Three protein factors (SF1, SF3 and U2AF) function in pre-splicing complex formation in addition to snRNPs

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The splicing of nuclear messenger RNA precursors can be reproduced *in vitro* with fractions obtained after chromatography of HeLa cell nuclear extracts. Here we report the chromatographic separation of three protein factors: SF1, SF3 and U2AF. All factors function early in the splicing reaction, in the assembly of a pre-splicing complex. Likewise, all factors are essential for the production of spliced RNA. In addition to their distinct chromatographic properties, the splicing factors can be distinguished by their sensitivities to heat and N-ethylmaleimide. All activities can be detected in a cytoplasmic S-100 fraction from HeLa cells. The fact that SF1, SF3 and U2AF are essential factors in pre-splicing complex formation raises the possibility that SF1 and/or SF3 participate in the interaction of U2 snRNP with the branch point in addition to U2AF.

Key words: pre-mRNA splicing/spliceosome assembly/splicing factors/snRNPs

Introduction

Our understanding of the structure and function of the splicing apparatus has been greatly improved by the development of *in vitro* systems (for reviews see Green, 1986; Padgett *et al.*, 1986; Sharp, 1987). The splicing of nuclear messenger RNA precursors (pre-mRNA) is initiated by the binding of a number of components to the RNA substrate, leading to the stepwise assembly of splicing complexes. Within these structures spliced mRNA is generated by two distinct cleavage and ligation reactions.

Factors that participate in the formation of the active spliceosome have been discovered by complementation experiments, chromatographic fractionation of cell-free extracts and genetic approaches. Major constituents of the spliceosomes are the small nuclear ribonucleoprotein particles (snRNPs) which interact with the pre-mRNA in a defined order (for reviews see Guthrie and Patterson, 1988; Steitz *et al.*, 1988; Lamond *et al.*, 1990). U1 and U2 snRNPs bind to the 5' splice site and the branch point, respectively, and both are present in the so-called pre-splicing complex. This complex is converted to the spliceosome by binding of U4, U5 and U6 snRNPs.

In addition to snRNPs, protein factors are also needed for the assembly of the pre-mRNA into splicing complexes. U2AF is required for the binding of U2 snRNP to the branch point and participates in the assembly of a pre-splicing complex (Ruskin *et al.*, 1988; Zamore and Green, 1989, 1991). Krainer *et al.* (1990) have purified a factor that is

essential at the same step in the reaction. Other proteins have been identified that specifically interact with sequences located at the 3' end of introns (Gerke and Steitz, 1986; Swanson and Dreyfuss, 1986; Tazi *et al.*, 1986; Garcia-Blanco *et al.*, 1989), with the 5' splice site (Mayeda *et al.*, 1986; Zapp and Berget, 1989; Heinrichs *et al.*, 1990) or are found associated with splicing complexes (Choi *et al.*, 1986; Chang *et al.*, 1988; Garcia-Blanco *et al.*, 1989; Pinto and Steitz, 1989; Fu and Maniatis, 1990; Garcia-Blanco *et al.*, 1990; Whittaker *et al.*, 1990). However, the exact function of these proteins in the splicing reaction remains to be established.

We have reported the separation of HeLa cell nuclear extracts into six chromatographic fractions that are required to splice an RNA substrate derived from the adenovirus major late (AdML) transcription unit (Krämer *et al.*, 1987; Krämer and Keller, 1990). Two of the fractions are enriched in the snRNPs which are required early in the splicing reaction. The other four fractions contain protein factors, designated SF1 to SF4, all of which are necessary for 5' splice site cleavage and lariat formation (Krämer *et al.*, 1987; Utans and Krämer, 1990).

SF1 and SF3 function in the splicing pathway in the formation of the pre-splicing complex (Krämer, 1988). Thus, these factors appear to be functionally related to U2AF (see above). Based on their similar chromatographic behavior on a Mono Q anion exchange column and because both SF3 and U2AF were detected only in nuclear extracts whereas SF1 was also found in a cytoplasmic S-100 fraction, we suspected previously that SF3 may be identical to U2AF (Krämer, 1988). However, the recently published details of the purification of U2AF activity (Zamore and Green, 1989) make this assumption less likely. We show here that U2AF is unrelated to SF1 and SF3 and that it represents a third protein factor that functions in the assembly of a pre-splicing complex.

