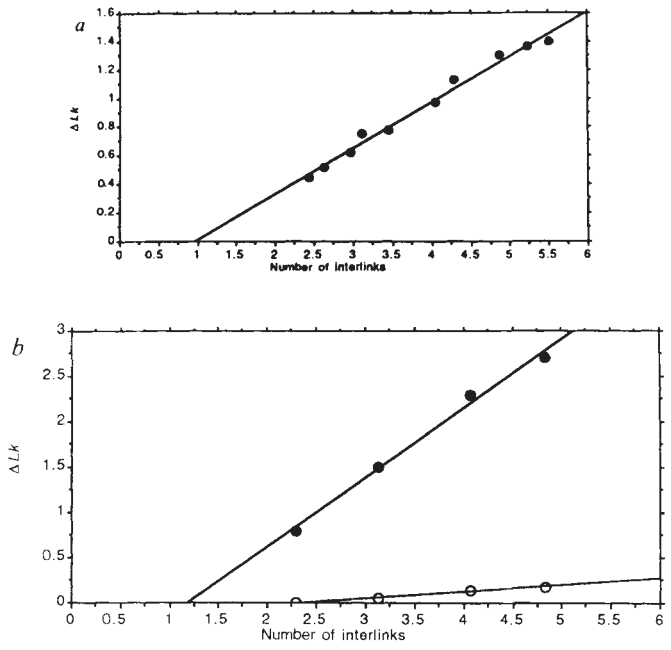

Fig. 4 Dependence of entrapped linking difference on catenane complexity. The abscissa indicates the average number of intertwinings for the catenated fraction of each product mixture. The ordinate plots the average shift in *Lk* observed for the uncut DNA ring. Data gathered as described in Figs 2 and 3 were quantitated with a Hoeffer Model GS3000 densitometer. ΔLk was calculated by subtracting the mean topoisomer number for each precut sample from the mean for the corresponding postcut DNA. *a*, pAB7.0d catenanes. The slope of the least squares regression line ($r^2 = 0.99$) is 0.32; the *x*-intercept is 0.95. *b*, pBNW3.8d catenanes. (●) ΔLk for 2.9 kb ring; the slope of the regression line is 0.76. (○) ΔLk for 0.9 kb ring; the regression line has a slope of 0.073.

That supercoiling *per se* can alter the helical repeat of duplex DNA independent of protein-DNA interactions was previously suggested on theoretical grounds by Levitt[13]. He argued that a decrease in the helical repeat would be needed to accommodate an increased base separation at the compacted inner side of a duplex bend. A decrease in helical repeat was subsequently confirmed for nucleosome-bound DNA[14], but does not hold true for the catenanes studied here in which the repeat actually increases. Although the supercoils modelled by Levitt had a handedness opposite to that of the right-handed catenanes, the need to relieve stress on the inner side of the bends in DNA should be about the same for both structures. If, however, the change in helical repeat with supercoiling results from the geometry of DNA winding, rather than steric interactions between bases, the observed opposite changes in helical repeat for right-handed catenanes and left-handed nucleosomal DNA are predicted[6].

The method we devised to measure helical repeat depends on the number of supercoils of each catenated ring being equal to the readily measurable number of intertwines between rings. The results apply only to toroidal supercoils as also occur in nucleosomal DNA, but not to the plectonemic (interwound) forms that predominate for underwound DNA in solution[15] But we expect on theoretical grounds that plectonemic supercoiling will also change the repeat of the double helix[6] and that there is thus a general relationship between helical parameters and DNA supercoiling. Bending of DNA can change helical repeat just as changes in the structure of the double helix can cause bending.

Supercoils, catenanes and knots, the three common higher-order forms of DNA intertwining[3], can have similar bends and crossings, despite differing in global connectivity. The results presented here support the idea that these forms share a common local structure in terms of altered helical repeat. That catenane or knot intertwinings can substitute for plectonemic supercoiling in directing DNA transposition has been demonstrated directly by Craigie and Mizuuchi[16]. Moreover, multiple (H. Benjamin, and N.R.C., unpublished data) but not single[10] intertwining of catenanes directs site specific recombination by Tn*3* resolvase, and we find an induced ΔLk in DNA only for multiply-intertwined catenanes.

