The Future

Work is under way on a new generation of helioseismology instruments that promise a tenfold or greater improvement in the accuracy of the measurements shown above. The Global Oscillation Network Group (GONG) (*30*), scheduled to begin operation in 1993, will consist of a network of six telescopes, spaced in longitude to provide continuous Doppler measurements of the solar surface for several years. Other networks of integrated sunlight instruments (*31*) will provide more accurate measurements of the properties of modes with $\ell \leq 3$. Meanwhile, the Solar Heliospheric Observatory (SOHO) spacecraft will contain several helioseismology instruments, including the GOLF (Global Oscillations at Low Frequencies) (*32*), and MDI (Michelson Doppler Imager) (*33*) instruments, which will provide precision measurements of low-frequency and high-ℓ oscillations, respectively.

These instruments, combined with theoretical advances yet to come, should provide an extremely detailed picture of many properties of the solar interior. Going beyond a basic understanding of the structure of the sun, these new helioseismological measurements are expected to turn the sun into a precision laboratory for learning about the physics of high-temperature plasmas and magnetohydrodynamics, neutrino oscillations, radiative transfer, and the dynamics of large-scale stratified convection and rotation.

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35. We thank N. Murray and P. Goldreich for enlightening conversations. This work was supported by NSF grants ATM-8907012 and AST-8657393.

Messenger RNA Splicing in Yeast: Clues to Why the Spliceosome Is a Ribonucleoprotein

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The removal of introns from eukaryotic messenger RNA precursors shares mechanistic characteristics with the self-splicing of certain introns, prompting speculation that the catalytic reactions of nuclear pre–messenger RNA splicing are fundamentally RNA-based. The participation of five small nuclear RNAs (snRNAs) in splicing is now well documented. Genetic analysis in yeast has revealed the requirement, in addition, for several dozen proteins. Some of these are tightly bound to snRNAs to form small nuclear ribonucleoproteins (snRNPs); such proteins may promote interactions between snRNAs or between an snRNA and the intron. Other, non-snRNP proteins appear to associate transiently with the spliceosome. Some of these factors, which include RNA-dependent adenosine triphosphatases, may promote the accurate recognition of introns.

LITTLE MORE THAN A DOZEN YEARS HAVE PASSED SINCE THE discovery of the "amazing" (*1*) process of RNA splicing. Although the removal of introns has already been comfortably assimilated into the canon of gene expression, the most basic questions about the molecular mechanisms of nuclear messenger RNA (mRNA) splicing remain unanswered. For example, we have only a limited understanding of how splice sites are chosen, and none as to how they are juxtaposed. Furthermore, the discovery of a class of self-splicing introns in organellar mRNAs has raised afundamental question. What catalyzes nuclear mRNA splicing: RNA, proteins, or a collaboration of the two? Here I review recent results from *Saccharomyces cerevisiae* that lend insight into why the spliceosome is made up of both RNA and protein. This yeast

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provides the opportunity to approach, by combined genetic and biochemical analyses, the challenging task of defining specific roles for individual components of the splicing machinery. I have drawn comparisons to other systems when the data are particularly relevant. These systems and other aspects of yeast splicing are reviewed in (*2, 3*).

Catalytic RNA and RNA Splicing

The discovery of self-splicing RNAs has had an inestimable impact on our conceptual thinking about the mechanism (and evolutionary origin) of nuclear mRNA splicing. A number of self-splicing introns have now been identified. Located primarily in organelles, these introns are of two types: Group I and Group II. In both groups, splicing proceeds by a two-step transesterification mechanism (*4*). In Group II introns (Fig. 1), 5′ splice-site cleavage results from nucleophilic attack by the 2′-end hydroxyl of an internal A residue located upstream of the 3′ splice site; this causes the release of the 5′ exon and the formation of a "lariat" intermediate, so called because of the branched structure of the 2′,5′ phosphodiester bond thus produced. In the second step the 3′-end hydroxyl of the upstream exon makes a nucleophilic attack on the 3′ splice site. This displaces the intron and joins the two exons together. Because the number of phosphodiester bonds is conserved in the reaction, no exogenous energy source is required.

The central significance of Group II autocatalytic splicing derives from the discovery that nuclear mRNA splicing also proceeds through a lariat intermediate in a two-step reaction (*2, 3*). In contrast to the highly conserved structural elements that reside within Group II introns (Fig. 2B), however, the only conserved features of nuclear mRNA introns are restricted to short regions at or near the splice junctions. In yeast these motifs are (i) a conserved hexanucleotide at the 5′ splice, (ii) an invariant heptanucleotide, the UACUAAC box, surrounding the branchpoint A (underlined), and (iii) a generally conserved enrichment for pyrimidine residues adjacent to the invariant AG dinucleotide at the 3′ splice site (Fig. 2A).

