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Review

PIAS/SUMO: new partners in transcriptional regulation

D. Schmidt and S. Müller*

Max Planck Institute of Biochemistry, Department of Molecular Cell Biology, Am Klopferspitz 18a, 82152 Martinsried (Germany), Fax: +49 89 8578 3022, e-mail: stmuelle@biochem.mpg.de

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Abstract. Protein inhibitor of activated STATs (PIAS) proteins were initially identified as negative regulators of cytokine signalling that inhibit the activity of STAT-transcription factors. Evidence is accumulating that PIAS proteins function as transcriptional coregulators in various other important cellular pathways, including Wnt signalling, the p53 pathway and steroid hormone signalling. Most interestingly, recent work from several laboratories revealed that PIAS proteins act as E3-like ligases that stimulate the attachment of the ubiquitin-like SUMO modifier to target proteins acting in these pathways. Since in most cases the SUMO ligase activity and the transcriptional coregulator activity are functionally correlated, the PIAS/SUMO pathway appears to be an important mechanism of transcriptional regulation. In this review we will discuss some key findings that exemplify the role of PIAS proteins in the regulation of transcriptional processes and propose a model how the PIAS/SUMO system may modulate transcriptional activities by mediating the assembly of coactivator or corepressor complexes within distinct subnuclear structures.

Key words. PIAS; SUMO; STAT; wnt; p53; nuclear hormone receptors; transcription; PML nuclear bodies.

The eukaryotic family of PIAS proteins

The eukaryotic family of protein inhibitor of activated STATs (PIAS) proteins represents a group of proteins that play a pivotal role in the control of various important cellular pathways. The acronym PIAS stems from the initial finding that members of this family act as inhibitors of STAT-transcription factors. Work by many research groups during the last few years has demonstrated that the cellular function of PIAS proteins goes far beyond inhibition of STATs. This is best illustrated by the growing number of cellular proteins that were identified as binding partners of PIAS proteins (table 1). In this review we will try to give a general overview of the PIAS protein family and dissect their function as E3-like SUMO ligases and transcriptional regulators.

* Corresponding author.

The eukaryotic family of PIAS proteins is evolutionarily conserved from yeast to humans. In the yeast *Saccharomyces cerevisiae* two members of the family (Siz1/Siz2) are found. In higher eukaryotes the family is more diversified. The human family of PIAS proteins consists of at least five members, PIAS1, PIAS3, the α and β splice variants of PIASx, and PIASy [1]. With a length of 651-amino acid residues PIAS1 is the largest protein within this family, whereas PIASy is the smallest with about 500 residues. With the exception of a variable C-terminal region the family members are highly homologous, showing an overall identity ranging from 50 to 60% at the amino acid level. Together with their orthologues from yeast or *Drosophila* all mammalian PIAS forms share a characteristic domain structure that is schematically depicted in figure 1. Within the N-terminus of PIAS a region of about 35 amino acids spans a so-called SAP module [2]. The acronym SAP refers to three of the defining members of the class of SAP-containing

Table 1. Currently known cellular interaction partners of mammalian PIAS proteins.

	Protein function	PIAS forms	SUMOylation	Functional role of PIAS	Reference
STAT1	transcription factor	PIAS1, PIASy	unknown	inhibition of transactivation	37, 42
STAT3	transcription factor	PIAS3	unknown	inhibition of transactivation	40
STAT5	transcription factor	PIAS3	unknown	inhibition of transactivation	41
IRF-1	transcription factor	PIAS3	yes	inhibition of transactivation	43
LEF1	transcription factor	PIASy	yes	inhibition of transactivation	5
MITF	transcription factor	PIAS3	binds Ubc9	inhibition of transactivation	45
duplin	inhibitor of β-catenin	unknown	yes	unknown	48
p53	transcription factor	all forms	yes	activation/inhibition of transactivation	24, 56, 57, 97
p73	transcription factor	PIASxα	yes	unknown	24
mdm2	E3 ubiquitin ligase for p53	PIAS1, PIASxβ	yes	unknown	59
AR, GR, PR	transcription factor	all forms	yes	activation/inhibition of transactivation	64–68, 74–76
GRIP1/TIF2	transcriptional co-activator	PIAS1, PIAS3, PIASx	yes	stimulation of coactivation	69–71
Sp3	transcription factor	PIAS1	yes	inhibition of transactivation	23
C/EBPα	transcription factor	PIASy	yes	inhibition of transactivation	88
c-Myb	transcription factor	PIASy	yes	inhibition of transactivation	86, 87
c-Jun	transcription factor	PIAS1,PIASx	yes	unknown	20, 21
Gfi-1	transcription factor	PIAS3	unknown	activation of STAT3 transactivation	29
TFII-I	transcription factor	PIASxα	unknown	activation of transcription	98
CRP2	transcriptional cofactor	PIAS1	unknown	unknown	99
Gu	RNA helicase	PIAS1	unknown	unknown	100
Msx-2	transcription factor	PIASxα	unknown	unknown	101
HMG1-C	transcriptional cofactor	PIAS3	unknown	inhibition of GR transactivation	102
mouse disabled 2	unknown	PIASxα	unknown	unknown	103
DJ-1	unknown	PIASxα	yes	activation of AR transactivation	104

proteins, scaffold attachment factor (SAF), acinus, and PIAS. The SAP domain shows a bipartite distribution of conserved hydrophobic and polar amino acids that are separated by a region containing an invariant glycine residue. Secondary structure modelling predicts that the module forms two amphiphatic helices that fold into a hooklike structure sharing significant homology with the orientation of helix 1 and helix 2 of the homeodomain [3]. A common feature of SAP-containing proteins is their ability to bind to chromatin. In particular, the SAP module in SAF recognises distinct A-T-rich DNA sequences known as matrix or scaffold attachment regions (MARs/SARs). MARs/SARs are operationally defined as DNA regions that are associated with the nuclear matrix, a proteinaceous meshwork, which mediates the organisation of higher-order chromatin structures. MAR sequences are involved in chromatin remodelling and transcriptional regulation. The N-termini of PIAS1 and PIASy containing the SAP module can bind to MAR/SAR-containing synthetic DNA sequences in vitro [4, 5]. Moreover, PIASy is associated with the nuclear matrix in vivo, suggesting that PIAS proteins participate in the organisation of higher-order chromatin structure [5]. This idea is supported by the recent finding that the *Drosophila* homologue of PIAS [Su(var)2-10] controls chromosome organisation in interphase nuclei [6]. The exact role of PIAS proteins in these processes and the impact on nuclear functions remain to be elucidated.

Another characteristic feature of Siz/PIAS proteins is the presence of a cysteine/histidine-rich domain, known as Miz-zinc finger or SP-RING domain [7, 8]. This domain is related to the classical zinc-binding RING motif, which is defined by the consensus sequence $CX_2CX_{(9-39)}CX_{(1-3)}HX_{(2-3)}C/HX_2CX_{(4-48)}CX_2C$ [9]. The RING-finger domain characterises a subclass of ubiquitin E3 ligases (see below). E3s or ubiquitin-protein ligases stimulate the attachment of ubiquitin to target proteins and are largely responsible for substrate selection. An alignment of the RING finger region from the c-cbl ubiquitin ligase with the SP-RING motif from PIAS is shown in figure 1. When compared with the canonical RING finger, the SP-RING motif lacks the third and sixth cysteine residues, that are part of the first and third pair of cysteine/histidine residues in the RING motif. In a so-called cross-brace arrangement this first and third pair of cysteine/histidine residues forms one zinc binding site, while the second and fourth pairs form the second binding site in the RING motif. It remains to be determined whether the SP-RING domain can acquire a similar conformation without two of the critical zinc coordinating residues. Noteworthy, however, the U-box motif, which also exerts ubiquitin ligase activity, repre-

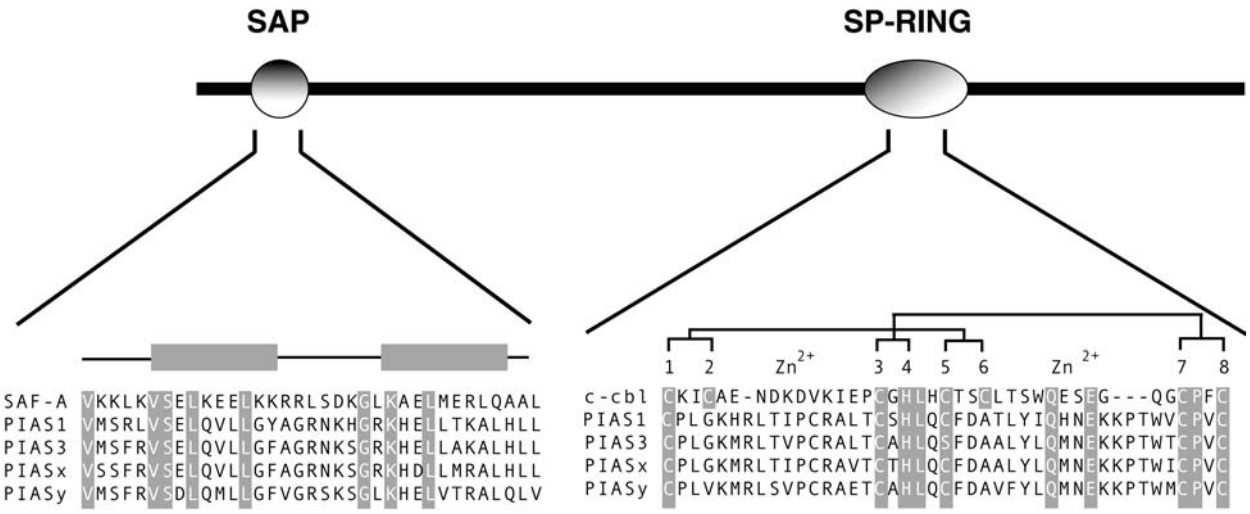

Figure 1. Domain structure of the Siz/PIAS family. On the left the N-terminal SAP module from SAF-A is aligned with the SAP region from the human PIAS-family members. A schematic representation of the predicted secondary structure (two amphiphatic helices) is shown above. On the right the RING-finger region from the c-cbl ubiquitin ligase is aligned with the SP-RING motif from the human PIAS-family members. The 'cross-brace' arrangment of the zinc coordinating residues in c-cbl is shown above.

sents an example of a domain that folds into a RING-finger-like structure, despite lacking most of the zinc-chelating cysteine/histidine residues [10, 11].

