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Review

Transcriptional coregulator SNW/SKIP: the concealed tie of dissimilar pathways

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Abstract. Eukaryotic gene expression requires that all the steps of messenger RNA production are regulated in concert to integrate the diverse inputs cells receive. We discuss the functioning of SNW/SKIP, an essential spliceosomal component and transcriptional coregulator, which may provide regulatory coupling of transcription initiation and splicing. SNW/SKIP potentiates the activity of important transcription factors, such as vitamin D receptor, CBF1 (RBP-Jκ), Smad2/3, and MyoD. It synergizes with Ski in overcoming pRb-mediated cell cycle arrest, and it is targeted by the viral transactivators EBNA2 and E7. SNW/SKIP may aid in conformational transition of the gene expression machine through its avidity to nuclear matrix fractions or by recruiting foldases such as the prolyl isomerase PPIL1. The extensive list of SNW/SKIP partners, its unique primary structure, conserved from yeast to humans, and its essential character suggest a distinct function of general importance.

Key words. Prp45p; Bx42; U2AF; cyclophilin; pRb; Ski oncoprotein; spliceosome; nuclear matrix.

Introduction

The inside of the eukaryotic nucleus houses a complex information-retrieval system. Because of the inherent importance of the timeliness, accuracy and completeness of the retrieval process, regulation of the way the information in genes is interpreted and eventually packed and exported in edited form is tightly linked to almost every function within the cell. It is the cell's ability to respond to composite sets of inputs which apparently made it necessary for the nuclear machinery to evolve to the current level of intricacy.

Coactivators and corepressors of transcription factors, called coregulators in the text, propagate the changes signaled by transcription factors themselves [1, 2]. Classical coregulators, such as the proteins of the SRC-1 family (steroid receptor coactivator-1; [3]), induce the assembly of histone acetyltransferases, e.g. p300/CBP [4], at the site of the preinitiation complex assembly. Context-directed acetylation within the amino termini of histones drives the changes in chromatin structure and serves as a switch for docking bromo-domain-containing proteins such as TAF250 [5]. An important group of coregulators is involved in posttranscriptional processes, participating in the regulation of alternative splice-site selection, splicing efficiency, and messenger RNA (mRNA) export [6, 7].

It is our aim to summarize the significance of a novel potential coregulator, the SNW/SKIP protein, for transcription regulation. SNW/SKIP is part of nuclear regulatory complexes and interacts with factors involved in preinitiation, splicing and polyadenylation. SNW/SKIP, which was shown to be required for pre-mRNA splicing [8], interacts directly with and potentiates the activity of certain types of transcription factors (table 1; [9, 10]). The coregulator may act by coupling transcription initiation to pre-mRNA processing steps, but there is probably more to learn. It is ancestral to its transcription factor partners in human cells and it is highly conserved, providing an es-

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Table 1. The list of tested employments and interaction partners of SNW/SKIP proteins.

Process	Pathway	Partners	References
Transcription initiation	steroid hormones	VDR/RXR heterodimer, other steroid receptors	9, 17, 25, 50, 51 17, 51
	TGF-β	Ski, Sno Smad2, Smad3	16, 57–59 57
	MyoD	MyoD	40
	Delta/Notch	NotchIC CBF1 (RBP-Jκ) EBV EBNA2*	52 10, 52, 77 10
Transcription repression		NcoR/SMRT HDAC2 mSin3A CIR	10, 77 10 10 10
Splicing, pre-mRNA processing		U2AF^{23} PPIL1 cyclophilin Prp46p Prp22p Clf1p (Syf3p) Syf1p PABP2	12 36, 38, 39 8 8 8 8 40
Cell cycle	E2F/pRb	pRb E2F1, E2F3 HPV E7*	59 85 87

SKIP, Ski interacting protein; VDR, vitamin D receptor; RXR, retinoid X receptor; TGF-β, transforming growth factor-β; Ski, Sloan-Kettering Institute oncoprotein; Sno, Ski-related novel oncoprotein; Smad, transcription factor of the TGF-β pathway; MyoD, myogenic transcription factor; NotchIC, intracellular component of receptor Notch; CBF1, Notch-responsive transcription factor; RBP-Jκ, J kappa recombination signal sequence-binding protein; EBV, Epstein Barr virus; EBNA2, Epstein Barr virus nuclear antigen 2; NCoR, nuclear receptor corepressor; SMRT, silencing mediator for retinoid and thyroid receptors; HDAC2, histone deacetylase 2; CIR, CBF1 interacting corepressor; U2AF^{23}, small subunit of U2 auxiliary splicing factor (23 kD); PPIL1, peptidylprolyl isomerase (cyclophilin)-like 1; PABP2, poly(A)-binding protein 2; pRb, Retinoblastoma protein; E2F1/3, transcription factor involved in cell cycle regulation; HPV E7, human papilloma virus E7 transactivator. The asterisks indicate viral origin.

sential function in both yeasts and probably in all Metazoa (see below). The engagement of SNW/SKIP in the signal transduction pathways may reflect a hitherto unknown mode of signal relay to, e.g. cell cycle checkpoints. We need to understand the logic behind SNW/SKIP-mediated interactions in order to discover more of the inputs and outputs of the networks of gene expression machines. Such knowledge may well be of therapeutic use, given that transcription factor coregulators have been identified as causal agents in the development of cancer [11].

