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The small subunit of the splicing factor U2AF is conserved in fission yeast

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ABSTRACT

The human splicing factor U2 auxiliary factor (hsU2AF) is comprised of two interacting subunits of 65 and 35 kDa. Previously we identified the *Schizosaccharomyces pombe* homolog, spU2AF^{59}, of the human large subunit. We have screened a fission yeast cDNA library in search of proteins that interact with spU2AF^{59} using the yeast two-hybrid system and have identified a homolog of the hsU2AF^{35} subunit. The *S.pombe* U2AF small subunit is a single copy gene that encodes a protein which shares 55% amino acid identity and 17% similarity with the human small subunit. Unlike the human protein, the yeast protein lacks an arginine/serine-rich region. The predicted molecular mass of the spU2AF small subunit is 23 kDa. The region of spU2AF^{59} that interacts with spU2AF^{23} is similar to the region in which the human small and large subunits interact.

INTRODUCTION

Splicing proceeds in a two-step pathway (1–3). The first step involves cleavage of the pre-mRNA at the highly conserved 5′ splice site (GU). The result of this step is a linear first exon and a lariat intron–second exon intermediate. The second step of splicing involves cleavage of the 3′ splice site (AG) and ligation of the 5′ phosphate of the second exon to the 3′ hydroxyl of the first exon, producing the mRNA and the lariat intron.

Splicing takes place in a dynamic complex termed the spliceosome (4–6). The spliceosome is thought to be comprised of at least 50 components, many which still need to be identified and characterized (7). The components that have been previously identified include small nuclear ribonucleoproteins (snRNPs) and non-snRNP splicing factors. These factors interact with the pre-mRNA as well as each other to drive splicing.

The first ATP-dependent step in spliceosome assembly involves binding of the U2 snRNP particle to the branch point sequence (8–10). In mammals this step is facilitated by the non-RNP auxiliary factor U2AF, which recognizes and binds to the 3′ splice site prior to U2 snRNP binding (11). Human U2AF consists of two subunits of 65 and 35 kDa (12). The specific interaction of the U2 snRNP with the branch point sequence requires the U2AF large subunit (11). hsU2AF^{65} binds *in vitro* to the characteristic polypyrimidine-rich region found in mammalian introns between the branch point sequence and the 3′ splice site (13). hsU2AF^{65} is essential for splicing *in vitro* and the fission yeast homolog, spU2AF^{59}, and the fruit fly homolog, dmU2AF^{50}, are essential splicing factors *in vivo* (14–16).

The small subunit of hsU2AF was identified through its co-purification with the large subunit (12,13). The gene for the small hsU2AF subunit has been cloned and sequenced (17). hsU2AF^{35} contains two serine/arginine (SR) motifs in its C-terminus separated by a stretch of 12 glycines. Deletion studies using *in vitro* translated proteins have determined that the central region of hsU2AF^{35} is necessary for binding to hsU2AF^{65}. This region does not include either of the S/R domains. In contrast to hsU2AF^{65}, hsU2AF^{35} has not been shown to be essential for splicing *in vitro*. The function of hsU2AF^{35} remains unknown.

Recently, Wu and Maniatis have identified protein–protein interactions that take place between hsU2AF and other known splicing factors (18). Specifically, they have shown that hsU2AF^{65} interacts with hsU2AF^{35} *in vivo* using the yeast two-hybrid system. hsU2AF^{35} in addition to binding with the large subunit can also interact with itself and the SR splicing factors SC35 and SF2/ASF. SC35 and SF2/ASF in turn bind through protein–protein interactions to the U1 70 kDa protein, which is part of the U1 snRNP complex that binds to the 5′ splice site of introns. The yeast two-hybrid system is a sensitive assay that allows rapid identification of protein interactions that are weak and transient.

Here we report the cloning of the fission yeast U2AF small subunit. The spU2AF small subunit was identified by its protein–protein interaction with spU2AF large subunit through screening of a fission yeast cDNA library using the yeast two-hybrid system. The predicted amino acid sequence shows a high degree of similarity with the hsU2AF small subunit. Through deletion studies, we have identified the region of spU2AF^{59} that interacts with the spU2AF small subunit.

