Spliceosome assembly is a dynamic process involving the sequential recruitment and rearrangement of small nuclear ribonucleoproteins (snRNPs) on a pre-mRNA substrate. Here we identify several spliceosome protein interactions with different domains of human splicing factor SPF30 that have the potential to mediate the addition of the tri-snRNP to the prespliceosome. In particular, we show that the C-terminal tails of SmD1, SmD3, and the protein Lsm4 interact with the central Tudor domain of SPF30. We identify a novel interaction between the N-terminal domain of SPF30 and U2AF35, a prespliceosome protein that has a role in recognizing the 3′ splice site and recruiting U2 snRNP. We also show that the C terminus of SPF30 interacts with a middle domain of hPrp3, a component of U4/U6 di-snRNP and the tri-snRNP. Importantly, we show that the U2AF35 and hPrp3 interactions with SPF30 can occur simultaneously, thereby potentially linking 3′ splice site recognition with tri-snRNP addition. Finally, we note that SPF30 and its partner-interacting domains are not conserved in yeast, suggesting this interaction network may play an important role in the complex splicing observed in higher eukaryotes.

The spliceosome is the large and dynamic macromolecular complex responsible for removing intron sequences that interrupt many eukaryotic gene transcripts. Spliceosomes are composed of five small nuclear ribonucleoproteins (snRNPs), known as U1, U2, U4, U5, and U6, and more than 100 additional proteins (1). In vitro splicing studies show that snRNPs and additional protein factors come together with a pre-mRNA transcript in an ordered assembly via short-lived intermediate complexes to carry out the chemistry of splicing (2). Precisely how the assembly of these larger complexes is executed and controlled and which factors are responsible are poorly understood.

Much of our knowledge about the identity and function of individual spliceosome proteins is the result of extensive genetic and biochemical analyses in the yeast *Saccharomyces cerevisiae* (3, 4). Although the yeast spliceosome is fundamentally similar to the mammalian spliceosome, the mammalian spliceosome must handle more complex splicing substrates and is subject to more complex regulation. Therefore, it is likely that some mammalian splicing factors have no functional correlate in yeast. Consistent with this idea, recent analyses of human spliceosomes by mass spectrometry identified a significant number of proteins not previously associated with splicing (1, 5–7). Many of these proteins do not have an obvious homolog in *S. cerevisiae*, and subsequent *in vitro* studies demonstrated a critical role for some of these factors in splicing (8–10). A major challenge is to determine the specific functions of these specialized mammalian splicing factors.

Human SPF30 (also known as SMNrp) protein was first identified by mass spectrometry as a component of purified spliceosomes (5) and initially characterized as a nuclear protein containing a Tudor domain, a motif found in RNA-binding proteins implicated in binding methylated arginines (11). Although the sequence of SPF30 protein is generally conserved within higher eukaryotes, there is no *S. cerevisiae* homolog. SPF30 does share ~51% sequence identity within the Tudor domain of SMN (survival of motor neuron), a protein important for snRNP biogenesis (40). Mutations in SMN are believed to be responsible for its role in the autosomal recessive disease spinal muscular atrophy (12). The Tudor domain of SMN is sufficient for binding four of the Sm/Lsm proteins that make up a core heptamer of the U snRNPs (SmB, SmD1, SmD3, and Lsm4). These four proteins contain arginine- and glycine-rich (RG-rich) motifs, and SMN protein has been shown to bind preferentially to dimethylarginine-modified residues within the RG-rich repeats of SmD1, SmD3 (13). In addition, the Tudor domains of both SMN and SPF30 have been shown to preferentially bind a synthetic symmetrically dimethylated (R<sup>dimA</sup>)<sup>4</sup> peptide identical to a sequence within the C terminus of the human SmD1 protein (14).

A critical role for SPF30 in splicing was demonstrated in a human *in vitro* system. Immunodepletion of the SPF30 protein from human nuclear cell extracts blocks spliceosome assembly on a minimal splicing substrate at the transition from prespliceosomes (A complex) to assembled spliceosomes (B complex) but does not appear to be necessary for tri-snRNP assembly (8, 9). Additionally, SPF30 was identified by mass spectrometry in purified human prespliceosomes and spliceosomes but was not detected in spliceosomes activated for catalysis in which the U4

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2 The abbreviations used are: snRNP, small nuclear ribonucleoprotein; RG-rich, arginine-rich; GST, glutathione S-transferase; Y2H, yeast two-hybrid.
and U1 snRNP association is destabilized (C complex) (6, 7, 15–17). The prespliceosome to spliceosome transition is characterized by the addition of the U4/U6-U5 tri-snRNP to a complex of U1 and U2 snRNPs bound, respectively, to the 5' splice site and branch point region of a pre-mRNA transcript. SPF30 has been shown to associate with both the U2 17 S snRNP and the U4/U6-U5 tri-snRNP, suggesting that it may act as a bridge between the prespliceosome and tri-snRNP (8, 9). A direct protein interaction between SPF30 and Prp3, a U4/U6 di-snRNP protein, was reported (8). However, the mechanism, including its specific interaction partners and regulation, by which SPF30 serves as this potential bridge is unknown.

