Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 848–852, February 1993
Biochemistry

Convergent transcripts of the yeast *PRP38–SMD1* locus encode two essential splicing factors, including the D1 core polypeptide of small nuclear ribonucleoprotein particles

BRIAN C. RYMOND

T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225

Communicated by Mary Edmonds, October 30, 1992 (received for review August 7, 1992)

ABSTRACT **The *PRP38* gene of *Saccharomyces cerevisiae* is necessary for the excision of intron sequences from pre-mRNA and required for the maintenance of maximal levels of U6 small nuclear RNA (snRNA). This report describes the identification of a gene of related function, *SMD1*, located immediately 3′ to *PRP38*. The *PRP38* and *SMD1* transcription units are configured in an unusual "tail-to-tail" arrangement with their respective open reading frames terminating on opposite strands of a common 6-bp region. The predicted *SMD1* polypeptide, Smd1p, is 40% identical to the D1 protein of human small nuclear ribonucleoprotein particles. Experimentally induced depletion of Smd1p blocks the first step of splicing and results in growth arrest. In addition, the levels of the trimethylguanosine-capped spliceosomal snRNAs, U1, U2, U4, and U5, but not the Prp38p-sensitive U6 snRNA, decrease in response to Smd1p depletion. The cap structures of snRNAs persisting in the absence of *SMD1* expression appear to be peculiar, as they are poorly recognized by an anti-trimethylguanosine antibody. These data establish Smd1p as a required component of the cellular splicing apparatus and a factor in snRNA maturation and stability.**

The eukaryotic spliceosome is a large, complex ribonucleoprotein. Chief among its subunits are five small nuclear ribonucleoprotein (snRNP) particles, the U1, U2, U4, U5, and U6 snRNPs (for review, see refs. 1 and 2). With the exception of U6, each snRNP is composed of a small nuclear RNA (snRNA) possessing a 2,2,7-trimethylguanosine (TMG) cap, a conserved set of small (10- to 27-kDa) common proteins (B, B′, D1, D2, D3, E, F, G), and a variable number of snRNP-specific polypeptides. U6 snRNA has a γ-monomethyl cap and does not directly bind the common proteins.

Among the earliest detectable events in the assembly of metazoan snRNP particles is the binding of a 6S protein complex composed of snRNP proteins D1 (plus perhaps D2 or D3), E, F, and G to the snRNA (3, 4). This highly stable snRNA–protein core complex forms in the cytoplasm (5) and requires a single-stranded region of the snRNA, the Sm binding site, which contains the consensus sequence $RA(U)_{>3}GR$ (6). Succeeding events in snRNP maturation—e.g., binding of the remaining common and snRNP-specific polypeptides, hypermethylation of the snRNA cap, and movement of the snRNP into the nucleus—are reliant upon (or facilitated by) the formation of this initial snRNA–protein core complex (1, 2).

Nuclease protection experiments have revealed that the core proteins shield ≈25 nt, including the Sm site, from nuclease attack (6). Mutations within the Sm site can have deleterious effects on snRNP assembly or stability and impede or block pre-mRNA splicing *in vitro* (1, 2, 7–9). *In vivo*, Sm-site mutations can be lethal (7, 10), presumably because they impair the processing of pre-mRNA. The identity of the snRNA-binding protein(s) that drives core complex formation remains uncertain. In RNA–protein binding studies, the D1 protein appears to have the greatest intrinsic affinity for RNA (11, 12), yet only the G protein is specifically crosslinked to the snRNA of purified core complexes (13).

This report describes the isolation of *SMD1*, the gene coding for *Saccharomyces cerevisiae* (yeast) core snRNP polypeptide D1 (Smd1p). Genetic and biochemical studies reveal an essential function for Smd1p in pre-mRNA splicing and implicate Smd1p and the product of a neighboring gene, *PRP38* (14), in the formation of stable, biologically active snRNP structures.*

MATERIALS AND METHODS

Yeast Strains. The yeast strains used included MGD353 46D (*MATα trp1-289 ura3-52 leu2-3,112 his3-Δ1 cyh*^{r}), MGD353 13D (*MATa trp1-289 ura3-52 leu2-3,112 arg4 ade2*), MGD407 (α/a diploid from an MGD353 46D × MGD353 13D cross), *SMD1* null [*MATα trp1-289 ura3-52 leu2-3,112 his3-Δ1, SMD1::LEU2*; pBM150(*URA3 GAL::SMD1*)], and *PRP38* null [*MATa trp1-289 ura3-52 leu2-3,112 ade2 PRP38::LEU2*; pBM150[(*URA3 GAL1::PRP38*)]. The *cyh*^{r} allele has not been scored for the null strains.