Two other characteristics of nuclear mRNA splicing in vitro that distinguish it from autocatalytic splicing are the dependence on added cell-free extracts and the requirement for adenosine triphosphate (ATP) (*2, 3*). Once in vitro systems had been established for mammalian and yeast pre-mRNA splicing, it was found that the size for the trans-acting machinery was unexpectedly large. When radiolabeled pre-mRNA was added to extracts in the presence of ATP, lariat intermediates were found to sediment at 40*S* to 60*S* on glycerol gradients in a large complex dubbed, inevitably, the spliceosome (*2, 3*). Sedimentation and electrophoretic fractionation techniques soon revealed that prominent components of this machine were ribonucleoprotein particles that had been identified by immunoprecipitation of mammalian extracts with certain autoantibodies (*5*). One particular type of antiserum (anti-Sm) brought down, in addition to a complex set of proteins, five snRNAs: U1, U2, U4, U5, and U6 (*5*). The RNA components of four of these snRNPs were shown to be essential for the in vitro splicing reaction by the technique of targeted ribonuclease (RNase) H degradation (*5*); the inaccessibility of U5 to oligonucleotides prevented a straightforward analysis of this RNA.

Together with the discovery of autocatalytic introns, the demonstration that snRNAs were essential, trans-acting components of the spliceosome argued strongly that Group II self-splicing and nuclear pre-mRNA splicing occurred by fundamentally equivalent mechanisms. According to this view (*6*), the snRNAs compensate for the low information content of nuclear introns and, by the formation of intermolecular RNA-RNA interactions, achieve the catalytic capability inherent in the intramolecular structure of autocatalytic introns (Fig. 2). One prediction of this hypothesis is that the protein components of the spliceosome do not serve in a catalytic capacity and thus will not be found to include enzymes such as the ligase and endonuclease, that mediate transfer RNA (tRNA) splicing (7). By the strictest interpretation of this view, the RNA moieties of the spliceosome (the snRNAs and pre-mRNA) will be shown to comprise the "active site" or sites.

Tests of both predictions must await biochemical and ultrastructural investigations of no doubt heroic proportions. What, then, can be gleaned from currently available data with respect to this hypothesis? I will summarize evidence for the RNA-based interactions in the splicing pathway, then review recent data on the function of spliceosomal proteins. Finally, the question of why nuclear pre-mRNA splicing is highly dependent on ATP will be considered. The data are consistent with the idea that RNA may act catalytically; in contrast, proteins may serve to modulate fidelity, as demanded by the evolution of regulated and alternative splicing.

The Spliceosomal snRNAs

How does the splicing machinery recognize the sites in the intron that will undergo covalent modification? Considerable evidence supports the conclusion that the U1 and U2 snRNPs bind to sequences at the sites of 5′ cleavage and branch formation, respectively; several experiments suggest that the U5 snRNP recognizes the 3′ splice site (*5*). In principle, both protein and RNA may contribute to snRNP-intron interactions. The prototype for the use of the base-pairing potential of the RNA component of the snRNPs is the interaction between the 5′ end of U1 RNA and the 5′ splice site (Fig. 2). The original proposal was based solely on the observed nine–base-pair complementarity between the two mammalian sequences (*8*). This model has since been extensively verified experimentally [reviewed in (*5*)]. An elegant genetic demonstration of the Watson-Crick interactions between these RNAs was provided by the construction of compensatory base-pair changes in mammalian cells (*9*). Subsequently, suppressor mutations were used to prove the interaction between U1 and the 5′ splice site in yeast (*10*).

The only other established case for a base-pairing interaction between an snRNA and the intron is that between U2 and sequences surrounding the branchpoint. This interaction was first tested in yeast (*11*), where the strict conservation of the branchpoint sequence readily revealed the potential for complementarity. The branchpoint nucleotide, which carries out nucleophilic attack

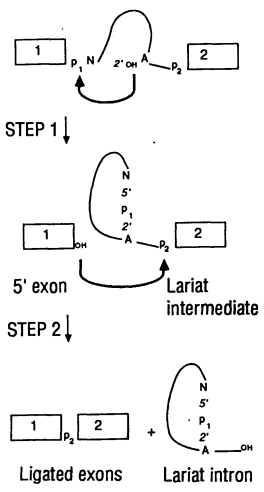

Fig. 1. Intron removal proceeds by two cleavage-ligation reactions. Group II introns and nuclear pre-mRNA introns are removed by the same mechanistic pathway (*4*). In the first catalytic step, a precursor RNA, containing two exons (boxed) separated by an intron (line), is cleaved at the phosphodiester bond at the 5′ splice junction (p_1) to form a lariat intermediate, releasing exon 1 (the 5′ exon). In the second step, cleavage at the 3′ junction (p_2) releases the lariat intron and the two exons are ligated. The lariat structure is due to the formation of a 2′,5′ phosphodiester bond to a conserved A upstream from the 3′ splice site; this residue is termed the branchpoint. Arrows indicate nucleophilic attack by the 2′-end hydroxyl of A and the 3′-end hydroxyl of the 5′ exon. In nuclear pre-mRNA, N must be a G.

