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Early Commitment of Yeast Pre-mRNA to the Spliceosome Pathway

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Pre-mRNA splicing in vitro is preceded by complex formation (spliceosome assembly). U2 small nuclear RNA (snRNA) is found in the earliest form of the spliceosome detected by native gel electrophoresis, both in *Saccharomyces cerevisiae* and in metazoan extracts. To examine the requirements for the formation of this early complex (band III) in yeast extracts, we cleaved the U2 snRNA by oligonucleotide-directed RNase H digestion. U2 snRNA depletion by this means inhibits both splicing and band III formation. Using this depleted extract, we were able to design a chase experiment which shows that a pre-mRNA substrate is committed to the spliceosome assembly pathway in the absence of functional U2 snRNP. Interactions occurring during the commitment step are highly resistant to the addition of an excess of unlabeled substrate and require little or no ATP. Sequence requirements for this commitment step have been analyzed by competition experiments with deletion mutants: both the 5′ splice site consensus sequence and the branch point TACTAAC box sequence are necessary. These experiments strongly suggest that the initial assembly process requires a *trans*-acting factor(s) (RNA and/or proteins) that recognizes and stably binds to the two consensus sequences of the pre-mRNA prior to U2 snRNP binding.

Splicing of eucaryotic, intron-containing pre-mRNAs takes place in the nucleus and is performed within a complex called a spliceosome (5, 7, 9). Multiple specific interactions occur between the substrate pre-mRNA and *trans*-acting factors, among them the small nuclear ribonucleoprotein particles (snRNPs) (for a review, see reference 18). The general features of spliceosome assembly have been characterized in vitro by several biochemical approaches including velocity gradients (5, 7, 9), native gel electrophoresis (11, 22), and affinity chromatography (3, 10, 28). The earliest complex detected in native gels contains, at least, U2 snRNP and pre-mRNA. A larger complex then forms which contains, in addition to U2 snRNP, U4/6 and U5 particles. Subsequently U4 leaves the complex and the mature spliceosome is constituted; the cleavage and ligation reactions take place within this spliceosome (6, 11, 23). U1 snRNP is necessary for efficient splicing, but its presence in the various complexes is controversial (3, 10, 12, 30, 31). This description applies to both metazoan and yeast spliceosome assembly; the pathway has therefore been conserved over a great evolutionary distance.

In metazoa introns are characterized by two consensus sequences (19): one located at the 5′ splice site (AG/GT PuAGT) and the other located upstream of the 3′ splice site ($[Py]_nNPyAG/G$). In *Saccharomyces cerevisiae* a similar 5′ splice site consensus (G/GTAPyGT) is found. Although the presence of the polypyrimidine stretch is questionable, a very conserved sequence (TACTAAC) defines the branch point position (16, 24). Base pairing between these pre-mRNA consensus sequences and some small nuclear RNAs (snRNAs) could act as early events in spliceosome formation. Genetic experiments have shown that the 5′ splice site sequence base pairs with U1 snRNA (29; B. Seraphin, L. Kretzner, and M. Rosbash, EMBO J., in press) and, in *S. cerevisiae*, that the TACTAAC box base pairs with U2 snRNA (21). In addition, a protein factor, U2AF, has been identified as a metazoan polypyrimidine-binding factor that is required for a stable interaction between U2 snRNP and the pre-mRNA (25). In *S. cerevisiae*, no step prior to the formation of the U2 snRNA-containing complex (band III) has been defined, yet the involvement of multiple *trans*-acting factors is strongly suggested by the presence of the two conserved consensus sequences.

Oligonucleotide-directed RNase H cleavage is a powerful means of depleting a splicing extract of a given RNA. It has been successfully used to demonstrate the involvement of U1 and U2 snRNAs in splicing reactions (2, 4, 13–15). We first analyzed the consequences of U2 snRNA depletion by RNase H cleavage on spliceosome assembly; no spliceosome formation was detected in the absence of a fully active U2 snRNP. Such a finding gave us the opportunity to address the question of the commitment of pre-mRNA to the spliceosome pathway in the absence of active U2 snRNP. We designed a two-step protocol which could be used to assay a commitment step: a radiolabeled substrate was incubated in the absence of functional U2 snRNP to allow stable complex formation to occur, and then a large excess of cold substrate and active U2 snRNPs was added and spliceosome assembly was detected by native gel electrophoresis. By using this assay, we indeed define a commitment step for spliceosome assembly that does not require active U2 snRNP. The characteristics of this commitment process are described.