Creation of the *SMD1* and *PRP38* Null and *GAL1* Conditional Alleles. Oligonucleotide-directed mutagenesis (oligonucleotides 5′-CCAAGAAGAGGTCGACTATGATTCGC-TGC-3′ and 5′-CCTACTGCAAGTCGACAACATAG-CAAG-3′) was used to introduce two *Sal* I restriction sites into the *SMD1* coding segment of a 2-kb *Xba* I–*Pst* I subclone of yeast DNA (14). After cleavage with *Sal* I, the *SMD1*-containing fragment was blunted with mung bean nuclease and ligated to a similarly blunted 2.2-kb *Sal* I–*Xho* I fragment containing the yeast *LEU2* gene. The resulting *SMD1::LEU2* null allele was released from the vector sequences by cleaving the DNA with *Xba* I and *Pst* I and used to replace one chromosomal copy of *SMD1* in strain MGD407 by homologous recombination. The construction of the yeast *PRP38::LEU2* null allele was previously described (14).

A *GAL1::SMD1* fusion was prepared by ligating a *Bgl* II/*Bam*HI-digested 526-bp polymerase chain reaction (PCR) fragment containing the *SMD1* coding sequence into the *Bam*HI site of pBM150 (15). The 5′ and 3′ *SMD1* PCR oligonucleotides included noncoding *Bgl* II (5′-TTTAGATC-TATTATGAAGTTGGTTAACTTT-3′) and *Bam*HI (5′-CCCGGATCCGAGTAGTTCTAGCGAAAGCG-3′) sites, respectively, to facilitate cloning. *GAL1::PRP38* was constructed similarly by using oligonucleotides 5′-CTTAGATC-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; TMG, 2,2,7-trimethylguanosine; orf, open reading frame.

*The sequences of *SMD1* and *PRP38* have been deposited in the GenBank data base (accession nos. L04669 and M95921, respectively).

TGGACTACAATGGCTGTCAATG-3′ and 5′-TTTAGATCTATTATGAAGTTGGTTAACTTT-3′. To obtain the conditional lethal haploids expressing the *GAL1* fusion constructs, plasmids containing *GAL1*::SMD1 or *GAL1*::*PRP38* were introduced by transformation into strain MGD407 made heterozygous for the appropriate cellular gene disruption, the transformants were sporulated, and the asci were dissected on rich galactose-based medium. Individual meiotic progeny were then scored on galactose-based medium without leucine for the presence of the *SMD1*::*LEU2* (or *PRP38*::*LEU2*) null allele and on uracil-deficient medium for the presence of the *URA3*-containing *GAL1*::*SMD1* (or *GAL1*::*PRP38*) plasmid. The correctness of these genetic assignments was confirmed by Southern analysis.

Immunoprecipitation. Fifteen microliters of the K121 anti-TMG antibody (16) and 0.5 μl of rabbit anti-mouse IgG (Zymed Laboratories) were bound to 50 μl of protein A-agarose (BRL–GIBCO) in buffer B (20 mM Hepes, pH 8.0/150 mM NaCl/0.05% Triton X-100) for 30 min at room temperature. Excess antibody was removed by washing the agarose four times with 1 ml of buffer B. Twenty micrograms of total cellular RNA was added in 50 μl of buffer B, and the antibody/RNA mixture was incubated at 23°C for 60 min with occasional gentle mixing. After five washes with 1 ml of buffer B, the specifically associating RNAs were released by the addition of 150 μl of buffer K (100 mM Tris·HCl, pH 7.5/150 mM NaCl/1% SDS with proteinase K at 0.2 mg/ml) at 37°C for 15 min. Residual proteins were removed by phenol extraction and the RNA was concentrated by ethanol precipitation. One-fifth of the recovered RNA was end-labeled with RNA ligase and [^{32}P]pCp prior to electrophoresis in a 7 M urea/5% polyacrylamide gel.