on the 5′ splice site, is thought to be unpaired (Fig. 2A). The analogous residue bulges out of an intramolecular helix in a conserved domain of self-splicing Group II introns; this nucleotide must be unpaired for proper function (*4*) (Fig. 2B). The base-pairing interaction between U2 and the intron has also been demonstrated genetically in mammalian systems (*12*). In fact, although mammalian branchpoint sequences are notable for their deviation from a strict consensus, it can be shown under the appropriate experimental conditions that the preferred sequence is identical to the invariant core of the yeast consensus, CUAAC (*13*).

Genetic and biochemical analyses have established that the U1 snRNP binds to the 5′ splice site and the U2 snRNP binds to the branchpoint. There is now reason to believe that the story is more complicated, however, because binding of the U1 snRNP to the pre-mRNA substrate appears to require branchpoint sequences yet does not require the U2 snRNP. With the use of affinity chromatography, it was shown that the kinetic order of snRNP binding to the pre-mRNA is U1 (in an ATP-independent step), then U2 (*14, 15*). The U1 snRNP can bind to wild-type substrates in extracts in which U2 snRNA has been inactivated by RNase H–targeted degradation, but binding is greatly reduced in the presence of point mutations in the branchpoint region (*15*). The order of the binding interactions was verified with the use of extracts prepared from strains genetically depleted of either U1 or U2 snRNPs (*16*). A complex between snRNPs and radioactive pre-mRNA was defined as "committed" if it could be chased into spliceosomes on addition of excess unlabeled substrate. Committed complexes could form in the absence of U2 but not in the absence of U1. Finally, substrate challenge experiments indicated that the branchpoint sequence is needed for U1-snRNP complex formation. A similar dependence of U2 snRNP binding on the presence of the U1 snRNP has recently been observed for HeLa extracts (*17*). Taken together, these results suggest that some component of the U1 snRNP, or an associated factor, is required for an early step in branchpoint recognition, before binding of the U2 snRNP.

Just as these experiments argue that the branchpoint is seen by more than the U2 snRNA, genetic evidence in yeast suggests that the intron base-pairing region at the 5′ end of U1 RNA per se is not sufficient to specify the site of 5′ cleavage. Mutation of the invariant G at position 5 of the 5′ splice site (Fig. 2A) not only depresses cleavage efficiency at the normal GU site but activates cleavage nearby; the precise location of the aberrant site varies depending on the surrounding context (*18, 19*). Introduction of a U1 RNA, the sequence of which has been changed to restore base-pairing capability at position 5, does not depress the abnormal cleavage event; it enhances the cleavage at both wild-type and aberrant sites (*10*). These results argue that the complementarity between U1 and the intron is important for recognition of the splice-site region but does not determine the specific site of bond cleavage (*20*). Molecules that have undergone cleavage at aberrant sites are not substrates for the second cleavage-ligation reaction and thus accumulate as "dead-end" lariat intermediates (*18, 19*). This defect appears to be due to the absence of a G residue 2′ to the branch A (Fig. 1, residue N), as conversion of the aberrant cleavage site residue to G in this mutant now permits the second step to proceed (albeit inefficiently) (*21*). As discussed more fully below, this behavior may be best explained as a proofreading mechanism in which important residues are inspected at multiple steps, and by multiple components, in the splicing pathway.

Recent genetic experiments in yeast have revealed that the U5 snRNP is an excellent candidate for a trans-acting factor that functions in the specification of the 5′ cleavage site and the proofreading of this choice. Mutation of the invariant G residue at the first position of the 5′ splice site to an A allows the first

