The "Spliceosome": Yeast Pre-Messenger RNA Associates with a 40*S* Complex in a Splicing-Dependent Reaction

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Abstract. *The in vitro splicing reactions of pre-messenger RNA (pre-mRNA) in a yeast extract were analyzed by glycerol gradient centrifugation. Labeled pre-mRNA appears in a 40*S *peak only if the pre-mRNA undergoes the first of the two partial splicing reactions. RNA analysis after extraction of glycerol gradient fractions shows that lariat-form intermediates, molecules that occur only in mRNA splicing, are found almost exclusively in this 40*S *complex. Another reaction intermediate, cut 5′ exon RNA, can also be found concentrated in this complex. The complex is stable even in 400 m*M *KCl, although at this salt concentration, it sediments at 35*S *and is clearly distinguishable from 40*S *ribosomal subunits. This complex, termed a "spliceosome," is thought to contain components necessary for mRNA splicing; its existence can explain how separated exons on pre-mRNA are brought into contact.*

The process of pre-messenger RNA (pre-mRNA) splicing is central to the understanding of eukaryotic gene expression. Pre-mRNA contains sequences that code for protein (exons), but this information is often interrupted by one or more sequences (introns), which must be removed to allow formation of a contiguous messenger RNA (mRNA) molecule. Splicing consists of removing intron RNA and rejoining appropriate exon RNA's into such a contiguous mRNA. This cutting and rejoining poses a number of problems that are not yet resolved. Introns can be very large, yet the two exonic RNA sequences must be brought into proximity in order to be spliced. Also, the splicing must be precise (and rapid). A 5′ exon RNA molecule (exon 1 RNA), once cut, must join with the appropriate 3′ exon (exon 2 RNA) and with no other. Clearly, binding pre-mRNA into a supramolecular complex and holding cut exon RNA intermediates in such a complex would be one way to facilitate accurate splicing.

The splicing of pre-mRNA in *Saccharomyces cerevisiae*, as in HeLa cell extracts, takes place via two apparently concerted reactions (*1*, *2*). In a first step, pre-mRNA is cut at the junction of exon 1 and the intron with simultaneous formation of a lariat RNA form containing the intron (IVS) and exon 2 (IVS–exon 2; we have previously referred to this as the "2/3 molecule"). The lariat in this reaction product is the consequence of a 2′,5′-phosphodiester bond formed between the 2′ OH group of the last A in the conserved UACUAAC (TACTAAC box) sequence and the pG of the 5′ end of the intron (Fig. 1) (A, adenine; C, cytosine; T, thymine; G, guanine, U, uracil). In a second step, IVS–exon 2 is cut at the intron–exon 2 junction, and exon 1 and exon 2 are ligated to give mRNA. This endonucleolytic cut releases intron RNA in a lariat form (Fig. 1). In whole cell extracts of yeast, these reactions can be carried out with fidelity (*3*). We have examined the fate of wild-type and mutant pre-mRNA during these splicing reactions by glycerol gradient sedimentation of the reaction mixtures and RNA analysis of each fraction.

Splicing of CYH pre-mRNA. Pre-mRNA precursors were synthesized after insertion of the intron and small portions of each exon from the CYH-2 gene of *S. cerevisiae* (*4*) into a phage SP6 transcription unit on a plasmid (*5*) (Fig. 2). The CYH-2 gene codes for the yeast ribosomal protein L29 (*4*). In a first series of experiments the pre-mRNA contained either (i) the wild-type CYH intron sequence, (ii) an intron in which the conserved TACTAAC box had been mutated to TACTACC, that is, an A → C mutation at the A, which is the base of the branch point in wild-type lariat RNA, or (iii) an intron sequence in which the conserved 5′ splice site sequence GTATGT had been mutated to ATATGT, that is, a G → A mutation at the 5′ end of the intron (*3*). ^{32}P-Labeled SP6-CYH pre-mRNA transcripts were gel purified, then used as substrates for in vitro splicing in a yeast whole cell extract (*3*). As Newman *et al.* have already shown (*3*), the wild-type intron-containing substrate undergoes both of the reactions outlined in Fig. 1. The A → C mutation at the branch point renders this transcript incapable of undergoing either reaction. The G → A mutation at the 5′ end of the intron yields an RNA that can be used as a substrate in reaction 1, but is inactive in reaction 2 of the splicing pathway.

