Genetic and Physical Interactions Between Factors Involved in Both Cell Cycle Progression and Pre-mRNA Splicing in *Saccharomyces cerevisiae*

Sigal Ben-Yehuda,*,[1] Ian Dix,†,[1] Caroline S. Russell,† Margaret McGarvey,† Jean D. Beggs† and Martin Kupiec*

*Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv 69978, Israel and †Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, United Kingdom

Manuscript received May 11, 2000
Accepted for publication August 7, 2000

ABSTRACT

The *PRP17/CDC40* gene of *Saccharomyces cerevisiae* functions in two different cellular processes: pre-mRNA splicing and cell cycle progression. The Prp17/Cdc40 protein participates in the second step of the splicing reaction and, in addition, *prp17/cdc40* mutant cells held at the restrictive temperature arrest in the G2 phase of the cell cycle. Here we describe the identification of nine genes that, when mutated, show synthetic lethality with the *prp17/cdc40*Δ allele. Six of these encode known splicing factors: Prp8p, Slu7p, Prp16p, Prp22p, Slt11p, and U2 snRNA. The other three, *SYF1*, *SYF2*, and *SYF3*, represent genes also involved in cell cycle progression and in pre-mRNA splicing. Syf1p and Syf3p are highly conserved proteins containing several copies of a repeated motif, which we term RTPR. This newly defined motif is shared by proteins involved in RNA processing and represents a subfamily of the known TPR (tetratricopeptide repeat) motif. Using two-hybrid interaction screens and biochemical analysis, we show that the *SYF* gene products interact with each other and with four other proteins: Isy1p, Cef1p, Prp22p, and Ntc20p. We discuss the role played by these proteins in splicing and cell cycle progression.

THE division of a cell into a pair of genetically identical daughters depends on accurate chromosome duplication and segregation. The regulation of timing is of capital importance: lack of coordination between different processes can lead to cell death, chromosomal aberrations, or cancerous growth. Precise coordination can be obtained only if a tight control is exerted on the expression of key genes to ensure that a particular event can take place only after a previous one has been completed (Elledge 1996). Much of our understanding of the basic mechanisms that govern the cell cycle and coordinate its different stages comes from studies in simple organisms, such as yeast. Many *cdc* (cell division cycle) mutants were isolated and found to identify genes with central roles in cell cycle progression. Most of these basic mechanisms are conserved from yeast to humans (Lamb *et al.* 1994).

The *CDC40* gene of the yeast *Saccharomyces cerevisiae* was first identified through the temperature-sensitive mutation *cdc40-1*, which affects both the mitotic and meiotic cell cycles. At the restrictive temperature, *cdc40-1* cells arrest with a large bud and one undivided nucleus (Kassir and Simchen 1978). A full deletion allele of the *CDC40* gene also shows a temperature-sensitive phenotype (Vaisman *et al.* 1995). Although DNA replication seems to be completed (as measured by FACS analysis), *cdc40*-arrested cells remain sensitive to hydroxyurea (HU), a well-characterized inhibitor of DNA synthesis (Kassir and Simchen 1978; Vaisman *et al.* 1995; Boger-Nadjar *et al.* 1998). In addition, the Cdc40 protein is needed for the maintenance of the mitotic spindle; in *cdc40*Δ cells held at the restrictive temperature (37°), the spindle, as detected by antitubulin antibodies, disappears and the cells arrest in the G2 phase of the cell cycle (Vaisman *et al.* 1995).

It was recently found that the *CDC40* gene is identical to *PRP17*, a gene encoding a pre-mRNA splicing factor (Boger-Nadjar *et al.* 1998). Pre-mRNA splicing takes place by two consecutive transesterification reactions. In the first step the border between the 5′ exon and the intron is cleaved, yielding the 5′ exon and lariat intron-exon intermediates. In the second step, the 3′ splice site is cleaved and the two exons are joined, creating a mature RNA and the lariat intron (Madhani and Guthrie 1994). Pre-mRNA splicing requires the activity of a large number of *trans*-acting factors (Will and Luhrmann 1997). Many of the proteins involved have been identified by genetic screens for conditional mutations in yeast (reviewed in Beggs 1995). In addition, a number of small nuclear RNA molecules (U1, U2, U4, U5, and U6 snRNAs) form small nuclear ribonucleoprotein particles (snRNPs) that play important roles in splicing, assembling onto the pre-mRNA substrate in a stepwise fashion (Madhani and Guthrie 1994).

The *PRP17/CDC40* gene encodes a protein that participates in the second step of the splicing reaction; in

Corresponding author: Martin Kupiec, Whitehead Institute, 9 Cambridge Ctr., Cambridge, MA 02142. E-mail: kupiec@wi.mit.edu

[1]These authors contributed equally to this study.

prp17/cdc40 mutants intermediates accumulate even at the permissive temperature (Ben-Yehuda *et al.* 1998). We have previously demonstrated a physical association of Prp17/Cdc40p with splicing intermediates, providing evidence that it is associated with spliceosomes (Ben-Yehuda *et al.* 1998). The Prp17/Cdc40 protein is conserved throughout the evolutionary scale and contains several copies of the WD repeat (Vaisman *et al.* 1995; Ben-Yehuda *et al.* 1998; Lindsey and Garcia-Blanco 1998; Zhou and Reed 1998). This repeated motif is found in proteins that play important roles in signal transduction, cell cycle progression, splicing, transcription, and development (for review see Smith *et al.* 1999).

The identity of a gene known to participate in splicing with one required for G2/M transition suggests that *PRP17/CDC40* plays a role in regulating cell cycle progression through splicing. For simplicity, we refer to the gene in this article as *PRP17*. In our study we looked for mutants that are synthetically lethal with a deletion of the *PRP17* gene. These represent nine genes, which affect splicing, cell cycle progression, or both processes. Out of these nine genes, three are novel: *SYF1*, *SYF2*, and *SYF3*. Here we present evidence that the products of the *SYF* genes exhibit extensive interactions among themselves and with other known cell cycle and splicing factors.

MATERIALS AND METHODS

Media, growth, and general procedures: Yeast cells were grown at 25° or 30° in YPD (1% yeast extract, 2% bacto peptone, 2% dextrose) or SD (0.67% yeast nitrogen base, 2% dextrose, and the appropriate nutrients added). Bacto agar (1.8%) was added for solid media. Selective media lacking one nutrient are designated SD − nutrient (*e.g.*, SD − Ura is SD with all the nutrients except uracil). Ura$^-$ colonies were selected on SD complete medium with uracil (50 mg/liter) and 5-fluoroorotic acid (5-FOA; 0.8 g/liter; Boeke *et al.* 1987).

Yeast strains and plasmids: Yeast strain YH2 (*MAT***a** *prp17*Δ*::LEU2 ade2 ade3 leu2 ura3 trp1 lys2*) was used for the synthetic lethality screen. Plasmid p1426 is a derivative of pRS416 (Sikorski and Hieter 1989) carrying the *URA3*, *ADE3*, and *PRP17* genes. pSBY18 is a derivative of the centromeric *TRP1* plasmid YCplac22 (Gietz and Sugino 1988), carrying the *PRP17* gene. pSBY19 was constructed by *in vivo* recombination in yeast, replacing the *URA3* gene of p1426 by the *TRP1* gene of pRS314 (Sikorski and Hieter 1989).

Mutagenesis, screening, and genetic characterization: YH2/p1426 was subjected to UV irradiation to yield a survival rate of 10% on YEPD plates. Survivors were screened for red, nonsectoring colonies, which, after restreaking, were tested for sensitivity to 5-FOA. All the nonsectoring 5-FOA^S colonies were transformed with pSBY18 (*TRP1*, *PRP17*) or the vector YCplac22 (*TRP1*). Only strains that showed red/white sectoring with pSBY18 but not with YCplac22 were studied further. The presence of *PRP17* on pSBY18 allowed loss of p1426, conferring sectoring and resistance to 5-FOA.

Each putative *syf* mutant was crossed to YH3 (*MAT*α, isogenic to YH2). All the diploids exhibited red/white sectoring, demonstrating that the mutations were recessive. The diploids were sporulated, and the 2:2 segregation of the sectoring/nonsectoring phenotype was verified. Derivatives of all the mutants were crossed in all the possible combinations to establish complementation groups. One member of each group was chosen for cloning of the complementing gene.

Cloning of the SYF genes: Nonsectoring *syf* mutants carrying plasmids p1426 (*URA3*, *ADE3*, *PRP17*) or pSBY19 (*TRP1*, *ADE3*, *PRP17*) were transformed with pRS314 (*TRP1*)- or YEp24 (*URA3*)-based yeast genomic libraries. The transformants were screened for the ability to form red/white sectors and tested for resistance to 5-FOA. The plasmids were isolated from sectoring or white transformants, subjected to partial DNA sequencing, and compared to the complete yeast genome. When several open reading frames (ORFs) were present in each complementing plasmid, a series of deletions and subclones was constructed to identify the complementing ORF. For each *syf* mutant the complementing ORF was also cloned into an integrative vector, integrated in the genome of a wild-type strain, and crossed to the *syf* mutant to confirm, by tetrad analysis, allelism between the integrated plasmid and the original mutation. Overexpression experiments were carried out using each gene cloned in YEp24, a 2 μ high copy-number vector.

Two-hybrid screens: Two-hybrid screens were performed as described by Fromont-Racine *et al.* (1997). LexA two-hybrid bait constructs were made by cloning a sequenced PCR-generated copy of the ORF in frame into the multiple cloning site of pBTM116 (Vojtek *et al.* 1993). 3-Aminotriazole (3-AT) was added to the selective plates in the Syf1 and Syf3 screens in concentrations of 10 and 30 mm, respectively, to increase the stringency. The numbers of diploids tested with each bait were as follows: Syf1p, 9×10^6; Syf2p, 11×10^6; Syf3p, 35×10^6; Isy1p, 60×10^6; Cef1p, 40×10^6; and Ntc20p, 47×10^6. The positive (His$^+$) clones were tested for β-galactosidase production by an overlay assay (Fromont-Racine *et al.* 1997). The prey plasmids were rescued from His$^+$ colonies that also showed β-galactosidase activity and subjected to DNA sequencing.