The distortion of helical geometry in multiply-intertwined DNAs could provide a directional energetic drive for many DNA transactions *in vivo* such as the activity of transcription and replication factors. The magnitude of unwinding caused by intertwining can be quite large. For pAB7.0d, helical repeat increased by 0.1 for every five supercoils. The analogous right-handed wrapping of 100 bp of DNA around a protein to give one positive supercoil would shift the helical repeat from 10.5 to 11.3. An increase in repeat of similar proportion in the transition from B-form to A-form duplex is accompanied by a 20° base-pair tilt. The results here therefore suggest that intertwining, whether in the form of catenation or supercoiling alone, profoundly alters the architecture, and hence the activity, of the DNA double helix.

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Internal sequences that distinguish yeast from metazoan U2 snRNA are unnecessary for pre-mRNA splicing

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U2 small nuclear RNA is a highly conserved component of the eukaryotic cell nucleus involved in splicing messenger RNA precursors[1–3]. In the yeast *Saccharomyces cerevisiae*, U2 RNA interacts with the intron by RNA-RNA pairing between the conserved branchpoint sequence UACUAAC and conserved nucleotides near the 5′ end of U2 (ref. 4). Metazoan U2 RNA is less than 200 nucleotides in length[5], but yeast U2 RNA is 1,175 nucleotides long[6]. The 5′ 110 nucleotides of yeast U2 are homologous to the

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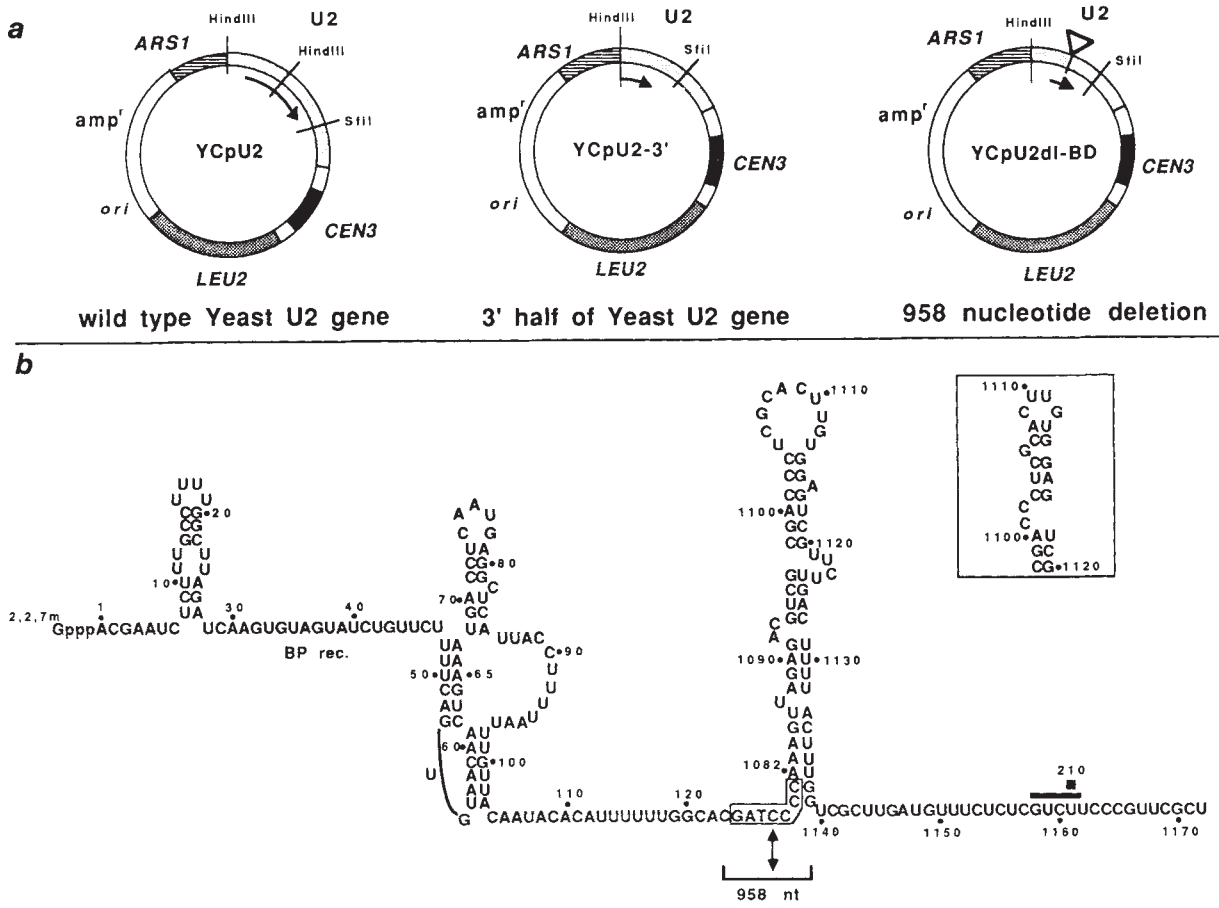