SNW structure

The genes encoding SNW proteins are present throughout eukaryotic phylla, including lower eukaryotes, plants, fungi and animals. There seems to be only one gene per genome, which codes for rare, ubiquitous mRNA and a 60–80 kDa protein of low abundance and preferentially nuclear localization. Detailed characterizations have been reported for the protein Prp45p of *Saccharomyces cerevisiae* [8], Snw1p of *Schizosaccharomyces pombe* [12], SnwA of *Dictyostelium discoideum* [13], CeSKIP of *Caenorhabditis elegans* [14], Bx42 of *Drosophila melanogaster* [15] and human SKIP/NCoA-62 [16, 17]. The acronym SNW stands for the absolutely conserved motif SNWKN [13], SKIP is the abbreviation of Ski interacting protein as the Ski oncoprotein was the first binding partner identified in human [16], and NCoA-62 describes SKIP as nuclear receptor coactivator of 62 kDa [17].

SNW primary structure can be subdivided into two domains, N- and C-terminal, separated by species-specific inserts (fig. 1). Multiple sequence alignments discern several highly conserved regions throughout the primary structure. Importantly, however, no part of the sequence is found as a part of other proteins. A number of tentative phosphorylation sites could be distinguished, which corresponds to preliminary findings that *D. discoideum* SnwA [Folk et al., unpublished results] or human SKIP [18] are phosphoproteins. Secondary structure prediction programs consistently identify the region between amino acids 299 and 360 as α helix. This region, which overlaps the area where most of SNW partners bind, is highly charged (pI > 9), and it is composed of three weakly recurring motifs. Modeling this sequence into the helical coordinates of, e.g., troponin C structure shows 'buttons' of negative charge interlaced with regions of basic amino acids.

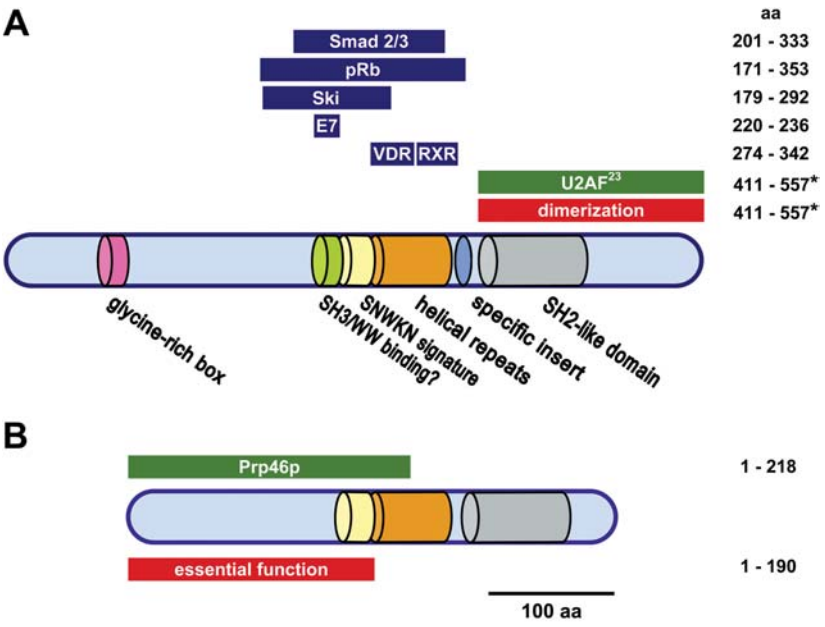

Figure 1. The overview of conserved, functional and interaction regions of SNW/SKIP proteins. (*A*) Canonical SNW/SKIP proteins. The numbers indicate amino acid residues in human SKIP or in Snw1p (*). (*B*) Prp45p, the divergent homolog of *Saccharomyces cerevisiae*. The primary structure contains, starting at the N-terminus, the following remarkable motifs: glycine-rich box, proline-rich box, which constitutes a plausible SH3 and WW domain binding site, SNWKN signature, identical in all genera, highly conserved amphiionic helical repeats, species-specific insert and the region of similarity to the N-terminal half of SH2 domains (between α-helix A and β-strand D of the SH2 domain) [13]. The sequence characteristics of the N-terminal half also fit the requirements for PEST sequences [93]. The mapped regions of interaction with transcriptional regulators and splicing factors are shown in blue and green, respectively. The regions implicated in the dimerization of Snw1p of *Schizosaccharomyces pombe* and the essential part of Prp45p are shown in red (see the text for references and detailed discussion).

All the proteins of the family tree show the same sequence features, with the exception of *S. cerevisiae* Prp45p. This protein lacks part of the N-terminus, including the highly conserved glycine-rich box, and the similarity of the remaining motifs to, e.g., human SKIP, is much lower than those of the other homologs. The gene encoding FUN20/Prp45p of *S. cerevisiae* was discovered during analysis of yeast chromosome I as an essential gene of unknown function – hence called Function UNknown 20 [19, 20]. The authors were unable to target the region encompassing *FUN20* with temperature-sensitive (Ts^-) lethal mutations, which may reflect structural features of the SNW protein itself. It implies that the key structural requirements of FUN20/Prp45p are met unless a limited domain or 'simple' structure is distorted. Such a condition may be compatible with a large set of amino acid substitutions, and the chance of recovering a Ts^- lethal phenotype with a single mutation was therefore low. Deletion analysis of *PRP45* showed that it is solely the N-terminal half of Prp45p (amino acids 1–190) that can support wild-type growth [21]. The truncation leaves little 'remarkable' in the Prp45(1–190) protein (see fig. 1) because the SNWK motif, which is absolutely conserved, can be replaced with alanines without consequence for cell viability [21]. Interestingly, SnwA of *D. discoideum*, Snw1p of *S. pombe* and human SKIP did not complement the PRP45 knockout. The simple sterical hindrance of the N-terminus is, however, unlikely, because N-terminal tagging of Prp45p (e.g. by LexA) is compatible with viability. It is thus possible that species-specific structural subtleties are required for Prp45p to work.

