Splicing of yeast nuclear pre-mRNA in vitro requires a functional 40S spliceosome and several extrinsic factors

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We have previously shown that extracts prepared from most of the yeast temperature-sensitive *rna* mutants are heat sensitive for pre-mRNA splicing in vitro, and that the products of the corresponding RNA genes are essential for the early stages of the splicing region. In this report, we demonstrate that most heat-inactivated mutant extracts do not form the spliceosome, suggesting that their gene products are likely to be involved in spliceosome formation. Heat-inactivated *rna2* extracts, on the other hand, do form a splicing-dependent 40S complex containing uncleaved pre-mRNA exclusively. The pre-mRNA in the 40S complex can be converted to the splicing products in the presence of ATP and complementing extracts. These results demonstrate that: (1) the 40S complex formed in heat-inactivated *rna2* extracts is a spliceosome (termed the *rna2Δ* spliceosome), (2) the spliceosome is a functional intermediate in the splicing pathway, and (3) the splicing process can be dissected into two steps, spliceosome formation and cleavage-ligation reactions. Additional results indicate that at least two extrinsic factors, as well as the *RNA2* gene product, are required for complementation of the *rna2Δ* spliceosome. A three-step mechanism for nuclear pre-mRNA splicing in yeast is proposed.

[Key Words: RNA splicing; spliceosome; *rna* mutants; yeast]

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RNA splicing, the precise deletion of intervening sequences (introns) from precursor RNA, is a key step in the regulation of many genes in all eukaryotes as well as in some eubacteria and archaebacteria (Abelson 1979; Belfort et al. 1985; Daniels et al. 1985). Two basic questions have arisen concerning the mechanism of splicing: (1) how are the two splice sites recognized and brought into proximity (recognition-alignment) and (2) what are the chemical mechanisms for cleavage and ligation at the molecular level (cleavage-ligation)? The various groups of splicing systems appear to solve these problems through distinct pathways (Cech and Bass 1986).

In tRNA splicing, recognition-alignment is achieved by the secondary and tertiary structure of the exons, while cleavage-ligation is catalyzed by a pair of protein enzymes, tRNA endonuclease and ligase (Greer et al. 1983; Peebles et al. 1983). In the case of *Tetrahymena* rRNA and group I mitochondrial RNAs, both recognition-alignment and cleavage-ligation can be mediated exclusively by the intron and a guanosine cofactor in the absence of external macromolecules (Cech 1983; Garriga and Lambowitz 1984; Van der Horst and Tabak 1985).

Splicing of nuclear mRNA and group II mitochondrial RNAs follows a common two-step mechanism (Cech 1986). The first step involves cleavage at the 5' splice

site and formation of a branched RNA through a 2'-5' phosphodiester linkage (the 5' splice site reaction). The second step involves cleavage at the 3' splice site and ligation of two exons (the 3' splice site reaction). Although these two types of RNA have similar splicing intermediates, their splicing requirements are strikingly different. Splicing of the group II mitochondrial RNAs can proceed by itself in the absence of any cofactor (Peebles et al. 1986; Van der Veen et al. 1986), while splicing of the nuclear mRNA requires numerous external RNAs, proteins, and complex protein-RNA interactions (Padgett et al. 1986).

At least five small nuclear (sn) RNAs (U1, U2, U5, U4, and U6) are required for nuclear mRNA splicing in mammalian systems (Krämer et al. 1984; Black et al. 1985; Chabot et al. 1985; Krainer and Maniatis 1985; Berget and Roberson 1986; Black and Steitz 1986). These snRNAs are associated with proteins and most likely function in the form of ribonucleoprotein complexes (RNPs) (Hinterberger et al. 1983; Kinlaw et al. 1983). In addition, one of the protein constituents of the heterogeneous nuclear (hn) RNPs, the C protein (Choi et al. 1986), as well as a number of factors, presumably proteins or RNPs (Krainer and Maniatis 1985), has been shown to be involved in the mRNA splicing process. Although the function of many of these splicing components remains obscure, U-snRNPs have been proposed to be involved in recognition-alignment of the pre-mRNA (Lerner et al. 1980; Rogers and Wall 1980). In-

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deed, recent evidence has indicated that U1-snRNP interacts with the 5' splice site (Mount et al. 1983; Black et al. 1985; Zhuang and Weiner 1986), U2-snRNP with the branch site (Black et al. 1985), and the U5-snRNP probably with the 3' splice site (Chabot et al. 1985). In yeast, snRNAs are also components of the splicing machinery, although no snRNP complexes have yet been identified (Cheng and Abelson 1986; Pikielny and Rosbash 1986; Z. Hostomsky, S. Ruby, and J. Abelson, in prep.; N. Riedel and C. Guthrie, in prep.).

The pre-mRNA and the splicing intermediates produced from the 5' splice site reaction are associated with a 40–60S RNP particle called the spliceosome (Brody and Abelson 1985; Frendewey and Keller 1985; Grabowski et al. 1985). Such a large particle could have the complexity of a ribosomal subunit and may contain as many as 30 proteins and several RNA molecules. Indeed, many of the sn (Grabowski et al. 1985; Cheng and Abelson 1986; Grabowski and Sharp 1986; Konarska and Sharp 1986; Pikielny and Rosbash 1986; Z. Hostomsky, S. Ruby, and J. Abelson, in prep.; N. Riedel and C. Guthrie, in prep.) and hn (Choi et al. 1986) splicing components mentioned above are present in the spliceosome. Although the function of the spliceosome remains unclear, it may function in recognition-alignment of the reactive sequences present in the pre-mRNA as well as in holding two splicing intermediates together.

Several laboratories have been attempting to dissect the components of splicing systems through biochemical fractionation. Fractionation of the in vitro splicing extracts has successfully identified unique components responsible for different steps of the splicing pathway (Krainer and Maniatis 1985; Cheng and Abelson 1986; Perkins et al. 1986). However, difficulties in identifying individual splicing components by further fractionation and reconstitution assays (S.-C. Cheng and J. Abelson, unpubl.), once again, suggest the complexity of the splicing machinery.

To elucidate the mechanism of nuclear mRNA splicing, it is necessary to enumerate the components of the spliceosome and determine their functions. We have combined the biochemical and genetic analyses available in the yeast *Saccharomyces cerevisiae* to approach this problem. Mutants in 10 complementation groups (*rna2-11*) in yeast originally identified by Hartwell and co-workers (Hartwell 1967; Hartwell et al. 1970) have subsequently been found to be defective in mRNA splicing. Yeast cells carrying temperature-sensitive mutations in these genes accumulate intron-containing pre-mRNA at the nonpermissive temperature (Fried et al. 1981; Rosbash et al. 1981; Larkin and Woolford 1983). We have shown that whole-cell extracts prepared from most of the *rna* mutants are heat-sensitive for mRNA splicing activity in vitro (Lustig et al. 1986). In vitro complementation, tetrad analysis, and reversion analysis have demonstrated that heat inactivation of each of these *rna* mutant extracts results in the specific loss of a splicing component. The products of the corresponding RNA genes are required for the early stages of mRNA splicing, since none of these heat-inactivated mutant

extracts can carry out the first step of the splicing reaction (the 5' splice site reaction).