The presumed structural relationship of PIAS proteins to the RING-finger family was one piece of evidence that led to the idea that both protein families may share functional properties. In a pioneering work Erica Johnson's and Yoshiko Kikuchi's laboratories independently showed that the yeast Siz proteins act in the pathway of SUMO conjugation that is highly related to the ubiquitin-pathway [12–14]. Siz proteins function as E3-like SUMO ligases that stimulate the attachment of the ubiquitin-like SUMO modifier to specific target proteins. Subsequent work by several laboratories demonstrated that the mammalian orthologues of Siz also exert SUMO-ligase activity on a variety of target proteins.

PIAS proteins as E3-like factors in the SUMO pathway

SUMO is a member of the growing family of ubiquitin-like modifiers that can be covalently attached to target proteins [15, 16]. SUMO is highly conserved from yeast to humans. In mammals three SUMO forms, SUMO-1, SUMO-2 and SUMO-3, are expressed from three different genes. Research has mainly focused on SUMO-1, a protein of 101 amino acids that shares 18% identity with ubiquitin. The conjugation of SUMO to target proteins, termed SUMOylation, proceeds via a multistep pathway that is mechanistically analogous to ubiquitination but uses a set of enzymes that is distinct from that acting on ubiquitin (fig. 2). Like ubiquitin, all SUMO forms are initially made as inactive precursor proteins that are processed by a C-terminal proteolytic cleavage event. This cleavage exposes two C-terminal glycine residues that are essential for the formation of an isopeptide bond between the C-terminus of SUMO with an ε-amino group of a lysine residue of a target protein. In most cases the lysine residue is embedded in a consensus sequence composed of a characteristic ψKxE motif, where ψ is a large hydrophobic residue. Subsequent to processing, the cascade of conjugation proceeds via an ATP-dependent activation step catalysed by an E1-activating enzyme. The SUMO-specific E1-activation enzyme is a heterodimer composed of the proteins AOS1 (SUA1) and UBA2 (SUA2). Remarkably, UBA2 bears clear sequence similarity to the C-terminal region of UBA1, the E1 enzyme for ubiquitin, whereas AOS1 is related to the amino-terminal part of UBA1. Following activation, SUMO is transferred to the SUMO-specific E2-conjugating enzyme UBC9. As mentioned above, in the ubiquitin pathway one additional factor, called E3 or ubiquitin-protein ligase, is required in most cases for formation of an isopeptide bond between ubiquitin and the target protein. E3 ubiquitin ligases are considered to trigger ubiquitination by recruiting ubiquitin-conjugating enzymes to the substrate proteins. Two major classes of ubiquitin ligases, HECT-domain ligases and RING-type ligases, have been identified [17]. HECT E3s catalyse ubiquitination by forming thiol-ester intermediates between ubiquitin and a conserved cysteine residue in the HECT domain. RING-type ligases merely function as adaptor molecules that have the ability to bind both the substrate and the E2. The RING family can further be subdivided into two groups. In the monomeric RING ligases, binding sites for E2 and substrate are found on a single protein, while in multi-

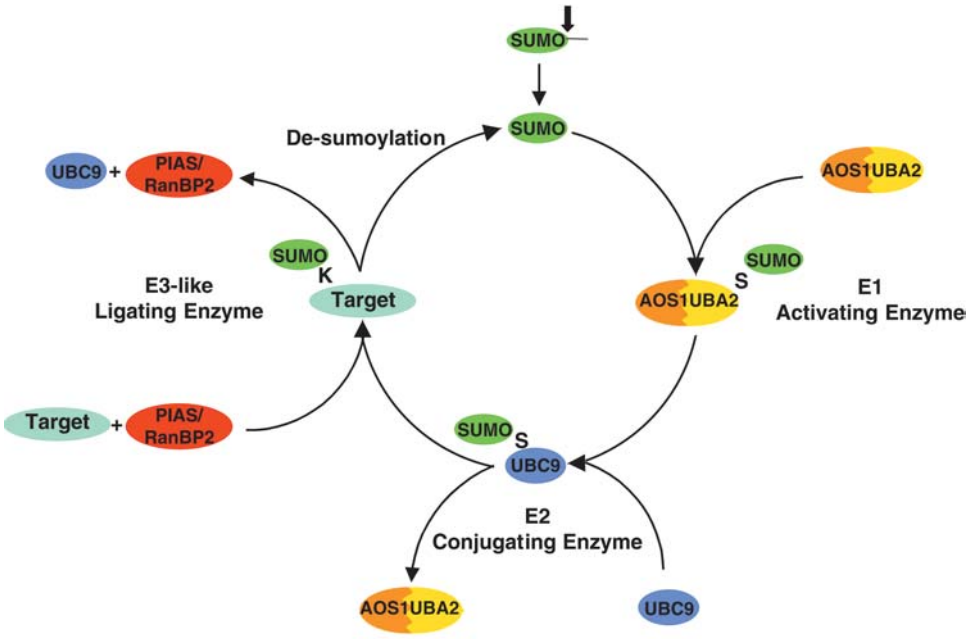

Figure 2. The pathway of SUMO conjugation/deconjugation. SUMO is synthesised as a precursor and C-terminally processed (arrowhead) by a class of SUMO-specific cysteine proteases, termed SUSPs or SENPs. Subsequently, the conjugation to proteins involves SUMO-activating E1 enzyme (AOS1/UBA2) and an E2-conjugating enzyme (Ubc9) that form thioesters (S) with the modifier. E3-like factors, such as PIAS and RanBP2, stimulate the attachment to specific lysine residues within a target protein. In most SUMO target proteins the lysine residue serving as the SUMO acceptor site resides within a short consensus sequence consisting of a ψKxE motif. The cleavage of SUMO from its target proteins, termed de-sumoylation, is catalysed by the SUSP/SENP isopeptidase family. In humans at least seven members of this family were identified.

meric ligases, such as the SCF complex, the RING-finger component serves as a platform for E2 binding, and additional associated factors recruit the substrate. Based on the observation that Ubc9 can directly associate with SUMO target proteins it had been proposed that in contrast to ubiquitination, no E3-like factors participate in the process of SUMO conjugation. This view had to be revised when it was demonstrated by genetic and biochemical approaches that the yeast Siz1 protein dramatically stimulates the conjugation of SUMO to septins, the major SUMO target proteins in yeast. Work by several laboratories later showed that all mammalian PIAS proteins also exert SUMO-ligase activity towards various mammalian SUMO-target proteins. The concept of E3-like activities in the SUMO system has been extended by identification of the PIAS unrelated nucleoporin, RanBP2, as another type of E3-like SUMO ligase [18]. The exact molecular mechanism of PIAS action in the SUMO pathway is still unclear. Remarkably, however, PIAS E3-SUMO ligases share important similarities with RING-type ubiquitin ligases. While RING-type ubiquitin ligases require the RING domain for their ligase activity, PIAS ligases depend on the integrity of the SP-RING domain for SUMO conjugation [5, 13, 14, 19–21]. Importantly, PIAS proteins themselves are covalently modified by SUMO in a process that also requires the integrity of the SP-RING domain [20, 21]. This automodification by SUMO is very reminiscent of the RING-dependent autoubiquitination of RING-type ubiquitin ligases. In summary, these findings further extend the analogy between the ubiquitin and SUMO pathways. Noteworthy, however, ubiquitin and SUMO ligases differ in some important aspects. One important difference concerns the substrate specificity of PIAS- and RING-type E3s. While in most cases a distinct RING-type E3 targets ubiquitination of a single substrate, a given PIAS protein can stimulate SUMOylation of several target proteins, indicating that PIAS proteins are less important determinants for substrate specificity in the SUMO pathway than ubiquitin ligases in the ubiquitin pathway. Moreover, in contrast to E3 ubiquitin ligases, PIAS proteins do not seem to be essentially required for the SUMOylation of substrates, but rather function as factors that stimulate SUMO conjugation, suggesting that at higher concentrations Ubc9 may be sufficient for substrate recognition. In line with this idea structural studies have recently shown that at least in the case of the RanGAP1 protein, Ubc9 makes direct contact with the ψKxE SUMOylation motif [22]. One possible interpretation of these data is that in the SUMO system it is primarily Ubc9 that contributes to substrate recognition, whereas the major role of PIAS proteins is to stabilise the interaction between Ubc9 and substrate. This notion is supported by the finding that PIAS proteins can make physical contact with Ubc9 and

most substrates. Importantly, it has been observed that SUMOylation of a target protein enhances its interaction with PIAS [5, 21, 23]. This effect is mediated by a region which has been proposed to function as a SUMO recognition motif [24]. This region adjacent to the SP-RING finger, which is composed of a central serine triplet flanked by a C-terminal stretch of acidic residues, can directly bind to SUMO. Hence, PIAS proteins seem to exert a dual function as E3-like SUMO ligases and SUMO-tethering factors. Considering that PIAS proteins promote their own modification and interact with many proteins that undergo SUMOylation, this could provide a self-perpetuating mechansim to assemble large protein complexes.

