REGULATION OF GENE-ACTIVATION PATHWAYS BY PIAS PROTEINS IN THE IMMUNE SYSTEM

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Abstract | The protein inhibitor of activated STAT (PIAS) family of proteins has been proposed to regulate the activity of many transcription factors, including signal transducer and activator of transcription proteins (STATs), nuclear factor-κB, SMA- and MAD-related proteins (SMADs), and the tumour-suppressor protein p53. PIAS proteins regulate transcription through several mechanisms, including blocking the DNA-binding activity of transcription factors, recruiting transcriptional corepressors or co-activators, and promoting protein sumoylation. Recent genetic studies support an *in vivo* function for PIAS proteins in the regulation of innate immune responses. In this article, we review the current understanding of the molecular basis, specificity and physiological roles of PIAS proteins in the regulation of gene-activation pathways in the immune system.

SMALL-UBIQUITIN-LIKE-MODIFIER E3 LIGASE (SUMO E3 ligase). An enzyme that catalyses the conjugation of SUMO to a protein substrate.

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doi:10.1038/nri1667

Cytokines regulate the functions of immune cells mainly by modifying the transcriptional profile of a cell to increase or decrease the expression of relevant genes. Signal transducer and activator of transcription proteins (STATs), nuclear factor-κB (NF-κB), and SMA (small body size)- and MAD (mothers against decapentaplegic)-related proteins (SMADs) are three key families of transcription factors that are widely used downstream of cytokine-mediated signalling to regulate gene expression. After a cytokine binds its cognate receptor, these transcription factors become activated in the cytoplasm and then translocate to the nucleus, where they bind DNA and regulate the transcription of specific genes (FIG. 1). The activity of STATs, NF-κB and SMADs is tightly regulated at several levels, and inappropriate regulation can result in diseases in humans, including cancers and immune disorders[1–9]. For example, constitutive activation of NF-κB has been reported in several inflammatory disorders, including asthma, inflammatory bowel disease and rheumatoid arthritis[10]. Individuals with a deficiency in STAT1 suffer from mycobacterial infections and die from virally induced disease[11].

Mammalian protein inhibitor of activated STAT (PIAS) proteins were initially identified as negative regulators of STAT signalling[12,13]. The PIAS family consists of PIAS1, PIAS3, PIASx (also known as PIAS2) and PIASy (also known as PIAS4)[2,14,15]. Except for PIAS1, two isoforms of each PIAS protein have been identified (FIG. 2a). Recent studies indicate that PIAS proteins have SMALL UBIQUITIN-LIKE MODIFIER (SUMO)-E3-LIGASE activity[16,17]. Biochemical studies by many research groups have now identified a rapidly growing list of more than 60 proteins, most of them transcription factors, that can be either positively or negatively regulated by members of the PIAS family through multiple mechanisms (TABLE 1; see Supplementary information S1 (table)). Gene-targeting studies have shown an important role for PIAS proteins in the regulation of the immune system and have uncovered an unexpected specificity of PIAS proteins in the regulation of a subset of cytokine-induced genes. This Review article focuses on the regulation of key transcription factors in the immune system by PIAS proteins and discusses the latest insights into the molecular mechanisms, specificity and physiological functions of PIAS proteins in immune regulation.

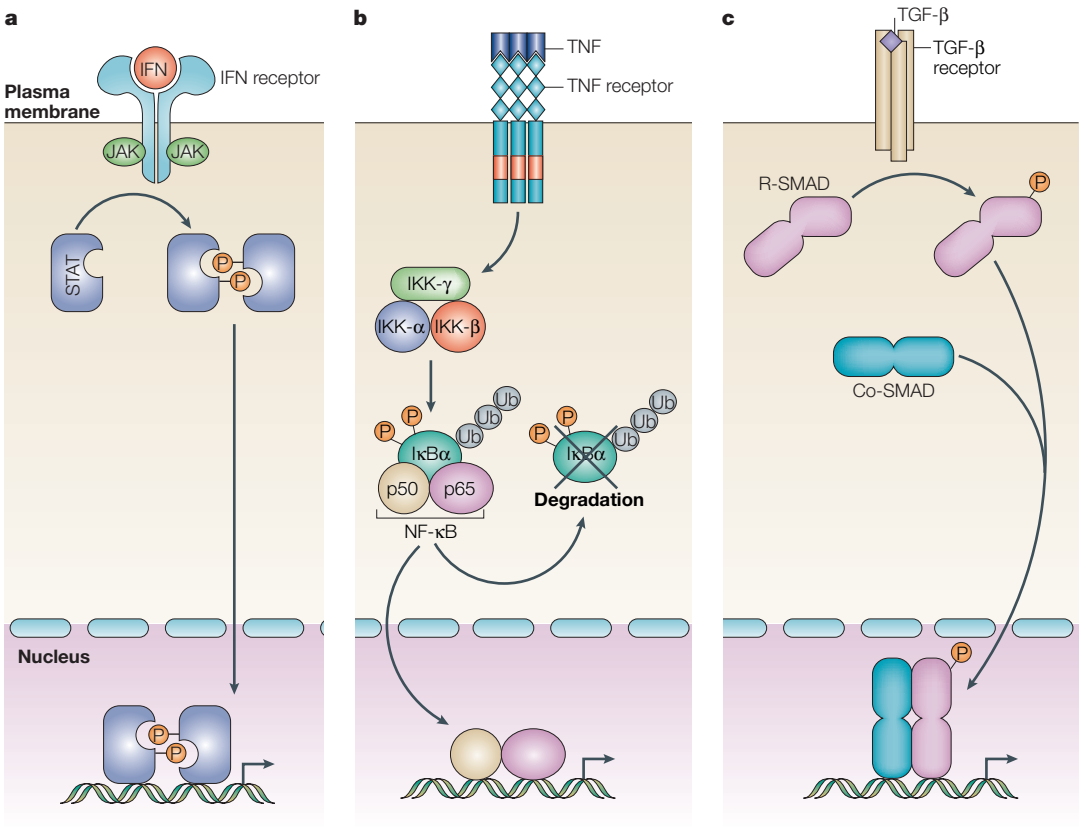


Figure 1 | **The cytokine-activated STAT, NF-κB and SMAD pathways.** Signal transducer and activator of transcription proteins (STATs), nuclear factor-κB (NF-κB) proteins, and SMA (small body size)- and MAD (mothers against decapentaplegic)-related proteins (SMADs) are three important families of latent cytoplasmic transcription factors. On stimulation of the cell with cytokine, these transcription factors become activated and translocate to the nucleus, where they bind DNA and activate the transcription of specific genes. **a** | STATs are latent cytoplasmic transcription factors that become phosphorylated by Janus activated kinases (JAKs) in response to various cytokines. Tyrosine-phosphorylated STATs then dimerize and translocate to the nucleus, where they activate transcription. **b** | NF-κB proteins are present in the cytoplasm in an inactive form as a result of their association with inhibitor of NF-κB (IκB) proteins. Signal-induced phosphorylation of IκB is mediated by the IκB kinase (IKK) complex. Phosphorylated IκB is then ubiquitylated, which targets it for degradation by the 26S proteasome, thereby releasing NF-κB dimers and allowing them to translocate to the nucleus. **c** | On stimulation with transforming growth factor-β (TGF-β), the receptor-regulated SMADs (R-SMADs) SMAD2 and SMAD3 become serine phosphorylated and form a heterodimeric complex with the common-mediator SMAD (Co-SMAD), SMAD4. The SMAD2–SMAD4 and SMAD3–SMAD4 heterodimers then translocate to the nucleus, where they cooperate with other transcription factors and co-regulators to regulate transcription. IFN, interferon; TNF, tumour-necrosis factor.

Domain structure and function of PIAS proteins

Members of the mammalian PIAS family have significant sequence identity (more than 40%), and several functional domains and motifs that are conserved between the PIAS-family members have been identified (FIG. 2a).