Here we report studies on spliceosome formation in extracts from those yeast *rna* mutants that exhibit heat sensitivity for mRNA splicing activity in vitro. We provide evidence that the yeast spliceosome can function as an intermediate in the splicing pathway and that additional components are required to complete the splicing process. We have also tentatively assigned the steps at which the RNA gene products are active in mRNA splicing. On the basis of these and other results, we propose a three-step model for nuclear mRNA splicing in yeast.

Results

Spliceosome formation analysis

It has been shown that a 40S complex (spliceosome), containing pre-mRNA and intermediates of the splicing reaction, will form in wild-type yeast extracts in the presence of a functional pre-mRNA and ATP (Brody and Abelson 1985; Vijayraghavan et al. 1986). To investigate the role of the RNA gene products in the mRNA splicing pathway, heat-sensitive extracts from the *rna* mutants were tested for their ability to form spliceosomes. Whole-cell extracts were prepared from *rna2*, *rna3*, *rna4*, *rna5*, *rna7*, *rna8*, and *rna11* mutants, all of which exhibit heat sensitivity for mRNA splicing in vitro (Lustig et al. 1986). The extracts were heat-inactivated at 30°C for 30–60 min under conditions in which *rna* mutant extracts are completely inactivated for splicing activity. The splicing activity can be partially restored in many cases by pairwise mixing of the heat-inactivated extracts from different *rna* mutants (data not shown). Thus, loss of splicing activity in *rna* mutant extracts is due, at least in part, to the specific heat-inactivation of an *rna* gene product.

These heat-inactivated *rna* mutant extracts were then tested for spliceosome formation. Extracts were incubated with ³²P-labeled pre-mRNA at 15°C for 20 min under splicing conditions, and the reaction mixtures were sedimented in glycerol gradients (Fig. 1). Complex formation was monitored by following the radioactivity in the RNA. Spliceosome formation in an active extract is readily detected by this assay.

Heat-inactivated rna2 extracts form a splicing-dependent 40S complex containing only uncleaved pre-mRNA

Extracts from a pair of isogenic strains carrying either an *rna2* mutation (SS304) or a reversion of that mutation (SS304r2) were tested for spliceosome formation (Fig. 1, B and A, respectively). A 40S peak at fraction 14 was detected in both active and heat-inactivated *rna2* mutant extracts (Fig. 1B). As in the wild-type, the formation of this 40S complex is ATP dependent. This complex sediments in nearly the same position as the wild-type spliceosome in control extracts (SS304r2, Fig. 1A). The slower sedimentation rate of the 40S complex (fraction

Figure 1. Glycerol gradient sedimentation analysis of spliceosome formation in *rna* mutant extracts. Extracts were incubated with ^{32}P -labeled pre-mRNA at 15°C for 20 min in a 10- μl reaction under appropriate conditions. The reaction mixture was sedimented in a 15–27% glycerol gradient for 100 min as described in Materials and methods. Fractions were collected and radioactivities were determined as Cerenkov counts per minute; 40S particles would appear in fractions 13–16. The strains used are: SS304r2 (*rna2* revertant) (A); SS304 (*rna2*) (B); RL173 (*rna5*) (C); and SPJ11.4 (*rna11*) (D). (Δ) Treatment of the extract under the heat inactivation conditions; ($\circ$) splicing reaction with the extract before inactivation and in the presence of ATP; ($\bullet$) after heat inactivation and in the presence of ATP; ($\square$) after heat inactivation and in the absence of ATP. Fractions 1–10 have radioactivities less than 100 cpm and are not plotted.

14 for SS304 versus fraction 15 for SS304r2) may be the result of slight differences in spliceosome structure (see Discussion). The reduced size of the 40S peak after heat treatment of both extracts is most likely due to some nonspecific loss of splicing components during the stringent heat treatment.

Since splicing activity is completely abolished in heat-inactivated *rna2* extracts, the 40S complex formed contains only uncleaved pre-mRNA (see below). To test

whether this complex is related to mRNA splicing, we used as substrate a mutant actin pre-mRNA which contains a G $\rightarrow$ C transversion at the first conserved nucleotide of the 5' splice site [the actin(Δ 6)C1 mutant; Vijayraghavan et al. 1986]. This actin(Δ 6)C1 mutation prevents both splicing activity and spliceosome formation in wild-type extracts [Vijayraghavan et al. 1986]. When the actin(Δ 6)C1 mutant and its wild-type counterpart, actin(Δ 6), pre-mRNAs were

incubated separately with the heat-inactivated *rna2* extract, the 40S complex is formed only with the wild-type pre-mRNA, but not with the C1 mutant pre-mRNA (data not shown). Therefore, the formation of this 40S complex is dependent on the presence of ATP and a functional pre-mRNA, as in the wild-type case. By these criteria and subsequent experiments (see below), we conclude that the 40S complex formed in the heat-inactivated *rna2* extract is a spliceosome. We have termed this complex the *rna2*Δ spliceosome to distinguish it from spliceosomes, formed in active splicing extracts, which bind pre-mRNA as well as lariat and exon 1 intermediates.

The fact that *rna2* extract is heat-sensitive for splicing but not spliceosome formation suggests that at least some activity of the *RNA2* gene product acts prior to the 5' splice site reaction but is dispensable for *rna2*Δ spliceosome formation.

Most RNA gene products are required for spliceosome formation

Active and heat-inactivated extracts from *rna3*, *rna4*, *rna5*, *rna7*, *rna8*, and *rna11* mutants were also tested for

their ability to carry out spliceosome formation. All of these mutant extracts were heat-sensitive for spliceosome formation, i.e., the spliceosome peaks at fraction 15 were detected before but not after heat-inactivation of extracts (*rna5* and *rna11* results are shown in Fig. 1, C and D, respectively, others not shown).

