A 10S galectin-3–U1 snRNP complex assembles into active spliceosomes

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ABSTRACT

In previous studies, we reported that fractionation of HeLa cell nuclear extracts on glycerol gradients revealed an endogenous ~10S particle that contained galectin-3 and U1 snRNP and this particle was sufficient to load the galectin polypeptide onto a pre-mRNA substrate. We now document that this interaction between the galectin-3–U1 snRNP particle and the pre-mRNA results in a productive splicing complex, leading to intermediates and products of the splicing reaction. Nuclear extracts were depleted of U1 snRNP with a concomitant loss of splicing activity. Splicing activity in the U1-depleted extract can be reconstituted by the galectin-3–U1 snRNP particle, isolated by immunoprecipitation of the 10S region (fractions 3–5) of the glycerol gradient with anti-galectin-3 antibodies. In contrast, parallel anti-galectin-3 immunoprecipitation of free galectin-3 molecules not in a complex with U1 snRNP (fraction 1 of the same gradient), failed to restore splicing activity. These results indicate that the galectin-3–U1 snRNP-pre-mRNA ternary complex is a functional E complex and that U1 snRNP is required to assemble galectin-3 onto an active spliceosome.

INTRODUCTION

Five splicesomal snRNAs (U1, U2, U4, U5 and U6), assembled as ribonucleoprotein (RNP) complexes with core Sm and U-specific proteins (1,2), are found in the nucleus as mono-particles (U1 and U2) or associated with other snRNPs as multimers (U4/U6 and U4/U6.U5). These snRNP complexes in conjunction with pre-messenger RNA (pre-mRNA), and many other proteins, orchestrate the chemistry of intron removal and exon ligation in the spliceosome to produce mature mRNA (3,4).

Kinetic studies of pre-mRNA splicing and spliceosome formation lend evidence to an ordered assembly of the snRNP components of the spliceosome (5,6). Recent studies in yeast have suggested an additional spliceosome assembly pathway, which appears to be dependent on splicing substrate (6). In the canonical assembly pathway, the critical first step in spliceosome formation is the binding of U1 to the 5′ splice site of the pre-mRNA in an ATP-independent manner (5,7,8). Following the loading of U1 snRNP onto the pre-mRNA, other snRNPs can then be added to the initial complex in an ATP-dependent manner, resulting in a catalytically active spliceosome that generates the mature mRNA product (8). However, this early U1-pre-mRNA complex is not necessarily committed to the splicing pathway as this complex may be an H particle that cannot enter the splicing pathway (5,7). A functional complementation assay that assesses the production of mature mRNA and splicing intermediates can distinguish between these two possibilities.

The galectin family of saccharide-binding proteins was initially defined on the basis of two key criteria: binding affinity for β-galactosides and significant sequence similarity in the carbohydrate recognition domain (9). There are now 15 mammalian galectins that show tissue-specific expression. In addition to binding to galactose-containing glycoconjugates, many members of the galectin family share another property. They exhibit dual localization (10), being found in both the intracellular (cytoplasm and nucleus) as well as the extracellular (cell surface and medium) compartments. The most extensively studied members of the galectin family are galectin-1 (Gal1) and galectin-3 (Gal3) and there is a large body of literature documenting the nuclear localization of these polypeptides, as well as finding them at the cell surface or in the extracellular medium (reviewed in ref. (11,12)). The literature on the galectins has been dominated by studies focused on their activity on the extracellular side, based on their binding to cell surface glycoconjugates and adhesive glycoproteins of the extracellular matrix (integrins, laminin, etc.). Thus, Gal1 and Gal3 have been shown to modulate, both positively and negatively, cell–cell or cell–substratum adhesion and the consequences of such activities on cell proliferation and cancer metastasis (11–13).

The nuclear localization of Gal3, coupled with its cosedimentation with hnRNPs and snRNPs, provided the initial hint that it might play a role in pre-mRNA splicing...
(14,15). When both Gal1 and Gal3 are depleted from HeLa nuclear extracts (NE), splicing is abrogated at the earliest stage of spliceosome assembly (16,17). Addition of either galectin to the depleted NE restores splicing activity. Both galectins have been found associated with spliceosomes (18). In addition, Gal1 and Gal3 are associated with snRNPs outside the splicing pathway (i.e. antibodies to either galectin precipitate snRNPs in the absence of splicing substrate, 19).