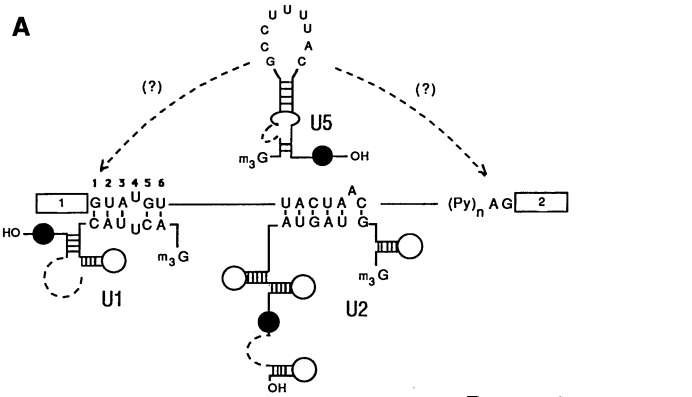

Fig. 2. (**A**) Interactions between spliceosomal snRNAs and the intron. A typical yeast intron contains conserved sequences (capitalized) at the 5′ and 3′ splice sites and surrounding the branchpoint A residue (Fig. 1) (*3*); a pyrimidine-rich tract, $(Py)_n$, is found at the 3′ splice site in many, but not all, cases (*49*). Base-pairing interactions between U1 and the 5′ splice site (*9, 10*) and between U2 and the branchpoint region (*11, 12*) have been demonstrated genetically. It is not known whether there are direct RNA-RNA interactions between U5 snRNA and the 5′ and 3′ splice sites. Sequences in the snRNAs indicate phylogenetically conserved sequences implicated in RNA-RNA interactions (*24*). Conserved stem-loop structures are shown; dotted lines indicate regions that differ between yeast and mammals (*24*). The binding sites for core proteins (Sm site) are indicated by filled circles; m_3 G indicates trimethylguanosine. (**B**) The structure of a self-splicing Group II intron is shown in simplified form. Arrows are as in Fig. 1. Note that the branchpoint A is unpaired (*4*), as is proposed for the U2-intron helix (*11*).

cleavage-ligation reaction to occur, although at reduced efficiency. Here again, however, the lariat intermediates contain an A residue 2′ to the branch nucleotide and cannot undergo the second step of splicing (*19, 22*). In a search for suppressors of this phenotype, Newman and Norman identified a single base substitution and a single base deletion in U5 snRNA within an evolutionarily conserved loop sequence of nine nucleotides (Fig. 2) (*23*). These suppressors appear to function by activating nearby cryptic 5′ splice sites. In a third case, multiple mutations within this nine-base loop generated a U5 snRNA which, in addition to activating a cryptic site, allows the lariat intermediates containing A to be spliced productively. These results argue that U5 is involved in the fidelity of the first and the second cleavage-ligation reactions. Each of the three U5 mutants exhibits a distinct spectrum of 5′ splice-site usage; this allele-specific pattern of suppression suggests that U5 suppresses by an "instructive" mechanism rather than by influencing the overall fidelity of splicing. The challenge now is to develop a testable molecular model because there are no obvious candidates for Watson-Crick interactions between U5 and the 5′ splice site (*24*).

At least one role of the U1, U2, and U5 snRNPs appears to be the recognition of consensus signals within the intron. In contrast, no specific binding site for the U4-U6 snRNP has been identified (*5, 14*). Possible insight into the role of these two snRNAs was provided when comparisons between *S. cerevisiae* and mammalian snRNAs revealed variations in size and sequence [reviewed in (*24*)]. The yeast U2, U1, and U5 snRNAs are much larger than their mammalian homologs (6.5-, 3.5-, and 2-fold larger, respectively). In contrast, U4 and U6 are well conserved in length between yeast and mammals and are found base-paired to one another in a single snRNP (*25*). Unlike U4, U6 is strikingly conserved at the primary sequence level: more than 80% of the nucleotides in the central third of the molecule are conserved (*26*). This region of U6 includes two

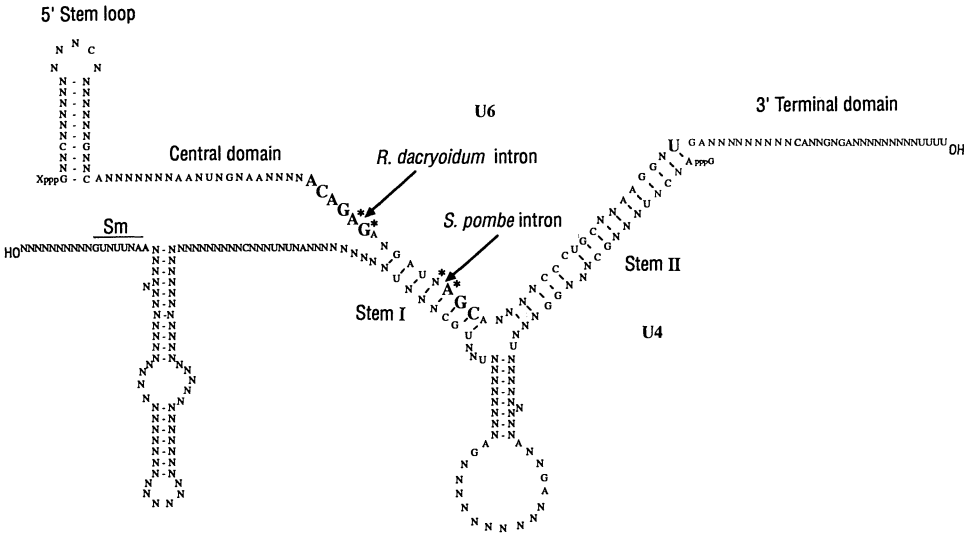

Fig. 3. Phylogenetically conserved and functionally important nucleotides in yeast U6 snRNA. N indicates nucleotides that vary among species (*24*). Mutations in nucleotides indicated in bold have strong detrimental effects on growth in vivo (*30*) and on splicing in vitro (*33, 82*). Additional residues may be important for U6 function. Arrows indicate the locations of mRNA-type introns in the U6 genes of *S. pombe* and *R. dacryoidum* (*31, 34*). Mutations in residues marked by asterisks result in partial or severe blocks to the second step of the splicing reaction in vitro (*33*).