We considered that due to the inherent difficulties in dealing with crude extracts, some of the splicing components may be inactivated nonspecifically during the stringent heat-inactivation process. Therefore, we tested the effects of different heat-inactivation conditions on spliceosome formation. Extracts from *rna2*, *rna5*, and *rna11* mutants were heat-inactivated under conditions in which nonspecific inactivation of splicing components is reduced to a minimum. In this case, the splicing activity was complemented to nearly the original level by pairwise mixing of different mutant extracts. Nonetheless, under these conditions, spliceosome formation was only detected with the heat-inactivated *rna2*, but not *rna5* or *rna11* extract (data not shown). Therefore, we conclude that the gene products of *RNA5* and *RNA11*, and likely *RNA3*, *RNA4*, *RNA7*, and *RNA8*, are involved in spliceosome formation.

Figure 2. Chasing the pre-mRNA in the heat-inactivated (Δ) *rna2* extract gradient fractions with *rna11*Δ extract. (A) The gradient profile of a 50-μl preparative splicing reaction containing 25 μl of *rna2*Δ extract and 0.1 pmole of 32 P-labeled pre-mRNA. The reaction mixture was sedimented for 120 min and analyzed as described in the Materials and methods. A peak of the *rna2*Δ spliceosome was detected at fraction 12. Degraded RNA would remain at the top of the gradient. (B) The autoradiogram of a gel assay of the chasing reaction products. The gradient fractions of interest were thawed on ice and mixed with *rna11*Δ extracts (SPJ11.4) under splicing conditions described in Materials and methods. The *rna2*Δ spliceosome alone (cf. lanes 1 and 2, Fig. 3) or the *rna11*Δ extract alone (data not shown) displayed no splicing activity. The fraction numbers as in A are given on top of the autoradiogram. Arrows indicate the reaction products: intron-exon 2 lariat (IVS*·E2), intron lariat (IVS*), pre-mRNA, mRNA, and exon 1 (EI).

Pre-mRNA on the *rna2Δ* spliceosome can be converted to splicing products with complementing extracts

If the spliceosome is a true splicing intermediate, the pre-mRNA in the 40S complex formed in the heat-inactivated *rna2* extract (*rna2Δ* spliceosome) should be converted to splicing intermediates and/or products by addition of complementing extracts. To test this, a large-scale reaction with a heat-inactivated *rna2* extract was prepared under conditions in which no detectable splicing intermediates or products were formed. The reaction mixture was then sedimented through a glycerol gradient, the fractions were collected, and ³²P-labeled pre-mRNA was monitored. As before, an *rna2Δ* spliceosome peak was detected (Fig. 2A).

To test the splicing activity of the *rna2Δ* spliceosome, individual gradient fractions were complemented with heat-inactivated *rna11* extracts under splicing conditions. The heat-inactivated *rna11* extract by itself does not have any detectable splicing activity, but can complement heat-inactivated *rna2* whole-cell extracts (Lustig et al. 1986). The products of the complementation reactions were analyzed by polyacrylamide gel electrophoresis (Fig. 2B). Only pre-mRNA sedimenting in the 40S region can be chased into intermediates and products (lanes 2–5). The complementation of the *rna2Δ* spliceosome is ATP dependent (data not shown). In contrast, all pre-mRNA in the gradient remains a substrate for splicing, since pre-mRNA isolated from fractions of other parts of the gradient, when tested, can be converted to spliced RNA in the presence of ATP and fully active extracts (data not shown).

Complementation of the *rna2Δ* spliceosome could be due either to chasing of the pre-mRNA on a preformed spliceosome or to the dissociation of the complex and subsequent reformation on the pre-mRNA in the presence of the complementing extract. To distinguish between these two possibilities, a second functional pre-mRNA which gives rise to different-sized products [yeast actin(Δ 6) pre-mRNA; Vijayraghavan et al. 1986] was added to the reaction mixture in the complementation experiment. Only the original pre-mRNA present on the *rna2Δ* spliceosome could be spliced (data not shown). A similar result was obtained when the order of addition of these two pre-mRNAs was reversed. Thus, only the preformed complex can be chased into splicing intermediates and products, indicating that the *rna2Δ* spliceosome is a functional intermediate. The failure of the newly added pre-mRNA to splice is probably due to the low concentration of the components from the *rna2Δ* spliceosome in the reaction.

We have further defined the requirements for complementation of the *rna2Δ* spliceosome (Fig. 3). Neither products nor intermediates are formed in the absence of complementing extract (lane 2). As expected, the heat-inactivated *rna2* extract does not contain the complementing activity, while the extract from a revertant of *rna2*, subjected to the same heat treatment, can complement the *rna2Δ* spliceosome (lanes 4 and 3, respectively). These results indicate that the product of the *RNA2* gene is required to complement the complex. It

Figure 3. Complementation of the *rna2Δ* spliceosome with various heat-treated extracts. Fractions 11 and 12 in Fig. 2A were pooled and used as the source for *rna2Δ* spliceosome containing only uncleaved pre-mRNA. The chasing reactions were performed under splicing conditions as described in Materials and methods. The reaction products were analyzed by gel electrophoresis and subjected to autoradiography. (Lane 1) No complementing extract was added and was left on ice for 60 min (no incubation at 15°C); (lanes 2–8) incubation at 15°C for 60 min in the absence (lane 2) or presence (lanes 3–8) of complementing extracts. The extracts were from SS304r2 (*rna2* revertant), SS304 (*rna2*), RL144 (*rna4*), RL173 (*rna5*), AHrna7 (*rna7*), and SPJ11.4 (*rna11*). Δ indicates treatment of the extracts under heat-inactivation conditions. Heat-inactivated *rna* mutant extracts displayed no splicing activity by themselves (data not shown). Arrows indicate the reaction products as described in Fig. 2B.

must act prior to the 5' splice site reaction, as no splicing intermediates were detected when the *RNA2* activity is heat inactivated.

Heat-sensitive extracts from *rna4*, *rna5*, *rna7*, and *rna11* mutants were tested for their ability to complement the *rna2Δ* spliceosome. All these heat-inactivated extracts contain full complementing activities (Fig. 3, lanes 5–8). When heat-inactivated *rna3* and *rna8* extracts were analyzed in a similar fashion, they complemented the *rna2Δ* spliceosome as well (data not shown). The fact that heat-inactivated extracts from *rna3*, *rna4*, *rna5*, *rna7*, *rna8*, and *rna11* mutants can complement

the *rna2Δ* spliceosome suggests that either (1) the products of *RNA3*, *RNA4*, *RNA5*, *RNA7*, *RNA8*, and *RNA11* genes are present in the spliceosome, or (2) these gene products have already functioned to form the spliceosome and are not required in further steps. We have not been able to distinguish between these two possibilities.

In summary, the *rna2Δ* spliceosome is an active splicing intermediate, and ATP and at least the *RNA2* gene product are required to complete the splicing process on the complex.