Results

In our previously published fractionation scheme, splicing activities from HeLa cell nuclear extracts were separated by chromatography on DEAE—Sepharose, heparin—Sepharose and Mono Q (Krämer *et al.*, 1987; Krämer and Keller, 1990; see Figure 1). After DEAE—Sepharose chromatography, which separates splicing factor SF1 from the remaining activities, efficient splicing of an AdML pre-mRNA was observed with a combination of DS100 (100 mM KCl flowthrough fraction) and DS500 (500 mM KCl step fraction). The DS500 fraction usually showed a low level of splicing in the absence of DS100, indicating that SF1 activity was only partially removed from this fraction. Consistent with this result we also observed the formation of pre-splicing complexes with the DS500 fraction alone (not shown, but see Perkins *et al.*, 1986), whereas only non-specific complexes were detected with the DS100 fraction
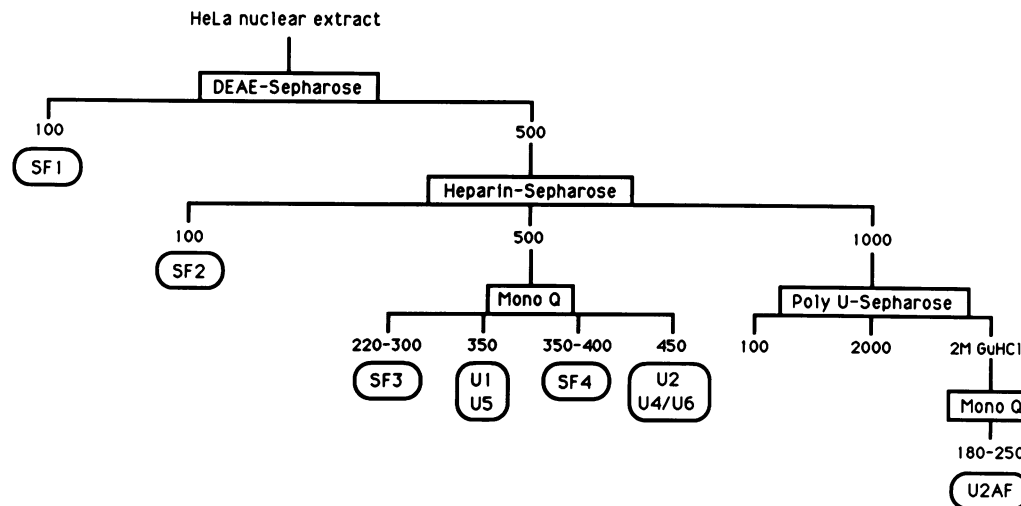
Fig. 1. Chromatographic fractionation of splicing activities from HeLa cell nuclear extracts. The column materials used are shown in boxes. Numbers designate the KCl concentration (in mM) at which the activities elute from a column, except for poly(U)−Sepharose chromatography where the last elution step was performed with 2 M guanidinium hydrochloride.

(Krämer, 1988). After chromatography on heparin−Sepharose splicing of the pre-mRNA depended on the presence of DS100, HS100 and HS500; no activity was observed in individual fractions or in combinations of two. When these fractions were tested for splicing complex formation, a low level of activity was detected in the HS500 fraction, indicating that residual SF1 in the DS500 bound to heparin−Sepharose and eluted together with SF3 and snRNPs (not shown). However, chromatography on Mono Q and elution of the column with a salt gradient separated SF1 and SF3 activities. Complex formation was not observed with individual column fractions but only in combination with DS100 (Krämer, 1988). Furthermore, SF4 and snRNPs were separated on this column and splicing of the pre-mRNA required a combination of DS100 (SF1), HS100 (SF2) and Mono Q fractions that contained SF3, SF4 and snRNPs (Krämer et al., 1987; Utans and Krämer, 1990).

As reported by Zamore and Green (1989) U2AF binds to DEAE−Sepharose at 0.15 M KCl and is eluted with 0.25 M KCl. It is then bound to heparin−Sepharose where it elutes between 0.5 and 1.0 M KCl and can be further purified to apparent homogeneity by subsequent poly(U)−Sepharose chromatography. When we included a 1.0 M KCl heparin−Sepharose step fraction (HS1000) in our assay system, no effect on splicing complex formation nor on the complete splicing reaction was observed (see Figure 4). The most likely explanation of this result was that the chromatographic behavior of U2AF in our fractionation scheme differed from that reported by Zamore and Green (1989).

