Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 411–415, January 1988
Biochemistry

Spliceosome assembly involves the binding and release of U4 small nuclear ribonucleoprotein

ANGUS I. LAMOND*, MARIA M. KONARSKA, PAULA J. GRABOWSKI†, AND PHILLIP A. SHARP

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Contributed by Phillip A. Sharp, October 2, 1987

ABSTRACT **Splicing complexes that form a rabbit β-globin precursor mRNA (pre-mRNA) have been analyzed for their small nuclear RNA (snRNA) content by both affinity chromatography and specific probe hybridization of replicas of native electrophoretic gels. A pathway of spliceosome assembly was deduced that has at least three stages. (*i*) U2 small nuclear ribonucleoprotein (snRNP) alone binds to sequences of mRNA upstream of the 3′ splice site. (*ii*) U4, U5, and U6 snRNPs bind, apparently simultaneously. (*iii*) U4 snRNP is released to generate a spliceosome that contains U2, U5, and U6 snRNPs together with the RNA intermediates in splicing. U1 snRNP was not detected in association with any of these complexes. A parallel analysis of the spliceosome found with an adenovirus precursor mRNA substrate yielded an identical snRNP composition with one additional, unidentified RNA species, called X. This latter RNA species was not detected in the spliceosome bound to the β-globin substrate.**

The splicing of precursors to eukaryotic mRNAs (pre-mRNAs) proceeds in a two-step reaction. First, cleavage occurs at the 5′ splice site to generate reaction intermediates of a free 5′ exon and a lariat form of the intervening sequence plus the 3′ exon. Second, the 5′ and 3′ exons are ligated, releasing the mRNA and fully excised intron lariat as reaction products. Both splicing reactions occur in a highly ordered structure called a spliceosome (1–5). The assembly of a functional spliceosome is dependent upon conserved sequences located at the 5′ and 3′ splice sites of the pre-mRNA (3, 6–9). The integrity of multiple snRNP particles in cell extracts originally designated U for unspecific, including U1, U2, and U4/6 small nuclear ribonucleoproteins (snRNPs), is essential for splicing (10–14).

Spliceosomes were initially identified as complexes containing the RNA splicing intermediates (1–5). Characterization of the mammalian spliceosome by affinity selection of biotinylated pre-mRNA on streptavidin-agarose beads revealed multiple snRNP components in the spliceosome, including U2, U4, U5, and U6 snRNPs, but not U1 snRNP (15). More recently, spliceosomes have been analyzed by electrophoresis in native polyacrylamide gels, and the snRNP composition of the splicing complexes has been studied by either RNA blotting or direct elution of the RNA species from the gel (16–20). These data established that an early step in spliceosome assembly involves the ATP-dependent binding of U2 snRNP to sequences upstream of the 3′ splice site. This is followed by the binding of U4, U5, and U6 snRNPs, possibly in the form of a preassembled poly(snRNP) particle (20). Yeast spliceosomes are also found to contain multiple snRNP components that probably correspond directly to the mammalian species (17, 18, 21).

We have previously shown that three forms of mammalian splicing complexes, α, β, and γ, can be detected with a pre-mRNA containing the second intron of rabbit β-globin (9). These three complexes are kinetically related and form in the order $\alpha \rightarrow \beta \rightarrow \gamma$. The γ complex contains the splicing intermediates and probably corresponds to a mature form of the spliceosome. In this work we analyze the snRNP composition of the splicing complexes that bind to the β-globin pre-mRNA using a combination of glycerol-gradient centrifugation and native gel electrophoresis.

MATERIALS AND METHODS

Materials. Radiochemicals ([^{32}P]pCp and [α-^{32}P]UTP) were purchased from New England Nuclear. T4 RNA ligase, SP6 RNA polymerase, and all restriction enzymes were from New England Biolabs. T7 RNA polymerase and RNasin were purchased from Promega Biotec (Madison, WI), and T3 RNA polymerase was purchased from Stratagene (La Jolla, CA). Biotinylated UTP (biotin-11-UTP) was purchased from Bethesda Research Laboratories.