A factor, in addition to the RNA2 gene product, is required for complementing the rna2Δ spliceosome

To ascertain whether factors in addition to the *RNA2* gene product are required for the complementation of the *rna2Δ* spliceosome, we took advantage of a yeast strain, RL92/pRN2-1(pJDB207), that overproduces the wild-type *RNA2* gene product 50- to 200-fold over the wild-type level (Last 1986). To test the splicing activity of the overproduced *RNA2* gene product in vitro, an ex-

tract from this overproducer strain (termed pR2 extract) was used to complement the splicing defect of heat-inactivated *rna2* whole-cell extracts. If the overproduced *RNA2* gene product is active in vitro, the pR2 extract, when compared to a wild-type extract, should complement heat-inactivated *rna2* extracts at a much higher dilution. As shown in Figure 4A, the pR2 extract complements strongly at a 1/400 dilution (lane 5), and weakly at a 1/4000 dilution (lane 4). In contrast, a control extract, from either a revertant of *rna2* (RL92r2, a strain isogenic to the overproducer strain) or a wild-type strain (EJ101), complements at a 1/40 dilution and very weakly at a 1/400 dilution (lanes 7–10). This titration experiment indicates that the pR2 extract contains about 50-fold more *rna2* complementing activity than does a wild-type extract. This result is consistent with the immunotitrations of *RNA2* gene product in this strain conducted by Last and Woolford (Last 1986). To demonstrate that the high complementing activity of the pR2 extract is due to the high titer of the *RNA2* gene product rather than a general increase in all splicing components, we measured the amount of the *RNA11* gene product in pR2

Figure 4. Titrating extract from a strain overproducing the *RNA2* gene product. Extracts were diluted in buffer D before being assayed for their ability to complement either heat-inactivated (Δ) *rna2* (SS304) or *rna11* (SPJ11.4) whole-cell extracts. The reactions were performed in mixtures containing 50% (vol/vol) *rna2Δ* or *rna11Δ* extracts, 25% (vol/vol) buffer D or diluted complementing extracts, and 32 P-labeled pre-mRNA under splicing conditions. *rna2* and *rna11* extracts without heat inactivation were also assayed as control (lane 1 in both panels). Autoradiograms of the gel assay of the reaction products from the *rna2Δ* series and *rna11Δ* series are shown in A and B, respectively. Final dilution factors are given on top of the autoradiogram. For example, 1/40 (lanes 6, 8, and 10 in A; lanes 4, 6, and 8 in B) indicates that 1 μ l of a 1:10 dilution of the complementary extract was assayed per 4 μ l of splicing reaction. pR2 indicates the extract prepared from the strain overproducing the *RNA2* gene product. Arrows indicate the reactions products as described in Fig. 2B.

Figure 5. Complementation of the *rna2Δ* spliceosome with diluted pR2 extracts in the presence of *rna2Δ* extracts. Extracts prepared from a strain overproducing the *RNA2* gene product [RL92/pRN2-1(pJDB207), designated as pR2 on the figure] or a wild-type strain (EJ101) were diluted in buffer D by factors 10, 100, or 500. One microliter of the diluted extract was used to complement the *rna2Δ* spliceosome (as described in Fig. 3) in an 8- μ l chasing reaction mixture supplemented with either 1 μ l of 20 mg/ml bovine serum albumin (BSA) solution or 1 μ l of the heat-inactivated (Δ) *rna2* (SS304) extract. Autoradiograms of the gel assay of the reaction products from the BSA series and *rna2Δ* series are shown in A and B, respectively. The final dilution factors are given on top of the autoradiograms. For example, 1/8 (lanes 1 and 4 in A; lanes 1 and 5 in B) indicates that 1 μ l of an undiluted complementing extract was assayed in an 8- μ l chasing reaction. Arrows indicate the reactions products as described in Fig. 2B.

extracts and two different control extracts (EJ101 and SS304). All three extracts complement heat-inactivated *rna11* extracts at a 1/40 dilution, but very weakly at a 1/400 dilution (Fig. 4B). Therefore, it is likely that the pR2 extract contains specifically high levels of *RNA2* gene product. These results are consistent with the notion that heat treatment of *rna2* whole-cell extracts specifically inactivates the mutant *rna2* gene product, and further suggest that inactivated *rna2* mutant extracts can be complemented by the wild-type *RNA2* gene product alone.

The property of the pR2 extract allowed us to determine whether factors in addition to the *RNA2* gene product are needed for the complementation of the *rna2Δ* spliceosome. If the *RNA2* gene product is the only extrinsic factor required for the 5' splice site reaction in the *rna2Δ* spliceosome, then the pR2 extract would have a higher titer for complementation of the complex than would a wild-type extract. This is, however, not the case. Both extracts failed to complement the isolated *rna2Δ* spliceosome at the same dilution (1/800) (Fig. 5A, lanes 3 and 6). Based on the titration experiment in whole-cell extracts described above, the pR2 extract would supply sufficient activity of the *RNA2* gene product at a 1/800 dilution (cf. Fig. 4A). Loss of complementation due to low concentration of protein can be ruled out, since bovine serum albumin was added to the reaction mixtures (Fig. 5A). If loss of complementation activity at a 1/800 dilution is due to limitation of some other factor, it should be supplied by addition of heat-inactivated *rna2* extracts to the complementation reaction. As shown in Figure 5B, addition of heat-inactivated *rna2* extracts does not rescue the EJ101 extract at a 1/800 dilution (lane 3). However, heat-inactivated *rna2* extracts can rescue the pR2 extract diluted up to 1/4000 (lane 8). These results indicate that, in the case of the EJ101 extract diluted 1/800 in the complementation reaction, the *RNA2* gene product is a limiting factor, while in the case of the pR2 extract at the same dilution, some factor other than the *RNA2* gene product becomes limiting. Therefore, we conclude that a factor b_n in addition to the *RNA2* gene product, is required for complementing the *rna2Δ* spliceosome, and is most likely to be extrinsic to the complex (an extrinsic factor).

Two factors are required for the 3' splice site reaction

The wild-type yeast extract has been separated into three fractions, I, II, and III (Cheng and Abelson 1986). Individual fractions have no splicing activity by themselves. The combination of fractions I and II allows the spliceosome to form and the 5' splice site reaction to occur, resulting in the accumulation of splicing intermediates. Addition of fraction III completes the splicing reaction indicating that a unique factor(s) (termed c_{III} , see below) present in fraction III is required for the 3' splice site reaction (Cheng and Abelson 1986; cf. Fig. 6A).

