Contents lists available at ScienceDirect

Cytokine & Growth Factor Reviews

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

Survey

The implication of SUMO in intrinsic and innate immunity

Zara Hannoun, Ghizlane Maarifi, Mounira K. Chelbi-Alix*

INSERM UMR-S 1124, Université Paris Descartes, Paris, France

ARTICLE INFO

Article history:

Received 19 April 2016
Received in revised form 21 April 2016
Accepted 26 April 2016
Available online xxx

Keywords:

SUMO
TRIM proteins
Innate immunity
Signal transduction
Restriction factors

ABSTRACT

Since its discovery, SUMOylation has emerged as a key post-translational modification involved in the regulation of host-virus interactions. SUMOylation has been associated with the replication of a large number of viruses, either through the direct modification of viral proteins or through the modulation of cellular proteins implicated in antiviral defense. SUMO can affect protein function via covalent or non-covalent binding. There is growing evidence that SUMO regulates several host proteins involved in intrinsic and innate immunity, thereby contributing to the process governing interferon production during viral infection; as well as the interferon-activated Jak/STAT pathway. Unlike the interferon-mediated innate immune response, intrinsic antiviral resistance is mediated by constitutively expressed antiviral proteins (defined as restriction factors), which confer direct viral resistance through a variety of mechanisms. The aim of this review is to evaluate the role of SUMO in intrinsic and innate immunity; highlighting the involvement of the TRIM family proteins, with a specific focus on the mechanism through which SUMO affects i- interferon production upon viral infection, ii-interferon Jak/STAT signaling and biological responses, iii-the relationship between restriction factors and RNA viruses.

© 2016 Published by Elsevier Ltd.

Contents

1. Introduction	00
2. SUMOylation, TRIM protein family and PML nuclear bodies	00
2.1. TRIM proteins as SUMO E3 ligases	00
2.2. PML nuclear bodies as hot spots for SUMOylation	00
3. SUMOylation of cellular proteins implicated in innate immunity (Table 1)	00
3.1. SUMO negatively regulates IFN production	00
3.2. SUMOylated PML positively regulates IFN- β synthesis	00
3.3. Effect of SUMO on STAT1 phosphorylation and transcription induced by type I and II IFNs	00
3.3.1. IFN signaling	00
3.3.2. SUMO inhibits IFN-induced STAT1 phosphorylation	00
3.3.3. Differential effects of SUMO-1 and SUMO-2/3 on ISG products	00
3.4. SUMOylated PML positively regulates signaling of type I and II IFNs	00
4. SUMOylation of restriction factors and intrinsic antiviral activity against RNA viruses	00
4.1. PML NBs, SUMO and RNA viruses	00
4.1.1. PML, SUMO and rhabdoviruses	00
4.1.2. PML, SUMO and picornaviruses	00
4.1.3. PML, SUMO and human foamy virus	00
4.2. P53	00
4.3. TRIM5 α	00
4.4. PKR	00
4.5. MxA	00
5. Conclusion and prospects	00

* Corresponding author.

E-mail address: mounira.chelbi-alix@parisdescartes.fr (M.K. Chelbi-Alix).

Acknowledgements	00
References	00

1. Introduction

Post-translational modifications (PTM) dynamically regulate the activity and/or broaden the functional spectrum of a given substrate protein, in response to specific signals. Ubiquitin-like proteins (Ubls) represent a growing list of modifiers, which are homologous to ubiquitin and display similar three-dimensional structures. These include interferon-stimulated gene 15 (ISG15), small ubiquitin-like modifier (SUMO) and NEDD8. Biochemically, Ubls are processed, activated, conjugated and deconjugated by a different set of enzymes, which are analogous to those implicated in the ubiquitin pathway [1,2].

The SUMO proteins are covalently conjugated to other proteins, regulating a range of biological processes, including protein subcellular localisation, transcription, DNA repair, chromosome dynamics, protein stability, innate immunity and antiviral defense. Despite the name, SUMO only shares ~18% homology with ubiquitin [3] and is approximately 11 kDa in size, comparable to the 8 kDa ubiquitin molecule [3]. SUMO has been found to bind to the lysine residue on the consensus sequence; ψ KxE (where ψ corresponds to a large hydrophobic amino acid, K is a lysine residue, x is any amino acid and E is a glutamic acid residue) found on the target protein [4]. Four homologues exist in mammals, SUMO-1, -2, -3 and -4. SUMO-2 and -3 share 95% homology with each other, but only share 50% identity with SUMO-1 [5]. SUMO-2

and -3 have the ability to form poly-SUMO chains, covalently binding to themselves via the lysine residue at the N terminus consensus motif ψ KxE. SUMO-1 lacks this consensus site and as a consequence is unable to form poly-chains [6] but can act as a SUMO chain terminator [7]. It should be noted that SUMO-2 and -3 cannot be distinguished with current antibodies and as such will be referred to as SUMO2/3 from this point onwards. SUMO-1 and SUMO-2/3 have been shown to have distinct functions, for example SUMO-1, can function as an antagonist of ubiquitin in the regulation of certain substrates. SUMO-1 conjugation to proteins such as I κ B α inhibits its subsequent degradation by preventing its ubiquitination [8,9]. In contrast, SUMO-2/3 can act as a signal for the recruitment of E3 ubiquitin ligases, thereby promoting ubiquitination and degradation of the target proteins [9–11].

The SUMO conjugation pathway involves three enzymes (Fig. 1); E1-activating enzyme, E2-conjugating enzyme and E3-ligase [5]. SUMO is bound to its target protein via an isopeptide bond formed between an α -amino group on the lysine residue on the target protein and the C terminal carboxyl group on the SUMO protein [12]. The pro-form of SUMO needs to be cleaved prior to protein conjugation [13]. This is carried out by isopeptidases, also known as the SENP SUMO deconjugating enzymes [14]. The SUMO activating enzyme (E1), SAE1/2, commences the reaction process by interacting with SUMO, to form a high-energy thioester bond. The SUMO conjugating enzyme (E2) then binds SUMO via its

Fig. 1. The SUMOylation Pathway 1. The pro-form of SUMO is first cleaved prior to protein conjugation (X is defined as any amino acid), carried out by isopeptidases, also known as the SENP SUMO deconjugating enzymes. 2. The SUMO activating enzyme (E1), SAE1/2, commences the reaction process by interacting with SUMO, to form a high-energy thioester bond. 3. This is followed by the transfer of SUMO to the SUMO conjugating enzyme (E2), forming a highly reactive species. 4. The final conjugation to the target protein can be carried out by an E3 ligase. 5. As SUMOylation is a reversible process, SUMO can be removed from its target substrate by SENPs.

cysteine residue in its active site. This intermediate provides a highly reactive species, important in the final conjugation, usually facilitated by an E3 ligase [6]. SUMO E3 ligases act to either activate Ubc9 or bring Ubc9 and the target protein within close proximity of each other, thus enhancing SUMOylation [7]. They can be regarded as E3 enzymes as they are able to bind to the E2 and the substrate and facilitate the formation of the bond formed between SUMO and the target protein. It has also been shown that a large number of proteins (~40%) can be SUMOylated without the presence of the consensus sequence (ψ KxE), demonstrating differences in substrate specificity [7].

SUMO interaction motifs (SIMs) are another method whereby SUMO is able to effect protein activity without being covalently attached. SIMs are usually a sequence of 10 amino acids (V/I-x-V/I-V/I or V/I-V/I-x-V/I/L) or less comprised of a hydrophobic core and an acidic region in close proximity [15]. The hydrophobic residues are responsible for binding to the β 2 strand of SUMO and the acidic residues strengthen the bond by interacting with the basic surface of the SUMO protein. A well-characterized protein with SIM regions is the RING finger protein 4 (RNF4), a SUMO-dependent ubiquitin E3 ligase, known to bind and ubiquitinate various proteins that have been poly-SUMOylated [10,16–18].

The covalent attachment of SUMO is more frequently emerging as a main regulatory system in a number of biological systems, including during viral infections, whereby the modifications lead to dramatic changes in the SUMO proteome [19–24]. Among the roles of SUMO in various cellular functions, a number of reports have also confirmed its role in regulating viral replication. SUMO either directly targets the viral proteins or alters the functions of cellular proteins involved in the antiviral defense mechanism [25]. Many viral proteins are SUMOylated or influence the SUMOylation of cellular proteins. Therefore, the infection ability of specific viruses is dependent on pathways regulated by SUMOylation [25]. To further support the implication of SUMO in antiviral defense, viruses have also evolved various strategies using the host SUMOylation processes to enhance viral replication and evade cellular immune responses (reviewed in [25–27]).

The establishment of an antiviral state in cells is defined by the functions of IFNs, this characteristic of IFNs lead to their discovery in 1957 by Isaacs and Lindenmann [28–30]. Every stage of viral replication may be a target for inhibition by IFNs, including entry, transcription, RNA stability, initiation of translation, maturation, assembly, and release. Three classes of IFNs have been identified, designated as types I, II and III. In humans, type I consists of IFN- α , IFN- β , IFN- ϵ , IFN- κ and IFN- ω , whereby a single gene, with the exception of IFN- α , which has 13 subtypes, encodes each type I IFN. IFN- γ is the only type II IFN and type III IFN is composed of four members (IFN- λ 1–4) [31].

The innate immune system constitutes the first line of host defense during infection and plays a crucial role in the early recognition process; triggering IFN synthesis and establishing an antiviral state. After the IFNs are produced, they are secreted and bind to cells, activating the Jak/STAT pathway, leading to the expression and antiviral activity of the IFN-stimulated gene (ISG) products [29,31]. There is increasing evidence that SUMOylation can regulate innate immunity by altering the production of type I IFNs upon viral infection, Jak/STAT pathway and the subsequent antiviral activity [2,32].

In contrast to innate immunity, the intrinsic antiviral resistance (also known as intrinsic immunity) is mediated by endogenously expressed antiviral proteins (also known as restriction factors), which directly confer viral resistance, in the absence of IFN synthesis, utilizing a variety of inhibitory mechanisms. These constitutive antiviral proteins can also be further induced through viral infection and/or IFN treatment. Individually, they can interfere with a particular stage of the viral life cycle. Some of

the restriction factors including members of TRIM protein family interact with and/or are modified by SUMO (see References within Table 2).

This review will focus on the implication of tripartite motif (TRIM) proteins, as many of them have emerged as SUMO ligases [33], and PML/TRIM19, the organizer of nuclear bodies (NBs), as these nuclear structures are sites enriched with SUMOylated proteins [34] implicated in both intrinsic and innate immunity. It will then focus on the mechanism through which SUMO affects i—IFN production upon viral infection, ii—IFN Jak/STAT signaling and subsequent biological responses, iii—the relationship between restriction factors and RNA viruses.

2. SUMOylation, TRIM protein family and PML nuclear bodies

2.1. TRIM proteins as SUMO E3 ligases

There are over 70 TRIM proteins in humans. These proteins all possess a common N-terminal tripartite RBCC motif, consisting of a RING (really interesting new gene) finger domain, one or two B-Box domains (which, like the RING finger domain, are zinc-binding domains) and a coiled-coil domain required for oligomerization of TRIM proteins. These domains are consistently arranged in the stated order in the N-terminal region followed by a C-terminal region specific to each TRIM protein. The variability of the C-terminal regions allows the proteins to be further classified into eleven different sub-groups [35]. One such subgroup has been reported to act as RING-finger E3 ubiquitin ligases [36]. Functionally, the RING finger domain has been found to play a crucial role in mediating the transfer of ubiquitin both to heterologous substrates as well as to the RING proteins themselves. Several TRIMs are induced by IFN [37,38] and are implicated in intrinsic and/or innate immunity [35,39,40]. Some members of this family of proteins have been found to promote innate immune responses (TRIM6, PML/TRIM19, TRIM25, TRIM56, to name a few), in contrast others (including TRIM8, TRIM11, TRIM13, TRIM21, TRIM24, TRIM27 and TRIM38) inhibit IFN production mainly by targeting signaling molecules for ubiquitin-mediated degradation [41]. Remarkably, a subset of this family is implicated in intrinsic antiviral activity (TRIM5 α , PML/TRIM19, TRIM22, TRIM32 and TRIM79 α) [42–48], which mediates IFN-induced antiviral activity [9,44,48,49].