***In Vitro* Splicing Assays.** HeLa cell nuclear extract was prepared as described by Dignam *et al.* (22). Transcription of uniformly labeled, capped β-globin pre-mRNAs (from template pBSAL4 that had been cleaved with *Eco*RI) and *in vitro* splicing assays were done as previously described (9). The 5′ splice site mutants were previously described (9). For RNA blot analysis, unlabeled pre-mRNA was prepared and used at a higher final concentration of ≈1 μg/ml. Biotinylated, uniformly labeled, capped pre-mRNAs were prepared essentially as described by Grabowski and Sharp (15). Biotinylated pre-mRNA was included in the splicing assays at a final concentration of ≈7 μg/ml. Standard splicing assays analyzed by native gel electrophoresis were done in a 10-μl reaction volume. Splicing assays with the biotinylated pre-mRNAs were scaled up to 300-μl reaction volume. Mobility retardation assays were done using 4% native polyacrylamide gels run in a 50 mM Tris·glycine buffer as described by Konarska and Sharp (20). In these experiments the gels were run at room temperature for 4–5 hr at a constant voltage of ≈35 V/cm.

Glycerol Gradients and Affinity Selection. Splicing assays done with biotinylated pre-mRNAs were stopped by addition of heparin to a final concentration of 4 mg/ml, incubated for a further 8 min at 30°C, and then loaded onto 10–30% (vol/vol) glycerol gradients and centrifuged for 14–16 hr at 24,000 rpm in a SW 41 rotor. Gradients were run at 5°C in a buffer containing 25 mM KCl, 1 mM $MgCl_2$, and 20 mM Hepes, pH 7.4. Twenty 0.55-ml fractions were collected from each gradient, and ^{32}P levels were measured. Affinity selection of designated fractions on streptavidin-agarose beads was done as described by Grabowski and Sharp (15).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: snRNP, small nuclear ribonucleoprotein; pre-mRNA, precursor mRNA; U1, U2, . . . , originally designated as unspecific complexes U1, U2, ...

*Present address: European Molecular Biology Laboratory, Meyerhofstrasse 1, 6900 Heidelberg, F.R.G.
†Present address: Department of Biochemistry, Brown University, Providence, RI 02912.

RNA Blots and RNA Elution from Native Gels. Native gels were electroblotted onto GeneScreen membrane (New England Nuclear) and hybridized to snRNA riboprobes as described by Konarska and Sharp (20). The β-globin pre-mRNA probe was prepared by transcription of *Hin*dIII-cut pBSAL4 with T7 RNA polymerase (9). RNA elutions were done by first electroblotting the native gels onto DEAE membrane (Schleicher & Schuell, NA-45). Transfer was in 40 mM Tris·glycine buffer, pH 8.8, for 14–16 hr at 4°C, 150 mA. After transfer, membranes were autoradiographed, and RNA was eluted from designated bands by incubation with 0.7 ml of BOB buffer (50% formamide/20 mM Tris·HCl, pH 7.5/1 M sodium acetate/0.1% $NaDodSO_4$/1 mM EDTA) containing 20 μg of glycogen at 68°C for 1 hr. Membrane was then removed, and the samples were spun for 5 min in a Microfuge to pellet residual fragments. Supernatants were extracted twice with phenol/chloroform, 1:1 (vol/vol) and once with chloroform, ethanol precipitated, and finally resuspended in 20 μl of 10 mM Tris, pH 7/1 mM EDTA. End-labeling reactions with [^{32}P]pCp were done as described (15).

RESULTS

Comparison of Pre-mRNAs from β-Globin and Adenovirus in Affinity Selection of Gradient-Fractionated Spliceosome. We have previously used affinity selection of biotinylated substrate RNAs to study the snRNP composition of splicing complexes that bind to a pre-mRNA substrate containing the first intron of adenovirus (15). The same protocol was used with a substrate RNA from the second intron in β-globin (see ref. 9 for structure of RNA substrates). Splicing complexes bound to biotinylated pre-mRNAs are first separated by velocity sedimentation in glycerol gradients and then affinity purified on streptavidin-agarose beads. Components, retained on the streptavidin-agarose beads, are subsequently eluted by treatment with high salt and detergent, and the selected RNA species are 3′ end-labeled and then analyzed on denaturing polyacrylamide gels. As a control for the nonspecific binding of snRNAs to the streptavidin-agarose matrix, splicing reactions done in the absence of exogenously added pre-mRNA are processed in parallel.