Fig. 1 Structure of plasmids and the U2-bd transcript. *a*, Plasmid constructs used in this study. Plasmids were derived by standard techniques[21] from plasmid R489 (gift of S. Roeder, Yale) which contains a *Sau*3A fragment of *CEN3* cloned in the *Bam*HI site, a *Hpa*I-*Sal*I fragment of the *LEU2* gene cloned between the *Sal*I and *Pvu*II sites, and an *Eco*RI-*Hin*dIII fragment of *ARS1* cloned between the (filled-in) *Eco*RI and *Hin*dIII sites of pBR322. A 1.3-kb *Hin*dIII fragment containing the 3′ half of the yeast U2 gene (known as *LSR1* (ref. 6) or *SNR20* (refs 4, 22)) was introduced into the *Hin*dIII site and then the downstream *Hin*dIII site was destroyed by filling in with Klenow, producing YCpU2-3′ (centre). YCpU2 was constructed by cloning the 0.95-kb *Hin*dIII fragment containing the promoter and 5′ half of the yeast U2 gene into the *Hin*dIII site of YCpU2-3′ in the correct orientation. YCpU2dl-BD was generated beginning with a subclone containing the *Dra*I-*Sma*I fragment in the *Sma*I site of mp10 (ref. 6). The *Bam*HI site adjacent to the fused *Dra*I-*Sma*I site was cleaved, filled in and the DNA was recut further down in the polylinker at the *Hin*dIII site. Into this site was ligated a U2 fragment cut at the *Ban*I site at position 119, filled in and recut upstream of the promotor with *Hin*dIII. This generated a subclone containing the promotor and coding sequences to position 123 fused to 7 bp derived from the polylinker (boxed sequence in Fig. 1*b*) fused to position 1,082 of the coding region (the *Dra*I site) and 3′ flanking sequences. The sequence was confirmed across the junction by dideoxy sequencing[23]. This gene was introduced into YCpU2-3′ using the unique *Hin*dIII and *Sfi*I sites. Arrows represent the U2 coding region. *b*, Secondary structure model of transcript coded by the U2dl-BD gene. Numbering begins with the first nucleotide. Pairings in the first 100 nt, including the pseudoknot, are phylogenetically conserved[14]. The 3′ sequences can be folded to resemble either of two stuctures found in other U2 RNAs, stem IV (shown) or stem III (inset). Nucleotides derived from the polylinker are boxed in the sequence. Bracket indicates the site of the 958 nucleotide deletion. Bar above the sequence indicates region of 3′ end formation of transcripts from the deletion gene. Square indicates position 210 of the U2-bd transcript (wild-type transcripts would extend past position 1,170 as shown).

5′ 100 nucleotides of metazoan U2 (ref. 6), and the very 3′ end of yeast U2 bears a weak stuctural resemblance to features near the 3′ end of metazoan U2. Internal sequences of yeast U2 share primary sequence homology with metazoan U4, U5 and U6 small nuclear RNA (ref. 6), and have regions of complementarity with yeast U1 (ref. 7). We have investigated the importance of the internal U2 sequences by their deletion. Yeast cells carrying a U2 allele lacking 958 nucleotides of internal U2 sequence produce a U2 small nuclear RNA similar in size to that found in other organisms. Cells carrying only the U2 deletion grow normally, have normal levels of spliced mRNA and do not accumulate unspliced precursor mRNA. We conclude that the internal sequences of yeast U2 carry no essential function. The extra RNA may have a non-essential function in efficient ribonucleoprotein assembly or RNA stability. Variation in amount of RNA in homologous stuctural RNAs has precedence in ribosomal RNA[8,9] and RNaseP[10].