MATERIALS AND METHODS

Yeast two-hybrid screen

The yeast two-hybrid screen for protein interactions with spU2AF^{59} was done using a *Schizosaccharomyces pombe* cDNA

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library subcloned into the *GAL4* activation domain (AD) of the plasmid pGADGH which carries the *LEU2* gene of *Saccharomyces cerevisiae* (Clonetech, Palo Alto, CA). Plasmid from the library was prepared as described in the Clonetech Matchmaker library protocol. spU2AF^{59} was subcloned in-frame using the *Bam*HI site downstream of the *GAL4* DNA binding domain in the plasmid pGBT9, which carries the *TRP1* gene. Yeast transformations were carried out sequentially as described in Schiestl *et al.* (20). Y190 (MAT**a** *ade2 gal4-Δgal80-Δhis3 leu2-3, 112 trp1-Δ901 ura3-52 LYS2*::*GAL1-HIS3 URA3*::*GAL1-lacZ*) was used as the host yeast strain for screening the library (21). HF7c [MAT**a**, *ura3-52 his3-200 lys2-801 ade2-101 trp1-901 leu2-3, 112 gal4-542 gal80-538 LYS2*::*GAL1-HIS3 URA3*::(*GAL4* 17mers)$_3$-*CYC1-lacZ*] was used as the host yeast strain for the promoter control experiments (22). Transformants carrying pGBT9/spU2AF^{59} were selected on minimal medium lacking tryptophan. Transformants containing pGBT9/spU2AF^{59} and the *S.pombe* cDNA (spcDNA) library were selected on minimal medium lacking leucine, tryptophan, uracil and histidine and containing 3-aminotriazole. β-Galactosidase filter assays were performed on colony lifts from the yeast transformation plates (Clonetech Matchmaker Protocol). spcDNA library plasmids that gave positive results in the β-galactosidase filter assay were recovered by growing the strain for several generations in minimal medium without leucine and plating the cells on minimal medium either without leucine or without leucine and tryptophan. This allowed the identification of strains that were missing the pGBT9/spU2AF^{59} plasmid but carrying the pGADGH/spcDNA library plasmid. The strains carrying only the pGADGH/spcDNA library plasmids were grown in minimal medium without leucine and plasmids were prepared after glass bead breakage according to the Clonetech Matchmaker protocol.

Construction of spU2AF^{59} and spU2AF^{23} deletion series

Full-length or shorter fragments of spU2AF^{59} and spU2AF^{23} were amplified by polymerase chain reaction (PCR) (23). Refer to Table 1 for oligonucleotides PRP2-*Bam*HI 5′, PRP2-Δ111 5′, PRP2-Δ284 3′ and PRP2-*Bam*HI 3′ used to subclone fragments of the large subunit gene and Clone A-*Bam*HI 5′, Clone A-*Bam*HI 3′, Clone A-Δ1–49 5′ and Clone A-Δ169–200 3′ for the small subunit gene. The PCR buffer contained 10 ng pIRT3/spU2AF^{59} plasmid, 20 pmol 5′ and 3′ oligonucleotides, 1 mM $MgCl_2$, 20 µmol dNTPs, 1× Taq polymerase buffer and 2.5 U Taq polymerase (Promega, Madison, WI) in a 100 µl reaction. Full-length or shorter fragments of spU2AF^{23} were amplified by PCR from wild-type *S.pombe* 972 genomic DNA. The PCR conditions were the same as above except 30 ng genomic DNA was used as template. The purified fragments were digested with *Bam*HI (New England Biolabs, Beverly, MA). pGBT9 and pGAD424 were digested with *Bam*HI and treated with 10 U calf alkaline phosphatase (New England Biolabs) according to the conditions suggested by the manufacturer. The PCR fragments were then subcloned in-frame into either pGBT9 or pGAD424. Correct orientation of the inserts was determined either through restriction enzyme analysis or dideoxy sequencing. All pairwise interactions were conducted with each construct in both the pGBT9 and pGAD424 plasmids.