We previously showed that an endogenous ~10S particle isolated by glycerol gradient fractionation of HeLa NE was composed of Gal3 and U1 snRNP. The Gal3–U1 snRNP particle bound to a splicing substrate in a snRNA-dependent and splice site-dependent manner (19), consistent with models of ordered spliceosome formation. Our hypothesis was that this U1–Gal3 loaded pre-mRNA complex can proceed into a catalytically active spliceosome. In this communication we show that indeed the Gal3–U1 snRNP–pre-mRNA complex is functional as it complements a U1 snRNP-depleted splicing extract (U1∆NE) and forms intermediates and products of the splicing pathway.

MATERIALS AND METHODS

General laboratory procedures and reagents

We have recently described in detail the following general procedures used in the cell-free splicing assays carried out in our laboratory (20): (i) preparation of NE from HeLa cells according to the method of Dignam et al. (21); (ii) preparation of the MINX splicing substrate (22); (iii) assembly of the splicing reaction mixture and (iv) digestion of the reaction mixture with proteinase K, extraction of RNA, and analysis by denaturing gel electrophoreses. In these procedures, the compositions of the buffers were: (i) buffer C is 0.42 M NaCl, 20 mM HEPES (pH 7.9), 0.2 mM EDTA, 0.5 mM DTT and 0.5 mM PMSF; (ii) buffer D is 0.1 M KCl, 20 mM HEPES (pH 7.9), 20% (v/v) glycerol, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT and 0.5 mM PMSF; (iii) buffer E is 0.1 M KCl, 20 mM HEPES (pH 7.9), 20% (v/v) glycerol, 0.2 mM EDTA, 0.5 mM DTT and 0.5 mM PMSF and (iii) 60% buffer D is 60% buffer D and 40% H2O.

The snRNAs were separated by denaturing gel electrophoreses as described (19). The gels were either stained with ethidium bromide or subjected to northern blotting. For ethidium bromide staining, the gels were briefly washed in H2O, then incubated with approximately 100 ml of an ethidium bromide solution (1 μg/ml) with gentle rotation for 30 min at room temperature. After staining, the gels were washed quickly in H2O, then destained for approximately 5 min using approximately 100 ml of H2O with gentle rotation. For Northern blotting, the procedures for transfer to Hybond-N nylon membranes, UV cross-linking, and hybridization, as well as the synthesis and labeling of oligonucleotide probes specific for each of the U-snRNAs, have all been detailed (19).

Polyclonal antisera directed against Gal3 were developed in two rabbits; the characterization of the antisera from rabbit #24 (23) and of the antisera from rabbit #49 (19) has been reported. When NE was subjected to immunoblotting with either of these antisera, a single polypeptide (~30 kDa) corresponding to Gal3 was obtained. More importantly, when NE is fractionated on a glycerol gradient (see below), both of these antisera immunoprecipitated a 10S particle containing Gal3 and U1 snRNP from fractions 3–5 of the gradient (19).

The following antibodies were used for immunoblotting: (i) affinity purified rabbit anti-PSF (pyrimidine track binding protein associated splicing factor) from Santa Cruz Biotechnology; (ii) mouse monoclonal anti-U1-70K protein from Synaptic Systems; (iii) human autoimmune sera ENA anti-Sm from The Binding Site and (iv) rat monoclonal anti-Mac2 specific for Gal3 (23). The details of the immunoblotting procedures have been described (16,17).

Glycerol gradient sedimentation of NE

This was carried out as previously described (19). Briefly, a 250 μl reaction mixture containing 150 μl of NE, 3 mM MgCl2, 0.5 mM ATP, and 20 mM creatine phosphate was incubated for 30 min at 30°C and loaded onto a 5 ml glycerol gradient (12-32% glycerol in 60% Buffer D without glyc- erol). Centrifugation was carried out in a Beckman SW50.1 rotor at 44 000 rpm at 4°C for 3.5–4 h. Gradient fractions (250 μl each) were collected from the top. We routinely fractionate freshly prepared NE on six parallel glycerol gradients in one centrifuge run. A small aliquot of one gradient of each centrifuge run was taken to monitor the profile of proteins across the gradient. We have found these gradients to be reproducible. The profiles of U1-70K protein and of Gal3 in the relevant fractions of the gradient used for the experiments of the present report are documented in Supplementary Figure S1 (see Supplementary data).