Similar gradient profiles were obtained with reactions containing biotin-labeled substrate RNAs from either the second intron of β-globin or the first intron of adenovirus (Fig. 1*A*). Affinity chromatography of material from the 35S peak of the β-globin substrate was enriched in snRNAs U2, U4, U5, and U6 relative to the equivalent fraction from the control gradient (Fig. 1*B*, lanes A and B, respectively). A similar enrichment of the same set of snRNAs was observed with the adenovirus pre-mRNA substrate (Fig. 1*B*, lanes C and D). Note that U1 snRNA was not enriched in the spliceosome formed on either substrate RNA. These results confirm previous analysis of the snRNA composition of the spliceosome formed on the adenovirus substrate (15) and generalize these to a second substrate RNA.

In the previous study on the snRNA composition of splicing complexes that bind to an adenovirus pre-mRNA substrate, we found that an additional small RNA species, termed X, was enriched in the spliceosome together with U2, U4, U5, and U6 (15). Comparison of lanes A and C of Fig. 1*B* shows that a RNA species with the mobility of X was affinity selected with the adenovirus substrate but not with the β-globin substrate. The X species migrated slightly faster than a common background RNA in the example shown in Fig. 1 and was observed in multiple experiments. We conclude from the data shown in Fig. 1 that the RNA species X either is not a component of the spliceosome that assembles on the β-globin pre-mRNA or else it binds significantly less avidly to this pre-mRNA than it does to the adenovirus substrate, such that it is quantitatively removed from the complex during the analytical procedure.

Resolution of Splicing Complexes on Native Polyacrylamide Gels. The β-globin pre-mRNA analyzed here in the affinity-purification studies has been previously shown to form at least three ATP-dependent complexes, α, β, and γ, that can be resolved by electrophoresis in native polyacrylamide gels (9). These three complexes are kinetically related and form, both temporally and in order of decreasing mobility, α → β → γ. To relate the affinity-purification data with the pattern of complexes separated by native gel electrophoresis, fractions taken from a velocity gradient were subsequently

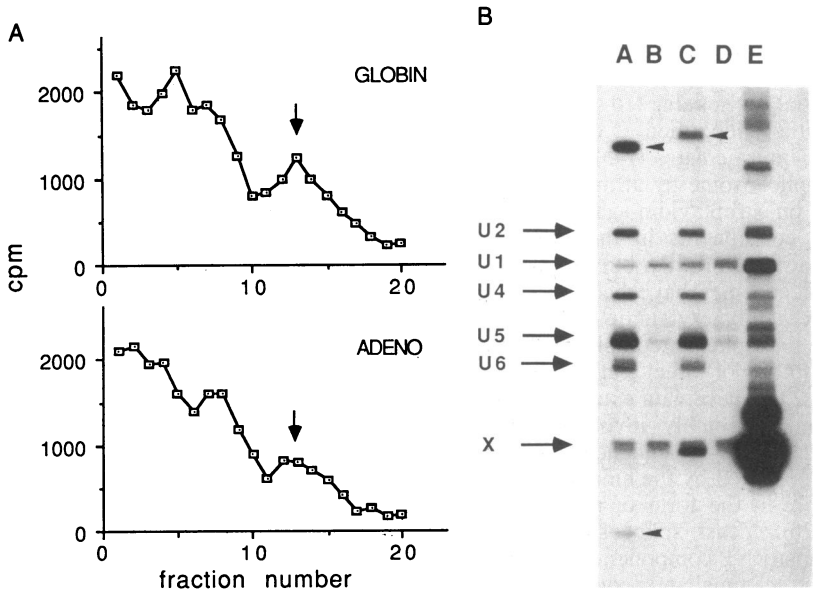

FIG. 1. Comparison of snRNAs in spliceosomes formed with β-globin and adenovirus pre-mRNAs. (*A*) Glycerol-gradient profiles. β-Globin and adenovirus (ADENO) pre-mRNAs were incubated in a splicing assay for 30 and 15 min, respectively, before loading onto the gradient. Heparin was added to reactions before sedimentation, and gradients were fractionated from top to bottom. Fractions 12 and 13 were analyzed by affinity selection in each case. Arrow indicates a sedimentation value of ≈35S. (*B*) Denaturing polyacrylamide gel showing affinity-selected, end-labeled snRNAs. Lanes: A, β-globin substrate; B, minus β-globin substrate pre-mRNA control; C, adenovirus substrate; D, minus adenovirus substrate pre-mRNA control; and E, HeLa cell nuclear RNA. Left-pointing arrowheads mark the positions of intact pre-mRNAs and of the free β-globin 5′ exon. Note that U5 snRNA is more efficiently end-labeled than the other snRNAs (15).