Oligonucleotides:

Syf1TnT F: 5′-GATAATACGACTCACTATAGGGAGCCACCA TGTCAGCATACATCGCAATG-3′
Syf1R: 5′-GATCAAGCGTAATCTGGAACATCGTATGGGTAT CAGTATAGCTTCTGAATACAC-3′
Syf2TnT F: 5′-GATAATACGACTCACTATAGGGAGCCACCA TGGATTTTTACAAATTAGACG-3′
Syf2 R: 5′-TTATTCTGATCCTTTTGA-3′
Syf2 *Bam*HI: 5′-AGCGGATCCTATTCGAAGAATGGATTTT TAC-3′
Syf3TnTF: 5′-GATCGGATCCTAATACGACTCACTATAGGG AGCCACCATGGACACTTTAGAGCCAAC-3′
Syf3 R: 5′-CTCAGTTGAGCTCCAAATATCTTC-3′
Isy1TnT F: 5′-GATAATACGACTCACTATAGGGAGCCACCA TGAGTAGAAATGTAGATAAGGC-3′
Isy1 R: 5′-ATCGCTGCAGCTAAAGGTTAAGTTCATCCAT-3′
Cef1TNT F: 5′-GCGCTAATACGACTCACTATAGGGAGCCA CCATGCCCCCCGTACCAATATAC-3′
Cef1 R: 5′- CTAAGTGCGCCCTTGGGTTATA-3′
Ntc20 TNT F: 5′-GCGCTAATACGACTCACTATAGGGAGCC ACCATGCCCTCTCTGCGAGATTTA-3′
Lex R: 5′- TTTTAAAACCTAAGAGTCAC-3′

***In vitro* translations:** *In vitro*-translated proteins were produced using the TnT-coupled transcription/translation kit (Promega, Madison, WI) and [^{35}S]methionine, according to the manufacturer's instructions. The templates were produced by PCR amplification using the oligonucleotide primers listed above. The *SYF1* PCR product represents the first 1300 bp of the gene, encoding the N-terminal 433 amino acids. The *SYF3* PCR product represents the first 1623 bp of the gene, encoding the N-terminal 541 amino acids. The *CEF1*

PCR product represents the first 1480 bp of the gene, encoding the N-terminal 493 amino acids. The *SYF2*, *ISY1*, and *NTC20* PCR products encode the full-length proteins.

His_6-Isy1p production in *Escherichia coli*: The pET19-Isy1 plasmid for production of His_6-Isy1p in *E. coli* was constructed by cloning a *Sma*I-*Eco*RV fragment (containing the entire *ISY1* sequence) from the LEX-Isy1 two-hybrid bait plasmid (Dix *et al.* 1999) into the *Xho*I site (blunt-ended using Klenow) of the expression vector pET19b (Novagen) and transformed into *E. coli* strain BL21-Gold(DE3) pLysS (Stratagene, La Jolla, CA). Cultures (1 liter) were grown at 30° in Luria broth (LB) medium containing 50 μg/ml ampicillin and 20 μg/ml chloramphenicol until the A_{600} reached 0.4; then expression was induced by the addition of isopropyl thiogalactoside (IPTG) to 0.75 mm. Cells were grown for a further 4 hr, then harvested and stored at −80°. Cell extracts were prepared essentially as described by Schwer and Gross (1998). The His_6-Isy1 protein was purified from cell extract by metal affinity chromatography as described by Plumpton *et al.* (1994).

Immunoprecipitations: Purified His_6-Isy1p (4–5 μg) and *in vitro*-translated protein (5-μl reaction; not purified) were incubated together for 1 hr at 4° before addition of 0.8 μg antipentahistidine antibodies (QIAGEN, Chatsworth, CA) and further incubation at 4° for 1 hr. Protein A-Sepharose beads (Sigma, St. Louis) in IP buffer [6 mm Hepes pH 7.9, 150 mm NaCl, 2.5 mm $MgCl_2$, 0.05% (v/v) Nonidet P-40] were added and incubation continued at 4° for 2 hr prior to precipitation of the beads. The precipitates were washed three times with IP buffer and the precipitated [^{35}S]methionine-labeled proteins were analyzed by SDS-PAGE and autoradiography. Control sample containing an unrelated $(His)_6$-tagged protein produced no signal above background (data not shown).

Bioinformatics: *Syf1:* Syf1 orthologs were identified in a BLASTP and a TBLASTN (Altschul *et al.* 1997) search of the nonredundant (nr) DNA and protein databases and the Human expressed sequence tag (EST) database through the National Center for Biotechnology (NCBI) at the National Institutes of Health (NIH). The complete human sequence was obtained by sequencing of an EST clone EST28504 (AA325290). The alignment was made using CLUSTALW (Thompson *et al.* 1994). All alignment manipulations were carried out using GeneDoc (Nicholas and Nicholas 1997). The *Caenorhabditis elegans* protein accession number is Entrez-|AAB37794.

Syf3: The protein accession numbers are as follows: for *Schizosaccharomyces pombe*, EMBL|Z97204|lSPBC31F10; for *C. elegans*, GenBank|AF016441|lCELM03F8; and for *Drosophila melanogaster*, SwissProt|P17886. No protein accession number was available for any of the *Arabidopsis thaliana* putative orthologs as they were only identified in a TBLASTN search of the nr DNA database. The DNA accession numbers are as follows: *A. thaliana*_5a, dbj|AB016871; *A. thaliana*_5b, dbj|AB006698; *A. thaliana*_2, dbj|AP000375. The protein sequence was determined using the TBLASTN output and the identification of putative exons within the DNA using the gene identification programs GenScan (Burge and Karlin 1997) and Grail (Xu *et al.* 1996). The partial human sequence was compiled from the ESTs gb|AI924865.1, gb|AI814570.1, emb|FO6818|HSC1LF121, gb|AA298104, gb|N83366, and gb|AA471150.

The tetratricopeptide repeat (TPR) consensus was generated from 127 annotated TPR repeats in 19 nonorthologous proteins in SwissProt: SP|P09798|CDC16, SP|P16522|CDC23, SP|P50502|HIP, SP|Q08168|HRP, SP|P31948|IEFS, SP|P32333|-MOT1, SP|P10505|NUC2, SP|P23231|TOM70, SP|P50542|PEX5, SP|P53041|PPP5, SP|P17883|SKI3, SP|P14922|SSN6, SP|P15-706|STI1, SP|P33339|TFC4, SP|P53804|TTC3, SP|P42460|YCOA, SP|P19737|YREC, SP|Q04364|YMP8, and SP|P17886|CRN. The crooked neck (CRN) consensus was generated from the 50 well-conserved CRN repeats in the *S. cerevisiae*, *S. pombe*, *D. melanogaster*, *C. elegans* and *A. thaliana*_5a putative orthologs. The RNA TPR (RTPR) consensus was generated in the following way: BLASTP analysis of Syf3p revealed 10 nonorthologous TPR-containing proteins with very low expected values (<0.001): KIAA01256 (*Homo sapiens*), Rrp5p orthologues (*S. cerevisiae*, *S. pombe*, *H. sapiens*, and *C. elegans*), Prp6p orthologues (*H. sapiens, A. thaliana*, and *S. pombe*), Prp39p (*S. cerevisiae*), and an Syf1p orthologue (*C. elegans*). ClustalX analysis of the TPR motifs from 5 nonorthologous representatives of these 10 proteins, combined with all the annotated TPRs in SwissProt (totaling 38 TPRs), revealed that the TPRs from these proteins cluster as a separate group, suggesting that they form a TPR subfamily. All the consensuses were generated using the program Motif Hunter (I. Dix, unpublished results).

RESULTS

Isolation of mutants that are synthetically lethal with *prp17*Δ: To identify genes that interact with *PRP17*, we performed a screen for mutants unable to survive in the absence of the Prp17 protein even at the permissive temperature. The screen made use of the fact that yeast *ade2 ADE3* cells produce red colonies because a red pigmented metabolite accumulates in the absence of Ade2p function, whereas *ade2 ade3* colonies are white.

Yeast strain YH2 (*MAT***a** *prp17*Δ*::LEU2 ade2 ade3 leu2 ura3 trp1*) carrying a centromeric plasmid (p1426) with the *PRP17*, *URA3*, and *ADE3* genes gives rise to white and red/white sectored colonies at the permissive temperature, due to spontaneous loss of the nonessential plasmid. YH2/p1426 cells were mutagenized using UV irradiation and a screen for uniformly red colonies (unable to lose the plasmid at the permissive temperature) was carried out. These colonies bear mutations that are lethal in the absence of the *PRP17* gene product and thus cannot lose the *PRP17*-containing plasmid.

Out of 25,000 colonies that survived the UV mutagenesis, 40 uniformly red, 5-fluoroorotic acid-sensitive colonies were isolated. The mutations were designated *syf* (*s*ynthetic lethal with cdc *f*orty). The mutants were crossed to a *MAT*α *prp17* strain (YH3, congenic to YH2); all the mutations were found to be recessive (the diploids *SYFX/syfx* showed red/white sectoring). The *syf* mutations were assigned to complementation groups, and the wild-type genes were cloned from a yeast genomic library by complementation of a representative mutant. The minimal complementing fragments for each gene were identified by subcloning analysis (see materials and methods). Among nine of the *SYF* genes identified in our genetic screen, six corresponded to known splicing genes previously isolated: *PRP8*, *SLU7*, *PRP16*, *PRP22*, *SLT11*, and *SNR20*, the gene encoding the U2 snRNA. The remaining three were previously uncharacterized genes: *SYF1* (YDR416w), *SYF2* (YGR129w), and *SYF3* (YLR117c). While this work was in preparation, the *SYF3* gene was independently isolated as *CLF1* and shown to be involved in spliceosome assembly (Chung

TABLE 1

***SYF* genes: Alleles of *SYF* genes are synthetically lethal with a deletion mutation of the *PRP17* gene**

Gene name	ORF name	Gene function	References
PRP8	YHR165c	Splicing factor involved in first and second steps	Teigelkamp *et al.* (1995)
PRP16	YKR086w	Second step-splicing factor	Schwer and Guthrie (1991)
SLU7	YDR088c	Second step-splicing factor	Frank and Guthrie (1992)
PRP22	YER013w	mRNA-releasing factor, role in the second step of splicing	Schwer and Gross (1998)
SLT11	YBR065c	Mutation blocks splicing prior to the first step	Xu *et al.* (1998)
SNR20		U2 SnRNA splicing factor-branchpoint recognition	Ares (1986)
SYF1	YDR416w	New gene	This work
SYF2	YGR129w	New gene	This work
SYF3	YLR117c	New gene	Chung *et al.* (1999); this work

et al. 1999). Table 1 summarizes the genes identified in this screen.

PRP8 encodes a large evolutionarily conserved protein that is an essential component of U5 snRNPs and of spliceosomes during both steps of the splicing reaction (Lossky *et al.* 1987; Teigelkamp *et al.* 1995). It is thought that Prp8p anchors the exons in the catalytic centers of the spliceosome and stabilizes interactions between the U5 snRNA and the exon sequences that align the splice sites for the second catalytic step (Beggs 1995; Teigelkamp *et al.* 1995; Dix *et al.* 1998; Reyes *et al.* 1999).