To characterize further the spliceosome and extrinsic splicing factors, these three fractions and fraction (II + III), which is the source of fractions II and III, were tested for their ability to complement the *rna2Δ* spliceosome (Fig. 6B). Fraction I actively complements the *rna2Δ* spliceosome (lane 1) for both the 5' and 3' splice site reactions. Fraction II complements the complex very weakly, if at all (lane 5). Three preparations of fraction (II + III) complement the *rna2Δ* spliceosome to different extents (lanes 2 to 4). Accumulation of the splicing intermediates, exon 1 and intron-exon 2, was observed in two out of three cases (lanes 2 and 4), suggesting fraction (II + III) complemented the 5' splice site reaction better than the 3' splice site reaction. More-

Figure 6. Complementation of the *rna2Δ* spliceosome with fractionated wild-type extracts. (A) A scheme representing the reconstitution of the splicing reaction from extract fractions I, II, and III. See text for details. (B) The autoradiogram of the reaction products from chasing the pre-mRNA on the *rna2Δ* spliceosome with either fraction I (lane 1), fraction (II + III) (lanes 2–4), fraction II (lane 5), or fraction III (lane 6). The chasing reaction was performed as described in Fig. 3. Fractions of the wild-type extract used to complement the *rna2Δ* spliceosome are indicated on top of the autoradiogram. Fraction (II + III), previously designated as 40W (Cheng and Abelson 1986), is the fraction obtained before further fractionation into fractions II and III. Three different preparations of fraction (II + III) were used (lanes 2, 3, and 4). Arrows indicate the reaction products as described in Fig. 2B. (The ratio of acrylamide to bis-acrylamide in lanes 3–6 is slightly higher than a typical 29:1, which resulted in the change of the relative mobility of the lariat molecules.)

over, the accumulation of splicing intermediates became even more obvious when fraction III was used to complement the complex (lane 6).

From the pattern of complementation of the fractions on the *rna2Δ* spliceosome and from previous results in the complementation pattern among fractions I, II, and III (Cheng and Abelson 1986; cf. Fig. 6A), we can deduce the existence of two factors involved in the 3' splice site reaction and their relation to the *rna2Δ* spliceosome. If a fraction complements the *rna2Δ* spliceosome, it suggests that all splicing components uniquely in other fractions are either present in the gradient fractions containing the complex, or are not involved in later steps of the splicing reaction.

First, the fact that fraction I can completely complement the *rna2Δ* spliceosome indicates that the c_{III} factor is present in the gradient fractions containing the *rna2Δ* spliceosome (cf. Fig. 6A). This c_{III} factor was previously found in fraction III and is required for the 3' splice site reaction but not spliceosome formation (Cheng and Abelson 1986). Either the c_{III} factor cosediments with, but is not part of, the *rna2Δ* spliceosome, or it is associated with the spliceosome but is not necessary for its assembly. In the latter event, it is interesting that a factor necessary for the second cleavage-ligation reaction can associate with an intermediate complex in the absence of the first cleavage-ligation reaction. We tentatively define this c_{III} factor as an intrinsic factor of the spliceosome.

Second, the fact that complementation with fraction III only allows the *rna2Δ* spliceosome to proceed through the 5' splice site reaction suggests that a factor required for the 3' splice site reaction is limiting. Clearly, this limiting factor is not the c_{III} factor, since it is present in both the *rna2Δ* spliceosome and in fraction III. Therefore, it is most likely that another factor has to be provided in the complementing extract to allow the 3' splice site reaction to occur on the *rna2Δ* spliceosome. This factor (c_n) is likely to be an extrinsic factor (not in the spliceosome) and is apparently enriched in fraction I (cf. Fig. 6B, lane 1).

Discussion

The RNA gene products and spliceosome formation

To understand the mechanism of mRNA splicing, it will be necessary to enumerate and characterize each component required for the reaction. We have previously demonstrated that the gene products of *RNA2*, *RNA5*, and *RNA11* are essential for the yeast pre-mRNA splicing (Lustig et al. 1986). In addition, several other RNA gene products (*RNA3*, *RNA4*, and *RNA8*), and perhaps *RNA7*, are also involved in the splicing process (Lustig et al. 1986). We have shown in this paper that the gene products of *RNA5* and *RNA11*, and likely *RNA3*, *RNA4*, *RNA7*, and *RNA8*, are involved in spliceosome formation. Heat inactivation of these *rna* mutant extracts abolishes their spliceosome formation activity. We cannot, however, exclude the possibility of an overlying nonspecific loss of splicing components during the heat inactivation process, which may in part eliminate the spliceosome formation activity in these *rna* mutant extracts. Nevertheless, the fact that many pairwise combinations of different *rna* mutant extracts result in *in vitro* complementation makes that possibility less likely (for a detailed argument, see Lustig et al. 1986). We should point out, however, that the requirement or involvement of a factor in complex formation does not indicate that the factor is present in the complex when the complex is isolated, and vice versa.

We also showed that at least some activities of the *RNA2* gene product are dispensable for spliceosome formation. It is possible that, after heat inactivation of the

rna2 extract, some domain of the mutant *rna2* gene product may remain functional, allowing formation of the spliceosome, while its splicing activity is completely abolished. In any case, a 40S complex containing exclusively uncleaved pre-mRNA was formed in heat-inactivated *rna2* extracts. We have called this 40S complex the *rna2Δ* spliceosome to distinguish it from spliceosomes, formed in active splicing extracts, that contain precursors as well as lariat and exon 1 intermediates. The formation of the *rna2Δ* spliceosome resembles that of the wild-type spliceosome, as tested by substrate and co-factor requirements. However, the *rna2Δ* spliceosome does not cosediment precisely with the wild-type spliceosome (cf. Fig 1, A and B), suggesting subtle differences between these two splicing complexes. Further studies have shown that several kinetic intermediate complexes sedimenting in the 40S region in glycerol gradients can be resolved into individual species by native gel electrophoresis; and that heat-inactivated *rna2* extracts only generate a subset of those species (S.-C. Cheng and J. Abelson, unpubl.). These results suggest that the *rna2Δ* spliceosome may be a true kinetic intermediate.

The rna2Δ spliceosome is a functional splicing intermediate

As mentioned above, the *rna2* extract is heat sensitive for splicing but not for spliceosome formation. This property allowed us to demonstrate that the *rna2Δ* spliceosome is a functional intermediate in the splicing pathway in vitro. The pre-mRNA incubated with a heat-inactivated *rna2* extract was fractionated by glycerol gradient sedimentation. Only the pre-mRNA sedimenting in the 40S region can be converted to splicing products in the presence of ATP and complementing extracts. The *rna2Δ* spliceosome is not active for splicing by itself but can be complemented by the *RNA2* gene product and two factors (b_n and c_n , see below). Moreover, only the prebound pre-mRNA is active under these conditions. These results indicate that (1) the *rna2Δ* spliceosome is a functional intermediate in the splicing pathway, and (2) the splicing process can be dissected into two steps, i.e., spliceosome formation and cleavage-ligation reactions.