Wild-type pre-mRNA was incubated with yeast extract for 5 minutes at 28°C; the reaction mixture was cooled on ice and immediately centrifuged through a 15 to 40 percent glycerol gradient. Two peaks that sediment faster than bare pre-mRNA are seen (Fig. 3; the ordinate of the plot is on a logarithmic scale). One of these sediments indistinguishably from yeast 40*S* ribosomal subunits; the other is a broad peak that sediments between 15 and 30*S*. About 70 percent of the input radioactivity stays near the top of the gradient. This represents degraded RNA, since, as we shall show below, it is not precipitated by ethanol. When we incubate yeast extract with pre-mRNA prepared from the A → C mutant, sedimentation analysis (Fig. 3) shows that the 40*S* peak is missing, although the 15 to 30*S* peak and the degraded RNA appear to be identical to those seen with wild-type RNA. We have incubated wild-type RNA with yeast extract in reactions in which adenosine 5′-triphosphate (ATP) was replaced with the nonhydrolyzable analog, β,γ-methylene adenosine 5′-triphosphate (AMPPCP). In the presence of this analog, both splicing reactions are inhibited (*3*, *6*). Glycerol gradient analysis of this reaction again shows the disappearance of the 40*S* peak. About twice as much pre-mRNA is found in the 15 to 30*S* peak as was found when ATP was used in the reaction. Pre-mRNA is found associated with the 40*S* complex after incubation with yeast extract only when splicing of the pre-mRNA takes place.

We next compared in vitro splicing of

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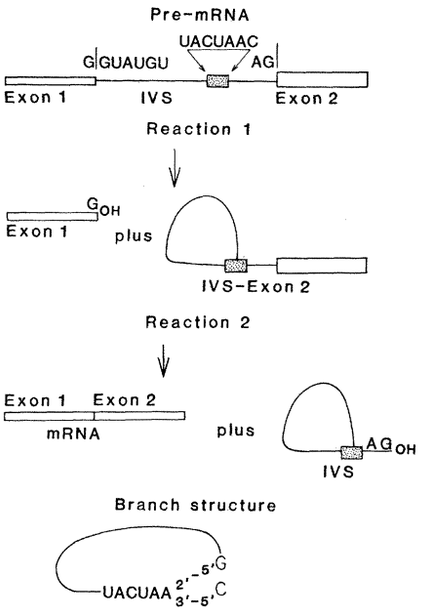
wild-type RNA and RNA from the G → A 5′ splice site mutant. Wild-type RNA was again incubated for 5 minutes at 28°C; the mutant RNA was incubated for 10 minutes at this temperature. In Fig. 4a, we show duplicate samples of each type of RNA sedimented through a glycerol gradient after in vitro splicing. The 40*S* peak is clearly visible with wild-type RNA; a smaller, but reproducible 40*S* peak is seen with RNA from the 5′ splice site mutant. Each fraction from these glycerol gradients was then extracted with a mixture of phenol and chloroform and precipitated with ethanol (Fig. 4b); 100 percent of the ^{32}P in the 40*S* fraction was recovered, as was 70 to 80 percent of the ^{32}P in the 15 to 30*S*