MATERIALS AND METHODS

Plasmids and RNAs. The construction of plasmids pSPrp51AΔ2 (wild type), pSPrp51A[5′o] (5′ splice site deletion mutant), and pSPrp51A[Δ3B] (TACTAAC box deletion mutant) and their runoff RNAs have been described previously (22). The total sizes of the RNAs are 237, 730, and 768 nucleotides, respectively. Substrate (hot) RNA and competitor (cold) RNA had specific activities of 70,000 and 70 dpm/ng, respectively. RNA was gel purified on 5% polyacrylamide gels before use in splicing reactions.

Splicing reactions and native gel electrophoresis. Splicing extracts and splicing reactions were performed as described

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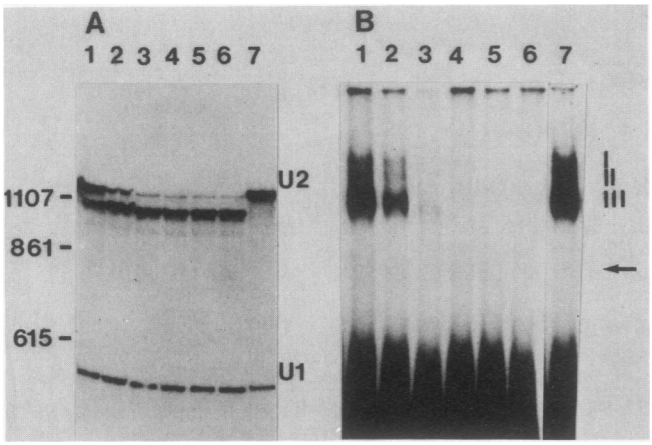

FIG. 1. RNase H-directed U2 snRNA cleavage. (A) Northern (RNA) blot analysis of U1 and U2 snRNA. Splicing extracts were incubated with increasing amounts of U2 snRNA-specific oligonucleotide (lanes 1 to 6, twofold increases starting from 0.3 μM; lane 7, no oligonucleotide added). On the left, the migration of single-stranded DNA markers is indicated. (B) Spliceosome assembly analyzed by native gel electrophoresis. After the incubation of the splicing extract with U2 snRNA-specific oligonucleotide, labeled splicing substrate was added for 10 min at 25°C. The lanes are the same as in panel A. Roman numerals indicate the different splicing complexes (23). The arrow indicates the migration position of a faint complex discussed in the text.

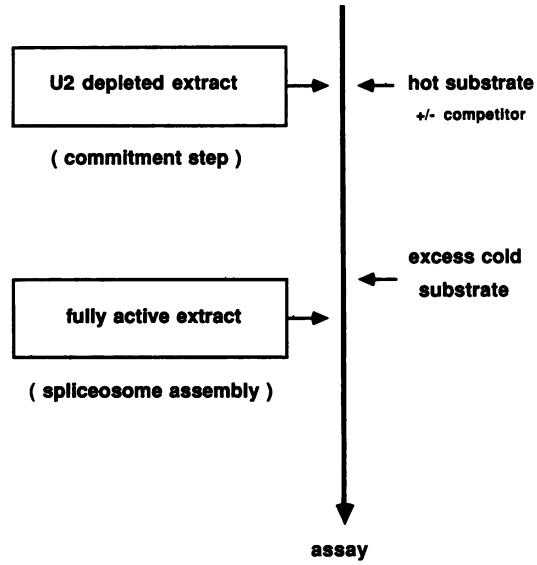

FIG. 2. Design of the chase experiment. Standard conditions were a 10-min incubation in the U2 snRNA-depleted extract and a 5-min incubation in the fully active extract. The isotopic dilution was performed 1 min before the addition of the second extract, except when otherwise stated (see text). For competition experiments presented in Fig. 6, competitor RNA in various amounts was added simultaneously with the hot substrate. Routinely, the fully active extract was preincubated at 30°C for 20 min as a mock incubation for the U2 snRNA depletion treatment and as a verification that the preincubation alone does not deplete the extract of any crucial factor required for spliceosome formation.

previously (17, 20). Substrates were used at 1 nM, which corresponds to a saturating amount of substrate for in vitro spliceosome formation and for the splicing reaction in our extracts. Native gel electrophoresis and RNA analyses were performed by the method of Pikielny and Rosbash (22). For the cleavage of U2 snRNA, splicing extracts were incubated for 20 min at 30°C with a U2 snRNA-specific oligonucleotide (15). Typical cleavage reactions were performed for 4 μl of splicing extract in 10-μl reaction mixtures containing the oligonucleotide at 4 μM.

snRNA blot. RNA samples were run on a 4% denaturing polyacrylamide gel. Electroblotting onto a Biotrans membrane (ICN Pharmaceuticals, Inc.) was performed for 4 h at 300 mA in 0.5× TBE (100 mM Tris, 100 mM boric acid, 2 mM EDTA). The membrane was then UV treated (800 μW/cm^2) for 2 min and baked under vacuum for 1 h at 80°C. Prehybridization, hybridization, and wash procedures were performed under standard high-stringency conditions. Nick-translated probes of U1 snRNA (15) and U2 snRNA (1) genomic clones were used at 2×10^5 cpm/ml.

