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REVIEW

PIAS proteins: pleiotropic interactors associated with SUMO

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Abstract The interactions and functions of protein inhibitors of activated STAT (PIAS) proteins are not restricted to the signal transducers and activators of transcription (STATs), but PIAS1, -2, -3 and -4 interact with and regulate a variety of distinct proteins, especially transcription factors. Although the majority of PIAS-interacting proteins are prone to modification by small ubiquitin-related modifier (SUMO) proteins and the PIAS proteins have the capacity to promote the modification as RING-type SUMO ligases, they do not function solely as SUMO E3 ligases. Instead, their effects are often independent of their Siz/PIAS (SP)-RING finger, but dependent on their capability to noncovalently interact with SUMOs or DNA through their SUMO-interacting motif and scaffold attachment factor-A/B, acinus and PIAS domain, respectively. Here, we present an overview of the cellular regulation by PIAS proteins and propose that many of their functions are due to their capability to mediate and facilitate SUMO-linked protein assemblies.

Keywords Protein inhibitor of activated STAT (PIAS) · Small ubiquitin-related modifier (SUMO) · SUMOylation (covalent SUMO modification) · SUMO-interacting motif (SIM) · E3 ligase · SP-RING domain · Transcription · Coregulator

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Discovery of PIAS family

The term protein inhibitor of activated STAT (PIAS) derives from the identification of two members of a mammalian protein family as inhibitors of signal transducer and activator of transcription-1 (Stat1) and -3 (Stat3) [1, 2]. After the initial characterization of PIAS1 and PIAS3 as inhibitors of STAT signaling, it has become clear that the interactions and functions of PIAS proteins are not specific for the STATs, but they have a wide role in cellular regulation, especially in the regulation of transcription (reviewed in [3]). Human PIAS proteins are encoded by four genes, *PIAS1*, *PIASx* (*PIAS2*), *PIAS3* and *PIASy* (*PIAS4*). To simplify the nomenclature, PIAS2 and PIAS4 are used instead of PIASx and PIASy, respectively, throughout this review. PIAS mRNAs are ubiquitously expressed, albeit PIAS2 and PIAS4 mRNAs are clearly more abundant in the testis than in other human tissues (http://ist.genesapiens.org/). PIAS orthologs are found in nonvertebrate animal species, plants and yeasts [4–6]. Proteins, such as non-structural maintenance of chromosomes element 2 homolog (NSE2), zinc finger MIZ domain-containing protein 1 (ZMIZ1) or ZMIZ2, that possess the PIAS RING (SP-RING) signature or its extended version but that are otherwise dissimilar are considered only very distantly related the PIAS proteins and not discussed here [7–9]. Since the regulatory role of PIAS proteins in the immune system and cytokine signaling has been recently extensively reviewed [10, 11], this review will focus on the function of PIAS proteins in cellular regulation apart from cytokine signaling. Most of

the PIAS functions are by some means linked to small ubiquitin-related modifier (SUMO) modifications. This review aims to underline SUMOylation-linked, but not necessarily E3 ligase activity-dependent, functions of the PIAS family for which the acronym might better stand for *P*leiotropic *I*nteractors *A*ssociated with *S*UMO.

SUMO modification system

Attachment of SUMO (SUMOylation) is a bulky covalent modification in which a protein, SUMO, is attached via an isopeptide linkage to lysine residues of target proteins [12, 13]. The modification has emerged as a significant regulatory mechanism in cell physiology, especially in transcription and signal transduction. In these processes, the modification can affect, in a target-specific fashion, protein's subcellular and subnuclear localization, its ability to interact with other proteins and/or its activity in transcription [12]. Mammals contain three ~100-amino acid SUMO proteins, SUMO-1, -2 and -3, that can form isopeptide linkages. SUMO-2 and SUMO-3 are nearly identical (from now on collectively called SUMO-2/3), whereas SUMO-1 is only ~50% identical with SUMO-2/3. SUMOs are similar to ubiquitin in their three-dimensional structure, but their amino acid sequences and surface charge distributions differ considerably from those of ubiquitin. In contrast to ubiquitylation, the SUMO attachment lysines are often found within a minimal consensus motif ΨKxE (where Ψ is I, L or V; x is any residue). The SUMO conjugation pathway is similar to that of ubiquitin, but cells contain distinct E1 (activating enzyme), E2 (conjugase) and E3 (ligase) activities for both processes (Fig. 1). SUMOs are first activated in an ATP-consuming fashion by SAE1 and -2 dimer and subsequently conjugated by Ubc9 [12, 13]. The presence of only one SUMO E2 enzyme contrasts with the ubiquitylation pathway, where multiple E2s participate in ubiquitylation of distinct sets of targets. SUMOylation is a highly dynamic modification that is reversed by a family of six SUMO-specific proteases (SENP1-3 and SENP5-7) (reviewed in [14, 15]). The pathway is conserved in eukaryotes, and the single SUMO genes in *Caenorhabditis elegans* and *Saccharomyces cerevisiae* are clearly essential for viability or normal embryogenesis [16–18]. Deletion of *Ubc9* in mouse results in embryonic lethality with severe abnormalities in nuclear architecture and chromosome segregation, but *Sumo-1* functions appear to be compensated for by *Sumo-2/3* [19, 20]. Moreover, mutation of the mouse *Senp1* gene indicates that a fine-tuned balance of SUMO conjugation–deconjugation is essential for normal mammalian development [21].

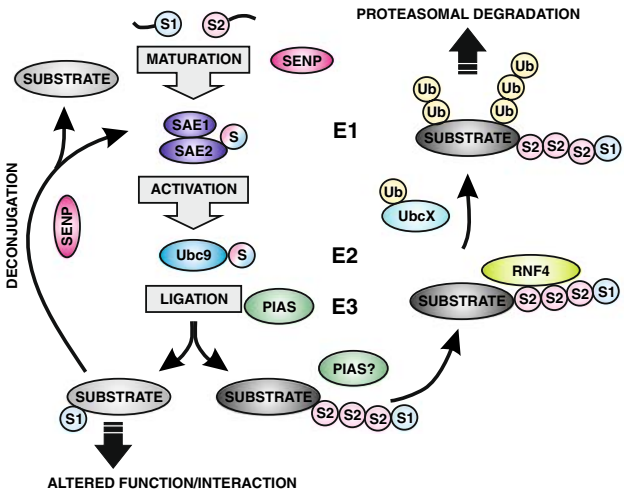

Fig. 1 SUMO modification pathway. SUMO proteins are C-terminally processed by a SUMO-specific protease (SENP) prior to attachment to the activating enzyme (SAE1/SAE2) and transfer to the conjugating E2 (Ubc9) enzyme. Ligation to target proteins is assisted by E3 ligases, such as PIAS proteins. Depending whether the target is modified by mono- or polySUMOylation, two alternative fates of the substrates are illustrated: MonoSUMOylation regulates the function, interaction or cellular localization of the substrate, whereas polySUMOylated protein is recognized by a SUMO-targeted ubiquitin ligase, such as RNF4, subsequently ubiquitylated and directed to proteasomal degradation

Modification with ubiquitin can have very different outcomes, depending on whether the substrate is conjugated by a single ubiquitin or a polymeric chain of ubiquitins [13, 22]. It is well established that addition of a polyubiquitin chain can target proteins for proteasomal degradation, whereas monoubiquitylation can have a regulatory function. In transcriptional regulation, for example, polyubiquitylation-targeted degradation of several transcription factors is important for the dynamic nature of signal-induced transcription [23], whereas specific monoubiquitylation of histone H2B and H2A regulates chromatin structure [24]. As with ubiquitin, SUMO-2/3 can form polymeric chains through a conserved lysine embedded in a consensus modification site [25]. In contrast, SUMO-1 is not thought to be modified to form a polymeric chain, but it can be linked to the end of a poly-SUMO-2/3 chain and thus terminate the chain growth. Interestingly, conjugation of SUMO-2/3, but not that of SUMO-1, has been reported to be induced in response to various stresses [26]. Different SUMO paralogs may thus have, at least in part, distinct targets and regulation.

SUMOylation and ubiquitylation have been long thought to be distinct pathways, but having antagonistic roles on a limited number of targets. Recent identification of RING domain-containing proteins Slx5/8 in *S. cerevisiae*, Rfp1/2 in *Schizosaccharomyces pombe* and RNF4 (also known as SNURF) in mammals as SUMO-targeted

ubiquitin E3 ligases (STUbLs) has directly connected SUMOylation to ubiquitylation and thereby changed our view dramatically [27]. The STUbLs use their SUMO-interacting motifs to recognize SUMO-modified proteins and direct them for degradation [28–30]. The mammalian STUbL RNF4 prefers SUMO-2-conjugated polySUMO chains over monomeric SUMO [30], and its depletion leads to accumulation of SUMO-2/3-modified proteins and chains, which resembles the situation in cells with inhibited proteasome function [31]. These findings imply an extensive cross-talk between SUMO-2/3 conjugation and the ubiquitin–proteasome system and suggest that, as in the case of ubiquitin, monomeric and polymeric chains of SUMO can have distinct biological functions.