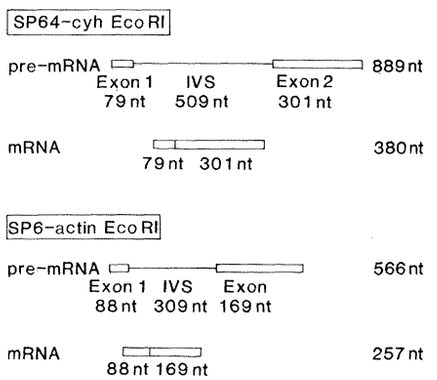


Fig. 1 (left). The splicing pathway of pre-mRNA. The reactions are described in the text. The intron-exon junctions are represented by vertical bars. This pathway of two apparently concerted reactions is found in *S. cerevisiae*, both in vivo and in vitro (*1*, *3*) as well as in extracts prepared from HeLa cells (*2*). Fig. 2 (right). SP64 CYH. A Hinf I–Eco RI fragment from the CYH gene (with the Hinf I site filled in) was first cloned into Sma I–Eco RI cut phage mp11. This 848-bp fragment contains 38 bp of exon 1, the 509-bp intron, and 301 bp of exon 2. This mp11 recombinant DNA was then cut with Hind III and Eco RI and cloned into Hind III–Eco RI cut pSP64. This yields the recombinant that has 41 additional base pairs at the beginning of exon 1 derived from the 6-bp SP6 leader and the 35-bp mp11 polylinker. The diagram shows the 889-nucleotide transcript that results when SP64-CYH DNA cut by Eco RI is transcribed by SP6 RNA polymerase. The transcription reaction contained 40 m*M* tris (*p*H 7.5; 5 m*M* $MgCl_2$; 2 m*M* spermidine; 20 m*M* NaCl; 10 m*M* dithiothreitol (DTT), RNasin in ribonuclease inhibitor (1 unit/μl) (Promega Biotec); 500 μ*M* ATP, GTP, and CTP; 25 μ*M* [α-^{32}P]UTP at 200 μCi/μmol; Eco RI cut SP64-CYH DNA at 200 μg/ml; SP6 RNA polymerase (Promega Biotec) at 7 units per 10 μl of reaction mixture. The total volume was 10 to 20 μl. The reaction time was 3 hours at 37°C. The reaction was stopped with proteinase K at 250 μg/ml, 25 m*M* EDTA, and 0.25 percent Sarkosyl, and then extracted twice with a mixture of phenol and chloroform; it was precipitated with ethanol, washed, dried, and analyzed on a polyacrylamide sequencing gel. Full-length transcripts were cut out of the gel and eluted from the gel slice with 50 m*M* sodium acetate, *p*H 5.2, 1 m*M* EDTA, 25 μg of *E. coli* RNA per milliliter, 0.1 percent sodium dodecyl sulfate, and 10 percent phenol. Full-length transcripts were precipitated with ethanol, washed twice with 80 percent ethanol, dried and resuspended in H_2O. These were then used as ^{32}P-labeled pre-mRNA in an in vitro splicing reaction (*3*). SP6 actin. A 544-bp Alu I–Alu I fragment of the actin gene, containing 73 bp of exon 1, the 309-bp intron, and 162 bp of exon 2 (*1*, *8*) was ligated and cloned into the Sma I site of plasmid proteus 6, a pBR322 derivative with an SP6 promoter followed by a polylinker cloning site (*5*). There are 15 bp between the SP6 start site and exon 1 (*5*) and 7 bp between the Sma I site and the Eco RI site of pSP6 (*5*). The diagram shows the transcript that results when pSP6-actin is cut with Eco RI and transcribed with SP6 RNA polymerase. Details of the transcription reaction are as given above.

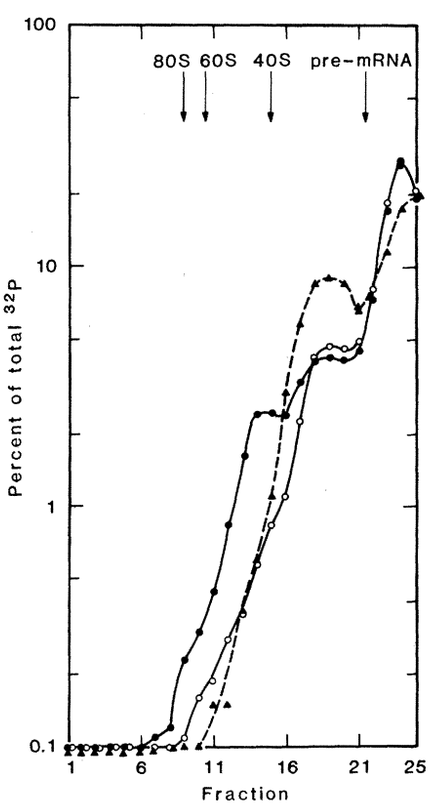


Fig. 3 (right). Glycerol gradient analysis of the splicing of CYH wild-type and mutant pre-mRNA. CYH pre-mRNA labeled with [^{32}P]UTP (uridine triphosphate) was prepared as given in the legend to Fig. 2. Either wild-type RNA (● and ▲) or A → C mutant RNA (○; see text) (about 5 × 10^5 cpm, counted as Cerenkov radiation) was incubated for 5 minutes at 28°C with yeast whole cell extract, which was prepared by the procedure of Newman and Cheng (*3*). Essentially, yeast cells were broken open by Zymolyase treatment and gentle homogenization, the homogenate was brought to 0.2*M* KCl, and the cell debris was removed by centrifugation at 30,000*g* for 30 minutes. The resulting supernatant was then centrifuged at 100,000*g* for 1 hour. The supernatant of this centrifugation was dialyzed against a buffer containing 20 m*M* Hepes buffer, *p*H 7.3, 0.5 m*M* DTT, 50 m*M* KCl, and 20 percent glycerol, and frozen in equal portions at −70°C. The in vitro splicing reaction contained 40 percent (by volume) yeast whole cell extract; 120 m*M* KCl, of which 20 m*M* is provided by the extract; 2 m*M* $MgCl_2$; 3 percent (weight to volume) PEG 8000; 1 m*M* ATP; and ^{32}P-labeled pre-mRNA. When the ATP dependence of the reaction was to be measured, we substituted 1 m*M* AMPPCP (β,γ-methylene adenosine 5′-triphosphate) for ATP. Each 30-μl reaction mixture was immediately put on ice at the end of the reaction, and layered over 12-ml (SW41) 15 to 40 percent glycerol gradients. The sterilized glycerol solutions usually contained 20 m*M* Hepes, *p*H 7.3, 100 m*M* KCl, 2 m*M* $MgCl_2$, and 0.2 m*M* DTT. The gradients were sedimented at 35,000 rpm for 12 hours in an SW41 rotor. The rotor temperature was 3°C. Separate gradients were used for analysis of yeast ribosomes and of wild-type ^{32}P-labeled CYH pre-mRNA (not incubated with extract). Fractions of each gradient were immediately incubated with proteinase K (250 μg/ml), 25 m*M* EDTA, and 0.25 percent Sarkosyl for 30 minutes at 37°C, and then extracted twice with an equal volume of the phenol-chloroform mixture. The ^{32}P distribution was measured (Cerenkov counting of fractions) and each fraction was precipitated with ethanol. In the experiments presented in Figs. 5 and 7, ethanol-precipitated samples were dried and resuspended in 3 μl of 95 percent formamide, after which they were subjected to electrophoresis on polyacrylamide, 8*M* urea sequencing gels. The peak fractions of 80*S* ribosomes, 60 and 40*S* ribosomal subunits, and pure CYH pre-mRNA are indicated by arrows. The normalized distribution of the radioactivity is plotted for each fraction. (●—●) Wild-type RNA incubated with ATP in the reaction mixture; (▲---▲) wild-type RNA incubated with AMPPCP; (○—○) A → C mutant RNA (see text) incubated with ATP.