The structure of SNW proteins may suit the need of binding an extensive list of partners (see below and table 1). Part of the structure may exist in a partially unfolded state, which could undergo refolding in response to the interacting molecule. It is noteworthy that (i) Prp45p lacks spots for thermosensitive mutations, (ii) the SNWK motif within the essential part of Prp45p can be replaced, (iii) SNW proteins contain extensively charged stretches of amino acids and (iv) *Dictyostelium* SnwA was found in vitro to have some properties resembling unfolded proteins. Are parts of SNW molecule 'natively unfolded', e.g. 'lacking ordered structure at neutral pH in vitro' [22]? Such regions are found in proteins that exhibit remarkable interaction-flexibility, for which the human papilloma virus (HPV)-16 E7 protein can serve as example (see below; [23]).

SNW and subnuclear compartments

Cell fractionations in *Dictyostelium* found the majority of SnwA in nuclei with ~10% or less in the cytosol. Cyto-

logical and biochemical studies strongly suggested their association with nuclear matrix fractions [Folk et al., unpublished results]. In *Drosophila*, the selective association of SNW homolog Bx42 with nuclear chromatin was demonstrated using the salivary glands' polytene chromosomes. Certain puffs were stained with the anti-Bx42 antibody, and the pattern varied during development [15]. Human SKIP was found among the ~100 protein constituents of splicing-involved speckles [24], a structure typically observed in the nuclei of higher eukaryotes. SKIP was retained in a nuclear matrix preparation from rat osteosarcoma cells and coprecipitated matrin 3, and other scaffold factors, from HeLa nuclear extracts [25]. The subnuclear compartmentalization of SNW/SKIP seems to agree with the protein's involvement in splicing related regulations (see below). Spliceosomal proteins show characteristic subnuclear distribution, which reflects multiple and extensive associations of splicing components with nuclear matrix [26, 27].

SNW in pre-mRNA processing

Since 1998, when SKIP was found to be the part of reconstituted spliceosomes [18], and 1999, when the associations of FUN20 with proteins involved in splicing prompted its renaming (Prp45p; Pre-mRNA Processing 45; [8]), the evidence for the engagement of SNWs in splicing has accumulated.

Two independent systematic two-hybrid searches found the interaction of Prp45p with the WD-domain-containing protein Prp46p [28, 29]. Prp45p, Prp46p and Prp22p (see below) were also found as part of the 'CDC5' complex. We refer to the complex as CDC5 because it contains, as its basic constituent, Cef1p, Cdc5p or CDC5L when analyzed in *S. cerevisiae*, *S. pombe* or *H. sapiens*, respectively [30–32]. Comparative mass spectrometry (MS) analyses in *S. cerevisiae* and *S. pombe* of the tandem affinity-purified 40S CDC5 complexes found the presence of Prp45p and Snw1p, respectively [31]. The composition of the complexes in both yeasts was nearly identical, with 24 components being conserved between species. The properties of the complexes, their avidity to U2, U4 and U5 small nuclear RNAs (snRNAs) and the connectivity maps of their components suggest that these novel particles represent part of the spliceosome or temporal assembly arising at certain stage of its function. The CDC5 complex in a human cell line (HeLa) was characterized by the Lamond laboratory, which also used MS technology, at various levels of purification stringency. The so-called core CDC5L complex was composed of CDC5L and, among others, PRLG1, the homolog of Prp46p. Multiple other proteins, including several U2 small nuclear ribonucleoprotein (snRNP) components, were found in the CDC5L complex(es), albeit less firmly bound or associating only transiently [32].

Prp45p, Prp46p and Prp22p were recently used in exhaustive two-hybrid screens to assess their mutual interactions in *S. cerevisiae* [8]. The authors confirmed that Prp45p interacted directly with Prp46p (N-terminal 218 amino acids of Prp45p). It seems possible that Prp46p interaction with Cef1p (not detected here but proved for the human homologs [32]) recruits the Prp46p/Prp45p/Prp22p complex to the CDC5 particle. Downregulation of Prp45p levels stalled splicing of two pre-mRNA probes, irrespective of their branchpoint-3′ splice site distance. Using tagged Prp45p, the authors showed that Prp45p associated with U2, U5 and U6 snRNA under nonsplicing conditions and with assembled spliceosomal complexes throughout the splicing process. The depletion of Prp45p, which impaired cell growth, correlated with the accumulation of unprocessed pre-mRNA probes.