U2AF activity is distinct from SF1 and SF3

A dependence of splicing complex formation and splicing on the HS1000 fraction was observed when the protein concentration of nuclear extract or DS500 that were loaded onto DEAE−Sepharose and heparin−Sepharose columns, respectively, was lowered from ~10−15 mg/ml of column volume to ~5−7 mg/ml. Under these conditions pre-splicing complexes are still assembled in the presence of DS500 only (not shown); however, no specific complexes are visible with the HS500 fraction alone (Figure 2). Efficient assembly of a pre-splicing complex (complex A) is now
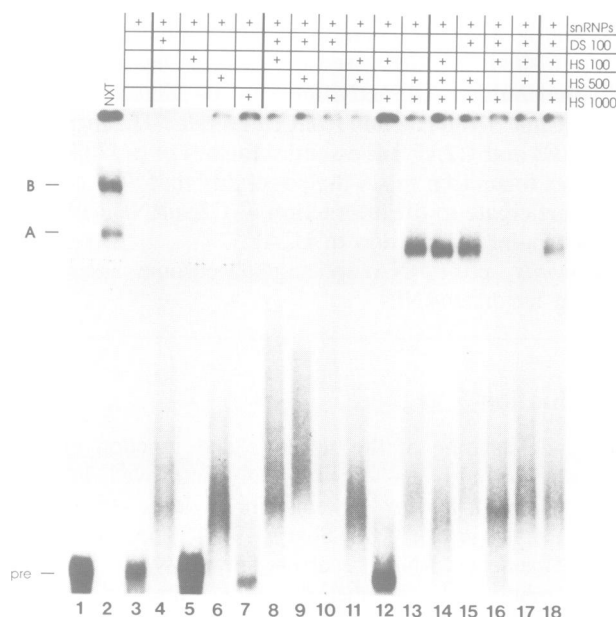
Fig. 2. Splicing complex formation with DEAE−Sepharose and heparin−Sepharose fractions. The reactions contained the following fractions: lane 1, 5 μl of buffer; lane 2, 3 μl of nuclear extract; lanes 4−13, 2 μl of the fractions indicated above the figure; lanes 14−17, 1.3 μl of each of the indicated fractions; lane 18, 1 μl of each of the fractions. In addition, the reactions shown in lanes 3−18 contained 0.5 μl each of U1 and U2 snRNP-enriched Mono Q fractions. Splicing complexes were allowed to form for 60 min at 30°C. The positions of free pre-mRNA, pre-splicing complex A and splicing complex B are indicated on the left side of the figure.

completely dependent upon the addition of the HS1000 fraction, whereas a combination of DS100 and HS500 only results in the formation of a minute amount of this complex. Also, addition of the DS100 fraction does not stimulate complex formation observed in the presence of HS500 and HS1000. (All of the reactions shown in Figure 2 were performed in the presence of partially purified snRNPs to allow optimal complex formation with fractions that are completely or partially devoid of these particles.) These
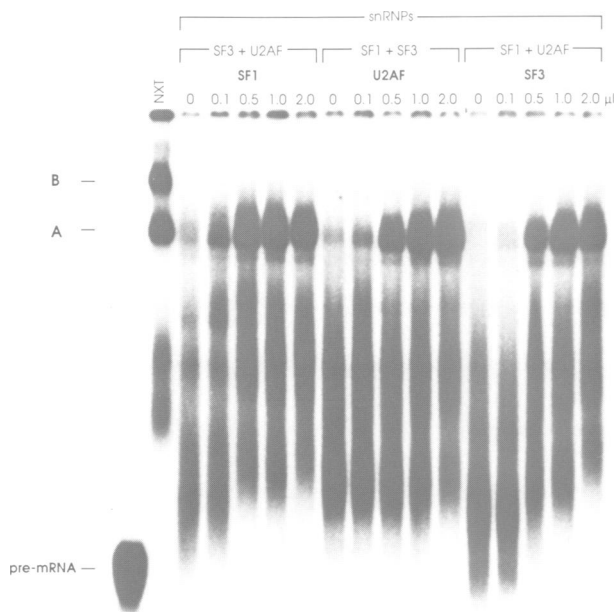
Fig. 3. Titration of SF1, SF3 and U2AF activities. Reactions (10 μl) contained 3 μl of nuclear extract (NXT), or 0.5 μl each of U1 and U2 snRNP-enriched Mono Q fractions and 1 μl each of SF1, SF3, and U2AF (as indicated). The fractions shown in bold letters were added at the concentration shown above the figure.

results demonstrate that an activity required for pre-splicing complex formation binds tightly to heparin – Sepharose and complements the HS500 fraction.