Here we present data identifying SPF30-interacting partners and map those interactions to individual protein domains. Our results support a model in which specific domains of SPF30 interact with components in both the prespliceosome and the tri-snRNP, potentially joining them together within the spliceosome. Furthermore, we note that these interactions are not conserved in yeast, suggesting they may be important for the more complex splicing of multi-intron pre-mRNAs found in humans and other multicellular organisms.

EXPERIMENTAL PROCEDURES

Recombinant Protein Expression and Purification—Full-length cDNA were cloned from HeLa cell total RNA (Ambion™). Recombinant GST-tagged proteins were expressed in Escherichia coli purified by glutathione affinity (GSTrap™; GE Healthcare) followed by size exclusion chromatography (Superdex 200™; GE Healthcare).

GST-SPF30 Chromatography and Detection of snRNPs from HeLa Nuclear Extract—Approximately 1.0 mg of purified GST-SPF30 protein was coupled to Affi-Gel 10™ affinity matrix (Bio-Rad). HeLa nuclear extract (18) equilibrated in 150 mM KCl, 20 mM Tris, pH 7.4, 0.2 mM EDTA, 10% glycerol, 0.1% Nonidet P-40, and 10 mM dithiothreitol was passed over the column. It has been observed that U4/U6-U5 tri-snRNP complex can be disrupted upon repeated freeze-thawing (19), so we used freshly prepared nuclear extract to avoid disruption of spliceosome subcomplexes. After washing, snRNPs were eluted from the column using a gradient from 0.15 to 2 M KCl. RNA was purified from eluates using phenol/chloroform isolation and ethanol precipitation and separated by urea-PAGE electrophoresis. Standard Northern blot analysis was carried out using antisense U1, U2, U4, U5, and U6 snRNA probes (20).

Yeast Two-hybrid Assay—cDNAs were cloned from HeLa total RNA into the Yeast Matchmaker™ GAL4 two-hybrid system 3 (Clontech). AH109 and Y187 yeast strains were transformed with bait and prey vectors, respectively. Whole cell lysates from yeast strains expressing each bait and prey vector were analyzed by immunoblotting with anti-c-Myc or anti-hemagglutinin antibodies, respectively, to ensure that each bait and prey protein was expressed and of the correct molecular weight. All of the possible combinations of bait and prey matings were tested, and diploid yeast strains were selected on Trp−Leu− plates. Diploids were then grown in Trp−Leu− liquid medium and plated onto either Trp−Leu− plates or onto Trp−Leu+His− plates to test for interaction between the bait and prey proteins.

GST-SPF30 Pulldown of 35S-Labeled in Vitro Translated Proteins—In vitro translated proteins generated using TnT® quick coupled transcription/translation system (Promega). For GST pulldown assays 25 µl in vitro TnT® reactions were split into two and added to glutathione agarose resin (Sigma) bound to 50 µg of purified GST-SPF30 or GST alone. These mixtures were allowed to incubate with rotation for 2 h at 4 °C, followed by three 10-min washes in buffer containing 150 mM KCl, 20 mM Tris, pH 7.4, 10% glycerol, 0.1% Nonidet P-40, 10 mM dithiothreitol. The washed glutathione resin was resuspended in 25 µl of sample buffer and boiled, and the samples were analyzed by 10% SDS-PAGE and phosphorimaging.

U2AF Depletion from Nuclear Extract and Immunoblotting—U2AF depletions were carried out as previously described (21). Briefly, 1 ml of HeLa nuclear extract was incubated with 2 ml of oligo(dT) cellulose (NEB) for 30 min at 4 °C in 1 M KCl, 20 mM HEPS, pH 7.9, 0.5 mM EDTA, 10% glycerol, 0.1% Nonidet P-40, and 1 mM dithiothreitol. The column was washed in the same buffer except with 2.5 M KCl followed by an elution containing 2 M guanidine HCl. Eluted fraction was dialyzed against 100 mM KCl, 20 mM HEPS, pH 7.9, 0.5 mM EDTA, 10% glycerol, 0.1% Nonidet P-40, and 1 mM dithiothreitol, concentrated, and stored at −80 °C. The presence of U2AF35 in depleted extracts and eluate was assayed by immunoblotting against U2AF35 (ProteinTech).