A functional splicing complex has also been suggested by a kinetic analysis of spliceosome formation in a HeLa cell-free system. A 60S complex, formed during the lag period of the splicing reaction and containing almost exclusively uncleaved pre-mRNA, has been isolated from glycerol gradients. When supplemented with fully active nuclear extract and ATP, the pre-mRNA in the complex can be converted to spliced RNA with accelerated kinetics and higher efficiencies than newly added pre-mRNA (Grabowski et al. 1985). It is possible that this 60S complex may be a mammalian counterpart of the yeast *rna2Δ* spliceosome. In both yeast and mammalian systems, the isolated splicing complex does not contain the full activity required for splicing, suggesting that some splicing components are not present in the com-

plex (see below). However, we cannot exclude the possibility that the yeast complex lacks some factors as the consequence of either the inactivation of the *RNA2* gene product or the isolation procedure.

Requirements for complementing the rna2Δ spliceosome

To obtain splice products from the *rna2Δ* spliceosome, the complementing extracts must contain at least the wild-type *RNA2* activity. By utilizing the extract from a strain overproducing the *RNA2* gene product in a titration experiment, we found that an additional factor (b_n), apparently extrinsic to the *rna2Δ* spliceosome, is required for the 5' splice site reaction to occur on the complex. At least two factors are involved in the 3' splice site reaction, as deduced by complementation studies with extract fractions. One, c_m , is present in the complex, while the other, c_n , is apparently an extrinsic factor. Interestingly, Krainer and Maniatis (1985), by utilizing selective inactivation and complementation studies in vitro, have also shown that two factors are required for the 3' splice site reaction in the HeLa cell-free system.

It is formally possible that the additional extrinsic factors, b_n and c_n , are required simply to stabilize the *rna2Δ* spliceosome or components in the spliceosome, but are not directly involved in the splicing reaction. Alternatively, these extrinsic factors may initially associate with the *rna2Δ* spliceosome but become dissociated or inactive upon freezing and thawing the gradient fractions containing the spliceosome prior to the chasing reactions. Nevertheless, purification and characterization of these splicing factors is required to determine whether they catalyze the cleavage-ligation reactions or are required for structuring the spliceosome to allow the reaction to take place.

A working model for nuclear mRNA splicing

Our present understanding of the requirements for nuclear mRNA splicing in yeast is summarized in Figure 7. There are three steps in splicing: spliceosome formation (step A); the 5' splice site reaction, i.e., formation of the exon 1 and intron-exon 2 lariat (step B); and the 3' splice site reaction, i.e., formation of the intron lariat and spliced mRNA (step C). It appears that each of these steps requires multiple factors. Step A, spliceosome formation, requires the products of the *RNA3*, *RNA4*, *RNA5*, *RNA7*, *RNA8*, and *RNA11* genes. We have termed these a_3 , a_4 , a_5 , a_7 , a_8 , and a_{11} , respectively. Cheng and Abelson (1986) have shown that a combination of fractions I and II from wild-type extracts can form the spliceosome, suggesting the presence of a factors in fractions I (a_I) and II (a_{II}). The a_3 , a_4 , a_5 , a_7 , a_8 , and a_{11} factors are a subset of the a_I factors, since fraction I complements all of the heat-inactivated *rna* mutant extracts (Lustig et al. 1986). Some of these a factors may be present in other fractions as well. The snRNAs are also likely to be required for spliceosome formation, since

Figure 7. A proposed three-step mechanism for nuclear mRNA splicing in yeast. a, b, and c indicate factors that are involved in the corresponding steps A, B, and C. Subscript numbers indicate the following: arabic for the product of the corresponding RNA gene, and roman for the factor(s) present in the corresponding fractions of the wild-type extract. For example, b₂ is the product of the *RNA2* gene, whose activity is involved in step B; a_I is a collection of the factors present in fraction I, which are involved in step A. See text for details.

micrococcal nuclease and anti-trimethyl-cap antibody abolish the splicing and spliceosome formation activities in extracts (Cheng and Abelson 1986), and several yeast snRNAs are found in the spliceosome (Pikielny and Rosbash 1986; Z. Hostomsky, S. Ruby, and J. Abelson, in prep.; N. Riedel and C. Guthrie, in prep.). Step B requires the *RNA2* gene product, which is designated b₂, and another factor designated b_n. Step C requires an intrinsic factor c_{III}, and an extrinsic factor c_n. It is, of course, possible that factors required for the early steps function at later steps as well. ATP is required both for spliceosome formation (step A) and for conversion of pre-mRNA within the complex to the splicing intermediates (step B). It is not yet clear whether ATP is involved in step C. In any event, ATP may have two different roles in mRNA splicing. It is possible that some of the splicing factors described above may be the yeast counterparts of the U-snRNPs or hnRNP proteins found in higher organisms.

The scheme shown in Figure 7 is clearly a tentative outline. For example, all components present in the spliceosome may interact with the pre-mRNA in a single step. Alternatively, there may be step-wise interaction of different components with the pre-mRNA. Isolation and characterization of the RNA genes and their products will aid in understanding the assembly of the spliceosome. This information will provide a more detailed view of the mechanism of the splicing reaction.

Materials and methods

Yeast strains

The yeast strains used in this study are briefly summarized as the following: wild-type strain, EJ101; *rna2* revertants, RL92r2 and SS304r2; *rna2* strains, RL92 and SS304; *rna3* strain, SPJ3.33; *rna4* strain, RL144; *rna5* strains, RL173 and AJL144-3B; *rna7* strain, AHrna7; *rna8* strain, AHrna8; *rna11* strains, SPJ11.4 and AJL145-9D. The genotypes of these strains have been described by Lin et al. (1985) or by Lustig et al. (1986). The strain, RL92/pRN2-1 (pJDB207), which overproduces the product of the *RNA2* gene approximately 50- to 200-fold over the wild-type level, was constructed and kindly provided by Last and Woolford. The pRN2-1 (pJDB207) (Last 1986) is comprised of a 4.4-kb restriction fragment containing the wild-type *RNA2* gene on a high-copy-number plasmid, pJDB207 (Beggs 1981).

SP6 transcription

The SP6-actin, as well as the SP65-actin(Δ6) and SP65-actin(Δ6)C1, transcription plasmids have been described by Lin et al. (1985) and Vijayraghavan et al. (1986), respectively. The actin(Δ6) construct has a 6-base deletion within the actin intron, but this deletion has no effect on splicing either in vitro or in vivo. ³²P-labeled transcripts were synthesized with the SP6 RNA polymerase (Promega) essentially as described by Lin et al. (1985), except that the UTP concentration was at 100 μM, and the [α-³²P]UTP (Amersham) at 2 mCi/ml. Synthesized transcripts were not capped.