peak. The bulk of the radioactivity at the top of the gradient (compare Figs. 3 and 4a with Fig. 4b) comes from degraded RNA, and it is not precipitated by ethanol.

RNA samples extracted from the glycerol gradient fractions were then fractionated by electrophoresis on 6 percent polyacrylamide, 8*M* urea gels (Fig. 5). The pre-mRNA [889 nucleotides (nt)] is found throughout the gradient, with a peak at 40*S*. RNA breakdown is evident, but less so in the rapidly sedimenting fractions, and more so in the slower sedimenting fractions of the glycerol gradient. In the profile of wild-type pre-mRNA splicing (Fig. 5a), the branched product of splicing reaction 1, intron–exon 2 (IVS–exon 2), and of splicing reaction 2, the intron in a lariat form (IVS), are both found almost exclusively in the fractions defining the 40*S* peak. Although RNA breakdown may have masked some of these branched intermediates in the 15 to 30*S* fractions, this could not be more than a minor fraction, since we can account for almost all the branched products of the total reaction in what we recover from the 40*S* peak (*7*). The other product of reaction 1, the cut 5′ exon (79 nucleotides), can be seen as well in Fig. 5a. It is broadly distributed in the gradient, from the top down to, and including, the 40*S* component. We suggest below that the cut exon 1 RNA, like the other product of reaction 1, the intron–exon 2 lariat RNA, was in the 40*S* complex, but dissociated from it during the centrifugation through glycerol. The other product of reaction 2, spliced mRNA, is not seen in these gradient fractions, although it is visible in the unfractionated reaction. It seems to be preferentially degraded during the course of our analyses.

The G → A 5′ splice site mutant RNA undergoes only reaction 1 of the splicing pathway (*3*). Glycerol gradient analysis (Fig. 4) shows a small 40*S* peak. All the

