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Proteolysis of splicing factors during rat and monkey cell fractionation

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ABSTRACT

We have investigated the ability of various rat and monkey cell lines to yield nuclear extracts that would allow splicing of a model adenovirus pre-mRNA substrate. Extracts from normal FR3T3, rat-1 and CV-1 fibroblasts were unable to assemble splicing complexes and displayed a dramatic reduction in the binding activity of the splicing factor 65 kD U2AF. These results correlated with reduced levels of 65 kD U2AF and the snRNP-associated B protein. When a battery of protease inhibitors was used during cell fractionation, increased levels of 65 kD U2AF and B proteins were detected. Most importantly, U2AF binding and complex formation were dramatically improved in FR3T3, rat-1 and CV-1 extracts. Interestingly, transformation of rat and monkey cells with the SV40 large T antigen yielded extracts active in complex formation. Similar extracts were generated following transformation of rat-1 cells with the Py middle T antigen but not with the v-*fos* oncogene. Only SV40-transformed FR3T3 extracts displayed splicing activity. Our results indicate that proteolysis is a major obstacle encountered during the preparation of active extracts from normal rat and monkey cells and suggest that cells transformed with T antigens manifest reduced proteolysis during fractionation.

INTRODUCTION

In vitro splicing systems derived from HeLa cells have greatly contributed to our understanding of the mechanism of RNA splicing (1). The importance of alternative RNA splicing is now well established and it can be expected that the identification and characterization of regulatory splicing factors will require the development of *in vitro* splicing systems from different cell lines. While few attempts have thus far been made in this direction (2–5), it is noteworthy that all cells successfully used in the preparation of splicing extracts were either transformed *in vitro* or derived from tumours.

In this paper, we report our initial attempts at using various rat and monkey cell lines for preparing splicing extracts. The cells used included phenotypically normal FR3T3, rat-1 and CV-1 fibroblasts, as well as transformed derivatives expressing the SV40 large T antigen, the polyoma (Py) middle T antigen or the v-*fos* oncogene. Splicing activity and complex formation were examined as well as binding of the 65 kD U2 snRNP auxiliary factor (U2AF). We find that nuclear extracts prepared from cells expressing either the SV40 large T or the Py middle T are strikingly more competent than extracts from normal rat and monkey fibroblasts or v-*fos*-transformed rat cells. Proteolysis of splicing factors was associated with normal extracts and extracts prepared from cells expressing T antigens contained higher levels of intact splicing factors.

MATERIALS AND METHODS

Cell lines

Fisher Rat 3T3 (FR3T3) and hy2Δ2005/11 cell lines were kindly provided by L.St-Onge and M.Bastin (Université de Sherbrooke). The hy2Δ2005/11 cell line expresses the SV40 large T antigen and also a truncated, non-transforming, Py middle T protein (6). The rat-1, 1302-4-1 and FBJ-VL3 cell lines were kindly provided by P.Jolicoeur (Institut de Recherches Cliniques, Montréal). V-*fos*-transformed rat-1 cell lines (1302-4-1 and FBJ-VL3) were obtained by transfection with plasmid FBJ-2 (7,8) and pFBJ-VL3 (9), respectively. The Rneo-1 cells were produced by transfection of rat-1 cells with the plasmid pSV2neo, whereas the RLT cell lines were generated by transfecting rat-1 cells with pneo-SV2Δ2005 (6). The RmT cells lines are rat-1 derivatives obtained either by transfecting with pneo-MT3 (10) or by co-transfecting pSV2neo with pPyMT3 (11).

Cell culture and transfection

FR3T3, rat-1, CV-1 and their transformed derivatives were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum. HeLa cells were grown in suspension in RPMI 1640 (GIBCO) supplemented with 10% fetal bovine serum.

Transfection of rat-1 cells was performed as described by Weber *et al.* (12). Foci of G418 resistant cells were isolated following 10–12 days of growth in the presence of 400 μg/ml

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of geneticin (GIBCO). Cells displaying a transformed phenotype were further analyzed for the expression of the appropriate oncogene by ^{35}S-methionine labeling followed by antibody-specific immunoprecipitation.

Nuclear extracts and SP6 transcription

HeLa cell nuclear and S100 extracts were prepared according to Dignam *et al.* (13). FR3T3, rat-1 and CV-1 extracts as well as extracts from their transformed derivatives were prepared according to Lee *et al.* (14) with an approximate yield, from 30 plates (100 mm), of 300 μl at protein concentration of ≈2−8 mg/ml. For extracts prepared in the presence of protease inhibitors, leupeptin (0.5 μg/ml) and pepstatin (0.7 μg/ml) were present throughout the extraction procedure. APMSF (10 μg/ml) and bestatin (40 μg/ml) were only present during extraction with buffers A and C (14). Proteases inhibitors were purchased from Boehringer-Mannheim.

pSPAd and pHβΔ6 were cut with *HincII* and *AccI*, respectively, and transcribed with SP6 RNA polymerase (Pharmacia) in the presence of 100 μCi of [α-^{32}P]UTP as previously described (15).

Splicing activity and complex formation

Splicing reactions were set up according to Krainer *et al.* (16). Splicing activity was assessed by separating labeled RNA molecules on gels as described (17). The electrophoretic separation of splicing complexes and ultraviolet crosslinking assays were performed as described in Krämer (18) and Zamore and Green (19), respectively.