RESULTS

Isolation of *SMD1*. Two low-abundance poly(A)$^+$ transcripts derived from the region of the *PRP38* locus (14) were detected by Northern hybridization (Fig. 1*A*). The larger, 1175-nt transcript corresponds to RNA transcribed in the same orientation as the *PRP38* orf and presumably represents the mRNA encoding this essential splicing factor. The smaller, 550-nt transcript is synthesized in the opposite orientation. As the hybridization probes were restricted to the *PRP38* coding and 3′ flanking regions, the smaller RNA either originates from within the *PRP38* gene or is the product of a convergently transcribed gene located immediately 3′ of *PRP38*. DNA sequence analysis of the region downstream of *PRP38* revealed the suspected second orf, 438 bp in length, which was designated *SMD1* (Fig. 1*B*). Curiously, the translational termination codons for *PRP38* and *SMD1* are directly adjacent on opposing DNA strands; the last codon in each orf encodes the final amino acid of the other.

Yeast and Human D1 Proteins Are Remarkably Similar. Comparison of the putative basic *SMD1*-encoded polypeptide (estimated pI, 11.2) with the GenBank protein data base (April 1991) revealed a striking similarity to the human core snRNP polypeptide and Sm antigen, D1 (ref. 17; see Fig. 2). Overall, the yeast and human proteins are ≈40% identical, with blocks of 70–80% identity at the amino terminus (amino acids 1–28, segment I) and internally at amino acids 88–109 (segment II). An extremely basic carboxyl terminus follows segment II in both proteins. The human D1 carboxyl terminus is composed of a lysine-rich segment followed by a simple Gly-Arg repeat similar to the repetitive Arg/Asp and Arg/Ser elements observed in a number of splicing factors (e.g., U1 70-kDa protein, ASF/SF2, U2AF; see references in ref. 18) and a recently identified family of nuclear phosphoproteins (19). The carboxyl terminus of the yeast protein is more complex but is comparable in length and charge characteristics to that present in human D1. Overall, the yeast protein,

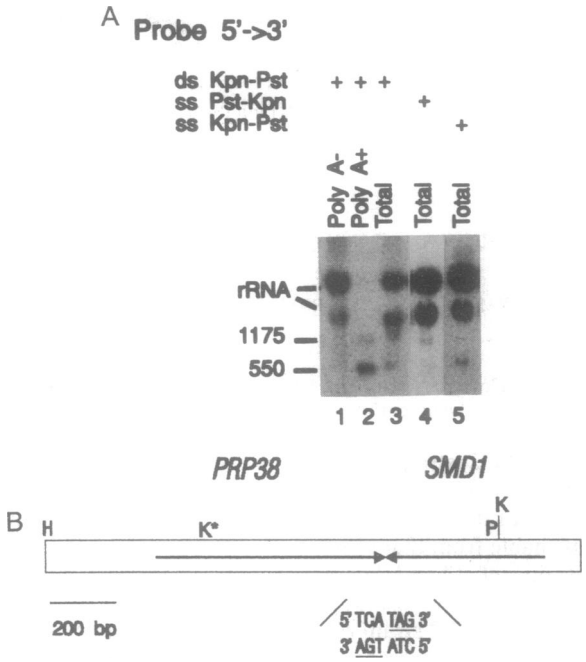

FIG. 1. Isolation of *SMD1*. (*A*) Northern hybridization of 20 μg of poly(A)$^-$ (lane 1), 2 μg of poly(A)$^+$ (lane 2), or 20 μg of total yeast RNA (lanes 3–5) with the uniformly labeled 880-bp *Kpn* I–*Pst* I double-stranded (ds) DNA fragment (lanes 1–3; see restriction map in *B*) or single-stranded (ss) RNA probes prepared 5′–3′ *Pst* I → *Kpn* I (lane 4) or *Kpn* I → *Pst* I (lane 5). The lengthy exposure time (7 days) required to detect these transcripts enhances the background hybridization of the abundant 25S and 18S rRNAs. Numbers at the left indicate the sizes (nt) of the *PRP38* and *SMD1* transcripts. Internal RNA size standards (U2, U1, and U5 snRNAs, *RP51A* mRNA) visualized after rehybridization of this membrane were used to estimate the *PRP38* and *SMD1* lengths (data not shown). (*B*) Map of the *PRP38*–*SMD1* locus. Arrows indicate the respective open reading frames (orfs). The expanded segment displays the 6-bp sequence containing the *PRP38* and *SMD1* translational termination codons (underlined). Restriction endonuclease recognition sites: H, *Hin*dIII; K, *Kpn* I; P, *Pst* I. The *Kpn* I site used to prepare the *Kpn* I–*Pst* I fragment for hybridization in *A* is noted by an asterisk.