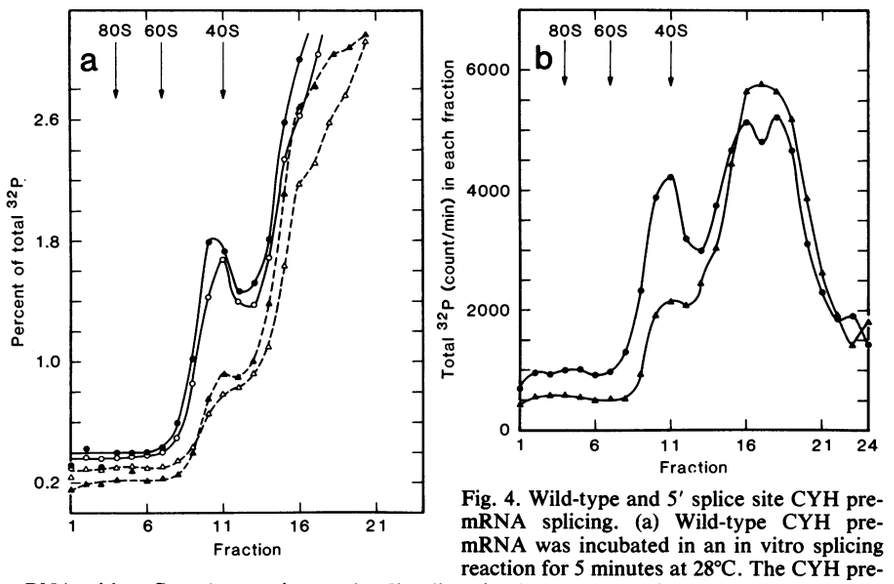


Fig. 4. Wild-type and 5′ splice site CYH pre-mRNA splicing. (a) Wild-type CYH pre-mRNA was incubated in an in vitro splicing reaction for 5 minutes at 28°C. The CYH pre-mRNA with a G → A mutation at the 5′ splice site (see text) was incubated in an in vitro splicing reaction for 10 minutes at 28°C. Analysis was carried out as described in the legend to Fig. 3. The scale of normalized counts per minute is now linear, so that the top few fractions are not represented in this figure. (● and ○) Duplicate samples of wild-type RNA; (▲ and △) duplicate samples of 5′ splice site mutant RNA. (b) The distribution of one wild-type RNA gradient (●) and one 5′ splice site mutant RNA (▲) are shown after ethanol precipitation and two washings of the precipitates with 80 percent ethanol.

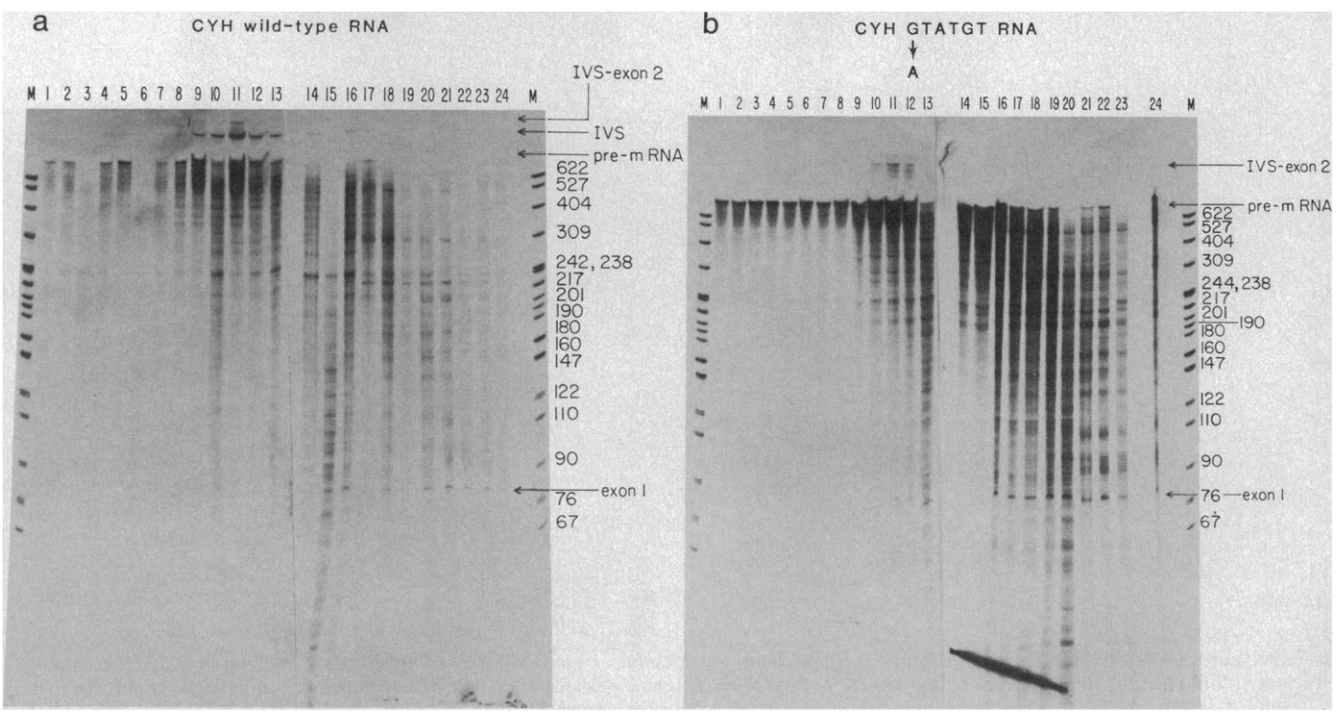


Fig. 5. (a) Fractions from the CYH wild-type pre-mRNA splicing reaction presented in Fig. 4b were subjected to electrophoresis on 6 percent polyacrylamide, 8*M* urea gels. One gel comprises markers and fractions 1 to 13 and the other, markers and fractions 14 to 24. Pre-mRNA, exon 1 RNA, IVS–exon 2 lariat RNA and IVS lariat RNA have been identified by cutting out these bands and doing T1 and pancreatic ribonuclease digests (*1*, *3*). (b) Fractions from the gradient shown in Fig. 4b depicting the CYH G → A mutant RNA splicing reaction were run on polyacrylamide gels as discussed in (a). The markers (lanes M) are ^{32}P-labeled fragments of Hpa II cut pBR322 DNA.