RESULTS

U2 snRNA cleavage precludes spliceosome assembly. Owing to the presence of a high level of RNase H activity in yeast splicing extracts (26), it is possible to cleave any accessible RNA simply by adding a specific complementary oligonucleotide to the extract. The results of such an experiment are presented in Fig. 1A, in which an oligonucleotide complementary to the U2 snRNA TACTAAC box binding sequence was used. Addition of this oligonucleotide at concentrations ranging from 0.3 to 10 μM cleaved U2 snRNA and did not affect U1 snRNA. The major cleavage product of U2 snRNA was an RNA species about 80 nucleotides shorter. A very small amount of U2 snRNA was resistant to the RNase H digestion. These partially U2 snRNA-depleted extracts were used in splicing reactions, and the spliceosome assembly was analyzed by native gel electrophoresis (Fig. 1B). Three specific splicing complexes are detected in this assay; the complex with the greatest mobility (III) is the first to form and contains only U2 snRNA (23). High levels of U2 snRNA-specific oligonucleotide completely inhibited the formation of all three complexes (lanes 4 to 6). No residual amounts of these complexes were detected, suggesting that the small amount of uncleaved U2 snRNA cannot support the formation of spliceosomes. Mock experiments with several different oligonucleotides unrelated to U2 snRNA have been performed, and none of these oligonucleotides (used at concentrations of up to 50 μM) had an inhibitory effect on spliceosome assembly (data not shown). Two light bands of greater electrophoretic mobility were often detected, but recovery of these complexes was variable from one experiment to another (compare Fig. 1B with Fig. 3A or 4). Hybridization of blots from native gels with a U2 snRNA-specific probe reveals the location of U2 snRNP. This position was only slightly modified in a U2 snRNA-depleted extract, suggesting that the cleaved U2 snRNP remains stable, yet is unable to support spliceosome assembly (data not shown).

A chase experiment defines a commitment step in spliceosome assembly. Since we were able to prevent spliceosome formation by U2 snRNA cleavage, we searched for an early commitment step. The design of the experiment is shown in Fig. 2. During a first incubation, radiolabeled splicing substrate was added to the U2 snRNA-depleted extract. A 100-fold excess of unlabeled (or low-specific activity) substrate was then added, followed by a fully active extract; the mixture was then incubated for 5 min (during which the U2 snRNA and U2 snRNP profiles from the fully active extract were unaffected; data not shown). Spliceosome assembly

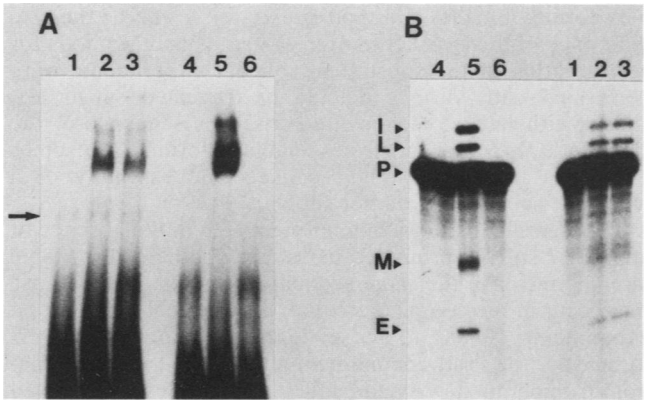

FIG. 3. Analysis of a chase experiment for spliceosome assembly (A) and splicing products (B). Labeled splicing substrate was incubated for 10 min in a U2 snRNA-depleted extract (lanes 1); alternatively, this incubation was followed by a second 10-min incubation after the addition of a fully active extract (lanes 2). For lanes 3, the same incubations as for lanes 2 were performed, but a 100-fold molar excess of cold substrate was added just before the active extract was added (chase experiment). Lanes 4 and 5 show 1 and 10 min, respectively, of incubation of a labeled substrate in an active splicing extract. Lanes 6 are the same as lanes 5, but labeled and cold (100-fold molar excess) substrates were mixed before the incubation in extract. The arrow (panel A) shows bands whose significance is discussed in the text. On the left of panel B are indicated the various RNA species detected in the gel: E, 5′ exon; M, mRNA; P, pre-mRNA; L, lariat intron; I, lariat intermediate.