A SAP domain (scaffold-attachment factor A (SAFA) and SAFB, apoptotic chromatin-condensation inducer in the nucleus (ACINUS) and PIAS domain) is located at the amino (N) terminus of PIAS proteins. This domain, which was initially identified through protein-sequence analysis, is present in many chromatin-binding proteins, such as SAFA and SAFB[18]. The SAP domain of SAFA and SAFB can recognize and bind (A+T)-rich DNA sequences that are present in SCAFFOLD-ATTACHMENT REGIONS (SARs; also known as matrix-attachment regions)[19], which might be involved in anchoring independent chromatin loops to a subnuclear structure known as the NUCLEAR SCAFFOLD or nuclear matrix. SARs are frequently found close to gene enhancers, and the interaction of SARs with nuclear-scaffold proteins creates a unique nuclear microenvironment for transcriptional regulation. Indeed, the SAP domain of PIASy or PIAS1 can bind non-specific (A+T)-rich DNA sequences *in vitro*[20]. So, the SAP domain might target PIAS proteins to the nuclear scaffold. The three-dimensional structure of the N-terminal region (amino acids 1–65) of PIAS1, which contains a SAP domain, has recently been determined using nuclear magnetic resonance (NMR) spectroscopy, and it shows a unique four-helix-bundle stucture[21]. NMR analysis of the SAP domain of PIAS1 in complex with a 16-base-pair DNA fragment indicates that one end of the four-helix bundle is the DNA-binding site.

SCAFFOLD-ATTACHMENT REGION
(SAR). Also known as matrix-attachment region. A DNA element in the eukaryotic genome that attaches the chromatin fibre to the nuclear scaffold (which is also known as the nuclear matrix).

NUCLEAR SCAFFOLD
Also known as nuclear matrix. A subnuclear structure that consists of the proteinaceous network of the nucleus.

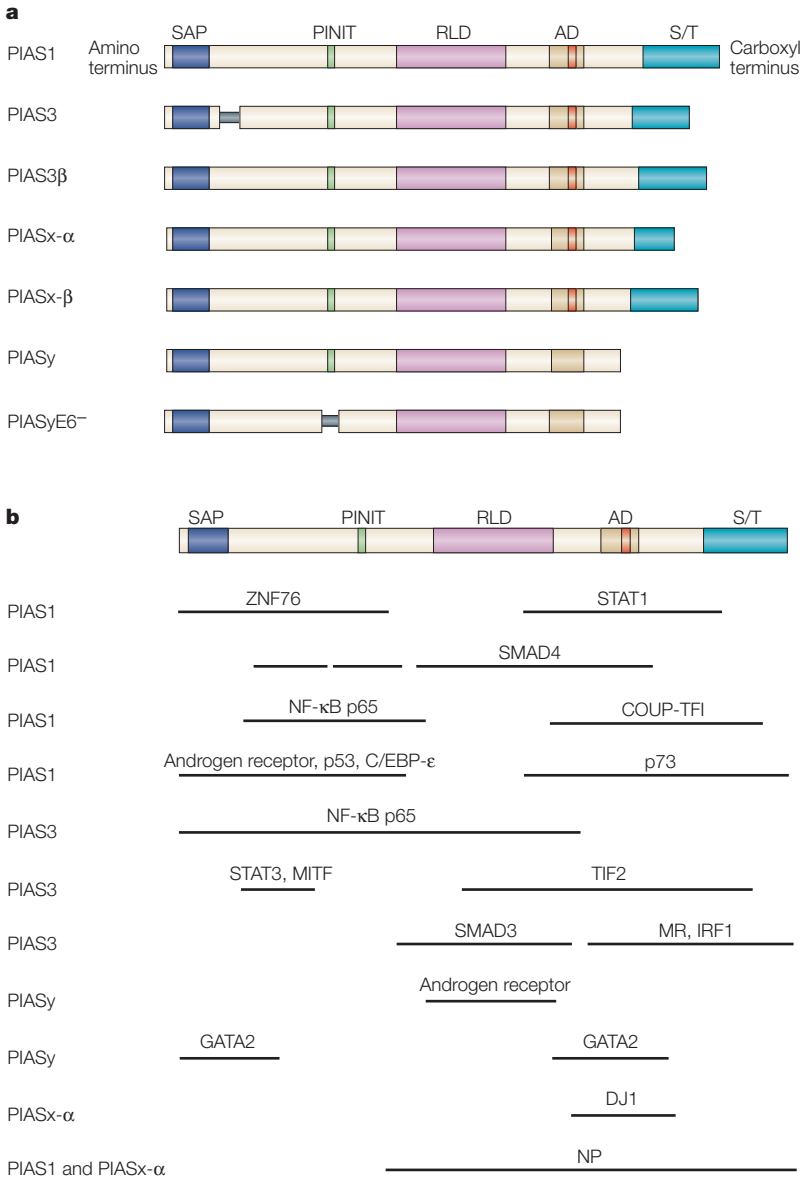


Figure 2 | **The domain structure of PIAS proteins. a** | Shown are the conserved domains of protein inhibitor of activated STAT (signal transducer and activator of transcription protein) proteins (PIAS proteins). Four mammalian PIAS-family members have been identified: PIAS1, PIAS3, PIASx and PIASy. Each PIAS protein, except PIAS1, has two isoforms. Compared with PIAS3, PIAS3β contains an insertion of 39 amino-acid residues. PIASx-α and PIASx-β are identical except for their carboxy-terminal region. PIASyE6⁻ is a splice variant of PIASy that lacks exon 6, which encodes amino-acid residues 218–260 of PIASy. Most PIAS proteins have five conserved domains or motifs: the SAP domain (scaffold-attachment factor A (SAFA) and SAFB, apoptotic chromatin-condensation inducer in the nucleus (ACINUS) and PIAS domain), which contains a conserved LXXLL amino-acid motif (where X denotes any amino acid); the PINIT amino-acid motif, which is present in all PIAS proteins except PIASyE6⁻; the RLD (RING-finger-like zinc-binding domain); the AD (highly acidic domain), which contains a SUMO1 (small ubiquitin-like modifier 1)-interaction motif (red) that is found in all PIAS proteins except PIASy and PIASyE6⁻; and the S/T region (serine- and threonine-rich region), which is also not present in PIASy and PIASyE6⁻. **b** | Shown are the PIAS-protein regions that are involved in protein–protein interactions (for further details, see main text and TABLE 1). C/EBP-ε, CCAAT/enhancer-binding protein-ε; COUP-TFI, chicken ovalbumin upstream-promoter transcription factor 1; GATA2, GATA-binding protein 2; IRF1, interferon-regulatory factor 1; MITF, microphthalmia-associated transcription factor; MR, mineralocorticoid receptor; NF-κB, nuclear factor-κB; NP, nucleocapsid protein, from hantavirus; p53, tumour-suppressor protein p53; p73, tumour protein p73; SMAD, SMA (small body size)- and MAD (mothers against decapentaplegic)-related protein; TIF2, transcriptional intermediary factor 2; ZNF76, zinc-finger protein 76.

An LXXLL amino-acid motif (where X denotes any amino acid) is located within the SAP domain of PIAS proteins. This LXXLL signature motif — which was previously identified in several nuclear-receptor co-regulators, such as steroid-receptor co-activator 1 (REF. 22) and nuclear-receptor corepressor 1 (REFS 23,24) — is an α-helical protein-interaction module that mediates interactions between nuclear receptors and their co-regulators[25]. The LXXLL motif of PIASy is required for PIASy-mediated transcriptional repression of STAT1 (REF. 26) or androgen receptor[27].

The PIAS family also contains a conserved CCCHCCCC-motif-type RING-finger-like zinc-binding domain (RLD). However, the spacing between potential zinc-coordinating residues in the PIAS RLD differs substantially from the genuine RING domains that are present in many ubiquitin E3 ligases[28]. In addition, the cysteine residue at the fifth position of the putative CCCHCCCC domain is absent in PIAS3; the significance of this difference is unknown. The PIAS RLD is required for the SUMO-E3-ligase activity of PIAS proteins, because replacement of a conserved tryptophan residue in the RLD with a phenylalanine residue abolished the SUMO-E3-ligase activity of PIAS proteins[29].