Interestingly, TRIM proteins were revealed as a new class of SUMO E3 ligases (TRIM1, TRIM19, TRIM22, TRIM 27, TRIM28, TRIM32, TRIM36) [33,50]. The SUMO E3 activity of TRIM proteins relies on the RING domain, extending the described ubiquitin E3 role of this domain. It also involves intact B1 and B2-boxes. Like other known SUMO ligases, TRIM proteins stimulate the conjugation of both SUMO-1 and SUMO-2/3 to target proteins [33]. Among the substrates of TRIM SUMO E3 ligases is the tumour suppressor p53. In addition, several TRIM proteins interact with p53, control its abundance and/or its activity and some of them are also p53 target genes [51], thereby implicating the importance of the cross-talk between p53 and TRIM proteins during viral infections.

Taken together these activities may also explain the diverse spectrum of functional roles ascribed to TRIM proteins. Some TRIM proteins modulate their target proteins through ubiquitination while others through SUMOylation. A TRIM protein may also attach ubiquitin and SUMO onto the same target simultaneously or sequentially [33]. One of the first extensively studied members of the TRIM family, TRIM19, known as ProMyelocytic Leukemia (PML) protein, has been shown to interact and to be conjugated to SUMO [52,53], and this SUMOylation is required for the formation of PML nuclear bodies (NBs) and the antiviral activity of PML [25,45, 54–56]. Moreover, it has recently been shown that TRIM5 α is

conjugated to SUMO at Lysine 10 [57,58] and possess three SIMs, whereby two of which are vital for its antiviral activity [57,59].

Additional investigations are required to determine: i—whether other members of TRIM protein family interact or are conjugated to SUMO and whether SUMO pathway is implicated in their intrinsic and/or innate immune activity, ii—the TRIM protein's SUMO E3 activity, its targets and the dynamic interplay with ubiquitin E3 activity, iii—the implication of SUMO pathway between the cross-talk of p53 and TRIM proteins and the consequences on apoptosis in infected cells.

2.2. PML nuclear bodies as hot spots for SUMOylation

PML/TRIM19 is the most well-characterized TRIM protein and is the first member of the TRIM family that has been shown to be directly induced by IFN [60,61] and to confer intrinsic viral resistance by interacting with a viral protein inhibiting its function [44]. PML is the organizer of the nuclear speckles, known as PML NBs [62], which are implicated in various cellular functions including senescence, apoptosis, protein degradation, IFN response and antiviral defense [54,55,63–66].

PML NBs are dynamic structures, which harbor a few permanently (PML, Sp100 and SUMO) and numerous transiently residing proteins depending on different surrounding conditions *i.e.* transformation, stress, IFN treatment and viral infections. The list of cellular and viral proteins recruited to PML NBs, based on co-localization studies, is continuously growing.

Several PML isoforms are generated by alternative splicing from a single gene and are designated PML1 to PMLVIIb [55,67]. They share the same N-terminal region (exons 1–3), which encodes the RBCC/TRIM motif, but differ in their C-terminal regions due to alternative splicing of exons 4–9. The variability of the C-terminal region of the PML isoforms is vital for the unique function of each

as it defines the recruitment of specific interacting partners [45,55,56,68].

SUMO is a key regulator of PML function. PML *via* its RING finger domain directly binds to Ubc9, resulting in its SUMOylation on at least three major lysine residues (K65, K160, K490) [22,53]. PML SUMOylation affects its cellular localization, stability and ability to interact with other partners. SUMO modification of PML is critical for PML NB formation as a PML SUMO deficient mutant exhibits a nuclear speckle pattern but has a defect in recruiting NB-associated partners [45,69–71]. In addition, the non-covalent interaction of SUMO with the SIM of PML only present in PML1 to PMLV (VVVI encoded by exon 7a at position 556–559) was proposed to mediate non-covalent interactions with other SUMOylated proteins and to promote their recruitment in PML NBs [71,72].

Based on database analyses, PML partners were mapped together in a comprehensive network that contained 166 proteins [34]. Of which, a common feature of PML partners found in NBs is their capacity to be SUMOylated. Various relationships between PML NBs and SUMO pathways are derived from the localization within PML NBs, including Ubc9, SUMO-specific proteases and SUMO E3 ligases: members of the PIAS family [73,74]. According to the current literature, almost 40% of PML partners have been confirmed to be SUMOylated [34]. This suggests that PML NBs are enriched sites for SUMOylated proteins and may function as nuclear SUMOylation hotspots [34].

More interestingly, PML itself has been shown to have SUMO E3 ligase activity [33], which may mediate the SUMOylation of various PML NB partners, including p53, a well-known interacting PML partner [51]. PML NBs control p53 stability, post-translational modification (SUMOylation, phosphorylation and acetylation) and activity [10,75–77]. Upon specific stimulation, PML recruits p53 and HIPK2 (homeodomain-interacting protein kinase 2)

Fig. 2. SUMOylation of factors implicating in IFN production Upon RNA virus infection, RIG-I/MDA5 bind to viral RNAs and undergo a conformational change exposing the N-terminal CARD domains, resulting in the association with the adaptor protein MAVS (also known as IPS-1, CARDIF or VISA), located on mitochondria, which then phosphorylates IRF3 and/or IRF7 through TRAF3, NAP1 and TBK1/IKKε. Phosphorylated IRF3 and IRF7 homodimerize and translocate into the nucleus where they induce the expression of type I IFN genes, resulting in the early production of IFNs. RIG-I, MDA5, IRF3 and IRF7 have been reported to be SUMOylated and to alter IFN synthesis. Pin1 interacts with phosphorylated IRF3 and promotes its degradation *via* the ubiquitin-proteasome pathway [99]. SUMOylated PMLIV is the only PML isoform able to recruit Pin1 within PML NBs, preventing the degradation of phosphorylated IRF3 and thus sustaining IFN-β synthesis [56]. Please refer to Table 1 detailing how SUMO affects the function of the individual signaling members.

responsible for the phosphorylation of p53 [76] or CBP (CREB-binding protein), which acetylates p53 resulting in an increase in the expression of p53 target genes and p53 activity [77]. PML NBs are also the site of SUMO-dependent proteasomal degradation of PML protein itself and other PML NB partners such as p53 or Sp100 [9,10,75,78,79].

3. SUMOylation of cellular proteins implicated in innate immunity (Table 1)

3.1. SUMO negatively regulates IFN production

A crucial component of the host defense system against viral infection is the activation of the type I IFN response, which is initiated by detection of viral RNA or DNA by host pattern recognition receptors (PRRs) (Fig. 2). The cytosolic helicases retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) are intracellular detectors of viral RNA that discriminate between different classes of RNA viruses. For example, RIG-I is responsible for detection of Newcastle Disease Virus, Vesicular Stomatitis Virus (VSV), Influenza A Virus (IAV), and Sendai Virus (SeV), while MDA5 is important for recognition of picornaviruses, including encephalomyocarditis virus (EMCV) [80]. Although the diverse PRRs induce distinct signaling pathways, their initiation results in the activation of the IFN regulatory factor (IRF) 3 and/or IRF7. Both RIG-I and MDA5 contain two N-terminal caspase recruitment domains (CARD) and a C-terminal RNA helicase domain. Upon RNA virus infection, RIG-I/MDA5 bind to dsRNA and undergo a conformational change exposing the N-terminal CARD domains and resulting in the association with the adaptor protein MAVS (also known as IPS-1, CARDIF or VISA), on the outer membrane of mitochondria [81]. In turn, this triggers MAV to recruit the TANK-Binding Kinase 1 (TBK1), which then phosphorylates IRF3 and IRF7 [82,83]. Once phosphorylated, IRF3 and IRF7 homodimerize and translocate to the nucleus to induce the activity of the promoter regions of type I IFNs [84,85] (Fig. 2), resulting in the early production of IFNs and subsequent establishment of the antiviral state.

There is increasing evidence that SUMOylation can influence the regulation of innate immunity and the synthesis of type I IFNs (IFN- α and IFN- β) in response to viral infection (Table 1). Given that IRF3 and IRF7 are central mediators of type I IFN production, many regulatory pathways target IRF activation and/or stability to control IFN production. Lysine residues 70 and 87 of human IRF3 are targeted for SUMOylation, whereas K87 and K152 are the two major residues targeted for SUMOylation on mouse IRF3 [86]. Ran et al. showed that the lysine 87 on mouse IRF3, also conserved in human IRF3, is critical for the SUMOylation, which attenuates the K48-linked polyubiquitination and the subsequent proteasomal degradation of IRF3 [86]. In contrast, in a previous study, Kubota et al. reported that SUMOylation of mouse IRF3 reduces its transcriptional response [87]. These observations imply that human and mouse cells use a common mechanism to regulate

IRF3 stability but may use different mechanisms to regulate IRF3 activity.

SUMOylation of mouse IRF3 at K152 (a residue not conserved in human IRF3) as well as IRF7 at K406 negatively regulates their transcriptional activities [87]. Accordingly, IRF3 and IRF7 mutants defective in SUMOylation led to higher levels of IFN mRNA induction after viral infection, compared to the wild-type IRFs, indicating a negative role for SUMOylation in IFN synthesis [87].

Interestingly, TRIM28 acts as a specific SUMO E3 ligase of IRF7 as it has little effect on the closely related IRF3 [50]. The lysine 444 and lysine 446 are reported to be the main SUMOylation sites of IRF7 in the presence of TRIM28. The specific IRF7 target by TRIM28 distinguishes it from the general SUMO E3 ligase PIAS1, which displays no preference between IRF7 and IRF3 [88].

Some RNA and DNA viruses utilize the host SUMO pathway to counteract the innate immune signaling. For example, the Ebola viral protein VP35 inhibits type I IFN production by promoting PIAS1-mediated IRF7 SUMOylation [88], thereby reducing its DNA-binding activity. Also, EBV latent membrane protein 1 (LMP1) enhances IRF7 SUMOylation resulting in the reduced activation of the IFN- β promoter [94]. These findings indicate that viruses exploit the cellular SUMOylation machinery for their advantage and explain how they are able to overcome host innate defense.

Another member of IRF family, IRF1 acts as a transcriptional activator of IFN- β and specific ISGs [95]. IRF2 binds the same recognition IFN-stimulated response element (ISRE) as IRF1, acting as a transcriptional repressor [96] (Table 1). The SUMO-1 modification of IRF1 by PIAS3 represses its transcriptional activity [89]. In addition, the transient co-expression of Ubc9 or SUMO-1 reduces the IRF-1-mediated activation of the IFN- β promoter, whilst the expression of both, synergistically increase their individual effects. These results suggest that the modification of IRF-1 by SUMO-1 via its association with Ubc9 may be an important determinant of its functional regulation [90]. SUMOylated IRF1 mimics IRF2, an antagonistic repressor, and shows oncogenic potential [97]. Furthermore, IRF2 interacts with the SUMO-E3 ligase, PIASy, and is SUMOylated at positions K137, K166, and K293. This IRF2 modification increases its ability to inhibit IRF1 transcriptional activity and decreases its ability to activate the ISRE [91].