The role of the PIAS/SUMO system in subnuclear compartmentalisation

An increasing number of cellular proteins are reported to undergo SUMOylation, yet the functional significance of this modification is rather unclear. The currently available data indicate that in contrast to ubiquitination, SUMOylation does not tag proteins for degradation but rather seems to enhance their stability or modulate protein-protein interactions. The role of SUMO in protein stabilisation has most convincingly been illustrated on the inhibitor of nuclear factor kappa B alpha (IκBα) [25]. In this particular case SUMO modification occurs at the same lysine residue that is used for ubiquitination, thus antagonising ubiquitination and proteasomal degradation of IκBα. The alternative modification by SUMO or ubiquitin at the same lysine residue has recently also been described for the PCNA (proliferating cell nuclear antigen) protein [26]. Ubiquitination of PCNA, however, is not implicated in protein degradation, but plays a pivotal role in DNA repair. Intriguingly, SUMOylation seems to inhibit the role of PCNA in DNA repair. This led to the idea that lysine residues that are affected by alternative modifications may function as regulatory switchboards that can direct proteins to alternate functions [26]. The switch of modifications may allow the exchange of binding partners and thus control protein-protein interactions. The SUMO-dependent regulation of protein-protein interactions is in many cases associated with changes in the subcellular localisation of the respective target proteins. This was first shown for the nuclear import factor RanGAP1, which can bind the nuclear pore complex protein RanBP2 upon SUMO conjugation and concomitantly is recruited from the cytosol to the nuclear envelope [27, 28]. Importantly, SUMO also seems to play a crucial role in compartmentalisation of proteins within the nucleus. In particular, SUMO-modified proteins are frequently found in distinct subnuclear foci, the so-called PML nuclear bodies (NBs, also known as ND10 or PODs), indicating that SUMO directs proteins to NBs or, alternatively, that NBs are sites for SUMOylation. Remarkably, all mammalian PIAS proteins concentrate in subnuclear structures that resemble PML NBs [5, 21, 29]. In the case of PIASy it has indeed been demonstrated that at least a subset of PIASy-containing foci colocalise with a subpopulation of PML nuclear bodies [5]. This suggests that for other PIAS forms, the PIAS-containing nuclear foci also overlap with PML NBs. Noteworthy, however, the number of PIAS foci generally exceeds the number of PML NBs, indicating that PIAS proteins also target nuclear structures distinct from NBs. PML NBs are a particular subtype of nuclear structures that are tightly associated with the nuclear matrix. Normal cells possess between 10 and 30 NBs per nucleus that appear as dense spherical particles, 0.3–0.5 µm in diameter [30]. They all contain the NB core components PML and Sp100, and a number of additional proteins that transiently associate with NBs. Among these are various transcription factors, such as the tumor suppressor p53, as well as transcriptional coregulators, such as the corepressor Daxx or the coactivator CBP. Many of these proteins, including PML, Sp100, p53 or Daxx, have been shown to undergo SUMOylation, and this modification appears to modulate their association with NBs. In particular, SUMOylation of PML has a pivotal role in controlling the assembly of NBs and is essential for recruitment of NB-associated proteins such as Daxx, CBP or Sp100 to these structures. Although NBs have been implicated in a number of cellular processes, their main biological function remains largely enigmatic. The presence of transcription factors and transcriptional coregulators clearly argues for a role of NBs in the control of transcriptional processes [31]. However, NBs do not seem to be active sites of transcription but rather represent storage or assembly sites for transcription factors and transcriptional coregulators. The localisation of the PIAS E3-like SUMO ligases to a subset of PML NBs supports the idea that SUMO modification plays an important role in regulating NB activity. Proper localisation of PIAS proteins to nuclear foci depends on the integrity of the SP-RING domain, indicating that the ligase activity is implicated in the targeting process [5, 21]. In line with this notion, coexpression of SUMO promotes recruitment to these structures. Interestingly, the SUMO interaction motif described above is also required for proper localisation [21]. Thus, it is tempting to speculate that targeting of PIAS to nuclear foci involves binding to SUMO-modified core components of the PML NBs. Importantly, as described in detail below, targeting of PIAS proteins to NBs can trigger recruitment of PIAS-associated transcriptional regulators to these structures. This compartmentalisation appears to be a critical determinant for regulation of these factors.

PIAS proteins as transcriptional coregulators

In the following section we will discuss the regulatory role of PIAS proteins in important cellular signalling pathways. Our particular focus will be to dissect the function of PIAS proteins as transcriptional regulators and to highlight the role of SUMO modification in these processes.

STAT pathway

The STAT family of transcription factors (signal transducer and activator of transcription) plays a central role in cytokine-dependent gene expression and regulates a variety of cellular processes, including immune response, differentiation, cell-survival and cell proliferation [32–34]. Currently seven mammalian STATs have been identified, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. In the unstimulated state STATs reside as latent transcription factors in the cytoplasm. Upon binding of cytokines to a receptor at the cell surface, STATs are activated via a cascade of phosphorylation that is initiated by the autophosphorylation of a receptor associated janus kinase (JAK). The activated JAK in turn phosphorylates the receptor at tyrosine residues, inducing the SH2-domain-dependent recruitment of STAT to the receptor. Subsequently, the receptor-bound STATs themselves undergo JAK-mediated phosphorylation on a conserved tyrosine residue, leading to formation of STAT dimers that translocate to the nucleus and bind to a cognate DNA binding motif in the promoter region of target genes. The nature of the cytokine stimulus determines the activation of a distinct STAT pathway. Here we will focus on pathways that activate STAT1, STAT2 and STAT3, respectively. Type I interferons (IFNα,β) signal through STAT1/STAT2 heterodimers that recruit as an additional factor IRF9/p48 to form the heterotrimeric ISGF3 complex. This complex recognises specific promoter elements termed ISRE (IFNα/β-stimulated response element). Type II interferons (IFNγ) or interleukin 6 family members induce formation of STAT1 homodimers that bind to a so-called GAS (γ-activated sequence) motif. Interleukin 6 can also activate the formation of STAT3 homodimers. STAT3 dimers form also in response to growth factors, including epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) that signal via a different class of tyrosine kinase receptors.

To allow tight control of cytokine signalling, the activity of the STAT pathway is downregulated at several levels [35, 36]. SOCS proteins are upstream negative regulators that block JAK function by either directly inhibiting their catalytic activity or promoting their degradation. In the nucleus a tyrosine phosphatase inhibits STAT activity by dephosphorylating the activated STAT dimer, allowing relocation to the cytosol in a CRM1-dependent nuclear export process. Work from the laboratory of Ke Shuai has illustrated that PIAS proteins function as another type of important negative regulator of STATs in the nucleus. The laboratory initially isolated PIAS1 as an interaction partner of STAT1 by performing a yeast two-hybrid screen using a STAT1 variant lacking the C-terminal transactivation domain as bait [37]. Subsequent experiments demonstrated that PIAS1 specifically interacts with STAT1, but not STAT2 or 3, in vivo in several cell lines. The interaction is mediated by a C-terminal fragment of PIAS1 that binds to the N-terminal region in STAT1. Recent data show that an arginine residue within this region of STAT1 undergoes methylation [38]. Inhibition of methylation by the drug methylthioadensine strongly enhanced binding of PIAS1 to STAT1, indicating that the interaction is negatively regulated by arginine methylation. The STAT1/PIAS1 interaction is strictly dependent on the presence of interferon and consistently requires the phosphorylation of STAT1 at tyrosine residue 701. Accordingly, PIAS1 binds to the activated STAT1 dimer [39]. Remarkably, overexpression of PIAS1 drastically downregulates the transcriptional activity of STAT1 in response to interferon γ on a luciferase reporter gene construct harboring a GAS element in its promoter. In vitro DNA binding experiments by electrophoretic mobility shift assay (EMSA) further demonstrated that recombinant PIAS1 inhibits the DNA binding of STAT1 to a probe containing a high-affinity STAT1 binding site. This led to a model where binding of PIAS1 to the active STAT1 dimer masks the DNA binding domain of STAT1, thereby blocking interaction with DNA. An alternative model might be that PIAS1 interacts transiently with STAT dimers to induce their dissociation and consequently the loss of DNA binding activity. The finding that after IFNα stimulation no STAT1/STAT2 heterodimers can be immunoprecipitated together with PIAS1 from cellular extracts may favor this second model.

Subsequent to the identification of PIAS1, database searches revealed the existence of four proteins homologous to PIAS1. Assuming that these proteins may also be involved in STAT signalling, Shuai and co-workers could show that one of these proteins, termed PIAS3, downregulates STAT3 activity in a manner analogous to the inhibition of STAT1 by PIAS1 [40]. PIAS3 associates specifically with STAT3 in cells that had been stimulated with ligands that signal via STAT3. Moreover, PIAS3 blocks the DNA binding activity of STAT3 homodimers, as well as STAT1/STAT3 heterodimers, but not STAT1 homodimers. Accordingly, overexpression of PIAS3 inhibited STAT3-mediated gene activation in transactivation assays. Hence, these results suggest that distinct PIAS proteins act as specific inhibitors on a given STAT transcription factor by specifically inhibiting its DNA-binding activity. This view has been somewhat challenged by later

findings that PIAS3 also serves as a repressor of STAT5 [41], while PIASy is able to inhibit STAT1 activity [42]. Surprisingly, however, PIASy does not block the DNA binding of STAT1, indicating that PIAS proteins may also use alternate mechanisms to downregulate STAT activity. Considering the SUMO ligase activity of PIAS proteins, an obvious question is whether PIAS-mediated SUMO modification of STATs may eventually be involved in the inhibitory process. Future studies will undoubtedly tackle this question to gain a full understanding of the role of PIAS proteins in controlling STAT activity. Intriguingly, however, recent data already revealed that PIAS-mediated SUMO modification is implicated in the regulation of a downstream target gene of STAT1 and STAT3, the interferon-regulated transcription factor IRF-1 [43]. STAT1 and STAT3 bind to GAS sites in the promoter region of IRF-1, thereby inducing the expression of IRF-1 in response to interferons. IRF-1 in turn controls the expression of numerous cell-cycle inhibitory proteins, such as p53 and p21, indicating that it mediates the antiproliferative and proapoptotic effects of interferons. PIAS3 binds to an N-terminal region of IRF-1 that exerts an inhibitory function on transactivation, and consequently PIAS3 suppresses the transcriptional activity of IRF-1. Importantly, the repressive activity requires the integrity of the SP-RING domain, suggesting that the SUMO-ligase function is required for repression. Consistently, PIAS3 induces the SUMO modification of IRF-1. Although it remains to be determined how PIAS-induced SUMOylation represses IRF-1 activity, these data illustrate that PIAS proteins can inhibit cytokine signalling not only by inhibiting STAT activity, but also by inhibiting downstream effectors. This dual function may have evolved to ensure a tight and immediate downregulation of cytokine action. In summary, these findings underscore the pivotal role of PIAS proteins as negative regulators in the STAT pathway.