The *PRP16* and *SLU7* genes were shown to play a role in the second step of the splicing reaction (Schwer and Guthrie 1991; Frank and Guthrie 1992; Jones *et al.* 1995; Umen and Guthrie 1995a). *PRP16* encodes an RNA-dependent ATPase of the DEAH-box family, which has been shown to unwind RNA duplexes *in vitro* (Wang *et al.* 1998). *SLU7* encodes a protein that was shown to interact directly with the 3′ splice site during the second catalytic step (Umen and Guthrie 1995a). *PRP22* is another DEAH-box splicing factor, which unwinds RNA duplexes and mediates ATP-dependent mRNA release from the spliceosome (Schwer and Gross 1998; Wagner *et al.* 1998). *SLT11* encodes a protein with two putative zinc-fingers and a domain that shares homology to the yeast ribosomal protein L25. It was found recently in a synthetic lethal screen with mutations in the U2 snRNA gene. Slt11p functions prior to the first step of the splicing reaction (Xu *et al.* 1998).

Three novel genes (*SYF1*, *SYF2*, and *SYF3*) were uncovered in this screen. *SYF1* and *SYF3* encode proteins of 859 and 687 amino acids, respectively. These proteins are structurally similar to each other and play roles in both pre-mRNA splicing and cell cycle progression. We have demostrated that depletion of each of the proteins causes a cell cycle arrest at the G2 phase of the cell cycle, and in addition we have evidence that both proteins are splicesome associated and required for pre-mRNA splicing (to be published elsewhere). The two proteins are conserved throughout the evolutionary scale and contain several copies of the TPR motif (Lamb *et al.* 1994; see below). *SYF2* encodes a small protein (215 amino acids long) with no similarities in the databases. Although it is not essential for splicing, we have shown that antibodies against a tagged version of Syf2 coprecipitate splicing intermediates, indicating an association of Syf2 with spliceosomes (our unpublished results).

Overproduction of the U2 snRNA partially suppresses the syf phenotype: A synthetic lethal interaction previously implicated Prp17p as a U5-interacting protein (Frank *et al.* 1992) although no physical association of Prp17p with U5 snRNA has been reported. Our screen uncovered genetic links with other genes whose products seem to interact directly or indirectly with the U5 snRNP, such as *SLU7*, *PRP16* (Frank *et al.* 1992), and *PRP8* (Lossky *et al.* 1987). The results of our screen show that the *PRP17* gene interacts with the U2 snRNA gene as well. Similar results were reported by Xu *et al.* (1998): mutations in *PRP17* were found to be lethal in combination with mutations that modify the stem I region of the U2 snRNA.

To further explore the genetic interactions among the nine genes isolated in our study, each gene was overexpressed in each of the *syf* mutants and screened for the presence of red/white sectors (indicating suppression of the synthetic lethal defect). The only gene able to suppress the other *syf* mutants was *SNR20*. Overproduction of U2 snRNA was able to suppress the *syf* phenotype of all the isolated mutants. Figure 1 shows examples of such suppression. The degree of suppression (sectoring) by U2 overproduction varied with different mutants and with distinct alleles of the same genes; however, in each case it was clearly different from

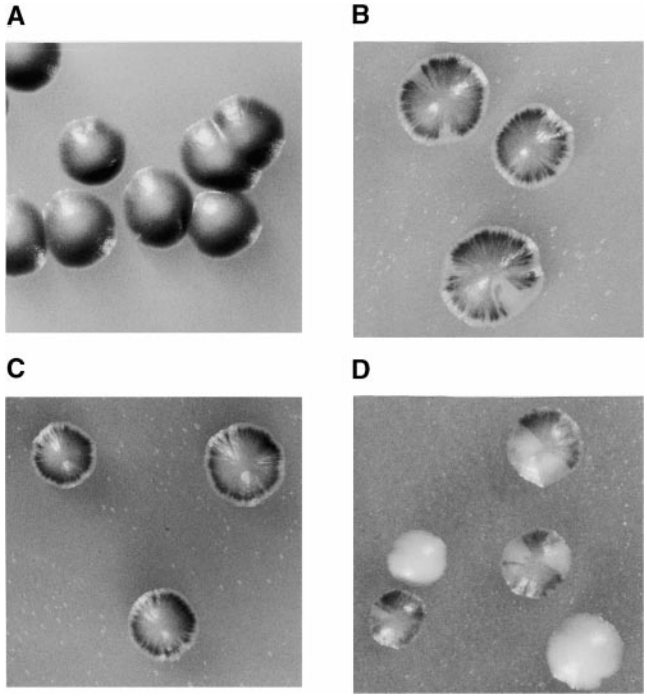


FIGURE 1.—Sectoring assay used in the *syf* screen. (A) *syf* phenotype (*syf1* strain). The cells are uniformly red due to the inability to lose the *PRP17, ADE3*-containing plasmid. (B and C) *syf1* and *slu7/syf5* strains, respectively, transformed with a plasmid overproducing the U2 snRNA. (D) *snr20/syf4* mutant transformed with the same plasmid.

the complementation of *snr20* (U2 snRNA) mutants. Full complementation of *snr20* allowed the cells to lose the *PRP17, ADE3* plasmid readily, generating white colonies and big white sectors; no white colonies were seen in any of the other suppressed mutants (Figure 1). In contrast, overproduction of the U5 snRNA did not suppress the *syf* phenotype (data not shown).

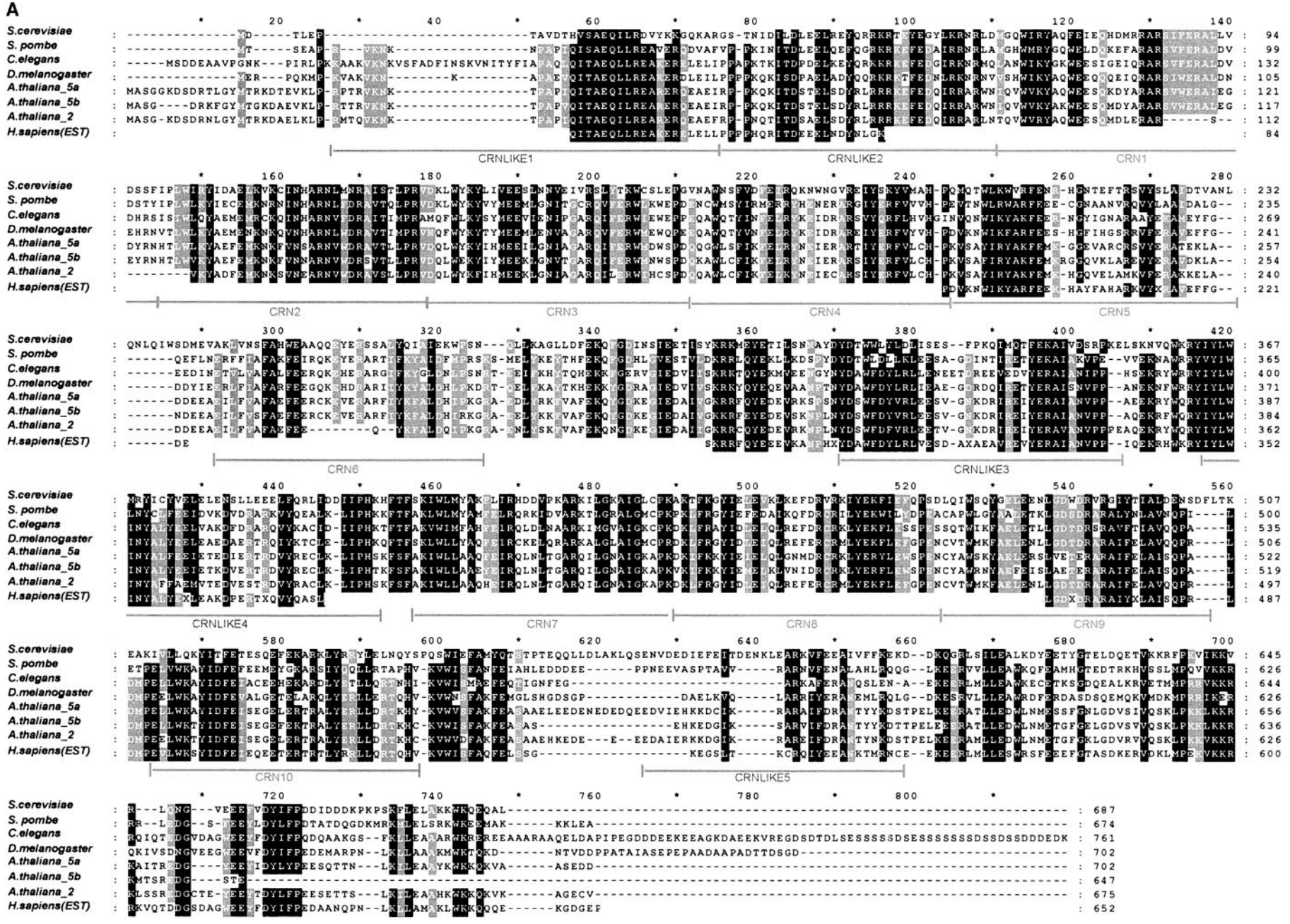
Syf1 and Syf3: two highly conserved proteins containing TPR motifs: To further characterize the novel Syf1, Syf2, and Syf3 proteins, their sequences were used to search the nonredundant DNA and protein databases of NCBI. No clear orthologs were identified for Syf2p, only a *S. pombe* protein that showed weak similarity (data not shown). In contrast, the search revealed putative ortholog proteins for Syf1 and Syf3 from different organisms (Figure 2), implying that the Syf1 and Syf3 proteins are highly conserved throughout the evolutionary scale.

As shown in Figure 2A, the search of the databases using the Syf1 protein sequence revealed *C. elegans* and human proteins that exhibit a high level of conservation with Syf1p. The yeast Syf1p shows 24% identity and 43% similarity to both proteins through the entire protein length, while the human and the *C. elegans* proteins share 48% identity. A search for known motifs in the Syf1 protein sequence identified nine semiconserved copies of the ubiquitous TPR motif (Figure 2A), that, in other systems, have been shown to be involved in protein-protein interactions (DAS *et al.* 1998).

A database search using the Syf3 protein sequence identified putative orthologs from *S. pombe, C. elegans*, and *D. melanogaster* (Figure 2B). In addition, three separate *A. thaliana* DNA contigs were found, which potentially encode proteins with good similarity to Syf3p. These three contigs represent different genes, since the sequences surrounding the loci are different and the proteins they encode are only 75–85% identical (Figure 2B and Table 2). This suggests that there are at least three paralogs with strong similarity to Syf3p in *A. thaliana*. The Syf3p orthologs show high similarity over their entire length (Figure 2B); the percentage of identity among the different Syf3 proteins is shown in Table 2. In addition, analysis of the human EST database identified a number of ESTs with good similarity to Syf3p, suggesting the existence of at least one Syf3p ortholog in humans (Figure 2B). The identification of Syf3p homologs with high levels of conservation in six different organisms suggests that the Syf3 protein plays an important role in all eukaryotic cells.