In contrast, the SUMO pathway has also been associated with the positive regulation of innate immunity. MDA5 and RIG-I can be SUMOylated in transient transfection assays and this is correlated with an increase in type I IFN induction (Table 1) [92,93]. Enhanced SUMOylation of MDA5 by exogenously expressed Ubc9 or in cells transfected with the polyinosinic-polycytidylic acid (polyI:polyC), a mimic of double stranded RNA virus recognized by MDA5 is correlated with upregulation of IFN- β expression and knockdown of the SUMO E3 ligase PIAS2 isoform β decreases both MDA5 SUMOylation and IFN- β production [93]. Additionally, SUMOylation of RIG-I has been reported to enhance type I IFN production through increased ubiquitination and interaction with its downstream adaptor molecule, Cardif [92]. These seemingly conflicting

Table 1
SUMOylation and innate immunity.

Target	SUMOylation Sites	SUMOylation	References
IRF1	ND	Repress its transcriptional activity	[89,90]
IRF2	K137, K166, K293	Increase inhibition of IRF1 transcriptional activity	[91]
IRF3	K152 (murine) K70, K87 (human)	Negative regulation of IFN- β synthesis	[86,87]
IRF7	K406 (murine) K444, K446 (human)	Negative regulation of type I IFN synthesis	[50,87]
RIG-I	ND	Positive regulation of type I IFN	[92]
MDA5	ND	Positive regulation of type I IFN	[93]
PML/TRIM19	K65, K160, K490	Positive regulation of IFN- β synthesis upon viral infection	[56]

results can be reconciled because MDA5 and RIG-I function upstream of IRF3/IRF7, and SUMOylation of MDA5 or RIG-I may override any downstream modifications.

Converse experiments show that SUMOylation deficient Ubc9 knockout cells is sufficient to initiate a spontaneous type I IFN response and the stimulation of these cells with toll-like receptors induces a massive IFN- β response when compared to wild-type cells [98]. Mechanistically, SUMO operates from a distal enhancer of the gene encoding IFN- β (*Ifnb1*) to silence both basal and stimulus-induced activity of the *Ifnb1* promoter. Furthermore, depleting the SUMO pathway, results in enhanced antiviral behaviour when infected with chikungunya virus in mice [98].

Taken together, these reports show that enhanced or decreased (in the absence of Ubc9) cellular SUMOylation reduces or boosts type I IFN synthesis in infected cells respectively, thus suggesting that SUMO negatively regulates the innate immune response by decreasing IFN production.

3.2. SUMOylated PML positively regulates IFN- β synthesis

A large number of studies have indicated that in addition to its intrinsic antiviral activity, PML also plays a critical role in innate immunity. Indeed, among all PML isoforms, only PMLIV enhances IRF3 phosphorylation in infected cells, resulting in the dramatic production of IFN- β . The positive regulation of IFN- β synthesis by PMLIV requires its SUMOylation, as a SUMO deficient mutant of PMLIV is unable to do so [56]. The boost by PMLIV of IFN- β synthesis occurs during infection with viruses from diverse viral families, such as VSV, EMCV, SeV, IAV and human T-lymphotropic virus 1 (HTLV-1); but also by synthetic double-stranded RNA following the activation of RIG-I [56]. Mechanistically, PMLIV enhances IFN- β synthesis by regulating the cellular distribution of Pin1 (peptidyl-prolyl *cis/trans* isomerase NIMA-interacting 1) (Fig. 2) (Table 1), inducing its recruitment to PML NBs where both proteins colocalize. Pin1 is known to interact with phosphorylated IRF3 and to promote its degradation via the ubiquitin-proteasome pathway [99]. The interaction of SUMOylated PMLIV with endogenous Pin1 and its recruitment within PML NBs prevent the degradation of activated IRF3, and thus potentiate IRF3-dependent production of IFN- β .

Remarkably, another member of Ubl, ISG15 also acts on IRF3 via Pin1. IRF3 is modified by ISG15, which is robustly induced by virus infection [100,101]. The ISGylation of IRF3 attenuates its interaction with Pin1, significantly impairing IRF3 ubiquitination and its proteasome-dependent degradation, thereby resulting in sustained IRF3 activation. These results show that SUMOylated PMLIV and ISGylated IRF3 alter Pin1 function by two different mechanisms resulting in a higher virus-induced IRF3 phosphorylation. It has been reported that Pin1 is SUMOylated on Lys6 in the WW

domain and on Lys63 in the PPIase domain and that Pin1 SUMOylation inhibits its substrate binding and catalytic activity [102]. It will be interesting to determine whether PMLIV could also act as a SUMO ligase able to modify Pin1, thereby inhibiting its cellular function.

Taken together, these results suggest that the modulation of IFN production by SUMO is complex and is dependent on the SUMOylation state of specific cellular proteins.

3.3. Effect of SUMO on STAT1 phosphorylation and transcription induced by type I and II IFNs

3.3.1. IFN signaling

IFNs act on cells by binding to their respective receptors (IFNAR for IFN α/β , IFNGR for IFN- γ or IFNLR for IFN λ) and activating the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathways to trigger the transcription of more than three hundred ISGs [31] (Figs. 3 and 4). Briefly, the interaction of IFN- α/β with its receptor, IFNAR, leads to the phosphorylation of STAT1 and STAT2 by activated JAK tyrosine kinases (Tyk2 and JAK1). Phosphorylated STATs heterodimerize and form with the DNA binding protein IRF9; a complex called IFN-stimulated growth factor 3 (ISGF3). ISGF3 translocates into the nucleus to induce ISGs containing an ISRE in their promoters. The binding of IFN- γ to its receptor, IFNGR, results in the phosphorylation of STAT1 by the activated JAK1 and JAK2. Phosphorylated STAT1 (pSTAT1) forms homodimers that migrate to the nucleus and bind to a DNA element termed GAS (gamma-activated sequence) in the promoter of specific ISGs. Accordingly, transcriptional responses to IFN- γ are dominated by the activity of phosphorylated STAT1 homodimers. Finally, type III IFNs that are structurally and genetically distinct from type I IFNs bind to different receptors, which expression is restricted to epithelial tissues [103], but activate the same signal transduction pathway.

3.3.2. SUMO inhibits IFN-induced STAT1 phosphorylation

Prior work has identified STAT1 to be conjugated to SUMO on Lys703 by protein inhibitor of activated STAT (PIAS) [104–106]. In addition, several studies have reported that the activity of STAT1 could be inhibited by SUMO conjugation, as a SUMOylation deficient STAT1 mutant is hyper-phosphorylated and exhibits increased DNA-binding on STAT1 responsive gene promoters [105,107,108]. More recently, the various roles of SUMO in IFN signaling, transcriptional response and IFN-induced biological effects have been reported [9]. The expression of each SUMO paralog leads to endogenous STAT1 SUMOylation and to a decrease in IFN γ -induced STAT1 phosphorylation. This process correlates with both lower levels of STAT1 binding to the GAS and the transcriptional response to IFN- γ (Fig. 3). Interestingly, IFN- γ

Table 2
The role of SUMO and SIM in the intrinsic antiviral activity of restriction factors against RNA viruses.

ISG products	SUMOylation Sites and SIMs	SUMOylation	References
PML/TRIM19	K65, K160, K490	Required for anti-VSV activity Required for anti-RABV activity Required for anti-EMCV activity Not required for anti-HFV activity Required for resistance to poliovirus in p53-dependent way	[53] [56] [119] [45] [44] [75]
TRIM5 α	1 SIM K10 3SIMs	Not required for anti-HIV activity 2 SIMs are required for anti-HIV activity	[72] [58] [57,59]
PKR	K60, K150, K440	Required for anti-VSV activity	[120]
p53	K386	Required for anti-VSV activity	[121]
MxA	K48	Not required for anti-VSV activity	[122]
	2 SIMs	MxA SIMs are required for anti-VSV activity	[122]

exerts a negative retro-control on its own signaling by enhancing STAT1 SUMOylation. Importantly, inhibition of IFN- γ signaling by SUMO is associated with a decrease of IFN- γ -induced apoptosis and cell growth inhibition, as well as a dramatic reduction of IP-10-induced chemotaxis. Conversely, inhibition of cellular SUMOylation by Ubc9 depletion in human cells enhances IFN- γ -induced STAT1 phosphorylation and biological responses. In conclusion, this work [9] allows the inclusion of SUMO to the list of negative regulators of IFN signaling known to date and postulates SUMO as a possible contributor to IFN- γ resistance.

Although, SUMO decreases the activation of STAT1 in response to IFN- α , it does not alter STAT2 phosphorylation, its formation of an ISGF3-like complex responsible for binding to ISRE and inducing a transcriptional response [9]. This is in agreement with accumulating evidence supporting the existence of alternative STAT2 signaling pathways that are independent from STAT1 [109,110]. Therefore, it is likely that SUMO overexpression has no effect on IFN- α signaling and transcription, because alternative complexes, composed of phosphorylated STAT2 homodimers, can compensate for the reduction of STAT1 phosphorylation (Fig. 4) [9].

3.3.3. Differential effects of SUMO-1 and SUMO-2/3 on ISG products

SUMO-1 and SUMO-2/3 modify both mutual and distinct substrates and several lines of evidence suggest that SUMO-1 and SUMO-2/3 may have individual functions [111]. Key differences between the subfamilies include; expression levels [112], susceptibility to deSUMOylating enzymes [113] and the ability to form

SUMO chains. As mentioned in the introduction SUMO-2/3, but not SUMO-1, can form polychains which are recognized by RNF4, a SUMO-dependent ubiquitin E3 ligase; leading to the ubiquitination of SUMO-2/3 conjugated substrates and their proteasomal degradation upon different stimuli [9,10].

The ISG products PML and Sp100 proteins are modified by SUMO and are the permanent constituents of PML NBs [60,61,114]. In line with the selective effect of SUMO-3, in SUMO-3 expressing cells, IFN- α and IFN- γ increase PML and Sp100 SUMOylation, induce their mRNA levels, without altering their protein levels due to the induction of their proteasomal mediated degradation, thus negating the IFN-mediated increase in PML and Sp100 protein expression [9]. Interestingly, RNF4 depletion in IFN- α and IFN- γ -treated cells overexpressing SUMO-3 prevents PML degradation, thus restoring an increase of PML protein expression in response to both IFNs (Figs. 3 and 4) [9]. In addition, SUMO-1 and SUMO-3 inhibit IFN- γ transcriptional response but the inhibition is higher in SUMO-3-expressing cells due to PML degradation, see paragraph below [9].

Further investigations are needed to determine whether targets for IFN-induced SUMOylation and degradation by SUMO-3 may also include other proteins.

Fig. 3. SUMO and type II IFN response The binding of IFN- γ to its receptor, IFNGR, results in the phosphorylation of STAT1 (pSTAT1) by JAK1 and JAK2. pSTAT1 homodimers migrate to the nucleus, and bind to GAS in the promoter regions of specific ISGs such as IRF1, PKR, IP-10, Sp100 and PML. In return, PML positively regulates IFN- γ signaling [115], possibly through the efficient accumulation of STAT1 transcripts [32]. SUMO-1 or SUMO-3 overexpression leads to an inhibition of STAT1 phosphorylation reducing the capacity of STAT1 to bind GAS and inhibiting the transcription. More precisely, SUMO selectively inhibits the levels of mRNAs induced with high concentrations of IFN- γ (requiring high amounts of pSTAT1) such as *IP-10* and *TAP1*; whereas the upregulation of *PML*, *STAT1*, *PKR* and *IRF1* mRNAs in response to IFN- γ is insensitive to SUMO, because they are induced with a low concentration of IFN- γ . In addition, SUMO-3, but not SUMO-1, impairs the IFN- γ -mediated increase of the PML protein due to its RNF4-dependent ubiquitination and to its subsequent degradation by the proteasome [9]. This process negates the positive activity of PML on IFN- γ signaling.