Wnt signalling

The evolutionarily conserved Wnt signalling pathway plays a crucial role during embryonic patterning and cell fate determination [44]. Wnts are secreted glycoproteins that activate receptor-mediated signal transduction pathways to control gene expression, cell behavior, cell adhesion and cell polarity. Here we will concentrate on the best-understood, so-called canonical Wnt pathway. The key downstream effector of this pathway is the bipartite transcription factor β-catenin/lymphoid enhancer factor-1 (LEF1). Members of the T cell factor (TCF)/LEF family of proteins are characterised by a high-mobility group (HMG) box in their DNA binding region. They act as architectural DNA-binding factors that upon binding induce bending of DNA. LEF1 does not act as transcriptional activator by its own, but requires collaboration with other factors, such as β-catenin, for transcriptional activation. In the absence of these factors LEF1 even acts as a transcriptional repressor by tethering corepressors like Groucho to the promoters of Wnt-responsive genes. Binding of β-catenin to LEF1 displaces these corepressors and generates a transcriptionally active complex. In the absence of Wnt signals β-catenin is kept inactive in the cytosol by constitutive degradation via the ubiquitin-proteasome pathway. Upon Wnt signalling a cascade is initiated that leads to stabilisation of β-catenin, which can then enter the nucleus and associate with the N-terminus of LEF1. LEF1 can also be activated, independent of Wnt signalling, by interacting with the cofactor ALY to activate enhancers of specific target genes, such as the T cell receptor-enhancer.

Again, it was the result of a yeast two-hybrid interaction screen that identified PIAS proteins as new important regulators of LEF activity and Wnt signalling. Sachdev et al. isolated PIASy as a binding partner of LEF1 [5]. The N-terminal domain of PIASy, including the SAP motif, specifically binds within the C-terminal region of LEF1, containing the HMG domain. Remarkably, in transient transfection experiments overexpression of PIASy dramatically repressed the activity of LEF1/β-catenin on various reporter genes harboring LEF1-responsive promoter elements. Similarly, PIASy could downregulate Wnt-independent LEF1 activity. Although this scenario is very reminiscent of the inhibitory role of PIAS in the STAT pathway, the underlying mechanism appears to be different. Rather than interfering with the DNA binding activity of the β-catenin/LEF1 complex, PIASy inhibits the function of LEF1 by altering its subnuclear distribution. Coexpression of PIASy with LEF1 induces a dramatic relocalisation of LEF1 from a predominantely nuclear diffuse localisation to distinct nuclear dots that correspond to a subset of PML nuclear bodies. Importantly, coexpression of PIASy also induces SUMO modification of LEF1, indicating that PIASy exerts SUMO-ligase activity towards LEF1. Although these data provided evidence for a direct link between SUMOylation of LEF1, NB recruitment and transcriptional repression, the scenario appears to be more complex. Mapping and mutation of the SUMO attachment sites within LEF1 revealed that SUMO-deficient LEF1 mutants can still be recruited to PML bodies upon overexpression of PIASy, indicating that SUMO modification of LEF1 is not essential for recruitment, at least under conditions where PIASy and LEF1 are overexpressed. This indicates that the binding of PIASy to LEF1, but not the SUMO modification of LEF1, determines sequestration to NBs. However, because SUMO modification of LEF1 augments the association with PIASy, SUMO may facilitate NB targeting. Consistent with this idea coexpression of SUMO together with LEF1 and PIASy promotes recruitment to NBs. In

summary, these results indicate that PIAS-mediated recruitment of LEF1 to nuclear bodies represents an important and novel mechanism to inhibit LEF1 activity.

Intriguingly, PIAS proteins seem to target additional components of the Wnt-signalling pathway. Hence, the activity of MITF (microphtalima-associated transcription factor), a member of the basic helix-loop-helix leucine zipper family of transcription factors, is inhibited by PIAS3 [45]. MITF has recently been shown to bind to LEF1 and functionally cooperate with LEF1 and β-catenin in the induction of a Wnt-responsive promoter [46]. Since MITF is a candidate SUMO-target protein, the inhibitory effect of PIAS3 on MITF is presumably also linked to SUMO modification [47]. Another PIAS target in the Wnt pathway is duplin, a nuclear inhibitor of β-catenin [48]. Duplin has also been reported to bind to PIAS. However, the functional outcome of this interaction has not yet been elucidated. These data clearly demonstrate that, as in cytokine signalling, PIAS proteins interfere at several levels, with Wnt signalling underlining the importance of the PIAS/SUMO system in this pathway.

p53 pathway

The p53 protein plays a key role in tumor suppression and in the cellular response to DNA damage. Its label 'guardian of the genome' or 'cellular gatekeeper' most adequately summarises the cellular function of p53 [49]. p53 mainly acts as a sequence-specific transcription factor on genes whose products regulate cell-cycle progression and apoptosis, such as the CDK inhibitor p21 and the proapoptotic Bax protein. The p53 protein can be divided structurally and functionally into four domains: the N-terminal transactivation domain, the central sequence-specific DNA binding domain, the tetramerization domain and the basic C-terminal negatively regulatory region. In normal cells, the amount of p53 protein is very low since interaction with the E3 ubiquitin ligase Mdm2 promotes its degradation through the ubiquitin-proteasome pathway [50]. Upon stress, such as DNA damage, the half-life and activity of p53 are dramatically increased. The tight and rapid regulation of p53 activity is achieved by the orchestrated action of multiple posttranslational modifications [51]. Although the exact function of specific modification sites remains somehow controversial, the most important sites appear to be clustered in the N-terminal and C-terminal regions. Phosphorylation of N-terminal residues upon DNA damage, including S15 and/or S20, attenuates the p53/Mdm2 interaction and thus induces the accumulation of p53. Acetylation of residues in the C-terminal region more likely directly activates transcription through recruitment of coactivators, such as the CBP/p300 acetyltransferases [52]. Within the C-terminal region a distinct lysine residue (K386) is also targeted by SUMO modification, and recent data implicate PIAS proteins in this process [19, 29, 53–55]. Although PIAS1, PIASxβ and PIASy were isolated as p53-interacting proteins in different independent two-hybrid screens some time ago [24, 56, 57], the connection to SUMOylation of p53 was only established after the E3-like activity of the yeast Siz proteins became apparent. Subsequently, we and others could show that PIAS proteins, such as PIAS1 and PIASxβ, strongly stimulate the attachment of SUMO to p53 in vivo and in a reconstituted in vitro SUMOylation system [19, 20]. The coexpression of PIAS1 and PIASxβ together with SUMO and p53 in transient expression experiments strongly induces the formation of SUMO-p53 conjugates. Remarkably, this is paralleled by inhibition of the transcripitional activity of p53 on a reporter gene, which harbors a synthetic p53 DNA binding site in its promoter [20]. The repressive activity requires the integrity of the SP-RING domain and at least partially depends on the coexpression of SUMO, suggesting that it is linked to the SUMO ligase activity of PIAS. Surprisingly however, the $p53^{K386R}$ mutant that has lost the major SUMOylation site was also repressed by PIAS/SUMO. This situation is very reminiscent of the inhibitory effect of PIASy on LEF1 activity, which also does not essentially depend on SUMOylation of LEF1. Considering that p53 is associated with PML NBs [58], it is tempting to speculate that as for LEF1, NB targeting of p53 by the PIAS/SUMO system could be implicated in transcriptional silencing. The role of SUMO modification of p53 may only be to facilitate the interaction with PIAS proteins and since SUMOylation may not be crucial under conditions where both p53 and PIAS proteins are overexpressed. Alternatively, the inhibition of p53 following expression of PIAS/SUMO may not be due to the enhanced SUMOylation of p53 itself, but result from the PIAS-mediated modification of another SUMO substrate. In support of this idea, recent work by Yasuda and co-workers has demonstrated that PIAS1 and PIASxβ not only stimulate SUMOylation of p53 but also induce SUMOylation of the mdm2 ubiquitin ligase [59]. Although the functional consequence on mdm2 ligase activity has not yet been investigated, the PIAS-induced SUMO modification of mdm2 may be involved in inhibition of p53 activity. Consistent with the idea that PIAS proteins function as negative regulators in the p53 pathway, PIASy has also been shown to inhibit the ability of p53 to activate the target genes p21 and Bax [56]. Moreover, expression of PIASy blocks the DNA-binding activity of p53 in EMSA experiments. Hence, it is tempting to propose a model where binding of PIAS, followed by SUMOylation of p53 or an associated regulatory factor, inhibits p53 by either directly interfering with its DNA-binding activity or by sequestering p53 in an inactive complex. Although this is an attractive model, a further

level of complexity has been added by recent findings that in other contexts, PIAS1 and PIAS3 may function as activators of p53-dependent gene expression [60, 61]. Cotransfection of PIAS1 together with p53 in a p53-negative cell line (H1299) stimulates expression of the p21 protein and consistently induces cell-cycle arrest. However, since PIAS1 expression alone can also activate a reporter gene construct driven by the p21 promoter and induce cell-cycle arrest in in the absence of functional p53, these effects are only partially mediated by p53. Moreover, SUMO modification does not appear to be involved, since a PIAS1 mutant lacking the SP-RING domain can also exert these effects. Activating effects on p53 have also been reported for a splice variant of PIAS3 termed KchAP or PIAS3β [61]. Expression of PIAS3β in a prostate cancer cell line harboring a wild-type p53 promotes phosphorylation of serine 15 in p53 and induces the accumulation of p53. This coincided with the induction of the p21 protein and cell-cycle arrest. It remains to be elucidated how PIAS proteins can integrate both negative and positive regulatory effects on p53 and what might be the particular role of SUMO in this process. One possibility is that distinct PIAS proteins exert differential effects on p53. Alternatively, PIAS proteins may exert cell-type specific effects on the p53 pathway. Because for some PIAS proteins a highly tissue specific expression has been reported, one possible interpretation is that tissue-specific factors modulate PIAS function.