The PINIT amino-acid motif, which is located in a highly conserved region of PIAS proteins, has recently been identified[30]. This motif is present in all PIAS proteins except PIASyE6⁻, which is a splice variant of PIASy that lacks exon 6 (REF. 31). Mutations in the PINIT motif of PIAS3, which is usually present in the nucleus, resulted in disruption of the restricted nuclear expression of PIAS3 (REF. 30), so it is possible that the PINIT motif of other PIAS proteins might also be involved in their nuclear retention.

The carboxy (C)-terminal region of PIAS proteins, which is the most diverse region, contains a highly acidic domain (AD) and a serine and threonine rich (S/T) region. In addition, a putative SUMO1-interaction motif (SIM) is present in the AD[32]. The functional roles of the AD, S/T region and SIM of PIAS proteins remain to be defined. It was reported that removal of the SIM of PIASx-α had no effect on the SUMO-E3-ligase activity of PIASx-α[29]. In addition, although PIASy and PIASyE6⁻ do not contain the conserved SIM, they remain functional in promoting the conjugation of SUMO to proteins[20,31]. Taken together, these results indicate that there are multiple SUMO-interaction domains in PIAS proteins.

More than 60 proteins, most of them transcription factors, have now been suggested to interact with members of the PIAS family (TABLE 1; see Supplementary information S1 (table)), and the regions of PIAS proteins that are involved in protein–protein interactions have been identified in many studies. Interestingly, various regions of PIAS proteins seem to be involved in different protein–protein interactions (FIG. 2b). For example, the N-terminal region of PIAS1 can interact with the p65 subunit of NF-κB[33], whereas the C-terminal region of PIAS1 can bind STAT1 (REF. 34). These findings raise the possibility that targeted mutational analysis

Table 1 | **Main PIAS-interacting proteins involved in immune regulation**

Interacting protein	PIAS	Interacting domain of PIAS*	Effect of interaction with PIAS	Refs
Interferon pathways				
STAT1	PIAS1 PIASy PIAS1, -3 , -x-α	392–541 ND ND	Repression of STAT1, by blocking DNA binding of STAT1 Repression of STAT1, by functioning as a STAT1 corepressor Sumoylation of STAT1	13,34 26 43–45
STAT3	PIAS3	82–156	Repression of STAT3, by blocking DNA binding of STAT3	12,84
STAT4	PIASx	ND	Repression of STAT4, by functioning as a STAT4 corepressor	37
STAT5	PIAS3	ND	Repression of STAT5-mediated transcription	102
GFI1	PIAS3	ND	Release of repression of STAT3 by PIAS3	103
ATBF1	PIAS3	ND	Repression of STAT3, working in synergy with PIAS3	104
IRF1	PIAS3	428–619	Repression of IRF1-mediated transcription	105
IRF3, -7	PIASy	ND	Repression of IRF3- and IRF7-mediated transcription	106
NF-κB pathways				
NF-κB p65	PIAS1 PIAS3	89–344 1–391	Repression of NF-κB p65, by blocking DNA binding of NF-κB p65 Repression of NF-κB p65, by blocking interaction of NF-κB p65 with CBP	33 56
TRIF	PIASy	ND	Repression of TRIF-mediated transcription	106
SMAD pathways				
SMAD3	PIAS3 PIASy	274–392 ND	Activation of SMAD3-mediated transcription, by recruiting p300 or CBP Repression of SMAD3-mediated transcription, by recruiting HDAC1; sumoylation of SMAD3 and SMAD4	46 38,58, 59
SMAD4	PIAS1 PIASx-β	120–480 ND	Activation of SMAD4-mediated transcription, by sumoylating SMAD4 Activation of SMAD4-mediated transcription, by sumoylating SMAD4	61 60
Oncoproteins and tumour-suppressor proteins				
p53	PIAS1 PIASx-β	1–318 ND	Possibly activation or repression of p53, independent of p53 sumoylation Repression of p53-mediated transcription, by sumoylating p53	68–70 69
p73	PIAS1 PIASx	401–651 ND	Regulation of cell cycle, by sumoylating p73 Regulation of subcellular localization of p73, by sumoylating p73	74 32
JUN	PIAS1, -x	ND	Sumoylation of JUN	69
MYB	PIASy	ND	Repression of MYB-mediated transcription, by sumoylating MYB	107
MDM2	PIAS1, -x-β	ND	Regulation of nuclear localization of MDM2, by sumoylating MDM2	78
PLAG1	PIAS1, -y	ND	Repression of PLAG1-mediated transcription, by sumoylating PLAG1	108
LEF1	PIASy	ND	Repression of LEF1-mediated transcription, by sequestering LEF1; sumoylation of LEF1	20
Viral proteins				
IE2	PIAS1	ND	Activation of IE2-mediated transcription; sumoylation of IE2	92
RTA	PIAS1	ND	Activation of RTA-mediated transcription; sumoylation of RTA	91
E1	PIAS1, -x	ND	Sumoylation of E1	94
NP	PIAS1, -x-β	284–651	ND	93
Others				
C/EBP-ε	PIAS1 PIASx	1–338 ND	ND Activation of C/EBP-ε-mediated transcription; sumoylation of C/EBP-ε	89 90
SATB2	PIAS1	ND	Repression of SATB2-mediated transcription; sumoylation of SATB2	79
MITF	PIAS3	82–156	Repression of MITF-mediated transcription	82–84

*Range indicates interacting amino-acid region. ATBF1, AT-binding transcription factor 1; CBP, cyclic-AMP-responsive-element-binding protein (CREB)-binding protein; C/EBP-ε, CCAAT/enhancer-binding protein-ε; E1, protein E1, from papillomavirus; GFI1, growth-factor independent 1; HDAC1, histone deacetylase 1; IE2, immediate-early protein 2, from human cytomegalovirus; IRF, interferon-regulatory factor; LEF1, lymphoid-enhancer-binding factor 1; MDM2, mouse double minute 2 homologue; MITF, microphthalmia-associated transcription factor; ND, not determined; NF-κB, nuclear factor-κB; NP, nucleocapsid protein, from hantavirus; p53, tumour-suppressor protein p53; p73, tumour protein p73; p300, a co-activator of transcription; PIAS, protein inhibitor of activated STAT; PLAG1, pleiomorphic adenoma gene 1; RTA, replication and transcription activator, from Epstein–Barr virus; SATB2, special (A+T)-rich-sequence-binding protein 2; SMAD, SMA (small body size)- and MAD (mothers against decapentaplegic)-related protein; STAT, signal transducer and activator of transcription protein; TRIF, Toll/interleukin-1-receptor-domain-containing adaptor protein inducing interferon-β.

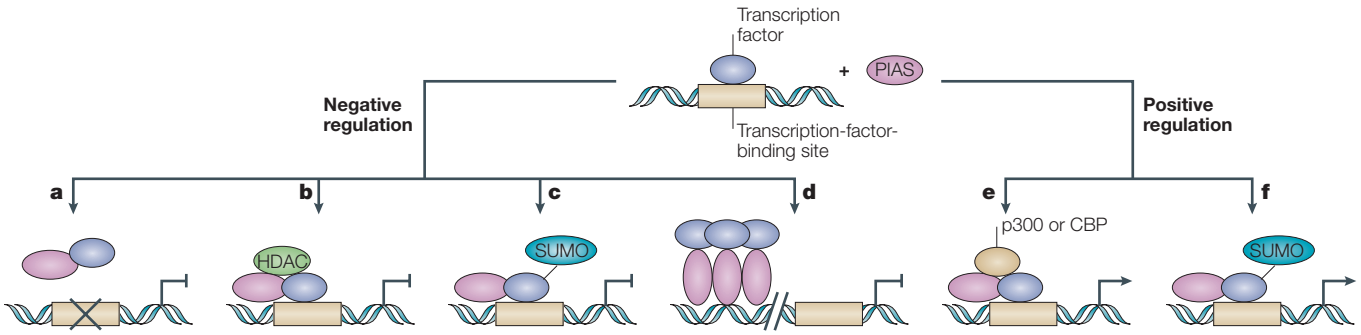


Figure 3 | **Proposed mechanisms of PIAS-mediated transcriptional regulation.** Protein inhibitor of activated STAT (signal transducer and activator of transcription protein) proteins (PIAS proteins) can regulate transcription either positively or negatively. Four mechanisms have been proposed for PIAS-mediated transcriptional repression. **a** | PIAS proteins block the DNA-binding activity of a transcription factor. **b** | PIAS proteins function as transcriptional corepressors by recruiting histone deacetylase (HDAC) to inhibit transcription. **c** | PIAS proteins promote sumoylation of the transcription factor, which inhibits its transcriptional activity. **d** | PIAS proteins inhibit transcription by sequestering transcription factors in distinct subnuclear structures. PIAS proteins have also been implicated in transcriptional activation through two mechanisms. **e** | PIAS proteins recruit the transcriptional co-activators p300 or CBP (cyclic-AMP-responsive-element-binding protein (CREB)-binding protein) to enhance transcription. **f** | PIAS proteins promote sumoylation of the transcription factor, which positively regulates its transcriptional activity. SUMO, small ubiquitin-like modifier.

could be used to dissect the functional role of PIAS proteins in various signalling events.