Preparation of NE depleted of U1 snRNP

To prepare U1∆NE, human autoimmune serum specific for U1 snRNP (Hu ENA-RNP, #33471 from The Binding Site) was covalently cross-linked (19) using 100 μl antiserum to 100 μl protein A-Sepharose CL-4B beads (Amersham Biosciences). After washing, 100 μl antibody-conjugated beads were incubated with HeLa NE (200 μl) in buffer C for 60 min at 4°C with rotation. The unbound fraction (U1∆NE) was removed, dialyzed against 60% buffer D and snap frozen. The beads were washed twice in 60% buffer D and materials bound (proteins and snRNAs) were removed with 2× SDS-PAGE sample buffer, extracted and analysed in terms of protein and RNA components. For use in the splicing complementation experiments (described below), the anti-U1 beads were washed once quickly in 0.5 ml of 60% buffer D to remove the high salt conditions, then added to the splicing reaction.

Reconstitution of splicing activity by Gal3-U1 snRNP and U1 snRNP

To assay for splicing activity, U1∆NE was incubated at 30°C with 32P-labeled MINX (22) splicing substrate, ATP and creatine phosphate. For splicing complementation experiments, protein A-Sepharose beads (50 μl) conjugated with anti-Gal3 rabbit serum were incubated with 60% buffer D, glycerol gradient fraction 1 or fractions 3–5 (150 μl) at 4°C for 60 min with rotation. After removal of the unbound material, U1∆NE (8 μl), 32P-labeled MINX, ATP and creatine phosphate were added to the beads to a final volume...
24 µl. After incubation at 30°C for 90 min to allow splicing, an equal volume of 2× SDS PAGE sample buffer was added. The mixture was heated at 100°C for 7 minutes to elute any materials of the beads. The eluted material was transferred to a new microtube followed by proteinase K digestion for 40 min at 37°C. RNA was then extracted and analyzed (20). Similar reconstitution experiments were performed with protein A-Sepharose beads conjugated with human autoimmune serum specific for U1 snRNP except these beads were incubated with NE as described for depletion of U1 snRNP. After removal of the unbound material and washing the beads in 60% buffer D, U1ΔNE (8 µl), 32P-labeled MINX, ATP and creatine phosphate were added to the beads to a final volume 24 µl and incubated at 30°C for 90 min to allow splicing to occur. As shown in Supplementary Figure S2 (lane 3), the bead bound U1 snRNP was able to reconstitute splicing activity in the U1ΔNE indicating that splicing complexes bound to beads could be used to complement a splicing deficient nuclear extract (Supplementary Figure S2, lane 2).

RESULTS

We have previously reported the fractionation of HeLa NE on glycerol gradients (19) with the following key findings: (i) fractions 3 through 5 (~10S) contain U1 snRNP some of which is bound to Gal3, and no other U-snRNPs are found in these fractions; (b) fraction 1 contains free Gal3 and no U1 snRNP. In these studies, we also showed that the 10S Gal3–U1 snRNP particle formed with a pre-mRNA substrate in a splice-site dependent manner (19). To distinguish whether this association with pre-mRNA resulted in a functional E complex as opposed to a dead end H complex (5,7), two reagents are needed: (i) a splicing extract specifically depleted of U1 snRNPs (U1ΔNE) and (ii) a fraction enriched with Gal3–U1 snRNPs but devoid of U1 snRNPs not associated with Gal3. These two components were prepared as follows.

To generate U1ΔNE, NE was prepared in Buffer C of Dignam et al. (21). Under this condition of ionic strength (0.42 M NaCl), the NE was incubated with protein A-Sepharose beads containing Hu ENA-RNP antibodies. Northern blotting of the material remaining bound to the beads after washing revealed U1 snRNA but little/no U2, U4, U5, U6 snRNAs (Figure 1A, lane 1) or 5S rRNA (not shown). In the material not bound to the beads (i.e. the U1ΔNE) the signal for U1 snRNA was greatly reduced while the signals for U2, U4, U5, U6 snRNAs (Figure 1A, lane 2) and 5S RNA (not shown) were comparable to those observed in the original NE (Figure 1A, lane 3). Quantitation of the northern blots documented that <10% of U1 remained in the U1ΔNE. Corroborating these results, the U1-70K protein, a polypeptide unique to U1 snRNP, was prominent in the western blots of the bound fraction (Figure 1B, lane 1) but greatly diminished in the unbound fraction (Figure 1B, lane 2). On the other hand, the core polypeptides of the snRNPs (e.g. Sm B'/B and D) were observed in both the bound (due to U1 snRNP) as well as the unbound fractions (due to U2, U4/U6 and U5 snRNPs) (Figure 1B, lanes 1 and 2). The splicing factor PSF, which has previously been shown to interact with snRNPs outside the spliceosome (24), was not removed by the #33471 autoimmune serum under these conditions. Finally, Gal3 was not detected in the bound material as it is dissociated from the U1 snRNP at this salt concentration (19). These results indicate that the unbound fraction represents a NE depleted of U1 snRNP (U1ΔNE).