Prp45p might differ from other SNW homologs in its splicing function, because several splicing factors were coeliminated in *S. cerevisiae* [33] and the splicing-involved interacting partners for Prp45p should be distinct from those of, e.g., *S. pombe* Snw1p. Snw1p was found in a two-hybrid screen of the *S. pombe* complementary DNA (cDNA) library to interact via its C-terminal part (amino acids 411–557) with spU2AF^{23}, the small subunit of the U2 auxiliary splicing factor (U2AF^{35} in human; [12]), which is absent in *S. cerevisiae*. Functional evidence that human SKIP can influence pre-mRNA splicing was provided most recently by MacDonald and coworkers [25]. The expression of truncated SKIP (amino acids 87–342) led to the accumulation of unspliced transcripts derived from a reporter minigene that was under the control of SKIP-interacting transcription factor (see below).

Comprehensive proteomic analyses of human spliceosomes confirmed the inclusion of SKIP at various stages of the spliceosomal cycle. The analyses found the spliceosomal proteome very large and with a significant portion of factors that couple splicing to other steps of gene expression. Two reports found SKIP as well as U2AF^{35} in spliceosomes from a mixture of all stages of assembly [34, 35]. Three reports analyzed spliceosomes at a defined stage of the catalytic cycle, which is characterized by complexes H, E, A, B, B*, C, and the postspliceosomal complexes. SKIP presence was indicated in the H/E complexes (together with PRLG1 and CDC5) where the pairing of pre-mRNA to U2 snRNA is loose [36]. After an ATP-dependent step the U2 snRNA-pre-mRNA pairing is established, and the prespliceosomal A complex is formed. When the A complexes were allowed to assemble on an affinity matrix and analyzed, SKIP was not found, whereas U2AF^{35} was [37]. This finding may not be conclusive, however, as the kinetics of the splicing reaction was severely slowed. It is possible that

the solid phase-assembly precluded proper involvement of SKIP or that the long incubation times led to the breakdown of SKIP, because SNWs are labile proteins. After the association of U4/U6.U5 tri-snRNP, the complex B is formed, which undergoes a dramatic rearrangement, releasing, among others, U1 and U4 snRNPs and yielding an activated B complex (denoted B*; [38]). The authors showed that the B* complex, as well as the post-spliceosomal intron complex and a novel 35S U5 snRNP particle, contained both SKIP and CDC5 and its associating proteins. The first transesterification step leads to the transformation of the B* complex into the C complex, where both SKIP and CDC5 were found again [36]. To sum up, the proteomic results suggest the association of SKIP with the CDC5 subcomplex, which is documented in the B* and C complexes and in the post-spliceosomal species [38], and is in agreement with the yeast data [8].

The rearrangements, which occur in the B to B* and B* to C transitions of the spliceosome, are very extensive, requiring concerted conformational transitions to take place [38]. Intriguingly, the prolyl isomerases of the evolutionarily conserved cyclophilin subfamily [PPIL1; peptidylprolyl isomerase (cyclophilin)-like 1] were identified as partners of SNW proteins in both *D. discoideum* and *S. pombe* [39]. The cyclophilin-SNW interaction did not occupy the prolyl isomerase catalytic site, which could mean that SNW serves as an adaptor for this cyclophilin, bringing the foldase to sites where extensive remodeling takes place. SNW/SKIP may thus facilitate the rearrangements of large nucleoprotein complexes (fig. 2C). This hypothesis seems now to be supported by the findings of SKIP together with PPIL1 and another four cyclophilins within the spliceosomal stages containing the CDC5 subcomplex [36, 38].

The effects of SNW/SKIP may not be limited to events occurring solely at the site of pre-mRNA generation, as the interaction partners of SNWs function at various stages of RNA processing. First, SKIP interacts with the poly(A) binding protein 2 (PABP2), and both proteins colocalize to nuclear speckles [40]. PABP2, which has high affinity to nuclear matrix [41], engages the nuclear export machinery and may contribute to mRNA export [42]. PABP2 accompanies spliced mRNAs on its route to cytoplasm, where it is replaced, in a still unknown way, by PABP1 [42]. Second, Snw1p binds the small subunit of the heterodimeric splicing factor U2AF [12], which was likewise shown to shuttle continuously between the nucleus and the cytoplasm by a mechanism that is independent of binding to mRNA [43]. U2AF shuttling may regulate the availability of splicing factors in the nucleus for, e.g., alternative splice site selection [44]. It would be interesting to learn what the role of the cytoplasmic pool of SNW/SKIP is and how the shuttling of SNW/SKIP contributes to the compartmentalization of both U2AFs and PABP2.