Domain structure of PIAS proteins

The four members of the mammalian PIAS family share a high degree of sequence homology within their ~430 N-terminal amino acids. Their sizes vary from the smallest, PIAS4 with 510 amino acid residues, to the largest, PIAS1 with 651 amino acids (human sequences). Splice variants have been functionally characterized for PIAS2, -3 and -4, but not yet for PIAS1. Overall, five different domains or motifs have been identified: N-terminal SAP (scaffold attachment factor-A/B, acinus and PIAS) domain, a PINIT motif, a RING-type zinc-binding structure, a SIM (SUMO-interacting motif) and a serine/threonine-rich C-terminal region (S/T) [32–35] (Fig. 2). The most conserved of these regions are the SAP domain and the central RING-type zinc finger, whereas the C-terminal S/T-rich region is the least conserved region within the family. Interestingly, the S/T region is considerably shorter in PIAS4 than in other PIAS proteins, and a splice variant of PIAS4 is devoid of the PINIT motif [36, 37]. Splice variants PIAS2a (PIASxα, androgen receptor-interacting protein 3, ARIP3) and PIAS2b (PIASxβ, Miz1) differ from each other with regard to the length of the S/T region [38, 39], while the isoforms of PIAS3 and PIAS3L (K^+ channel-associated protein, Kchap) differ with respect to a 35-amino acid stretch between the SAP and the PINIT motif. Non-vertebrate PIAS orthologs contain essentially the same motifs and domains as their mammalian counterparts.

The SAP domain is found in many chromatin-associated proteins and is involved in sequence- or structure-specific DNA binding [40]. Interestingly, the N-terminal region of PIAS4 has been reported to bind to nuclear matrix attachment region (MAR) DNA, playing a role in nuclear architecture and often residing close to enhancer regions [37, 41]. The three-dimensional structure of the PIAS1 N-terminal domain (amino acids 1–65) revealed the presence of a four-α-helix bundle that has a strong affinity towards A–T rich DNA but can also recognize proteins, such as p53 [42]. Within the SAP domain, PIAS proteins harbor an LxxLL motif that is often found in proteins interacting with nuclear receptors [43, 44]. C-terminal to the SAP domain, there is the PINIT motif that, at least for PIAS3L, has been shown to be essential for the nuclear retention [34].

Between the SP-RING and the S/T-rich regions, PIAS proteins harbor the SIM. The SIM was initially identified in the PM-Scl75 protein and defined as a sequence $\psi\psi$xSxS/Taa (ψ, hydrophobic amino acid; x, any amino acid; a, acidic amino acid) [33]. The motif interacted noncovalently with SUMO proteins, and its deletion abolished the

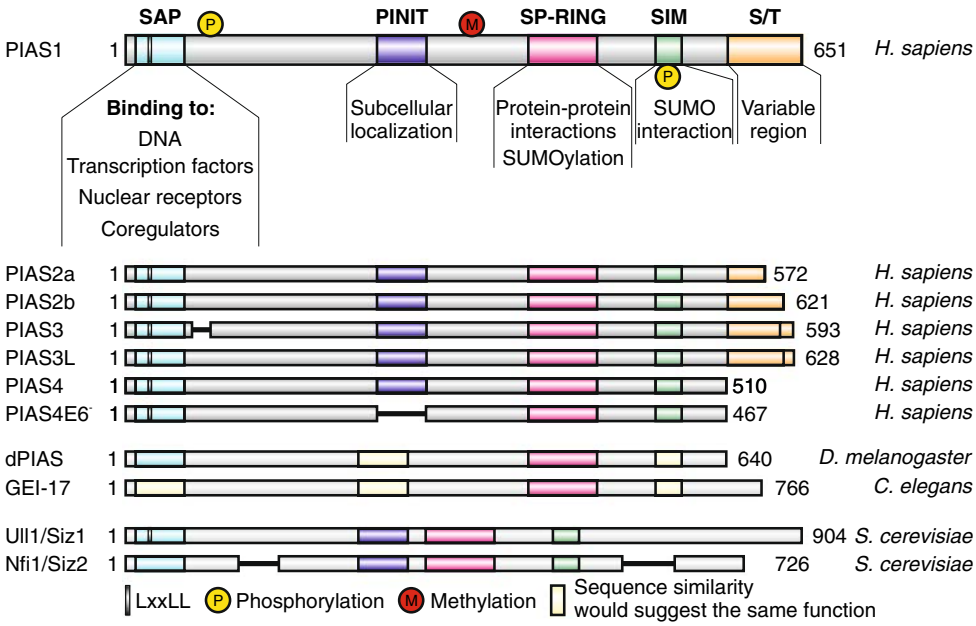

Fig. 2 Schematic structures of PIAS proteins. The domain structures and post-translational modifications of the different human PIAS proteins are illustrated. Orthologous PIAS proteins from *S. cerevisiae*, *C. elegans* and *D. melanogaster* are also shown. *Numbers* on the right depict the amino acids in each PIAS protein

interaction. Later, the motif was shown to be more variable and have a less stringent consensus sequence, consisting of an essential hydrophobic core of $\psi x\psi\psi$ or $\psi\psi x\psi$ (where ψ is often V or I) usually next to a C-terminal cluster of negatively charged residues. Interestingly, the acidic cluster is considerably longer in PIAS4 than in other members of the PIAS family (16 E/D residues vs. 4 residues) [45–48]. SIMs are found in a large number of nuclear proteins, typically in those with known roles in SUMOylation, including the STUbL RNF4 [30], many of which can associate with promyelocytic leukemia (PML) bodies [49, 50]. Interestingly, a lot of the physiological consequences of SUMO modifications seem to be mediated by 'effector' proteins that recognize SUMO through SIMs.

In the central part of PIAS proteins, there is a cysteine-rich region forming a putative RING-type of zinc finger termed Siz/PIAS RING (SP-RING) [32]. The classical RING domain contains eight zinc-binding cysteine or histidine residues [51]. The residues can coordinate two zinc ions in an interleaved fashion, creating a globular domain that can mediate protein–protein interactions. Many RING proteins mediate E2 enzyme-dependent attachment of ubiquitin to target proteins, i.e., function as ubiquitin E3 ligases. The ubiquitin E3s bind substrates with their RING directly interacting with the E2s and thereby govern the specificity of ubiquitylation. They are thought to facilitate the transfer of ubiquitin from the E2 to the substrate, probably without formation of covalent intermediates. The SP-RING motif is suggested to have a similar fold as in the RING finger, although it lacks two zinc-coordinating cysteines found in the classical RING domain.

PIAS proteins as SUMO E3 ligases

In contrast to ubiquitylation, where the E3 ligase activities are mandatory for a specific substrate modification to occur [51], SUMO E1 and E2 enzymes are sufficient for covalent attachment of SUMO to specific substrates, at least in vitro. Ubc9 is able to bind via its catalytic cleft to the SUMOylation consensus motifs present in many substrates and apparently donate SUMO directly to the substrates. Due to the apparent lack of need of E3 activity for SUMOylation in vitro, the existence of SUMO E3 ligases was initially doubted. However, it is now clear that several, structurally surprisingly different types of proteins can exhibit E3-type ligase activity in SUMOylation reactions [12]. In addition to PIAS and other SP-RING-containing proteins [5, 52–56], nuclear pore protein RanBP2 (Nup358) [57, 58] and polycomb protein 2 (Pc2) [59], which are both non-RING proteins, and TOPORS (topoisomerase I-binding, arginine/serine-rich) [60], which harbors a RING domain, all display SUMO E3 ligase activity. Interestingly, the TOPORS appears to be capable of functioning also as a ubiquitin ligase [61]. Also histone deacetylase 4 (HDAC4) has been recently listed as a SUMOylation-enhancing protein [62–65]. Compared to the PIAS proteins, these other proteins display SUMO ligase-like activity only on a limited number of substrates. In the case of Pc2, this may derive from a restricted subnuclear localization and mobility. Moreover, it is not completely clear whether Pc2 and HDAC4 function as bona fide SUMO ligases or whether they stimulate SUMO modifications merely as 'stoichiometric interaction partners' [12].

A large number of the SUMOylated proteins identified to date are directly recognized at least by one member of the PIAS family. The SP-RING domain interacts with the SUMO conjugase Ubc9 and is essential for the PIAS E3 function both in vivo and in vitro [5, 37, 54, 56, 66]. However, PIAS4-mediated SUMOylation of Yin Yang1 (YY1) has been reported to be independent on the SP-RING integrity [67]. Since the SP-RING resembles the RING domain, the mechanism of E3 function of PIAS proteins is thought to be similar to that of the RING-type ubiquitin ligases [51]. They are thought to promote the modification by acting as adaptors that stabilize the interaction between the SUMO thioester-loaded Ubc9 and the acceptor protein. Analysis of a (non-RING) RanBP2 E3 ligase domain co-crystallized with Ubc9 and a RanGAP1-SUMO-1 conjugate revealed that the E2 is the central component interacting with the three other proteins in the complex and that the E3 ligase domain contacts both SUMO and Ubc9. These and other biochemical data support a model in which RanBP2 acts as an E3 by binding both SUMO and Ubc9 to position the SUMO-E2-thioester in an optimal orientation to enhance conjugation [68, 69]. The PIAS SIM does not seem to be needed for the E3 ligase activity; for example, in the case of PIAS2a, deletion of the SIM actually leads to augmented E3 activity [54]. In addition to the SP-RING, the PINIT domain plays a role in the ligase function with some substrates [70, 71]. Even though both of the latter domains appear to contribute to substrate selectivity [36, 54, 70, 71], the SUMO target lysine seems to be mainly specified by the Ubc9. However, crystal structures of PIAS-containing SUMOylation complexes are needed to expose the exact mechanism by which PIAS proteins facilitate SUMOylation processes.