This U1ΔNE lacked splicing activity when incubated with 32P-labeled MINX (Figure 1C, lane 2), consistent with the requirement of U1 snRNP-mediated recognition of 5′-splice site in the early step of spliceosome assembly (25). When a small amount of complete NE (0.7 µl) was added to the U1ΔNE, splicing activity was restored (Figure 1C, lane 3). The same amount of complete NE (0.7 µl) did not exhibit splicing activity when incubated alone (Figure 1C, lane 4). The lack of splicing activity in the small amount of complete NE (0.7 µl) cannot be ascribed to a simple ‘mass effect,’ inasmuch as addition of bovine serum albumin (BSA) equivalent to the total amount of protein found in U1ΔNE also failed to exhibit splicing activity (Supplementary Figure S3, lane 3). Finally and most importantly, we determined that U1ΔNE could be complemented with bead-bound U1 snRNP derived from the depletion procedure (Supplementary Figure S2, lane 3).

Previous studies had documented that a Gal3–U1 snRNP complex can be isolated by anti-Gal3 immunoprecipitation of the ~10S region (fractions 3–5) when NE is fractionated over a glycerol gradient (19). Immunoprecipitation of fractions 3–5 with anti-Gal3 serum showed that the U1-70K and Sm B'/B' and D polypeptides, as well as the cognate antigen Gal3, are bound to the beads along with a portion of the U1 snRNA found in these fractions. Thus, the anti-Gal3-bound material from fractions 3–5 contained Gal3–U1 snRNP complexes. In contrast, the corresponding anti-Gal3 bound material from fraction 1 of the gradient contained Gal3 but no U1 snRNA or associated proteins (19), serving as one control in the splicing complementation assays (see below).

Using these two reagents (U1ΔNE and immunoprecipitated Gal3–U1 snRNP complexes from fractions 3–5), we tested whether the Gal3–U1 snRNP complex (bound to anti-Gal3- Sepharose beads) was functional in assembling a catalytically active spliceosome and producing spliced products and intermediates. The immobilized Gal3–U1 snRNP complex was incubated with 32P-labeled MINX and U1ΔNE under splicing conditions. Splicing activity in the U1ΔNE was reconstituted by the bead-bound complex (Figure 2A, lane 6). Both products (ligated exons and excised intron lariat [denoted by arrows to right of lane 6]) as well as intermediates (exon 1 and lariat-exon 2) of the splicing reaction were observed. Several controls were critical in arriving at this conclusion. First, the precipitated fraction of a sham immunoprecipitation procedure, carried out with anti-Gal3 in 60% Buffer D, failed to reconstitute splicing in the U1ΔNE (Figure 2A, lane 2). This suggests that the anti-Gal3 beads were not responsible for reconstitution of splicing activity. Second, anti-Gal3 immunoprecipitation of fraction 1 of the glycerol gradient did not reconstitute splicing activity in the U1ΔNE (Figure 2A, lane 4). The compositions of the snRNAs in the splicing reaction mixtures were analyzed by ethidium bromide staining. This analysis revealed that the anti-Gal3 bound material from fraction 1
Figure 1. Characterization of U1ΔNE. Panel A. Northern analysis of snRNAs in material bound to autoimmune serum specific for U1 snRNP (Hu ENA-RNP #33471) beads (lane 1), in unbound material after adsorption to Hu ENA-RNP #33471 beads (U1ΔNE, lane 2) and in complete NE (lane 3). Lane 1 represents material from twice the volume of unbound material and NE. Panel B. Western analysis of representative proteins in material bound to Hu ENA-RNP #33471 beads (lane 1), unbound material after adsorption to Hu ENA-RNP #33471 beads (U1ΔNE, lane 2) and complete NE (lane 3). Note that Gal3 is not detected in the bound fraction as it is released from U1 snRNP at 0.42 M NaCl used for the depletion. Panel C. Splicing activity of 3 μl complete NE (lane 1); 3 μl U1ΔNE (lane 2); 3 μl U1ΔNE plus 0.7 μl NE (lane 3) and 0.7 μl NE alone (lane 4).