The putative *D. melanogaster* ortholog of Syf3p [the *crooked neck* (*crn*) protein] was previously identified as being involved in the proliferation of brain neuroblasts. Loss of zygotic expression of *crn* causes a defect in embryogenesis, affecting primarily cell lineages in the nervous system still undergoing cell division (ZHANG *et al.* 1991). The *crn* protein contains 16 copies of a TPR-like motif; since this repeated sequence is slightly different from other TPR motifs, it was termed the CRN repeat (ZHANG *et al.* 1991). The CRN motifs are spread throughout the entire length of the protein, with the exception of the C terminus, and are well conserved in all the Syf3 orthologous proteins (Figure 2B).

The degenerate TPR motif has no one position characterized by an invariant residue, but a consensus pattern of hydrophobic amino acids has been defined (Figure 2C; LAMB *et al.* 1995). Despite this degenerate primary structure, analysis of the crystal structure of protein phosphatase 5 (DAS *et al.* 1998) showed that each of its three TPR motifs consists of a pair of antiparallel, amphipathic α-helices (domains A and B) of equivalent length. DAS *et al.* (1998) went on to postulate that tandem TPRs form a right-handed superhelical structure with a continuous groove suitable for interaction with an α-helix of a target protein. This suggests that both Syf1p and Syf3p may function as scaffold proteins, binding multiple proteins. An analysis of nonorthologous TPR-containing proteins (see MATERIALS AND METHODS) revealed that the TPRs of Syf1 and Syf3 form part of a separate group within the TPR family. All the proteins in this cluster are involved in RNA processing [*e.g.*, the Prp6 and Prp39 splicing factors (LOCKHART and RYMOND 1994; URUSHIYAMA *et al.* 1997)] and the rRNA processing protein [*e.g.*, Rrp5p (VENEMA and


A
S.cerevisiae
S. pombe
C.elegans
D.melanogaster
A.thaliana_5a
A.thaliana_5b
A.thaliana_2
H.sapiens(EST)
CRNLIKE1
CRNLIKE2
CRN1
CRN2
CRN3
CRN4
CRN5
CRN6
CRNLIKE3
CRNLIKE4
CRN7
CRN8
CRN9
CRN10
CRNLIKE5

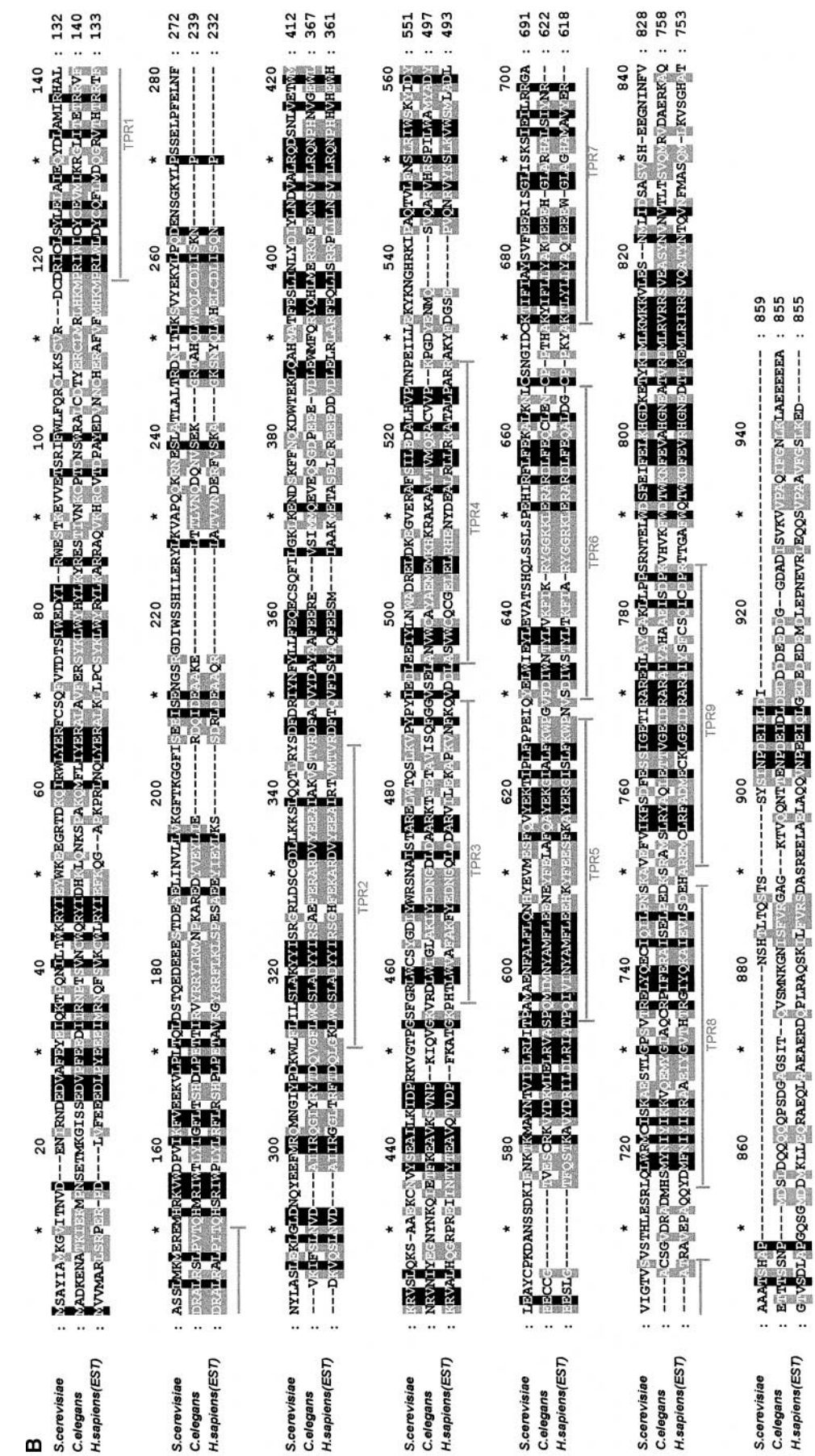


FIGURE 2—*Continued.*

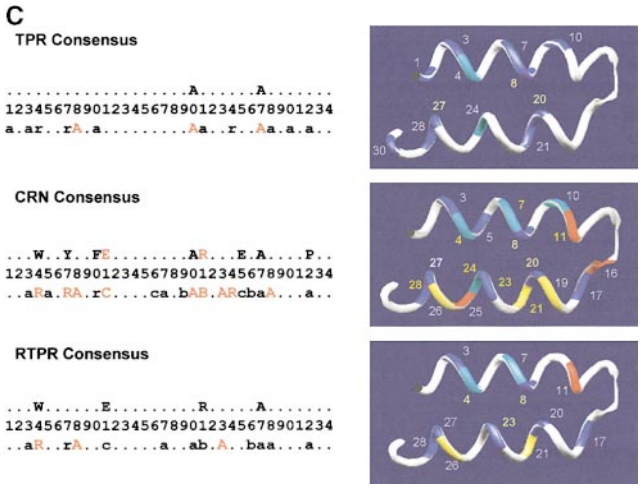


FIGURE 2.—(A) Amino acid alignment of the *S. cerevisiae* Syf1p sequence and putative orthologs from *C. elegans* and *H. sapiens*. Identical and chemically related residues that occur at the same position in all three sequences are shown white on black. Where the conservation is only in two sequences out of three they are shown white on gray. (B) Amino acid alignment of the *S. cerevisiae* Syf3p sequence and putative orthologs from *S. pombe*, *C. elegans*, *D. melanogaster*, *A. thaliana*, and *H. sapiens*. The CRN-TPR motifs annotated below the alignments were identified using Prosite Profiles. (C) Consensus sequences generated for the TPR, CRN, and RTPR repeats and their superimposition on the crystal structure of TPR1 of PP5 (DAS *et al.* 1998). The consensuses were generated using MotifHunter with amino acids conserved in >50% of the motifs drawn in black while amino acids conserved in >75% of the motifs were drawn in red. The top consensus shows individual amino acids while the bottom consensus represents amino acid type [a, aliphatic (AGILPV); r, aromatic (FWY); b, basic (HKR); c, acidic (DE)]. The consensus amino acid type was superimposed on the predicted structure (dark blue, hydrophobic aliphatic; light blue, hydrophobic aromatic; red, acidic; yellow, basic); white writing indicates conservation of >50% while bold yellow writing indicates conservation of >75%).

TOLLERVEY 1996)]. We refer to this subfamily as the RTPR motif (Figure 2C).

Figure 2C also shows that the semiconserved hydrophobic amino acids of the RTPR motif are slightly different in position from the normal TPR consensus, but still lie within the predicted nonpolar core of the motif, suggesting a similar overall structure. Interestingly, the RTPR, and especially the CRN-TPR motifs from Syf3p, have numerous charged residues conserved in the B domain helix, which DAS *et al.* (1998) have predicted lie on the outside face of the predicted superhelix. These residues may be important in protein-protein interactions, or may be important in interactions with nucleic acids, thus explaining their conservation in proteins involved in RNA metabolism.

Extensive protein-protein interactions involving the SYF gene products: To define the cellular context in which the newly identified proteins act, we searched for proteins that physically interact with Syf1p, Syf2p, and Syf3p. Two-hybrid interaction screens were performed with a yeast genomic library (FROMONT-RACINE *et al.* 1997), using each protein as a bait. The results show interactions among the products of the three novel *SYF* genes and between them and four additional spliceosomal proteins: Cef1p, Ntc20p, Isy1p, and Prp22p (Figure 3). Two-hybrid screens performed in turn with the four proteins as baits supported the existence of a network of interactions (Figure 3). The statistical significance of each interaction is indicated according to the two-hybrid criteria of FROMONT-RACINE *et al.* (1997), with the most significant being A1 (different, overlapping fragments of the same ORF) and A2 (N-terminally located fragments). Interestingly, Prp17p was not identified as a prey in any of these screens, and a screen with Prp17p as bait did not connect with this network of proteins (A. COLLEY and J. D. BEGGS, unpublished results).

