ligase, interacting with the E2 enzyme, bringing it in proximity to the substrate. The second RING domain, also called required for catalysis (Rcat), then accepts the ubiquitin from the E2 enzyme before transferring it to the substrate, similar to the action of a HECT ligase (Spratt et al.,

Please cite this article as: Davis, M.E., Gack, M.U., Ubiquitination in the antiviral immune response. Virology (2015), http://dx.doi.org/10.1016/j.virol.2015.02.033