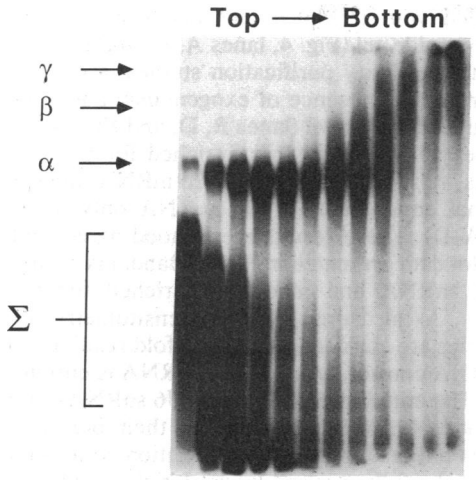

FIG. 2. Separation of β-globin splicing complexes on native polyacrylamide gels. Glycerol gradient fractions 3–14, taken from a gradient similar to that shown in Fig. 1*A* for the wild-type β-globin pre-mRNA, were analyzed by native gel electrophoresis. These samples were heparin treated before loading onto the glycerol gradient, causing a decrease in the proportion of β and γ bands relative to the other species (A.I.L., unpublished observations).

analyzed on a native polyacrylamide gel (Fig. 2). This two-dimensional analysis shows that the spliceosome peak formed with the wild-type β-globin pre-mRNA, which was analyzed by affinity purification in Fig. 1, is heterogeneous and consists of a mixture of the complexes that migrate in the positions of the β and γ bands on a native gel. By contrast, the other bands detected on the native gel, Σ and α, are well resolved from β and γ and also well resolved from one another in the top and middle fractions of the gradient, respectively. Additional affinity-purification experiments, done with the gradient fractions that predominantly contain either Σ or α, show no detectable enrichment for an RNA species in Σ fractions and enrichment for only U2 snRNP in α fractions (data not shown). By affinity purification we have also analyzed fractions from the bottom of the gradients, which are sedimenting faster than the spliceosome peak. These rapidly sedimenting fractions showed no specific enrichment for an RNA species relative to the minus precursor control.

snRNA Components of Complexes Separated on Native Gels. The snRNA composition of the β and γ complexes was determined after separation in a native polyacrylamide gel by a combination of (*i*) direct RNA blotting (20) and (*ii*) elution of RNA from the native gel and subsequent detection by end-labeling and electrophoresis on a denaturing polyacrylamide gel.

Fig. 3 shows an RNA blot analysis of splicing complexes that have been separated in a native polyacrylamide gel. Splicing complexes formed on both the wild-type β-globin pre-mRNA and two 5′ splice site mutants were assayed in parallel on the same gel. One of these mutants possesses a G → A mutation at the 5′ splice site, whereas the second is deleted for all sequences at the 5′ splice site (see ref. 9 for structure of mutants). A probe, complementary to β-globin pre-mRNA, was used to determine the distribution of pre-mRNA-containing complexes across the gel. This latter probe shows that the wild-type and mutant substrates were all assembled into complexes that separate as bands α, β, and γ (compare with Fig. 2). With the substrate from the 5′ splice-site deletion mutant, the three splicing complexes migrated more rapidly than the corresponding complex formed on either the wild-type or 5′ point mutant substrates. This is primarily due to the reduced length of the mutant pre-mRNA substrate (ref. 9, data not shown). The same RNA blot was sequentially probed with uniformly labeled RNA probes complementary to each of the snRNA species, U1, U2, U4, U5, and U6, to determine their location in each of the splicing complexes (20). The distribution of snRNAs between the three complexes was identical for each pre-mRNA. In agreement with the affinity purification data, U1 snRNA was not detected in either the α, β, or γ complex. However, in each case U2 snRNA was seen in all α, β, and γ bands. For band α, no additional snRNAs were present. By contrast with α, band β contained a full set of U4, U5, and U6 snRNAs as well as U2 snRNA. Interestingly, the slowest mobility complex, γ, contained U2, U5, and U6 snRNAs, but no U4 snRNA. Thus, assuming a precursor–product relationship between the β and γ complexes, U4 snRNA has either been lost from γ or else its binding affinity