stretches that form intermolecular helices with U4 (stem I and stem II) (Fig. 3). The conservation of primary sequence within a helix is unusual; typically, a phylogenetically conserved structure exhibits compensatory base-pair changes. One could rationalize this otherwise paradoxical sequence conservation in U6 by postulating that the role of U4 is primarily to base-pair with U6, but that U6 has a second function in splicing in addition to pairing with U4. In fact, the interaction between U4 and U6 is markedly destabilized specifically at a late stage in spliceosome assembly, before the first nucleolytic step of the reaction (*27, 28*); on electrophoresis under nondenaturing conditions, U4 is no longer associated with the spliceosome. This temporal correlation, together with the unusual size and sequence conservation of U6, led to the hypothesis that the unwinding of U4 from U6 activates U6 for participation in catalysis. In this view, U4 would function as an antisense negative regulator, sequestering U6 in an inert conformation until it is appropriate to act (*24*). Recent mutational studies demonstrate a functional role for U6 residues in the U4-U6 interaction domain in addition to base-pairing (*29, 30*).

Further evidence consistent with a catalytic role for U6 snRNA came from an unexpected source: the sequence of the U6 gene from the yeast *Schizosaccharomyces pombe* revealed the presence of an mRNA-type intron in the stem I region (*31*). To explain the unprecedented occurrence of an intron in a spliceosomal snRNA, it was proposed that the *S. pombe* U6 intron arose through an aberrant event during splicing whereby an intron integrated into the closely juxtaposed catalytic machinery of the spliceosome, namely U6 snRNA (*32*). One prediction of this model is that the region in U6 interrupted by the intron (the active site) should be functionally important. This has been borne out by recent studies. Two groups have performed extensive mutagenesis of yeast U6; one assayed the function of the mutated RNA with an in vitro reconstitution system (*33*), and the other transformed the mutagenized U6 gene into yeast and identified mutants by their in vivo phenotype (*30*). Whereas most mutations in U6 have little or no functional consequence (even when conserved residues were altered), two regions that are particularly sensitive to nucleotide changes were identified: a short sequence in stem I (CAGC) that is interrupted by the *S. pombe* intron, and a second, six-nucleotide region (ACAGAG) upstream of stem I (Fig. 3). Like the stem I sequence, this second region is interrupted by an mRNA-type intron, in this case in the yeast *Rhodosporidium dacryoidum* (*34*).

Mutations in the nucleotides that immediately flank both of these intron insertion sites block splicing at the second catalytic step in vitro; all other deleterious mutations in U6 block the first catalytic step (*33*) (Fig. 3). This correlation could indicate that the U6 residues required for the second step are spatially juxtaposed with the 3′ splice site and that in (presumably rare) reverse splicing accidents, the targets of the excised intron were the neighboring U6 residues rather than the ligated exons. An intron insertion reaction has been reported for the mechanistic cousins of nuclear introns, the Group II self-splicing introns (*35*).

Mutational analysis of the other spliceosomal RNAs has also revealed a tolerance of substitutions or, in some cases, deletion, even of phylogenetically conserved residues (*36, 37*). Conversely, the clusters of lethal point mutations at the 5′ end of U1 (*10*) and in the branchpoint recognition region (*11*) and adjacent stem-loop domain of U2 (*38*) appear to reflect critical and highly constrained interactions between these snRNAs and the intron. The question remains as to whether these mutationally sensitive residues are functioning in a catalytic capacity, either by contributing to the structure or chemical reactivity of the active sites, or more indirectly, by providing binding sites for protein factors, which in turn mediate catalysis. Alternatively, the enzymatic functions may be the product of a complex collaboration between amino acid and nucleic acid constituents.

Spliceosomal Proteins

Two classes of proteins have been distinguished. The snRNP proteins bind tightly to one or more species of snRNA and are sometimes referred to as integral proteins. The non-snRNP proteins, or extrinsic factors, are not stably associated with snRNAs and, in at least some cases, appear to interact only transiently with spliceosomal components.