CEF1 (YMR213w) is the *S. cerevisiae* homologue of the *S. pombe* cell cycle gene *Cdc5*$^{+}$. Cef1p is an evolutionarily conserved protein containing myb domains. In fission and budding yeasts, Cdc5p and Cef1p are essential for G2/M progression (OHI *et al.* 1994, 1998). In addition, the two yeast proteins have been shown to play a role in pre-mRNA splicing (MCDONALD *et al.* 1999; TSAI *et al.* 1999) and their human counterpart is spliceosome associated (NEUBAUER *et al.* 1998). Cef1p was also isolated as part of a large protein complex that includes the Prp19 spliceosomal protein (TSAI *et al.* 1999). Our two-hybrid screen also identified Ntc20p, another member of this protein complex, as an interactor with Syf3p. *NTC20* is a nonessential gene whose product associates with spliceosomes (TSAI *et al.* 1999). The *ISY1* gene

TABLE 2

Percentage homology among the different *SYF3* orthologs

	S. pombe	*C. elegans*	*D. melanogaster*	*A. thaliana 5a*	*A. thaliana 5b*	*A. thaliana 2b*
S. cerevisiae	40.2	35.8	37.1	33.9	34.4	33.1
S. pombe		48.1	49.3	49.9	48.8	45.6
C. elegans			57.7	47.8	49.9	46.3
D. melanogaster				55.0	54.8	53.9
A. thaliana 5a					84.6	77.8
A. thaliana 5b						75.5

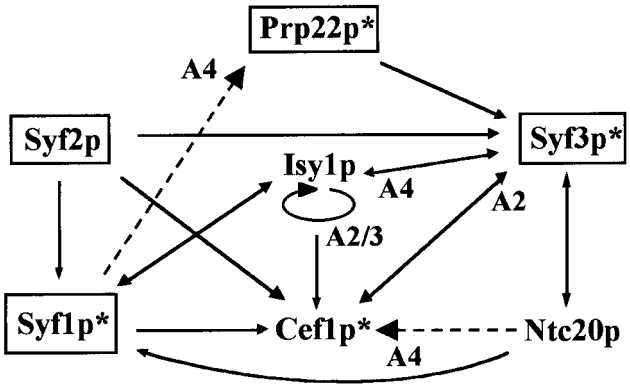


FIGURE 3.—Extensive protein-protein interactions involving the *SYF* gene products identified in genomic two-hybrid screens. Arrows extend from the bait protein toward the interacting protein. The categories of interaction are as defined by FROMONT-RACINE *et al.* (1997). A1 is the most statistically significant: different, overlapping fragments of the same ORF. A2, fragments encoding the N terminus of the protein, which are likely to be found rarely, and are therefore significant; A3, fragments of 1 kb or more, which are underrepresented in the library; A4, all other coding fragments. Where the category of the interaction is not indicated, it is A1. The components isolated as mutants that show synthetic lethality with *prp17*Δ are boxed. Proteins for which mutant alleles exist that cause cell cycle defects are indicated by an asterisk.

(*I*nteractor of *S*yf1p) encodes a spliceosome-associated protein that when used as a bait in a two-hybrid screen identified significant interactions with a number of proteins including the splicing factors Prp39p and Lea1p (DIX *et al.* 1999). Null *isy1* mutants are viable and show subtle splicing defects (DIX *et al.* 1999). Finally, the splicing factor, Prp22p, which was identified in our two-hybrid screens as well as in the synthetic lethal screen (Table 1 and Figure 3), interacts with both Syf3p and Syf1p (Figure 3). Interestingly, a *prp22* mutation has been identified that causes cell cycle arrest in G2/M (HWANG and MURRAY 1997).

In summary, the Syf1, Syf2, Syf3, Cef1, Ntc20, Isy1, and Prp22 proteins form a network of interactions that are suggestive of a multiprotein complex. The Syf3 and Cef1 proteins, in particular, show many connections (Figure 3). Alternatively, these results may reflect transient interactions between the proteins, rather than the existence of a stable complex. CHUNG *et al.* (1999) reported that in a direct two-hybrid test, Syf3/Clf1p showed interactions with Prp40p and Mud2p, two first-step splicing factors. We did not find evidence for such interactions in our two-hybrid screens.

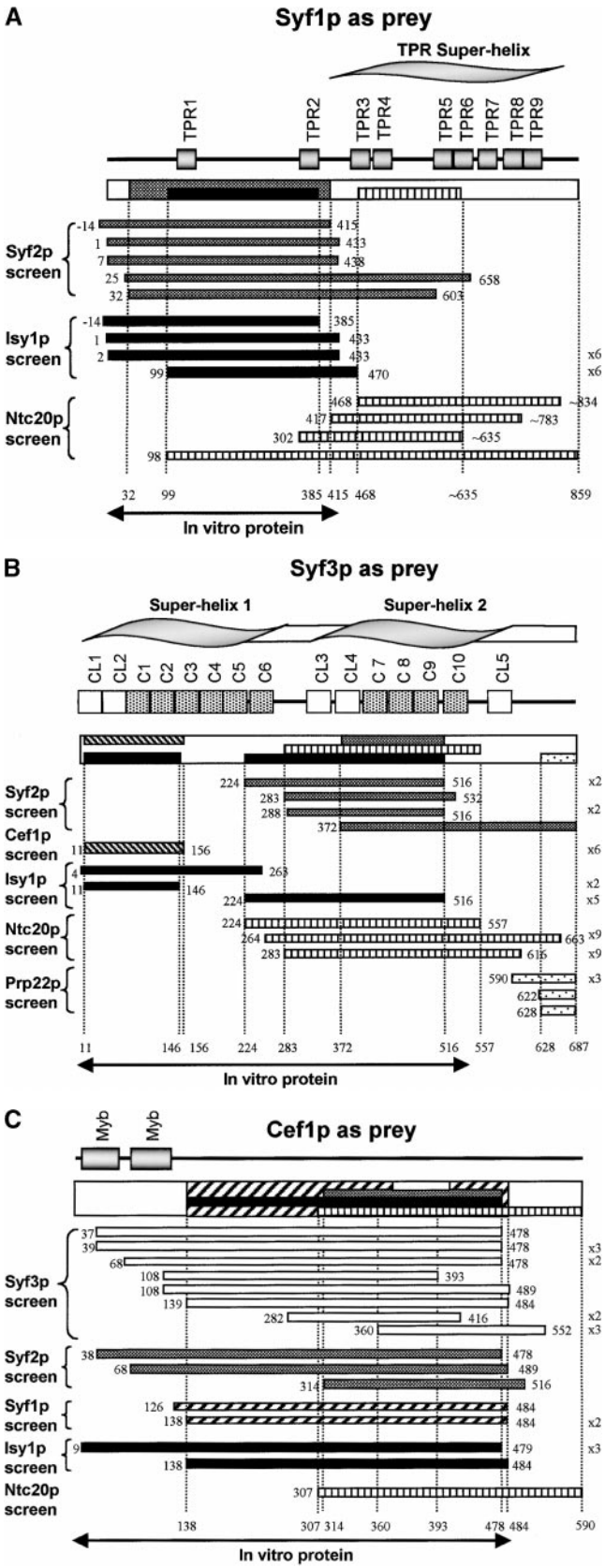
In addition to the four splicing factors described, the two-hybrid screens identified two protein kinases: (1) Syf2p as bait interacted with Kin3p (A2 category), and (2) Pkc1p showed A1 interactions with Cef1p and an A4 interaction (a single clone isolated several times) with Syf2p as bait. Kin3p is a nonessential kinase whose role remains unknown (JONES and ROSAMOND 1988), and Pkc1p is involved in osmoregulation and budding, among other processes (LEVIN and BARTLETT-HEUBUSCH 1992). These results suggest that splicing could be regulated by phosphorylation and dephosphorylation activities, modifications that could also provide a connection between the cell cycle and splicing (see DISCUSSION).

Protein regions involved in interactions: Functionally meaningful interactions found in the genomic two-hybrid method (FROMONT-RACINE *et al.* 1997) usually produce several interacting clones containing different overlapping fragments of the same gene (A1 category prey). A gene segment that is common to all prey fusions thus represents the minimum region involved in the interaction with the bait protein, although such a region is not necessarily sufficient for the interaction. Figure 4 shows the interacting regions in the Syf1, Syf3, and Cef1 proteins.

Syf1p: Both the Syf2p and the Isy1p two-hybrid screens isolated the N-terminal half of Syf1p, a section of the protein containing few TPR repeats, whereas the Ntc20p screen isolated a distinct region in the C-terminal half, rich in TPR motifs (Figure 4A). These results thus suggest that the TPR-rich C-terminal region of Syf1p is involved in different protein-protein interactions compared to its N terminus. X-ray crystallization studies of three TPR motifs present in the human PP5 protein led DAS *et al.* (1998) to postulate that tandem TPR repeats form a regular, right-handed superhelix with a helical repeat of about seven TPRs. In their model they hypothesize that the hydrophobic groove on the inside of the helix is involved in protein-protein interactions, contacting α-helices from adjacent proteins. Ntc20p, which is predicted to contain α-helices (data not shown) and binds the C terminus of Syf1p, a putative TPR superhelix, is a good candidate for this type of interaction (Figure 4A).

Syf3p: Bioinformatic analysis of the Syf3 protein family suggests that Syf3p consists of two complete TPR-like superhelices separated by a short linker, with a semiconserved non-TPR C terminus (Figure 4B). This predicted structure correlates well with the fragments isolated in two-hybrid screens, which identified three clear regions of interaction: (1) The N-terminal region (superhelix 1) interacts with Cef1p and Isy1p; (2) superhelix 2 mediates interactions with Syf2p, Isy1p, and Ntc20p; and (3) the TPR-free C terminus interacts solely with Prp22p. The interaction of Isy1p, Ntc20p, and Syf2p with both Syf1p and Syf3p may suggest that these proteins bind TPR-like motifs nonspecifically; this is probably not the case, however, since no other TPR-containing proteins were isolated in our two-hybrid screens. Thus, the interactions of these proteins, whether direct or indirect, appear specific. In addition, Ntc20p, Isy1p, and Cef1p fragments were found in the reciprocal Syf3p screen, further indicating that these interactions are significant.

Cef1p: The Cef1 protein contains Myb repeats located at its N terminus (Ohi *et al.* 1994, 1998; Figure 4C). This region is not required for interaction with any of the other identified proteins. Although the myb motif of other proteins has been shown to interact with DNA, no evidence has been found that Cef1p or its *S. pombe* homolog Cdc5p acts as a DNA-binding factor (Ohi *et al.* 1998). The involvement of Cef1p and its homologs in pre-mRNA splicing (Neubauer *et al.* 1998; McDonald *et al.* 1999; Tsai *et al.* 1999) suggests that the myb-containing domain may be involved in RNA binding. The central region of Cef1p was isolated as numerous fragments in the Syf1p, Syf2p, Syf3p, Isy1p, and Ntc20p screens (Figure 4C). The finding of interactions between Cef1p and Syf3p in the reciprocal two-hybrid screens (Cef1p or Syf3p as bait) indicates a close interaction between these two proteins.