Table 1. The nucleotide sequences of the deoxyoligonucleotides used in this study are listed

Oligonucleotides	Sequence	Experiments
Clone A-1 sense	5′-TTTAGTCAAACGATC-3′	Sequencing
Clone A-1 antisense	5′-GATCGTTTGACTAAA-3′	Sequencing
Clone A-2 sense	5′-CGGTGAAGTTGAACA-3′	Sequencing
Clone A-2 antisense	5′-TGTTCAACTTCACCG-3′	Sequencing
Clone A-5 sense	5′-GCATGCCAAAAAACC-3′	Sequencing
Clone A-5 antisense	5′-GGTTTTTTGGCATGC-3′	Sequencing
Clone A-6 sense	5′-GCCTTTGTAATTTTA-3′	Sequenicng
Clone A-11 antisense	5′-CGCGTAAAAGACTGAGGACTTGG-3′	PCR, 5′-end cloning
Adaptor primer 1	5′-CCATCCTAATACGACTCACTATAGGGC-3′	PCR, 5′-end cloning
M13 universal primer	5′-GTAAAACGACGGCCAT-3′	Sequencing
M13 reverse –20	5′-TTCACACAGGAAACAG-3′	Sequenincg
PRP2-*Bam*HI 5′	5′-TTGGATCCCAATGGATTTGTCTTCCAGA-3′	PCR, full-length, deletion series
PRP2-*Bam*HI 3′	5′-TTGGATCCCTTTTCATTGAAACTG-3′	PCR, full-length
PRP2-Δ111 5′	5′-TTGGATCCCAATCGAACAGGAACTC-3′	PCR, deletion series
PRP2-Δ284–518 3′	5′-TTGGATCCGATGTAATTTTGAATTCTT-3′	PCR, deletion series
Clone A-*Bam*HI 5′	5′-TTGGATCCCAATGGCAAGTCATTTGGCAAGTATTT-3′	PCR, full-length
Clone A-*Bam*HI 3′	5′-TTGGATCCTCGTGGAATCACTATTGGG-3′	PCR, full-length
Clone A-Δ1–49	5′-TTGGATCCCAAACATGTATAAAAACCCAATTCAT-3′	PCR, deletion series
Clone A-Δ169–200	5′-TTGGATCCTGAAAACTCACAAAACATGTCCT-3′	PCR, deletion series

The experiment(s) in which the oligonucleotides were used are listed in the third column.

Oligonucleotides

Oligonucleotides were synthesized by Gibco BRL (New York, NY). The oligonucleotides used for sequencing and PCR experiments are listed in Table 1.

5′-End cloning

The 5′-end of spU2AF^{23} was obtained using the Clonetech Marathon cDNA Amplification Kit. Total RNA was prepared from *S.pombe* 972 (h$^-$) as described (24). Poly(A)$^+$ RNA was isolated using the Poly(A)$^+$ Quick kit (Stratagene, La Jolla, CA). Reverse transcription of mRNA, second strand synthesis, adaptor ligation and PCR were according to the Marathon cDNA protocol using oligonucleotides Clone A-11 antisense and adaptor primer (Table 1). PCR products were ligated into pCR II cloning vector (Invitrogen San Diego,CA) using T4 DNA ligase (New England Biolabs). Ligations were transformed into *Escherichia coli* XL Blue and transformants with plasmids containing PCR inserts were selected by blue/white screening on LB-amp plates containing 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-gal) (25). The 5′-end of spU2AF^{23} was determined by dideoxy sequencing using oligonucleotides M13 reverse and Clone A1 antisense (Table 1).

Sequencing and sequence analysis

Sequencing of full-length spU2AF^{23} and the spU2AF^{59} deletions was completed using the dideoxy sequencing method (26). [α-^{35}S]dATP (1000 Ci/mmol; Amersham Arlington Heights, IL) and Sequenase version 2.0 DNA polymerase (US Biochemicals, Cleveland, OH) were used for sequencing according to the suppliers kit and protocol.