Each of the four spliceosomal snRNPs (U1, U2, U4-U6, and U5) in metazoans is constructed from a set of common core proteins and a variable number of proteins unique to the particular snRNP (*39*). U1 snRNP, for example, contains three unique polypeptides, whereas U5 snRNP may contain seven or more. Ironically, although substantial biochemical information about mammalian snRNP proteins has accumulated, few of the more than 20 yeast splicing genes currently identified [pre-mRNA processing (*PRP*) genes] (*3*) appear to encode obvious analogs. Yeast must contain related core proteins because spliceosomal snRNPs can be immunoprecipitated from yeast with the use of mammalian antibodies to Sm (anti-Sm) (*40*). In yeast and mammals, the core proteins are critical for the synthesis

of stable snRNAs (*37, 41*). In metazoans, the Sm proteins are required for nuclear localization (*41*). Whether the functions of the core proteins are restricted to roles in snRNP biosynthesis and metabolism remains unknown.

The general function of snRNP-specific proteins may be to promote the interaction between two snRNAs or between an snRNA and the intron. These interactions probably involve protein-RNA and protein-protein contacts, as suggested by the presence in several snRNP proteins of motifs implicated in binding to RNA or to protein (Table 1). Moreover, the presence in some proteins of multiple binding domains has suggested the possibility that snRNP-specific proteins may form bridges between multiple components of the splicing machinery (*42*).

It is likely that one or more U5-specific proteins bridge the association between this snRNA and the intron. Mammalian cells contain intron binding protein (IBP), a U5-associated protein that binds to the polypyrimidine tract and 3′ splice site (*43*). Moreover, it has recently been shown that antibodies to a yeast U5 protein, PRP8 (280 kD) (*44*), cross-react with mammalian spliceosomes (*45*) and can immunoprecipitate from mammalian extracts a 220-kD protein that has been cross-linked to a pre-mRNA substrate; the ultraviolet cross-link is dependent on an intact 3′ splice site (*46*). There are no apparent sequence-specific requirements 3′ to the branchpoint for the first cleavage-ligation reaction in yeast (*47*), unlike in HeLa extracts (*48*). However, recent experiments have uncovered a role for a U-rich element in the second step of splicing in yeast (*49*). A parsimonious hypothesis explaining these experiments and recent results on natural and manipulated mammalian introns (*50*) is that the polypyrimidine tract in most mammalian introns plays a dual role, first in aiding branch site recognition and then in identifying 3′ splice sites. In yeast, there is evidence only for the latter event. It remains to be seen if different sets of trans-acting factors are required to mediate both recognition events in mammals, only one of which will be shared by yeast.

Proteins that mediate snRNP-snRNP associations would seem to be involved in the complex set of ATP-responsive interactions between U4-U6 and U5. In vitro studies in both yeast and mammals suggest that U5 joins the spliceosome as part of a "tri-snRNP" with the U4-U6 snRNP (*28, 51*). In yeast, several proteins that interact with the U4-U6 snRNP have been identified genetically (Table 1). Antibodies to one of these, PRP4, inhibit spliceosome assembly (*52*). A deletion mutant of U4 snRNA that lacks the 5′ stem-loop allows efficient formation of the U4-U6 snRNP but fails to bind PRP4 and cannot form the triple complex with the U5 snRNP (*53*). The indication that PRP4 functions by promoting the binding of the U4-U6 snRNP to the U5 snRNP and the fact that PRP4 contains five repeated amino acid segments found in the β subunits of the heterotrimeric G proteins (*54*) suggest that this motif may be involved in a protein-protein interaction.

The role of snRNP proteins may also be inferred from dynamic changes in their association with other snRNPs or spliceosomal components. Immunological studies in yeast suggest that PRP8 is associated with the U5 snRNP, the active spliceosome, and the post-splicing complex containing the excised intron (*55*). In contrast, antibodies to PRP24 (which was identified genetically as a suppressor of a cold-sensitive mutation in U4 that disrupts the U4-U6 base-pairing interaction) immunoprecipitate only U6 from wild-type strains and both U4 and U6 snRNAs when the U4-U6 complex is destabilized (*56*). This pattern may suggest that PRP24 interacts transiently with U4 and U6 to promote the formation of the U4-U6 complex.

Spliceosomal Dynamics and ATP

It is now apparent that a number of proteins required for splicing work as extrinsic factors. As with the ribosome, the distinction between snRNP proteins and so-called extrinsic factors may become blurred for proteins with weak inherent affinity for the ribonucleoprotein particle. Perhaps a reasonable operational definition of an extrinsic factor is its association with spliceosomal components at a particular step in the pathway. The existence of this type of factor was first suggested by the demonstration that yeast spliceosomes assemble in heat-inactivated extracts from a temperature-sensitive

Table 1. Yeast spliceosomal proteins. Sequence motifs may reflect the capabilities of the proteins to interact with RNA (RNP, zinc finger–like, S1-like), interact with another protein (G protein), or mediate RNA helicase activity (DEAD) or RNA-dependent ATPase activity (DEAH). ND, no data. "Step 1" and "step 2" refer to Fig. 1.