RESULTS

Distinct Domains of SPF30 Interact with Specific snRNPs—SPF30 is a critical splicing factor in human extracts and interacts with multiple spliceosome snRNPs (8, 9). To gain insight into the role of SPF30 in splicing, we asked whether the snRNP interactions are distinct and mediated by different domains of SPF30. Using limited trypsin digests of purified SPF30 protein and in silico protein structure modeling, we predicted three distinct structural domains of SPF30 (data not shown). We expressed and purified GST-tagged versions of these predicted domains from bacteria (Fig. 1A) and obtained very pure soluble protein (Fig. 1B). We then tested the association of the SPF30 domains with snRNPs by an in vitro binding assay in which we passed HeLa nuclear extract over columns made from different SPF30 domains covalently linked to beads. After washing extensively, we eluted interacting species with a salt gradient and detected spliceosomal U snRNAs in elution fractions by Northern blotting (Fig. 2). We also show by Western analysis that the common snRNP protein SmB/B′ is present in the pooled column eluate, supporting the hypothesis that snRNA detection is indicative of the corresponding snRNP (Fig. 2A).

Previous studies showed that full-length SPF30 pulls down U4 and U6 snRNPs and to a lesser extent U2 and U5 snRNPs from HeLa nuclear extract (8, 9). Notably, no U1 association was detected. In contrast, we readily detect all five snRNAs (U1, U2, U4, U5, and U6) in the pooled column eluate with full-length SPF30 (1–238) (Fig. 2B). Interestingly, full-length SPF30 differentially binds the snRNPs in a salt-dependent manner (Fig. 2C). The most robust interaction is with U4 and U6 snRNPs, which both elute at ~1.5 M KCl. It is likely that the coelution of U4 and U6 represents a U4/U6 di-snRNP. U5 snRNP elutes in a peak that coincides with the peak of U4 and
U6, likely as a member of the tri-snRNP. U1 and U2 snRNPs show a weaker interaction, with both peaking at ~0.6 M KCl. As expected, with GST alone we detect neither SmB/B’ nor snRNA association (Fig. 2, A, B, and H). We conclude that SPF30 interacts either directly or indirectly with all of the snRNPs, but with differential affinities.

To dissect the SPF30 domains responsible for the snRNP interactions, we repeated this analysis with columns carrying fusions of GST to distinct structural domains of SPF30. An N-terminal region of SPF30 (amino acids 1–65) binds primarily U2 and U1 snRNPs (Fig. 2D). Surprisingly, the central Tudor domain of SPF30 (amino acids 66–126) binds only U1 snRNP (Fig. 2E). Using a C-terminal domain (amino acids 127–238), mainly U4 and U6 snRNPs are bound, and, to a much lesser extent, U5 snRNP (Fig. 2F). SPF30 lacking the Tudor domain (Δ66–126) binds U2, U4, and U6 snRNPs and appears to have minimal interactions with U1 and U5 (Fig. 2G).

These results clearly show that distinct domains of SPF30 are responsible for interacting with different snRNPs. The N-terminal domain binds specifically with both U2 and U1 or with a complex that contains both snRNPs. The C-terminal domain interacts with U4 and U6 snRNPs, likely as a di-snRNP. U1 and U5 associations cannot be assigned to SPF30 domains in a straightforward manner. U1 interacts strongly with the Tudor domain of SPF30 but also appears to interact with the N-terminal domain in some contexts, possibly indirectly through U2 snRNP. We see strong U5 association only with full-length SPF30, likely as a member of the U4/U6-U5 tri-snRNP.

SPF30 Interacts with Proteins in the Prespliceosome and Tri-snRNP—To identify which snRNP-associated protein factors might interact directly with SPF30, we carried out a yeast two-hybrid (Y2H) screen with U1, U2, U4, and U6 snRNPs, respectively. Using this assay we identified several proteins that potentially interact with SPF30: SmD1, SmD3, the U6-specific protein Lsm4, hPrp3 (U4/U6 90-kDa protein), and, surprisingly, U2AF35, a key protein factor involved in 3’ splice site recognition and prespliceosome assembly (22–25) (Figs. 3A, 4A, and 5A, respectively). The interaction with hPrp3, which is
a component of both the U4/U6 di-snRNP and tri-snRNP, was not unexpected given previous studies that showed in vitro binding of this protein with SPF30 (8). However, the interactions with Sm, Lsm, and, in particular, U2AF35 are novel. To further confirm these interactions and map them to specific domains within the proteins, we applied both the Y2H system and in vitro pulldowns as described below.