Smd1p, is larger than human D1 (16.3 kDa vs. 13.3 kDa), owing to a 27-amino acid expansion of the region between segments I and II.

***SMD1* Is Required for Pre-mRNA Splicing.** A conditional allele was created in which the native *SMD1* promoter was replaced by the nutritionally regulated *GAL1* promoter (see *Materials and Methods*). Plasmids bearing this *GAL1*::*SMD1* fusion successfully complemented a chromosomal null allele, *SMD1*::*LEU2*, in which the yeast *LEU2* gene replaced the *SMD1* sequence coding for the final 70 amino acids of Smd1p. This effect was gene-specific, as the *GAL1*::*SMD1* construct failed to complement a *PRP38*::*LEU2* null allele (see below) or the temperature-sensitive *prp38-1* allele (14), which contains a G → A transition within *PRP38* codon 66 (data not shown). Haploid strains containing *GAL1*::*SMD1* as the sole source of Smd1p grew indistinguishably from the parental wild-type strain on inducing medium (galactose-based) and for at least 4 hr after shift to repressing medium (glucose-based). At later times the growth of the strain bearing the chromosomal *SMD1*::*LEU2* disruption (but not the wild type) slowed and essentially ceased 8–12 hr after *GAL1*::*SMD1* repression.

RNA extracted before and at various times after *GAL1*::*SMD1* repression was assayed for splicing by using a probe composed of the intron and exon sequences of the yeast ribosomal protein 51 gene (*RP51A*; Fig. 3*A*). In galactose medium, the RNA profiles of the wild-type and

```
                10        20        30        40        50        60
H-D1    MKLVRFLMKLSHETVTIELKNGTQVHGTITGVDVSMNTHLKAVKMTLKNREPVQLETLSI----------
        :::: :: ::  : ::::::::: : ::   :   ::  :  :: ::       :    :
Y-SMD1  MKLVNFLKKLRNEQVTIELKNGTTVWGTLQSVSPQMNAILTDVKLTLPQPRLNKLNSNGIAMASLYLTGGQQPTASDNIASLQYINI
                10        20        30        40        50        60        70        80

                70        80        90        100       110
H-D1    RGNNIRYFILPDSLPLDTLLVDVEPKVKSKKREAVAGRGRGRGRGRGRGRGRGGPRR
        ::: ::  :::::: :: ::::             :       :        :
Y-SMD1  RGNTIRQIILPDSLNLDSLLVDQKQLNSLRRSGQIANDPSKKRRRDFGAPANKRPRRGL
          90        100       110       120       130       140
```

FIG. 2. Comparison of the yeast and human D1 polypeptides. Segments I and II, containing regions of high sequence identity (:), are underlined, the basic carboxyl-terminal region is in bold. Y-SMD1, yeast Smd1p; H-D1, human D1.

SMD1::*LEU2* strains transformed with the *GAL1*::*SMD1* plasmid were similar (compare lanes 1 and 7). The pre-mRNA to mRNA ratio, a measure of splicing efficiency, was indistinguishable from that observed in the untransformed parental strain (ref. 14 and data not shown). In contrast, 4 hr after *GAL1*::*SMD1* repression, pre-mRNA levels were elevated in the *SMD1*::*LEU2* strain relative to wild type. Splicing in the mutant strain progressively worsened throughout the period of *GAL1*::*SMD1* repression. Transcripts originating from the intron-containing *ACT1* and *CYH2* genes were similarly affected by Smd1p depletion; no differences were observed in the abundance or migration of the intronless *ADE3* transcript or in the pattern of rRNA (Fig. 3*B* and data not shown). Consequently, the block to cell proliferation associated with *GAL1*::*SMD1* repression can be attributed to pre-mRNA splicing inhibition; after 8–12 hr of growth without Smd1p synthesis the cells become limited for the product of one or more indispensable, intron-containing genes.