Protein	Gene*	MW (kD)†	Sequence motif	Function
snRNP proteins	*PRP8*	280	ND	U5 snRNP protein (*44*)
	PRP4	52	G protein (β subunit) (*54*)	U4-U6 snRNP protein (*52, 75*); required for U5 snRNP binding to U4-U6 snRNP (*53*)
	PRP6	104	Zinc finger–like (*76*)	U4-U6 snRNP protein (77)
	PRP24	51	RNP (*56*)	U6 snRNP protein (*56*); required for formation of U4-U6 (*56*); may facilitate PRP28 function (*72*)
Non-snRNP proteins (extrinsic factors)	*PRP11*	30	Zinc finger–like (*78*)	Present in spliceosome (*78*)
	PRP9	63	Zinc finger–like (*76*)	Promotes U2 binding (77)
	PRP2	100	DEAH (*64*)	Required for step 1 (*57*)
	PRP16	120	DEAH (*60*)	Required for step 2 (*59*); RNA-dependent ATPase (*59*); suppresses intron branchpoint mutation (*66*)
Proteins of unknown location	*PRP22*	130	DEAH; S1-like (*65*)	Required for mRNA release from spliceosome (*65*)
	PRP5	96	DEAD (*79*)	Required for step 1 (*71*)
	PRP28	67	DEAD (*72*)	Required for step 1; suppressed by mutation in PRP8; may facilitate PRP24 function (*72*)
	SPP81/DED1	65	DEAD (*73*)	Suppresses mutation in PRP8 (*73*)
	PRP17	52	G protein (β subunit) (*3*)	Required for step 2 (*80*)
	PRP18	28	ND	Required for step 2 (*81*)

*The *PRP* genes have been reviewed recently (*3* and references therein). †Molecular weight is predicted from the amino acid sequence of the cloned gene.

mutant strain, *prp2*, but fail to carry out the first catalytic reaction; this defect could be complemented by the addition of heat-treated extracts from a different mutant strain plus ATP (*57*). Recent immunoprecipitation experiments show that the PRP2 protein is associated with pre-mRNA (and, to a lesser extent, with intermediates), but not with spliced products (*58*). Thus PRP2 appears to bind just before the first cleavage step and is released at or before the second cleavage.

A detailed biochemical analysis of the PRP16 protein demonstrates that it associates with spliceosomes containing lariat intermediates but not with splicing products or with precursor RNA (*59*). PRP16 is completely dispensable for the first cleavage-ligation reaction in vitro because extracts immunodepleted for PRP16 accumulate intermediates that can be chased into products by the addition of purified PRP16, in an ATP-dependent reaction. The purified protein has been shown to hydrolyze ATP in response to RNA but not single-stranded DNA. This catalytic activity was anticipated (*60*) from sequence similarities between PRP16 and a family of proteins for which the prototype, eukaryotic initiation factor 4A (eIF4A), is an RNA-dependent adenosine triphosphatase (ATPase) (*61*) and an ATP-dependent RNA helicase (*62*). Within most of these motifs, however, the sequence of PRP16 exhibits variations from the consensus; for example, the sequence of the so-called DEAD-box for which this eIF4A-like family was named [according to the one-letter amino acid code for the consensus tetrapeptide (*63*)] is DEAH in PRP16 (*60*).

Sequence analysis of PRP2 (*64*) and of another essential splicing protein, PRP22 (*65*), reveals that they have extensive homologies with PRP16. This homology is highest (about 50%) in the 320–amino acid region encompassing the putative ATP binding domain. The identity is strongest for the sequences that distinguish the eIF4A-like motifs from those of PRP16. In addition to these homologies in the central domain, a region of 280 amino acids in the COOH-terminus is 42% and 35% identical to PRP22 and PRP2, respectively. Each of these proteins appears to have a unique role in the splicing pathway; PRP2 is required for step 1, PRP16 for step 2, and PRP22 for a later stage in the pathway, when the ligated mRNA is released from the spliceosome (*65*). It seems likely that all three proteins are RNA-dependent ATPases, differing in their ligands and effector functions. PRP2 and PRP22, like PRP16, may undergo transient high-affinity interactions with the spliceosome. It remains to be established whether release of PRP16 from the spliceosome requires the hydrolysis of ATP.