Unlike in ubiquitylation cascades, where target discrimination is governed by an interplay between hundreds of different E3 activities and a number of distinct E2 enzymes, the specificity of SUMOylation is not likely to be specified solely by the Ubc9 and the SUMO E3 activities known thus far. The increase in the number of SUMO acceptors showing conjugation to sites that considerably deviate from the SUMOylation consensus also clearly hints that additional activities or mechanisms are needed to

ensure the specificity [72–74]. In some cases, SUMO substrates themselves may actually act as E3s via an intramolecular reaction [75, 76]. Even though the number of SUMO E3 ligases looks to be gradually increasing, it is evident that target site discrimination involves additional determinants of specificity, such as stoichiometric interacting partners or extended SUMOylation motifs, like phosphorylation-dependent ones (PDSMs) regulated by extracellular signals [77–79]. The roles of PIAS proteins with PDSM-harboring substrates have not been systematically elucidated.

There is only limited information about SUMO paralog selectivity of PIAS proteins or their capability to promote formation of SUMO-2/3 polymers. In the case of cardiac progenitor Nkx2.5, PIAS1, PIAS2 and PIAS4 mediated SUMO-1 attachment to the substrate, whereas only PIAS2 was able to conjugate SUMO-2 to the protein [80]. Interestingly, the PINIT domain seems to dictate the capability of PIAS4 to promote modification of cellular proteins by different SUMO isoforms; the PIAS4 devoid of the PINIT domain is still able to stimulate modification by SUMO-2, while the capability to enhance modification by SUMO-1 is apparently lost [36]. Even though the SIMs of PIAS proteins do not generally seem to discriminate between SUMO paralogs, PIAS proteins show selectivity in their own SUMOylations in intact cells; e.g., PIAS2a is preferentially modified by SUMO-1, whereas PIAS3 is equally modified by SUMO-1 and -2 (S. Kaikkonen, unpublished results). However, the biological significance of these intra- or intermolecular 'automodifications' is currently not known. SUMOylation of Ubc9 has interestingly been shown to influence its target discrimination [72]. All in all, due to the significant sequence homology within the PIAS family and their often redundant interactions, it is likely that other post-translational modifications and/or their local concentrations largely govern their specificity in vivo.

PIAS proteins in cellular regulation

Although there are examples of cytoplasmic regulation in which PIAS proteins are involved, such as SUMOylation of septins by PIAS ortholog Siz1 in yeast bud neck, and functional interaction of PIAS3 with metabotropic glutamate receptor-8 and voltage-gated potassium channel Kv1.5 [56, 81, 82], the majority of thus far reported interactions of PIAS proteins occurred with transcription factors or other proteins linked to nuclear regulation (Fig. 3). The bulk of these interactions have been discovered through yeast 2-hybrid screens. These screens also often revealed more than one PIAS family member and other components of the SUMOylation machinery, such as Ubc9 and/or SUMOs, probably reflecting the tendency of the PIAS-interacting partners towards SUMOylation. Since not all of these interactions have been confirmed in other experimental systems than yeast, some of them may represent false positives that derive from binding of the PIAS SIM to the SUMO residue in a conjugate. However, in most cases, the PIAS–partner interactions have been validated by using additional assays and proven to be functionally relevant.

SP-RING-dependent mechanisms

According to an E3 SUMO ligase model, PIAS proteins promote SUMOylation of their interaction partners, subsequently altering the function, interaction or localization of the interaction partner (Fig 4a–c). Even though this type of mechanism does not always explain the effects of PIAS proteins in transcriptional regulation, there are notable cases with several PIAS proteins. For example, induction of cellular senescence requires the E3 activity of PIAS4, which stimulates SUMOylation and transcriptional activity of p53 [83] (Fig. 4a). PIAS3 interacts with and promotes SUMOylation of the photoreceptor-specific transcription factor Nr2e3 when bound to the promoters of cone-specific genes, which converts the factor to a transcriptional repressor [84]. PIAS1-mediated SUMOylation of p73 in turn targets the p73 to nuclear matrix, subsequently repressing its transcriptional activity [85] (Fig. 4b). Even though PIAS4 promotes SUMOylation of LEF1 (lymphoid enhancer-binding factor 1), its repressive activity is not dependent on the SUMOylation of LEF1, but mediated by targeting of LEF1 to subnuclear bodies [37]. However, since the integrity of PIAS4 SP-RING is essential for the targeting, PIAS4-facilitated SUMOylation of a currently unknown nuclear body protein may be involved in the process. Similarly, SP-RING-dependent interaction between PIAS4 and FIP200 (focal adhesion kinase family-interacting protein of 200 kDa) results in recruitment of the FIP200, not within the nucleus, but from the cytoplasm to the nucleus, even though the FIP200 is not SUMOylated [86].

SUMO modifications of nuclear receptors (NR), androgen receptor (AR, NR3C4), glucocorticoid receptor (GR, NR3C1), estrogen receptor (ER) α (NR3A1), mineralocorticoid receptor (MR, NR3C2) and peroxisome proliferator-activated receptor (PPAR) γ (NR1C3) have been correlated with impaired transcriptional activation on promoters containing compound binding sites [87–91]. These receptors all interact with PIAS proteins, most often with PIAS1. Overexpression of PIAS proteins enhances SUMOylation of NRs without influencing their subnuclear localizations. In contrast to what one would assume, overexpressed PIAS proteins can in many promoter contexts coactivate these transcription factors instead of repressing

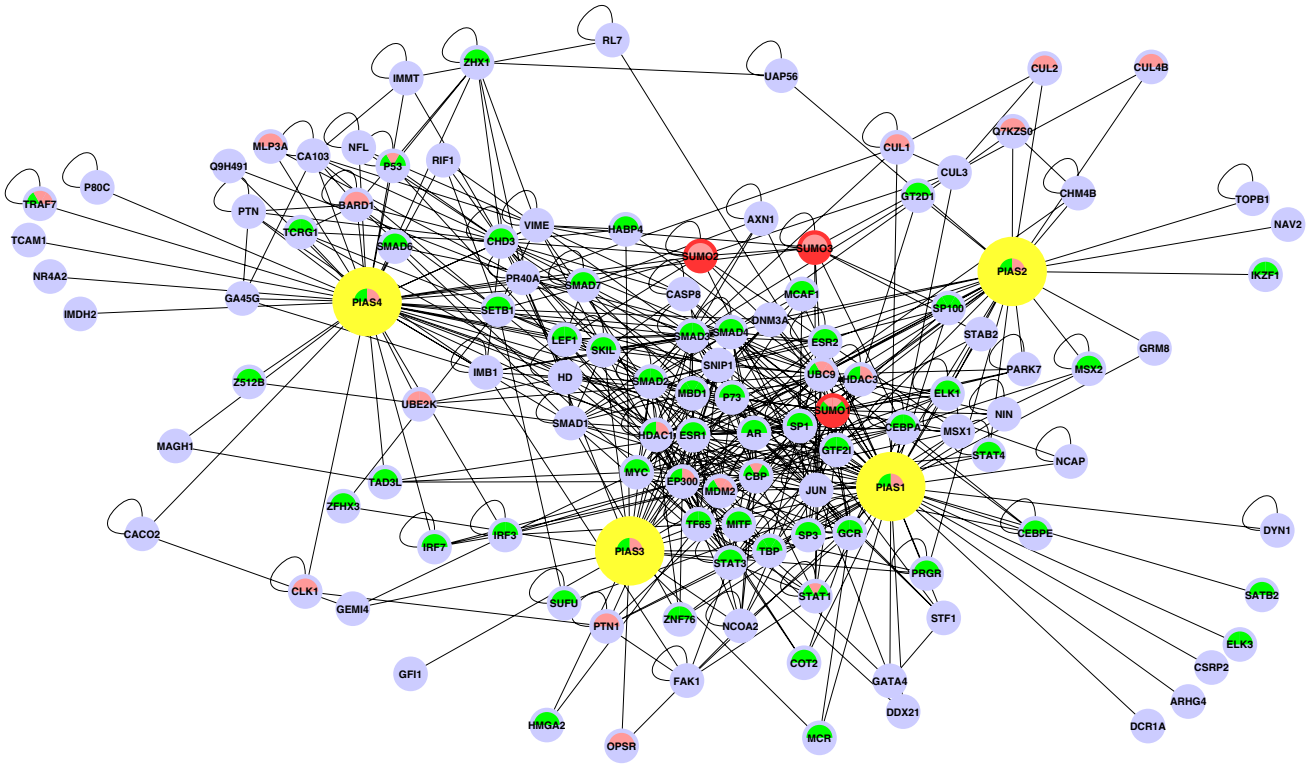

Fig. 3 Network of experimentally observed protein–protein interactions between the four human PIAS proteins and their first interacting partners. *Yellow* and *red* nodes represent the PIAS proteins and the three SUMO proteins, respectively. Proteins that participate in the biological processes related to post-translational modifications and transcription have been marked with *pink* and *green* sectors, respectively. The graph was created using the APID2NET plugin [132] included in the Cytoscape software [133], which integrates BIND [134], BioGrid [135], DIP [136], HPRD [137], IncAct [138] and MINT [139] interaction databases. The graph is based on 120 proteins and 596 protein–protein interactions. The interacting proteins are listed in Supplementary Table 1