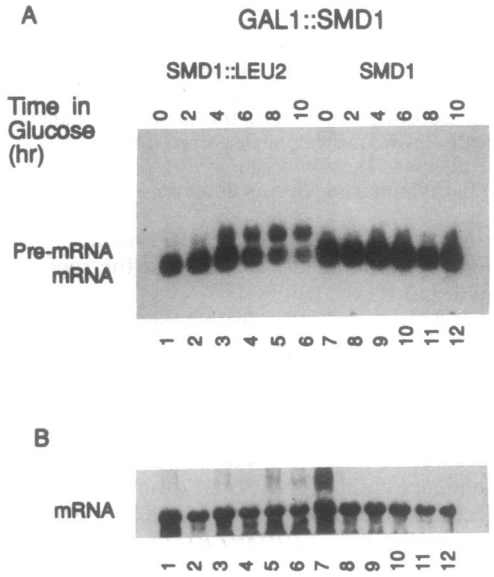

FIG. 3. Northern analysis of pre-mRNA splicing. RNA was extracted from the *SMD1*::*LEU2* mutant (lanes 1–6) and wild-type cells (lanes 7–12) transformed with the *GAL1*::*SMD1* fusion gene during growth in galactose medium ($t = 0$ hr) and various times after shift to glucose medium. (*A*) Hybridization with a double-stranded DNA probe prepared from an *RP51A* PCR fragment containing 398 bp of intron and ≈200 bp of exon II. The positions of precursor mRNA (pre-mRNA) and mRNA are noted. Primer extension analysis using an oligonucleotide complementary to *RP51A* intron positions 21–37 confirmed that the RNA designated as precursor represents full-length unprocessed *RP51A* pre-mRNA (data not shown). (*B*) Hybridization of the same filter with a double-stranded DNA probe prepared by random primer labeling of plasmid pBRYE2 (a gift of C. Staben, University of Kentucky) and containing the 2.8-kb *ADE3* coding sequence and ≈2.0 kb of flanking sequences.

Distinct Spliceosomal snRNAs Decrease in Response to Smd1p and Prp38p Loss. To evaluate whether intracellular Smd1p depletion influences the levels of spliceosomal snRNAs, a Northern transfer was probed for U1, U2, U4, U5, and U6 snRNAs (Fig. 4*A*). With the exception of U6, the abundance of each of these snRNAs decreased in the *SMD1*::*LEU2* mutant upon *GAL1*::*SMD1* repression. Densitometric traces of multiple gel exposures in three separate experiments showed that ≈45% of U2 and 30% of U1, U4, and U5S snRNAs remained after 8 hr of incubation in glucose. For unknown reasons U5L levels decreased more substantially than U5S levels, and became virtually undetectable after 6 hr of *GAL1*::*SMD1* repression. Increasing the time of incubation in glucose to 20 hr resulted in only a minor (<50%) further reduction in the Smd1p-sensitive snRNAs (data not shown). While not directly tested, it is likely that the initial rapid decrease in snRNA results from (*i*) dilution of a fixed Smd1p pool through cell division and (*ii*) instability of newly synthesized snRNAs in the absence of sufficient free Smd1p. After cell division ceases the rate of snRNA loss slows.

Depletion of Prp38p, achieved by 14 hr of *GAL1*::*PRP38* transcriptional repression in a strain containing a chromosomal *PRP38*::*LEU2* null allele (see *Materials and Methods*), caused a marked reduction of U6 snRNA (Fig. 4*A*, compare lanes 13 and 14). As indicated by the complementation specificity of the *GAL1* gene fusions and the fact that diploids heterozygous for both gene disruptions are viable (data not shown), neither the *SMD1*::*LEU2* nor the *PRP38*::*LEU2* mutation blocks the expression of the adjacent splicing factor. Thus the products of two neighboring genes, *PRP38* and *SMD1*, are required for splicing and influence the abundance of nonoverlapping subsets of spliceosomal snRNAs.