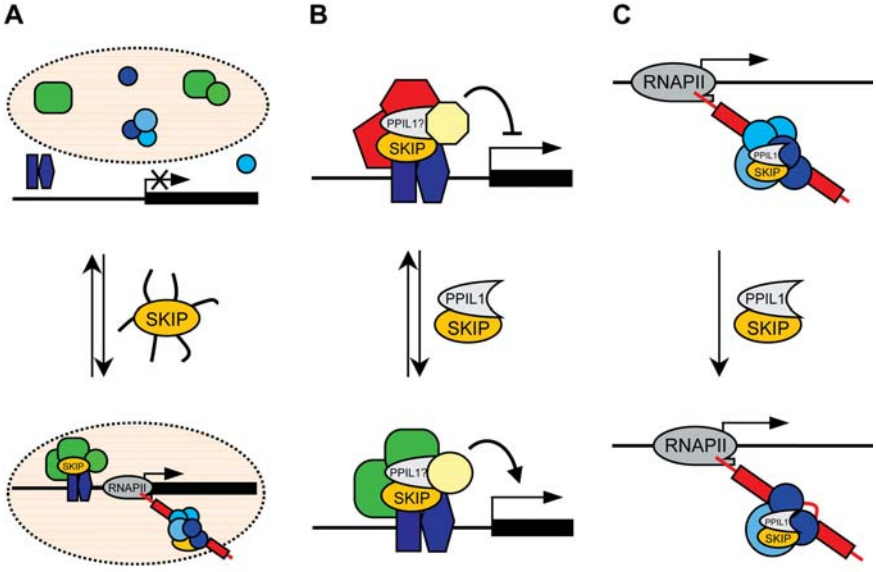

Figure 2. Three nonexclusive hypotheses of SNW/SKIP function. (*A*) SNW/SKIP provides a means of compartmentalization, bringing the complexes of transcription regulators and/or complexes of splicing factors to the appropriate sites via its association with 'nuclear matrix' structures [82]. (*B*) SNW/SKIP assists the exchange of components and/or conformational transitions between repressing and activating complexes of transcription regulators. SNW/SKIP may do so by serving as an interaction platform or by sequestering appropriate effector molecules, such as the prolyl isomerase PPIL1 [39]. (*C*) SNW/SKIP, which is present in several stages of the active spliceosome [36, 38], may regulate the efficiency of splicing by serving as an interaction platform or via targeting of the spliceosome associated prolyl isomerase PPIL1 [36, 38]. See the text for more details.

SNW/SKIP as transcriptional coregulator

Recent reviews reconcile the available data on pre-mRNA generation, processing, export and quality control in favor of an interconnected and jointly regulated process [45, 46]. It follows that factors that influence or mediate cross-talk between the distinct processing steps would have an effect on reporter gene activity/mRNA production in cotransfection experiments, which measure the performance of transcription factors (e.g. the studies described below). One such example is the PABP2 protein, which is important for proper poly(A) tail synthesis and adjustment [47]. It was found to bind the myogenic transcription factor MyoD and function as its coactivator [40]. This binding could reflect the propensity of transcription factors to sequester molecules involved in pre-mRNA processing [26]. It is perhaps for similar benefits that the transcription factors listed below bind SNW/SKIP (table 1).

Steroid receptors

The first implication that SNW proteins were involved in steroid hormone signaling was made in the Saumweber Laboratory, when the *Drosophila* homolog Bx42 was found [15, 48]. One of the chromosome loci that recruited Bx42 harbors an ecdysone receptor heterodimer binding site in the 5′-regulatory region of the Sgs4 gene [49]. Human SNW (SKIP/NCoA-62) was found as partner of the vitamin D receptor (VDR) using two-hybrid and in vitro pull-down techniques [17, 9]. The authors demonstrated the interaction between both VDR and retinoid X receptor (RXR) and two highly conserved regions of SKIP (amino acids 274–309 and 309–342, respectively). They showed that SKIP interacts preferentially, and in a ligand-dependent manner, with the VDR-RXR heterodimer and documented the presence of ternary complexes composed of VDR-RXR, SKIP and the SRC-type coactivators SRC-1 or GRIP-1 (glucocorticoid receptor interacting protein-1). SKIP and GRIP-1 did not interact directly and docked with distinct regions on the heterodimer [9]. The interaction between SKIP and VDR was later shown to include the conserved region of helix H10 of the VDR ligand binding domain, which is distinct from the activation function-2 domain, where the SRC-type coactivators interact [50].

SKIP potentiated vitamin D-dependent transactivation when coexpressed with VDR in COS-7 cells. Simultaneous presence of RXR markedly increased the effect. Cotransfection experiments also showed that SKIP can specifically boost the ligand-dependent activity of other steroid receptors (GR, RAR and ER). The removal of amino acids 487–536 of SKIP substantially reduced its coactivation potential [9]. In addition, the authors used a Gal4-responsive reporter system in COS-7 cells and demonstrated that the C-terminal domain of SKIP (amino acids 388–536) activated transcription when targeted to DNA via Gal4 [51]. Likewise, DNA targeted LexA-Prp45p activated transcription in *S. cerevisiae*, but the capacity was dependent on both the C-terminal domain and the charged helical repeats between amino acids 190 and 254 [21]. These observations are in contrast with the results of Hayward group, which found the full-length SKIP to mediate transcriptional repression when expressed as Gal4-fusion in the presence of the Gal4-responsive reporter in HeLa cells [52]. The availability of other endogenous partners, namely those related to the presence of human papilloma virus (HPV) sequences in the HeLa cell line, may have been important for the outcome of the assay (see the information on HPV E7 below). The capacity of SKIP to tether NCoR/SMRT ([10], see below) and to bind the ternary complexes of VDR/RXR/SRC-1 encouraged Gardiner and co-workers to suggest that SKIP can regulate the transition from the unliganded/repressing VDR complex to the liganded/activating VDR complex [50].

Smad factors and MyoD

Shortly before the link between the transforming growth factor-β (TGF-β) signaling pathway and the oncoproteins-turned-transcription cofactors Ski/Sno was established, SKIP was found to interact with Ski [16]. The Ski segment that was capable of binding SKIP (amino acids 79–185) was required for the transforming ability of Ski. Subsequently, both Ski and Sno were shown to bind Smad transcription factors and attenuate TGF-β-dependent transcription through the recruitment of the nuclear receptor corepressor NCoR and its mSin3A/HDAC histone deacetylase complex [53, 54]. Blocking TGF-β signaling by recruiting histone deacetylases proved to be the underlying cause of Ski/Sno-induced oncogenic transformation or of the enhancement of muscle differentiation, which were the two well-documented effects of these oncoproteins [55, 56]. Finally, SKIP was found to interact with Smad2/Smad3 proteins in glutathione S-transferase (GST) pull-downs, Far Western and two-hybrid experiments, and to augment TGF-β-dependent transcription [57]. Ski as well as Sno attenuated the SKIP-mediated augmentation of TGF-β-dependent gene reporter activity.