Mechanisms of PIAS-mediated gene regulation

Negative regulation. Since the initial description of a role for PIAS proteins in the negative regulation of STATs[12], PIAS proteins have been suggested to repress the activity of many other transcription factors (TABLE 1; see Supplementary information S1 (table)). Four molecular mechanisms have been proposed to explain how PIAS proteins might negatively regulate transcription (FIG. 3).

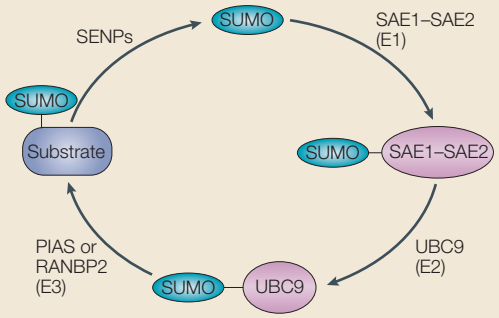
First, a PIAS protein might block the DNA-binding activity of a transcription factor, although the precise molecular basis of such inhibition remains to be determined. For example, PIAS1 can inhibit the DNA-binding activity of STAT1 or NF-κB p65 *in vitro*[13,33]. Consistent with this, the ability of STAT1 or NF-κB p65 to bind the promoters of endogenous genes was markedly increased in PIAS1-deficient cells, as examined by CHROMATIN-IMMUNOPRECIPITATION (ChIP) ASSAY[33,35].

Second, a PIAS protein might recruit other co-regulators, such as histone deacetylases (HDACs), to repress transcription. HDACs, which are enzymes that catalyse the removal of acetyl groups from lysine residues in both histone and non-histone proteins, have an important role in the regulation of gene transcription, through modifying chromatin. For example, PIASx-β has been reported to interact with HDAC3 (REF. 36). The inhibitory effect of PIASx on interleukin-12 (IL-12)-induced STAT4-dependent gene activation was abolished by the HDAC inhibitor trichostatin A (TSA)[37]. Similarly, PIASy can interact with HDAC1 and HDAC2 (REFS 38,39), and the ability of PIASy to repress the transcriptional activity of SMAD3 or androgen receptor was abolished by TSA[38,39].

Third, a PIAS protein might repress transcription by promoting the sumoylation of a transcription factor. This hypothesis is based on the recent finding that PIAS proteins have SUMO-E3-ligase activity[16]. Sumoylation — that is, the post-translational modification of proteins with SUMO — has been suggested to regulate a wide variety of cellular processes, including targeting of proteins to the nucleus, interactions between proteins, stability of proteins, formation of subnuclear structures, and modulation of transcription factors[40]. Sumoylation occurs through a pathway that is distinct from, but analogous to, protein ubiquitylation, and it involves the sequential actions of three enzymes: an activating enzyme (E1), a conjugating enzyme (E2) and a ligase (E3) (BOX 1). The first identification of SUMO E3 ligases came from an elegant study in yeast, by Johnson and Gupta[41]. They showed that the removal of Siz1 and Siz2 proteins (SAP- and MIZ (MSH-homeobox-homlogue-2-interacting zinc finger)-domain-containing proteins) almost completely abolished protein sumoylation in yeast. In addition, Siz1 could promote the sumoylation of yeast septins *in vitro*. These biochemical and genetic studies indicated that Siz1 and Siz2 are SUMO E3 ligases. Siz1 and Siz2 are homologous to the mammalian PIAS proteins, and it has subsequently been shown that members of the PIAS family can promote the sumoylation of many proteins, including the tumour-suppressor protein p53, STAT1 and SMAD4 (TABLE 1; see Supplementary information S1 (table)). Biochemical studies indicate that the PIAS-mediated sumoylation of transcription factors has different effects on the activity of these transcription factors. Whereas PIAS-mediated sumoylation positively or negatively regulates the activity of some transcription factors, it has no effect on the activity of many others. For example, studies in cell culture indicate that PIASy can repress the transcriptional activity of androgen receptor[27]; however, a SUMO-E3-ligase-defective mutant of PIASy was as efficient as wild-type PIASy at repressing the transcriptional activity of androgen

CHROMATIN-IMMUNOPRECIPITATION ASSAY
(ChIP assay). A technique for the detection of proteins bound to specific regions of chromatin. These assays involve chemically crosslinking bound proteins to the underlying DNA sequences, followed by immunoprecipitation with an antibody that is specific for the crosslinked protein.

Box 1 | **The SUMO–protein conjugation pathway**



SUMO (small ubiquitin-like modifier) is a molecule that is similar to ubiquitin. The mammalian SUMO family consists of SUMO1, SUMO2 and SUMO3. The covalent conjugation of SUMO to substrate proteins is known as protein sumoylation, and this occurs through a pathway that is distinct from, but analogous to, protein ubiquitylation. Sumoylation involves three discrete steps — activation, conjugation and ligation — each of which is mediated by a specific enzyme (E1, E2 and E3, respectively) (see figure). Before the activation step, it is essential that the carboxy (C)-terminal extension of SUMO is removed by specific proteases to generate a C terminus that ends in two glycine residues (not shown). SUMO is activated by the SUMO E1 activating enzyme (E1), which is a heterodimer that consists of SAE1 (SUMO-activating enzyme subunit 1) and SAE2, in an ATP-dependent manner. E1 uses ATP to adenylate the C-terminal glycine residue of SUMO, and a thioester bond is then formed between this residue of SUMO and a cysteine residue in SAE2. Next, SUMO is transferred from E1 to the cysteine residue of the SUMO E2 conjugating enzyme (E2) ubiquitin-conjugating enzyme 9 (UBC9). The SUMO E3 ligase (E3) then promotes the transfer of SUMO from E2 to the target substrate, forming an isopeptide bond between the C terminus of SUMO and the ε-amino group of a lysine residue in the target protein[95,96]. Several SUMO E3 ligases, including PIAS (protein inhibitor of activated signal transducer and activator of transcription protein) proteins and RAN-binding protein 2 (RANBP2), have been identified.

Unlike ubiquitylation, SUMO modification preferentially occurs at a conserved amino-acid motif, ΨKXE (where Ψ denotes L, I, V or F, and X denotes any amino acid)[97], although recent studies indicate that SUMO can also be attached to lysine residues that are not located within this motif[98–100]. Proteins can be targeted for either mono- or polysumoylation[101], but most proteins that have been characterized do not undergo polysumoylation.

SUMO modification is a highly dynamic process in which protein substrates undergo rapid conjugation (sumoylation) and deconjugation (desumoylation). In the mammalian system, SUMO-specific proteases (SENPs) proteolytically remove SUMO from target substrates[95]. These desumoylating enzymes are cysteine proteases that can be inhibited by *N*-ethylmaleimide.