Residual Spliceosomal snRNAs Are Largely Refractory to Anti-TMG Immunoprecipitation. The common snRNP proteins bind a limited number of nonspliceosomal snRNAs, including U7 snRNA and, at least in yeast, U3 snRNA (20). The TMG-capped yeast snRNAs were assayed by immunoprecipitation using the K121 anti-TMG monoclonal antibody (16) before and after 10 hr of Smd1p depletion (Fig. 4*B*). As expected from the Northern blot analysis of the same samples (Fig. 4*A*, lanes 1 and 6), each of the spliceosomal snRNAs was reduced by Smd1p depletion. Curiously, however, the diminutions appeared more pronounced when measured by the immunoprecipitation assay. For instance, the loss of U1 precipitability was essentially complete even though ≈30% of normal U1 snRNA levels persisted in the sample (compare Fig. 4*A*, lanes 1 and 6, with Fig. 4*B*, lanes 1 and 2). Since 5-fold-longer exposures of the autoradiogram in Fig. 4*B* failed to detect any of the residual U1 (data not shown), one can

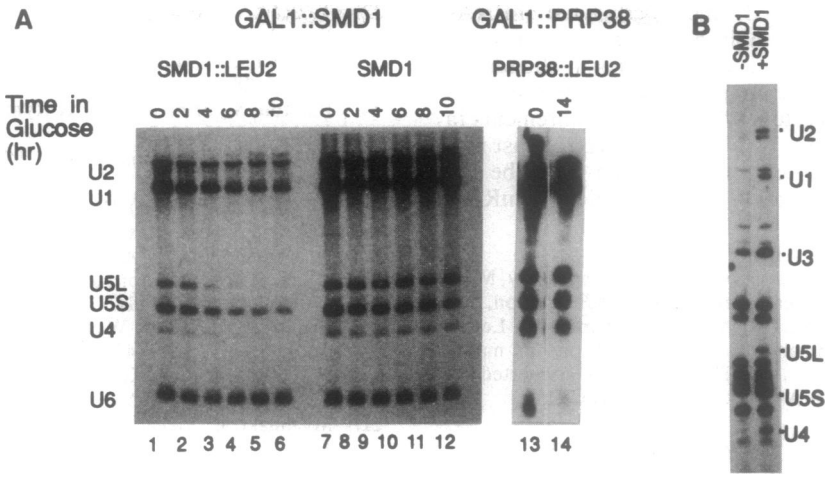

FIG. 4. snRNA profile during Smd1p and Prp38p depletion. (*A*) RNA was isolated as described in Fig. 3 and was resolved in a denaturing 5% polyacrylamide gel. The Northern transfer was probed for each of the spliceosomal snRNAs (U1, U2, U4, U5L, U5S, and U6) by using uniformly labeled double-stranded DNA fragments as described (14). Lanes 1–12, RNA extracted after the indicated time of *GAL1::SMD1* repression; lanes 13 and 14, RNA extracted before and after *GAL1::PRP38* repression, respectively. (*B*) Anti-TMG antibodies were used to precipitate yeast snRNAs before (+SMD1) or 10 hr after (−SMD1) transcriptional repression of *GAL1::SMD1*. The recovered RNAs were subsequently 3′-end-labeled by using [^{32}P]pCp and RNA ligase, fractionated by gel electrophoresis, and visualized by autoradiography. The positions of the splicing-associated snRNAs (U1, U2, U4, U5L, and U5S) and the yeast U3 are indicated at right.

estimate that <5% of the anti-TMG-precipitable U1 snRNA remained.

Yeast U3 snRNA decreased modestly (by a factor of ≈2) in response to Smd1p depletion. As both genes encoding yeast U3 snRNA contain an intron (21), it is unclear whether this decrease was a direct consequence of Smd1p loss or an outcome of the general inhibition of splicing. None of the other resolvable, anti-TMG-precipitable snRNAs were affected by the loss of Smd1p. The bulk of yeast snRNAs, therefore, either do not associate with Smd1p or are quite stable in the absence of ongoing Smd1p synthesis. An Sm-independent pathway for cap modification and nuclear import must exist, as many nonspliceosomal snRNAs possess a TMG cap (reviewed in ref. 22) but lack an Sm site and are not sensitive to Smd1p depletion.