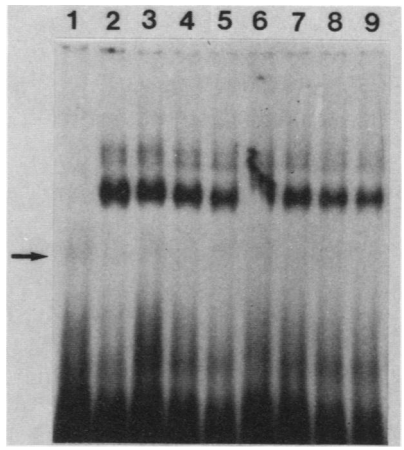

FIG. 4. Resistance of the committed splicing substrate to the subsequent addition of excess substrate. Lanes 1 and 2 are identical to lanes 1 and 2 of Fig. 3. Lanes 3 to 9 show chase experiments with increasing lengths of time between the additions of the cold substrate and the fully active extract (15 and 40 s and 1, 3, 6, 10, and 20 min, respectively). The arrow shows bands whose significance is discussed in the text.

was then assayed by native gel electrophoresis. If the absence of active U2 snRNP prevents the stable binding of substrate to splicing factors, the extensive isotopic dilution of the substrate will preclude the detection of radiolabeled spliceosomes. On the contrary, if stable commitment of the radioactive splicing substrate occurs during the first incubation, the subsequent addition of U2 snRNP will allow detectable spliceosome assembly, even in the presence of an excess of cold substrate.

The results of this experiment are shown in Fig. 3A. A splicing substrate incubated for 10 min in a U2 snRNA-depleted extract did not undergo spliceosome assembly (lane 1). When this incubation was followed by the addition of a fully active extract and a subsequent 5-min incubation, normal spliceosome assembly occurred (lane 2). The addition of an excess of cold substrate just before the second incubation did not modify this profile (lane 3), whereas the addition of a mixture of both labeled and unlabeled RNAs to a standard splicing reaction completely prevented the detection of labeled spliceosomes (lane 6 compared to lane 5). In the experiments performed with U2 snRNA-depleted extracts, a rapidly migrating doublet was detected as described above. These bands did not disappear in the chase experiment (lane 3), indicating that they are not the structures chased into spliceosomes after the addition of fully active extract. We conclude either that the committed substrate is present in an unstable complex under our electrophoresis conditions or that it comigrates with the nonspecific complexes at the bottom of the gel.

The splicing reaction products obtained in the same experiments were also assayed (Fig. 3B). A comparison between lane 3 and lane 2 shows that splicing products were obtained in the chase experiment. Both for spliceosome formation (Fig. 3A) and for splicing (Fig. 3B), the two-step reactions (lanes 2 and 3) were less efficient than a one-step reaction (lane 5). This was routinely observed and was independent of the addition of the cold substrate, indicating that some inhibition occurs as a consequence of the incubation in the presence of the U2 snRNA-complementary oligonucleotide. In conclusion, these results indicate that a splicing substrate can be committed to the spliceosome assembly pathway in a U2 snRNA-depleted extract. The subsequent addition of active U2 snRNP allows the completion of spliceosome assembly and splicing.

Characteristics of the commitment step. The initial chase experiments were performed by adding excess cold substrate and the active extract in very close succession. This procedure permits an isotopic dilution of the labeled substrate but does not directly address the question of the stability of the committed, labeled substrate. For that purpose, chase experiments were performed with various lengths of time between the addition of cold substrate and the addition of fully active extract (Fig. 4). Incubating the committed labeled substrate with a 100-fold excess of cold substrate for 20 min before adding active extract did not significantly decrease the level of radioactive spliceosome (compare lanes 3 and 9). The slight decrease observed was due mainly to general degradation of the pre-mRNA, which occurred after a relatively long incubation in a yeast splicing extract. The result shows that the committed substrate is resistant to the addition of excess unlabeled substrate, suggesting that the factor(s) is tightly bound to the substrate. Furthermore, this experiment indicates that there is a limiting factor(s) in the first incubation step. Otherwise, commitment of the unlabeled substrate would decrease the recovery of spliceosomes (compare lane 2 with lanes 3 to 9). In further experiments, the chase was routinely performed by adding cold substrate 1 min prior to the addition of the fully active extract.

We also examined the ATP requirement of the commitment step (Fig. 5). Spliceosome assembly requires ATP, since the omission of exogenous ATP leads to a low level of complex formation (Fig. 5A), probably owing to the presence of residual endogenous ATP. We compared the time course of the commitment step of the chase experiment in the presence and in the absence of exogenous ATP. (ATP was always added simultaneously with the fully active