Steroid hormone receptor signalling

The steroid hormone receptor family represents a subgroup of the nuclear receptor superfamily [62]. It is composed of the androgen (AR), estrogen (ER), progesterone (PR), mineral (MR) and glucocorticoid (GR) receptors. Steroid hormone receptors act as ligand-dependent transcription factors that bind as homodimers to distinct hormone response elements in the promoter region of their respective target genes. All members of this family share a characteristic modular structure that consists of an N-terminal transactivation domain (AF-1), a central DNA binding domain and the C-terminal ligand binding domain harboring a second activation domain (AF-2). In the absence of ligand the receptors are kept in an inactive state by binding to a large inhibitory complex mainly consisting of heat shock proteins. Binding of ligand induces a conformational change that displaces this inhibitory complex and allows the receptors to bind to the regulatory regions of target genes. Subsequent to DNA binding, distinct coactivator complexes are recruited to the promoter to initiate transcription. The best-characterised coactivator family is the p160 family, which is recruited to the receptor via the AF-2 domain. This family includes SRC1 (also termed NcoA-1), GRIP1 (also termed TIF2) and pCIP (also termed TRAM-1 or Rac) [63]. A characteristic feature of this family is the presence of a LXXLL motif in the nuclear receptor interaction domain. Via this motif p160 family members interact with the AF-2 domain in nuclear hormone receptors. Binding of p160 to the receptor seems to provide a platform for docking of chromatin-modifying enzymes. Thus, p160 recruits the histone acetyltransferases p300/CBP and the arginine methyltransferase CARM1 to promoters to induce local decondensation of chromatin via an acetylation-methylation cascade of histone tails.

Yeast two-hybrid interaction screens uncovered a link between PIAS proteins and nuclear hormone receptor signalling. Initially, two laboratories independently reported the interaction of the androgen receptor with PIAS1 and PIASxα (also termed ARIP3), respectively [64, 65]. In both cases the interaction domain in the AR was mapped to the central region, including the DNA binding domain. Interestingly, the interaction was greatly stimulated by ligand binding to AR. Subsequently, interactions of AR with all other PIAS family members were reported [66, 67]. Likewise, other steroid hormone receptors, such as GR, PR and ER, were shown to bind to PIAS proteins [68]. Several independent studies showed that PIAS proteins modulate the ligand-dependent transactivation potential of the receptors. Depending on the receptor type, the cell lines and promoters used in transactivation assays, both activating and repressing effects on transcription were observed upon expression of a distinct PIAS family member, indicating that PIAS proteins play a context-dependent dual role as activators or repressors in steroid hormone signalling. In an attempt to study the mechanism underlying the action of PIAS proteins in steroid hormone signalling, Kotaya and co-workers could show that PIASx and PIAS1 not only bind to the hormone receptors but also interact with the GRIP1/TIF2 coactivator [69]. Independently, PIAS3 was identified as a binding partner of GRIP1/TIF2 [70]. PIAS proteins act synergistically with GRIP1/TIF2 to activate GR- and AR-dependent transactivation, indicating that PIAS and GRIP1/TIF2 are components of the same coactivator complex. Intriguingly, GRIP1/TIF2 undergoes SUMOylation at three lysine residues, two of which (K731, 788) are located in the LXXLL nuclear receptor interaction domain, and very recent data demonstrate that PIAS1 and PIASxβ stimulate the SUMO modification of GRIP1/ TIF2 [70, 71]. Mutation of the SUMO-attachment sites impairs the ability of GRIP1/TIF2 to coactivate AR-dependent transactivation and to synergise with PIAS-mediated activation. In addition, the mutant has lost its ability to colocalize with AR-containing nuclear foci. Together, these data suggest that PIAS-mediated SUMOylation of GRIP1/TIF2 facilitates its interaction with the androgen receptor, enhancing its transcriptional activity. In support of this model SUMOylation of the related p160 member SRC-1 has been shown

to enhance binding to the progesterone receptor [72]. While such a scenario can explain the observed coactivator function of PIAS proteins in androgen and steroid hormone signalling, how might the negative effects of PIAS proteins be explained? The answer comes from new findings demonstrating that PIAS1 and PIASx exert SUMO-ligase activity not only towards p160 coactivators but also towards the androgen receptor and presumably other steroid receptors [21, 73]. AR, GR and PR undergo SUMOylation at lysine residues that reside in a region that functions as a transcriptional inhibitory domain [74–76]. Blocking SUMO modification by substituting these SUMO-attachment residues to arginines enhances the transactivation potential of the receptors. Conversely, inducing SUMO modification by overexpression of PIAS together with SUMO inhibits AR activity, indicating that modification of the AR (and likely the other receptors) negatively regulates transcriptional activity.

The picture that emerges from these data is that the effects of PIAS on AR-dependent gene activity likely reflect combinatorial effects of PIAS proteins on the SUMOylation of both coactivators and receptors. This is most convincingly illustrated by the differential effects of the two PIASx splice variants on AR-dependent transactivation [73]. Overexpression of PIASxα, which exerts strong ligase activity on AR but only weak activity on GRIP1, represses AR-dependent transactivation when co-expressed with SUMO. PIASxβ, in contrast, which has a preference for SUMOylation of GRIP1, activates AR-dependent gene expression. The function of PIAS1 appears to be more complex since it can stimulate SUMO modification of both GRIP1 and the receptor. Kotaja and co-workers propose a model where SUMO modification of GRIP1 and AR occur sequentially at different stages of the AR transcription complex formation. Initially SUMOylation of GRIP-1 may promote the recruitment of the coactivator to the receptor. Subsequent modification of the receptor may later turn down the activity after successful initiation of the transcription process [21].

A model for PIAS/SUMO function in transcriptional regulation

The examples described above underline the crucial role of the PIAS/SUMO pathway in the regulation of transcriptional processes. Depending on the context, PIAS proteins either function as transcriptional coactivators or repressors. Since SUMO ligase activity and transcriptional regulatory function are correlated, SUMO modification is likely be important for this function. In the following section we will try to develop a general model for how the PIAS/SUMO system could integrate both negative and positive functions by mediating the assembly of coactivator or corepressor complexes.

PIAS/SUMO-mediated targeting to subnuclear structures and transcriptional regulation

An important mechanism for controlling the activity of transcriptional regulators appears to be their targeting to specific subnuclear sites. Accumulating evidence indicates that the PIAS/SUMO system is involved in this process. Hence, as describd above, PIASy has been shown to inactivate LEF1 by inducing its compartmentalisation into PML NBs. Considering that NBs are unlikely active sites of transcription, the most simple interpretation of these data is that recruitment to NBs via PIAS/SUMO sequesters LEF1 away from its target genes, thus neutralising its transcriptional activity. Recent data on the GC box-binding transcription factor Sp3 extend this concept and provide evidence that NB targeting via the PIAS/SUMO system not only neutralises transcriptional activity but actively induces transcriptional silencing. The Sp3 protein can act both as a transcriptional activator and repressor. Sapetschnig and co-workers could demonstrate that the repressor function of Sp3 resides in a small inhibitory domain that serves as an interface for binding to PIAS1 and harbors a SUMO attachment site [23]. PIAS1 promotes the SUMO modification of Sp3, and similar to what has been observed for the interaction between PIASy and LEF1, this further enhances the interaction of PIAS1 with Sp3. In an independent study Ross and co-workers reported that SUMO triggers the targeting of Sp3 to PML nuclear bodies, acting as a major regulatory switch that converts Sp3 from a transcriptional activator to a repressor [77]. Combining these data, one can again delineate a pathway where the PIAS/SUMO system initiates the recruitment of a transcriptional regulator to NBs. But how might this convert Sp3 to a repressor? Transcriptional repression is in many cases mediated by corepressor complexes that contain histone deacetylase activity. Accordingly, the inhibitory function of Sp3 is at least partially mediated by the Sin3A/HDAC transcriptional repressor complex [78, 79]. Since components of this complex are associated with PML [80], a very likely scenario is that recruitment of Sp3 to the NBs facilitates the assembly of Sp3 with this corepressor complex. The recent finding that PIAS proteins can directly interact with histone deacetylase (HDACs) provides additional evidence for a role of PIAS proteins in the assembly of such a complex [81]. Moreover, considering that p53 is associated with PML NBs and is transcriptionally inhibited by repressor complexes containing histone deacetylase activity [82–84], NB targeting and tethering of deacteylases could also account for the inhibitory effects of PIAS proteins on p53. Strikingly, very recent data have also provided a link between the inhibition of STAT signalling and PML NBs [85]. PML was reported to form a complex with STAT3, thereby inhibiting its DNA-binding activity. In light of

this finding it is tempting to speculate that PIAS3 may control STAT3 activity by regulating its subnuclear localisation.