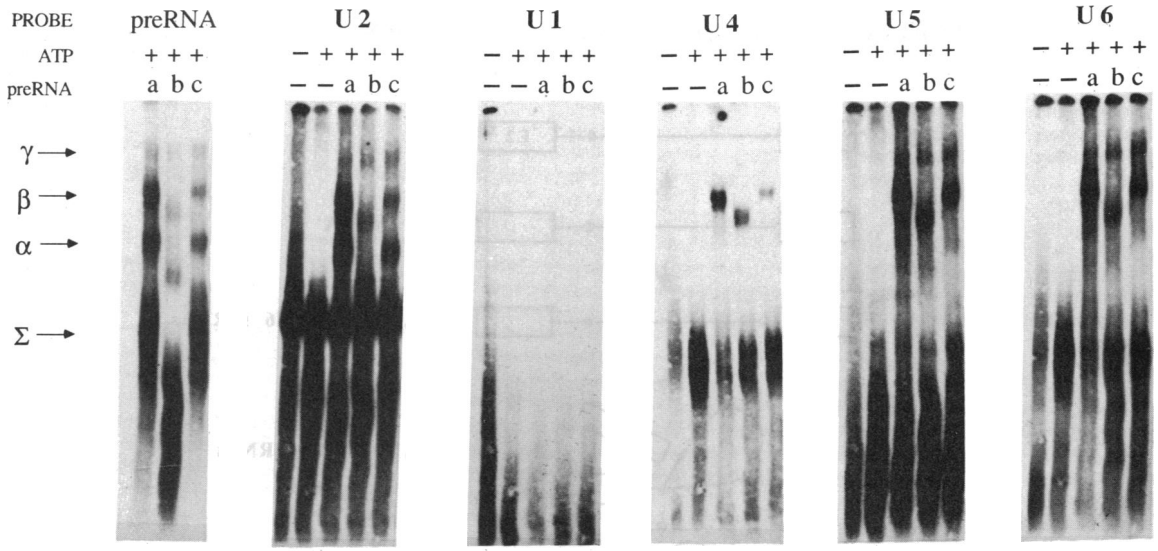

FIG. 3. RNA blot analysis of splicing complexes separated on a native polyacrylamide gel. The same blot was hybridized sequentially to each of the probes indicated. The three β-globin pre-mRNA substrates analyzed were wild type (lanes a), 5′ deletion mutant (lanes b) (9), and 5′ G → A mutant (lanes c) (9). The wild-type pre-mRNA was incubated in a splicing assay for 45 min, and the two mutant pre-mRNAs were incubated for 75 min before loading on the gel.

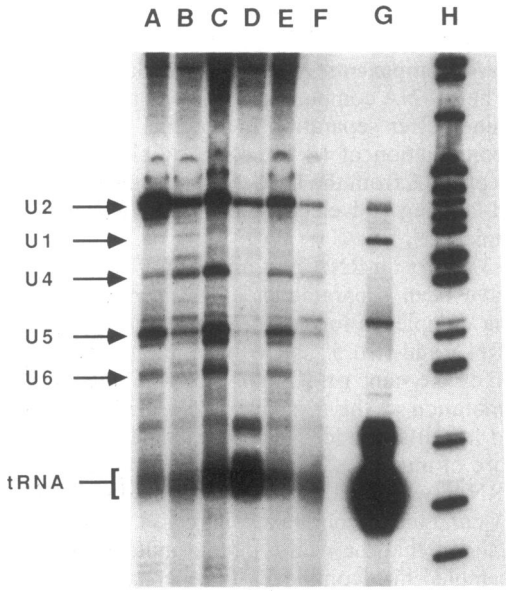

FIG. 4. Analysis of snRNAs eluted from β-globin splicing complexes. Lanes A, C, and E show the snRNAs eluted from the γ, β, and α bands, respectively, formed with the wild-type β-globin pre-mRNA. Lanes B, D, and F show the snRNAs eluted from equivalent regions of a gel run without pre-mRNA (control). Both plus and minus pre-mRNA samples were incubated in a splicing assay for 50 min before loading onto the native gels. Lane G shows total HeLa cell nuclear RNA, and lane H shows DNA markers made by end-labeling an *Msp* I digest of pBR322.

has decreased to such an extent that it no longer remains stably associated during electrophoresis in a native gel.