The SPF30 Tudor Domain Is Sufficient to Interact with SmD1, SmD3, and Lsm4—Starting with SmD1 and SmD3, we tested both the N- and C-terminal domains of these proteins using boundaries identified in a structural study of Sm proteins (26). The N-terminal domain of SmD1 and SmD3 contains the characteristic SmI and SmII β-strand motifs required for heptameric ring formation, whereas the C-terminal domain contains RG-rich repeats that can be dimethylated. As observed by Y2H, the central Tudor domain of SPF30 is sufficient to interact with SmD1, SmD3, and Lsm4 (Fig. 3, A, C, and E). Interestingly, both the full length and the Tudor domain alone of SPF30 interact with the C-terminal domains of both SmD1 and SmD3. In contrast, deletion of the C-terminal from either SmD1 or SmD3 provides no or very weak Y2H signal (Fig. 3, A and C). These results suggest that SPF30 interacts with a subset of the Sm/Lsm proteins. Given previous studies showing that the isolated Tudor domain of SPF30 can bind an RG-rich repeat containing peptides (14), it is very likely that the Sm/Lsm protein interactions with SPF30 are mediated by its Tudor domain and the RG-rich C-terminal tails of SmD1, SmD3, and Lsm4. Interestingly, of the 14 spliceosomal Sm/Lsm proteins, only SmD1, SmD3, and Lsm4 contain extensive RG-rich C-terminal tails.

To confirm the interaction between these proteins, we performed in vitro GST pulldown experiments using recombinant GST-tagged SPF30 and 35S-labeled in vitro translated SmD1, SmD3, and Lsm4. With full-length GST-SPF30 we readily detect all three proteins in the pulldowns and observe little or no 35S-labeled protein in pulldowns with GST alone (Fig. 3, B, D, and F). These results further support a direct interaction of the SPF30 Tudor domain with the RG-rich repeat-containing Sm/Lsm proteins.

Specific Domains of SPF30 Interact with the U4/U6 90-kDa Protein hPrp3 and the 17 S U2 snRNP Protein U2AF35—To further delineate the interaction of SPF30 with hPrp3, we tested several hPrp3 domains using domain boundaries for hPrp3 described previously in a study exploring protein interactions between hPrp3 and hPrp4 (27). We observe a positive Y2H signal when full-length hPrp3 is tested with either full-length SPF30 or SPF30ΔTudor, but not with SPF30 Tudor domain alone (Fig. 4A). All of the hPrp3 domains containing amino acids 195–442 give a positive Y2H signal with both SPF30 and SPF30ΔTudor, but not those lacking these central residues. These results suggest that it is the central region of hPrp3 that interacts with SPF30. To demonstrate a direct interaction, we performed pulldown experiments using recombinant GST-SPF30 and in vitro translated hPrp3. Using either the full length or the C-terminal domain (127–238) of GST-tagged SPF30, we readily detect in vitro translated hPrp3 in pulldowns (Fig. 4B). As observed in the yeast two-hybrid assays, hPrp3
proteins containing residues 195–442, but not those lacking these residues, are pulled down by SPF30. GST alone does not pull down any of the hPrp3 protein constructs. These results show that the C-terminal domain of SPF30 is sufficient to interact with the central domain of hPrp3.

Of the 17 S U2 snRNP proteins we tested in the Y2H screen, only U2AF35 gives a positive signal. We used an approach similar to that described for hPrp3 above to map the regions of U2AF35 and SPF30 that interact with each other. Using the N-terminal (amino acids 1–146) and C-terminal domains (amino acids 147–240) of U2AF35 based on earlier structural studies of the protein (28), we assayed for interactions with SPF30. Both SPF30 full-length and SPF30ΔTudor show positive Y2H signal with full-length or the N-terminal domain of U2AF35 (Fig. 5A). This result suggests that the N-terminal domain of U2AF35 interacts with SPF30 residues outside of the Tudor domain. To confirm a direct interaction, we performed in vitro GST pulldown experiments using either GST-tagged full-length or N-terminal domain SPF30. We readily detect both in vitro translated U2AF35 full length (1–240) and truncated constructs containing the N-terminal domain residues 1–146 and residues 43–146, the latter of which lacks the zinc knuckle motif of U2AF35 (Fig. 5B). No U2AF35 pulldown is observed with GST alone.