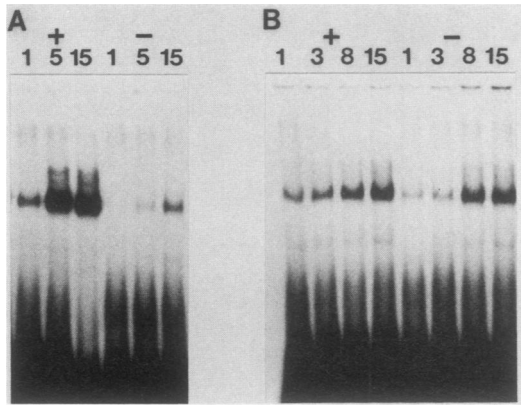

FIG. 5. Kinetic analysis of the commitment step. The time courses of spliceosome assembly (panel A) and of the commitment step of a chase experiment (panel B) were analyzed. Incubations were performed with (+) or without (−) the addition of ATP. Numbers indicate minutes of incubation. For the chase experiments, ATP was added in each case simultaneously with the fully active extract and the subsequent incubation was continued for 5 min.

extract.) The absence of ATP during the commitment step did not affect the time course of spliceosome formation, although the results for the short time points may suggest a slight decrease in the absence of ATP (Fig. 5B). This experiment also shows that substrate commitment is quite slow with this two-step experimental protocol, since the recovery of splicesomes increased between 1 and 15 min of incubation. Changing the length of the second incubation did not modify the recovery of spliceosomes, suggesting strongly that the second step is faster than the first (data not shown). Also, the total recovery of spliceosomes in the chase experiment was lower than that obtained with a simple one-step incubation in an active splicing extract (15-min time points with ATP in Fig. 5B compared with Fig. 5A; also see Fig. 3A, lanes 3 and 5). These results indicate that factors involved in the second step (especially U2 snRNP) are not limiting. These experiments suggest that commitment of a substrate to the splicing pathway in the absence of active U2 snRNP is a relatively slow process which requires very little ATP. A greater concentration of ATP is required thereafter and might be related to the binding of U2 snRNP.

Sequence requirements for commitment of a substrate to the spliceosome pathway. Yeast spliceosome assembly requires a substrate with both a functional 5′ splice site consensus sequence and a branch point (TACTAAC box) sequence (22); substrates deleted for either one of these sequence elements fail to undergo spliceosome assembly. To determine whether any of these deleted substrates were able to associate stably with *trans*-acting factors in the extract (thereby inhibiting spliceosome assembly of a bona fide substrate), we performed competition experiments. Cold competitor RNA was incubated for 5 min in a splicing extract; a small amount of labeled substrate was then added, and spliceosome assembly was assayed by native gel electrophoresis (Fig. 6A). When a wild-type substrate was used as competitor, a decrease in the recovery of labeled spliceosomes was observed (lanes 1 to 3); in contrast, a TACTAAC deletion mutant was unable to inhibit spliceosome formation (lanes 4 to 6). The new band which appears in these experiments is the nonspecific complex formed by the large excess of competitor substrate of low specific activity. Its slow migration, compared with that of the nonspecific complex formed by the wild-type substrate, is due to the size difference of the two RNAs (see Materials and Methods for a description of the substrates). Similar experiments were performed with various deleted or truncated substrates, lacking either the 5′ splice site consensus sequence or the TACTAAC box. All substrates unable to form spliceosomes were also unable to inhibit spliceosome assembly of a wild-type substrate (data not shown).

The identification of a commitment step in the absence of active U2 snRNP prompted us to ask whether these stable interactions have the same sequence requirements as those that occur in the complete extract. To this end, competition experiments similar to those described above were performed for the first, commitment step of the two-step reaction. Competitor RNAs and splicing substrates were mixed and incubated in U2 snRNA-depleted extracts; subsequently a chase incubation was performed (the protocol is depicted in Fig. 2; results are shown in Fig. 6B and C). Wild-type RNA was a good competitor (Fig. 6B, lanes 1 to 4), whereas mutants with deletions in either the 5′ splice site consensus (lanes 5 to 8) or the TACTAAC box sequence (Fig. 6C, lanes 1 to 4) were unable to inhibit the commitment of a wild-type substrate (see legend to Fig. 6). (Electrophoresis was carried out for longer in the experiment depicted in Fig. 6B than in those in Fig. 6A and C, leading to the apparent difference in migration of the complexes.) These experiments establish the fact that both yeast intron consensus sequences are required (in this in vitro assay) to commit an RNA to the spliceosome pathway.