Sequence data for spU2AF^{23} was assembled using Assembly-align version 1.0.7 (Eastman Kodak Co., New Haven, CT). The nucleic acid sequence was translated and analyzed with MacVector version 4.0.1 (Eastman Kodak Co.). GenBank was searched using the BLASTP program version 1.4.8. The Profile Gap program (GCG Sequence Analysis Software Package version 8.0.1; GCG, Madison, WI) was used to align the hsU2AF^{35} and spU2AF^{23} peptide sequences.

Southern blot analysis

Genomic DNA was prepared from *S.pombe* 972 (h$^-$) as described (27) and 20 µg DNA were digested with either *Bam*HI (New England Biolabs), *Eco*RI (New England Biolabs), *Pst*I (New England Biolabs), *Hin*dIII (Promega) or *Xba*I (Promega) according to the conditions suggested by the manufacturer. Digests were run on a 0.8% agarose gel and transferred overnight onto a Gene Screen Plus (New England Nuclear, Boston, MA) filter. The filter was prehybridized in 4× SSCP (60 mM sodium citrate, 0.48 M sodium chloride, 32 mM sodium phosphate and 18 mM sodium phosphate monobasic), 1× Denhardts, 1% SDS and 0.5 µg/ml denatured salmon sperm DNA at 48°C and hybridized as above without salmon sperm DNA. spU2AF^{23} cDNA was random primer-labeled with [α-^{32}P]dCTP (3000 Ci/mmol; New England Nuclear) using the Random Primer Plus Extension Labeling System kit (New England Nuclear). Aliquots of 4.5×10^6 c.p.m. of labeled cDNA was used to probe the Southern blot. The filter was washed at room temperature in 2× SSC (25), 1% SDS and 0.1× SSC, 1% SDS for 30 min each with a final wash in 0.1× SSC, 1% SDS at 65°C for 1 h. The filter was exposed to X-Omat AR film (Eastman Kodak Company) at –70°C overnight.

RESULTS

Identification of protein–protein interactions

To identify interactions between spU2AF^{59} and other proteins, we used the yeast two-hybrid system. The cDNA encoding spU2AF^{59} was subcloned into the yeast expression vector pGBT9 in-frame with the sequence encoding the DNA binding domain of the GAL4 protein. The pGBT9/spU2AF^{59} construct was the bait for our library screen. pGBT9/spU2AF^{59} was transformed into the yeast strain Y190 (21), which contains two reporter genes, *HIS3* and *LacZ*, under the control of a promoter containing the GAL1 binding site. The pGBT9/spU2AF^{59} fusion protein can bind the promoter of these reporter genes but lacks a transcription activation domain and thus cannot activate transcription. An *S.pombe* cDNA library containing cDNAs subcloned into a yeast expression vector in-frame with a sequence encoding the transcription activation domain of the GAL4 protein was transformed into the Y190 strain carrying pGBT9/spU2AF^{59}. Interaction between the spU2AF^{59} protein and a protein encoded by the cDNA library vector was identified by growth on medium lacking histidine and the expression of β-galactosidase in a X-gal filter assay.

Approximately 6×10^6 independent transformants of the *S.pombe* library were screened. Sixty two transformants that showed both growth on medium lacking histidine and production of β-galactosidase in a filter assay were identified. A series of controls were run to determine whether positives identified in the initial library screen were false positives. cDNA library clones were isolated as described in Materials and Methods. The cDNA plasmids were transformed into Y190 alone and assayed for β-galactosidase activity using X-gal filter assays to determine if they contained any intrinsic DNA binding ability. The cDNA plasmids were also transformed into strain Y190 with pGBT9 containing no inserts or with pVA3, the murine *p53* gene (amino acids 72–390), subcloned into pGBT9, to rule out the possibility of non-specific protein interactions (28). cDNA plasmids that have β-galactosidase activity only in the presence of pGBT9/spU2AF^{59} fusion protein were considered true positives. These cDNA plasmids were then transformed into strain HF7c carrying pGBT9/spU2AF^{59} (22). HF7c contains a *LacZ* reporter gene under the control of a promoter containing the GAL4 binding site. The transformants were assayed for β-galactosidase activity. Positives indicated that the activation of the reporter gene by the interaction of pGBT9/spU2AF^{59} and the cDNA plasmid was promoter independent. Any clones that produced a positive β-galactosidase assay in the presence of pVA3 or without pGBT9/spU2AF^{59} were not pursued further. Thirty of the cDNA clones were true positives according to these criteria. These clones were sequenced from their 3′-end and this preliminary sequence analysis was used to determine that the 30 clones encoded seven distinct cDNA clones. The cDNAs were designated A–G, with clone A represented five times, clone B represented 19 times, clone F represented twice and all others represented once each. Preliminary sequence analysis of clones B and F indicates that these are novel proteins that are not represented in GenBank.