Because U2AF35 exists as a stable heterodimer with U2AF65, we also tested whether SPF30 interacts with U2AF35 in the presence of U2AF65 (Fig. 5C). We find that although SPF30 does not interact with U2AF65 alone by yeast two-hybrid or by in vitro binding (data not shown), it can pull down both U2AF35 and U2AF65 when present in an in vitro GST binding assay. Combined, these data show that the N-terminal domain of SPF30 is sufficient to interact with the N-terminal domain of U2AF35.

U2AF35 Is Insufficient Alone to Mediate the SPF30 Interaction with U2 snRNP—In nuclear extracts SPF30 protein previously was shown by glycerol gradient centrifugation to associate with the 17 S U2 snRNP but not U4/U6 di-snRNP or U4/U6/U5 tri-snRNP (8). This observation prompted us to ask whether the SPF30 interaction with U2AF35, a 17 S U2 snRNP associated protein, is sufficient to mediate the SPF30 association with U2 snRNP. For these experiments, we depleted nuclear extracts of U2AF35 protein using oligo(dT) cellulose chromatography (Fig. 6A) (22, 29). This technique efficiently depletes extracts of both
U2AF65 and U2AF35 (U2AF) while allowing for the subsequent purification of the U2AF heterodimer. We incubated U2AF-depleted nuclear extracts with GST-SPF30 N-terminal domain bound to glutathione resin in the presence or absence of purified U2AF. Surprisingly, the N-terminal domain of SPF30 efficiently pulls down U1 and U2 snRNPs from extracts depleted of U2AF35 (Fig. 6B). Similarly, SPF30 association with 17 S U2 snRNP is not affected in U2AF-depleted extracts as observed by glycerol gradient centrifugation (data not shown). SPF30 association with U1 snRNP observed in these pulldown experiments remains to be further characterized. These results suggest that factor(s) additional to U2AF35 facilitate the SPF30 association with U2 snRNP.

**SPF30 Bridges an Indirect Interaction between U2AF35 and hPrp3**—Previous studies suggested that SPF30 may serve as a bridge between presplicosomes and tri-snRNP, thereby allowing splicosomes to assemble (8, 9). In this study we identified and characterized interactions via two distinct domains of SPF30 with both U2AF35 and Prp3, two proteins associated with presplicosomes and tri-snRNP, respectively. Given these results, we tested whether SPF30 can associate with both U2AF35 and hPrp3 simultaneously. We bound recombinant GST-Prp3 (196–402) protein to glutathione resin and incubated with 35S-labeled U2AF35 (1–146) in the presence or absence of recombinant SPF30. GST alone either in the presence or absence of SPF30 did not precipitate U2AF35, whereas GST-Prp3 in the presence of SPF30 significantly enhanced the precipitation of U2AF35 (Fig. 7). These results suggest that SPF30 may promote an indirect interaction between hPrp3, a core tri-snRNP component, with U2AF35, an essential splicing factor present in presplicosomes.

**DISCUSSION**

Splicosome assembly is a highly complex and dynamic process involving a myriad of proteins and RNAs. Although a number of specific interactions that mediate splicosome assembly and function have been elucidated (primarily by genetic studies in *S. cerevisiae*), a large portion remains to be characterized. In particular, the interactions that govern tri-snRNP association with splicosomes are not fully understood. Furthermore, there are likely many additional interactions required by the more complex splicing machinery in more complex eukaryotes. In this study, we focused on the human protein SPF30 and its interactions with splicosome components.