Fig. 4. SUMO and type I IFN response The interaction of IFN- α with IFNAR leads to the activation of the JAK tyrosine kinases (Tyk2 and JAK1) that phosphorylate STAT1 and STAT2. Phosphorylated STATs heterodimerize and form with IRF9 the ISGF3 complex that translocates into the nucleus to induce ISGs harboring an ISRE such as PKR, STAT1, Sp100 and PML. In turn, PML acts as a positive regulator of type I IFN-induced ISG expression by regulating ISGF3 [32]. SUMO1 or SUMO3 overexpression leads to an inhibition of STAT1 phosphorylation in response to IFN- α . The IFN- α -transduction pathway and transcription are unaffected by this inhibition, because pSTAT2 homodimers can compensate for the lack of STAT1 phosphorylation. However, SUMO-3, but not SUMO-1, impairs the IFN- α -mediated increase of PML protein due to its RNF4-dependent ubiquitination and to its subsequent degradation by the proteasome [9]. This process could diminish the positive activity of PML on IFN- α signaling.

3.4. SUMOylated PML positively regulates signaling of type I and II IFNs

All IFNs directly induce PML gene expression through the response elements ISRE and GAS present in its promoter region [61], resulting in the increase of different PML isoforms, and in the subsequent enhancement of the number and size of PML NBs [60]. The absence of PML in mouse or human cells results in a defect of IFN- γ signaling. Indeed, in response to IFN- γ , PML $^{-/-}$ mouse embryonic fibroblasts (MEFs) have lower levels of phosphorylated STAT1 and reduced STAT1 DNA binding, compared to wild-type cells [115]. Additionally, the depletion of PML by RNA interference in various human cells is accompanied by a decrease of IFN- γ -induced STAT1 phosphorylation, STAT1 DNA binding, transcription of ISGs and synthesis of their products, [115]. These results are consistent with the incapacity of IFN- γ to induce apoptosis in PML $^{-/-}$ cells [116].

All nuclear PML isoforms (PMLI to PMLVI), but not the cytoplasmic isoform PMLVIIb, function as positive regulators of IFN- γ signaling and this process requires PML SUMOylation, as the PMLIII SUMO deficient mutant abrogates its positive regulation of IFN- γ -induced STAT1 phosphorylation [115]. More recently, it has been reported that PML promotes STAT1, but not STAT2, transcription in unstimulated cells, suggesting that PML is required for the efficient accumulation of STAT1 transcripts [32]. Although, further investigation is needed to elucidate the mechanism underlying this PML activity, this process could account for the boost by PML of

IFN- γ -induced STAT1 activation. In addition, stabilization of endogenous PML by RNF4 depletion leads to enhanced STAT1 phosphorylation in response to IFN- γ and IFN- α , resulting in an increase of IFN- γ , but not IFN- α , transcriptional response [9] (Fig. 3).

The critical role of PML in innate immunity is further demonstrated by the recent findings that PML also acts as a positive regulator of type I IFN-induced ISG expression by regulating ISGF3. In IFN- β -treated cells, PML is required for the efficient accumulation of the activated forms of STAT1 and STAT2 [32] (Fig. 4). Remarkably, this regulation of type I IFN signaling by PML is counteracted by human cytomegalovirus (HCMV) IE1 protein [32], which is known to mediate loss of SUMO-conjugated forms of PML in HCMV-infected cells resulting in PML NB disruption [117]. These results again suggest that SUMOylated PML is required for the increase of type I IFN-induced ISG expression and are in agreement with the reduced capacity of IFN- α to induce apoptosis in PML $^{-/-}$ cells [116].

Overall, these findings show that the positive regulations of both type I and type II IFN signaling by PML requires its SUMOylation. Moreover, because PML is induced by all IFNs, these activities demonstrate a positive feedback loop for IFN signaling.

4. SUMOylation of restriction factors and intrinsic antiviral activity against RNA viruses

IFNs activate Jak/STAT pathway leading to the induction of several ISGs, the products of which mediate the IFN-induced

biological responses. Different restriction factors have been implicated in the resistance to viral infection in IFN-treated cells. Namely, PML/TRIM19, TRIM5 α , p53, PKR, and Mx proteins; play a key role in antiviral defense and are also modified by and/or interact with SUMO (Table 2).

4.1. PML NBs, SUMO and RNA viruses

The implication of PML in antiviral defense against RNA viruses from different families has been demonstrated in cells stably expressing individual PML isoforms or in cells depleted for PML using RNA interference [54,118]. In addition to its implication in innate immunity [32,56,63] (Table 1), PML exerts an intrinsic antiviral activity against RNA viruses from different families, which requires its SUMOylation and localization within PML NBs (except in the case of HFV) (Table 2). PML confers resistance to RNA viruses in a p53-independent manner by interacting with viral proteins inhibiting their functions or in a p53-dependent manner by inducing apoptosis in infected cells (Table 2).

In both cases the interaction with SUMO is required. Furthermore, as previously mentioned, there is added evidence that specific RNA and DNA viruses utilize the cellular SUMOylation machinery to their advantage in order to counteract PML NB functions and/or to induce PML degradation in SUMO-dependent manner [25,78].

4.1.1. PML, SUMO and rhabdoviruses

Rhabdoviridae is a family of negative sense, single stranded RNA viruses that replicate in the cytoplasm and include VSV, a member of the *Vesiculovirus* genus, and rabies virus, a member of the *Lyssavirus* genus.

The antiviral effect of PML has been observed *in vivo*, as PML deficiency renders mice more susceptible to VSV infection [123]. A study performed with all PML isoforms revealed that only the stable expression of PMLIII and PMLIV confer resistance to VSV infection and their SUMOylation is required for their antiviral activities [56]. The anti-VSV activity of PMLIII requires its SUMOylation and is strictly IFN-independent, whereas resistance to VSV exerted by PMLIV occurs *via* two independent mechanisms. Indeed, PMLIV is able to inhibit VSV replication in an IFN-independent manner, and also positively regulates IFN- β synthesis *via* a higher IRF3 phosphorylation. Both activities of PMLIV require its SUMOylation. However, how SUMOylated PMLIII and PMLIV inhibit intrinsically the VSV is still unknown.

In the case of rabies virus (RABV), among all PML isoforms tested only PMLIV confers intrinsic resistance to the virus by inhibiting viral transcription, resulting in reduction of viral titer [119]. The anti-RABV effect exerted by PMLIV requires its SUMOylation and is independent of IFN production. Further investigations are needed to elucidate the mechanism by which SUMOylated PMLIV confers resistance to RABV.

4.1.2. PML, SUMO and picornaviruses

Picornaviridae is a family of positive strand RNA viruses that replicate in the cytoplasm and they include EMCV and poliovirus. Cells derived from PML knockout mice are more sensitive to EMCV infection when compared to wild-type cells. The stable expression of PMLIV isoform in p53 inactive cells inhibits viral replication and protein synthesis, leading to a reduction of EMCV production [45]. This anti-EMCV activity is not observed with other nuclear PML isoforms (I, II, III, V, and VI) or with the cytoplasmic PMLVIIb and requires PMLIV to be SUMOylated at the three sites K65, K160 and K490. Mechanistically, only PMLIV, but not the SUMOylation deficient PMLIV mutant, interacts with EMCV 3D polymerase (3Dpol) and sequesters it within PML NBs [45]. Also, PMLIV depletion by RNA interference significantly increases EMCV

production in IFN- α -treated cells. These findings indicate that SUMOylated PMLIV acts as an anti-EMCV mediator of IFN, preventing viral replication in the cytoplasm through sequestration of the viral 3Dpol in PML NBs.

PMLIII confers resistance to many RNA viruses [54] but not to EMCV (in p53 inactive cells) [124], which counteracts the antiviral property of PMLIII by inducing its degradation [78]. Mechanistically, early post-infection, EMCV induces PMLIII transfer from the nucleoplasm to the nuclear matrix and PML conjugation to SUMO-1, SUMO-2, and SUMO-3, leading to an increase in PML body size where the viral protease 3C and the proteasome component colocalize with PML within the NBs. PML is then degraded in a proteasome- and SUMO-dependent manner, without the involvement of the SIM of PML [78].

In p53 inactive cells, PMLIV, but not PMLIII, confers resistance to poliovirus ([75], unpublished data). However, PMLIII expression can confer resistance to poliovirus in p53 dependent manner [75]. Infection with poliovirus induces ERK activation resulting in PML phosphorylation. This modification necessary for poliovirus-induced PML SUMOylation, results in the shift of PML from the nucleoplasm to the nuclear matrix and in an increase in PML NB size. This process leads to the recruitment of p53 to PML NBs and its phosphorylation on Ser15 in a PML-dependent manner [75]. Infection by poliovirus of p53 wild-type cells induces the activation of p53 target genes, *Mdm2* and *Noxa*, leading to the induction of apoptosis and the inhibition of viral replication. All of these effects are abolished when endogenous PML expression is downregulated by RNA interference. In contrast, these effects are increased by exogenous expression of PMLIII. Moreover, the knockdown of PML or p53 by RNA interference results in higher poliovirus replication, indicating that PML and p53 are involved in antiviral defense and that PML NBs are a site for p53 phosphorylation, triggering apoptosis in infected cells. In addition, it should be mentioned that later during infection, poliovirus counteracts this antiviral defense by inducing both the recruitment of 20S and MDM2 to PML NBs and subsequent p53 degradation in an MDM2- and proteasome-dependent manner [75].

This report demonstrates that both PML and p53 cooperate in a SUMO-dependent manner to inhibit poliovirus replication, whether other viruses may be inhibited in a similar mechanism is unknown. It should be noted that none of the overexpressed human PML isoforms confer resistance to HIV-1 in PML-/- MEFs [125] immortalized by the SV40 large T antigen [44], whereas overexpression of human PML restricts HIV-1 in an isoform-specific fashion [126] in PML-/- MEFs immortalized by a 3T9 protocol where p53 function is normal [127]. Further investigations are required to determine whether the anti-HIV-1 effect of PML requires p53 and SUMO. There is significant cross-talk between PML and p53, both are ISG products [61,128], PML is a p53 target gene [129] and PML NBs, which require SUMO for their function, control p53 stability and activity [65,75,130]. Therefore, it is important to determine the intrinsic antiviral effects of PML in the context of p53 inactive and p53 wild-type cells.

4.1.3. PML, SUMO and human foamy virus

Foamy viruses are complex retroviruses isolated from different animal species, mainly nonhuman primates. Human Foamy Virus (HFV) encodes an auxiliary protein, Tas (trans activating spuma), which transactivates viral gene expression by binding directly to the long-terminal repeat (LTR) and an internal promoter (IP). The replication of HFV is similar in wild-type MEFs and PML-/- MEFs, immortalized by the SV40 large T antigen indicating that endogenous PML expression is not sufficient to inhibit this virus [44]. Furthermore, PML does not play an important role in mediating HFV latency [131]. However, overexpression of PMLIII represses HFV transcription by interacting with the HFV

transactivator, Tas, PML inhibiting the complex formation between LTR and Tas and IP and Tas [44]. This interaction requires the N-terminal region of Tas and the RING finger of PML, but does not necessitate the covalent modification of PML by SUMO. Importantly, Tas-mediated LTR and IP transactivation is repressed by IFN- α in wild-type MEFs, but not in PML-/- MEFs and the expression of PML in PML-/- MEFs rescues the inhibition of transactivation [44]. These results indicate that PML is an essential mediator of HFV inhibition by IFN but does not require its SUMOylation to do so. Whether the SIM of PML is required for its anti-HFV activity is unknown.