The central region of SKIP between amino acids 171 and 336 is the protein's focal interaction platform (fig. 1). Originally, the binding of Ski was narrowed to the area between amino acids 179 and 292 [58], but was later suspected to be indirect [59]. The region between amino acids 201 and 333 of SKIP served as docking site for Smad3, which bound SKIP through the MH2 domain –

the same domain that interacted with Ski/Sno [57]. Other regions within the Smad molecule might have joined in the interaction, as the authors failed to show that GST-tagged MH2 domain alone was capable of SKIP binding. It is important to note that when the truncated SKIP variants were analyzed, full-length SKIP, SKIP(201–333) and SKIP(334–536) activated the reporter gene to the same extent, arguing that direct Smad3-SKIP binding was perhaps not required for SKIP to function. The authors presented the possibility that SKIP may have worked by sequestering corepressors (such as SMRT, which was found to bind SKIP by others [10]).

TGF-β was shown to act in synergy with vitamin D on certain promoters, supposedly through SRC-1- and ligand-potentiated physical interaction between VDR and Smad3 [60, 61]. Subramaniam and co-workers were later unable to confirm the physical interaction between VDR and Smad3, but reiterated that crosstalk between these pathways occurred when both VDR and Smad3 were allowed to bind to their cognate binding elements positioned in close proximity [62]. SKIP bound and coactivated both VDR/RXR [9] and Smad3/Smad4, becoming a conceivable candidate for (yet another) link between the two pathways.

TGF-β inhibits muscle differentiation through functional repression of myogenic transcription factors, such as the MyoD/E-protein heterodimer, which regulates E-box sequence motifs within muscle genes enhancers. This transcriptional repression seems to be mediated by Smad3 interaction with MyoD [63]. Ski itself has long been studied because of its involvement in muscle differentiation [64], and the discovery of Ski-Smad interaction in 1999 [53] provided a plausible molecular explanation for the effect of Ski on MyoD-regulated genes. Additional interactions were soon discovered, however, suggesting that 'induced proximity' of multiple factors needs to be taken into account. SKIP and the poly(A)-binding protein 2 (PABP2) were two such proteins, found to form a ternary complex with MyoD and cooperatively stimulating its transcriptional activity [40]. Both SKIP and PABP2 coprecipitated MyoD independently and, in addition, interacted via the N-terminal domain of PABP2 with each other.

Crosstalk in the TGF-β/Smad pathway is extensive, and interactions with other signal transduction networks occur at virtually every step [65]. Many transcription factors interact with Smad proteins at DNA binding elements in apposition (e.g. AP1; [66]). Ligand-induced interactions of Smads/transcription factor complexes may lead to the recruitment of either coactivators, such as CBP/p300, or corepressors, such as TGIF [67] or Ski. Similarly to its proposed role in VDR complexes, SKIP may regulate the rearrangements between repressing and activating complexes, both at or in the vicinity of the promoter DNA.

CBF1 (RBP-Jκ)/NotchIC

Delta/Notch signal transduction regulates differentiation and proliferation responses by reprogramming the transcriptional repressors of the CSL family (CBF1, Supressor of Hairless, Lag-1) to activators [68, 69]. After ligand binding, the intracellular portion of the Notch receptor (NotchIC) is cleaved off and translocated to the nucleus, where it binds to the CSL family members such as CBF1 (RBP-Jκ) and replaces the SMRT/HDAC corepressor complex with itself. It is this replacement that allows transcription to start from CBF1-controlled promoters. The replacement, a complicated refurbishing of multiple interactions within a ternary complex, seems to be assisted by SKIP [52]. When C2C12 myoblast differentiation was blocked by overexpression of NotchIC, expressing antisense SKIP relieved this block and the cells resumed the differentiation program. The authors inferred that the presence of SKIP was required for NotchIC to efficiently activate CBF1-bound promoters. Notch activity was abrogated when the fourth ankyrin repeat, a site of SKIP binding, was mutated. SKIP was shown to interact with several components of the CBF1 ternary complex, using yeast and mammalian two-hybrid assays and coimmunoprecipitations. SKIP bound the N-terminal part of CBF1 (exons 1–5), the fourth ankyrin repeat of NotchIC, SMRT, mSin3A, the CBF1 corepressor CIR and mHDAC2 [10, 52].

In *Drosophila*, the role of the SNW homolog Bx42 in the Notch pathway was clearly demonstrated using wing disc-specific induction of RNA interference (RNAi) [70]. Overexpression of the CBF1 homolog Suppressor of Hairless resulted in specific defects in the vein architecture of the wing; these defects were strongly enhanced by concurrent downregulation of Bx42 mRNA, suggesting a repressive function for Bx42 in the Notch pathway.