Interestingly, the presence of SUMO-modification sites within inhibitory or negative regulatory domains in transcription factors appears to be a common theme. Remarkably, in most cases PIAS proteins promote the modification of these factors. In addition to Sp3, IRF-1 and steroid hormone receptors, PIAS-triggered SUMO modification in inhibitory domains has been observed for the c-Jun and c-myb protooncogenes and the CAAT enhancer binding protein C/EBPα [86–89]. In all cases, mutation of the SUMO attachment sites enhances the transcriptional activity, indicating that SUMO modification exerts an inhibitory role on these factors. Although it remains to be elucidated whether NBs are implicated in this process, several lines of evidence support this idea. Expression of a SUMO isopeptidase (SuPr-1), for instance, induces disaggregation of NBs and concomitantly stimulates the transcriptional activity of c-Jun [90]. Furthermore, PIASy-mediated SUMOylation of c-myb causes a shift in the nuclear distribution of c-myb towards the insoluble matrix fraction [87]. In summary, these data strengthen the idea that PIAS binding and SUMOylation are a more widespread mechanism to downregulate the activity of transcription factors and suggest that assembly of transcriptional corepressors by the PIAS/SUMO system within subnuclear structures, such as PML NBs, could provide a more general mechanism for transcriptional silencing (fig. 3). In this model PIAS proteins play a dual role as E3-like factors for SUMOylation and components or assembly factors of a transcriptional coregulatory complex. Although it is not yet clear how these two functions are connected, a similar duality was described for several E3 ubiquitin ligases that bind within transactivation domains and can act as transcriptional coactivators [91]. While these findings connect transcriptional activation to the ubiquitin-proteasome pathway, it is intriguing to speculate that the PIAS/SUMO system may act antagonistically to counterbalance these effects. It remains to be determined whether SUMO may directly interfere with ubiquitin at distinct lysine residues in these cases.

Noteworthy, however, PIAS proteins can also exert activating functions on transcription factors in some contexts. As described above, activating functions have been reported in steroid hormone signalling and in the p53 pathway. At least in steroid hormone signalling this effect is linked to the assembly of a functional receptor coactivator complex, since PIAS-mediated SUMOylation of GRIP-1 or SRC-1 facilitates the interaction with the androgen and progesterone receptor, respectively. This process is likely connected to PML NBs, because GRIP1 and SRC-1 as well as the associated coactivator CBP are found in nuclear substructures that overlap with PML NBs [92, 93]. Hence, in analogy to the scenario described above for transcriptional repression, recruitment of PIAS and PIAS-associated transcription factors to PML NBs or related matrix-associated structures could trigger the assembly of coactivator complexes. An obvious question is what determines whether the PIAS/SUMO system ultimately leads to the formation of a coactivator or corepressor complex on a given transcription factor. One possibility might be that distinct PIAS forms function as targeting factors to specific subpopulations of NBs, which may contain either corepressors or coactivators. This idea is supported by the observation that at least upon overexpression distinct PIAS forms show a different pattern of subnuclear localisation. Hence, PIAS1 exhibits a microgranular localisation in small foci, whereas PIAS3 concentrates in fewer, but larger granules [21]. More detailed localisation studies on the endogenous PIAS proteins must elucidate the identity of the respective subnuclear structures.

Conclusions and perspectives

Research on PIAS proteins during the last few years has provided exciting new insights into the cellular function of this protein family. In particular, the discovery of the E3-like activity in the SUMO pathway has opened up new

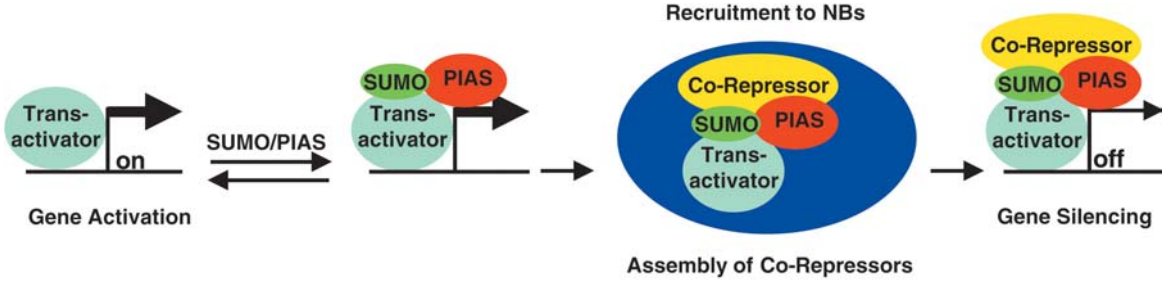

Figure 3. A model for the role of the PIAS/SUMO system in transcriptional silencing. PIAS-triggered SUMOylation induces the transient recruitment of a transcription factor to PML NBs or related subnuclear structures. In these structures a corepressor complex is assembled on the transactivator, leading to inhibition of its activity and gene silencing. As described in detail in the text, an analogous model can explain the context-dependent coactivator role of PIAS proteins.

perspectives in the study of PIAS proteins. The examples described above illustrate the importance of the PIAS/SUMO system as a regulatory mechanism of individual transcription factors. However, the control of gene expression is in most cases not achieved by a single transcription factor but results from the interplay among many factors at composite promoter/enhancer elements. Interestingly, some of the PIAS-interacting factors described above cooperate in the expression of selected target genes. Thus, STATs are engaged in transcriptional cross-talk with nuclear hormone receptors to synergise in gene activation [94]. C/EBPs in turn can cooperate with c-myb on promoter/enhancer elements of myeloid-specific genes [95]. PIAS proteins may thus not only control the activity of transcription factors individually, but may orchestrate the complex interplay of several transcription factors at a given promoter [96]. To understand the exact role of the SUMO system in these processes will be one major challenge in the field. Given the pace of recent research on the PIAS/SUMO system it can be anticipated that we will soon learn more about this exciting new pathway.

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Note added in proof. While this manuscript was under review PIAS mediated SUMOylation of STAT1 has been reported:
Ungureanu D., Vanhatupa S., Kotaja N., Yang J., Aittomaki S., Janne O. A., Palvimo J. J. and Silvennoinen O. (2003) PIAS proteins promote SUMO-1 conjugation to STAT1. Blood, in press
Rogers R. S., Horvath C. M. and Matunis M. J. (2003) SUMO Modification of STAT1 and its role in PIAS-mediated inhibition of gene activation. J. Biol. Chem. **278:** 30091–30097

1 Shuai K. (2000) Modulation of STAT signaling by STAT-interacting proteins. Oncogene **19:** 2638–2644
2 Aravind L. and Koonin E. V. (2000) SAP – a putative DNA-binding motif involved in chromosomal organization. Trends Biochem. Sci. **25:** 112–114
3 Kipp M., Gohring F., Ostendorp T., van Drunen C. M., van Driel R., Przybylski M. et al. (2000) SAF-Box, a conserved protein domain that specifically recognizes scaffold attachment region DNA. Mol. Cell Biol. **20:** 7480–7489
4 Tan J. A., Hall S. H., Hamil K. G., Grossman G., Petrusz P. and French F. S. (2002) Protein inhibitors of activated STAT resemble scaffold attachment factors and function as interacting nuclear receptor coregulators. J. Biol. Chem. **277:** 16993– 17001
5 Sachdev S., Bruhn L., Sieber H., Pichler A., Melchior F. and Grosschedl R. (2001) PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. Genes Dev. **15:** 3088–3103
6 Hari K. L., Cook K. R. and Karpen G. H. (2001) The *Drosophila* Su(var)2-10 locus regulates chromosome structure and function and encodes a member of the PIAS protein family. Genes Dev. **15:** 1334–1348
7 Hochstrasser M. (2001) SP-RING for SUMO: new functions bloom for a ubiquitin-like protein. Cell **107:** 5–8
8 Jackson P. K. (2001) A new RING for SUMO: wrestling transcriptional responses into nuclear bodies with PIAS family E3 SUMO ligases. Genes Dev. **15:** 3053–3058
9 Joazeiro C. A. and Weissman A. M. (2000) RING finger proteins: mediators of ubiquitin ligase activity. Cell 102: 549–552
10 Aravind, L. and Koonin, E. V. (2000) The U box is a modified RING finger – a common domain in ubiquitination. Curr. Biol. **10:** R132–134
11 Ohi M. D., Vander Kooi C. W., Rosenberg J. A., Chazin,W. J. and Gould K. L. (2003) Structural insights into the U-box, a domain associated with multi-ubiquitination. Nat. Struct. Biol. **3:** 3
12 Johnson E. S. and Gupta A. A. (2001) An E3-like factor that promotes SUMO conjugation to the yeast septins. Cell **106:** 35–744
13 Takahashi Y., Kahyo T., Toh E. A., Yasuda H. and Kikuchi Y. (2001) Yeast Ull1/Siz1 is a novel SUMO1/Smt3 ligase for septin components and functions as an adaptor between conjugating enzyme and substrates. J. Biol. Chem. **276:** 48973–48977
14 Takahashi Y., Toh-e A. and Kikuchi Y. (2001) A novel factor required for the SUMO1/Smt3 conjugation of yeast septins. Gene **275:** 223–231
15 Melchior F. (2000) Sumo nonclassical ubiquitin. Annu. Rev. Cell. Dev. Biol. **16:** 591–626
16 Muller S., Hoege C., Pyrowolakis G. and Jentsch S. (2001) SUMO, ubiquitin's mysterious cousin. Nat. Rev. Mol. Cell Biol. **2:** 202–210
17 Weissman A. M. (2001) Themes and variations on ubiquitylation. Nat. Rev. Mol. Cell Biol. **2:** 169–178
18 Pichler A., Gast A., Seeler J. S., Dejean A. and Melchior F. (2002) The nucleoporin RanBP2 has SUMO1 E3 ligase activity. Cell **108:** 109–120
19 Kahyo T., Nishida T. and Yasuda H. (2001) Involvement of PIAS1 in the sumoylation of tumor suppressor p53. Mol. Cell **8:** 713–718
20 Schmidt D. and Muller S. (2002) Members of the PIAS family act as SUMO ligases for c-Jun and p53 and repress p53 activity. Proc. Natl. Acad. Sci. USA **99:** 2872–2877
21 Kotaja N., Karvonen U., Janne O. A. and Palvimo J. J. (2002) PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. Mol. Cell Biol. **22:** 5222–5234
22 Bernier-Villamor V., Sampson D. A., Matunis M. J. and Lima C. D. (2002) Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. Cell **108:** 345–356
23 Sapetschnig A., Rischitor G., Braun H., Doll A., Schergaut M., Melchior F. et al. (2002) Transcription factor Sp3 is silenced through SUMO modification by PIAS1. EMBO J. **21:** 5206–5215
24 Minty A., Dumont X., Kaghad M. and Caput D. (2000) Covalent modification of p73alpha by SUMO-1. Two-hybrid screening with p73 identifies novel SUMO-1-interacting proteins and a SUMO-1 interaction motif. J. Biol. Chem. **275:** 36316–36323
25 Desterro J. M., Rodriguez M. S. and Hay R. T. (1998) SUMO-1 modification of IκBα inhibits NF-κB activation. Mol. Cell **2:** 233–239
26 Hoege C., Pfander B., Moldovan G. L., Pyrowolakis G. and Jentsch S. (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature **419:** 135–141
27 Matunis M. J., Wu J. and Blobel G. (1998) SUMO-1 modification and its role in targeting the Ran GTPase-activating protein, RanGAP1, to the nuclear pore complex. J. Cell. Biol. **140:** 499–509
28 Mahajan R., Delphin C., Guan T., Gerace L. and Melchior F. (1997) A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. Cell **88:** 97–107