To examine further whether the activity present in the HS1000 fraction represented a third factor necessary for complex formation or whether the previously characterized factors SF1 or SF3 exhibit a different behavior when chromatographed at lower protein concentration, we fractionated the HS500 fraction on a Mono Q column. The fractions were tested for SF3 activity either in the presence of snRNPs and DS100 (SF1) or in the presence of snRNPs, DS100 and HS1000. SF3 activity was found only in assays containing a combination of snRNPs, DS100 and HS1000 and it eluted at a salt concentration of 0.22 – 0.3 M KCl (not shown) consistent with previous observations (Krämer *et al.*, 1987).

This result did not exclude the possibility that SF1 and U2AF were identical. We therefore performed an experiment in which DS100, HS1000 and a Mono Q fraction containing SF3 activity were titrated (Figure 3). With combinations of two of the fractions and snRNPs, pre-splicing complexes are assembled inefficiently or not at all. However, when all components are combined in one reaction, complexes are formed with high efficiency. This result clearly shows that DS100, Mono Q fractions and HS1000 contain different factors that all participate in pre-splicing complex formation. Thus, the previously described splicing factors SF1 and SF3 are distinct from the activity that is found in the HS1000.

The HS1000 fraction was further purified on poly(U) – Sepharose (not shown). Fractions active in complex formation contain two polypeptides of 65 and 35 kd, as expected for purified U2AF (Zamore and Green, 1989). Consistent with the results of Zamore and Green (1989), the 65 kd polypeptide can be cross-linked to the pre-mRNA substrate by irradiation with ultraviolet light (not
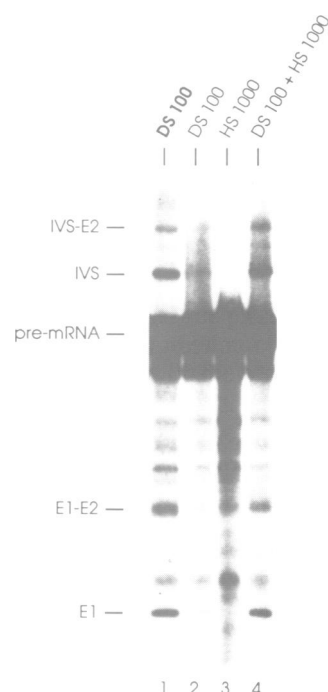

Fig. 4. Dependence of *in vitro* splicing on DS100 and HS1000 fractions. Splicing reactions (25 μl) were performed in the presence of 2.5 μl SF2 (HS100), 2.5 μl SF3 (Mono Q), 2.5 μl SF4 (Mono Q), 1.5 μl U1 snRNP and 0.5 μl U2 snRNP (Mono Q). In addition, the reactions contained: lane 1, 2.5 μl DS100 (prepared according to Krämer *et al.*, 1987); lanes 2 and 4, 2.5 μl DS100 (prepared as indicated in Materials and methods); lanes 3 and 4, 1.5 μl HS1000. Reactions were performed for 2 h at 30°C. The positions of the reaction products are indicated on the left of the figure: IVS-E2, intron–exon 2-lariat; IVS, intron-lariat; pre-mRNA, unprocessed RNA substrate; E1-E2, spliced RNA; E1, cleaved exon 1.

shown). We therefore conclude that the activity present in the HS1000 fraction represents U2AF and that this protein factor participates in pre-splicing complex formation in addition to SF1, SF3 and snRNPs.

U2AF is required for the complete splicing reaction

Pre-splicing complexes are believed to represent intermediates in the formation of the active spliceosome (Frendewey and Keller, 1985; Konarska and Sharp, 1986; Barabino *et al.*, 1989; Pruzan *et al.*, 1990). Therefore, U2AF activity should also be required for the cleavage and ligation reactions that generate spliced mRNA. Figure 4. shows that splicing in the presence of partially purified splicing factors is dependent on the presence of the HS1000 fraction containing U2AF (lanes 3 and 4). A similar result was obtained with purified U2AF that was prepared by poly(U) – Sepharose and Mono Q chromatography (not shown). Thus, U2AF not only functions in pre-splicing complex assembly, but is also necessary for the complete splicing reaction. This supports the idea that pre-splicing complexes are true intermediates in the splicing pathway.