PRP16 was initially identified by the isolation of a dominant suppressor of a splicing defect conferred by an A to C branchpoint mutation; cells containing the suppressor allele (*prp16-1*) use the mutant branchpoint nucleotide with increased efficiency (*66*). The mutation that confers the suppressor phenotype resides within the putative ATP binding domain, which suggests that the accuracy of branchpoint utilization is coupled to the binding or hydrolysis of ATP (*60*). Specifically, it was proposed that the *prp16-1* mutation might cause a change in a "proofreading clock." Mutations that increase the rate of formation of a committed (productive) complex in a branched kinetic pathway can reduce fidelity by reducing the time available for dissociation of the incorrect complex through the "discard" branch of the proofreading pathway. As has been shown for the decoding of the ternary elongation factor–Tu (EF-Tu)/tRNA/guanosine triphosphate complex at the ribosomal A site in *Escherichia coli*, certain mutations in the translational apparatus decrease accuracy by decreasing the time for noncognate tRNAs to dissociate from the ribosome (*67*). According to this hypothesis (*60*), the decreased fidelity of the *prp16-1* mutant may be reflected in an altered affinity for, or rate of hydrolysis of, ATP in the in vitro assays (*59*) described above.

EF-Tu and several other ribosomal factors appear to require nucleoside triphosphate (NTP) hydrolysis for factor release, rather than for actual binding or activity (*68*); thus they are thought to use nucleotide hydrolysis to direct a series of unidirectional conformational switches. In contrast, eIF4A is reported to use the energy of ATP hydrolysis to unwind secondary structure (*62, 69*), which presumably explains how the eIF4A complex scans from the mRNA cap to the first AUG. The PRP16 family differs from eIF4A at a number of consensus positions, as described above; it is yet to be determined if PRP16 (or any of the other DEAH family members) has RNA helicase activity or, perhaps like EF-Tu, functions as a simpler RNA-dependent ATP-driven conformational switch. However, a large and distinct set of yeast proteins contain perfect matches to motifs of the DEAD family (*70*). The function of at least three of these proteins [PRP5 (*71*), PRP28 (*72*), and SPP81 (*73*)] has been implicated in splicing. Strains containing conditional lethal alleles of either *prp5* (*71*) or *prp28* (*72*) are defective in the first step of splicing at the nonpermissive temperature in vitro or in vivo, respectively. Moreover, suppressor studies provide a genetic link between PRP8, which is a U5 snRNP protein, and both PRP28 (*72*) and SPP81 (*73*). In addition, the synthetic lethal phenotype found in double mutants between *prp28* and *prp24*, which is a U6 protein (Table 1), suggests PRP28 might unwind the U4-U6 helix (*72*). The hypothesis that U4 must be displaced from U6 to enable the catalytic activation for the spliceosome makes the destabilization of U4 and U6 a critical target for a potential helicase. In fact, there must be a multitude of opportunities for the melting of inter- and intramolecular base-pairs formed by the snRNA and pre-mRNA components of the spliceosome. In addition to the proven helices formed between U1 and U2 snRNAs and the intron, which must be disrupted before another round of splicing can initiate, it is likely that many other base-pairing interactions undergo dynamic changes during the spliceosome cycle (the set-size of potential interactions is enormous if duplexes as short as several base pairs are considered). Conceivably, each of these conformational rearrangements could contribute to the accuracy of splicing by dictating a strict ordering of assembly events and thus providing multiple, sequential opportunities for fidelity checks. The time is opportune for the biochemical analysis of these proteins.

Why Spliceosomes?

Why does nuclear mRNA splicing require such a complicated machinery, whereas Group I and Group II introns attest so powerfully to the potential for RNA self-splicing? To put the question another way, if the catalytic reactions of nuclear mRNA splicing prove to be RNA-based, why are so many proteins essential for splicing, even in an organism with the limited developmental complexity of yeast? The best answer seems to come from a comparison of the elaborate structural conservation of autocatalytic introns with the remarkably featureless architecture of pre-mRNA introns. That is, the low information content within all nuclear introns may demand mechanisms that ensure accuracy in the processing of pre-mRNA. Ultimately, the most compelling rationalization for the movement of information from the intron to the external machinery rests on the existence of regulated and alternative splicing, which generate genetic diversity (*74*). Because self-splicing introns carry the structural requirements for catalysis internally, they are poor candidates for regulation. In contrast, if splicing information is moved to the external machinery, the intron is free to diverge in size and sequence, and intron recognition can then be differentially regulated. But there is an unavoidable cost to be paid for intron variation: as constraints for structural conservation are re-

laxed, fidelity is compromised. Thus, the splicing pathway must have evolved strategies to maintain accuracy while encouraging plasticity. An elucidation of the molecular basis of fidelity in the constitutive splicing machinery will be critical for understanding the regulated events that enrich the biology of higher eukaryotes.

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83. I thank the members of my laboratory for help in all aspects of the preparation of this article; E. Blackburn, N. Craig, I. Herskowitz, and C. Kenyon for critical evaluation of the manuscript; and J. Piccini for expert editorial assistance. Supported by NIH grant GM21119.