DISCUSSION

In *S. cerevisiae* both the 5′ splice site consensus sequence and the TACTAAC box are required for spliceosome assembly and for branchpoint protection (27). These findings parallel genetic evidence that U1 snRNA base pairs with the 5′ splice site sequence (29; Seraphin et al., in press) and that U2 snRNA base pairs with the TACTAAC box (21). It is therefore tempting to attribute early recognition functions to these base-pairing interactions. Consistent with this interpretation are results presented in Fig. 1, which show that yeast spliceosomes do not assemble when U2 snRNA is cleaved at the crucial complementary positions. Different results have been obtained with metazoan systems; complex A formation still takes place, despite the cleavage of U2 snRNA at the positions precisely expected to base pair with the branch point sequence (8, 31). However, there is some evidence in both systems that spliceosome assembly is markedly affected when oligonucleotides complementary to the 5′ end of U2 snRNA are used for the RNase H oligonucleotide-directed cleavage (data not shown; 8, 31). We cannot rule out the possibility that yeast spliceosomes form in the absence of the U2 TACTAAC binding sequence but are unstable under our native gel electrophoresis conditions. However, all attempts to detect such complexes by changing the experimental conditions (e.g., electrophoresis temperature, salt concentration, and EDTA concentration) have failed. Alternatively, metazoan U2 snRNP could retain the small 5′ piece of the cleaved RNA and thus allow spliceosome assembly, whereas the same cleavage of yeast U2 snRNA would release the 5′ end of the RNA from the snRNP, precluding spliceosome assembly. In either case, these RNase H cleavage experiments suggest strongly that U2 snRNP is required for spliceosome assembly in *S. cerevisiae* and in metazoa.

The ability to block spliceosome assembly by U2 snRNA cleavage gave us the opportunity to design an experiment in

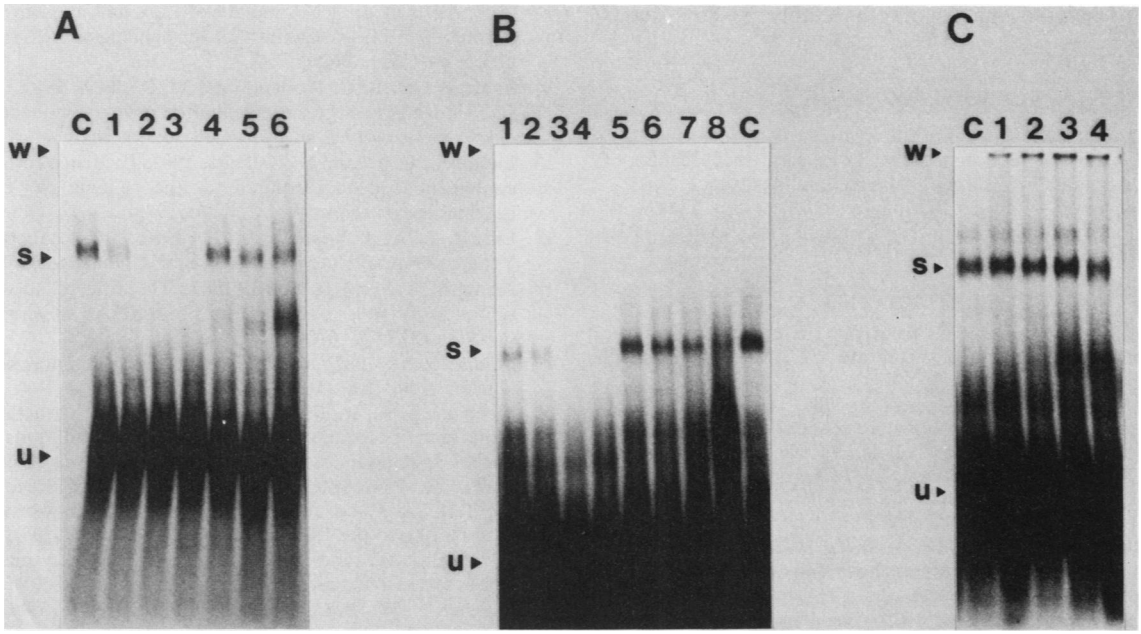

FIG. 6. (A) Competition experiments for spliceosome assembly. Competitor RNAs (either the full-length substrate [lanes 1 to 3] or the TACTAAC deletion substrate [lanes 4 to 6]) were added to splicing extracts for 5 min. Then, full-length labeled substrate was added for a further 5 min and spliceosome assembly was analyzed by native gel electrophoresis. In lane C, no competitor was added. The molar ratio of competitor to labeled RNAs was 10 (lanes 1 and 4), 30 (lanes 2 and 5), or 100 (lanes 3 and 6). (B) Competition experiments for the commitment step. Competitor RNAs (either the full-length substrate [lanes 1 to 4] or the 5′ splice site deletion mutant [lanes 5 to 8]) were mixed with the labeled substrate before the U2 snRNA-depleted extract was added, and a chase experiment was performed (Fig. 2). The molar ratio of competitor to labeled substrate RNAs was 1 (lanes 1 and 5), 5 (lanes 2 and 6), 25 (lanes 3 and 7), or 125 (lanes 4 and 8). Lane C, no competitor added. (C) Same as panel B, except that the competitor RNA was the TACTAAC box deletion mutant and the molar ratios of competitor to labeled substrate RNA were different: 1 (lane 1), 3 (lane 2), 9 (lane 3), or 30 (lane 4). In other experiments, a molar ratio of 100 did not give rise to a significant inhibition of complex formation (within a factor of 2, which is the experimental error of such an assay). In all panels, the complete gels, from wells to bottom, are shown (w, well; s, spliceosome complexes; u, unspecific complexes). When large competitor RNAs of low specific activity (see Materials and Methods) were added in increasing amounts, new unspecific complexes formed: panel A, lanes 4 to 6; panel B, lanes 5 to 8; panel C, lanes 1 to 4.