29 Rodel B., Tavassoli K., Karsunky H., Schmidt T., Bachmann M., Schaper F. et al. (2000) The zinc finger protein Gfi-1 can enhance STAT3 signaling by interacting with the STAT3 inhibitor PIAS3. EMBO J. **19:** 5845–5855

30 Seeler J. S. and Dejean A. (2001) SUMO: of branched proteins and nuclear bodies. Oncogene **20:** 7243–7249

31 Zhong S., Salomoni P. and Pandolfi P. P. (2000) The transcriptional role of PML and the nuclear body. Nat. Cell Biol. **2:** E85–90

32 Levy D. E. and Darnell J. E. Jr (2002) Stats: transcriptional control and biological impact. Nat. Rev. Mol. Cell Biol. **3:** 651–662

33 Aaronson D. S. and Horvath C. M. (2002) A road map for those who know JAK-STAT. Science **296:** 1653–1655

34 O'Shea J. J., Gadina M. and Schreiber R. D. (2002) Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell **109:** S121–131

35 Starr R. and Hilton D. J. (1999) Negative regulation of the JAK/STAT pathway. Bioessays **21:** 47–52

36 Hilton D. J. (1999) Negative regulators of cytokine signal transduction. Cell. Mol. Life Sci. **55:** 1568–1577

37 Liu B., Liao J., Rao X., Kushner S. A., Chung C. D., Chang D. D. et al. (1998) Inhibition of Stat1-mediated gene activation by PIAS1. Proc. Natl. Acad. Sci. USA **95:** 10626–10631

38 Mowen K. A., Tang J., Zhu W., Schurter B. T., Shuai K., Herschman H. R. et al. (2001) Arginine methylation of STAT1 modulates IFNalpha/beta-induced transcription. Cell **104:** 731–741

39 Liao J., Fu Y. and Shuai K. (2000) Distinct roles of the NH2- and COOH-terminal domains of the protein inhibitor of activated signal transducer and activator of transcription (STAT) 1 (PIAS1) in cytokine-induced PIAS1-Stat1 interaction. Proc. Natl. Acad. Sci. USA **97:** 5267–5272

40 Chung C. D., Liao J., Liu B., Rao X., Jay P., Berta P. et al. (1997) Specific inhibition of Stat3 signal transduction by PIAS3. Science **278:** 1803–1805

41 Rycyzyn M. A. and Clevenger C. V. (2002) The intranuclear prolactin/cyclophilin B complex as a transcriptional inducer. Proc. Natl. Acad. Sci. USA **99:** 6790–6795

42 Liu B., Gross M., ten Hoeve J. and Shuai K. (2001) A transcriptional corepressor of Stat1 with an essential LXXLL signature motif. Proc. Natl. Acad. Sci. USA **98:** 3203–3207

43 Nakagawa K. and Yokosawa H. (2002) PIAS3 induces SUMO-1 modification and transcriptional repression of IRF-1. FEBS Lett. **530:** 204–208

44 Moon R. T., Bowerman B., Boutros M. and Perrimon N. (2002) The promise and perils of Wnt signaling through beta-catenin. Science **296:** 1644–1646

45 Levy C., Nechushtan H. and Razin E. (2002) A new role for the STAT3 inhibitor, PIAS3: a repressor of microphthalmia transcription factor. J. Biol. Chem. **277:** 1962–1966

46 Yasumoto K., Takeda K., Saito H., Watanabe K., Takahashi K. and Shibahara S. (2002) Microphthalmia-associated transcription factor interacts with LEF-1, a mediator of Wnt signaling. EMBO J. **21:** 2703–2714

47 Xu W., Gong L., Haddad M. M., Bischof O., Campisi J., Yeh E. T. et al. (2000) Regulation of microphthalmia-associated transcription factor MITF protein levels by association with the ubiquitin-conjugating enzyme hUBC9. Exp. Cell Res. **255:** 135–143

48 Kobayashi M., Kishida S., Fukui A., Michiue T., Miyamoto Y., Okamoto T. et al. (2002) Nuclear localization of Duplin, a beta-catenin-binding protein, is essential for its inhibitory activity on the Wnt signaling pathway. J. Biol. Chem. **277:** 5816–5822

49 Vogelstein B., Lane D. and Levine A. J. (2000) Surfing the p53 network. Nature **408:** 307–310

50 Prives C. (1998) Signaling to p53: breaking the MDM2-p53 circuit. Cell **95:** 5–8

51 Jayaraman L. and Prives C. (1999) Covalent and noncovalent modifiers of the p53 protein. Cell. Mol. Life Sci. **55:** 76–87

52 Barlev N. A., Liu L., Chehab N. H., Mansfield K., Harris K. G., Halazonetis T. D. et al. (2001) Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. Mol. Cell **8:** 1243–1254

53 Rodriguez M. S., Desterro J. M., Lain S., Midgley C. A., Lane D. P. and Hay R. T. (1999) SUMO-1 modification activates the transcriptional response of p53. EMBO J. **18:** 6455–6461

54 Muller S., Berger M., Lehembre F., Seeler J. S., Haupt Y. and Dejean A. (2000) c-Jun and p53 activity is modulated by SUMO-1 modification. J. Biol. Chem. **275:** 13321–13329

55 Gostissa M., Hengstermann A., Fogal V., Sandy P., Schwarz S. E., Scheffner M. et al. (1999) Activation of p53 by conjugation to the ubiquitin-like protein SUMO-1. EMBO J. **18:** 6462–6471

56 Nelson V., Davis G. E. and Maxwell S. A. (2001) A putative protein inhibitor of activated STAT (PIASy) interacts with p53 and inhibits p53-mediated transactivation but not apoptosis. Apoptosis **6:** 221–234

57 Gallagher W. M., Argentini M., Sierra V., Bracco L., Debussche L. and Conseiller E. (1999) MBP1: a novel mutant p53-specific protein partner with oncogenic properties. Oncogene **18:** 3608–3616

58 Fogal V., Gostissa M., Sandy P., Zacchi P., Sternsdorf T., Jensen K. et al. (2000) Regulation of p53 activity in nuclear bodies by a specific PML isoform. EMBO J. **19:** 6185–6195

59 Miyauchi Y., Yogosawa S., Honda R., Nishida T. and Yasuda H. (2002) Sumoylation of Mdm2 by protein inhibitor of activated STAT (PIAS) and RanBP2 enzymes. J. Biol. Chem. **277:** 50131–50136

60 Megidish T., Xu J. H. and Xu C. W. (2002) Activation of p53 by protein inhibitor of activated Stat1 (PIAS1). J. Biol. Chem. **277:** 8255–8259

61 Wible B. A., Wang L., Kuryshev Y. A., Basu A., Haldar S. and Brown A. M. (2002) Increased K+ efflux and apoptosis induced by the potassium channel modulatory protein KChAP/PIAS3β in prostate cancer cells. J. Biol. Chem. **277:** 17852–17862

62 Beato M., Herrlich P. and Schutz G. (1995) Steroid hormone receptors: many actors in search of a plot. Cell **83:** 851–857

63 Rosenfeld M. G. and Glass C. K. (2001) Coregulator codes of transcriptional regulation by nuclear receptors. J. Biol. Chem. **276:** 36865–36868

64 Moilanen A. M., Karvonen U., Poukka H., Yan W., Toppari J., Janne O. A. et al. (1999) A testis-specific androgen receptor coregulator that belongs to a novel family of nuclear proteins. J. Biol. Chem. **274:** 3700–3704

65 Junicho A., Matsuda T., Yamamoto T., Kishi H., Korkmaz K., Saatcioglu F. et al. (2000) Protein inhibitor of activated STAT3 regulates androgen receptor signaling in prostate carcinoma cells. Biochem. Biophys. Res. Commun. **278:** 9–13