In the experiment shown in Figure 4 we also tested a DS100 fraction that was prepared according to the previously published protocol (Krämer *et al.*, 1987). This fraction is fully active in splicing (lane 1), whereas a DS100 fraction obtained by the modified procedure is inactive in the absence of HS1000 (lane 2). Thus, in our previous fractionation
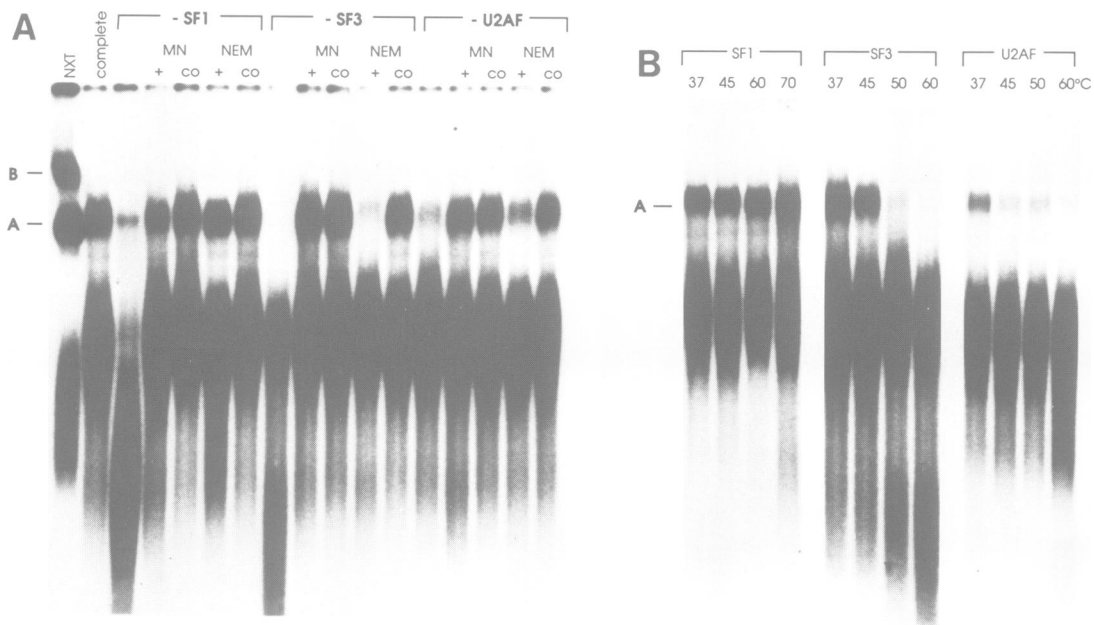
Fig. 5. Characterization of splicing factors SF1, SF3 and U2AF. **A.** The reactions contained the following components: 3 μl of nuclear extract (NXT); 0.5 μl each of U1 and U2 snRNP, and 1 μl each of SF1 (DS100), SF3 (Mono Q) and U2AF (poly(U)−Sepharose). As indicated above the figure, single fractions were either omitted from the reaction, or added after treatment with micrococcal nuclease (MN +), mock-treatment with micrococcal nuclease (MN co), or after treatment with N-ethylmaleimide (NEM +), or mock-treatment with N-ethylmaleimide (NEM co). **B.** Reaction parameters were as in (A). The fractions shown above the figure were incubated for 15 min at the indicated temperature prior to their addition to the remaining constituents of the reaction.

scheme (at least part of) U2AF was present in the DS100 fraction together with SF1.

Characterization of SF1, SF3 and U2AF activities

To characterize SF1, SF3 and U2AF further, each factor was subjected to treatment with micrococcal nuclease, N-ethylmaleimide or elevated temperature and then added to the remaining components of the reaction. Consistent with previous results (Ruskin et al., 1988; Krämer, 1988) neither of the factors is susceptible to micrococcal nuclease digestion and therefore not associated with an essential RNA moiety (Figure 5A). In support of this assumption neither of the fractions contains detectable levels of snRNAs or other RNA species (not shown). Treatment of the fractions with N-ethylmaleimide results in a marked reduction of the complex-forming activity of SF3 and U2AF; however, SF1 activity is not affected. This suggests that SF3 and U2AF, but not SF1 require sulfhydryl groups for their activity. When the fractions are treated at elevated temperature (Figure 5B), it is apparent that SF1 activity is heat-stable up to 70°C, SF3 activity is abolished at temperatures above 45°C, and U2AF activity is markedly decreased when preincubated at 45°C or higher. These results suggest that all three factors are proteins which exhibit different sensitivities to heat.