4.2. P53

The tumor suppressor protein p53 is a direct transcriptional target of type I IFNs contributing to the antitumor and antiviral activities mediated by IFN pathway [128]. The properties of p53 are controlled by post-translational modifications and by the regulation of its turnover rate. P53 is covalently modified by SUMO-1 at lysine 386 resulting in an increased transactivation ability of p53 [132–134]. MDM2 has also been shown to promote the SUMO-1 modification of p53, an activity enhanced by ARF and related to the relocalization of p53 to the nucleolus [135]. In addition to MDM2, a number of SUMO-E3-ligases that mediate the SUMO-1 conjugation of p53 have been identified, including the PIAS [136] and TRIM proteins [33]. Furthermore, MDM2 can also enhance conjugation of SUMO-2/3 to p53 [137].

Interestingly, either treatment with type I IFN or infection with VSV induces p53 SUMOylation. IFN-induced senescence in H1299 cells stably expressing p53 was dependent, to a great extent, upon p53 SUMOylation. In addition, a role for SUMO modification in p53 antiviral activity was suggested by the fact that the p53 SUMOylation deficient mutant has, compared to p53 wild-type, a reduced ability to induce apoptosis in response to infection with VSV, which results in enhanced viral replication [121]. Therefore, increased p53 SUMOylation could play a role in type I IFN response and VSV resistance.

4.3. TRIM5 α

TRIM5 α is a restriction factor that interferes with an early step of retroviral replication in a species-dependent manner and thus constitutes an effective barrier of cross-species transmissions of retroviruses. Rhesus macaque (RM) TRIM5 α is a potent inhibitor of HIV-1 infection, whereas the human TRIM5 α can block N-MLV and equine infectious anemia virus but is less effective against HIV-1 [138,139]. TRIM5 α interferes with the replication of retroviruses, including HIV and N-MLV, inhibiting an early step of viral replication in the cytoplasm by binding to viral nucleocapsids resulting in a deficiency in the delivery of the viral genome into the nucleus [42,43].

Human and RM TRIM5 α have one SUMO consensus conjugation site at K10 and three putative SIMs. Mutation of the consensus SUMO conjugation site, K10R, abrogates SUMOylation at this position, but does not disrupt TRIM5 α antiviral activity [58], suggesting that non-covalent interaction with SUMO or SUMO-modified proteins rather than direct SUMOylation of TRIM5 α may be responsible for TRIM5 α restriction. The SIMs that bind SUMO-1 within the TRIM5 α PRYSPRY domain are required for capsid recognition and mutation of the first two hydrophobic residues of SIM1 and SIM2 to lysine are shown to contribute to the restriction of N-MLV infection by human TRIM5 α [57,59].

SUMO-1 overexpression enhances restriction of N-MLV and HIV-1 by human and RM TRIM5 α , respectively, while knockdown of SUMO-1 or Ubc9 reduces HIV-1 restriction by RM TRIM5 α [57–59]. RNA interference-mediated knockdown of human TRIM5 α or

RM TRIM5 α in cells overexpressing SUMO-1 showed that the increased inhibition of N-MLV infection is due to TRIM5 α activity [57,59]. Furthermore, it has been suggested that these results may be due, in part, to the ability of SUMO-1 to stabilize RM TRIM5 α protein expression, as SUMO-1 knockdown increases RM TRIM5 α turnover and the mutations in SIM1 and SIM2 results to more rapid degradation than the wild-type protein [59].

Taken together, these results confirm that the SUMO pathway is implicated in TRIM5 α -mediated retroviral restriction, and demonstrate that TRIM5 α is a definitive SUMO substrate. The inability to abrogate TRIM5 α antiviral activity by mutating its main SUMO conjugation motif supports the notion that non-covalent interaction with SUMO or SUMOylated proteins is required.

4.4. PKR

Among the ISG products with significant biological functions induced by type I IFN is the ubiquitous and constitutively expressed double-stranded RNA (dsRNA)-dependent protein kinase (PKR), an enzyme with multiple effects in cells; playing a key role in the antiviral defense mechanism of the host [140]. PKR is a 68 kDa serine/threonine kinase that exerts two kinase activities, one for its own phosphorylation and the other for the phosphorylation of other substrates, including the α subunit of the protein synthesis initiation factor eIF-2 [140,141]. Phosphorylated eIF-2 α impairs the activity of the guanine nucleotide exchange factor eIF-2B, resulting in a shut-off of translation at the initiation step [142]. Viral replication can produce highly structured viral transcripts in the form of dsRNA that can bind and activate PKR, which then phosphorylates eIF2 α .

In addition to being phosphorylated, PKR was also identified as being a target of SUMOylation and ISGylation. PKR is modified by SUMO-1 and SUMO-2 [120] at lysine residues Lys-60, Lys-150 (located at the dsRNA binding domain of PKR) and Lys-440 (located at the C-terminal domain). SUMO and ISG15 [143] may compete for PKR modification as the triply PKR-SUMO deficient mutant is more ISGylated when compared to PKR wild-type. SUMO is required for efficient PKR-dsRNA binding, PKR dimerization, and eIF2 α phosphorylation, thus suggesting that SUMO may contribute to the full activity of PKR. In addition, the triply PKR-SUMO deficient mutant has reduced capability to regulate VSV replication when compared to PKR wild-type [120]. Future studies are required to evaluate the role of each PKR SUMO site on its various biological functions.

4.5. MxA

Myxovirus resistance (Mx) proteins are evolutionarily conserved dynamin-like large GTPases involved in viral resistance triggered by types I and III IFNs [144]. The human MxA is a cytoplasmic protein that confers resistance to a large number of viruses such as VSV and IAV [145]. Crystallography studies have confirmed that MxA is comprised of three sections, whereby a central region connects the two other domains. The first domain is the N-terminal GTP-binding domain responsible for GTPase activity, the second domain is known as the stalk (CID-GED) and is vital for oligomer formation and the third connector domain is called the Bundle-Signaling Element, also important for oligomer formation [146]. The MxA protein is known to self-assemble into oligomers vital for its general function, as its GTPase activity is dependent on its oligomerization.

Using yeast two-hybrid screens, 27 MxA binding partners were identified, some of which are related to the SUMOylation machinery; including SUMO and Ubc9 [122]. The interaction between MxA and SUMO-1 occurs through the stalk domain while the GTPase domain establishes the interaction with Ubc9. MxA

contains one SUMOylation site (K48) used for the covalent conjugation to SUMO-2 or SUMO-3 and two putative SIM sequences, SIMa and SIMb in the MxA GTPase domain.

The MxA oligomerization capacity is important for its interaction with SUMO-1 and Ubc9. Accordingly, the monomeric mutant MxA (L612 K) has a reduced interaction with SUMO and Ubc9 [122] and is rapidly degraded in cells when compared to wild-type MxA [147].

Interestingly, the SIMa-MxA and SIMb-MxA mutants located in the GTPase domain have reduced antiviral activity [122]. However, the MxA SUMOylation-deficient mutant (MxA-K48R) retains its capacity to inhibit VSV and IAV, at a lower capacity when compared to the wild-type MxA [122]. Therefore, it cannot be excluded that MxA SUMOylation is required for full antiviral activity.

Collectively, all these findings lead to the following conclusions presented in the illustration presented in Fig. 5. 1- SUMO enhances IRF3 and IRF7 SUMOylation resulting in a decrease in their viral-induced activation and in a significant reduction of type I IFN production, 2- SUMO enhances STAT1 SUMOylation, resulting in its reduced activation in response to type I and II IFNs, 3- the antiviral ISG restriction factors, PML/TRIM19, TRIM5 α , p53, PKR and MxA require SUMO for their antiviral activities. 4- viruses exploit the host SUMOylation machinery for their advantage by altering the expression of the restriction factors.

5. Conclusion and prospects

Post translational modifications of a large number of signaling proteins and restriction factors provide vital mechanisms for the regulation of intrinsic and innate immune responses. The actions of SUMO on IFN pathways are both dynamic and complex as differential effects are observed on IFN production, signaling of type I and II IFNs and restriction factors. Enhancing SUMOylation through overexpression and inhibiting its level through Ubc9 depletion, reduces and boosts IFN synthesis in infected cells respectively, suggesting that SUMO can negatively regulate the innate immune response by decreasing type I IFN production.

SUMO acts on the Jak/STAT signaling pathway by reducing STAT1 phosphorylation in response to type I (IFN- α) and type II (IFN- γ) IFNs. However, the transcriptional response to IFN- α is not affected, whereas that of IFN- γ is highly reduced, suggesting that SUMO can act as a possible contributor to IFN- γ resistance.

SUMO-3, but not SUMO-1, impairs the increase in PML and Sp100 protein expression in response to type I and II IFNs by inducing their SUMO-3 conjugation and subsequent proteasomal degradation. Whether SUMO-3 targets other ISG products distinct from PML and Sp100 is unknown and requires further investigation. Therefore, SUMOylation may alter other factors involved in signaling and transcription processes through altering the stability of the target proteins.

Furthermore, the ISG p53 protein is SUMOylated and many restriction factors such as PKR, PML/TRIM19 and other members of TRIM protein family interact with p53 and can in certain circumstances, such as viral infection, directly or indirectly induce p53 phosphorylation and activation of its target genes, leading to apoptosis of infected cells. Therefore it is important to compare the intrinsic antiviral activity of the restriction factors in the context of p53 inactive and p53 wild-type cells.

Significant advances have been made over the last 20 years in field of SUMO, however there are many unresolved questions. The intrinsic antiviral activity of many key restriction factors requires their interaction and/or their modification by SUMO. Therefore, it will be interesting to determine how SUMO is implicated in the intrinsic antiviral activity mediated by the various restriction factors and whether infection with viruses from different families could counteract these processes and how the effects differ between viral families. Elucidating such mechanisms may provide novel insight on targets that could be used for the development of effective antiviral therapies.

Over time, it will be interesting to identify the various proteins that are conjugated to SUMO in response to IFN and during viral infection and how the type of infection may alter the array of SUMOylated proteins. Further understanding the SUMO complex network of cellular antiviral processes and SUMO-dependent virus-host interactions will identify new targets and permit

Fig. 5. SUMO alters different stages in the innate immune response to viral infection.

understanding how viruses manipulate the host SUMOylation system and how SUMO affects viral functions.

Acknowledgements

This work was supported by grants from the Agence Nationale de la Recherche (ANR 11BSV3002803) and the Agence Nationale de Recherches sur le sida et les hépatites virales (ANRS).