66 Gross M., Liu B., Tan J., French F. S., Carey M. and Shuai K. (2001) Distinct effects of PIAS proteins on androgen-mediated gene activation in prostate cancer cells. Oncogene **20:** 3880–3887

67 Li P., Yu X., Ge K., Melamed J., Roeder R. G. and Wang Z. (2002) Heterogeneous expression and functions of androgen receptor co-factors in primary prostate cancer. Am. J. Pathol. **161:** 1467–1474

68 Kotaja N., Aittomaki S., Silvennoinen O., Palvimo J. J. and Janne O. A. (2000) ARIP3 (androgen receptor-interacting protein 3) and other PIAS (protein inhibitor of activated STAT) proteins differ in their ability to modulate steroid receptor-dependent transcriptional activation. Mol. Endocrinol. **14:** 1986–2000

69 Kotaja N., Vihinen M., Palvimo J. J. and Janne O. A. (2002) Androgen receptor-interacting protein 3 and other PIAS proteins cooperate with glucocorticoid receptor-interacting pro-

tein 1 in steroid receptor-dependent signaling. J. Biol. Chem. **277:** 17781–17788
70 Jimenez-Lara A. M., Heine M. J. and Gronemeye, H. (2002) PIAS3 (protein inhibitor of activated STAT-3) modulates the transcriptional activation mediated by the nuclear receptor coactivator TIF2. FEBS Lett. **526:** 142–146
71 Kotaja N., Karvonen U., Janne O. A. and Palvimo J. J. (2002) The nuclear receptor interaction domain of GRIP1 is modulated by covalent attachment of SUMO-1. J. Biol. Chem. **277:** 30283–30288
72 Chauchereau A., Amazit L., Quesne M., Guiochon-Mantel A. and Milgrom E. (2003) Sumoylation of the progesterone receptor and of the steroid receptor coactivator SRC-1. J. Biol. Chem. **278:** 12335–12343
73 Nishida T. and Yasuda H. (2002) PIAS1 and PIASxα function as SUMO-E3 ligases toward androgen receptor and repress androgen receptor-dependent transcription. J. Biol. Chem. **277:** 41311–41317
74 Poukka H., Karvonen U., Janne O. A. and Palvimo J. J. (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc. Natl. Acad. Sci. USA **97:** 14145–14150
75 Abdel-Hafiz H., Takimoto G. S., Tung L. and Horwitz K. B. (2002) The inhibitory function in human progesterone receptor N termini binds SUMO-1 protein to regulate autoinhibition and transrepression. J. Biol. Chem. **277:** 33950–33956
76 Tian S., Poukka H., Palvimo J. J. and Janne O. A. (2002) Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. Biochem. J. **367:** 907–911
77 Ross S., Best J. L., Zon,L. I. and Gill G. (2002) SUMO-1 modification represses Sp3 transcriptional activation and modulates its subnuclear localization. Mol. Cell **10:** 831–842
78 Won J., Yim J. and Kim T. K. (2002) Sp1 and Sp3 recruit histone deacetylase to repress transcription of human telomerase reverse transcriptase (hTERT) promoter in normal human somatic cells. J. Biol. Chem. **277:** 38230–38238
79 Sun J. M., Chen H. Y., Moniwa M., Litchfield D. W., Seto E. and Davie J. R. (2002) The transcriptional repressor Sp3 is associated with CK2-phosphorylated histone deacetylase 2. J. Biol. Chem. **277:** 35783–35786
80 Khan M. M., Nomura T., Kim H., Kaul S. C., Wadhwa R., Shinagawa T. et al. (2001) Role of PML and PML-RARalpha in Mad-mediated transcriptional repression. Mol. Cell **7:** 1233– 1243
81 Tussie-Luna M. I., Bayarsaihan D., Seto E., Ruddle F. H. and Roy A. L. (2002) Physical and functional interactions of histone deacetylase 3 with TFII-I family proteins and PIASxβ. Proc. Natl. Acad. Sci. USA **99:** 12807–12812
82 Luo J., Su F., Chen D., Shiloh A. and Gu W. (2000) Deacetylation of p53 modulates its effect on cell growth and apoptosis. Nature **408:** 377–381
83 Juan L. J., Shia W. J., Chen M. H., Yang W. M., Seto E., Lin Y. S. et al. (2000) Histone deacetylases specifically down-regulate p53-dependent gene activation. J. Biol. Chem. **275:** 20436–20443
84 Langley E., Pearson M., Faretta M., Bauer U. M., Frye R. A., Minucci S. et al. (2002) Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. EMBO J. **21:** 2383–2396
85 Kawasaki A., Matsumura I., Kataoka Y., Takigawa E., Nakajima K. and Kanakura Y. (2002) Opposing effects of PML and PML/RARα on STAT3 activity. Blood **27:** 27
86 Bies J., Markus J. and Wolff L. (2002) Covalent attachment of the SUMO-1 protein to the negative regulatory domain of the c-Myb transcription factor modifies its stability and transactivation capacity. J. Biol. Chem. **277:** 8999–9009
87 Dahle O., Andersen T., Nordgard O., Matre V., Del Sal G. and Gabrielsen O. S. (2003) Transactivation properties of c-Myb are critically dependent on two SUMO-1 acceptor sites that are conjugated in a PIASy enhanced manner. Eur. J. Biochem. **270:** 1338–1348
88 Subramanian L., Benson M. D. and Iniguez-Lluhi J. A. (2003) A synergy control motif within the attenuator domain of CCAAT/enhancer-binding protein alpha inhibits transcriptional synergy through Its PIASy-enhanced modification by SUMO-1 or SUMO-3. J. Biol. Chem. **278:** 9134–9141
89 Kim J., Cantwell C. A., Johnson P. F., Pfarr C. M. and Williams S. C. (2002) Transcriptional activity of CCAAT/enhancer-binding proteins is controlled by a conserved inhibitory domain that is a target for sumoylation. J. Biol. Chem. **277:** 38037–38044
90 Best J. L., Ganiatsas S., Agarwal S., Changou A., Salomoni P., Shirihai O. et al. (2002) SUMO-1 protease-1 regulates gene transcription through PML. Mol. Cell **10:** 843–855
91 Muratani M. and Tansey W. P. (2003) How the ubiquitin-proteasome system controls transcription. Nat. Rev. Mol. Cell Biol. **4:** 192–201
92 Baumann C. T., Ma H., Wolford R., Reyes J. C., Maruvada P., Lim C. et al. (2001) The glucocorticoid receptor interacting protein 1 (GRIP1) localizes in discrete nuclear foci that associate with ND10 bodies and are enriched in components of the 26S proteasome. Mol. Endocrinol. **15:** 485–500
93 Rivera O. J., Song C. S., Centonze V. E., Lechleiter J. D., Chatterjee B. and Roy A. K. (2003) Role of the promyelocytic leukemia body in the dynamic interaction between the androgen receptor and steroid receptor coactivator-1 in living cells. Mol. Endocrinol. **17:** 128–140
94 Gottlicher M., Heck S. and Herrlich P. (1998) Transcriptional cross-talk, the second mode of steroid hormone receptor action. J. Mol. Med. **76:** 480–489
95 Tahirov T. H., Sato K., Ichikawa-Iwata E., Sasaki M., Inoue-Bungo T., Shiina M. et al. (2002) Mechanism of c-Myb-C/EBP beta cooperation from separated sites on a promoter. Cell **108:** 57–70
96 Courey A. J. (2001) Cooperativity in transcriptional control. Curr. Biol. **11:** R250–252
97 Kirsh O., Seeler J. S., Pichler A., Gast A., Muller S., Miska E. et al. (2002) The SUMO E3 ligase RanBP2 promotes modification of the HDAC4 deacetylase. EMBO J. **21:** 2682–2691
98 Tussie-Luna M. I., Michel B., Hakre S. and Roy A. L. (2002) The SUMO ubiquitin-protein isopeptide ligase family member Miz1/PIASxβ/Siz2 is a transcriptional cofactor for TFII-I. J. Biol. Chem. **277:** 43185–43193
99 Weiskirchen R., Moser M., Weiskirchen S., Erdel M., Dahmen S., Buettner R. et al. (2001) LIM-domain protein cysteine- and glycine-rich protein 2 (CRP2) is a novel marker of hepatic stellate cells and binding partner of the protein inhibitor of activated STAT1. Biochem. J. **359:** 485–496
100 Valdez B. C., Henning D., Perlaky L., Busch R. K. and Busch H. (1997) Cloning and characterization of Gu/RH-II binding protein. Biochem. Biophys. Res. Commun. **234:** 335–340
101 Wu L., Wu H., Ma L., Sangiorgi F., Wu N., Bell J. R. et al. (1997) Miz1, a novel zinc finger transcription factor that interacts with Msx2 and enhances its affinity for DNA. Mech. Dev. **65:** 3–17
102 Zentner M. D., Lin H. H., Deng H. T., Kim K. J., Shih H. M. and Ann D. K. (2001) Requirement for high mobility group protein HMGI-C interaction with STAT3 inhibitor PIAS3 in repression of alpha-subunit of epithelial Na+ channel (alpha-ENaC) transcription by Ras activation in salivary epithelial cells. J. Biol. Chem. **276:** 29805–29814
103 Cho S. Y., Jeon J. W., Lee S. H. and Park S. S. (2000) p67 isoform of mouse disabled 2 protein acts as a transcriptional activator during the differentiation of F9 cells. Biochem. J. **3:** 645–650
104 Takahashi K., Taira T., Niki T., Seino C., Iguchi-Ariga S. M. and Ariga H. (2001) DJ-1 positively regulates the androgen receptor by impairing the binding of PIASxa to the receptor. J. Biol. Chem. **26:** 26