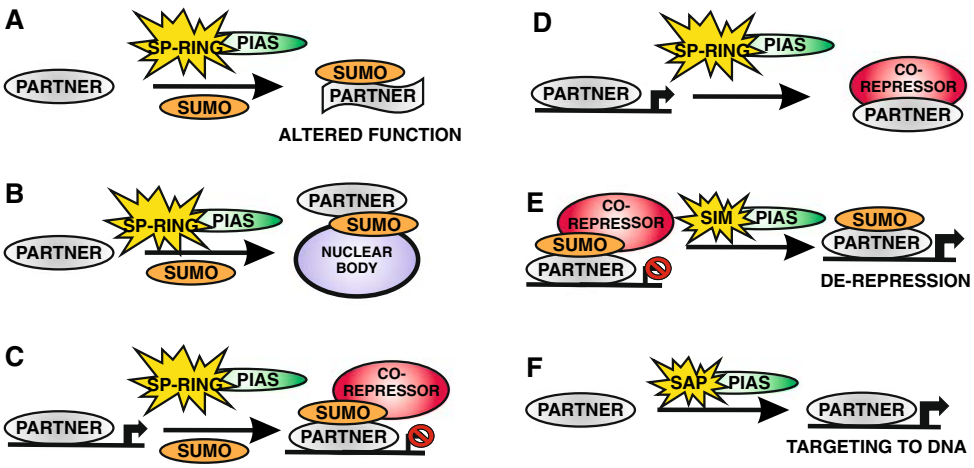

Fig. 4 Functional modes of PIAS proteins in transcriptional regulation. Schematic models for different SP-RING-dependent mechanisms (**a**–**d**) and SIM- or SAP-dependent mechanisms (**e** and **f**, respectively) utilized by PIAS proteins. See text for examples of PIAS-interacting partners and details

them [54, 89–92]. Interestingly, the activating effects of, for example, PIAS1 on AR- or GR-dependent transcription are largely dependent both on the SP-RING and the SIM and also require intact SUMOylation sites in the receptors [54, 93] (S. Kaikkonen, unpublished results). The mechanism underlying the latter phenomenon is not well understood, but it may in part relate to the ability of PIAS proteins to reverse repression that is caused by SUMO attached to a transcription factor (see [94]). In the case of STAT1, STAT3 and NF-κB, PIAS1 and PIAS3 exert their repressive action by blocking the DNA-binding of the factors without influencing their SUMOylation [1, 2, 95] (Fig. 4d). The E3 ligase function of PIAS1 is however essential for its ability to repress transcription mediated by NF-κB and STAT1 [96], suggesting that SUMOylation of other proteins is needed for the transcriptional repression to occur.

"Classic" coactivator and corepressor proteins have also been implicated in the function of PIAS proteins. PIAS3 is reported to activate transcriptional responses of Smad (mothers against decapentaplegic homolog) proteins through forming a complex with Smads and coactivator p300/CBP (cAMP response element binding protein-binding protein) [97]. PIAS proteins, except for PIAS4, may also enhance steroid receptor-dependent transcription through an SP-RING-mediated interaction and SUMOylation of coactivator protein GRIP1/SCR2 (glucocorticoid receptor-interacting protein 1/steroid receptor coactivator 2) [93]. In contrast, PIAS1-promoted SUMOylation of PPARγ can target the receptor to nuclear receptor corepressor (NCoR)-histone deacetylase-3 (HDAC3) complexes on inflammatory gene promoters, resulting in transcriptional repression [98] (Fig. 4c).

SIM- or SAP domain-dependent mechanisms

Interestingly, PIAS proteins, such as PIAS2a, seem to be also capable of reversing SUMO-mediated repression. Activation of ETS-domain transcription factor Elk-1 by PIAS2a requires the integrity of the SIM, but not that of the SP-RING, and is dependent on the SUMOylation sites of Elk-1 [94] (Fig. 4e). The de-repression process is linked to alterations in the histone acetylase and deacetylase activities associated with Elk-1. PIAS2 proteins and PIAS4 appear to stimulate also the activity of neutrophil-specific transcription factor C/EBPε (CCAAT/enhancer-binding protein ε) and that of GATA-binding protein 2 (GATA-2), respectively, through a similar de-repression mechanism [99, 100].

As mentioned above, the functional outcome of an interaction with a PIAS protein does not necessarily require SUMOylation of the interacting partner, but in many cases derives from the changed subcellular or subnuclear localization of the interacting partner. Moreover, the latter effects are often dependent on the SAP domain instead of the SP-RING. For example, the SAP domain-dependent interaction of PIAS1 with Msx1 (Msh homeobox 1-like protein) controls the homeoprotein's DNA-binding activity by altering its subnuclear localization and proximity to target genes [101] (Fig. 4f). This is needed for the Msx1 to function as an inhibitor of myoblast differentiation through repression of myogenic regulatory genes. Even though PIAS2a enhances SUMO modification of ETS family member FLI-1, its repressive activity on FLI-1 is independent of SUMOylation, but dependent on the SAP domain, and the repression involves nuclear relocalization of FLI-1 [102]. All three PIAS family members repress the transcriptional activity of C/EBPδ (CCAAT/enhancer-binding protein δ), with the PIAS4 being the most potent PIAS [103]. The repression by PIAS4 is independent of its ligase activity or the SUMOylation status of C/EBPδ, but again requires the SAP domain of PIAS4 and involves translocation of C/EBPδ from nuclear foci to the nuclear periphery [103]. Similarly, PIAS4 interacts via its SAP domain with POU domain transcription factor Oct4 and acts as a potent inhibitor of Oct4-mediated transcriptional activation without enhancing SUMOylation of Oct4, but sequestering it from the vicinity of Cajal bodies and splicing speckles to the nuclear periphery [104].

Regulation of PIAS proteins by post-translational modifications

In addition to SUMOylation, PIAS proteins have been recently shown to be modified by other post-transcriptional modifications and exhibit crosstalk with other signaling pathways. PIAS2a and -b are direct targets of p38 MAP kinase signaling and are phosphorylated at S113 and S116 [105]. Interestingly, these sites are not conserved in the other PIAS family members that also do not show signs of stress-activated phosphorylation. The MAPK-catalyzed phosphorylation of PIAS2a or -b does not have an apparent effect on their SUMO ligase activity or autoSUMOylation. Interestingly, however, the activating and repressing effects of PIAS2a on the Elk-1-dependent transcription are, at least in part, determined via its p38 MAPK-catalyzed phosphorylation. In addition to physical and chemical stress, the MAPK pathway is activated by certain growth factors and proinflammatory cytokines, and it is involved in the regulation of various cellular processes, such as growth, inflammation, differentiation, progression of the cell cycle and apoptosis (reviewed in [106]). The MAPK signaling mediated phosphorylation of PIAS2 thus links the SUMOylation machinery to several types of extracellular signals and regulatory processes. Phosphorylation of yeast PIAS orthologs Siz1 and Siz2 has also been reported [5, 56].

Interestingly, the SIM of PIAS proteins harbors three potential phosphorylation sites for the S/T kinase CK2. NMR analyses of a monophosphorylated peptide corresponding to the SIM of PIAS2 implied that the phosphorylation favors binding to SUMO-1 over SUMO-2 and may modulate the spatial orientation of SUMO-SIM interactions [48]. Recently, CK2 was indeed reported to phosphorylate S466, S467 and S468 of PIAS1 adjacent to the hydrophobic core of the SIM. The phosphorylation governed binding of SUMO and SUMO conjugates to PIAS1 in intact cells, albeit without an apparent SUMO paralog selectivity [107]. In keeping with the nonessential role of SIM for the E3 function, the CK2-catalyzed phosphorylation had no apparent effect on the function, but

interestingly regulated the ability of PIAS1 to function as a transcriptional coregulator.