Isy1p: Isy1p was isolated as a strong interactor with Syf1p (Dix *et al.* 1999). When *ISY1* was used as a bait, a clone containing the whole *ISY1* gene was isolated (data not shown; Dix *et al.* 1999), suggesting that Isy1p may function as a homodimer or multimer.

Ntc20p: Ntc20p was isolated as many fragments with Syf3p as bait but not in any of the other screens (data not shown). Ntc20p is a component of the Prp19 complex, which also includes the Cef1 protein (Tsai *et al.* 1999). The association with Syf3p may provide a link between this complex and the network of interactions described here.

It is uncertain whether any of these results represent direct or indirect interactions between the proteins, as transcriptional activation in two-hybrid experiments can, theoretically, be mediated by third party component(s) interacting with both the bait and the prey. To confirm the direct physical interaction between the proteins, we carried out *in vitro* studies.

Demonstration of protein interactions *in vitro*: We previously showed that *in vitro*-translated Isy1 protein was coimmunoprecipitated with protein A-tagged Syf1p in a yeast splicing extract (Dix *et al.* 1999). This result confirmed that the proteins associate, although it could not be ascertained from this assay whether it was a direct interaction or mediated by other yeast component(s). We therefore investigated whether these interactions are direct, using polyhistidine-tagged Isy1p (His_6-Isy1p) isolated from *E. coli*, in coprecipitation assays with the other proteins produced by *in vitro* translation (Figure 5). The *in vitro*-translated Syf2p, Isy1p, and Ntc20p were full length, whereas C-terminally truncated versions of Syf1p, Syf3p, and Cef1p were used (see Figure 4), since we were unable to obtain full-length versions of these much larger proteins.

Figure 4.—Regions involved in the interactions between proteins, as deduced from two-hybrid studies. (A) Syf1p; (B) Syf3p; (C) Cef1p. Numbers represent amino acid positions. Vertical lines delineate minimal regions found in common in overlapping prey fusions. The region of each protein that was produced by *in vitro* translation to test for a direct protein interaction (Figure 5) is indicated by an arrow at the bottom.

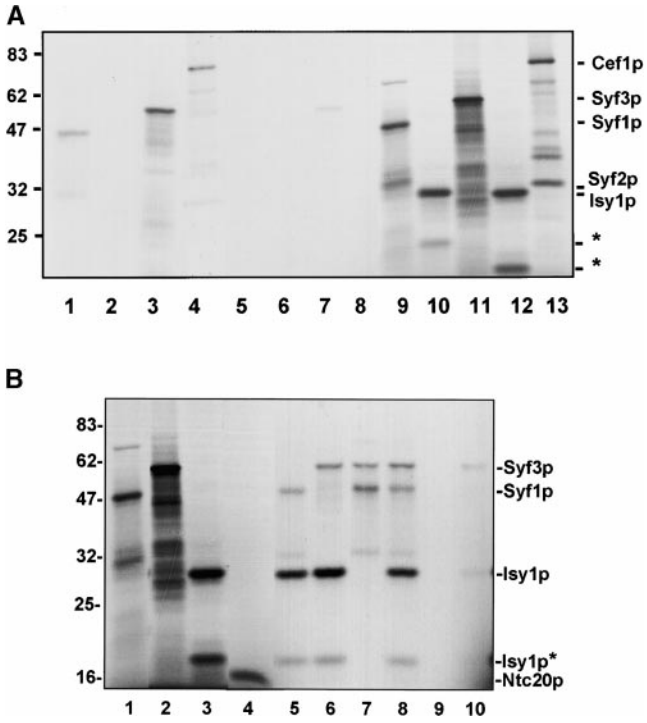


FIGURE 5.—Protein interactions *in vitro*. Coprecipitation of *in vitro*-translated Syf1p, Syf3p, Isy1p, and Cef1p with His_6-Isy1p. (A) Purified His_6-Isy1 protein (4–5 μg) was incubated with [^{35}S]methionine-labeled polypeptides produced by translation *in vitro* (6-μl reactions) and then with anti-pentahistidine antibodies and protein A-Sepharose to precipitate the His_6-Isy1p. The precipitated proteins were fractionated by SDS-PAGE and analyzed by autoradiography. Lane 1, Syf1p; lane 2, Syf2p; lane 3, Syf3p; lane 4, Cef1p. As controls, duplicate incubations were performed without His_6-Isy1p (lanes 5–8). Lanes 9–13 contain 1-μl aliquots of the *in vitro*-produced proteins (a shorter exposure is shown). Lane 9, Syf1p; lane 10, Syf2p; lane 11, Syf3p; lane 12, Isy1p; lane 13, Cef10p. The positions of the individual proteins are marked. The two bands marked with asterisks are abundant truncated or breakdown products of Syf2p and Isy1p obtained in the *in vitro* reaction. (B) Purified His_6-Isy1 protein was incubated with [^{35}S]-methionine-labeled polypeptides produced by translation *in vitro*: lane 5, Syf1p, Isy1p, and Ntc20p; lane 6, Syf3p, Isy1p, and Ntc20p; lane 7, Syf1p, Syf3p, and Ntc20p; lane 8, Syf1p, Syf3p, Isy1p, and Ntc20p; lane 9, Ntc20p. Lane 10 is a control with Syf1p, Syf3p, Isy1p, and Ntc20p but no His_6-Isy1p. Lanes 1–4 are aliquots of the *in vitro*-produced proteins. The positions of the individual proteins are indicated. Isy1p* represents an abundant truncated or breakdown product of Isy1p.

Figure 5A shows that *in vitro*-translated Syf1, Syf3, and Cef1 polypeptides were specifically coprecipitated with His_6-Isy1p (Figure 5A, lanes 1, 3, and 4, respectively), indicating a direct interaction, whereas *in vitro*-translated Syf2p (either alone as shown in Figure 5A, lane 2, or when mixed with all the other *in vitro*-translated proteins; data not shown) was not coprecipitated with His_6-Isy1p. The signals for coprecipitated Syf1p and Isy1p were always strongest. Nevertheless, His_6-Isy1p reproducibly coprecipitated levels of Syf3p and Cef1p that were significantly above background [levels obtained with no His_6-tagged protein (Figure 5A, lanes 5 and 8) or with an unrelated His_6-tagged protein (data not shown)]. *In vitro*-translated Ntc20p also did not coprecipitate with His_6-Isy1p, either alone (Figure 5B, lane 9) or in the presence of the other five proteins tested (Figure 5B, lanes 5–8, and data not shown). These data indicate that Isy1p is able to make direct interactions with Syf1p, Syf3p, Cef1p, and with itself but not with Syf2 or Ntc20 *in vitro*, strongly supporting the two-hybrid interaction results (Figure 3).

The question still arises whether these six proteins may all interact in a complex. The fact that Syf2p and Ntc20p did not coprecipitate with His_6-Isy1p even in the presence of the other five *in vitro*-translated proteins (data not shown and Figure 5B, lanes 5–9) may be taken as evidence against such a complex. However, the *in vitro*-produced Syf1p, Syf3p, and Cef1p were incomplete proteins, and the Syf1p and Cef1p fragments lacked regions that were indicated in the two-hybrid screens to be involved in interactions with Ntc20p (Figure 4, A and C). Therefore, it seems likely that these incomplete proteins may have limited the ability to form a heteromeric complex *in vitro*. Alternatively, other component(s) may be required for complex formation.

DISCUSSION

Synthetic lethal interactions: In an effort to identify genes that interact with the splicing and cell cycle gene *PRP17* we have isolated mutants that are inviable in the absence of the Prp17 protein. Six of the genes identified in our screen encode characterized splicing factors: Prp8p, Slu7p, Prp16p, Prp22p, Slt11p, and U2 snRNA; the remaining three genes (*SYF1-3*) are novel. While this work was in preparation, the *SYF3* gene was independently isolated as *CLF1* and shown to be involved in spliceosome assembly (CHUNG *et al.* 1999).

Previous work (JONES *et al.* 1995) has shown that *SLU7*, *PRP16*, *PRP18*, and *PRP17* act in concert in the second step of the splicing reaction. This step could be further separated into an ATP-dependent stage, which requires the activity of Prp16p and Prp17p, and a subsequent, ATP-independent stage at which the Slu7 and Prp18 proteins participate (JONES *et al.* 1995). Both Slu7p and Prp16p were shown to physically interact with the 3′ splice site during the second catalytic step of splicing (UMEN and GUTHRIE 1995a). Synthetic lethal interactions between mutations in *PRP17*, *PRP16*, *SLU7*, and *PRP18* have already been demonstrated (FRANK *et al.* 1992; SESHADRI *et al.* 1996). Our results are consistent with these results and show that synthetic lethality can be obtained in the complete absence of the Prp17 protein.

Prp8p is essential for the splicing reaction both *in vivo* and *in vitro*. It interacts with both splice sites, contacting the 3′ splice site after the first transesterification reaction (TEIGELKAMP *et al.* 1995; UMEN and GUTHRIE 1995a). Mutagenesis of Prp8p has shown that it is in-

volved in the recognition of the polypyrimidine tract and the 3′ splice site (Umen and Guthrie 1995b). We have recently identified extensive genetic interactions between *PRP8* and *PRP17*. In addition to the synthetic lethal alleles described here, we have uncovered *prp8* alleles that suppress the temperature sensitivity conferred by the absence of Prp17p. These mutations are also capable of suppressing alterations in the conserved PyAG trinucleotide at the 3′ splice site, suggesting that both proteins cooperate in recognizing this motif (Ben-Yehuda *et al.* 2000). It has been shown that Prp17p enhances the 3′ splice site interactions between Prp8p and Slu7p (Umen and Guthrie 1995a). Taking these observations together, it is reasonable to speculate that the Prp8 and Prp17 proteins are involved in the efficient recognition of the 3′ splice site, while Prp8p and Slu7p are required for executing the catalytic reaction. Therefore, the synthetic lethality observed may be a consequence of decreasing the efficiency of these two steps, leading to reduced splicing and cell death.

Recently, it was demonstrated that, in addition to mediating the release of mRNA from the spliceosome, Prp22p interacts directly with the 3′ splice site and is also required for the second step of the splicing reaction (Schwer and Gross 1998; McPheeters *et al.* 2000). Thus, five of the known genes isolated in our screen (including the U2 snRNA, see below), play a role during the second step of the splicing reaction. Preliminary characterization of the *slt11*, *syf1*, and *syf3* mutants suggests that they act prior to the first step of splicing (Xu *et al.* 1998; Chung *et al.* 1999; our unpublished results); however, a role in the second step of the splicing reaction has not been excluded for these factors.