Notch signaling is targeted by the Epstein Barr virus (EBV), which induces B cell immortalization in a process that mimics the early steps of the Notch pathway ([52] and references therein). EBV-encoded transcriptional coactivator EBNA2 binds to CBF1 via the CR6 region, the mutation in which was shown to abolish CBF1 binding and the ability of the corresponding virus to immortalize cells [71]. Remarkably, also, EBNA2 shares with NotchIC the ability to block muscle cell differentiation [72]. Amino-terminally to the CR6 region of EBNA2 is the region CR5, which, when deleted, produces EBNA2 with no transforming activity but with retained ability to bind CBF1 [73]. Hayward and co-workers showed that EBNA2 interacted via CR5 with SKIP and demonstrated that the two proteins colocalized intranuclearly. The authors correlated the inability of the CR5 mutant E2(II307) to efficiently activate reporters containing CBF1 binding sites with the inability of this mutant to bind SKIP. Hayward suggested a model in which CBF1,

SKIP and either NotchIC, SMRT or EBNA2 form a tripartite complex held by three binary interactions (see fig. 9 in [52]). According to the model, the disrupted EBNA2-SKIP interaction brings about the lack of transactivation ability, while CBF1-EBNA2 binding is retained.

SNW as a common link

SNW is being used by a number of complexes that form at, or close to, the sites of preinitiation complex assembly. Using chromatin immunoprecipitation in osteoblasts, MacDonald and co-workers showed that SKIP is physically present at a VDR-driven promoter together with VDR and SRC-1 [25]. Temporal cycling of VDR, SRC-1 and SKIP after vitamin D treatment was shifted relative to each other, implying differences between the two coregulators. SKIP showed a weak tendency to form higher molecular weight complexes in the presence of Smad 3/Smad4 and Smad binding element in COS-1 nuclear extract in gel shift assays [57].

It seems that there are more interactions possible within the SNW/SKIP complexes than those which would be needed just to explain their existence. SKIP-CBF1-NotchIC, SKIP-CBF1-SMRT, SKIP-VDR-RXR and SKIP-pRb-E7 (see below) are examples of tripartite complexes with three bipartite interactions documented experimentally. Larger particles, such as the CBF1-targeted SMRT complex, could theoretically be held together by many more bipartite interactions. However, the stoichiometry of these particles was not yet solved, which allows for several interpretations. First, the quaternary structure may employ various interactions depending on its functional status or way of assembly. Second, the associations may succeed each other rather than coexist at one particular time, which is what was demonstrated for the binding of ATP-dependent chromatin remodellers SWI/SNF and acetyltransferase complexes to transcription factors [74]. Third, the CBF1-SMRT-CIR-HDAC2-mSin3A complex, where each partner has a binding site for SKIP, may bind more than one molecule of SKIP. The dimerization (and perhaps oligomerization) capability of Snw1p/SnwA is to be noted [12].

The complexes participating in the regulation of transcription initiation are continuously changing objects, which must undergo error-free conformational changes and rearrangements during each functional cycle. The events of binding/dissociation of regulatory proteins are interlaced with events of covalent modification, peptide bond isomerization, or switches in conformation. SKIP can function by regulating a transition step between the repressing and activating assembly of subunits, which is what was proposed first by the Hayward group for CBF1 [10] and by analogy by the Gardiner group for VDR [50].

Three modes of SNW/SKIP activity can be hypothesized (fig. 2). In mode one, SNW/SKIP may facilitate the correct sequence of events at the site of preinitiation complex formation by bringing signaling partners in the vicinity of the appropriate target (fig. 2A). When the binding affinities are limiting, nuclear proteins need to reach above-average concentrations in order to assemble efficiently into complexes. Targeting those proteins at, or in the vicinity of, the assembly site might be of importance. SNW/SKIP could serve as decelerator for the regulatory components on their way through the nucleus, providing a means of compartmentalization [75, 76]. The partition of SNW proteins into 'matrix' fractions may reflect such a function [25]. In their last report, Zhou and Hayward suggested that SKIP and SMRT are important for intranuclear targeting of CBF1 on its way to specific sequences in the nucleus [77].

In mode two, SNW/SKIP can function by recruiting the prolyl isomerases of the PPIL1 type, which then facilitate conformational changes and increase the 'conformational plasticity' of the protein complexes (fig. 2B), similarly to the SWI/SNF remodeling activities on nucleosomes [74]. This is a proposal in analogy to the model of SKIP/PPIL1 function in the spliceosome and can be experimentally tested (fig. 2C). The example that prolyl isomerase is important for the functioning of large protein complexes can be found with the RNA polymerase II carboxy-terminal domain, where the activity of such an enzyme (Ess1/Pin1) is essential for the proper association of RNA-processing factors [78].

In mode three, SNW/SKIP works by coupling transcription initiation to splicing. The 'coactivating' effects of SNW/SKIP, which were measured at the level of reporter gene(s) products, could be explained by such an ability [9, 21, 51]. Several unrelated proteins are now implicated in coupling transcription and splicing, constituting a growing class of coregulators (see supplementary table in [6]). Coupling the trans-acting DNA-binding proteins to the splicing machinery apparently represents a complex way of regulating gene expression (e.g. WT1 [79], p52 [80] or PGC-1 [81]). The partition of SNW/SKIP in nuclear-matrix pellets [25] may well be of importance, as many splicing-related proteins associate with 'nuclear matrix' [82]. Finally, considering the involvement of U2AFs and PABP2 in mRNA transport [42–44], the SNW-coupling may also include the more downstream steps of pre-mRNA processing.