The post-translational modification of proteins with SUMO has been suggested to regulate a wide variety of cellular processes, including targeting of proteins to the nucleus, interactions between proteins, stability of proteins, formation of subnuclear structures and modulation of transcription factors[40].

receptor. In addition, mutation of all of the known possible sumoylation sites on androgen receptor had no effect on the repressive activity of PIASy on androgen receptor[39]. Similar findings have also been made for PIASy-mediated repression of the transcriptional activity of lymphoid-enhancer-binding factor 1 (LEF1), a WNT-responsive transcription factor[20]. However, other studies indicate that the SUMO-E3-ligase activity of PIAS1 and PIASx-α might be important for the regulation of androgen-receptor activity[42], indicating that different PIAS proteins use different mechanisms of transcriptional regulation. Controversial results on the effect of PIAS-mediated sumoylation have also been reported. For example, it has been shown that PIAS proteins can promote the sumoylation of STAT1 on the lysine residue at position 703 (REFS 43–45). Whereas one research group showed that the mutation of this lysine residue to an arginine residue increased the transcriptional activity of STAT1 (REFS 43,45), another group suggested that the same mutation had no effect on the PIAS1-mediated inhibition of STAT1 activity[44]. It is possible that the effect of sumoylation on the activity of a given transcription factor depends on the promoter context of individual target genes, because these studies[43–45] used different promoter constructs to measure transcriptional activity. Clearly, the physiological importance of the SUMO-E3-ligase activity of PIAS proteins in gene regulation needs to be clarified, and further studies are required to understand how the modification of proteins with SUMO can regulate the activity of a transcription factor *in vivo*.

Fourth, PIAS proteins might repress transcription by sequestering transcription factors in certain subnuclear structures that are enriched for corepressor complexes. For example, PIASy, when overexpressed, was shown to localize mainly to punctate structures in the nucleus[20]. When co-expressed with LEF1, PIASy could target LEF1 to NUCLEAR BODIES, a process that requires the SAP domain of PIASy. So, it was proposed that PIASy-mediated subnuclear sequestration of LEF1 might account for the repression of LEF1 activity by PIASy[20]. It should be noted, however, that endogenous PIAS1 and PIASy were found to be expressed uniformly in the nucleus[26,33]. It remains to be determined whether endogenous PIAS proteins can localize to nuclear bodies when challenged with certain stimuli.

Positive regulation. Although PIAS proteins are mainly known as transcriptional repressors, they have also been shown to positively regulate the activity of several transcription factors. For example, whereas PIASy represses the activity of androgen receptor, other PIAS proteins — PIAS1, PIAS3 and PIASx — can increase the activity of androgen receptor under the

NUCLEAR BODY
A subnuclear structure that is implicated in transcriptional repression, transcriptional activation and protein degradation.

same conditions[27]. Similarly, PIAS3 activates, whereas PIASy represses, the transcriptional activity of SMAD3 (REF. 46). Two possibilities have been proposed to explain the positive effect of PIAS proteins on transcription (FIG. 3). It has been reported that PIAS3 can recruit p300 or CBP (cyclic-AMP-responsive-element-binding protein (CREB)-binding protein) to activate transcription[46]. Alternatively, the PIAS-mediated sumoylation of transcription factors might positively regulate their activity through unknown mechanisms. So, although PIAS proteins are mostly involved in gene repression, they might also activate transcription under certain conditions.

PIAS proteins in cytokine signalling

The STAT-, NF-κB- and SMAD-signalling pathways are widely used by cytokines to regulate gene expression. Biochemical and genetic studies indicate that PIAS proteins are involved in regulating the transcriptional activity of these three protein families.

STAT signalling. STATs are latent cytoplasmic transcription factors that become phosphorylated by Janus activated kinases (JAKs) in response to various cytokines. Tyrosine-phosphorylated STATs then dimerize and translocate to the nucleus to activate transcription. The mammalian STAT family contains seven members: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6. Gene-targeting studies have shown important roles for STATs in the regulation of immune responses and other cellular responses[1,47].

STAT signalling can be negatively regulated through three main mechanisms: the dephosphorylation of JAKs or STATs by various protein tyrosine phosphatases, such as T-cell specific 45 (TC45)[48,49]; the inactivation of JAKs by the suppressor of cytokine signalling (SOCS) family of proteins; and the inhibition of the transcriptional activity of STATs by PIAS proteins[2,15].

Each member of the PIAS family has now been shown to be involved in the regulation of STAT signalling, and *in vivo* co-immunoprecipitation assays have been used to examine endogenous PIAS–STAT interactions. PIAS1, PIAS3 and PIASx interact with STAT1, STAT3 and STAT4, respectively[12,13,37], and PIASy interacts with STAT1 also[26]. These interaction studies indicate that there is both specificity and redundancy in PIAS–STAT interactions. Interestingly, PIAS–STAT interactions are cytokine dependent, which might result from the ability of PIAS proteins to interact with the dimeric form, but not the monomeric form, of STATs[34], which only occurs after JAK-mediated phosphorylation. Each member of the PIAS family negatively regulates the activity of the STAT(s) to which it binds. It has been proposed that PIAS1 and PIAS3 function by blocking the DNA-binding activity of STAT1 and STAT3, respectively[12,13]. The detailed molecular basis of PIAS-mediated inhibition of STAT DNA-binding activity has not been determined. By contrast, PIASy and PIASx repress the transcriptional activity of STAT1 and STAT4, respectively, by recruiting other corepressor molecules, such as HDACs[26,37].

A single PIAS protein, known as PIAS or ZIMP, has been identified in *Drosophila melanogaster*[50]. The functional integrity of the *D. melanogaster* JAK–STAT pathway is essential for eye development, and hyperactive JAK–STAT signalling leads to blood-cell tumour formation. The removal of PIAS from *D. melanogaster* resulted in defects in eye development and increased tumour formation[51] (TABLE 2). These results indicate that PIAS is a negative regulator of the *D. melanogaster* JAK–STAT pathway. A separate study by Hari and colleagues[52] showed that PIAS is also required for maintaining chromosome structure in *D. melanogaster*.

Recently, gene-targeting studies in mice have been carried out to understand the physiological functions of PIAS proteins in cytokine signalling (TABLE 2). *Pias1*$^{-/-}$ mice are produced at a frequency that is half that of the expected Mendelian ratio, as a consequence of partial perinatal lethality. The surviving PIAS1-deficient mice are runted compared with their wild-type litter-mates, but they have no gross histological defects and do not die prematurely. Both PIAS1-deficient males and females are fertile[35].

Detailed gene-activation studies using wild-type and *Pias1*$^{-/-}$ bone-marrow-derived macrophages (BMMs), as well as primary mouse embryonic fibroblasts, uncovered an unexpected specificity in PIAS1-mediated gene regulation[35]. DNA-microarray analyses of RNA samples prepared from wild-type and *Pias1*$^{-/-}$ BMMs treated with interferon-β (IFN-β) or IFN-γ, which signal through STAT1, indicate that the removal of PIAS1 resulted in the increased expression of only a subset of IFN-induced genes (which are designated as PIAS1-sensitive genes)[35] (FIG. 4). So, unlike other general negative regulators of JAKs or STATs, PIAS1 does not inhibit the entire IFN response. These studies indicate that cytokine-activated genes can be negatively regulated in subgroups by inhibitory molecules such as PIAS1. These interesting findings raise two important questions. How is such specificity of PIAS1 in the control of cytokine gene expression achieved? And as PIAS1 regulates only a subset of cytokine-responsive genes, what is its biological function?

Table 2 | ***In vivo* functions of PIAS proteins**

Gene	Phenotype of *Pias* deletion	Refs
Mouse *Pias1*	Increased transcription of a subset of STAT1- and NF-κB-induced genes, resistance to viral and bacterial infections, hypersensitivity to LPS-induced endotoxic shock, increased serum levels of pro-inflammatory cytokines, retardation of growth, and perinatal lethality	33,35
Mouse *Piasy*	On pure 129 background, no obvious phenotype; on mixed 129 and C57BL/6 background, normal distribution of lymphocytes and small reduction in expression of genes downstream of interferon-γ- and WNT-mediated signalling	31,53
Drosophila melanogaster Pias	Defective development of eyes, instability of chromosomes, and formation of melanotic tumours	51,52

LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PIAS, protein inhibitor of activated STAT; STAT1, signal transducer and activator of transcription protein 1.