References

- [1] M. Hochstrasser, Evolution and function of ubiquitin-like protein-conjugation systems, *Nat. Cell Biol.* 2 (2000) E153–E157.
- [2] X. Liu, Q. Wang, W. Chen, C. Wang, Dynamic regulation of innate immunity by ubiquitin and ubiquitin-like proteins, *Cytokine Growth Factor Rev.* 24 (2013) 559–570.
- [3] S. Muller, C. Hoege, G. Pyrowolakis, S. Jentsch, SUMO, ubiquitin's mysterious cousin, *Nat. Rev. Mol. Cell Biol.* 2 (2001) 202–210.
- [4] M.S. Rodriguez, C. Dargemont, R.T. Hay, SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting, *J. Biol. Chem.* 276 (2001) 12654–12659.
- [5] E.S. Johnson, Protein modification by SUMO, *Annu. Rev. Biochem.* 73 (2004) 355–382.
- [6] M.B. Kroetz, SUMO: a ubiquitin-like protein modifier, *Yale J. Biol. Med.* 78 (2005) 197–201.
- [7] H.D. Ulrich, The SUMO system: an overview, *Methods Mol. Biol.* 497 (2009) 3–16.
- [8] J.M. Desterro, M.S. Rodriguez, R.T. Hay, SUMO-1 modification of IκBα inhibits NF-κB activation, *Mol. Cell* 2 (1998) 233–239.
- [9] G. Maarifi, M.A. Maroui, J. Dutrieux, L. Dianoux, S. Nisole, M.K. Chelbi-Alix, Small ubiquitin-like modifier alters IFN response, *J. Immunol.* 195 (2015) 2312–2324.
- [10] M.H. Tatham, M.C. Geoffroy, L. Shen, A. Plechanovova, N. Hattersley, E.G. Jaffray, et al., RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation, *Nat. Cell Biol.* 10 (2008) 538–546.
- [11] F. Aillet, F. Lopitz-Otsoa, I. Egana, R. Hjerpe, P. Fraser, R.T. Hay, et al., Heterologous SUMO-2/3-ubiquitin chains optimize IκBα degradation and NF-κB activity, *PLoS One* 7 (2012) e51672.
- [12] J.M. Desterro, J. Thomson, R.T. Hay, Ubch9 conjugates SUMO but not ubiquitin, *FEBS Lett.* 417 (1997) 297–300.
- [13] J.M. Desterro, M.S. Rodriguez, G.D. Kemp, R.T. Hay, Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1, *J. Biol. Chem.* 274 (1999) 10618–10624.
- [14] D. Mukhopadhyay, M. Dasso, Modification in reverse: the SUMO proteases, *Trends Biochem. Sci.* 32 (2007) 286–295.
- [15] C.M. Hickey, N.R. Wilson, M. Hochstrasser, Function and regulation of SUMO proteases, *Nat. Rev. Mol. Cell Biol.* 13 (2012) 755–766.
- [16] W. Zhou, Z. Hannoun, E. Jaffray, C.N. Medine, J.R. Black, S. Greenhough, et al., SUMOylation of HNF4α regulates protein stability and hepatocyte function, *J. Cell Sci.* 125 (2012) 3630–3635.
- [17] R.T. Hay, Decoding the SUMO signal, *Biochem. Soc. Trans.* 41 (2013) 463–473.
- [18] L. Guo, B.I. Giasson, A. Glavis-Bloom, M.D. Brewer, J. Shorter, A.D. Gitler, et al., A cellular system that degrades misfolded proteins and protects against neurodegeneration, *Mol. Cell* 55 (2014) 15–30.
- [19] F. Lamoliatte, D. Caron, C. Durette, L. Mahrouche, M.A. Maroui, O. Caron-Lizotte, et al., Large-scale analysis of lysine SUMOylation by SUMO remnant immunoaffinity profiling, *Nat. Commun.* 5 (2014) 5409.
- [20] T. Tammsalu, I. Matic, E.G. Jaffray, A.F. Ibrahim, M.H. Tatham, R.T. Hay, Proteome-wide identification of SUMO modification sites by mass spectrometry, *Nat. Protoc.* 10 (2015) 1374–1388.
- [21] I.A. Hendriks, R.C. D'Souza, B. Yang, M. Verlaan-de Vries, M. Mann, A.C. Vertegaal, Uncovering global SUMOylation signaling networks in a site-specific manner, *Nat. Struct. Mol. Biol.* 21 (2014) 927–936.
- [22] F. Galisson, L. Mahrouche, M. Courcelles, E. Bonnell, S. Meloche, M.K. Chelbi-Alix, et al., A novel proteomics approach to identify SUMOylated proteins and their modification sites in human cells, *Mol. Cell. Proteomics* 10 (2011) (M110 004796).
- [23] E. Sloan, M.H. Tatham, M. Gros Lambert, M. Glass, A. Orr, R.T. Hay, et al., Analysis of the SUMO2 proteome during HSV-1 infection, *PLoS Pathog.* 11 (2015) e1005059.
- [24] P. Domingues, F. Golebiowski, M.H. Tatham, A.M. Lopes, A. Taggart, R.T. Hay, et al., Global reprogramming of host SUMOylation during influenza virus infection, *Cell Rep.* 13 (2015) 1467–1480.
- [25] R.D. Everett, C. Boutell, B.G. Hale, Interplay between viruses and host sumoylation pathways, *Nat. Rev. Microbiol.* 11 (2013) 400–411.
- [26] D. Mattoscio, C.V. Segre, S. Chioica, Viral manipulation of cellular protein conjugation pathways: the SUMO lesson, *World J. Virol.* 2 (2013) 79–90.
- [27] P. Wimmer, S. Schreiner, Viral mimicry to usurp ubiquitin and SUMO host pathways, *Viruses* 7 (2015) 4854–4872.
- [28] A. Isaacs, J. Lindenmann, Virus interference I. The interferon, *Proc. R. Soc. London Ser. B Biol. Sci.* 147 (1957) 258–267.
- [29] M.K. Chelbi-Alix, J. Wietzerbin, Interferon, a growing cytokine family: 50 years of interferon research, *Biochimie* 89 (2007) 713–718.
- [30] E.C. Borden, G.C. Sen, G. Uze, R.H. Silverman, R.M. Ransohoff, G.R. Foster, et al., Interferons at age 50: past, current and future impact on biomedicine, *Nat. Rev. Drug Discovery* 6 (2007) 975–990.
- [31] W.M. Schneider, M.D. Chevillotte, C.M. Rice, Interferon-stimulated genes: a complex web of host defenses, *Annu. Rev. Immunol.* 32 (2014) 513–545.
- [32] Y.E. Kim, J.H. Ahn, Positive role of promyelocytic leukemia protein in type I interferon response and its regulation by human cytomegalovirus, *PLoS Pathog.* 11 (2015) e1004785.
- [33] Y. Chu, X. Yang, SUMO E3 ligase activity of TRIM proteins, *Oncogene* 30 (2011) 1108–1116.
- [34] E. Van Damme, K. Laukens, T.H. Dang, X. Van Ostade, A manually curated network of the PML nuclear body interactome reveals an important role for PML-NBs in SUMOylation dynamics, *Intl. J. Biol. Sci.* 6 (2010) 51–67.
- [35] G.A. Versteeg, S. Benke, A. Garcia-Sastre, R. Rajsbaum, InTRIMsic immunity: positive and negative regulation of immune signaling by tripartite motif proteins, *Cytokine Growth Factor Rev.* 25 (2014) 563–576.
- [36] G. Meroni, G. Diez-Roux, TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases, *BioEssays News Rev. Mol. Cell. Dev. Biol.* 27 (2005) 1147–1157.
- [37] L. Carthagen, A. Bergamaschi, J.M. Luna, A. David, P.D. Uchil, F. Margottin-Goguet, et al., Human TRIM gene expression in response to interferons, *PLoS One* 4 (2009) e4894.
- [38] R. Rajsbaum, J.P. Stoye, A. O'Garra, Type I interferon-dependent and-independent expression of tripartite motif proteins in immune cells, *Eur. J. Immunol.* 38 (2008) 619–630.
- [39] K. Ozato, D.M. Shin, T.H. Chang, H.C. Morse 3rd., TRIM family proteins and their emerging roles in innate immunity, *Nat. Rev. Immunol.* 8 (2008) 849–860.
- [40] G.A. Versteeg, R. Rajsbaum, M.T. Sanchez-Aparicio, A.M. Maestre, J. Valdiviezo, M. Shi, et al., The E3-ligase TRIM family of proteins regulates signaling pathways triggered by innate immune pattern-recognition receptors, *Immunity* 38 (2013) 384–398.
- [41] R. Rajsbaum, A. Garcia-Sastre, G.A. Versteeg, TRIM immunity: the roles of the TRIM E3-ubiquitin ligase family in innate antiviral immunity, *J. Mol. Biol.* 426 (2014) 1265–1284.
- [42] M. Stremlau, C.M. Owens, M.J. Perron, M. Kiessling, P. Autissier, J. Sodroski, The cytoplasmic body component TRIM5α restricts HIV-1 infection in old world monkeys, *Nature* 427 (2004) 848–853.
- [43] M. Stremlau, M. Perron, M. Lee, Y. Li, B. Song, H. Javanbakht, et al., Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5α restriction factor, *Proc. Nat. Acad. Sci. U. S. A.* 103 (2006) 5514–5519.
- [44] T. Regad, A. Saib, V. Lallemand-Breitenbach, P.P. Pandolfi, H. de The, M.K. Chelbi-Alix, PML mediates the interferon-induced antiviral state against a complex retrovirus via its association with the viral transactivator, *EMBO J.* 20 (2001) 3495–3505.
- [45] M.A. Maroui, M. Pampin, M.K. Chelbi-Alix, Promyelocytic leukemia isoform IV confers resistance to encephalomyocarditis virus via the sequestration of 3D polymerase in nuclear bodies, *J. Virol.* 85 (2011) 13164–13173.
- [46] P. Eldin, L. Papon, A. Oteiza, E. Brocchi, T.G. Lawson, N. Mechti, TRIM22 E3 ubiquitin ligase activity is required to mediate antiviral activity against encephalomyocarditis virus, *J. Gen. Virol.* 90 (2009) 536–545.
- [47] B. Fu, L. Wang, H. Ding, J.C. Schwamborn, S. Li, M.E. Dorf, TRIM32 senses and restricts influenza A virus by ubiquitination of PB1 polymerase, *PLoS Pathog.* 11 (2015) e1004960.
- [48] R.T. Taylor, K.J. Lubick, S.J. Robertson, J.P. Broughton, M.E. Bloom, W.A. Bresnahan, et al., TRIM79α, an interferon-stimulated gene product, restricts tick-borne encephalitis virus replication by degrading the viral RNA polymerase, *Cell Host Microb.* 10 (2011) 185–196.
- [49] S.D. Barr, J.R. Smiley, F.D. Bushman, The interferon response inhibits HIV particle production by induction of TRIM22, *PLoS Pathog.* 4 (2008) e1000007.
- [50] Q. Liang, H. Deng, X. Li, X. Wu, Q. Tang, T.H. Chang, et al., Tripartite motif-containing protein 28 is a small ubiquitin-related modifier E3 ligase and negative regulator of IFN regulatory factor 7, *J. Immunol.* 187 (2011) 4754–4763.
- [51] S. Elabd, G. Meroni, C. Blattner, TRIMming p53's anticancer activity, *Oncogene* (2016), doi: <http://dx.doi.org/10.1038/ncr.2016.33> [Epub ahead of print].
- [52] M.N. Boddy, K. Howe, L.D. Etkin, E. Solomon, P.S. Freemont, PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukaemia, *Oncogene* 13 (1996) 971–982.
- [53] T. Kamitani, K. Kito, H.P. Nguyen, H. Wada, T. Fukuda-Kamitani, E.T. Yeh, Identification of three major sentrinization sites in PML, *J. Biol. Chem.* 273 (1998) 26675–26682.
- [54] M.C. Geoffroy, M.K. Chelbi-Alix, Role of promyelocytic leukemia protein in host antiviral defense, *J. Interferon Cytokine Res.* 31 (2011) 145–158.
- [55] S. Nisole, M.A. Maroui, X.H. Mascle, M. Aubry, M.K. Chelbi-Alix, Differential roles of PML isoforms, *Front. Oncol.* 3 (2013) 125.
- [56] F. El Asmi, M.A. Maroui, J. Dutrieux, D. Blondel, S. Nisole, M.K. Chelbi-Alix, Implication of PMLIV in both intrinsic and innate immunity, *PLoS Pathog.* 10 (2014) e1003975.
- [57] G. Arriagada, L.N. Muntean, S.P. Goff, SUMO-interacting motifs of human TRIM5α are important for antiviral activity, *PLoS Pathog.* 7 (2011) e1002019.
- [58] J. Dutrieux, D.M. Portillo, N.J. Arhel, U. Hazan, S. Nisole, TRIM5α is a SUMO substrate, *Retrovirology* 12 (2015) 28.