Preparation of the splicing whole cell extracts

Cells were grown to mid-to-late logarithmic phase at permissive temperature (23°C), or at semipermissive temperature (30°C), in YPD media essentially as described by Lustig et al. (1986). To prepare extract from the *RNA2* gene product overproducer, RL92/pRN2-1(pJDB207), the strain was initially inoculated in leucine drop-out minimal medium and incubated at 23°C overnight. The overnight culture with A₆₀₀ of 1 was diluted 40-fold into rich YPD medium and grown at 23°C. The cells were harvested at approximately A₆₀₀ of 1. Under these growth conditions, less than 10% of the cells lost the plasmid after five generations in the rich medium. Extracts were prepared as previously described by Newman et al. (1985) and Lin et al. (1985), except that spheroplast formation was performed at 23°C with 200 μg/ml of zymolyase 100,000 (Miles Labs). The formation of spheroplasts was monitored by reading the A₈₀₀ of 1:100 dilution of culture in 10% SDS. Usually, the A₈₀₀ dropped to less than 20% of the initial level within 90 min of incubation. The extracts were finally dialyzed against buffer D [20 mM HEPES (pH 7.9), 0.2 mM EDTA, 0.5 mM DTT, 50 mM KCl, 20% (vol/vol) glycerol]. The protein concentration of the extracts prepared was approximately 20 mg/ml.

Heat inactivation and splicing reaction in vitro

Heat inactivation of the extracts was performed at 30°C for 0–60 min, essentially as described by Lustig et al. (1986), in mixtures containing 90% (vol/vol) extract. For complete inactivation of *rna* mutant extracts for splicing, MgCl₂ was added to a final concentration of 2 mM; KPO₄ (pH 7) to 30 mM; and PEG8000 to 2% (wt/vol). In some cases, potassium phosphate was omitted from the inactivation mixture to reduce nonspecific inactivation of splicing components (Lustig et al. 1986). Heat-treated extracts (extractΔ) were quickly frozen in liquid nitrogen and stored at –70°C. Splicing activity of extracts was assayed in a mixture containing 50% (vol/vol) extractΔ, 2.5 mM

MgCl₂, 60 mM KPO₄ (pH 7), 2% PEG8000, 2 mM ATP, 0.1–0.4 nM ³²P-labeled pre-mRNA. After incubation at 15°C for 60 min, the reaction mixture was phenol extracted and ethanol precipitated. The ethanol precipitates were recovered and analyzed by polyacrylamide gel electrophoresis under denaturing conditions as described by Lin et al. (1985). Typically, extracts prepared from *rna* mutants are inactivated for splicing activity after 30–60 min incubation at 30°C (Lustig et al. 1986). Specific heat inactivation of *rna* extracts was tested by pairwise mixing of equal volumes of two extracts from different *rna* mutants in an in vitro complementation analysis (Lustig et al. 1986).

Glycerol gradient sedimentation analysis

The glycerol gradient sedimentation analysis of the spliceosome formation was modified from the procedure of Brody and Abelson (1985). After incubation, the splicing reaction mixture was chilled on ice and diluted with gradient buffer without glycerol [100 mM KCl, 2 mM MgCl₂, 20 mM HEPES (pH 7.4)], so that the density of the sample was lower than the gradient solution, and then layered on a 15–27% glycerol gradient. Typically, a sample of 50–60 µl was layered on a ½-in. by 2-in. polyallomer centrifuge tube (Beckman Instruments) containing 4.6 ml of gradient solution. The sedimentation was performed at 50,000 rpm for 100–120 min at 2°C in an SW55 rotor. Fractions were collected and radioactivities were determined as Cerenkov counts per minute. Fractions of interest were frozen in liquid nitrogen and kept at –70°C until further analysis.

Chasing reaction

The chasing reaction, i.e., conversion of the pre-mRNA in the individual gradient fraction to the splicing intermediates and products, was performed in a 10-µl reaction mixture containing 7 µl of the gradient fraction of interest, 1 µl 10× chasing buffer (200 mM KPO₄ (pH 7), 11 mM MgCl₂, 20 mM ATP, 14% PEG8000), 1–2 µl of complementing extract in buffer D, at 15°C for 60 min. Usually, heat-inactivated *rna* mutant extracts were added at 1 µl per 10-µl reaction. Fractions of the wild-type extract (Cheng and Abelson 1986) were mixed with equal volume of buffer D before adding at 1–2 µl per 10-µl reaction. In some cases, reactions as small as 5 µl were carried out. The reaction products were recovered and analyzed by polyacrylamide gel electrophoresis as described above.

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References

Abelson, J. 1979. RNA processing and the intervening sequence problem. *Annu. Rev. Biochem.* **48**: 1035–1069.