PIAS1 becomes rapidly phosphorylated on S90 close to the SAP domain in response to various inflammatory stimuli, with IKKα mediating the phosphorylation [95]. The S90 phosphorylation is essential for the capability of PIAS1 to repress transcription mediated by NF-κB and STAT1, and the E3 ligase function (i.e., intact SP-RING domain) of PIAS1 is needed for the phosphorylation. This may be due to SUMOylation of PIAS1 itself or other proteins involved in the process. Interestingly, the serine residue is conserved in PIAS2 and PIAS3, but it is currently not known if they are also targeted by IKKα. PIAS1 was recently also found to be dimethylated on R303, located between the PINIT motif and SP-RING domain, by protein arginine methyltransferase 1 (PRMT1) in response to interferon [108], which resulted in repression of STAT1 signaling. It is possible that also PIAS2 and PIAS3 are targeted by PRMT1, since the region encompassing the R residue is fully conserved in them.

Relatively little is known about the regulation of PIAS protein levels, but both PIAS1 and PIAS4 have been reported to be by ubiquitylated by ubiquitin E3 ligases hSiah2 (seven in absentia homologue 2) and Trim32, respectively, and thereby targeted for degradation [109, 110]. The hSiah2 also interacts with PIAS2 and PIAS3, and overexpression of hSiah2 influences their cellular levels.

Studies in model organisms

Knowledge about the biological roles of PIAS proteins is mainly based on experiments in mammalian or yeast cell cultures. *S. cerevisiae* contains two PIAS orthologs Siz1 (SAP and Miz1)/Ull1 (ubiquitin-like ligase 1) and Siz2/Nfi1 (neck filament interacting 1) [5]. Genetic studies indicate that they have some overlapping functions and are required for most SUMOylation in *S. cerevisiae*. In spite of growth defects and problems with chromosome maintenance after disruption of the Siz1 and the Siz2 function, their genes are not essential for the yeast [70, 111, 112]. *S. pombe* harbors only one PIAS ortholog Pli1, which is required for the majority of SUMOylation in the organism. Similar to the situation in baker's yeast, deletion of the fission yeast PIAS gene results in no dramatic growth defects, but milder defects in genome and chromosome stability, including increased telomere length and heterochromatin silencing [113, 114]. The other SUMO E3 ligase Nse2 (Mms21 in *S. cerevisiae*) may be able to compensate for the loss of PIAS ligase function [115, 116].

Multicellular organisms *Drosophila melanogaster* and *C. elegans* both contain only one PIAS ortholog. Interestingly, *Drosophila* PIAS protein dPIAS (Zimp) is encoded by the *Su(var)2–10* (*suppressor of variegation 2–10*) locus which deletion leads to severe abnormalities in the condensation and segregation of chromosomes and embryonic lethality [4, 117]. The result suggests that the dPIAS plays a role in heterochromatin-induced gene silencing. A conditional overexpression approach in turn indicates that the proper dose of dPIAS is critical for normal eye and blood cell development in *D. melanogaster* [118]. *C. elegans* PIAS ortholog GEI-17 interacts with the T-box factor TBX-2 that is required for normal pharyngeal development [119]. Depletion of GEI-17 by RNAi resulted in defects in the pharyngeal development and retarded the growth and development of the nematode. GEI-17 has also been shown to be important for the embryonic DNA damage response in *C. elegans* [120]. It is needed for the protection of POLH-1 translesion synthesis DNA polymerase from destruction until it has executed its task during the replication of damaged DNA in the nematode embryos [121]. Interestingly, the sole PIAS ortholog of *Arabidopsis thaliana* AtSIZ1 regulates in addition to stress responses, such as cold tolerance, growth and flowering of the plant [122, 123].

SUMOylation in general is important for normal mammalian development, as deletion of *Ubc9* leads to embryonic lethality caused by severe disorganization in nuclear architecture and abnormalities in chromosomal segregation [19]. Data of *Pias3* knockouts are not available, but results from a study employing electroporation-mediated overexpression Pias3 in developing mouse retina suggest that Pias3-dependent SUMOylation of a photoreceptor-specific transcription factor is involved in rod photoreceptor development [84]. Knockout mouse models have been constructed for *Pias1*, *Pias2* and *Pias4*, but no clear defects in overall cellular SUMOylation have been observed in the single gene mutants. Mice that lack Pias4 are phenotypically normal, but they have a modest reduction in their interferon and Wnt signaling [36, 124]. Interestingly, the cellular senescence response in PIAS4$^{-/-}$ murine embryo fibroblasts (MEFs) is also significantly delayed compared to PIAS4$^{+/+}$ MEFs [125]. *Pias1*$^{-/-}$ mice appear at a frequency lower than the expected Mendelian ratio, and especially males are smaller than their wild-type littermates [126]. The mice show enhanced immune responses to viral or microbial challenges, which is consistent with a negative regulatory role of PIAS in interferon signaling. Despite of the abundance of Pias2 in the rodent testis [127], *Pias2*$^{-/-}$ mice are fertile and have qualitatively normal spermatogenesis, albeit their sperm counts and testes are reduced compared to their wild-type littermates [128]. In contrast to the mild phenotypes of the single *Pias* knockouts, *Pias1*$^{-/-}$*Pias4*$^{-/-}$ embryos died before day 11.5 [129]. Taken together, these data imply a

considerable biological overlap in the functions of mammalian PIAS proteins.

Future challenges

Modification of proteins with mono- or polyubiquitin is already established as an important, multifaceted means of regulating cell growth and physiology [22, 130]. Also mono- and polySUMOylation are emerging as modifications having distinct biological consequences. Given the large number of proteins involved in transcription, chromatin-packaging, DNA replication/repair, apoptosis, tumorigenesis and neuronal inclusions that can be targeted both by ubiquitin and SUMOs [12, 13, 82, 131], the role of PIAS proteins in the emerging cross-talk between ubiquitin and SUMO pathways emphasizes the importance of further studies. In transcriptional activation and repression, PIAS proteins seem to employ several mechanisms. Even though PIAS proteins are more versatile than mere SP-RING-type SUMO ligases, their regulatory functions are still in many cases linked to SUMO, frequently through their noncovalent SUMO-tethering function. Although the direct interaction partners of PIAS proteins may not always be subjected to SUMOylation, the PIAS proteins may promote SUMO modifications of other targets in the same protein complexes. Alternatively, the complex formation may be facilitated via the SAP domain-mediated interaction with the chromatin. The latter domain appears to be particularly important for the ability of PIAS proteins to regulate subcellular and subnuclear localization of their interaction partners. Knockout mouse models have been constructed for *Pias1*, *Pias2* and *Pias4*, but no clear defects in overall cellular SUMOylation have been observed in the single gene mutants, which is consistent with the possibility that PIAS proteins possess overlapping functions. On the other hand, PIAS family members seem to have the capability to interact with each other and form multicomponent PIAS nuclear scaffolds [35]. Silencing of the PIAS function in model organisms, such as *C. elegans*, containing only one PIAS-type SUMO ligase and affinity-tagged SUMO as a transgene should enable elucidation of the role of the PIAS function in mono- and polySUMOylation reactions at the whole proteome level. Moreover, studies utilizing chromatin immunoprecipitations coupled with massively parallel sequencing (ChIP seq) can provide us with unbiased views of PIAS interactions with the chromatin.

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References

1. Chung CD, Liao J, Liu B, Rao X, Jay P, Berta P, Shuai K (1997) Specific inhibition of Stat3 signal transduction by PIAS3. Science 278:1803–1805
2. Liu B, Liao J, Rao X, Kushner SA, Chung CD, Chang DD, Shuai K (1998) Inhibition of Stat1-mediated gene activation by PIAS1. Proc Natl Acad Sci USA 95:10626–10631
3. Schmidt D, Muller S (2003) PIAS/SUMO: new partners in transcriptional regulation. Cell Mol Life Sci 60:2561–2574
4. Hari KL, Cook KR, Karpen GH (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
5. Johnson ES, Gupta AA (2001) An E3-like factor that promotes SUMO conjugation to the yeast septins. Cell 106:735–744
6. Miura K, Rus A, Sharkhuu A, Yokoi S, Karthikeyan AS, Raghothama KG, Baek D, Koo YD, Jin JB, Bressan RA, Yun DJ, Hasegawa PM (2005) The *Arabidopsis* SUMO E3 ligase SIZ1 controls phosphate deficiency responses. Proc Natl Acad Sci USA 102:7760–7765
7. Beliakoff J, Sun Z (2006) Zimp7 and Zimp10, two novel PIAS-like proteins, function as androgen receptor coregulators. Nucl Recept Signal 4:e017
8. Rodriguez-Magadan H, Merino E, Schnabel D, Ramirez L, Lomeli H (2008) Spatial and temporal expression of Zimp7 and Zimp10 PIAS-like proteins in the developing mouse embryo. Gene Expr Patterns 8:206–213
9. Potts PR, Yu H (2007) The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins. Nat Struct Mol Biol 14:581–590
10. Shuai K, Liu B (2005) Regulation of gene-activation pathways by PIAS proteins in the immune system. Nat Rev Immunol 5:593–605
11. Shuai K (2006) Regulation of cytokine signaling pathways by PIAS proteins. Cell Res 16:196–202
12. Geiss-Friedlander R, Melchior F (2007) Concepts in sumoylation: a decade on. Nat Rev Mol Cell Biol 8:947–956
13. Kerscher O, Felberbaum R, Hochstrasser M (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. Annu Rev Cell Dev Biol 22:159–180
14. Hay RT (2007) SUMO-specific proteases: a twist in the tail. Trends Cell Biol 17:370–376
15. Kim JH, Baek SH (2009) Emerging roles of desumoylating enzymes. Biochim Biophys Acta 1792:155–162
16. Jones D, Crowe E, Stevens TA, Candido EP (2002) Functional and phylogenetic analysis of the ubiquitylation system in Caenorhabditis elegans: ubiquitin-conjugating enzymes, ubiquitin-activating enzymes, and ubiquitin-like proteins. Genome Biol 3:RESEARCH0002
17. Broday L, Kolotuev I, Didier C, Bhoumik A, Gupta BP, Sternberg PW, Podbilewicz B, Ronai Z (2004) The small ubiquitin-like modifier (SUMO) is required for gonadal and uterine-vulval morphogenesis in *Caenorhabditis elegans*. Genes Dev 18:2380–2391
18. Johnson ES, Schwienhorst I, Dohmen RJ, Blobel G (1997) The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. EMBO J 16:5509–5519
19. Nacerddine K, Lehembre F, Bhaumik M, Artus J, Cohen-Tannoudji M, Babinet C, Pandolfi PP, Dejean A (2005) The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev Cell 9:769–779
20. Zhang FP, Mikkonen L, Toppari J, Palvimo JJ, Thesleff I, Janne OA (2008) Sumo-1 function is dispensable in normal mouse development. Mol Cell Biol 28:5381–5390