One caveat with the interpretation of the above RNA blotting data is that snRNA species that appear to be absent from specific complexes, such as U1 and U4, may actually be present but in a configuration that denies them interaction with hybridization probes. Although we consider this possibility unlikely,we have nonetheless employed an additional method that does not involve filter hybridization to characterize the snRNA composition of complexes separated on a native gel.

As an independent method of analyzing the complexes, snRNAs were eluted from each of the γ, β, and α bands, endlabeled with [^{32}P]pCp and resolved on a denaturing polyacrylamide gel (Fig. 4, lanes A, C, and E, respectively). As with the affinity purification studies, a control reaction was done in the absence of exogenously added pre-mRNA and analyzed in parallel (lanes B, D, and F). This shows that both the γ and β bands are enriched for U2, U5, and U6 snRNAs, relative to the minus pre-mRNA controls with an additional enrichment for U4 snRNA only in the β band (lanes A–D). These results are in good agreement with the RNA blot data presented in Fig. 3. Band α is clearly enriched for U2 snRNA and modestly enriched for U5 and U6 snRNAs. As measured by microdensitometry, the U5 and U6 species are enriched less than 2-fold relative to the minus pre-mRNA control, whereas U2 snRNA is enriched by 6- to 7-fold. The enrichment of U5 and U6 snRNAs in this region of the gel is probably not due to their presence in the α complex but results from contamination with a complex that contains the fully excised intron lariat, which migrates just above the α band (9). An equivalent complex, formed with an adenovirus pre-mRNA substrate, has been shown to contain only U2, U5, and U6 snRNAs (20). We note that no U1 snRNA was detected in any of the complexes, despite the high U1 snRNA signal seen when total HeLa cell nuclear RNA was end-labeled with [^{32}P]pCp (Fig. 4, lane G). We also note that the close correspondence between results obtained by RNA blotting (Fig. 3) and elution of snRNAs from the native gel (Fig. 4) argues strongly that RNA species that were not detected by RNA analysis are indeed absent from the complexes and not simply inaccessible to the hybridization probes.

DISCUSSION

We have used a combination of affinity purification, RNA blotting, and elution of snRNAs from native polyacrylamide gels to analyze the snRNP composition of splicing complexes formed on a pre-mRNA substrate containing the second intron of the rabbit β-globin gene. Taken together, the data indicate that the spliceosome assembles in a stepwise fashion involving the sequential binding of U2, U4, U5, and U6 snRNPs (Fig. 5). Complexes that correspond to each of the three stages of spliceosome assembly can be resolved by electrophoresis on a native polyacrylamide gel. First, U2 snRNP alone binds in the formation of α. This is followed by the apparently simultaneous binding of U4, U5, and U6

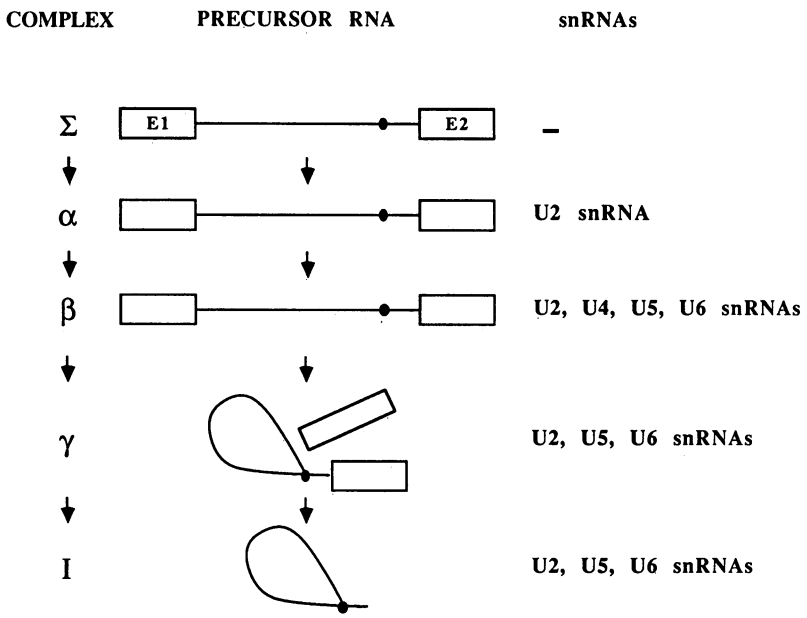

FIG. 5. A schematic summary of the pathway of mammalian spliceosome assembly.

snRNPs in the formation of β. The subsequent release of U4 snRNA generates a mature form of the spliceosome γ that contains the splicing intermediates. The concerted binding of U4, U5, and U6 snRNPs to form the β complex may result from a prior substrate-independent association of these species as described by Konarska and Sharp (20). The intron in lariat form is released as part of a complex containing U2, U5, and U6 snRNPs (9, 20).