Sequence analysis

The 5′-end of clone A was isolated using the Marathon cDNA amplification system from Clonetech. Both strands of clone A

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spU2AF23  MASHLASIYGTEQDKVNCSFYYKIGACRHGERCSRKHVKPNFSQTILCPN 50
hsU2AF35  MAEYLASIPGTEKDKVNCSFYFKIGACRHGDRCSRLHNKPTFSQTIALLN 50
spU2AF23  MYKNPIHEPNGKK.....FTQRELAEQFDAFYEDMFCEF.SKYGEVEQLV 94
hsU2AF35  IYRNPQNSSQSADGLRCAVSDVEMQEHYDEFFEEVFTEMEEKYGEVEEMN 100
spU2AF23  VCDNVGDHLVGNVYVRFKYEESAQNAIDDLNSRWYSQRPVYAELSPVTDF 144
hsU2AF35  VCDNLGDHLVGNVYVKFRREEDAEKAVIDLNNRWFNGQPIHAELSPVTDF 150
spU2AF23  REACCRQHETSECQRGGLCNFMHAKKPSPQSFTRFGTSTKKVFGLKCSRR 194
hsU2AF35  REACCRQYEMGECTRGGFCNFMHLKPISRELRRELYGRRRKKHRSRSRSR 200
spU2AF23  NEKGAQ*                                          201
hsU2AF35  ERRSRSRDRGRGGGGGGGGGGGRERDRRRSRDRERSGRF*          241
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Figure 1. Comparison between the predicted amino acid sequence of the spU2AF^{23} gene product and the 35 kDa subunit of hsU2AF. Solid bars indicate identical amino acids and colons indicate amino acids that have similar physicochemical properties. The asterisk indicates the stop codon. The GenBank accession no. for spU2AF^{23} is U48234.

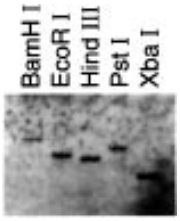

Figure 2. Southern blot analysis of wild-type fission yeast genomic DNA. A DNA blot of *S.pombe* wild-type DNA digested with *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I and *Xba*I was probed as described in Materials and Methods. The DNA blot was exposed to film overnight at –70°C with an intensifying screen.

were sequenced using the dideoxy sequencing method (26). The open reading frame of clone A encodes a putative protein of 200 amino acids if translation begins at the first ATG codon. The predicted molecular mass of the protein encoded by clone A is 23 kDa. The sequence of clone A was compared with those in the GenBank database and found to be similar to the human splicing factor U2AF small subunit (Fig. 1; 17). An alignment of spU2AF^{23} and hsU2AF^{35} indicates 55% of the amino acids are identical and 17% differ by conserved changes. The putative protein encoded by spU2AF^{23} is smaller than hsU2AF^{35} and does not contain the arginine/serine (R/S)-rich motifs or glycine run found at the C-terminus of the human protein. There are three regions of the small subunit that have been highly conserved throughout evolution, amino acids 14–35, 100–109 and 136–151.

We have cloned a genomic copy of spU2AF^{23} by PCR amplification. Sequence analysis of the genomic clone indicates that there are no introns present within the coding region. Southern blot analysis of wild-type fission yeast genomic DNA digested with several restriction enzymes and probed with ^{32}P-labeled spU2AF^{23} cDNA shows a single band in each digest, suggesting that spU2AF^{23} is represented by a single copy gene (Fig. 2).