21. Yamaguchi T, Sharma P, Athanasiou M, Kumar A, Yamada S, Kuehn MR (2005) Mutation of SENP1/SuPr-2 reveals an essential role for desumoylation in mouse development. Mol Cell Biol 25:5171–5182
22. Huang TT, D'Andrea AD (2006) Regulation of DNA repair by ubiquitylation. Nat. Rev. Mol. Cell Biol. 7:323–334
23. Dennis AP, O'Malley BW (2005) Rush hour at the promoter: how the ubiquitin–proteasome pathway polices the traffic flow of nuclear receptor-dependent transcription. J Steroid Biochem Mol Biol 93:139–151
24. Weake VM, Workman JL (2008) Histone ubiquitination: triggering gene activity. Mol. Cell 29:653–663
25. Vertegaal AC (2007) Small ubiquitin-related modifiers in chains. Biochem Soc Trans 35:1422–1423
26. Vertegaal AC, Andersen JS, Ogg SC, Hay RT, Mann M, Lamond AI (2006) Distinct and overlapping sets of SUMO-1 and SUMO-2 target proteins revealed by quantitative proteomics. Mol. Cell. Proteomics 5:2298–2310
27. Perry JJ, Tainer JA, Boddy MN (2008) A SIM-ultaneous role for SUMO and ubiquitin. Trends Biochem Sci 33:201–208
28. Prudden J, Pebernard S, Raffa G, Slavin DA, Perry JJ, Tainer JA, McGowan CH, Boddy MN (2007) SUMO-targeted ubiquitin ligases in genome stability. EMBO J 26:4089–4101
29. Sun H, Leverson JD, Hunter T (2007) Conserved function of RNF4 family proteins in eukaryotes: targeting a ubiquitin ligase to SUMOylated proteins. EMBO J 26:4102–4112
30. Tatham MH, Geoffroy MC, Shen L, Plechanovova A, Hattersley N, Jaffray EG, Palvimo JJ, Hay RT (2008) RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation. Nat Cell Biol 10:538–546
31. Schimmel J, Larsen KM, Matic I, van Hagen M, Cox J, Mann M, Andersen JS, Vertegaal AC (2008) The ubiquitin–proteasome system is a key component of the SUMO-2/3 cycle. Mol. Cell. Proteomics 7:2107–2122
32. Hochstrasser M (2001) SP-RING for SUMO: new functions bloom for a ubiquitin-like protein. Cell 107:5–8
33. Minty A, Dumont X, Kaghad M, 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
34. Duval D, Duval G, Kedinger C, Poch O, Boeuf H (2003) The 'PINIT' motif, of a newly identified conserved domain of the PIAS protein family, is essential for nuclear retention of PIAS3L. FEBS Lett 554:111–118
35. Tan JA, Hall SH, Hamil KG, Grossman G, Petrusz P, French FS (2002) Protein inhibitors of activated STAT resemble scaffold attachment factors and function as interacting nuclear receptor coregulators. J Biol Chem 277:16993–17001
36. Wong KA, Kim R, Christofk H, Gao J, Lawson G, Wu H (2004) Protein inhibitor of activated STAT Y (PIASy) and a splice variant lacking exon 6 enhance sumoylation but are not essential for embryogenesis and adult life. Mol Cell Biol 24:5577–5586
37. Sachdev S, Bruhn L, Sieber H, Pichler A, Melchior F, Grosschedl R (2001) PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. Genes Dev 15:3088–3103
38. Moilanen AM, Karvonen U, Poukka H, Yan W, Toppari J, Janne OA, Palvimo JJ (1999) A testis-specific androgen receptor coregulator that belongs to a novel family of nuclear proteins. J Biol Chem 274:3700–3704
39. Wu L, Wu H, Ma L, Sangiorgi F, Wu N, Bell JR, Lyons GE, Maxson R (1997) Miz1, a novel zinc finger transcription factor that interacts with Msx2 and enhances its affinity for DNA. Mech Dev 65:3–17
40. Aravind L, Koonin EV (2000) SAP—a putative DNA-binding motif involved in chromosomal organization. Trends Biochem Sci 25:112–114
41. Kipp M, Gohring F, Ostendorp T, van Drunen CM, van Driel R, Przybylski M, Fackelmayer FO (2000) SAF-Box, a conserved protein domain that specifically recognizes scaffold attachment region DNA. Mol Cell Biol 20:7480–7489
42. Okubo S, Hara F, Tsuchida Y, Shimotakahara S, Suzuki S, Hatanaka H, Yokoyama S, Tanaka H, Yasuda H, Shindo H (2004) NMR structure of the N-terminal domain of SUMO ligase PIAS1 and its interaction with tumor suppressor p53 and A/T-rich DNA oligomers. J Biol Chem 279:31455–31461
43. Liu B, Gross M, ten Hoeve J, Shuai K (2001) A transcriptional corepressor of Stat1 with an essential LXXLL signature motif. Proc Natl Acad Sci USA 98:3203–3207
44. Heery DM, Kalkhoven E, Hoare S, Parker MG (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387:733–736
45. Song J, Durrin LK, Wilkinson TA, Krontiris TG, Chen Y (2004) Identification of a SUMO-binding motif that recognizes SUMO-modified proteins. Proc Natl Acad Sci USA 101:14373–14378
46. Song J, Zhang Z, Hu W, Chen Y (2005) Small ubiquitin-like modifier (SUMO) recognition of a SUMO binding motif: a reversal of the bound orientation. J Biol Chem 280:40122–40129
47. Hannich JT, Lewis A, Kroetz MB, Li SJ, Heide H, Emili A, Hochstrasser M (2005) Defining the SUMO-modified proteome by multiple approaches in *Saccharomyces cerevisiae*. J Biol Chem 280:4102–4110
48. Hecker CM, Rabiller M, Haglund K, Bayer P, Dikic I (2006) Specification of SUMO1- and SUMO2-interacting motifs. J Biol Chem 281:16117–16127
49. Kerscher O (2007) SUMO junction-what's your function? New insights through SUMO-interacting motifs. EMBO Rep 8:550–555
50. Hakli M, Karvonen U, Janne OA, Palvimo JJ (2005) SUMO-1 promotes association of SNURF (RNF4) with PML nuclear bodies. Exp Cell Res 304:224–233
51. Weissman AM (2001) Themes and variations on ubiquitylation. Nat Rev Mol Cell Biol 2:169–178
52. Potts PR, Yu H (2005) Human MMS21/NSE2 is a SUMO ligase required for DNA repair. Mol Cell Biol 25:7021–7032
53. Cheng CH, Lo YH, Liang SS, Ti SC, Lin FM, Yeh CH, Huang HY, Wang TF (2006) SUMO modifications control assembly of synaptonemal complex and polycomplex in meiosis of *Saccharomyces cerevisiae*. Genes Dev 20:2067–2081
54. Kotaja N, Karvonen U, Janne OA, Palvimo JJ (2002) PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. Mol Cell Biol 22:5222–5234
55. Kahyo T, Nishida T, Yasuda H (2001) Involvement of PIAS1 in the sumoylation of tumor suppressor p53. Mol Cell 8:713–718
56. Takahashi Y, Toh-e A, Kikuchi Y (2001) A novel factor required for the SUMO1/Smt3 conjugation of yeast septins. Gene 275:223–231
57. Pichler A, Gast A, Seeler JS, Dejean A, Melchior F (2002) The nucleoporin RanBP2 has SUMO1 E3 ligase activity. Cell 108:109–120
58. Pichler A, Knipscheer P, Saitoh H, Sixma TK, Melchior F (2004) The RanBP2 SUMO E3 ligase is neither HECT- nor RING-type. Nat Struct Mol Biol 11:984–991
59. Kagey MH, Melhuish TA, Wotton D (2003) The polycomb protein Pc2 is a SUMO E3. Cell 113:127–137
60. Weger S, Hammer E, Heilbronn R (2005) Topors acts as a SUMO-1 E3 ligase for p53 in vitro and in vivo. FEBS Lett 579:5007–5012