DISCUSSION

SMD1, the gene coding for the likely yeast equivalent of the metazoan core snRNP protein D1, has been isolated. The data demonstrate a remarkable degree of sequence conservation between the yeast and human proteins and provide direct experimental evidence that D1 is essential for pre-mRNA splicing.

The "tail to tail" arrangement of *PRP38* and *SMD1* is curious and perhaps relevant to the expression of one or both genes. The *PRP38* mRNA is ≈1175 nt long. Primer extension analysis of the 5′ end of *PRP38* indicates that transcription initiates at two sites 14 and 27 bp upstream of its translational initiation codon (unpublished observations). Given the 726-nt *PRP38* orf and assuming a 5′ noncoding segment of 27 nt and a poly(A) tail characteristic of yeast (50–100 nt), the transcription of *PRP38* progresses well into *SMD1* to generate mRNA containing at least 300 nt of antisense *SMD1* mRNA. The *SMD1* orf (438 nt) is closer in size to its measured mRNA product (550 nt) and most likely carries <50–100 nt of antisense *PRP38* RNA. Although the issue has not been pursued, this overlapping arrangement of two essential genes involved in the same physiological process is intriguing from the perspective of possible cellular transcriptional or translational control strategies.

Yeast Smd1p and human D1 share at least three segments of outstanding similarity, conserved regions that presumably interact with other evolutionarily constrained components of the splicing apparatus. The extremely basic carboxyl-terminal tail is a strong candidate to mediate RNA binding. Indeed, human D1 has considerable affinity for RNA, although this binding lacks obvious substrate specificity (11, 12). D1 might act as a charged protein scaffold to promote snRNP assembly or strengthen snRNP–snRNP interactions through nonspecific electrostatic contacts with RNA (31). At a superficial level, the conservation of the highly charged nature but not the primary structure of the carboxyl-terminal region supports this view. Alternatively, the D1 polypeptide may interact uniquely with a defined RNA sequence but display rather subtle differences in its specific vs. nonspecific RNA binding properties when assayed *in vitro*.

The coordinate diminution of the U1, U2, U4, and U5 snRNAs upon *GAL1::SMD1* repression is not a simple consequence of a block to cellular proliferation or splicing, as neither the addition of cycloheximide (S. Zhu and B.R.C., unpublished work) nor the inhibition of Prp38p synthesis or U5 snRNA synthesis (23) has this effect. Subsets of the spliceosomal snRNAs are destabilized upon removal or inactivation of specific splicing factors, however. For example, temperature inactivation of any of the known U6 (or U4/U6) snRNP-associated proteins (Prp3p, Prp4p, Prp6p, Prp24p) or the Prp19p or Prp38p splicing factors leads to a decrease in intracellular U6 snRNA (14); U4, U5, and U6 snRNA levels decrease upon depletion of the U5 snRNP Prp8p polypeptide (24). Likewise, direct snRNA perturbations such as mutations at the Sm binding site (7) and elsewhere (8, 25–29) can cause individual snRNA levels to decrease, presumably by blocking the assembly of stable snRNP structures. This conclusion is reinforced by the observation that strong heterologous promoters increase intracellular snRNA abundance by only modest amounts (7, 30); limiting stabilizing factors appear to restrict snRNA levels and hence the abundance of the corresponding snRNP particles. Nevertheless, some if not all of the spliceosomal snRNAs are found in excess over what is necessary for efficient splicing (30). Therefore, it is possible that the primary splicing block imposed by Smd1p depletion results not from diminished snRNA levels but from the failure of splicing complexes to assemble or function properly in the absence of this polypeptide.

In metazoa, the binding of core proteins to the Sm site of spliceosomal snRNAs is a prerequisite for cap hypermeth-

ylation (5). Newly synthesized, undermethylated snRNAs are apt to remain in the cytoplasm and may decay with accelerated kinetics. The observed decreases in abundance and anti-TMG precipitability of spliceosomal snRNAs upon Smd1p depletion are consistent with similar requirements in yeast. With the serendipitous isolation of *SMD1*, yeast molecular genetic techniques can now be used to probe the details of core particle function in eukaryotic pre-mRNA splicing.