Taking these observations together, the synthetic lethality of these mutations seems to be related to a failure in carrying out the second step of the splicing reaction. Even at the permissive temperature, *prp17* mutants have a detectable splicing defect, which is not severe enough to prevent growth (Ben-Yehuda *et al.* 1998). Mutations affecting interacting proteins may reduce the efficiency or accuracy of the second step of splicing below the minimal level needed for survival.

Genetic interactions with the U2 snRNA: Mutations in both *PRP17* and *SLU7* were isolated as synthetically lethal with mutations in the highly conserved loop I sequence of the U5 snRNA (Frank *et al.* 1992), although there is no evidence for their physical association with U5 RNA. Prp8p is a component of the U5 snRNP (Lossky *et al.* 1987) and directly contacts the U5 snRNA (Dix *et al.* 1998). We show here that mutations in the U2 snRNA can be lethal in the absence of the Prp17 protein. In addition, overproduction of U2 snRNA, but not of U5 snRNA, partially suppresses the synthetic lethality of all the *syf* mutants. This suggests that U2 is more important than U5 in the critical aspect of splicing that requires the presence of Prp17p and its interacting proteins. Other mutations in *PRP17*, *PRP8*, *SLU7*, and *SLT11* were recently found to be synthetically lethal with mutations in the U2 snRNA that perturb the U2–U6 snRNA helix II interactions (Xu *et al.* 1998). In addition, Prp8p was found to be closely associated with the intron branch site (and hence in close proximity to U2) in active spliceosomes (Teigelkamp *et al.* 1995).

Xu *et al.* (1998) have suggested that the 5′ end of the U2 snRNA may provide a substrate for RNA interactions that serve to anchor the U5 snRNA and/or assist the U5 snRNA in the alignment of the two exons. According to this model, we speculate that the proteins identified in our screen may participate in stabilizing this interaction between the two snRNAs and the exons during step 2. In the absence of Prp17p this interaction is less stable, and mutation of any of the other factors leads to further destabilization, resulting in synthetic lethality. The observation that overproduction of U2 snRNA is able to partially suppress all the synthetic lethal mutations found in the screen may be explained by the production of increased numbers of spliceosomal complexes that could enable at least some splicing to occur.

Proteins involved in cell cycle progression and in pre-mRNA splicing interact genetically and physically: The *PRP17* gene was found to play a role in two seemingly unrelated processes: cell cycle progression and pre-mRNA splicing (see Introduction). Similarly, some of the genes uncovered in our synthetic lethality screen have roles in both pre-mRNA splicing and cell cycle progression. For example, some mutant alleles of *PRP8* (*dbf3* and *dna39*) were isolated as cell cycle-specific mutants (Dumas *et al.* 1982; Shea *et al.* 1994). The phenotype of *dbf3-1* strongly resembles that of *prp17* strains, showing a delayed DNA replication and a G2/M arrest at the restrictive temperature (Shea *et al.* 1994). Similarly, a *prp22* allele was recently found that causes a cell cycle arrest at the G2/M transition (Hwang and Murray 1997). Finally, the newly identified *SYF1* and *SYF3* genes were also found to be required for progression through the same cell cycle stage (our unpublished results).

In this study we uncovered extensive two-hybrid and physical interactions among a group of splicing factors: Syf1, Syf3, Prp22, Isy1, Ntc20, Syf2, and Cef1 (Figures 3–5). In addition to the first three proteins, Cef1p has also been shown to affect both cell cycle progression and pre-mRNA splicing (Ohi *et al.* 1998; Tsai *et al.* 1999). The nature of the intricate interactions observed among this group of proteins is still under investigation and currently the results could be interpreted in two alternative ways: (1) These proteins could be part of a protein complex with a role in both cell cycle progression and pre-mRNA splicing; or (2) the two-hybrid and the biochemical results may reflect transient interactions between the different proteins, not necessarily in the context of a protein complex. In support of the first possibility, a 40S complex from *S. pombe*, which includes the Cef1, Syf1, Syf3, Prp8, Slt11, and Prp19 homologues,

was recently characterized (McDonald *et al.* 1999). Moreover, a complex containing the Ntc20, Cef1, and Prp19 proteins has also been isolated from *S. cerevisiae* (Tsai *et al.* 1999). It is still unclear whether the interactions we have observed, which include the Ntc20 and Cef1 proteins, take place in the context of the same complex. We speculate that such complexes might act as mediators that link splicing to cell cycle progression (see below). However, the second hypothesis, suggesting that the interactions observed are transient, cannot be ruled out at present.

The nature of the connection between Prp17p and the interacting group of proteins is still unknown, since no physical interactions were detected between Prp17p and any of the proteins involved either in two-hybrid screens or in direct two-hybrid assays (data not shown). However, the identification of *SYF1*, *SYF2*, *SYF3*, and *PRP22* in the synthetic lethal screen with the *prp17*Δ allele suggests that this group of genes and *PRP17* may affect the same cellular activity.

The observation that Cef1p and Syf2p can associate with the Pkc1 and Kin3 protein kinases suggests a mechanism by which the interacting proteins could be regulated through phosphorylation and dephosphorylation activities. There are several examples of regulation of the splicing process by phosphorylation in mammalian cells; for example, many proteins that belong to the serine-arginine-rich (SR) family of splicing factors undergo phosphorylation, which affects their subnuclear localization (Colwill *et al.* 1996). Phosphorylation could regulate the distribution, localization, and/or activity of the Cef1 and Syf2 proteins and other proteins that may interact with them. Phosphorylation could also be responsible for the coordination between cell cycle progression and splicing. This link was recently strengthened by the finding of a physical association between Cyclin E-Cdk2 and components of the U2 snRNA-associated proteins SAP114, SAP145, and SAP155 in mammalian cells (Seghezzi *et al.* 1998).

Progression through the cell cycle could be regulated by the timely splicing of key pre-mRNAs at different cell cycle stages. We hypothesize that a small number of pre-mRNAs coding for proteins important for cell cycle progression may undergo splicing in a regulated manner. An example of such a regulation in yeast is the controlled splicing of the *MER2* recombination gene in a meiosis-specific fashion (Nandabalan and Roeder 1995). Much progress has been achieved lately in the understanding of how the cell cycle is regulated by the degradation of a small number of key proteins (Zachariae and Nasmyth 1999). These studies have shown that a housekeeping function, such as protein degradation, may be used by the cell to control other major processes. We propose that pre-mRNA splicing may act in a similar fashion, leading to the expression or inhibition of a subset of genes, thus controlling cell cycle progression.

All the genes identified in our screens are conserved throughout evolution. The human homologues of the following proteins have been identified: *PRP17* (Ben-Yehuda *et al.* 1998; Lindsey and Garcia-Blanco 1998; Zhou and Reed 1998), *PRP8* (Anderson *et al.* 1989), *PRP22* (Ohno and Shimura 1996), *CEF1* (Neubauer *et al.* 1998), and *ISY1* (Dix *et al.* 1999). In addition, we have identified orthologs of *SYF1* and *SYF3* from different species. The high conservation of these interacting genes throughout evolution emphasizes the importance of their function in eukaryotic cells and suggests that the connection between splicing and cell cycle progression is universal.

We thank Yona Kassir and Andrew Newman for their generous gifts of reagents. We also thank Rina Jaget and Rivka Steinlauf for excellent technical assistance, Michael Mellor-Clark for the Ntc20p screen, and Michael Albers and Kathy Gould for sharing unpublished information. This work was supported by grants to M.K. by the Israel Cancer Association, the Israel Cancer Research Fund, and the Recanati Foundation; and to J.D.B. by a Wellcome Trust Programme Grant (no. 047685). S.B.Y. was recipient of a travel scholarship from the British Council. J.D.B. was supported by a Royal Society Cephalosporin Fund Senior Research Fellowship.

LITERATURE CITED

Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang *et al.*, 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:** 3389–3402.

Anderson, G. J., M. Bach, R. Luhrmann and J. D. Beggs, 1989 Conservation between yeast and man of a protein associated with U5 small nuclear ribonucleoprotein. Nature **342:** 819–821.

Ares, M., Jr., 1986 U2 RNA from yeast is unexpectedly large and contains homology to vertebrate U4, U5, and U6 small nuclear RNAs. Cell **47:** 49–59.

Beggs, J. D., 1995 Yeast splicing factors and genetic strategies for their analysis, pp. 79–95 in *Pre-mRNA Processing*, edited by A. I. Lamond. R. G. Landes Company, Austin, TX.

Ben-Yehuda, S., I. Dix, C. S. Russell, S. Levy, J. D. Beggs *et al.*, 1998 Identification and functional analysis of hPRP17, the human homologue of the *PRP17* yeast gene involved in splicing and cell cycle control. RNA **4:** 1304–1312.

Ben-Yehuda, S., C. S. Russell, I. Dix, J. D. Beggs and M. Kupiec, 2000 Extensive genetic interactions between *PRP8* and *PRP17/CDC40*, two yeast genes involved in pre-mRNA splicing and cell cycle progression. Genetics **154:** 61–71.

Boeke, J. D., J. Truehart, G. Natsoulis and G. R. Fink, 1987 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:** 165–175.

Boger-Nadjar, E., N. Vaisman, S. Ben-Yehuda, Y. Kassir and M. Kupiec, 1998 Efficient initiation of S-phase in yeast requires Cdc40p, a protein involved in cell cycle progression and pre-mRNA splicing. Mol. Gen. Genet. **260:** 232–241.

Burge, C., and S. Karlin, 1997 Prediction of complete gene structures in human genomic DNA. J. Mol. Biol. **268:** 78–94.

Chung, S., M. R. McLean and B. C. Rymond, 1999 Yeast ortholog of the *Drosophila* crooked neck protein promotes spliceosome assembly through stable U4/U6.U5 snRNP addition. RNA **5:** 1042–1054.

Colwill, K., T. Pawson, B. Andrews, J. Prasad, J. L. Manley *et al.*, 1996 The Clk/Sty protein kinase phosphorylates SR splicing factors and regulates their intracellular distribution. EMBO J. **15:** 265–275.

Das, A. K., P. T. W. Cohen and D. Barford, 1998 The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. EMBO J. **17:** 1192–1199.

Dix, I., C. S. Russell, R. T. O'Keefe, A. Newman and J. D. Beggs, 1998 Protein-RNA interactions in the U5 snRNP of *Saccharomyces cerevisiae*. RNA **4:** 1239–1250.

Dix, I., C. S. Russell, S. Ben-Yehuda, M. Kupiec and J. D. Beggs, 1999 The identification and characterization of a novel splicing protein, Isy1p, of *Saccharomyces cerevisiae*. RNA **5:** 360–368.

Dumas, L. B., J. P. Lusky, E. J. McFarland and J. Shampay, 1982 New temperature-sensitive mutants of *Saccharomyces cerevisiae* affecting DNA replication. Mol. Gen. Genet. **187:** 42–46.