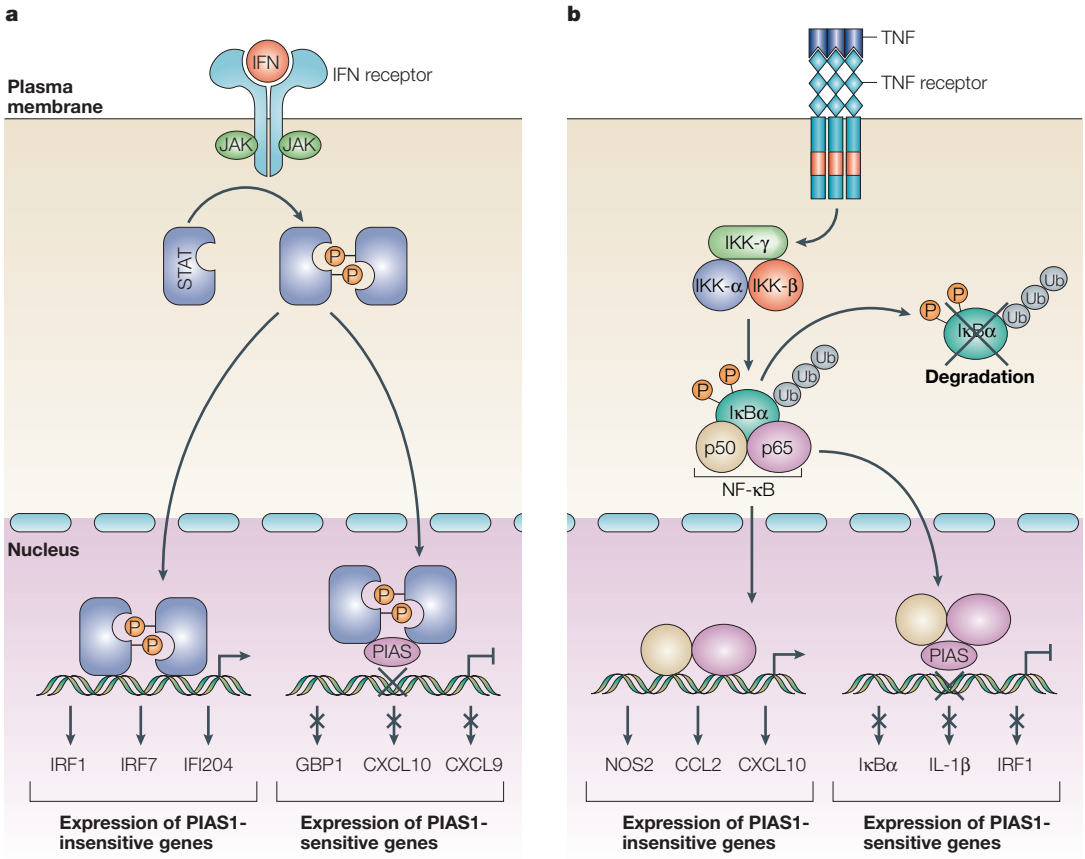


Figure 4 | **The specificity of PIAS1-mediated transcriptional repression in STAT1 and NF-κB signalling.** PIAS1 (protein inhibitor of activated STAT1 (signal transducer and activator of transcription protein 1)) selectively inhibits a subset of STAT1- or nuclear factor-κB (NF-κB)-dependent genes in response to treatment with interferon (IFN), or tumour-necrosis factor (TNF) or lipopolysaccharide, respectively[33,35]. PIAS1-insensitive genes are defined as genes that are not affected by deletion of *Pias1*, and PIAS1-sensitive genes are defined as genes that are affected by deletion of *Pias1*. CCL2, CC-chemokine ligand 2; CXCL, CXC-chemokine ligand; GBP1, guanylate-binding protein 1; IFI204, IFN-activated gene 204; IκBα, inhibitor of NF-κB α; IKK, IκB kinase; IL-1β, interleukin-1β; IRF, IFN-regulatory factor; JAK, Janus activated kinase; NOS2, nitric-oxide synthase 2.

In answer to the first question, it needs to be pointed out that the STAT1-binding sequences that are present in the promoters of IFN-responsive genes have different affinities for STAT1. It was found that a higher concentration of PIAS1 was required to inhibit the binding of STAT1 to a high-affinity DNA sequence than to a low-affinity sequence[35]. The level of endogenous PIAS1 protein that is expressed by macrophages might not be sufficient to affect the binding of STAT1 to high-affinity DNA sequences. As a result, in *Pias1*$^{-/-}$ macrophages, the expression of genes that contain weak STAT1-binding sequences, but not those with strong STAT1-binding sequences, was preferentially upregulated in response to treatment with IFN. Consistent with this model, ChIP assays revealed increased binding of STAT1 to the promoters of PIAS1-sensitive genes, but not PIAS1-insensitive genes, in PIAS1-deficient macrophages[35]. So, the efficiency of recruitment of STAT1 to the promoters of STAT1 target genes contributes to the specificity of PIAS1 in STAT1-mediated gene regulation. It remains to be determined whether the possible redundancy of PIAS proteins in gene regulation might also contribute to the observed specificity of PIAS1 in its selective regulation of a subgroup of IFN-responsive genes.

Consistent with the inhibitory role of PIAS1 in STAT1 signalling, the antiviral activity of IFN-β and IFN-γ were both markedly increased in *Pias1*$^{-/-}$ cells[35]. In addition, *Pias1*$^{-/-}$ mice showed increased protection against infection with either *Listeria monocytogenes* or vesicular stomatitis virus[35]. These results identify a crucial role for PIAS1 in innate immune responses, and they support a role for PIAS1 as a physiologically important negative regulator of STAT1 signalling.

Piasy$^{-/-}$ mice, which were generated independently by two research groups, showed no obvious developmental defects[31,53]. Both groups also showed that the basal level of protein sumoylation was not altered in the absence of PIASy (TABLE 2). Wong and colleagues[31] showed that the transcriptional activation of several IFN-responsive genes was not altered in *Piasy*$^{-/-}$ cells. Moreover, no marked differences were observed between wild-type and *Piasy*$^{-/-}$ cells after infection with Moloney murine leukaemia virus[31]. However, Roth and co-workers[53] observed a small reduction in IFN-γ- and WNT-induced gene activation in *Piasy*$^{-/-}$ cells, using reporter-gene

assays. It remains to be determined whether the lack of an obvious phenotype of *Piasy*$^{-/-}$ mice results from a functional redundancy in PIAS proteins.

NF-κB signalling. The NF-κB family contains dimeric transcription factors that are composed of members of the REL family of DNA-binding proteins, including p50 (also known as NF-κB1), p52 (also known as NF-κB2), REL (also known as cREL), p65 (also known as REL-A) and REL-B[54,55]. NF-κB can be activated by numerous signals, including the following: pro-inflammatory cytokines, such as tumour-necrosis factor (TNF) and IL-1; growth factors; bacterial lipopolysaccharide (LPS); viruses; and stress-inducing signals, such as ultraviolet-light irradiation, γ-irradiation and hypoxia. As a result, NF-κB is involved in mediating a wide range of cellular processes, including inflammation, cellular proliferation, transformation, apoptosis and responses to infection[3–5].

Two members of the PIAS family have been suggested to regulate NF-κB signalling[33,56]. Both PIAS1 and PIAS3 can interact with p65. But whereas PIAS3 binds the N-terminal region of p65 (REF. 33), PIAS1 binds the C-terminal region of p65 (REF. 56). Interestingly, PIAS1 selectively interacts with p65 but not its related family member p50 (REF. 33). It is not known whether PIAS1 or PIAS3 can interact with other members of the NF-κB family. Both PIAS1 and PIAS3 can repress the transcriptional activity of p65 in NF-κB-reporter assays[33,56]. Furthermore, PIAS1 overexpression can repress the induction of endogenous NF-κB target genes, such as those encoding inhibitor of NF-κB α (IκBα) and B-cell-lymphoma-2-related protein A1 (also known as BFL1), in response to stimulation with TNF[33]. It remains to be determined whether PIAS3 can inhibit the transcriptional activation of endogenous NF-κB-dependent genes. Several pieces of evidence strongly indicate that PIAS1 inhibits p65-mediated transcription by interfering with the DNA-binding activity of p65. For example, when examined by MOBILITY GEL-SHIFT ANALYSIS, PIAS1 purified from bacteria can inhibit the binding of p65 to NF-κB-binding sites in DNA. Furthermore, in LUCIFERASE-REPORTER ASSAYS, PIAS1 can repress the transcriptional activity of a fusion protein of GAL4 (which is involved in galactose metabolism) and p65 when assayed using an NF-κB-binding-site-containing reporter but not a GAL4-binding-site-containing reporter. Consistent with this, ChIP assays show that the recruitment of p65 to the endogenous promoters of NF-κB target genes, such as the gene encoding IκBα, is inhibited in cells that overexpress PIAS1 but is increased in cells that are deficient in PIAS1 (REF. 33).