intron–exon 2 lariat RNA (IVS–exon 2) formed during the reaction is associated with the 40*S* complex (Fig. 5b). The cut exon 1 RNA product of reaction 1 is again found distributed from the 40*S* complex upward to the top of the gradient. Not only does pre-mRNA enter into a 40*S* complex in a splicing-dependent manner, but we also find almost all the lariat form RNA products of the two splicing reactions in this complex.

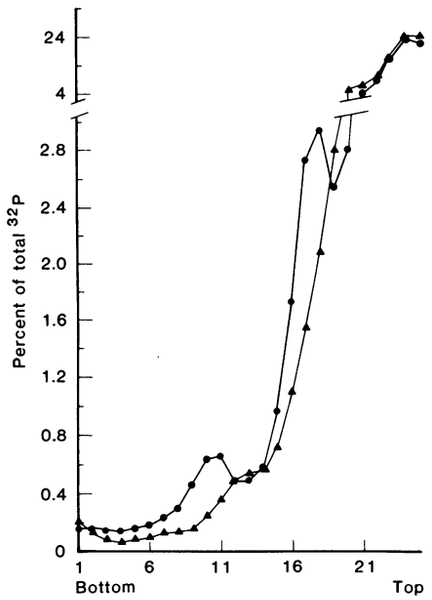


Fig. 6. Actin pre-mRNA splicing. ^{32}P-labeled actin pre-mRNA was incubated in an in vitro splicing reaction (legend to Fig. 3) for 10 minutes at 28°C. Equal portions of the reaction mixture were layered over 15 to 40 percent glycerol gradients, made up either with our standard buffer (containing 100 m*M* KCl; legend to Fig. 3) or with 400 m*M* KCl. Centrifugation and fractionation procedures were the same as for Fig. 3. (●—●) Glycerol gradient, 100 m*M* KCl, (▲--▲) glycerol gradient, 400 m*M* KCl. Separate gradients of ribosomal subunits at each salt concentration are not shown. In both cases 40*S* subunits sedimented identically with peaks at fraction 11.

Splicing of actin pre-mRNA. The splicing-dependent formation of a 40*S* complex is not limited to pre-mRNA from genes coding for ribosomal proteins. The glycerol gradient fractionation of an in vitro splicing reaction with an actin pre-mRNA is shown in Fig. 6 (*1*, *8*). The 309-base-pair (bp) actin intron and small pieces of the surrounding exons were cloned into a phage SP6 transcription unit on a plasmid. Transcription of linearized plasmid DNA by SP6 RNA polymerase again provided the labeled actin pre-mRNA. The 40*S* peak and 15 to 30*S* peak seen previously are again evident. Incubation with AMPPCP replacing ATP again inhibits 40*S* complex formation and increases the amount of material in the 15 to 30*S* fraction (*7*). An equal portion of the reaction mixture was sedimented through a glycerol gradient containing 400 m*M* KCl instead of our standard 100 m*M* KCl (Fig. 6). Although 40*S* ribosomal subunits sediment identically in the two types of gradients (*7*), the splicing-dependent 40*S* complex disappears at the higher salt concentration. Instead, we find a 35*S* shoulder of material at the higher salt concentration; the 15 to 30*S* material is replaced by a peak around 10 to 20*S*. By phenol-chloroform extraction and ethanol precipitation of