has no U1 snRNA (Figure 2B, lanes 3 and 4), confirming the notion that the Gal3 protein in fraction 1 represents free Gal3 and is not in an snRNP complex (19). These results suggest that the pre-mRNA substrate can be bound to the immobilized Gal3-U1 snRNP from gradient fractions 3–5 to initiate the splicing pathway. Immunoprecipitates from fraction 1 and fractions 3–5 did not exhibit splicing activity when assayed in the absence of U1ΔNE (Figure 2A, lanes 3 and 5, respectively). Ethidium bromide staining of the splicing gel reveals that U1 snRNA was absent in the U1ΔNE (Figure 2B, lanes 2 and 4) and nearly normal levels were found in the U1ΔNE complemented with the anti-Gal3 precipitate of fractions 3–5 (Figure 2B, lane 6).

All experiments documented above were conducted with polyclonal antibodies against Gal3 derived from rabbit #49. A key consideration in the significance of these results is the generality and specificity of the anti-Gal3 antibodies in precipitating the Gal3-U1 snRNP complex to reconstitute splicing activity in the U1-depleted NE. We found that another rabbit antiserum (#24) yielded the same results as the antiserum from rabbit #49 (Figure 3, compare lanes 3 and 4). Finally, pre-immune serum from rabbit #49 (PI 49) was unable to reconstitute splicing in the U1ΔNE following incubation with fractions 3–5 (Figure 3, lane 5).

Together, the present results indicate that an endogenous Gal3-U1 snRNP ∼10S particle isolated from splicing extracts can form a productive complex with a pre-mRNA substrate, initiating spliceosome assembly that progresses into the intermediates and products of the splicing reaction dependent on factors supplied by the U1ΔNE. Thus, Gal3 can enter the splicing pathway through its association with U1 snRNP.

DISCUSSION

The key conclusion derived from the experiments documented in the present report is that the Gal3–U1 snRNP binds to a pre-mRNA splicing substrate to form a functional E complex. Splicing is a complex series of nuclear events requiring a pre-mRNA substrate, five snRNP particles and numerous proteins. The criterion used to determine whether a newly isolated/characterized protein/RNA is a required splicing factor relies on depletion-reconstitution experiments. Gal1 and Gal3 have been shown to be required splicing factors using this criterion (16,17). Splicing activity and initiation of spliceosome complex formation are abrogated when both galectins are removed. Both activities are restored when either galectin is added to the double galectin-depleted NE suggesting that Gal1 and Gal3 are functionally redundant. Both galectins are associated with spliceosomal complexes throughout the splicing pathway as antibodies to either galectin immunoprecipitated the pre-mRNA substrate, splicing intermediates and products (18).

Initiation of pre-mRNA splicing requires several components – notably a U1 snRNP particle and a splicing substrate with an intron having a splice site complementary to the 5′-end of U1 snRNP. When either partner is absent (see Figure 1C, lane 2; and 26) or complementarity does not exist (19,27) splicing is not initiated. Gal3 is another component necessary for splicing initiation as depletion from nuclear extracts abolished splicing at an early step (16). We
have shown that Gal3 associates with snRNPs in the absence of spliceosome formation (i.e., outside the splicing pathway, 19). Specifically, Gal3 and U1 snRNP are assembled into an ~10S complex in nuclear extracts and this complex binds to a pre-mRNA splicing substrate (19). Is this association of Gal3 with free nucleoplasmic U1 snRNP the mechanism for initiation of spliceosome formation and entry of Gal3 into the splicing pathway?

The data presented document that this Gal3-U1 snRNP binds to the pre-mRNA substrate and this complex complements a U1 snRNP-depleted NE. This functional E complex initiates spliceosome formation and the splicing cascade, which results in the production of splicing intermediates and products. Thus, Gal3’s association with spliceosomes is mediated through its binding to the U1 snRNP.