61. Pungaliya P, Kulkarni D, Park HJ, Marshall H, Zheng H, Lackland H, Saleem A, Rubin EH (2007) TOPORS functions as a SUMO-1 E3 ligase for chromatin-modifying proteins. J Proteome Res 6:3918–3923
62. Zhao X, Sternsdorf T, Bolger TA, Evans RM, Yao TP (2005) Regulation of MEF2 by histone deacetylase 4- and SIRT1 deacetylase-mediated lysine modifications. Mol Cell Biol 25:8456–8464
63. Gregoire S, Yang XJ (2005) Association with class IIa histone deacetylases upregulates the sumoylation of MEF2 transcription factors. Mol Cell Biol 25:2273–2287
64. Ghisletti S, Huang W, Ogawa S, Pascual G, Lin ME, Willson TM, Rosenfeld MG, Glass CK (2007) Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. Mol Cell 25:57–70
65. Stankovic-Valentin N, Deltour S, Seeler J, Pinte S, Vergoten G, Guerardel C, Dejean A, Leprince D (2007) An acetylation/deacetylation-SUMOylation switch through a phylogenetically conserved psiKXEP motif in the tumor suppressor HIC1 regulates transcriptional repression activity. Mol Cell Biol 27:2661–2675
66. Takahashi Y, Kahyo T, Toh-E A, Yasuda H, 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
67. Deng Z, Wan M, Sui G (2007) PIASy-mediated sumoylation of Yin Yang 1 depends on their interaction but not the RING finger. Mol Cell Biol 27:3780–3792
68. Reverter D, Lima CD (2005) Insights into E3 ligase activity revealed by a SUMO-RanGAP1-Ubc9-Nup358 complex. Nature 435:687–692
69. Tang Z, Hecker CM, Scheschonka A, Betz H (2008) Protein interactions in the sumoylation cascade: lessons from X-ray structures. FEBS J 275:3003–3015
70. Takahashi Y, Kikuchi Y (2005) Yeast PIAS-type Ull1/Siz1 is composed of SUMO ligase and regulatory domains. J Biol Chem 280:35822–35828
71. Reindle A, Belichenko I, Bylebyl GR, Chen XL, Gandhi N, Johnson ES (2006) Multiple domains in Siz SUMO ligases contribute to substrate selectivity. J Cell Sci 119:4749–4757
72. Knipscheer P, Flotho A, Klug H, Olsen JV, van Dijk WJ, Fish A, Johnson ES, Mann M, Sixma TK, Pichler A (2008) Ubc9 sumoylation regulates SUMO target discrimination. Mol Cell 31:371–382
73. Lin DY, Huang YS, Jeng JC, Kuo HY, Chang CC, Chao TT, Ho CC, Chen YC, Lin TP, Fang HI, Hung CC, Suen CS, Hwang MJ, Chang KS, Maul GG, Shih HM (2006) Role of SUMO-interacting motif in Daxx SUMO modification, subnuclear localization, and repression of sumoylated transcription factors. Mol. Cell 24:341–354
74. Karvonen U, Jaaskelainen T, Rytinki M, Kaikkonen S, Palvimo JJ (2008) ZNF451 is a novel PML body- and SUMO-associated transcriptional coregulator. J Mol Biol 382:585–600
75. Ivanov AV, Peng H, Yurchenko V, Yap KL, Negorev DG, Schultz DC, Psulkowski E, Fredericks WJ, White DE, Maul GG, Sadofsky MJ, Zhou MM, Rauscher FJ III (2007) PHD domain-mediated E3 ligase activity directs intramolecular sumoylation of an adjacent bromodomain required for gene silencing. Mol Cell 28:823–837
76. Zeng L, Yap KL, Ivanov AV, Wang X, Mujtaba S, Plotnikova O, Rauscher FJ 3rd, Zhou MM (2008) Structural insights into human KAP1 PHD finger-bromodomain and its role in gene silencing. Nat Struct Mol Biol 15:626–633
77. Hietakangas V, Anckar J, Blomster HA, Fujimoto M, Palvimo JJ, Nakai A, Sistonen L (2006) PDSM, a motif for phosphorylation-dependent SUMO modification. Proc Natl Acad Sci USA 103:45–50
78. Yang SH, Galanis A, Witty J, Sharrocks AD (2006) An extended consensus motif enhances the specificity of substrate modification by SUMO. EMBO J 25:5083–5093
79. Anckar J, Sistonen L (2007) SUMO: getting it on. Biochem Soc Trans 35:1409–1413
80. Wang J, Zhang H, Iyer D, Feng XH, Schwartz RJ (2008) Regulation of cardiac specific nkx2.5 gene activity by small ubiquitin-like modifier. J Biol Chem 283:23235–23243
81. Wible BA, Yang Q, Kuryshev YA, Accili EA, Brown AM (1998) Cloning and expression of a novel K+ channel regulatory protein. KChAP J Biol Chem 273:11745–11751
82. Martin S, Nishimune A, Mellor JR, Henley JM (2007) SUMOylation regulates kainate-receptor-mediated synaptic transmission. Nature 447:321–325
83. Bischof O, Schwamborn K, Martin N, Werner A, Sustmann C, Grosschedl R, Dejean A (2006) The E3 SUMO ligase PIASy is a regulator of cellular senescence and apoptosis. Mol Cell 22:783–794
84. Onishi A, Peng GH, Hsu C, Alexis U, Chen S, Blackshaw S (2009) Pias3-dependent SUMOylation directs rod photoreceptor development. Neuron 61:234–246
85. Munarriz E, Barcaroli D, Stephanou A, Townsend PA, Maisse C, Terrinoni A, Neale MH, Martin SJ, Latchman DS, Knight RA, Melino G, De Laurenzi V (2004) PIAS-1 is a checkpoint regulator which affects exit from G1 and G2 by sumoylation of p73. Mol Cell Biol 24:10593–10610
86. Martin N, Schwamborn K, Urlaub H, Gan B, Guan JL, Dejean A (2008) Spatial interplay between PIASy and FIP200 in the regulation of signal transduction and transcriptional activity. Mol Cell Biol 28:2771–2781
87. Poukka H, Karvonen U, Janne OA, Palvimo JJ (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc Natl Acad Sci USA 97:14145–14150
88. Tian S, Poukka H, Palvimo JJ, Janne OA (2002) Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. Biochem J 367:907–911
89. Sentis S, Le Romancer M, Bianchin C, Rostan MC, Corbo L (2005) Sumoylation of the estrogen receptor alpha hinge region regulates its transcriptional activity. Mol Endocrinol 19:2671–2684
90. Ohshima T, Koga H, Shimotohno K (2004) Transcriptional activity of peroxisome proliferator-activated receptor gamma is modulated by SUMO-1 modification. J Biol Chem 279:29551–29557
91. Tallec LP, Kirsh O, Lecomte MC, Viengchareun S, Zennaro MC, Dejean A, Lombes M (2003) Protein inhibitor of activated signal transducer and activator of transcription 1 interacts with the N-terminal domain of mineralocorticoid receptor and represses its transcriptional activity: implication of small ubiquitin-related modifier 1 modification. Mol Endocrinol 17:2529–2542
92. Kotaja N, Aittomaki S, Silvennoinen O, Palvimo JJ, Janne OA (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
93. Kotaja N, Vihinen M, Palvimo JJ, Janne OA (2002) Androgen receptor-interacting protein 3 and other PIAS proteins cooperate with glucocorticoid receptor-interacting protein 1 in steroid receptor-dependent signaling. J Biol Chem 277:17781–17788
94. Yang SH, Sharrocks AD (2005) PIASx acts as an Elk-1 coactivator by facilitating derepression. EMBO J 24:2161–2171