Although nuclear precursor mRNA (pre-mRNA) splicing in *S. cerevisiae* and metazoans share many features, the yeast spliceosome appears to contain much more RNA than the metazoan spliceosome[7,11], due primarily to the greater length of yeast U1 and U2 RNA. In yeast, U1 is 568 nucleotides (nt)[7,11] and U2 is 1,175 nt[6] long, whereas in metazoans U1 is 165 nt and U2 is 189 nt[5]. The discovery of the yeast homologues of U5 (ref. 12) and U4 plus U6 small nuclear RNAs (snRNA)[13] has made the notion obsolete that the extra RNA found in yeast U2 is arranged as a 'poly-snRNA' (ref. 6), but the function of the extra RNA remains obscure.

Conservation of U2 sequences in different organisms allowed the alignment of the 5′ 120 nt of yeast U2 with the 5′ 110 nt of metazoan U2 (ref. 6), but the relation of the remaining 1,050 nt of yeast U2 to the remaining 78 nt of metazoan U2 was not clear. Our analysis of the 3′ end of yeast U2 using phylogenetic comparisons[14] or computer programs (also D. Konings; E. Shuster and C. Guthrie, personal communication) showed that the very 3′ end of yeast U2 could fold into either of two potential

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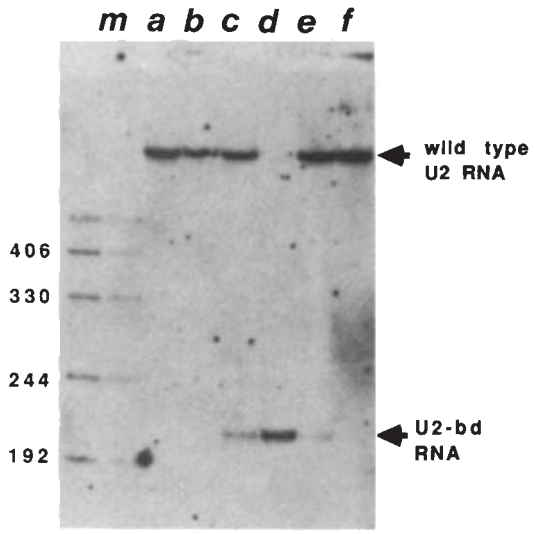

Fig. 2 Size of U2 RNAs in different yeast strains. RNA was extracted, separated on a 0.4 mm thick 6% polyacrylamide-7M urea gel, transferred to nylon, and probed as described previously with kinase-labelled L15 oligonucleotide[6]. Samples are *a*, wild type, diploid MA3: *HIS4/his4*-619 *trp1/TRP1*, *leu2*-3,112/*leu2*-3,112,*ura3*-52/*ura3*-52; *b*, MA31: MA3 containing the U2::*URA3* disruption allele; *c*, MA31/YCpU2dl-BD: MA31 containing the YCpU2dl-BD plasmid; *d*, 503d: meiotic spore of MA31/YCpU2dl-BD containing the U2::*URA3* disruption in the chromosome and the YCpU2dl-BD plasmid; *e*, 503a: spore (from same tetrad as 503d) containing the wild-type U2 allele in the chromosome and YCpU2dl-BD plasmid; *f*, 503a-LP: mitotic segregrant of 503a that has lost the plasmid; *m*, markers (two lanes), *Hpa*II fragments of pUC13 labelled by filling in with Klenow. The genetic constitutions described for each strain used in these studies was confirmed by hybridization of blots of genomic DNA from each strain with a U2 probe.