Previous analysis has shown that U2, U4, U5, and U6 snRNAs are present in the mammalian spliceosome that is formed with an adenovirus pre-mRNA substrate, as assayed by both affinity purification and RNA blotting (15, 16, 20). Recently it has also been reported that spliceosomes formed on a pre-mRNA substrate containing the first intron of human β-globin are enriched for U1 snRNP, as well as for U2, U4, U5, and U6 (19). U1 snRNP association with the spliceosome was found dependent upon sequences at the 5′ splice site and significantly weaker than the other snRNA components, U2, U4, U5, and U6. In this latter study affinity purification in the presence of high concentrations of salt was used to assay the snRNA composition of spliceosome complexes (19). Probably these less stringent chromatographic conditions account for the selection of U1 snRNA with the spliceosome. In all previous studies with the adenovirus pre-mRNA (15, 16, 20) and in the present work, U1 snRNP was not detected as a stable component of the splicing complexes. A probable involvement of U1 snRNA in promoting binding of the U2 snRNP complex was suggested by the effect of 5′ splice-site mutations on the rate of forming the α complex (9). Thus, while *in vivo* and *in vitro* suppression studies have clearly established that U1 snRNP interacts functionally with 5′ splice-site sequences during the splicing of nuclear pre-mRNA (23, 24), whether U1 snRNP behaves as an integral component of the spliceosome, or rather as a loosely associated or transiently interacting species, remains unclear.

Previous native gel analysis of splicing complexes formed on an adenovirus pre-mRNA only resolved two bands, termed A and B. In this case band A contained only U2 snRNP and kinetically preceded formation of band B, which contained U2, U4, U5, and U6 (16, 20). These data can be reconciled with the present study by postulating that the B band seen with the adenovirus pre-mRNA consisted of a mixture of complexes equivalent to the β and γ forms that are resolved with the β-globin substrate. This view is supported by the detection of a complex, containing the fully excised intron lariat of the adenovirus pre-mRNA, which appears to form as a product of splicing through breakdown of the spliceosome. This postsplicing complex contains U2, U5, and U6 snRNAs but no U4 snRNA (20). These data indicate that U4 snRNA is also dissociated from a splicing complex bound to the adenovirus substrate but that this event was only detected in the previous study at a later stage of the reaction.

An intriguing comparison can be made between this work and recent studies on the snRNA composition of spliceosomes in the budding yeast *Saccharomyces cerevisiae*. Mobility retardation assays in native polyacrylamide gels with yeast have shown that the yeast spliceosome assembles in a similar, stepwise pathway to that described here, involving the formation of either three or four separable complexes (18, 21). Interestingly, a yeast snRNA, snR14, is found to bind and then be released from splicing complexes before formation of the mature spliceosome that contains the splicing intermediates. The gene for snR14 RNA has now been cloned, and the RNA is structurally similar to the mammalian U4 snRNA. Like U4 snRNA, snR14 RNA is found in a particle together with a second yeast snRNA, snR6, which lacks a trimethyl cap structure and is homologous to the mammalian U6 snRNA (25). The striking similarity between the observed binding and release of U4 snRNA and snR14 during spliceosome assembly and splicing strongly suggests that these species are functionally as well as structurally related. As U4 and U6 snRNAs are believed to stably associate through hydrogen bonding (26, 27), the release of U4 most likely involves a significant rearrangement of ribonucleoprotein structure and possibly requires ATP hydrolysis. Thus, while the significance to the splicing mechanism of the displacement of U4 (snR14) is still unknown, the conservation of this step between organisms as diverse as yeast and mammals indicates that this step plays an important role in spliceosome function.