Presence of U2AF, SF1 and SF3 in the cytoplasmic S-100 fraction

U2AF activity was initially assayed by complementation of a post-nuclear S-100 fraction (Ruskin et al., 1988; Zamore and Green, 1989). We have shown that an S-100 fraction can substitute for SF1 but not for SF3 activity (Krämer, 1988). To re-examine the distribution of the three factors in the S-100 fraction, two experiments were performed. First, the S-100 fraction was used in a splicing complex reaction as a substitute for either SF1, SF3 or U2AF (Figure
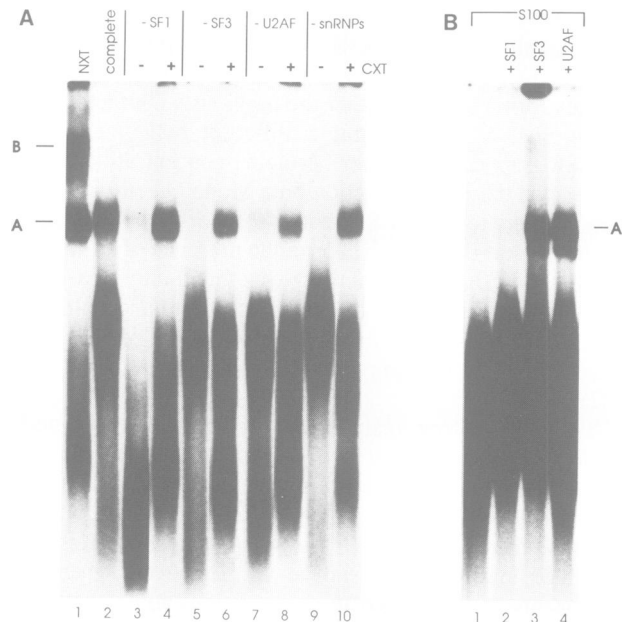
Fig. 6. Presence of splicing factors in the postnuclear S-100. **A.** Reactions were assembled in the presence of 3 μl of nuclear extract (NXT; lane 1), or 0.5 μl each of U1 and U2 snRNP-enriched Mono Q fractions and 1.3 μl each of SF1 (DS100), SF3 (Mono Q), and U2AF (Mono Q) (lane 2). Single fractions were omitted from the reactions as shown above the figure and substituted with 1.3 μl of S-100 where indicated. **B.** Reactions were performed in the presence of 2.5 μl of S-100, and 2.5 μl of SF1 (DS100), SF3 (Mono Q) or U2AF (Mono Q) were added as indicated.

6A). The result shows that all factors are present in the S-100 fraction, however at different concentrations. It is also apparent that the S-100 fraction contains active snRNPs
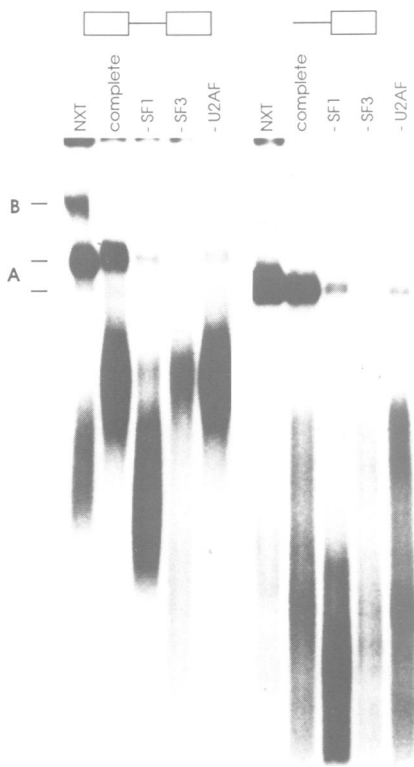
Fig. 7. Splicing complex formation with altered RNA substrates. Reactions were performed in the presence of 3 μl of nuclear extract (NXT), or 0.5 μl each of U1 and U2 snRNP-enriched Mono Q fractions and 1.3 μl each of SF1 (DS100), SF3 (Mono Q), and U2AF [poly(U)−Sepharose]. Single fractions were omitted from the reactions as indicated. The RNA substrates (see Materials and methods) are schematically shown above the figure. Boxes and lines indicate exons and introns, respectively.

(lanes 9 and 10). In the second experiment the S-100 fraction was complemented with the single factors (Figure 6B). Pre-splicing complexes are formed in the presence of SF3 or U2AF (lanes 3 and 4); however, the addition of SF1 does not result in the assembly of a specific complex (lane 2). Thus, SF1 is present in the S-100 in a concentration sufficient to allow the formation of a pre-splicing complex when either SF3 or U2AF are added. Hence SF3 and U2AF appear to be present in limiting quantities such that complex assembly can only be detected when one or the other is added to the reaction.