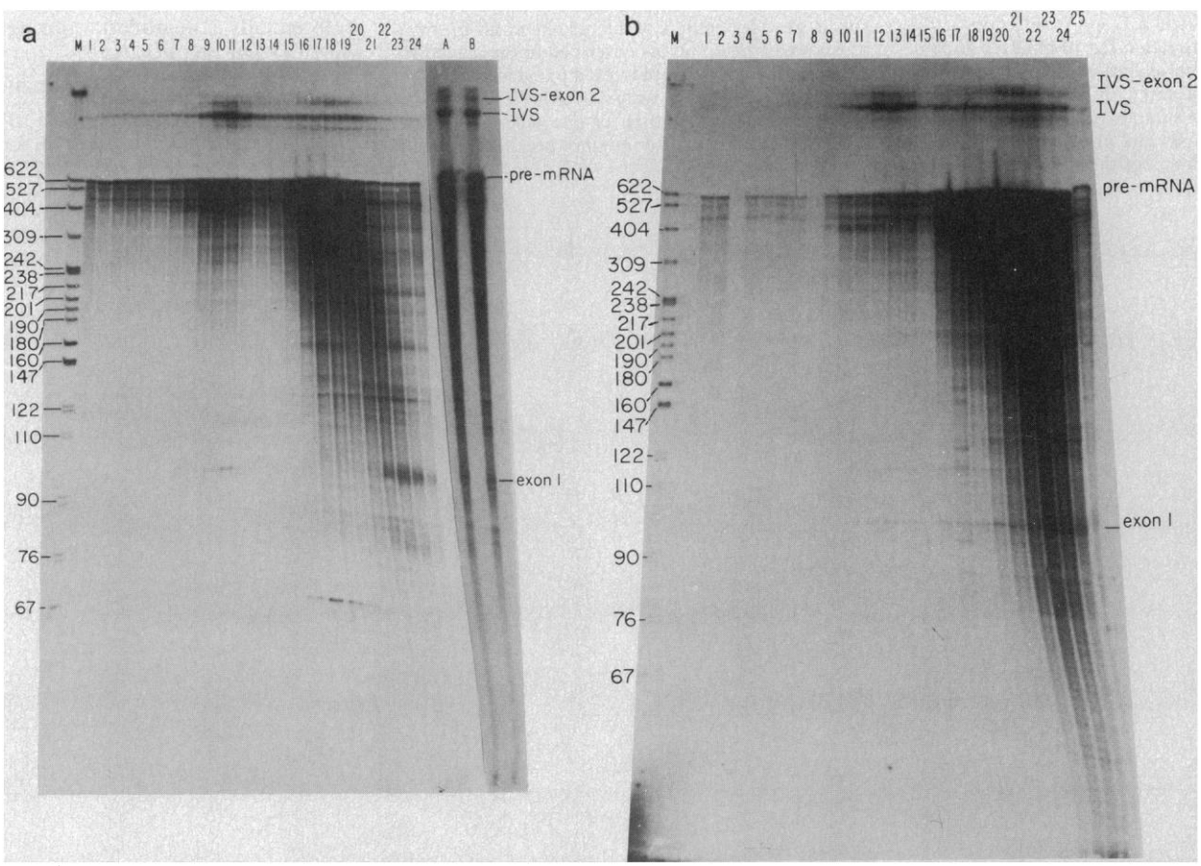


Fig. 7. Polyacrylamide gels of actin pre-mRNA splicing products. (a) Fractions from the 100 m*M* KCl glycerol gradient shown in Fig. 6 were extracted with the phenol-chloroform mixture, precipitated with ethanol, and washed, dried, and resuspended in 3 μl of 95 percent formamide (see legend to Fig. 3). These samples were analyzed on an 8 percent polyacrylamide, 8*M* urea sequencing gel. Markers were ^{32}P-labeled DNA from Hpa II cut pBR322. Fractions were marked above the corresponding lane. Fraction 25 of this gradient was lost and is not on this polyacrylamide gel. Pre-mRNA, IVS–exon 2 lariat RNA, IVS lariat RNA, and exon 1 RNA were all previously identified by cutting out bands and analyzing the T1 and pancreatic ribonuclease digestion products. On this 8 percent polyacrylamide gel the linear pre-mRNA (566 nucleotides) and linear 5′ exon RNA (88 nucleotides) run 9.8 percent more slowly than the corresponding DNA markers. (b) This is the polyacrylamide gel analysis (see above) of the fractions from the 400 m*M* KCl glycerol gradient shown in Fig. 6.

gradient fractions, we eliminate degraded RNA from the gradient profile, and find that the high salt 35*S* shoulder is, in fact, a distinct 35*S* peak. Polyacrylamide gel analyses of all fractions from both gradients are shown in Fig. 7. At 100 m*M* KCl, the actin lariat RNA forms are primarily (67 percent of total recovered from gradient) in the 40*S* peak, although some excised intron and intron–exon 2 RNA are associated with the 15 to 30*S* sedimenting material (14 percent). The cut actin 5′ exon RNA (88 nucleotides) is clearly cosedimenting with the 40*S* complex (Fig. 7a). This association of both products of reaction 1 with the 40*S* complex leads us to believe that in the CYH pre-mRNA splicing reaction as well, both products are associated with the 40*S* complex, but that during the centrifugation, a subcomponent of the complex containing exon 1 RNA gradually dissociates. Lane A represents an aliquot of the same in vitro splicing reaction that was run on this gradient, but had been immediately incubated with proteinase K at 250 μg/ml, 25 m*M* EDTA, and 0.25 percent Sarkosyl for 30 minutes at 37°C, and then put through the standard protocol. Lane B represents an identical aliquot immediately removed after the 10-minute in vitro splicing reaction, but diluted into ten volumes of the 15 percent glycerol solution used to make up the gradient shown in Fig. 6 (100 m*M* KCl). This mixture was kept at 3°C until the gradients had all been collected and put into the "stop" solution given above. Aliquot B was then subjected to the standard protocol. Comparison of lanes A, B, and the gradient fractions demonstrates that no breakdown occurs between the end of the 10-minute reaction and the work-up of gradient fractions. We have cut out and counted the radioactivity in the IVS lariat RNA in each lane; we find that lanes A and B have identical amounts, and that the sum of the gradient fractions accounts for 46 percent of the lariat IVS present at the end of the 10-minute reaction.