which we might detect commitment of the pre-mRNA in the absence of active U2 snRNP. This chase protocol does in fact define a commitment step (Fig. 3) that is resistant to the addition of a large excess of cold RNA and that requires little, if any, ATP (Fig. 4 and 5). Competition experiments with substrates deleted for either consensus sequence showed that both the 5′ splice site sequence and the TAC TAAC box are required to interact in a stable fashion with the factor(s) that commits a substrate to the spliceosome pathway (Fig. 6). U2 snRNA base pairing with the TAC TAAC box (at least the interaction that has been previously described) cannot be involved in this recognition process, since U2 snRNA has been cleaved at the key pairing location. U1 snRNA base pairing with the 5′ splice site, on the other hand, may be required to form this stable, committed complex. To test this possibility, we have carried out similar chase experiments with U1 snRNA-depleted extracts (cleavage at the 5′ end of U1 snRNA). However, U1 snRNA-complementary oligonucleotide must be used at higher concentrations (10 times greater than that needed to inactivate U2 snRNA) to cleave a substantial fraction of the U1 snRNA (data not shown). At these concentrations, there are substantial inhibitory effects on spliceosome assembly when such a U1 snRNA-depleted extract is mixed with a fully active extract. Therefore we cannot use these means to address the question of U1 snRNA involvement in the stable committed complex. We are aware that this assay for commitment to the spliceosome pathway is quite indirect. The many attempts to visualize directly the committed complex, including the use of various splicing substrates and of various native gel electrophoresis parameters, were unsuccessful. Since splicing reactions must be stopped in high-salt concentrations (above 150 mM potassium; P. Legrain, unpublished observations) to be analyzed by native gel electrophoresis, the committed complex might be very sensitive to high salt concentrations.

We are also aware that even in the absence of base pairing with the pre-mRNA, U2 snRNP may play a role in this commitment step through a direct interaction of the snRNP proteins with the pre-mRNA. If so, our data indicate that this interaction is likely to be ATP independent and that the cleaved U2 snRNP exchanges with an intact snRNP during the second incubation, leading to spliceosome formation. Also, the U2 snRNP exchange must not leave the substrate uncommitted; otherwise, it would be diluted by the excess of cold RNA. Given these considerations, we favor the hypothesis that in *S. cerevisiae* the factors responsible for initial intron recognition and stable complex formation act prior to U2 snRNP binding. The inability to titrate a factor which might bind independently to one of the two consensus sequences (Fig. 6) suggests either that there is only one key factor with two binding sites or that two or more factors act cooperatively.

At a minimum, the commitment assay described in this report should constitute a unique tool for the identification and purification of important and novel factors for in vitro spliceosome assembly. Further biochemical characterization of the commitment step, as well as the evaluation of its

relevance to in vivo spliceosome assembly, is now under way.