- [59] Z. Lukic, S.P. Goff, E.M. Campbell, G. Arriagada, Role of SUMO-1 and SUMO interacting motifs in rhesus TRIM5alpha-mediated restriction, *Retrovirology* 10 (2013) 10.
- [60] M.K. Chelbi-Alix, L. Pelicano, F. Quignon, M.H. Koken, L. Venturini, M. Stadler, et al., Induction of the PML protein by interferons in normal and APL cells, *Leukemia* 9 (1995) 2027–2033.
- [61] M. Stadler, M.K. Chelbi-Alix, M.H. Koken, L. Venturini, C. Lee, A. Saib, et al., Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element, *Oncogene* 11 (1995) 2565–2573.
- [62] A.M. Ishov, A.G. Sotnikov, D. Negorev, O.V. Vladimirova, N. Neff, T. Kamitani, et al., PML is critical for ND10 formation and recruits the PML-interacting protein daxx to this nuclear structure when modified by SUMO-1, *J. Cell Biol.* 147 (1999) 221–234.
- [63] G. Maarifi, M.K. Chelbi-Alix, S. Nisole, PML control of cytokine signaling, *Cytokine Growth Factor Rev.* 25 (2014) 551–561.
- [64] R. Bernardi, A. Papa, P.P. Pandolfi, Regulation of apoptosis by PML and the PML-NBs, *Oncogene* 27 (2008) 6299–6312.
- [65] E. Kriehoff-Henning, T.G. Hofmann, Role of nuclear bodies in apoptosis signaling, *Biochim. Biophys. Acta* 1783 (2008) 2185–2194.
- [66] N. Tavalai, T. Stamminger, New insights into the role of the subnuclear structure ND10 for viral infection, *Biochim. Biophys. Acta* 1783 (2008) 2207–2221.
- [67] K. Jensen, C. Shiels, P.S. Freemont, PML protein isoforms and the RBCC/TRIM motif, *Oncogene* 20 (2001) 7223–7233.
- [68] M. Reichelt, L. Wang, M. Sommer, J. Perrino, A.M. Nour, N. Sen, et al., Entrapment of viral capsids in nuclear PML cages is an intrinsic antiviral host defense against varicella-zoster virus, *PLoS Pathog.* 7 (2011) e1001266.
- [69] V. Lallemand-Breitenbach, J. Zhu, F. Puvion, M. Koken, N. Honore, A. Doubekovskiy, et al., Role of promyelocytic leukemia (PML) sumoylation in nuclear body formation, 11S proteasome recruitment, and As203-induced PML or PML/retinoic acid receptor alpha degradation, *J. Exp. Med.* 193 (2001) 1361–1371.
- [70] Y. Percherancier, D. Germain-Desprez, F. Galisson, X.H. Mascle, L. Dianoux, P. Estephan, et al., Role of SUMO in RNF4-mediated promyelocytic leukemia protein (PML) degradation: sumoylation of PML and phospho-switch control of its SUMO binding domain dissected in living cells, *J. Biol. Chem.* 284 (2009) 16595–16608.
- [71] M.A. Maroui, S. Kheddache-Atmane, F. El Asmi, L. Dianoux, M. Aubry, M.K. Chelbi-Alix, Requirement of PML SUMO interacting motif for RNF4- or arsenic trioxide-induced degradation of nuclear PML isoforms, *PLoS One* 7 (2012) e44949.
- [72] T.H. Shen, H.K. Lin, P.P. Scaglioni, T.M. Yung, P.P. Pandolfi, The mechanisms of PML-nuclear body formation, *Mol. Cell* 24 (2006) 331–339.
- [73] S. Sachdev, L. Bruhn, H. Sieber, A. Pichler, F. Melchior, R. Grosschedl, PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies, *Genes Dev.* 15 (2001) 3088–3103.
- [74] J.L. Best, S. Ganiatsas, S. Agarwal, A. Changou, P. Salomoni, O. Shiriha, et al., SUMO-1 protease-1 regulates gene transcription through PML, *Mol. Cell* 10 (2002) 843–855.
- [75] M. Pampin, Y. Simonin, B. Blondel, Y. Percherancier, M.K. Chelbi-Alix, Cross talk between PML and p53 during poliovirus infection: implications for antiviral defense, *J. Virol.* 80 (2006) 8582–8592.
- [76] A. Moller, H. Sirma, T.G. Hofmann, S. Rueffer, E. Klimczak, W. Droge, et al., PML is required for homeodomain-interacting protein kinase 2 (HIPK2)-mediated p53 phosphorylation and cell cycle arrest but is dispensable for the formation of HIPK domains, *Cancer Res.* 63 (2003) 4310–4314.
- [77] M. Pearson, R. Carbone, C. Sebastiani, M. Cioce, M. Fagioli, S. Saito, et al., PML regulates p53 acetylation and premature senescence induced by oncogenic Ras, *Nature* 406 (2000) 207–210.
- [78] B. El McHichi, T. Regad, M.A. Maroui, M.S. Rodriguez, A. Aminev, S. Gerbaud, et al., SUMOylation promotes PML degradation during encephalomyocarditis virus infection, *J. Virol.* 84 (2010) 11634–11645.
- [79] V. Lallemand-Breitenbach, M. Jeanne, S. Benhenda, R. Nasr, M. Lei, L. Peres, et al., Arsenic degrades PML or PML-RARalpha through a SUMO-triggered RNF4/ubiquitin-mediated pathway, *Nat. Cell Biol.* 10 (2008) 547–555.
- [80] C. Wilkins, M. Gale Jr., Recognition of viruses by cytoplasmic sensors, *Curr. Opin. Immunol.* 22 (2010) 41–47.
- [81] S.M. Belgnaoui, S. Paz, J. Hiscott, Orchestrating the interferon antiviral response through the mitochondrial antiviral signaling (MAVS) adaptor, *Curr. Opin. Immunol.* 23 (2011) 564–572.
- [82] K.A. Fitzgerald, S.M. McWhirter, K.L. Faia, D.C. Rowe, E. Latz, D.T. Golenbock, et al., IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway, *Nat. Immunol.* 4 (2003) 491–496.
- [83] S. Sharma, B.R. tenOever, N. Grandvaux, G.P. Zhou, R. Lin, J. Hiscott, Triggering the interferon antiviral response through an IKK-related pathway, *Science* 300 (2003) 1148–1151.
- [84] R. Lin, C. Heylbroeck, P.M. Pitha, J. Hiscott, Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation, *Mol. Cell Biol.* 18 (1998) 2986–2996.
- [85] W.C. Au, P.A. Moore, D.W. LaFleur, B. Tombal, P.M. Pitha, Characterization of the interferon regulatory factor-7 and its potential role in the transcription activation of interferon A genes, *J. Biol. Chem.* 273 (1998) 29210–29217.
- [86] Y. Ran, T.T. Liu, Q. Zhou, S. Li, A.P. Mao, Y. Li, et al., SENP2 negatively regulates cellular antiviral response by deSUMOylating IRF3 and conditioning it for ubiquitination and degradation, *J. Mol. Cell Biol.* 3 (2011) 283–292.
- [87] T. Kubota, M. Matsuoka, T.H. Chang, P. Taylor, T. Sasaki, M. Tashiro, et al., Virus infection triggers SUMOylation of IRF3 and IRF7, leading to the negative regulation of type I interferon gene expression, *J. Biol. Chem.* 283 (2008) 25660–25670.
- [88] T.H. Chang, T. Kubota, M. Matsuoka, S. Jones, S.B. Bradfute, M. Bray, et al., Ebola Zaire virus blocks type I interferon production by exploiting the host SUMO modification machinery, *PLoS Pathog.* 5 (2009) e1000493.
- [89] K. Nakagawa, H. Yokosawa, PIAS3 induces SUMO-1 modification and transcriptional repression of IRF-1, *FEBS Lett.* 530 (2002) 204–208.
- [90] E.J. Kim, J.S. Park, S.J. Um, Ubc9-mediated sumoylation leads to transcriptional repression of IRF-1, *Biochem. Biophys. Res. Commun.* 377 (2008) 952–956.
- [91] K.J. Han, L. Jiang, H.B. Shu, Regulation of IRF2 transcriptional activity by its sumoylation, *Biochem. Biophys. Res. Commun.* 372 (2008) 772–778.
- [92] Z. Mi, J. Fu, Y. Xiong, H. Tang, SUMOylation of RIG-I positively regulates the type I interferon signaling, *Protein Cell* 1 (2010) 275–283.
- [93] J. Fu, Y. Xiong, Y. Xu, G. Cheng, H. Tang, MDA5 is SUMOylated by PIAS2beta in the upregulation of type I interferon signaling, *Mol. Immunol.* 48 (2011) 415–422.
- [94] G.L. Bentz, J. Shackelford, J.S. Pagano, Epstein-Barr virus latent membrane protein 1 regulates the function of interferon regulatory factor 7 by inducing its sumoylation, *J. Virol.* 86 (2012) 12251–12261.
- [95] N. Tanaka, M. Ishihara, M. Kitagawa, H. Harada, T. Kimura, T. Matsuyama, et al., Cellular commitment to oncogene-induced transformation or apoptosis is dependent on the transcription factor IRF-1, *Cell* 77 (1994) 829–839.
- [96] H. Harada, T. Fujita, M. Miyamoto, Y. Kimura, M. Maruyama, A. Furia, et al., Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes, *Cell* 58 (1989) 729–739.
- [97] S.M. Park, M. Chae, B.K. Kim, T. Seo, I.S. Jang, J.S. Choi, et al., SUMOylated IRF-1 shows oncogenic potential by mimicking IRF-2, *Biochem. Biophys. Res. Commun.* 391 (2010) 926–930.
- [98] A. Decque, O. Joffre, J.G. Magalhaes, J.C. Cossec, R. Blecher-Gonen, P. Lapaquette, et al., Sumoylation coordinates the repression of inflammatory and anti-viral gene-expression programs during innate sensing, *Nat. Immunol.* 17 (2016) 140–149.
- [99] T. Saitoh, A. Tun-Kyi, A. Ryo, M. Yamamoto, G. Finn, T. Fujita, et al., Negative regulation of interferon-regulatory factor 3-dependent innate antiviral response by the prolyl isomerase Pin1, *Nat. Immunol.* 7 (2006) 598–605.
- [100] H.X. Shi, K. Yang, X. Liu, X.Y. Liu, B. Wei, Y.F. Shan, et al., Positive regulation of interferon regulatory factor 3 activation by Herc5 via ISG15 modification, *Mol. Cell Biol.* 30 (2010) 2424–2436.
- [101] G. Lu, J.T. Reinert, I. Pitha-Rowe, A. Okumura, M. Kellum, K.P. Knobeloch, et al., ISG15 enhances the innate antiviral response by inhibition of IRF-3 degradation, *Cell. Mol. Biol.* 52 (2006) 29–41.
- [102] C.H. Chen, C.C. Chang, T.H. Lee, M. Luo, P. Huang, P.H. Liao, et al., SENP1 deSUMOylates and regulates Pin1 protein activity and cellular function, *Cancer Res.* 73 (2013) 3951–3962.
- [103] M. Li, X. Liu, Y. Zhou, S.B. Su, Interferon-lambdas: the modulators of antiviral, antitumor, and immune responses, *J. Leukocyte Biol.* 86 (2009) 23–32.
- [104] R.S. Rogers, C.M. Horvath, M.J. Matunis, SUMO modification of STAT1 and its role in PIAS-mediated inhibition of gene activation, *J. Biol. Chem.* 278 (2003) 30091–30097.
- [105] D. Ungureanu, S. Vanhatupa, J. Gronholm, J.J. Palvimo, O. Silvennoinen, SUMO-1 conjugation selectively modulates STAT1-mediated gene responses, *Blood* 106 (2005) 224–226.
- [106] D. Ungureanu, S. Vanhatupa, N. Kotaja, J. Yang, S. Aittomaki, O.A. Janne, et al., PIAS proteins promote SUMO-1 conjugation to STAT1, *Blood* 102 (2003) 3311–3313.
- [107] J. Gronholm, S. Vanhatupa, D. Ungureanu, J. Valiaho, T. Laitinen, J. Valjakka, et al., Structure-function analysis indicates that sumoylation modulates DNA-binding activity of STAT1, *BMC Biochem.* 13 (2012) 20.
- [108] A. Begitt, M. Droscher, K.P. Knobeloch, U. Vinkemeier, SUMO conjugation of STAT1 protects cells from hyperresponsiveness to IFNgamma, *Blood* 118 (2011) 1002–1007.
- [109] H.C. Steen, A.M. Gamero, The role of signal transducer and activator of transcription-2 in the interferon response, *J. Interferon Cytokine Res.* 32 (2012) 103–110.
- [110] K. Blaszczyk, A. Olejnik, H. Nowicka, L. Ozgyn, Y.L. Chen, S. Chmielewski, et al., STAT2/IRF9 directs a prolonged ISGF3-like transcriptional response and antiviral activity in the absence of STAT1, *Biochem. J.* 466 (2015) 511–524.
- [111] A. Flotho, F. Melchior, Sumoylation: a regulatory protein modification in health and disease, *Annu. Rev. Biochem.* 82 (2013) 357–385.
- [112] H. Saitoh, J. Hinchey, Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3, *J. Biol. Chem.* 275 (2000) 6252–6258.
- [113] N. Kolli, J. Mikolajczyk, M. Drag, D. Mukhopadhyay, N. Moffatt, et al., Distribution and paralogue specificity of mammalian deSUMOylating enzymes, *Biochem. J.* 430 (2010) 335–344.
- [114] T. Grotzinger, K. Jensen, H. Will, The interferon (IFN)-stimulated gene Sp100 promoter contains an IFN-gamma activation site and an imperfect IFN-