95. Liu B, Yang R, Wong KA, Getman C, Stein N, Teitell MA, Cheng G, Wu H, Shuai K (2005) Negative regulation of NF-kappaB signaling by PIAS1. Mol Cell Biol 25:1113–1123
96. Liu B, Yang Y, Chernishof V, Loo RR, Jang H, Tahk S, Yang R, Mink S, Shultz D, Bellone CJ, Loo JA, Shuai K (2007) Proinflammatory stimuli induce IKKalpha-mediated phosphorylation of PIAS1 to restrict inflammation and immunity. Cell 129:903–914
97. Long J, Wang G, Matsuura I, He D, Liu F (2004) Activation of Smad transcriptional activity by protein inhibitor of activated STAT3 (PIAS3). Proc Natl Acad Sci USA 101:99–104
98. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature 437:759–763
99. Kim J, Sharma S, Li Y, Cobos E, Palvimo JJ, Williams SC (2005) Repression and coactivation of CCAAT/enhancer-binding protein epsilon by sumoylation and protein inhibitor of activated STATx proteins. J Biol Chem 280:12246–12254
100. Chun TH, Itoh H, Subramanian L, Iniguez-Lluhi JA, Nakao K (2003) Modification of GATA-2 transcriptional activity in endothelial cells by the SUMO E3 ligase PIASy. Circ Res 92:1201–1208
101. Lee H, Quinn JC, Prasanth KV, Swiss VA, Economides KD, Camacho MM, Spector DL, Abate-Shen C (2006) PIAS1 confers DNA-binding specificity on the Msx1 homeoprotein. Genes Dev 20:784–794
102. van den Akker E, Ano S, Shih HM, Wang LC, Pironin M, Palvimo JJ, Kotaja N, Kirsh O, Dejean A, Ghysdael J (2005) FLI-1 functionally interacts with PIASxalpha, a member of the PIAS E3 SUMO ligase family. J Biol Chem 280:38035–38046
103. Zhou S, Si J, Liu T, DeWille JW (2008) PIASy represses CCAAT/enhancer-binding protein delta (C/EBPdelta) transcriptional activity by sequestering C/EBPdelta to the nuclear periphery. J Biol Chem 283:20137–20148
104. Tolkunova E, Malashicheva A, Parfenov VN, Sustmann C, Grosschedl R, Tomilin A (2007) PIAS proteins as repressors of Oct4 function. J Mol Biol 374:1200–1212
105. Yang SH, Sharrocks AD (2006) PIASxalpha differentially regulates the amplitudes of transcriptional responses following activation of the ERK and p38 MAPK pathways. Mol Cell 22:477–487
106. Ono K, Han J (2000) The p38 signal transduction pathway: activation and function. Cell Signal 12:1–13
107. Stehmeier P, Muller S (2009) Phospho-regulated SUMO interaction modules connect the SUMO system to CK2 signaling. Mol Cell 33:400–409
108. Weber S, Maass F, Schuemann M, Krause E, Suske G, Bauer UM (2009) PRMT1-mediated arginine methylation of PIAS1 regulates STAT1 signaling. Genes Dev 23:118–132
109. Depaux A, Regnier-Ricard F, Germani A, Varin-Blank N (2007) A crosstalk between hSiah2 and Pias E3-ligases modulates Pias-dependent activation. Oncogene 26:6665–6676
110. Albor A, El-Hizawi S, Horn EJ, Laederich M, Frosk P, Wrogemann K, Kulesz-Martin M (2006) The interaction of Piasy with Trim32, an E3-ubiquitin ligase mutated in limb-girdle muscular dystrophy type 2H, promotes Piasy degradation and regulates UVB-induced keratinocyte apoptosis through NFkappaB. J Biol Chem 281:25850–25866
111. Chen XL, Reindle A, Johnson ES (2005) Misregulation of 2 microm circle copy number in a SUMO pathway mutant. Mol Cell Biol 25:4311–4320
112. Chen XL, Silver HR, Xiong L, Belichenko I, Adegite C, Johnson ES (2007) Topoisomerase I-dependent viability loss in *Saccharomyces cerevisiae* mutants defective in both SUMO conjugation and DNA repair. Genetics 177:17–30
113. Watts FZ, Skilton A, Ho JC, Boyd LK, Trickey MA, Gardner L, Ogi FX, Outwin EA (2007) The role of Schizo*saccharomyces pombe* SUMO ligases in genome stability. Biochem Soc Trans 35:1379–1384
114. Xhemalce B, Seeler JS, Thon G, Dejean A, Arcangioli B (2004) Role of the fission yeast SUMO E3 ligase Pli1p in centromere and telomere maintenance. EMBO J 23:3844–3853
115. Andrews EA, Palecek J, Sergeant J, Taylor E, Lehmann AR, Watts FZ (2005) Nse2, a component of the Smc5–6 complex, is a SUMO ligase required for the response to DNA damage. Mol Cell Biol 25:185–196
116. Zhao X, Blobel G (2005) A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. Proc Natl Acad Sci USA 102:4777–4782
117. Mohr SE, Boswell RE (1999) Zimp encodes a homologue of mouse Miz1 and PIAS3 and is an essential gene in *Drosophila melanogaster*. Gene 229:109–116
118. Betz A, Lampen N, Martinek S, Young MW, Darnell JE Jr (2001) A *Drosophila* PIAS homologue negatively regulates stat92E. Proc Natl Acad Sci USA 98:9563–9568
119. Roy Chowdhuri S, Crum T, Woollard A, Aslam S, Okkema PG (2006) The T-box factor TBX-2 and the SUMO conjugating enzyme UBC-9 are required for ABa-derived pharyngeal muscle in *C. elegans*. Dev Biol 295:664–677
120. Holway AH, Kim SH, La Volpe A, Michael WM (2006) Checkpoint silencing during the DNA damage response in *Caenorhabditis elegans* embryos. J Cell Biol 172:999–1008
121. Kim SH, Michael WM (2008) Regulated proteolysis of DNA polymerase eta during the DNA-damage response in *C. elegans*. Mol Cell 32:757–766
122. Catala R, Ouyang J, Abreu IA, Hu Y, Seo H, Zhang X, Chua NH (2007) The *Arabidopsis* E3 SUMO ligase SIZ1 regulates plant growth and drought responses. Plant Cell 19:2952–2966
123. Miura K, Jin JB, Hasegawa PM (2007) Sumoylation, a post-translational regulatory process in plants. Curr Opin Plant Biol 10:495–502
124. Roth W, Sustmann C, Kieslinger M, Gilmozzi A, Irmer D, Kremmer E, Turck C, Grosschedl R (2004) PIASy-deficient mice display modest defects in IFN and Wnt signaling. J Immunol 173:6189–6199
125. Bischof O, Dejean A (2007) SUMO is growing senescent. Cell Cycle 6:677–681
126. Liu B, Mink S, Wong KA, Stein N, Getman C, Dempsey PW, Wu H, Shuai K (2004) PIAS1 selectively inhibits interferon-inducible genes and is important in innate immunity. Nat Immunol 5:891–898
127. Yan W, Santti H, Janne OA, Palvimo JJ, Toppari J (2003) Expression of the E3 SUMO-1 ligases PIASx and PIAS1 during spermatogenesis in the rat. Gene Expr Patterns 3:301–308
128. Santti H, Mikkonen L, Anand A, Hirvonen-Santti S, Toppari J, Panhuysen M, Vauti F, Perera M, Corte G, Wurst W, Janne OA, Palvimo JJ (2005) Disruption of the murine PIASx gene results in reduced testis weight. J Mol Endocrinol 34:645–654
129. Tahk S, Liu B, Chernishof V, Wong KA, Wu H, Shuai K (2007) Control of specificity and magnitude of NF-kappa B and STAT1-mediated gene activation through PIASy and PIAS1 cooperation. Proc Natl Acad Sci USA 104:11643–11648
130. Thompson SJ, Loftus LT, Ashley MD, Meller R (2008) Ubiquitin–proteasome system as a modulator of cell fate. Curr Opin Pharmacol 8:90–95
131. Dorval V, Fraser PE (2007) SUMO on the road to neurodegeneration. Biochim Biophys Acta 1773:694–706
132. Hernandez-Toro J, Prieto C, De las Rivas J (2007) APID2NET: unified interactome graphic analyzer. Bioinformatics 23:2495–2497

133. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504
134. Bader GD, Betel D, Hogue CW (2003) BIND: the Biomolecular interaction network database. Nucleic Acids Res 31:248–250
135. Stark C, Breitkreutz BJ, Reguly T, Boucher L, Breitkreutz A, Tyers M (2006) BioGRID: a general repository for interaction datasets. Nucleic Acids Res 34:D535–D539
136. Salwinski L, Miller CS, Smith AJ, Pettit FK, Bowie JU, Eisenberg D (2004) The database of interacting proteins: 2004 update. Nucleic Acids Res 32:D449–D451
137. Keshava Prasad TS, Goel R, Kandasamy K, Keerthikumar S, Kumar S, Mathivanan S, Telikicherla D, Raju R, Shafreen B, Venugopal A, Balakrishnan L, Marimuthu A, Banerjee S, Somanathan DS, Sebastian A, Rani S, Ray S, Harrys Kishore CJ, Kanth S, Ahmed M et al (2009) Human protein reference database–2009 update. Nucleic Acids Res 37:D767–D772
138. Kerrien S, Alam-Faruque Y, Aranda B, Bancarz I, Bridge A, Derow C, Dimmer E, Feuermann M, Friedrichsen A, Huntley R, Kohler C, Khadake J, Leroy C, Liban A, Lieftink C, Montecchi-Palazzi L, Orchard S, Risse J, Robbe K, Roechert B et al (2007) IntAct—open source resource for molecular interaction data. Nucleic Acids Res 35:D561–D565
139. Chatr-aryamontri A, Ceol A, Palazzi LM, Nardelli G, Schneider MV, Castagnoli L, Cesareni G (2007) MINT: the Molecular INTeraction database. Nucleic Acids Res 35:D572–D574