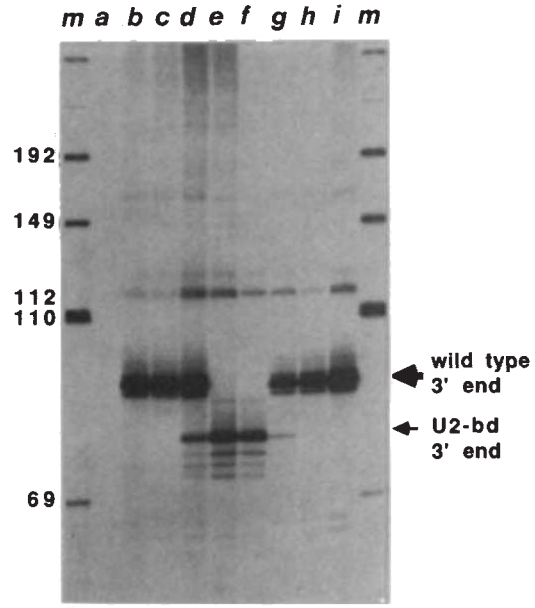

Fig. 3 Mapping 3′ ends of U2 transcripts in different yeast strains. An anti-sense probe transcript spanning from downstream of the gene and extended to position 1,082 in the coding region was synthesized using ^{32}P-labelled rGTP, and purified as described previously[6]. Two μg total yeast RNA was annealed to 5×10^4 c.p.m. of probe and was digested with 2 μg ml^{-1} RNase T1 at 30 °C, extracted and run out on a gel as before[6]. Samples are: (see Fig. 2 legend) *a*, digestion of probe after mock annealing with no RNA; after annealing to RNA from strains *b*, MA3; *c*, MA31; *d*, MA31/YCpU2dl-BD; *e*, 503d; *f*, HI2: a derivative of 503d that has replaced the U2::*URA3* disruption allele with the U2dl-BD allele (a mitotic gene conversion event using the plasmid as a donor, obtained by selection against *URA3* on 5-fluoro orotic acid[24]) and then lost the plasmid; *g*, 503a; *h*, 503a-LP; *i*, 50a: a spore containing the U2::*URA3* allele on the chromosome and the wild-type U2 gene plasmid YCpU2; *m*, markers are *Hpa*II fragments of pUC13. The presence of the 120-nt fragment is variable and its origin has not been determined.

structures, each similar to those found near the 3′ end of vertebrate U2 (see Fig. 1). This suggests that the extra RNA could interrupt an otherwise typical U2 snRNA.

To test the functional importance of the internal sequences, we constructed a deletion to remove them. This deleted U2 gene (U2dl-BD) lacks 958 base pairs (bp) between positions 123 and 1,082 of the wild-type U2 gene and would be expected to encode a 224-nucleotide U2 molecule containing the highly conserved 5′ end of U2 and the less well conserved 3′ end (Fig. 1*b*). Three U2 gene constructs were incorporated into a yeast centromere plasmid (Fig. 1*a*): the complete wild-type yeast U2 gene (YCpU2), a U2 gene lacking the promoter and 5′ half of the coding region (YCpU2-3′), and the U2dl-BD allele (YCpU2dl-BD).

We tested the ability of the three plasmids to rescue cells containing a lethal U2 mutation by introducing them into a diploid strain carrying one wild-type U2 gene, and one disrupted by deletion and insertion of the *URA3* gene[6]. We sporulated the diploids containing the plasmids and dissected tetrads. During meiosis, a centromere plasmid behaves in many respects like a normal chromosome[15]. If the plasmid-borne U2 gene is not functional, two of the meiotic products (spores) receive the chromosomal wild-type allele and survive (and are ura$^-$), whereas the two that receive the disrupted U2 allele[6] die regardless of whether or not they receive the plasmid. If the plasmid-borne U2 gene is functional, spores that receive the chromosome with the lethal U2 allele have a chance to survive if they also receive the plasmid at meiosis. The results (not shown) reveal that the plasmid lacking the U2 promoter and 5′ half of the U2 gene did not complement the disruption (no tetrads with >2 spores, no ura$^+$ spores), but both the wild-type U2 gene and the internal deletion did complement the disruption (tetrads with >2 spores, some ura$^+$). This genetic test shows that 958 nt of yeast U2 are not essential for function. Cells carrying only the deletion U2 gene grew normally. (Similar results on the dispensability of internal segments of the yeast U2 gene have been obtained by E. Shuster and C. Guthrie, personal communication.)