- stimulated response element which mediate type I IFN inducibility, *J. Biol. Chem.* 271 (1996) 25253–25260.
- [115] J. El Bougrini, L. Dianoux, M.K. Chelbi-Alix, PML positively regulates interferon gamma signaling, *Biochimie* 93 (2011) 389–398.
- [116] Z.G. Wang, D. Ruggero, S. Ronchetti, S. Zhong, M. Gaboli, R. Rivi, et al., PML is essential for multiple apoptotic pathways, *Nat. Genet.* 20 (1998) 266–272.
- [117] H.R. Lee, D.J. Kim, J.M. Lee, C.Y. Choi, B.Y. Ahn, G.S. Hayward, et al., Ability of the human cytomegalovirus IE1 protein to modulate sumoylation of PML correlates with its functional activities in transcriptional regulation and infectivity in cultured fibroblast cells, *J. Virol.* 78 (2004) 6527–6542.
- [118] R.D. Everett, M.K. Chelbi-Alix, PML and PML nuclear bodies: implications in antiviral defence, *Biochimie* 89 (2007) 819–830.
- [119] D. Blondel, S. Kheddache, X. Lahaye, L. Dianoux, M.K. Chelbi-Alix, Resistance to rabies virus infection conferred by the PMLIV isoform, *J. Virol.* 84 (2010) 10719–10726.
- [120] C.F. de la Cruz-Herrera, M. Campagna, M.A. Garcia, L. Marcos-Villar, V. Lang, M. Baz-Martinez, et al., Activation of the double-stranded RNA-dependent protein kinase PKR by small ubiquitin-like modifier (SUMO), *J. Biol. Chem.* 289 (2014) 26357–26367.
- [121] L. Marcos-Villar, J.V. Perez-Giron, J.M. Vilas, A. Soto, C.F. de la Cruz-Herrera, V. Lang, et al., SUMOylation of p53 mediates interferon activities, *Cell Cycle* 12 (2013) 2809–2816.
- [122] C.E. Brantis-de-Carvalho, G. Maarifi, P.E. Goncalves Boldrin, C.F. Zanelli, S. Nisole, M.K. Chelbi-Alix, et al., MxA interacts with and is modified by the SUMOylation machinery, *Exp. Cell Res.* 330 (2015) 151–163.
- [123] W.V. Bonilla, D.D. Pinschewer, P. Klennerman, V. Rousson, M. Gaboli, P.P. Pandolfi, et al., Effects of promyelocytic leukemia protein on virus-host balance, *J. Virol.* 76 (2002) 3810–3818.
- [124] M.K. Chelbi-Alix, F. Quignon, L. Pelicano, M.H. Koken, H. de The, Resistance to virus infection conferred by the interferon-induced promyelocytic leukemia protein, *J. Virol.* 72 (1998) 1043–1051.
- [125] J. Dutrieux, G. Maarifi, D.M. Portilho, N.J. Arhel, M.K. Chelbi-Alix, S. Nisole, PML/TRIM19-dependent inhibition of retroviral reverse-transcription by Daxx, *PLoS Pathog.* 11 (2015) e1005280.
- [126] N. Masroori, N. Merindol, L. Berthou, The interferon-induced antiviral protein PML (TRIM19) promotes the restriction and transcriptional silencing of lentiviruses in a context-specific, isoform-specific fashion, *Retrovirology* 13 (2016) 19.
- [127] R. Bernardi, P.P. Scaglioni, S. Bergmann, H.F. Horn, K.H. Vousden, P.P. Pandolfi, PML regulates p53 stability by sequestering Mdm2 to the nucleolus, *Nat. Cell Biol.* 6 (2004) 665–672.
- [128] A. Takaoka, S. Hayakawa, H. Yanai, D. Stoiber, H. Negishi, H. Kikuchi, et al., Integration of interferon- α/β signalling to p53 responses in tumour suppression and antiviral defence, *Nature* 424 (2003) 516–523.
- [129] E. de Stanchina, E. Querido, M. Narita, R.V. Davuluri, P.P. Pandolfi, G. Ferbeyre, et al., PML is a direct p53 target that modulates p53 effector functions, *Mol. Cell* 13 (2004) 523–535.
- [130] M. Pearson, P.G. Pelicci, PML interaction with p53 and its role in apoptosis and replicative senescence, *Oncogene* 20 (2001) 7250–7256.
- [131] C.D. Meiering, M.L. Linial, The promyelocytic leukemia protein does not mediate foamy virus latency in vitro, *J. Virol.* 77 (2003) 2207–2213.
- [132] M. Gostissa, A. Hengstermann, V. Fogal, P. Sandy, S.E. Schwarz, M. Scheffner, et al., Activation of p53 by conjugation to the ubiquitin-like protein SUMO-1, *EMBO J.* 18 (1999) 6462–6471.
- [133] M.S. Rodriguez, J.M. Desterro, S. Lain, C.A. Midgley, D.P. Lane, R.T. Hay, SUMO-1 modification activates the transcriptional response of p53, *EMBO J.* 18 (1999) 6455–6461.
- [134] S. Muller, M. Berger, F. Lehembre, J.S. Seeler, Y. Haupt, A. Dejean, c-Jun and p53 activity is modulated by SUMO-1 modification, *J. Biol. Chem.* 275 (2000) 13321–13329.
- [135] L. Chen, J. Chen, MDM2-ARF complex regulates p53 sumoylation, *Oncogene* 22 (2003) 5348–5357.
- [136] O. Bischof, K. Schwamborn, N. Martin, A. Werner, C. Sustmann, R. Grosschedl, et al., The E3 SUMO ligase PIASy is a regulator of cellular senescence and apoptosis, *Mol. Cell* 22 (2006) 783–794.
- [137] M.H. Stindt, S. Carter, A.M. Vigneron, K.M. Ryan, K.H. Vousden, MDM2 promotes SUMO-2/3 modification of p53 to modulate transcriptional activity, *Cell Cycle* 10 (2011) 3176–3188.
- [138] P.D. Bieniasz, Intrinsic immunity: a front-line defense against viral attack, *Nat. Immunol.* 5 (2004) 1109–1115.
- [139] B. Song, H. Javanbakht, M. Perron, D.H. Park, M. Stremlau, J. Sodroski, Retrovirus restriction by TRIM5 α variants from old world and new world primates, *J. Virol.* 79 (2005) 3930–3937.
- [140] M.A. Garcia, J. Gil, I. Ventoso, S. Guerra, E. Domingo, C. Rivas, et al., Impact of protein kinase PKR in cell biology: from antiviral to antiproliferative action, *Microbiol. Mol. Biol. Rev.* 70 (2006) 1032–1060.
- [141] C.G. Proud, PKR: a new name and new roles, *Trends Biochem. Sci.* 20 (1995) 241–246.
- [142] M. Gale Jr., M.G. Katze, Molecular mechanisms of interferon resistance mediated by viral-directed inhibition of PKR, the interferon-induced protein kinase, *Pharmacol. Ther.* 78 (1998) 29–46.
- [143] F. Okumura, A.J. Okumura, K. Uematsu, S. Hatakeyama, D.E. Zhang, T. Kamura, Activation of double-stranded RNA-activated protein kinase (PKR) by interferon-stimulated gene 15 (ISG15) modification down-regulates protein translation, *J. Biol. Chem.* 288 (2013) 2839–2847.
- [144] O. Haller, P. Staeheli, M. Schwemmle, G. Kochs, Mx GTPases: dynamin-like antiviral machines of innate immunity, *Trends Microbiol.* 23 (2015) 154–163.
- [145] O. Haller, S. Stertz, G. Kochs, The Mx GTPase family of interferon-induced antiviral proteins, *Microbes Infect.* Institut. Pasteur 9 (2007) 1636–1643.
- [146] O. Haller, G. Kochs, Interferon-induced mx proteins: dynamin-like GTPases with antiviral activity, *Traffic* 3 (2002) 710–717.
- [147] C. Janzen, G. Kochs, O. Haller, A monomeric GTPase-negative MxA mutant with antiviral activity, *J. Virol.* 74 (2000) 8202–8206.

Zara Hannoun completed her PhD at the University of Edinburgh focusing on the role of SUMOylation of hepatic differentiation of human embryonic stem cells. She then moved into industry and carried out a number of clinical studies aimed at elucidating statistically relevant determinants of diabetes. Due to her interest in fundamental research, she moved to join Dr. Chelbi-Alix to work on the effects of SUMOylation in intrinsic and innate immunity.

Ghizlane Maarifi is a PhD student, her thesis will be defended in June 2016. She is investigating the role of SUMO on interferon signaling and antiviral defense.

Mounira K. Chelbi-Alix was trained in cellular and molecular biology at the Institut de Biologie Physico-Chimique, Paris and obtained a PhD in the field of interferon research from the University of Paris VII in 1981. She became a CNRS (Centre National de la Recherche Scientifique) staff scientist in 1981 and heads a laboratory as a CNRS director of research since 1998. Her current interests concern the antiviral action of interferon and the role of SUMO and PML nuclear bodies in intrinsic and innate immunity.