SF1, SF3 and U2AF are required for an interaction at the 3' splice site

The assembly of a functional spliceosome requires consensus sequences at the 5' and 3' splice sites, whereas pre-splicing complex formation only requires the presence of a 3' splice site (Frendewey and Keller, 1985; Konarska and Sharp, 1986). In previous experiments we have shown that SF1, SF3 and snRNPs are essential for the formation of a pre-splicing complex on an RNA substrate that lacks a 5' splice site (Krämer, 1988). Since the SF1 fractions used in those experiments also contained U2AF, we examined splicing complex formation with the fractionated protein factors to investigate whether all three are required for pre-splicing complex assembly with an RNA substrate containing only a 3' splice site. The results shown in Figure 7 indicate that pre-splicing complexes with this substrate are assembled

efficiently only when all factors are combined in one reaction. Thus, SF1, SF3, U2AF and snRNPs have to interact with the pre-mRNA and/or with one another to initiate splicing complex assembly. Furthermore, the site of interaction lies within the sequences encompassing the 3' splice site and binding of these components does not require a 5' splice site.

Discussion

Here we report the separation of U2AF (Ruskin et al., 1988) from two activities (SF1 and SF3) that were shown to function early in the splicing reaction in the formation of a pre-splicing complex (Krämer, 1988). In the absence of detailed information about the chromatographic properties of U2AF, we assumed that U2AF and SF3 may be identical. Both activities could be detected in the nuclear but not in the cytoplasmic fraction prepared from HeLa cells and eluted from a Mono Q column at similar salt concentrations (Ruskin et al., 1988; Krämer, 1988). When the purification protocol for U2AF was published (Zamore and Green, 1989), however, a relationship between these factors became less likely.

We subsequently found that it is necessary to decrease the protein concentration of the material that is loaded onto DEAE− and heparin−Sepharose to bind U2AF efficiently. As shown by Zamore and Green (1989) U2AF can be eluted from DEAE−Sepharose with 250 mM KCl, i.e. it does not bind very tightly to the resin. This may explain why U2AF appears in the DEAE−Sepharose flowthrough fractions when the column is loaded at protein concentrations exceeding 5 mg/ml of column volume. With the modified protocol, U2AF activity can clearly be separated from SF1 and SF3. Hence, after fractionation on heparin−Sepharose the HS1000 fraction (containing U2AF) is essential for pre-splicing complex assembly and no specific complexes are formed in its absence.

The DS100 fraction, which was previously shown to contain at least one activity (SF1) required for pre-splicing complex formation, does not contribute to complex formation before fractionation on Mono Q. Thus, part of SF1 activity binds to both the DEAE− and heparin−Sepharose columns. SF1 can however be separated from SF3 by chromatography on Mono Q, where it elutes in the flowthrough fractions (data not shown); SF3 activity elutes between 0.22 and 0.3 M KCl (Krämer et al., 1987; Krämer, 1988). At this stage of the purification, pre-splicing complex formation only occurs in the presence of the DS100 fraction, U2AF (HS1000) and SF3-containing Mono Q fractions. Thus, SF1, SF3 and U2AF represent three distinct factors that participate in early complex assembly. In support of this conclusion, the factors can be distinguished by their sensitivity to heat and to treatment with N-ethylmaleimide.

Previous work demonstrated that U2AF mediates the interaction of U2 snRNP with the branch site located upstream of a 3' splice site (Ruskin et al., 1988). This conclusion was based on results obtained by immunoprecipitation of RNase-resistant pre-mRNA fragments with antibodies that recognize snRNP particles. From the available data it is not clear whether the interaction between U2AF, U2 snRNP and the branch point seen in the RNase protection assay corresponds to the formation of a pre-splicing complex that is detected upon electrophoresis in native polyacrylamide gels. Additional steps may exist that convert a complex consisting of U2 snRNP, U2AF and RNA substrate into the

pre-spliceosome. It has, for example, been shown that purified U1 snRNP binds to the pre-mRNA in the absence of other components (Mount *et al.*, 1983); however, this interaction escapes detection in most electrophoretic systems, including the one used here (Konarska and Sharp, 1986; unpublished data). In our hands, purified U2AF and U2 snRNP do not cause a retardation of the pre-mRNA substrate in native polyacrylamide gels (unpublished results), which may indicate that the initial binding of U2 snRNP and U2AF to the pre-mRNA is indeed disrupted during gel electrophoresis. Alternatively, this could mean that factors in addition to U2AF are required for the association of U2 snRNP with the RNA substrate.