Similar to the regulation of STAT1 signalling by PIAS1, disruption of *Pias1* resulted in the upregulation of a subgroup of NF-κB-dependent genes, including those encoding the pro-inflammatory cytokines IL-1β and TNF, in response to stimulation with LPS or TNF[33] (FIG. 4). It remains to be determined whether such specificity of PIAS1 in the regulation of NF-κB signalling is achieved by a similar molecular mechanism to that described for STAT1.

Consistent with the inhibitory role of PIAS1 in NF-κB signalling, serum levels of pro-inflammatory cytokines, such as IL-1β and TNF, were increased in *Pias1*$^{-/-}$ mice, and *Pias1*$^{-/-}$ mice were hypersensitive to LPS-induced endotoxic shock[33]. So, gene-targeting studies have established a physiological role for PIAS1 in the negative regulation of NF-κB. It will be interesting to investigate whether other members of the PIAS family also have a role in the regulation of NF-κB signalling.

SMAD signalling. The transforming growth factor-β (TGF-β) superfamily, which signals through the SMAD family of proteins, regulates various biological processes, including cellular differentiation and proliferation, normal development, tumorigenesis and immune responses[6–9]. After stimulation with TGF-β, the receptor-regulated SMADs (R-SMADs) SMAD2 and SMAD3 become phosphorylated on serine residues and form a heterodimeric complex with the common-mediator SMAD (Co-SMAD), SMAD4. The SMAD2–SMAD4 and SMAD3–SMAD4 heterodimers then translocate to the nucleus, where they cooperate with other transcription factors and co-regulators to regulate transcription. SMAD6 and SMAD7 are inhibitory SMADs (I-SMADs), which function by preventing the activation of R-SMADs[57] (FIG. 1).

Members of the PIAS family interact with SMADs to either negatively or positively regulate their transcriptional activity[38,46,58–60] (TABLE 1). PIASy was identified as a SMAD-interacting protein in a YEAST THREE-HYBRID SCREEN using SMAD3 and SMAD4 as bait[38]. PIASy can interact with either SMAD3 or SMAD4, and the formation of a ternary complex consisting of PIASy, SMAD3 and SMAD4 has been detected[38]. Treatment with TGF-β can enhance the PIASy–SMAD3 interaction. Interestingly, PIASy overexpression inhibits only a subset of endogenous TGF-β-responsive genes, which includes those that encode the cyclin-dependent kinase inhibitor p15 and plasminogen-activator inhibitor 1 (REF. 38). The molecular basis of the selective inhibitory effect of PIASy on TGF-β-responsive genes has not been determined. It has been proposed that PIASy inhibits SMAD-mediated transcription by recruiting HDAC1, which regulates transcription by modifying chromatin[38]. In a separate study, PIASy was also found to promote the sumoylation of SMAD3, and PIASy inhibited SMAD3-mediated transcription[58]. However, a positive-regulatory role for PIASy in TGF-β-mediated signalling has also been reported[59]. It was shown that PIASy promoted the sumoylation of SMAD4 and could target SMAD4 to subnuclear structures. Increased sumoylation of SMAD4 increased its stability and transcriptional activity. Further studies are required to clarify these contradictory results on the role of PIASy in TGF-β-mediated gene activation.

PIAS1, PIAS3 and PIASx have been proposed to positively regulate the transcriptional activity of SMAD2 and SMAD3. Long and colleagues[46] showed that PIAS3 increases the transcriptional activity of SMAD3 by recruiting either p300 or CBP, which are general

MOBILITY GEL-SHIFT ANALYSIS
A technique to detect the DNA-binding activity of a protein *in vitro*. This assay involves the mixing of proteins with a specific DNA sequence, followed by the separation of this mixture by electrophoresis.

LUCIFERASE-REPORTER ASSAY
A method to measure the transcriptional response. This assay uses a promoter from a gene of interest fused to the gene that encodes luciferase.

YEAST THREE-HYBRID SCREEN
A system that is used to study ternary protein complexes. This technique involves three proteins that allow or prevent the formation of a functional transcriptional-activator complex.

transcriptional co-activators. PIAS1 and PIASx-β have been reported to positively regulate TGF-β-mediated signalling by promoting the sumoylation and therefore the stabilization of SMAD4 (REFS 60,61).

Interestingly, TGF-β was found to induce expression of mRNA that encodes PIASy in the human hepatocellular-carcinoma cell line Hep3B[58]. In addition, the mitogen-activated protein kinase (MAPK) p38 signalling pathway, which can activate SMAD-dependent transcription, has been proposed to stabilize PIASx-β protein and to increase expression of the gene encoding PIASx-β[60]. These studies indicate that PIAS proteins might function as negative-feedback controls in the regulation of TGF-β-mediated signalling.

A role for PIAS proteins in the regulation of TGF-β-mediated signalling is further supported by studies in *Xenopus laevis*, in which the SMAD family of transcription factors has an important role in mesoderm formation. PIASy from *X. laevis* was first cloned using a yeast two-hybrid screen with *X. laevis* SMAD2 as bait[62]. Inhibition of *X. laevis* PIASy by MORPHOLINO OLIGONUCLEOTIDES induced elongation of ectodermal explants (animal caps), a process that is mediated by the SMAD2-signalling pathway, and it also induced expression of mesoderm genes even in the absence of morphogen-mediated activation, indicating that *X. laevis* PIASy is a physiological inhibitor of SMAD2 (REF. 62).

PIAS proteins in the cell cycle and apoptosis

The tumour-suppressor protein p53 and its two homologues p63 and p73 form a family of transcription factors. The activity of the p53 family is regulated by several mechanisms, including post-translational modification and interactions with specific and common regulatory proteins. The p53 family of proteins activates overlapping, as well as specific, sets of genes that have important roles in the regulation of the cell cycle and apoptosis[63,64]. As discussed in this section, the PIAS family has been suggested to regulate p53, the p53-related protein p73 and the p53 regulator MDM2 (mouse double minute 2 homologue).

p53. The tumour-suppressor protein p53 is often mutated in human cancers. Under normal conditions, p53 is short-lived and undergoes proteasome-mediated degradation. After exposure to various forms of stress and DNA damage, p53 is activated by post-transcriptional modifications, which leads to p53 accumulation, downstream gene activation and, ultimately, cell-cycle arrest or apoptosis[65–67].

It has been reported that PIAS1 can regulate the transcriptional activity of p53. However, the function of PIAS1 in the regulation of p53 activity is controversial. Whereas Megidish and colleagues[68] showed that PIAS1 increased p53-mediated transcription and p53-dependent arrest in the G1 (gap 1) phase of the cell cycle, Schmidt and Muller[69] found that PIAS1 repressed the transcriptional activity of p53. Future studies, such as analysing the effects of PIAS proteins on the expression of endogenous p53 target genes, might help to clarify the role of PIAS proteins in the regulation of p53.

How PIAS1 regulates the activity of p53 is still unclear. Earlier studies indicated that PIAS1 can promote the sumoylation of p53 in *in vitro* assays or when co-expressed in cells[69,70]. However, the ability of PIAS1 to regulate p53 activity seems to be independent of its SUMO-E3-ligase activity. Megidish and colleagues[68] showed that a C-terminal portion of PIAS1 lacking the RLD, which is defective in promoting protein sumoylation, was sufficient to activate p53 in luciferase-reporter assays[68]. In addition, Schmidt and Muller[69] showed that a mutant form of p53 lacking the sumoylation sites was repressed by PIAS1 as efficiently as wild-type p53. As discussed earlier, SUMO-E3-ligase-independent transcriptional regulation by PIAS proteins has also been observed for the regulation of androgen receptor and LEF1 (REFS 20,39).