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LITERATURE CITED

1. **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.
2. **Berget, S. M., and B. L. Robberson.** 1986. U1, U2 and U4/U6 small nuclear ribonucleoproteins are required for in vitro splicing but not polyadenylation. Cell **46**:691–696.
3. **Bindereif, A., and M. R. Green.** 1987. An ordered pathway of snRNP binding during mammalian pre-mRNA splicing complex assembly. EMBO J. **6**:2415–2424.
4. **Black, D. L., B. Chabot, and J. A. Steitz.** 1985. U2 as well as U1 small nuclear ribonucleoproteins are involved in premessenger RNA splicing. Cell **42**:737–750.
5. **Brody, E., and J. Abelson.** 1985. The "spliceosome": yeast pre-messenger RNA associates with a 40S complex in a splicing-dependent reaction. Science **228**:963–967.
6. **Cheng, S. C., and J. Abelson.** 1987. Spliceosome assembly in yeast. Genes Dev. **1**:1014–1027.
7. **Frendewey, D., and W. Keller.** 1985. Stepwise assembly of a pre-mRNA splicing complex requires U-snRNPs and specific intron sequences. Cell **42**:355–367.
8. **Frendewey, D., A. Kramer, and W. Keller.** 1987. Different small nuclear ribonucleoprotein particles are involved in different steps of splicing complex formation. Cold Spring Harbor Symp. Quant. Biol. **52**:287–298.
9. **Grabowski, P. J., S. R. Seiler, and P. A. Sharp.** 1985. A multicomponent complex is involved in the splicing of messenger RNA precursors. Cell **42**:345–353.
10. **Grabowski, P. J., and P. A. Sharp.** 1986. Affinity chromatography of splicing complexes: U2, U5, and U4+U6 small nuclear ribonucleoprotein particles in the spliceosome. Science **233**: 1294–1299.
11. **Konarska, M. M., and P. A. Sharp.** 1986. Electrophoretic separation of complexes involved in the splicing of precursors to mRNAs. Cell **46**:845–855.
12. **Konarska, M. M., and P. A. Sharp.** 1987. Interactions between small nuclear ribonucleoprotein particles in formation of spliceosomes. Cell **49**:763–774.
13. **Krainer, A. R., and T. Maniatis.** 1985. Multiple factors including the small nuclear ribonucleoproteins U1 and U2 are necessary for pre-mRNA splicing in vitro. Cell **42**:725–736.
14. **Kramer, A., W. Keller, B. Appel, and R. Luhrmann.** 1984. The 5′ terminus of the RNA moiety of U1 small nuclear ribonucleoprotein particles is required for the splicing of messenger RNA precursors. Cell **38**:299–307.
15. **Kretzner, L., B. C. Rymond, and M. Rosbash.** 1987. S. cerevisiae U1 RNA is large and has limited primary sequence homology to metazoan U1 snRNA. Cell **50**:593–602.
16. **Langford, C. J., and D. Gallwitz.** 1983. Evidence for an intron-contained sequence required for the splicing of yeast RNA polymerase II transcripts. Cell **33**:519–527.
17. **Lin, R.-J., A. J. Newman, S.-C. Cheng, and J. Abelson.** 1985. Yeast mRNA splicing *in vitro*. J. Biol. Chem. **260**:14780–14792.
18. **Maniatis, T., and R. Reed.** 1987. The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing. Nature (London) **325**:673–678.
19. **Mount, S. M.** 1982. A catalogue of splice junction sequences. Nucleic Acids Res. **10**:459–472.
20. **Newman, A. J., R.-J. Lin, S.-C. Cheng, and J. Abselson.** 1985. Molecular consequences of specific intron mutations on yeast mRNA splicing in vivo and in vitro. Cell **42**:335–344.
21. **Parker, R., P. G. Siliciano, and C. Guthrie.** 1987. Recognition of the TACTAAC box during mRNA splicing in yeast involves base pairing to the U2-like snRNA. Cell **49**:229–239.
22. **Pikielny, C. W., and M. Rosbash.** 1986. Specific small nuclear RNAs are associated with yeast spliceosomes. Cell **45**:869–877.
23. **Pikielny, C. W., B. C. Rymond, and M. Rosbash.** 1986. Electrophoresis of ribonucleoproteins reveals an ordered assembly pathway of yeast splicing complexes. Nature (London) **324**:341–345.
24. **Pikielny, C. W., J. L. Teem, and M. Rosbash.** 1983. Evidence for the biochemical role of an internal sequence in yeast nuclear mRNA introns: implications for U1 RNA and metazoan mRNA splicing. Cell **34**:395–403.
25. **Ruskin, B., P. D. Zamore, and M. R. Green.** 1988. A factor, U2AF, is required for U2 snRNP binding and splicing complex assembly. Cell **52**:207–219.
26. **Rymond, B. C., and M. Rosbash.** 1985. Cleavage of 5′ splice site and lariat formation are independent of 3′ splice site in yeast mRNA splicing. Nature (London) **317**:735–737.
27. **Rymond, B. C., and M. Rosbash.** 1986. Differential nuclease sensitivity identifies tight contacts between yeast pre-mRNA and spliceosomes. EMBO J. **5**:3517–3523.
28. **Rymond, B. C., D. D. Torrey, and M. Rosbash.** 1987. A novel role for the 3′ region of introns in pre-mRNA splicing of *Saccharomyces cerevisiae*. Genes Dev. **1**:238–246.
29. **Zhuang, Y., and A. M. Weiner.** 1986. A compensatory base change in U1 snRNA suppresses a 5′ splice site mutation. Cell **46**:827–835.
30. **Zillmann, M., S. D. Rose, and S. M. Berget.** 1987. U1 small nuclear ribonucleoproteins are required early during spliceosome assembly. Mol. Cell. Biol. **7**:2877–2883.
31. **Zillmann, M., M. L. Zapp, and S. M. Berget.** 1988. Gel-electrophoretic isolation of splicing complexes containing U1 snRNPs. Mol. Cell. Biol. **8**:814–821.