Interaction between spU2AF^{59} and spU2AF^{23}

The identification of spU2AF^{23} from the library screen which used spU2AF^{59} as bait suggests that the small and large subunits interact. A polyclonal antibody that recognizes spU2AF^{23} co-immunoprecipitates the large subunit in a wild-type strain of fission yeast (data not shown). To determine the region of interaction between the spU2AF^{59} and spU2AF^{23} proteins, a series of deletions was designed. Two deletions of spU2AF^{59} were constructed and subcloned in-frame into the DNA binding domain vector. One deletion (Δ284–518) removed the second and third RNA recognition motifs (RRM) at the C-terminus of spU2AF^{59} (spU2AF^{59}ΔRRM2,3) and the other removed both RRM 2 and 3 and the N-terminus R/S-rich region (Δ1–111 and Δ284–518, spU2AF^{59}ΔR/S, ΔRRM 2,3). Each deletion was transformed into strain Y190 carrying pGBT9/spU2AF^{59}. The interaction of the spU2AF large subunit deletions with the spU2AF small subunit was tested using β-galactosidase filter assays. The results of the filter assays are shown in Figure 3. The results show that spU2AF^{59}ΔRRM 2,3 interacts with spU2AF^{23}, but spU2AF^{59}ΔR/S, ΔRRM 2,3 does not.

A deletion of spU2AF^{23} was constructed and subcloned in-frame into the transcription activation domain vector that contained only amino acids 49–169 (spU2AF^{23}Δ1–49, Δ169–200). The ΔspU2AF^{23} construct was transformed into strain Y190 carrying spU2AF59, spU2AF^{59}ΔRRM 2,3 or spU2AF^{59}ΔR/S, ΔRRM 2,3. Interaction between spU2AF^{23}Δ1–49, Δ169–200 and spU2AF^{59} or its deletions was determined using the β-galactosidase filter assay. spU2AF^{23}Δ1–49, Δ169–200 interacts with full-length spU2AF^{59} and the spU2AF^{59}ΔRRM 2,3 deletion, but not with the spU2AF^{59}ΔR/S, ΔRRM 2,3 deletion (Fig. 3).

spU2AF^{59} and spU2AF^{23} were also assayed for the ability to form dimers (Fig. 4). spU2AF^{59} does not display β-galactosidase activity when assayed with itself. In contrast, spU2AF^{23} does produce a positive β-galactosidase assay, indicating that it is capable of forming a homodimer. This interaction is lost, however, when spU2AF^{23} is assayed for interaction with the ΔspU2AF^{23} construct.

DISCUSSION

We have screened a fission yeast cDNA library using the yeast two-hybrid system to identify proteins that interact with spU2AF^{59}. Through this screen we found a fission yeast homolog of the small subunit of the human splicing factor U2AF. The region of interaction between spU2AF^{59} and spU2AF^{23} was also determined using the yeast two-hybrid system by testing deletions within both the large and small subunits for their interaction with either full-length or shortened versions of the other subunit. Our results show that this region has been conserved over the billion years of evolution that separate yeast and man (29).

spU2AF^{23} and hsU2AF^{35} share 55% amino acid identity and 17% conserved amino acid changes. There are three regions of the two proteins that are highly conserved, corresponding to spU2AF^{23} amino acids 14–35, 100–109 and 136–151. By contrast, the two proteins diverge at their C-terminus. It is interesting that spU2AF^{23} lacks both R/S motifs and the glycine run that are present in the C-terminus of hsU2AF^{35}. The large hsU2AF subunit has an R/S domain that is located in the N-terminus. Both hsU2AF subunits contain the type of R/S domains in which arginine and serine are enriched but dispersed among other amino acids. This arrangement differs from SR proteins, in which the serines and arginines alternate in dinucleotide repeats, share a common epitope and co-purify in an ammonium sulfate/magnesium chloride two-step precipitation (30,31). R/S motifs similar to that found in hsU2AF are found in a number of other splicing factors, including U1 snRNP-specific