On the basis of the position of the 5′ and 3′ ends of wild-type U2 (ref. 6), we expected the U2dl-BD gene to encode a 220–224-nt transcript. Figure 2 shows that YCpU2dl-BD directs the accumulation of a transcript (U2-bd) that is close to this size, but significantly shorter at ~210 nt (Fig. 2). The 1,175-nt wild-type U2 transcript is absent from cells carrying U2dl-BD as the sole functional U2 gene, and the 210-nt transcript is the only detectable U2 RNA (Fig. 2, lane *d*), thus confirming the genetic conclusion that the central 958 nt are dispensable.

To determine the basis for the size difference, we mapped the 3′ ends of U2 RNA contained in different strains using an RNase protection assay (Fig. 3). As expected, RNA from cells containing the wild-type U2 gene protects an 89-nt fragment of the probe. RNA from cells containing the U2dl-BD gene protects a fragment ~78 nt long, indicating that U2-bd RNA is 10–15 nucleotides shorter than wild type at the 3′ end. This phenomenon is not due to a difference between the resident wild-type U2 genes and the cloned U2 gene we are using as a paradigm. Strains containing only a copy of the cloned wild-type gene have wild-type 3′ ends (Fig. 3, lane *i*). We have also recovered the plasmid from yeast by transformation into *E. coli*, and confirmed that the sequence through the 3′ end of the U2dl-BD gene is intact (not shown). Primer extension using the

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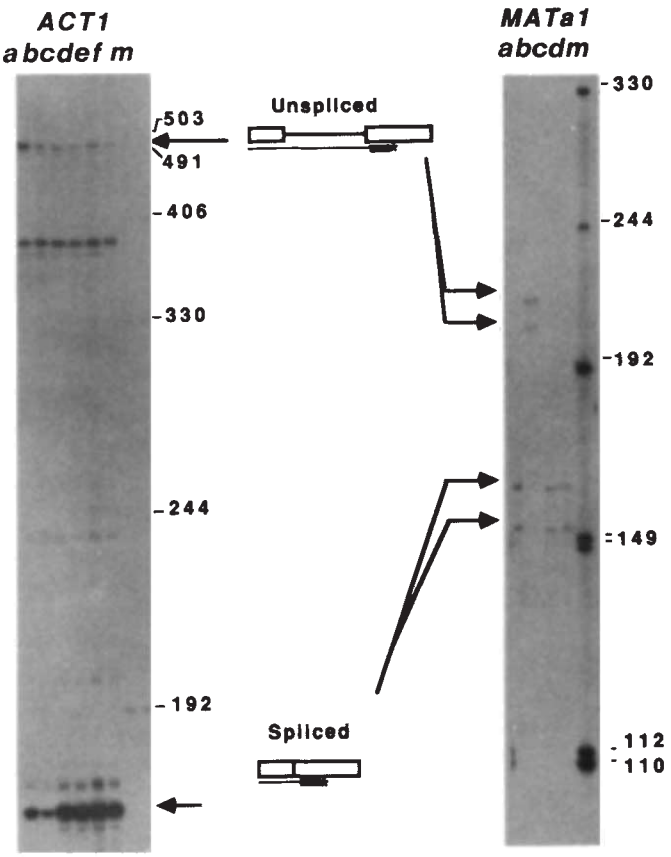