In the initial characterizations of the U-snRNPs, buffers of high ionic strength (250 mM salt or greater) were used for chromatographic separation to isolate mono-particles as well as stable RNA-protein complexes (28–30). Similar studies on spliceosomal complexes have established their core components (31–34). Splicing reactions were initiated (60 mM salt is optimal for splicing [see Dignam et al. (21)]) and then stopped at various steps. In these studies, complexes were isolated (usually via affinity adsorption to an immobilized ligand/antibody) and then washed with high salt buffers (again 250 mM or higher) before analysis of

Figure 2. Analysis of complementation of splicing activity in U1 ∆NE.

Panel A: Beads containing anti-Gal3 (αGal3) were used to immunoprecipitate glycerol gradient fractions 1 (Fr 1), fractions 3–5 (Fr 3–5), or 60% Buffer D (60%D). The presence or absence of each of these precipitates in reaction mixtures attempting to complement the splicing activity of U1 ∆NE (60%D). The presence or absence of each of these precipitates in reaction mixtures attempting to complement the splicing activity of U1 ∆NE is indicated by the + or – signs above the bold solid line. The presence of NE or U1 ∆NE in the splicing reaction mixture is indicated by the + or – signs below the bold solid line. Lane 1: 4 μl NE in a splicing assay volume of 12 μl, all of which were subjected to gel analysis. For lanes 2–6, the total splicing assay volume was 24 μl, half of which were subjected to gel analysis. Lane 2: 8 μl U1 ∆NE and beads from precipitation of Fr 1; Lane 3: 8 μl U1 ∆NE and beads from precipitation of Fr 3–5; Lane 4: 8 μl U1 ∆NE and beads from precipitation of Fr 3–5; Lane 5: 8 μl U1 ∆NE and beads from precipitation of Fr 3–5 by preimmune serum of rabbit #49. Arrows point to products of the splicing reaction.

Panel B: The snRNA composition of splicing reactions for each lane shown in Panel A, as revealed by ethidium bromide staining. The positions of migration of U1, U2, U4, U5 and U6 snRNAs are indicated on the left.

Figure 3. Analysis of complementation of splicing activity in U1 ∆NE using various antisera for selection of Gal3–U1 snRNP particles. Splicing reactions were carried out in the presence or absence of immunoprecipitates as indicated by the + or – signs above the bold solid line. The presence of NE or U1 ∆NE in the splicing reaction mixture is indicated by the + or – signs below the bold solid line. Lane 1: 4 μl NE in a splicing assay volume of 12 μl, all of which were subjected to gel analysis. For lanes 2–5, the total splicing assay volume was 24 μl, half of which were subjected to gel analysis. Lane 2: 8 μl U1 ∆NE and beads from anti-Gal3 (αGal3) rabbit #49 precipitation of 60% Buffer D (60%D). Lane 3: 8 μl U1 ∆NE and beads from αGal3 rabbit #49 precipitation of Fr 3–5. Lane 4: 8 μl U1 ∆NE and beads from αGal3 rabbit #49 precipitation of Fr 3–5. Lane 5: 8 μl U1 ∆NE and beads from precipitation of Fr 3–5 by preimmune serum of rabbit #49. Arrows point to products of the splicing reaction.
their components. These spliceosomal core particles have not been shown to be functional in a splicing assay following their isolation.

The association of galectins with spliceosome complexes is salt-sensitive (18). At 60 mM salt (the concentration used for splicing), anti-galectin antibodies immunoprecipitated spliceosomes. Increasing the salt after the splicing reaction to 130 mM diminishes dramatically anti-galectin precipitation of spliceosomes while no splicing complexes are precipitated at 250 mM salt. In contrast, the five spliceosomal snRNPs are associated with spliceosomes at all three salt concentrations (i.e. are salt-resistant, 18).

Thus, under conditions used to characterize stable snRNA–protein complexes and core spliceosome particles, galectins and other proteins would have been released from the stable U1-snRNP complexes (as shown by the absence of Gal3 in the material bound to an Hu ENA-RNP antibody column [Figure 1B, lane 1]). We (18) and others have documented additional polypeptides associated with spliceosomes (35, 36) and snRNPs (24) isolated under salt conditions optimal for in vitro splicing. Having now demonstrated that the Gal3–U1 snRNP forms a productive splicing complex with pre-mRNA, the question is raised whether the binding partner of Gal3 in the U1 snRNP is another salt-labile component or is it one of the salt-resistant core components already characterized.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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