In Fig. 7b, we show the RNA analysis of the fractions from the 400 m*M* KCl glycerol gradient. A number of striking results are seen. First of all, the 35*S* peak contains both lariat forms of RNA, and cut 5′ exon RNA, although the fraction of these forms in the complex is lower (37 percent of total IVS recovered from gradient) than the fraction found in the 40*S* complex at 100 m*M* KCl. The fact that splicing-associated intermediates are retained in the 35*S* complex at 400 m*M* KCl suggests that the 40*S* complex is a stable and physiologically significant particle containing the splicing machinery. The high salt gradient analysis also suggests that the 40*S* splicing complex is not derived from a 40*S* ribosomal subunit, since ribosomal subunits still sediment at 60*S* and 40*S* in 400 m*M* KCl. About 35 percent of the lariat IVS RNA is recovered from the gradient found in the 10 to 20*S* peak at 400 m*M* KCl, presumably by dissociation from the 40*S* complex. The sum of the lariat IVS RNA in these fractions represents 62 percent of the amount present at the end of the 10-minute reaction.

The "spliceosome." Pre-mRNA is found in a 40*S* complex when that pre-mRNA undergoes at least the first of the two reactions of yeast in vitro splicing. Inhibiting the first splicing reaction, either by mutation of one base, A → C, in the pre-mRNA, or by substituting AMPPCP for ATP, greatly diminishes the amount of pre-mRNA sedimenting at 40*S*. When splicing of pre-mRNA occurs, the lariat form RNA products of reactions 1 and 2 (Fig. 1) are associated almost entirely with this complex. We have argued that a subcomponent containing the cut exon 1 RNA is also in this complex, but bound less tightly than the lariat forms of RNA. Pre-mRNA also associates with material sedimenting at 15 to 30*S*, but this is a splicing-independent association. It is possible that association with the 15 to 30*S* material is a prerequisite for formation of the 40*S* complex, but we have no direct evidence for this. The complex is stable enough to be only slightly modified at 400 m*M* KCl, at which salt concentration the splicing intermediates are concentrated in a 35*S* instead of a 40*S* complex, and we think that this stability reflects the importance of this structure. We propose that this particle be called a "spliceosome." The spliceosome would be composed of those components necessary for folding the pre-mRNA into the correct conformation for splicing and for holding the products of reaction 1 in place so that reaction 2 can take place in a rapid and precise manner. It would ensure that cut exon 1 RNA is always in place, ready to be joined to the appropriate exon 2 as soon as the second partial reaction takes place.

It is interesting that we find one of the products of reaction 2, lariat IVS RNA, in the 40*S* complex. The other product, mRNA, is not found, and is presumed to be released as soon as it is formed. Why should lariat IVS RNA not be released as quickly? This is perhaps related to the finding of discrete unbranched intron RNA molecules in vivo (*1*), and to an enzyme in HeLa cells which specifically debranches the 2′,5′ bond of lariat RNA molecules (*9*). We suggest that the lariat IVS RNA participates in holding some "spliceosome" components together, and that debranching these lariat RNA molecules functions as a signal to recycle the spliceosome components for a new round of pre-mRNA splicing.

In mammalian splicing systems, U1 small nuclear ribonucleoprotein particles participate in pre-mRNA splicing (*10*), and U2 small nuclear ribonucleoprotein particles may participate in pre-mRNA splicing (*11*). In yeast, small nuclear RNA molecules exist, but their participation in splicing has not yet been demonstrated (*12*). The purification of the spliceosome could identify which, if any, of these yeast small nuclear RNA molecules participate in pre-mRNA splicing. It will also lead to the identification of other components of the splicing apparatus. A fundamental question not addressed by our experiments is whether or not the "spliceosome" is pre-formed in yeast cells. Because small nuclear ribonucleoprotein particles are known components of the mammalian pre-mRNA splicing systems (*10*), we argue by analogy that components of the yeast pre-mRNA splicing machinery are assembled into a large complex by interaction with the conserved RNA sequences in yeast introns.

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