I thank A. Krainer for his generous gift of the K121 antibody, Mary Porada for her assistance in creating the *GAL1::PRP38* fusion, and Susie Zhu for excellent technical support. The comments of Lester Goldstein, Martha Peterson, and Michael Rosbash on this manuscript were gratefully appreciated. This work was supported by National Institutes of Health Award GM42476 to B.C.R.

1. Luhrmann, R., Kastner, B. & Bach, M. (1990) *Biochim. Biophys. Acta* **1087**, 256–292.
2. Zieve, G. W. & Sauterer, R. A. (1990) *Crit. Rev. Biochem. Mol. Biol.* **25**, 1–46.
3. Fisher, D. E., Conner, G. E., Reeves, N. H., Wisniewolski, R. & Blobel, G. (1985) *Cell* **42**, 751–758.
4. Sauterer, R. A., Goyal, A. & Zieve, G. W. (1990) *J. Biol. Chem.* **265**, 1048–1058.
5. Mattaj, I. W. (1986) *Cell* **46**, 905–911.
6. Liautard, J. P., Sri-Widada, J., Brunel, C. & Jeanteur, P. (1982) *J. Mol. Biol.* **162**, 623–643.
7. Jones, M. H. & Guthrie, C. (1990) *EMBO J.* **9**, 2555–2561.
8. McPheeters, D. S., Fabrizio, P. & Abelson, J. (1989) *Genes Dev.* **3**, 2124–2136.
9. Wersig, C. & Bindereif, A. (1992) *Mol. Cell. Biol.* **12**, 1460–1468.
10. Siliciano, P. G., Kivens, W. J. & Guthrie, C. (1991) *Nucleic Acids Res.* **19**, 6367–6372.
11. Gerke, V. & Steitz, J. A. (1986) *Cell* **47**, 973–984.
12. Tazi, J., Alibert, C., Temsamani, J., Reveillaud, I., Cathala, G., Brunel, C. & Jeanteur, P. (1986) *Cell* **47**, 755–766.
13. Heinrichs, V., Hackl, W. & Luhrmann, R. (1992) *J. Mol. Biol.* **227**, 15–28.
14. Blanton, S., Srinivasan, A. & Rymond, B. C. (1992) *Mol. Cell. Biol.* **12**, 3939–3947.
15. Johnston, M. & Davis, R. W. (1984) *Mol. Cell. Biol.* **4**, 1440–1448.
16. Krainer, A. R. (1988) *Nucleic Acids Res.* **16**, 9415–9429.
17. Rokeach, L. A., Haselby, J. A. & Hoch, S. O. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4832–4836.
18. Zamore, P. D., Patton, J. G. & Green, M. R. (1992) *Nature (London)* **355**, 609–614.
19. Zahler, A. M., Lane, W. S., Stolk, J. A. & Roth, M. B. (1992) *Genes Dev.* **6**, 837–847.
20. Hughes, J. M. X., Koning, D. A. M. & Cesareni, G. (1987) *EMBO J.* **6**, 2145–2155.
21. Myslinski, E., Segault, V. & Branlant, C. (1990) *Science* **247**, 1213–1216.
22. Guthrie, C. (1988) in *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*, ed. Birnstiel, M. L. (Springer, New York), pp. 196–211.
23. Seraphin, B., Abovich, N. & Rosbash, M. (1991) *Nucleic Acids Res.* **19**, 3857–3860.
24. Brown, J. D. & Beggs, J. D. (1992) *EMBO J.* **11**, 3721–3729.
25. Shuster, E. & Guthrie, C. (1988) *Nature (London)* **334**, 450–453.
26. Ares, M., Jr., & Igel, A. H. (1990) *Genes Dev.* **4**, 2132–2145.
27. Bordonne, R., Banroques, J., Abelson, J. & Guthrie, C. (1990) *Genes Dev.* **4**, 1185–1196.
28. Liao, X., Kretzner, L., Seraphin, B. & Rosbash, M. (1990) *Genes Dev.* **4**, 1766–1774.
29. Madhani, H. D., Bordonne, R. & Guthrie, C. (1990) *Genes Dev.* **4**, 2264–2277.
30. Seraphin, B. & Rosbash, M. (1989) *Cell* **59**, 349–358.
31. Woppmann, A., Rinke, J. & Luhrmann, R. (1988) *Nucleic Acids Res.* **16**, 10985–11004.