A second point that emerges from this work is the conclusion that spliceosomes formed on different pre-mRNA substrates can differ in their composition. Although splicing complexes bound to either β-globin or adenovirus pre-mRNA substrates contain as stable components a common core of U2, U4, U5, and U6 snRNPs, an additional small RNA of ≈75 nucleotides, termed X, was only found as a stable component of the spliceosome formed with the adenovirus pre-mRNA. The identity of X has not yet been established, though it appears from an initial characterization that it is not a subfragment of either U1, U2, U4, U5, or U6 snRNAs and also not derived from the adenovirus substrate (ref. 15; P.J.G., unpublished observations). X is present in HeLa cells that have not been infected by adenovirus, and this species possibly corresponds to a minor snRNA species that can modulate spliceosome activity.

We are grateful to Drs. A. Virtanen and A. Gil for helpful comments on the manuscript. A.I.L. acknowledges postdoctoral support from the Science and Engineering Council of Great Britain; P.J.G., from the Medical Foundation; and M.M.K., from the Jane Coffin Childs Memorial Fund and the Lucille P. Markey Charitable Trust. This work was supported by a grant from the National Institutes of Health (GM34277) and partially by a National Cancer Institute core grant (CA14051) to P.A.S.

1. Brody, E. & Abelson, J. (1985) *Science* **228**, 963–967.
2. Grabowski, P. J., Seiler, S. R. & Sharp, P. A. (1985) *Cell* **42**, 345–353.
3. Frendewey, D. & Keller, W. (1985) *Cell* **42**, 355–367.
4. Bindereif, A. & Green, M. R. (1986) *Mol. Cell. Biol.* **6**, 2582–2592.
5. Perkins, K. K., Furneaux, H. M. & Hurwitz, J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 887–891.
6. Aebi, M., Hornig, H., Padgett, R. A., Reiser, J. & Weissmann, C. (1986) *Cell* **47**, 555–565.
7. Vijayraghavar, U., Parker, R., Tamm, J., Imura, Y., Rossi, J., Abelson, J. & Guthrie, C. (1986) *EMBO J.* **5**, 1683-1695.
8. Newman, A. J., Lin, R.-J., Cheng, S.-C. & Abelson, J. (1985) *Cell* **42**, 335–344.
9. Lamond, A. I., Konarska, M. M. & Sharp, P. A. (1987) *Genes Dev.* **1**, 532–543.
10. Krämer, A., Keller, W., Appel, B. & Lührmann, R. (1984) *Cell* **38**, 299–307.
11. Black, D. L., Chabot, B. & Steitz, J. A. (1985) *Cell* **42**, 737–750.
12. Krainer, A. R. & Maniatis, T. (1985) *Cell* **42**, 725–736.
13. Berget, S. M. & Robberson, L. (1986) *Cell* **46**, 691-696.
14. Black, D. L. & Steitz, J. A. (1986) *Cell* **46**, 697–704.
15. Grabowski, P. J. & Sharp, P. A. (1986) *Science* **233**, 1294–1299.
16. Konarska, M. M. & Sharp, P. A. (1986) *Cell* **46**, 845–855.
17. Pikielny, C. W. & Rosbash, M. (1986) *Cell* **45**, 869–877.
18. Pikielny, C. W., Rymond, B. C. & Rosbash, M. (1986) *Nature (London)* **324**, 341–345.
19. Bindereif, A. & Green, M. R. (1987) *EMBO J.* **6**, 2415–2424.
20. Konarska, M. M. & Sharp, P. A. (1987) *Cell* **49**, 763–774.
21. Cheng, S.-C. & Abelson, J. (1987) *Genes Dev.* **1**, 1014–1027.
22. Dignam, J. D., Lebovitz, R. M. & Roeder, R. D. (1983) *Nucleic Acids Res.* **11**, 1475–1489.
23. Zhuang, Y. & Weiner, A. M. (1986) *Cell* **46**, 827–835.
24. Aebi, M., Hornig, H. & Weissmann, C. (1987) *Cell* **50**, 237–246.
25. Siliciano, P. G., Brow, D. A., Roiha, H. & Guthrie, C. (1987) *Cell* **50**, 585–592.
26. Bringmann, P., Appel, B., Rinke, J., Reuter, R., Theissen, H. & Lührmann, R. (1984) *EMBO J.* **3**, 1357–1363.
27. Hashimoto, C. & Steitz, J. A. (1984) *Nucleic Acids Res.* **12**, 3283–3293.