Elledge, S. J., 1996 Cell cycle checkpoints: preventing an identity crisis. Science **274:** 1664–1672.

Frank, D., and C. Guthrie, 1992 An essential splicing factor *SLU7*, mediates 3′ splice site choice in yeast. Genes Dev. **6:** 2112–2124.

Frank, D., B. Patterson and C. Guthrie, 1992 Synthetic lethal mutations suggest interactions between U5 small nuclear RNA and four proteins required for the second step of splicing. Mol. Cell. Biol. **12:** 5197–5205.

Fromont-Racine, M., J.-C. Rain and P. Legrain, 1997 Towards a functional analysis of the yeast genome through exhaustive two-hybrid screens. Nat. Genet. **16:** 277–282.

Gietz, R. D., and A. Sugino, 1988 New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:** 527–534.

Hwang, L. H., and A. W. Murray, 1997 A novel yeast screen for mitotic arrest mutants identifies *DOC1*, a new gene involved in cyclin proteolysis. Mol. Biol. Cell **8:** 1877–1887.

Jones, D. G. L., and J. Rosamond, 1988 Identification of a gene encoding a novel protein kinase in yeast. Yeast **4:** 45.

Jones, M. H., D. N. Frank and C. Guthrie, 1995 Characterization and functional ordering of Slu7p and Prp17p during the second step of pre-mRNA splicing in yeast. Proc. Natl. Acad. Sci. USA **92:** 9687–9691.

Kassir, Y., and G. Simchen, 1978 Meiotic recombination and DNA synthesis in a new cell cycle mutant of *Saccharomyces cerevisiae*. Genetics **90:** 49–58.

Lamb, J. R., W. A. Michaud, R. S. Sikorski and P. A. Hieter, 1994 Cdc16p, Cdc23p and Cdc27p form a complex essential for mitosis. EMBO J. **13:** 4321–4328.

Lamb, J. R., S. Tugendreich and P. Hieter, 1995 Tetratricopeptide repeat interactions: to TPR or not to TPR? Trends Biochem. Sci. **20:** 257–259.

Levin, D. E., and E. Bartlett-Heubusch, 1992 Mutants in the *S. cerevisiae PKC1* gene display a cell cycle-specific osmotic stability defect. J. Cell Biol. **116:** 1221–1229.

Lindsey, L. A., and M. Garcia-Blanco, 1998 Functional conservation of the human homologue of the yeast pre-mRNA splicing factor Prp17p. J. Biol. Chem. **273:** 32771–32775.

Lockhart, S. R., and B. C. Rymond, 1994 Commitment of yeast pre-mRNA to the splicing pathway requires a novel U1 small nuclear ribonucleoprotein polypeptide, Prp39p. Mol. Cell. Biol. **14:** 3623–3633.

Lossky, M., G. J. Anderson, S. P. Jackson and J. D. Beggs, 1987 Identification of a yeast snRNP and detection of snRNP-snRNP interactions. Cell **51:** 1019–1026.

Madhani, H. D., and C. Guthrie, 1994 Dynamic RNA-RNA interactions in the spliceosome. Annu. Rev. Genet. **28:** 1–26.

McDonald, W., H. R. Ohi, N. Smelkova, D. Frendewey and K. L. Gould, 1999 Myb-related fission yeast cdc5p is a component of a 40S snRNP-containing complex and is essential for pre-mRNA splicing. Mol. Cell. Biol. **19:** 5352–5362.

McPheeters, D. S., B. Schwer and P. Muhlenkamp, 2000 Interaction of the yeast DexH-box RNA helicase Prp22p with the 3′ splice site during the second step of nuclear pre-mRNA splicing. Nucleic Acids Res. **28:** 1313–1321.

Nandabalan, K., and G. S. Roeder, 1995 Binding of a cell-type-specific RNA splicing factor to its target regulatory sequence. Mol. Cell. Biol. **15:** 1953–1961.

Neubauer, G., A. King, J. Rappsilber, S. C. Calvio, M. Watson *et al.*, 1998 Mass spectrometry and EST database searching allows characterization of the multi-protein spliceosome complex. Nat. Genet. **20:** 46–50.

Nicholas, K. B., and H. B. Nicholas, Jr., 1997 Genedoc, a tool for editing and annotating multiple sequence alignments, Ver 2.4. Distributed by the author.

Ohi, R., D. McCollum, B. Hirani, G. J. Den Haese, X. Zhang *et al.*, 1994 The *S. pombe* cdc5+ gene encodes an essential protein with homology to c-Myb. EMBO J. **13:** 471–483.

Ohi, R., A. Feoktistova, S. McCann, V. Valentine, A. T. Look *et al.*, 1998 Myb-related *Schizosaccharomyces pombe* cdc5p is structurally and functionally conserved in eukaryotes. Mol. Cell. Biol. **18:** 4097–4108.

Ohno, M., and Y. Shimura, 1996 A human helicase-like protein, HRH1, facilitates nuclear export of spliced mRNA by releasing the mRNA from the spliceosome. Genes Dev. **10:** 997–1007.

Plumpton, M., M. McGarvey and J. D. Beggs, 1994 A dominant negative mutation in the conserved RNA helicase motif "SAT" causes splicing factor PRP2 to stall in spliceosomes. EMBO J. **13:** 879–887.

Reyes, J. L., E. H. Gustafson, H. R. Luo, M. J. Moore and M. M. Konarska, 1999 The C-terminal region of hPrp8 interacts with the conserved GU dinucleotide at the 5′ splice site. RNA **5:** 167–179.

Russell, C. S., S. Ben-Yehuda, I. Dix, M. Kupiec and J. D. Beggs, 2000 Characterization of novel factors involved in both pre-mRNA splicing and cell cycle progression in *Saccharomyces cerevisiae*. RNA (in press).

Schwer, B., and C. H. Gross, 1998 Prp22, a DExH box RNA helicase, plays two distinct roles in yeast pre-mRNA splicing. EMBO J. **17:** 2086–2094.

Schwer, B., and C. A. Guthrie, 1991 *PRP16* is an RNA-dependent ATPase that interacts transiently with the spliceosome. Nature **349:** 494–499.

Seghezzi, W., K. Chua, F. Shanahan, O. Gozani, R. Reed *et al.*, 1998 Cyclin E associates with components of the Pre-mRNA splicing machinery in mammalian cells. Mol. Cell. Biol. **18:** 4526–4536.

Seshadri, V., V. C. Vaidya and U. Vijayraghavan, 1996 Genetic studies of the *PRP17* gene of *Saccharomyces cerevisiae*: a domain essential for function maps to a nonconserved region of the protein. Genetics **143:** 45–55.

Shea, J. E., J. H. Toyn and L. H. Johnston, 1994 The budding yeast U5 snRNP Prp8 is a highly conserved protein which links RNA splicing with cell cycle progression. Nucleic Acids Res. **22:** 5555–5564.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *S. cerevisiae*. Genetics **122:** 19–27.

Smith, T. F., C. Gaitatzes, K. Saxena and E. J. Neer, 1999 The WD repeat: a common architecture for diverse functions. Trends Biochem. Sci. **24:** 181–185.

Teigelkamp, S., A. J. Newman and J. D. Beggs, 1995 Extensive interactions of PRP8 protein with the 5′ and 3′ splice sites during splicing suggest a role in stabilization of exon alignment by U5 snRNA. EMBO J. **14:** 2602–2612.

Thompson, J. D., D. G. Higgins and T. J. Gibson, 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:** 4673–4680.

Tsai, W. Y., Y. T. Chow, H. R. Chen, K. T. Huang, R. I. Hong *et al.*, 1999 Cef1p is a component of the prp19p-associated complex and essential for pre-mRNA splicing. J. Biol. Chem. **274:** 9455–9462.

Umen, J. G., and C. Guthrie, 1995a Prp17p, Slu7p, and Prp8p interact with the 3′ splice site in two distinct stages during the second catalytic step of pre-mRNA splicing. RNA **1:** 584–597.

Umen, J. G., and C. Guthrie, 1995b A novel role for a U5 snRNP protein in 3′ splice site selection. Genes Dev. **9:** 855–868.

Urushiyama, S., T. Tani and Y. Ohshima, 1997 The *prp1+* gene required for pre-mRNA splicing in *Schizosaccharomyces pombe* encodes a protein that contains TPR motifs and is similar to Prp6p of budding yeast. Genetics **147:** 101–115.

Vaisman, N., A. Tzoulade, K. Robzyk, S. Ben-Yehuda, M. Kupiec *et al.*, 1995 The role of *S. cerevisiae* Cdc40p in DNA replication and mitotic spindle function. Mol. Gen. Genet. **247:** 123–136.

Venema, J., and D. Tollervey, 1996 *RRP5* is required for formation of both 18S and 5.8S rRNA in yeast. EMBO J. **15:** 5701–5714.

Vojtek, A. B., S. M. Hollenberg and J. A. Cooper, 1993 Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell **74:** 205–214.

Wagner, J. D., E. Jankowsky, M. Company, A. M. Pyle and J. N. Abelson, 1998 The DEAH-box protein PRP22 is an ATPase

that mediates ATP-dependent mRNA release from the spliceosomes and unwinds RNA duplexes. EMBO J. **17:** 2926–2937.

Wang, Y., J. D. O. Wagner and C. Guthrie, 1998 The DEAH-box splicing factor Prp16 unwinds RNA duplexes *in vitro*. Curr. Biol. **8:** 441–451.

Will, C. L., and R. Luhrmann, 1997 Protein functions in pre-mRNA splicing. Curr. Opin. Cell Biol. **9:** 320–328.

Xu, Y., R. J. Mural, J. R. Einstein, M. B. Shah and E. C. Uberbacher, 1996 Grail—a multiagent neural-network system for gene identification. Proc. IEEE **84:** 1544–1552.

Xu, D., D. J. Field, S. Tang, A. Moris, B. Bobchenko *et al.*, 1998 Synthetic lethality of yeast *slt* mutations with U2 small nuclear RNA mutations. Mol. Cell. Biol. **18:** 2055–2066.

Zachariae, W., and K. Nasmyth, 1999 Whose end is destruction: cell division and the anaphase-promoting complex. Genes Dev. **13:** 2039–2058.

Zhang, K., D. Smouse and N. Perrimon, 1991 The *crooked neck* gene of *Drosophila* contains a motif found in a family of yeast cell cycle genes. Genes Dev. **5:** 1080–1091.

Zhou, Z., and R. Reed, 1998 Human homologs of yeast Prp16 and Prp17 reveal conservation of the mechanism for catalytic step II of pre-mRNA splicing. EMBO J. **17:** 2095–2106.

Communicating editor: F. Winston