We find that full-length SPF30 interacts directly and/or indirectly with all five U snRNPs. Using truncated SPF30 proteins, we assigned specific snRNP interactions to distinct domains of SPF30. The N-terminal domain of SPF30 interacts predominantly with U2 and U1 snRNPs. The Tudor domain alone appears to interact exclusively with U1 snRNP, whereas the C-terminal domain interacts with the U4/U6 di-snRNP. Importantly, these observations correspond with the specific snRNP proteins that we subsequently found interact with SPF30 domains. The N-terminal domain of SPF30 binds U2AF35, a protein associated with the presplicosome and 17 S U2 snRNP. We also showed that SPF30 interacts with U2AF35 in the presence of U2AF65, suggesting that SPF30 interacts with an intact U2AF heterodimer. However, this interaction alone is not sufficient to explain SPF30 association with U2 snRNP. Most studies on U2AF35 focus on its role in early splicosome formation (22–25). U2AF35 interacts with its partner U2AF65 via residues 47–172 (N-terminal domain) (30). Together, the U2AF65/ U2AF35 dimer recognizes the pre-mRNA polypurimidine tract and 3′ splice site, respectively. U2AF65 has been shown to interact with SF3b155, a component of U2 snRNP, and this interaction may facilitate the recruitment and stable association of U2 snRNP with the pre-mRNA (31, 32). Similarly, SPF30 interaction with U2AF35 may facilitate recruitment and stable association of tri-snRNP with the presplicosome. U2AF35 is still present in fully assembled splicosomes, although its role at this later stage of assembly is not understood (17). It is intriguing to speculate that U2AF may play a critical role in both presplicosome formation and the transition to assembled splicosomes.

The C-terminal domain of SPF30 interacts with hPrp3. hPrp3 is a component of the U4/U6 di-snRNP and tri-snRNP, contacting hPrp4, hPrp6, and hSnu66 (33). It appears to join the splicosome with tri-snRNP during the formation of B complex (17). It is possible that tri-snRNP recruitment to the splicosome is mediated in part by the interaction of the C-terminal domain of SPF30 and hPrp3. Thus, when SPF30 is depleted from extracts, splicosomes arrest at presplicosomes as observed previously (8, 9). Importantly, we show that the U2AF35 and hPrp3 interactions with SPF30 can take place simultaneously. Previously, both Meister et al. (8) and Rappsilber et al. (9) hypothesized that SPF30 serves as a bridge between U2 snRNP and tri-snRNP at the transition to assembled splicosomes. Our results support this model and provide a potential mechanism where SPF30 may function as a link between 3′ splice site recognition in the presplicosome and recruitment of the tri-snRNP (Fig. 8), although more studies are needed to establish this function concretely.

It is also plausible that U2AF35/SPF30 and SPF30/hPrp3 binding events are temporally ordered. Based on mass spectrometry analysis of purified splicing complexes, SPF30 appears to join the splicosome at presplicosome assembly and prior to tri-snRNP addition (6, 15). Interestingly, we only observe a strong U4/U6 di-snRNP association with recombinant SPF30 in pulldown experiments. In contrast, endogenous SPF30 in nuclear extracts appears exclusively associated with U2 17 S snRNP and not U4/U6 di-snRNP or tri-snRNP. These observations suggest that there may be a mode to regulate the association of SPF30 with hPrp3. Upon splicosome activation, both U1 and U4 snRNP association with the splicosome is destabi-
interaction observed between SPF30 Tudor domain and U1 snRNP may simply be due to the accessibility of the Sm heptamer of U1 snRNP, whereas the Sm/Lsm heptamer of U2, U4, U5, and U6 snRNPs may be inaccessible in their assembled nuclear state.

Alternatively, the ability of the SPF30 Tudor domain to preferentially bind SmD1, SmD3, and Lsm4 may suggest an additional role in nuclear snRNP subcomplex assembly. The Tudor domain of SPF30 shares 51% identity to SMN protein, which plays an important role in snRNP biogenesis by assembling the Sm proteins into the nascent snRNPs (36). Like SPF30, SMN also binds directly to SmB/D1/D3 and Lsm4 via its Tudor domain (35). Furthermore, SPF30 colocalizes with SMN protein at Cajal bodies (8), a site of snRNP accumulation and putative subcomplex assembly (37–39).

Finally, we note that the SPF30 and its interaction partner domains are not conserved in S. cerevisiae. As with SPF30, there is no yeast homolog of U2AF35. Yeast Prp3 does not have a similar central domain. Yeast Prp3 and human Prp3 primarily show conservation in the C-terminal domain. Moreover, the C-terminal tails of SmD1, SmD3, and Lsm4 are not present in yeast. The presence of these elements in humans, but not in S. cerevisiae may be reflective of the differences in splicing in these organisms. In general, S. cerevisiae pre-mRNAs have single, relatively short introns, whereas human pre-mRNAs feature multiple introns that can be megabases in length and are often alternatively spliced. The spliceosome assembly process in humans, therefore, likely requires additional protein machinery not found in yeast. It is intriguing to speculate that an assembly of protein interactions, including SPF30, evolved in higher eukaryotes to coordinate the task of assembling spliceosomes on complex, multi-intron transcripts.

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