SNW and cell cycle regulation

Recent data made it likely that SNW proteins serve the same essential function in all eukaryotes. The knockout of *PRP45* and *snw1* gene is lethal in the haploid in *S. cerevisiae* and *S. pombe*, respectively [19, 12]. The

Drosophila and *Caenorhabditis* homologs are required for embryonic development of many tissues [14, 70].

The essential function of SNW proteins may be related to the requirement of SKIP for the functioning of the cell cycle regulator pRb [59]. SKIP synergized with Ski in overcoming the transcription repressing ability of pRb and a related pocket protein p130. The effects were demonstrated both on the activity of recombinant reporter genes and on the growth phenotype of pRb-null Saos-2 cells. SKIP, together with Ski, released the cells from G1 arrest induced by pRb expression. When bacterially expressed proteins were used, pRb-SKIP binding was strong (it mapped to amino acids 171–353 of SKIP), whereas Ski-SKIP binding was nil to negligible [59]. The lack of Ski-SKIP interaction in vitro may have reflected the lack of posttranslational modifications, defects in conformation or simply the absence of direct interaction. The authors suggested that the protein responsible for bringing Ski and SKIP in complex was pRb, but more careful scrutiny of the list of SNW/SKIP binding partners is probably needed. Their results may imply that pRb is behind the reported effects of SKIP on Smad-based complexes, Ski, MyoD and E7 (see below), where the participation of pRb was demonstrated before [83, 84]. At the same time, the evidence of direct interactions between SNW/SKIP and several proteins seems very solid, and the range of SNW/SKIP partners is clearly distinct from the range of pRb involvements (see fig. 1 and table 1).

SKIP binds not only to pRb, but also to the E2F transcription factors, as suggested by two hybrid experiments (E2F1 and E2F3; [85]). It could be argued that the binding of E2F1/3 is mediated through pRb, but then the other E2F members would also be expected to bind, which is not the case (see table 1 in [85]). E2F transcription factors can act both as repressors and activators, orchestrating cell cycle checkpoint-dependent changes in gene expression [86]. The cell cycle-regulatory effects of pRb are believed to occur mainly through E2Fs, which it binds in its unphosphorylated state, turning them into repressors. The phosphorylation of pRb by cyclin-dependent kinases releases pRb and induces E2Fs to serve as activators. Intriguingly, the change from repressing to activating E2F complexes is the type of exchange for which SKIP seems to be needed (see above).

The pRb tumor suppressor is one of the targets of E7, the immortalizing protein of human papillomavirus (HPV; [87]). E7 resembles the Ski oncoprotein in its binding to pRb [88]. Banks and co-workers showed that one other cellular target for E7 is SKIP and identified two E7 mutants, p31/32 and Δ52–56, that showed both greatly reduced transforming ability and no SKIP binding ([87] and references therein). The carboxy-terminal part of E7 and the central portion of SKIP, containing the proline-rich motif (amino acids 220–236), were involved in the interaction, which was proved to be direct. E7 attenuated reporter gene activity, which was induced cooperatively by Ski and Skip in both pRb-containing and -deficient cells. The E7 mutants lost this ability. Other E7 mutants exist, however, that are transformation deficient and positive for SKIP binding, indicating that SKIP interaction is only part of the scenario of E7-mediated cellular immortalization. Nevertheless, SNW/SKIP may become relevant as a target for blocking the viral life cycle and associated cellular transformation.

SNW/SKIP aids distinct transcription factors in various systems, but not quite in a manner of general transcription factors or TAFs (TBP-associated factors). Human SKIP was found to coregulate only at certain promoters [58], and in *Drosophila* it associated only with a subset of transcriptionally active puffs of salivary polytene chromosomes [15]. The employment in mammals of SKIP by pathways that didn't exist at the time the SNW structure was functioning in, e.g., *S. pombe* prompts the question – why just these transcription factors? The target genes may require specific function during pre-mRNA processing [89]. Such a function could (i) support promoter-specific alternative splicing [90], (ii) selectively regulate splicing efficiency (see below) or (iii) serve certain type of cell cycle or cell division checkpoint(s) [91]. The proteins of the so-called cell cycle and splicing complex (CSC) are required for efficient splicing of specific introns in two genes for yeast tubulin. The depletion of some of the CSC factors leads to the decrease in the levels of tubulin mRNA and to cell cycle stalling via the spindle checkpoint. The CSC mechanism is likely to be conserved, as human homologs of the CSC proteins have been identified ([89] and references therein). The authors propose that linking pre-mRNA splicing to spindle integrity may be a novel regulatory network for guarding genome stability [92]. It is of consideration that most of the CSC components are included within the CDC5 complexes, together with Prp45p/Snw1p (see above; [30, 31]).

Concluding remarks

We interpret the accumulated evidence to mean that the SNW proteins work by aiding the assembly or regulating the rearrangements of 'nuclear machines', such as the ternary complexes of transcription factors or spliceosomes. The inclusion of SNW proteins in the residual 'nuclear matrix' preparations and their interaction with the PPIL cyclophilins suggest two nonexclusive hypotheses for future testing. (i) SNW interacts with the less mobile nuclear components to serve as an interaction platform or to coordinate the spatiotemporal changes of nucleoprotein complexes. (ii) SNW recruits the PPIL-type foldases to the complexes, increasing the rate of conformational changes and exchange of components. By virtue of its selective involvement in the complexes of tran-

scription initiation and splicing, SNW may functionally couple both processes.

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