Fig. 4 Measurement of actin and *MATa1* pre-mRNA and mRNA levels in isogenic strains differing only at the U2 locus. Left panel, actin pre-mRNA and mRNA. Total RNA from each strain was annealed at 55 °C to a synthetic oligonucleotide (5′-ACACATAC-CAGAACCGTTATC-3′) complementary to the second exon of the actin mRNA[16,17]. Top arrow indicates migration of complementary DNA derived from unspliced pre-mRNA. Bottom arrow indicates migration of cDNA derived from mature mRNA. Lane *a*, *rna11* strain grown at 23 °C; lane *b*, *rna11* strain shifted to 37 °C for 45 minutes; *c*, MA5c; haploid *MATα*, *his4*-619, *ura3*-52, *leu2*-3,112; *d*, MA5c1B: same as MA5c, except that it carries U2::*URA3* disruption in the chromosome and the YCpU2dl-BD centromere plasmid; *e*, 503d; *f*, HI2 (see Fig. 3 legend); *m*, markers, *Hpa*II fragments of pUC13. Right panel, *MATa1* mRNA and pre-mRNA. Total RNA from each strain was annealed at 57 °C to a synthetic oligonucleotide (5′-TGTATGTTGCTCTACTTTAGTC-3′) complementary to the second exon of the *MATa1* mRNA[18]. Top two arrows indicate cDNAs derived from transcripts containing the first intron, bottom two arrows indicate cDNAs derived from transcripts from which the first intron has been removed. Lane *a*, *rna11* strain grown at 23 °C; *b*, *rna11* strain grown at 37 °C; *c*, MA2c; *MATa*, *his4*-619, *ura3*-52, *leu2*-3,112; *d*, MA2c1B: same as MA2c except contains the U2::*URA3* disruption in the chromosome and the YCpU2dl-BD centromere plasmid; *m*, markers, *Hpa*II fragments of pUC13.

Methods. RNA from *rna11* strain ts382 (Yeast Genetic Stock Center) was used as a control to aid in identification of cDNAs derived from unspliced precursors. This mutant is a member of a class of mutants defective in splicing[25]. The expected cDNAs derived from unspliced pre-mRNA were either elevated relative to wild-type *RNA11* strains at permissive temperature, as in the case of actin, or accumulated upon shift to restrictive temperature, as in the case of *MATa1*. Isogenic strain pairs differing at the U2 locus were constructed by introducing YCpU2dl-BD into the strain, and then replacing the chromosomal U2 locus with the U2::*URA3* allele[6] by transformation with a linear DNA fragment[26]. As one end of the linear U2::*URA3* DNA fragment used for the transformation lies within the U2dl-BD deletion, this DNA is efficiently incorporated into the chromosome rather than the plasmid. To measure spliced and unspliced transcripts, total RNA (10 μg) was annealed to 0.2 ng oligonucleotide end-labelled with ^{32}P, in 16 μl 125 mM Tris-Cl, *p*H 8.3, 17.5 mM KCl for 30 min at the indicated temperature, and then cooled rapidly on ice. The reaction mixture was made up to 1.25 mM each dNTP, 10 mM $MgCl_2$, 5 mM dithiol-tireitol, 40 μg ml^{-1} actinomycin D, and 3–4 units of AMV reverse transcriptase (Life Sciences) in a volume of 20 μl and incubated for 30 min at 42 °C.

LI5 oligonucleotide revealed no differences at the 5′ end of any of the U2 transcripts (not shown). We conclude that the shortened 3′ end of the U2-bd transcript is a consequence of the internal deletion, and that the missing 3′ end sequences are probably also dispensable to U2 function. Our data suggest a function for the dispensable sequences in snRNP assembly or RNA stability, because wild-type U2 seems to be preferentially accumulated in cells containing both wild-type and deletion U2 alleles (Fig. 3, lanes *d* and *g*), although a direct effect on 3′ end formation is not excluded.

As the cells carrying only the U2dl-BD allele displayed no growth defects, we tested the effect of the U2 deletion on the splicing of both the yeast actin intron[16,17] and the first intron of the *MATa1* gene[18]. There was no detectable increase in the level of pre-mRNA and no significant decrease in the level of spliced mRNA in either case (Fig. 4).

The 210-nt yeast U2 may be folded to resemble other U2 RNAs (Fig. 1*b*). The highly conserved 5′ 110 nt have the potential to form a pseudoknot[19], like all other U2 RNAs[14]. The 3′ end sequences can be folded to resemble stem III or stem IV (Fig. 1*b*, inset). We prefer the stem IV folding because trypanosome U2 (ref. 20) also seems to lack stem III (ref. 14). In this sense, the dispensable RNA in yeast U2 may be equivalent to the variable regions of ribosomal RNA[8,9] or RNaseP RNA[10].

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