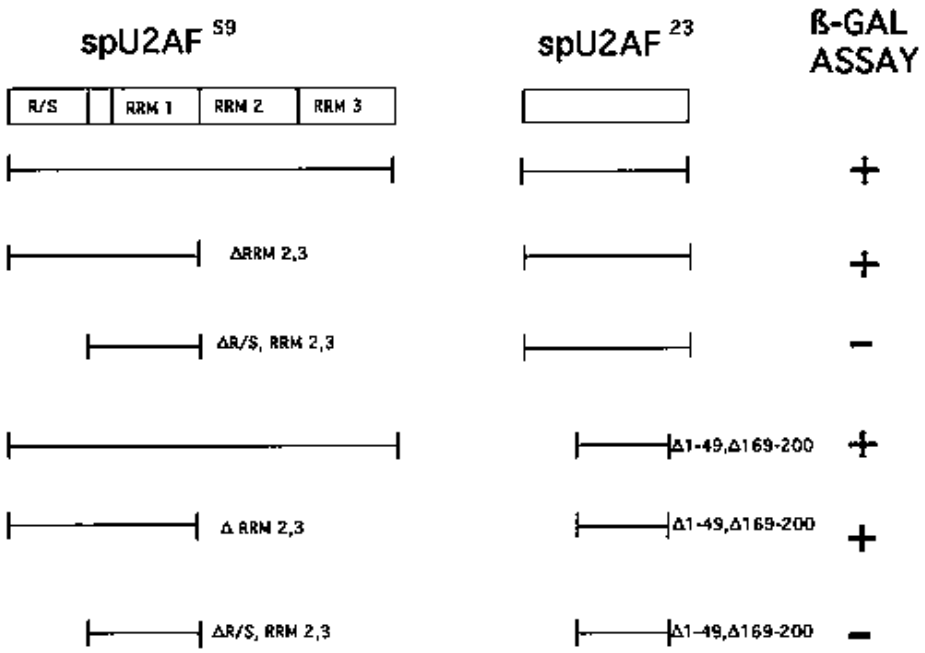

Figure 3. Heterodimer formation between the large and small subunits of fission yeast U2AF. Interaction between spU2AF^{59} and spU2AF^{23} was assayed by the β-galactosidase filter assay. A + denotes blue color production, indicating an interaction between the subunits. A– indicates there was no color production and therefore the two subunits do not interact. The spU2AF^{59}ΔRRM 2,3 deletion has amino acids 284–518 removed and spU2AF^{59}ΔR/S, ΔRRM 2,3 has amino acids 1–111 (R/S) and 284–518 (RRM 2,3) removed. The spU2AF^{23} deletion has amino acids 1–49 and 169–200 removed.