Although biochemical studies have indicated that PIAS1 regulates the transcriptional activity of p53, p53-mediated apoptosis in response to γ-irradiation was not altered in *Pias1*$^{-/-}$ thymocytes[35]. However, it is possible that the apparent lack of an effect on p53 in *Pias1*$^{-/-}$ mice might be a consequence of the redundant function of other PIAS proteins. Further genetic studies are required to understand the role of PIAS proteins in the regulation of p53 signalling.

p73. The tumour protein p73 is related to p53 and is involved in cell-cycle regulation and apoptosis. In contrast to p53, the level of p73 is not increased after genotoxic stress, and the activity of p73 is not regulated by MDM2. Recent studies indicate that ITCH, an E3 ligase of the HECT (homology to the E6-associated protein C terminus) family, binds p73 and mediates the ubiquitylation and degradation of p73 (REF. 71). In addition, it has been shown that, through interaction with the protein kinase ABL, p73 mediates an apoptotic response to γ-irradiation and cisplatin treatment[72,73].

Recent studies have shown that PIAS1 promotes the sumoylation of p73, which negatively regulates its transcriptional activity[74]. Interestingly, sumoylated p73 was found to be associated with the nuclear scaffold, indicating that sumoylation of p73 might regulate its subcellular localization and thereby its transcriptional activity.

MDM2. MDM2, which mediates negative-feedback control of p53, can repress the transcriptional activity of p53 and target it for degradation[75–77]. It has been shown that PIAS1 and PIASx-β can promote the conjugation of SUMO to MDM2 both in intact cells and in *in vitro* assays[78]. Whereas wild-type MDM2 is present in the nucleus, a sumoylation-defective mutant form of MDM2 (in which the lysine residue at position 182 is replaced by an arginine residue) is localized in the cytoplasm. These results indicate that, similar to p73, the sumoylation of MDM2 might regulate its cellular localization and thereby its activity.

MORPHOLINO OLIGONUCLEOTIDE
A 25-base-pair DNA analogue that operates by blocking mRNA translation or mRNA splicing and thereby inducing antisense effects. These oligonucleotides operate only when they are complementary either to a sequence that is located between the 5′ untranslated region and the first 25 bases 3′ of the AUG start site or to the sequence at a splice junction.

PIAS proteins in other transcriptional responses

PIAS proteins have been shown to participate in the regulation of several other transcription factors that have important roles in the immune system.

SATB2. Special (A+T)-rich-sequence-binding protein 2 (SATB2) is a precursor-B-cell-specific SAR-binding protein that modulates immunoglobulin μ gene expression. Studies by Dobreva and co-workers[79] showed that PIAS1 interacts with SATB2 and increases the SUMO modification of SATB2. Mutations of SATB2 that abolished the sumoylation sites resulted in increased binding of SATB2 to the SAR sequences in the endogenous immunoglobulin heavy-chain locus and increased SATB2-mediated transcriptional activation of this locus. Furthermore, wild-type SATB2 was located at the nuclear periphery, whereas mutant SATB2 that lacked SUMO-modification sites showed a diffuse nuclear localization. These studies indicate that PIAS1-mediated sumoylation of SATB2 might negatively regulate the activity of SATB2 and thereby immunoglobulin μ gene expression.

MITF. Microphthalmia-associated transcription factor (MITF) is a basic helix–loop–helix leucine-zipper DNA-binding factor that has an important regulatory role in tissue-specific gene expression in several cell types, including melanocytes, osteoclasts and mast cells. For example, in mast cells, MITF regulates the expression of mast-cell protease 5 (MCP5)[80] and MCP6 (REF. 81). It has been shown that PIAS3 interacts with MITF and represses MITF-mediated transcriptional activation by blocking its DNA-binding activity[82]. Interestingly, the interaction between MITF and PIAS3 is controlled by serine phosphorylation of MITF, which inhibits the PIAS3–MITF interaction[83]. In the nucleus of resting cells, unphosphorylated MITF is inactivated through its association with PIAS3. After activation of gp130 (glycoprotein 130)-containing receptors or KIT receptors by exposure of bone-marrow-derived mast cells to IL-6 or stem-cell factor (also known as KIT ligand), respectively, MITF becomes phosphorylated, and this results in the release of PIAS3, which then binds STAT3. These results indicate that there might be crosstalk between MITF, PIAS3 and STAT3 (REF. 84).

C/EBP-ε. CCAAT/enhancer-binding protein-ε (C/EBP-ε) is a neutrophil-specific transcription factor that is implicated in the regulation of several neutrophil- and macrophage-specific genes[85–87]. Targeted disruption of the gene that encodes C/EBP-ε in mice resulted in a block in neutrophil differentiation and in production of morphologically and functionally abnormal neutrophils[88]. It has been shown that PIAS1 and PIASx interact with C/EBP-ε[89,90]. PIASx promotes the sumoylation of C/EBP-ε and might function as a co-activator of C/EBP-ε[89,90].

Viral proteins. Interactions between viral proteins and host-cell machinery have important roles in the regulation of immune functions. It has recently been recognized that viral proteins can be sumoylated and that sumoylation is involved in regulating their activity. For example, RTA (replication and transcription activator), the immediate-early protein of Epstein–Barr virus (EBV), interacts with ubiquitin-conjugating enzyme 9 and PIAS1, which results in sumoylation of RTA[91], and this leads to increased transcriptional activity of RTA. Because RTA is a lytic-switch protein, these results indicate that sumoylation might have a role in regulating the lytic activation of EBV. Similarly, PIAS1 has also been shown to promote sumoylation of the human cytomegalovirus (CMV) protein IE2 (immediate-early protein 2)[92]. Sumoylation of IE2 increases its transcriptional activation of the CMV promoter, as well as the cellular cyclin E promoter. The activation of cyclin E is thought to be a viral strategy to drive host cells into the S (synthesis) phase of the cell cycle, thereby creating a more favourable environment for replication of CMV[92]. In both of these infections, the sumoylation of viral proteins by PIAS1 seems to benefit the virus. Two other viral proteins, the nucleocapsid protein (NP) from hantavirus and E1 from papillomavirus, have also been shown to interact with PIAS proteins and to become modified by SUMO. However, the functional significance of PIAS-mediated sumoylation of NP and E1 has not been determined[93,94].

Future directions

Great progress has been made in the PIAS field during the past few years. The rapidly growing number of PIAS-interacting proteins that have been identified in biochemical studies strongly indicates the involvement of PIAS proteins in various cellular-signalling events, but the biological significance of PIAS proteins in the regulation of these target proteins remains to be established. In addition, the physiological role of PIAS SUMO-E3-ligase activity in the regulation of cellular-signalling pathways is largely unknown. It will be important to identify the physiological SUMO-modification substrates of PIAS proteins and to characterize the substrate specificity of SUMO-E3-ligase activity of PIAS proteins. Clearly, the recently established PIAS gene-knockout model systems will be of great value to these studies. Four members of the mammalian PIAS gene family have been identified, and it is probable that there is specificity, as well as redundancy, in the functions of PIAS proteins. Therefore, new genetic models that involve the disruption of multiple PIAS genes should be established to help us to fully understand the biological functions of PIAS proteins in cellular signalling. Finally, an important aspect of research into PIAS proteins is to investigate how PIAS proteins are regulated under both normal and pathological conditions.

In summary, PIAS proteins regulate immune responses and other cellular functions through the modulation of transcription factors, using multiple molecular mechanisms. Future research in the PIAS field will improve our ability to design strategies for the treatment of human cancers and immune disorders that involve signalling through these pathways.

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Acknowledgements
K.S. is supported by grants from the National Institutes of Health (United States). B.L. is a special fellow of The Leukemia & Lymphoma Society (United States).

Competing interests statement
The authors declare no competing financial interests.

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