The experimental design employed earlier for the analysis of U2AF function does not exclude this possibility. Ruskin *et al.* (1988) used either micrococcal nuclease-treated nuclear extracts or protein-containing fractions obtained after CsCl-gradient centrifugation as a source of U2AF. Conceivably, these fractions could contain additional protein factors that may be involved in the interaction of U2 snRNP with the branch point. This also applies to the Mono Q fractions (obtained after chromatography of nuclear extract) that were used in some of their experiments. U2AF and SF3 elute from the column at 0.18−0.25 and 0.22−0.3 M KCl, respectively (unpublished data; Ruskin *et al.*, 1988; Krämer, 1988), and therefore these activities probably overlap in certain fractions when nuclear extract is directly applied to a Mono Q column.

To demonstrate that U2AF functions in pre-splicing complex assembly Zamore and Green (1989) used an S-100 fraction as a source of U2 snRNP. Our S-100 complementation experiments support their conclusion; however, apart from active snRNPs we also find SF1 and SF3 activities in this fraction. Although different preparations of S-100 vary in the amount of SF3 and U2AF that can be detected, we consistently find SF1 activity in this extract. Apparently this activity is easily lost from the nuclei during extract preparation (Krämer and Keller, 1985). In light of the fact that all three protein factors are necessary for the formation of a pre-splicing complex, it is highly likely that SF1 and/or SF3 are essential for the binding of U2 snRNP to the branch site in addition to U2AF or are required to stabilize the interaction.

Recent evidence accumulated both in mammalian and yeast splicing systems has revealed that an interaction of U2 snRNP with the branch point depends on the presence of U1 snRNP (Zillmann *et al.*, 1988; Legrain *et al.*, 1988; Ruby and Abelson, 1988; Seraphin and Rosbash, 1989; Barabino *et al.*, 1990). These observations indicate that U1 snRNP could also play a role in branch site recognition. By performing RNase protection assays such as those employed by Ruskin *et al.* (1988) it should be possible to determine whether SF1, SF3 and/or U1 snRNP also contribute to the interaction of U2 snRNP with the pre-mRNA substrate.

Materials and methods

Extract preparation and fractionation of splicing activities
Splicing activities were fractionated from HeLa cell nuclear extracts (Dignam *et al.*, 1983) as described (Krämer *et al.*, 1987; Krämer and Keller, 1990) except that the DEAE−Sepharose Fast Flow and heparin−Sepharose columns were loaded with ~5−7 mg protein/ml column volume.

Fractionation of U2AF
For poly(U)−Sepharose (Pharmacia/LKB) chromatography of the HS1000 fraction, 50 mg of protein were loaded onto a 10 ml column. Fractionation

was performed in buffer B (Krämer and Keller, 1990) according to Zamore and Green (1989). Poly(U)−Sepharose fractions were further chromatographed on a 1 ml Mono Q column (Pharmacia/LKB) which was developed with a 20 ml gradient of 100−400 mM KCl in buffer B. U2AF activity elutes at a concentration of 180−250 mM KCl.

In vitro splicing assays
Splicing substrates were prepared by *in vitro* transcription with SP6 RNA polymerase (Krämer, 1988). All splicing reactions were performed with a pre-mRNA derived from the AdML transcription unit containing 5′ and 3′ splice sites of intron 1 (RNA 1; Frendewey and Keller, 1985). For the reactions shown in Figure 7, an RNA substrate that lacks the 5′ splice site (RNA 10; Frendewey and Keller, 1985) was used in addition.

Assays for splicing complex formation and splicing have been described (Krämer, 1988; Utans and Krämer, 1990). The total volume of the reactions was 10 μl when splicing complexes were analyzed and 25 μl when a complete splicing reaction was performed. The amount of extract or column fractions used is indicated in the figure legends. Incubation was at 30°C for 30 min unless noted otherwise. Splicing complexes were separated in a 4% polyacrylamide gel (acrylamide:bis-acrylamide = 80:1) containing 25 mM Tris, 25 mM boric acid and 1 mM EDTA. Splicing reaction products were analyzed in a 12% polyacrylamide/8.3 M urea gel.

Protein determination
The protein content of extracts and column fractions was determined according to Bradford (1976) with gamma-globulin as a standard (BioRad).

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