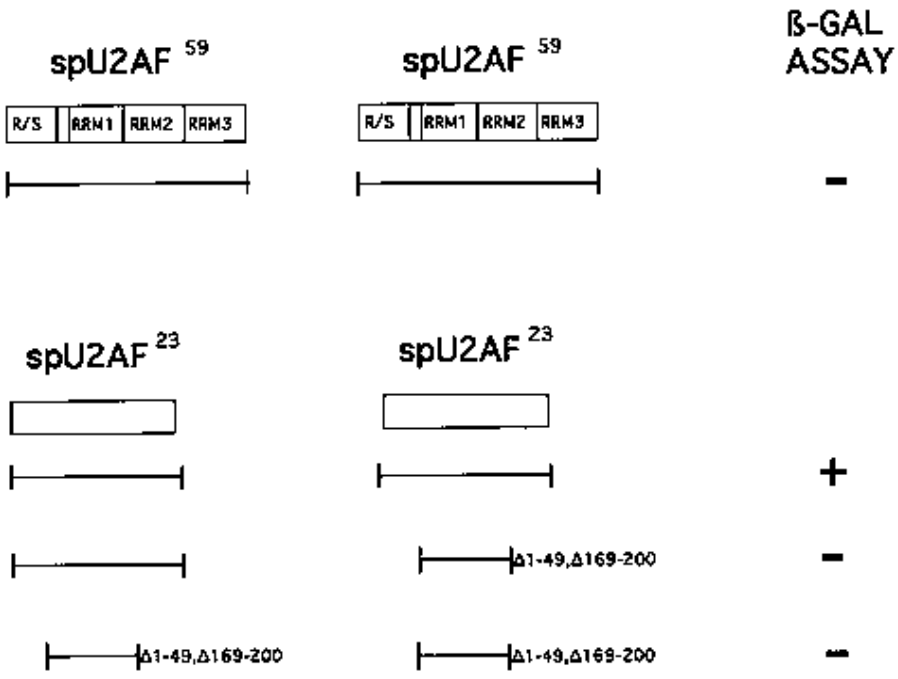

Figure 4. Homodimer formation between the large and small subunits of fission yeast U2AF. Protein–protein interaction was assayed by theβ-galactosidase filter assay. A + denotes blue color production, indicating an interaction. A– indicates there was no color production and therefore no interaction. The spU2AF^{23} deletion has amino acids 1–49 and 169–200 removed.

70 kDa protein (32), *Drosophila* tra (33), tra-2 (34), dmU2AF^{50} (16) and spU2AF^{59} (15).

The results from several experiments suggest possible roles for R/S domains in splicing factors. Deletion and substitution experiments with the *Drosophila* su(w^{a}) and tra protein R/S domains indicate that this motif is necessary for the localization of these splicing factors to the subnuclear region which is enriched with splicing factors (35). The R/S domain is also required for

some protein–protein interactions. A number of splicing factors containing R/S domains are present in the bridge of proteins that link the 5′ splice site to the 3′ splice site and the exon bridge that extends from the 3′ splice site to the downstream 5′ splice site (18,36,37). In the light of these studies, it is interesting that neither of the R/S regions of the small subunit of hsU2AF is required for its interaction with the large subunit (17). The function of the R/S regions of hsU2AF^{35} remains unknown. The absence of R/S motifs in spU2AF^{23} may indicate that some of the protein–protein interactions that have been identified in the mammalian spliceosome are not present in fission yeast. It is also possible that the same protein–protein interactions do exist in fission yeast but these interactions might be independent of the R/S domain.

We have identified the region of interaction between spU2AF^{59} and spU2AF^{23}. This interaction does not require the second and third RRMs of spU2AF^{59} but is dependent on the presence of its R/S motif. hsU2AF^{65} and hsU2AF^{35} interaction is similar in that it is autonomous of the second and third RRM. However, the human subunit interaction is only dependent on the existence of the linker region and the first RRM of the large subunit and does not require the R/S motif (17). This difference in the region of interaction between the small and large subunits may be either a true difference between fission yeast and human U2AF interaction or the result of the deletions that were designed. The R/S domain of the large subunits of U2AF in fission yeast and humans are scattered and ill defined. As a result, the amino acids that we have chosen to remove in the R/S deletion may include amino acids that are actually part of the linker region as defined through mammalian studies. This arbitrary distinction between the R/S motif and the linker region could account for the difference that we observe in regions of spU2AF^{59} required for the interaction with spU2AF^{23}.

The region of the small subunit that is required for interaction with the large subunit is contained within amino acids 49–169. This fragment of the spU2AF small subunit is capable of interacting with both the full-length large subunit and the deletion lacking RRMs 2 and 3. This region is similar to amino acids 47–172 of the human small subunit that are required for interaction with hsU2AF^{65} (17). The existence of similar regions of interaction between the fission yeast and human U2AF subunits suggests that other protein–protein interactions involving these subunits may also be evolutionarily conserved in fission yeast. In addition, we have also shown that the ability of the small subunit of U2AF to form a dimer with itself has been conserved in fission yeast. This suggests the possibility that the bridge between the 5′ and 3′ splice sites of mammalian introns proposed by Wu and Maniatis (18) may also exist in fission yeast. In this regard it will be interesting to determine whether homologs of the U1 snRNP 70 kDa protein, SC35 and SF2/ASF also exist in fission yeast.

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