- Beggs, J.D. 1981. Multi-copy plasmid vectors. In *Alfred Benzon Symp.* (ed. D. von Wettstein, J. Friis, M. Kielland-Brandt, and A. Stenderup), pp. 383–395. Munksgard, Copenhagen, Denmark.
- Belfort, M., J. Pedersen-Lane, D. West, K. Ehrenman, G. Maley, F. Chu, and F. Maley. 1985. Processing of the intron-containing thymidylate synthase (*td*) gene of phage T4 is at the RNA level. *Cell* **41**: 375–382.
- 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.
- Black, D.L. and J.A. Steitz. 1986. Pre-mRNA splicing in vitro requires intact U4/U6 small nuclear ribonucleoprotein. *Cell* **46**: 697–704.
- Black, D.L., B. Chabot, and J.A. Steitz. 1985. U2 as well as U1 small nuclear ribonucleoproteins are involved in pre-messenger RNA splicing. *Cell* **42**: 737–750.
- 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.
- Cech, T.R. 1983. RNA splicing: Three themes with variations. *Cell* **34**: 713–716.
- . 1986. The generality of self-splicing RNA: Relationship to nuclear mRNA splicing. *Cell* **44**: 207–210.
- Cech, T.R. and B.L. Bass. 1986. Biological catalysis by RNA. *Annu. Rev. Biochem.* **55**: 599–629.
- Chabot, B., D.L. Black, D.M. DeMaster, and J.A. Steitz. 1985. The 3' splice site of pre-messenger RNA is recognized by a small nuclear ribonucleoprotein. *Science* **230**: 1344–1349.
- Cheng, S.-C. and J. Abelson. 1986. Fractionation and characterization of yeast mRNA splicing extract. *Proc. Natl. Acad. Sci.* **83**: 2387–2391.
- Choi, Y.D., P.J. Grabowski, P.A. Sharp, and G. Dreyfuss. 1986. Heterogeneous nuclear ribonucleoproteins: Role in RNA splicing. *Science* **231**: 1534–1539.
- Daniels, C.J., R. Gupta, and W.F. Doolittle. 1985. Transcription and excision of a large intron in the tRNA^{Trp} gene of an archaeobacterium, *Halobacterium volcanii*. *J. Biol. Chem.* **260**: 3132–3134.
- Frederewey, D. and W. Keller. 1985. Stepwise assembly of a pre-mRNA splicing complex requires U-snRNPs and specific intron sequences. *Cell* **42**: 355–367.
- Fried, H.M., N.J. Pearson, C.H. Kim, and J.R. Warner. 1981. The genes for fifteen ribosomal proteins of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **256**: 10176–10183.
- Garriga, G. and A.M. Lambowitz. 1984. RNA splicing in *Neurospora* mitochondria: Self-splicing of a mitochondrial intron in vitro. *Cell* **39**: 631–641.
- 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.
- 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.
- Greer, C., C. Peebles, P. Gegenheimer, and J. Abelson. 1983. Mechanism of action of a yeast ligase in tRNA splicing. *Cell* **32**: 537–546.
- Hartwell, L.H. 1967. Macromolecule synthesis in temperature-sensitive mutants of yeast. *J. Bacteriol.* **93**: 1662–1670.
- Hartwell, L.H., C.S. McLaughlin, and J.R. Warner. 1970. Identification of ten genes that control ribosome formation in yeast. *Mol. Gen. Genet.* **109**: 42–56.
- Hinterberger, M., I. Pettersson, and J.A. Steitz. 1983. Isolation of small nuclear ribonucleoproteins containing U1, U2, U4, U5, and U6 RNAs. *J. Biol. Chem.* **258**: 2604–2613.
- Kinlaw, C.S., B.L. Robberson, and S.M. Berget. 1983. Fraction-

- ation and characterization of human small nuclear ribonucleoproteins containing U1 and U2 RNAs. *J. Biol. Chem.* **258**: 7181–7189.
- Konarska, M. and P. Sharp. 1986. Electrophoresis separation of complexes involved in splicing of precursors to mRNAs. *Cell* **46**: 845–855.
- Krainer, A.R. and T. Maniatis. 1985. Multiple factors including the small ribonucleoproteins U1 and U2 are necessary for pre-mRNA splicing in vitro. *Cell* **42**: 725–736.
- Krämer, 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.
- Larkin, J.C. and J.L. Woolford. 1983. Molecular cloning and analysis of the *CRY1* gene: A yeast ribosomal protein gene. *Nucleic Acids Res.* **11**: 403–420.
- Last, R.L. 1986. "Isolation and characterization of the RNA genes of yeast and their products: Components of the pre-mRNA splicing apparatus." Ph.D thesis, Carnegie-Mellon University, Pittsburgh, Pennsylvania.
- Lerner, M.R., J.A. Boyle, S.M. Mount, S.L. Wolin, and J.A. Steitz. 1980. Are snRNPs involved in splicing? *Nature* **283**: 220–224.
- Lin, R.-J., A.J. Newman, S.-C. Cheng, and J. Abelson. 1985. Yeast mRNA splicing in vitro. *J. Biol. Chem.* **260**: 14780–14792.
- Lustig, A.J., R.-J. Lin, and J. Abelson. 1986. The yeast RNA gene products are essential for mRNA splicing in vitro. *Cell* **47**: 953–963.
- Mount, S.M., I. Pettersson, M. Hinterberger, A. Karmus, and J.A. Steitz. 1983. The U1 small nuclear RNA-protein complex selectively binds a 5' splice site in vitro. *Cell* **33**: 509–518.
- Newman, A.J., R.-J. Lin, S.-C. Cheng, and J. Abelson. 1985. Molecular consequences of specific intron mutations on yeast mRNA splicing in vivo and in vitro. *Cell* **42**: 335–344.
- Padgett, R.A., P.J. Grabowski, M.M. Konarska, S. Seler, and P.A. Sharp. 1986. Splicing of messenger RNA precursors. *Annu. Rev. Biochem.* **55**: 1119–1150.
- Peebles, C., P. Gegenheimer, and J. Abelson. 1983. Precise excision of intervening sequences from precursor tRNAs by a membrane-associated yeast endonuclease. *Cell* **32**: 525–536.
- Peebles, C.L., P.S. Perlman, D.L. Mecklenburg, M.L. Pettillo, J.H. Tabor, K.A. Jarrell, and H.-L. Cheng. 1986. A self-splicing RNA excises an intron lariat. *Cell* **44**: 213–223.
- Perkins, K.K., H.M. Furneaux, and J. Hurwitz. 1986. RNA splicing products formed with isolated fractions from HeLa cells are associated with fast-sedimenting complexes. *Proc. Natl. Acad. Sci.* **83**: 887–891.
- Pikielny, C.W. and M. Rosbash. 1986. Specific small nuclear RNAs are associated with yeast spliceosomes. *Cell* **45**: 869–877.
- Rogers, J. and R. Wall. 1980. A mechanism for RNA splicing. *Proc. Natl. Acad. Sci.* **77**: 1877–1879.
- Rosbash, M., P.K.W. Harris, J.L. Woolford, and J.L. Teem. 1981. The effect of temperature-sensitive *rna* mutants on the transcription products from cloned ribosomal protein genes of yeast. *Cell* **24**: 679–686.
- Van der Horst, G. and H.F. Tabak. 1985. Self-splicing of yeast mitochondrial ribosomal and messenger RNA precursors. *Cell* **40**: 759–766.
- Van der Veen, R., A.C. Arnberg, G. Van der Horst, L. Bonen, H.F. Tabak, and L.A. Grivell. 1986. Excised group II introns in yeast mitochondria are lariats and can be formed by self-splicing in vitro. *Cell* **44**: 225–234.
- Vijayraghavan, U., R. Parker, J. Tamm, Y. Iimura, J. Rossi, J. Abelson, and C. Guthrie. 1986. Mutations in conserved intron sequences affect multiple steps in the yeast splicing pathway, particularly assembly of the spliceosome. *EMBO J.* **5**: 1683–1695.
- Zhuang, Y. and A.M. Weiner. 1986. A compensatory base change in U1 snRNA suppresses a 5' splice site mutation. *Cell* **46**: 827–835.

Splicing of yeast nuclear pre-mRNA in vitro requires a functional 40S spliceosome and several extrinsic factors.

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