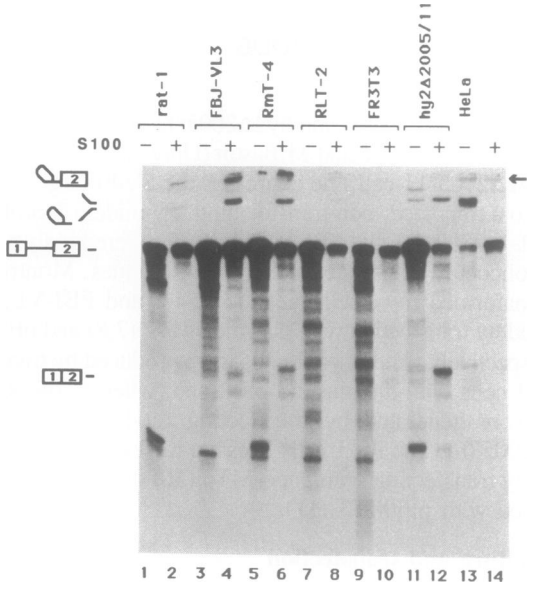

Figure 1. *In vitro* splicing of ^{32}P-labeled adenovirus pre-mRNA in rat nuclear extracts. Splicing reactions (12.5 μl) contained 25 μg (total protein) of rat extracts and were incubated in the presence (+) or absence (−) of 15 μg of a HeLa S100 fraction for 2 hours at 30°C. As controls, splicing reactions using 50 μg of standard HeLa nuclear (lane 13) or 15 μg of S100 (lane 14) extracts were prepared. The RNA was extracted and analyzed on a 10% acrylamide/7M urea gel. The uppermost band seen in several lanes represents material retained at the origin of the gel (indicated by an arrow). The position of the pre-mRNA, splicing intermediates and products is indicated. The free 5′ exon was not retained on the gel. Although splicing intermediates and products are difficult to detect in lane 10 (FR3T3 + S100), they were readily seen following a longer exposure of the gel (not shown).

Detection of spliceosomal proteins

Immunoblot analysis was performed by incubating nitrocellulose filters in buffer M (0.5% Tween-20, 5% w/v dry milk, 130 mM NaCl and 40 mM Tris-HCl pH 7.4) containing either the anti-65 kD U2AF antibodies (1:2700) or the Y12 antibodies (anti-snRNP B protein; 1:200) for 2 hours at room temperature. Following three 5 min washes in buffer M, antibody binding was detected by using 1 μCi/ml of ^{125}I-Protein A (Amersham) in buffer M for 1 hour. Filters were washed and exposed overnight on X-ray films.

RESULTS

Splicing activity and complex formation in rat extracts

Using the procedure developed by Lee *et al.* (14) for monolayers of HeLa cells, nuclear extracts were first prepared from normal FR3T3 and rat-1 cells as well as from several transformed derivatives. The latter included an SV40-transformed FR3T3 cell line (hy2Δ2005/11), rat-1 fibroblasts transformed with either the v-*fos* oncogene (FBJ-VL3 and 1302-4-1), the SV40 large T antigen (RLT-2 and -4), or the Py middle T antigen (RmT-3, -4 and -5). We also prepared extracts from a rat-1 cell line that stably expressed the neomycin resistance gene (Rneo-1). As verified by ^{35}S-methionine labeling followed by antibody immunoprecipitation, all transformed cell lines expressed the appropriate oncogene whereas normal cell lines did not produce the corresponding oncoprotein (data not shown).

To examine splicing activity in rat extracts, we used as substrate a standard ^{32}P-labeled adenovirus (Ad) pre-mRNA derived from the major late transcription unit (20, 21). The results indicate that hy2Δ2005/11 extracts were the only rat extracts which spliced Ad RNA (Figure 1, lane 11). However, splicing activity in all the other rat extracts was restored by supplementing with a HeLa post-nuclear (S100) fraction (Figure 1).

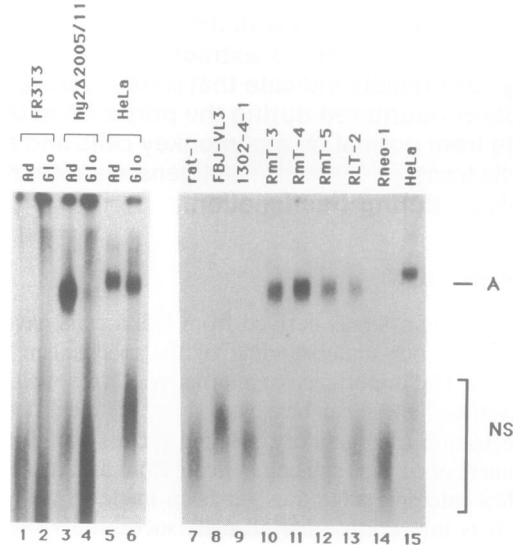

Figure 2. Formation of splicing complexes in rat nuclear extracts. Complex reactions were performed with the adenovirus pre-mRNA (lanes 1, 3, 5, 7−15) or a human β-globin substrate (lanes 2, 4 and 6) for 10 min at 30°C. Reactions contained equivalent amounts of total protein for each rat extract and were terminated by addition of 1 μl of heparin (1 mg/ml) to 4 μl samples. RNP complexes were analyzed on 4% polyacrylamide gels. The position of complex A and of non-specific complexes (NS) is indicated.

To identify the step at which splicing was blocked in most rat extracts, we looked at the ability of our extracts to assemble splicing complexes using native gel assays. The results shown in Figure 2 indicate that FR3T3, rat-1, Rneo-1, 1302-4-1 and FBJ-VL3 nuclear extracts failed to form splicing complexes on Ad RNA. The same effect was seen with a human β-globin pre-mRNA (Glo; lane 2 and data not shown). In contrast, extracts prepared from SV40- and Py-transformed rat cells readily assembled splicing complex A on Ad RNA (Figure 2, lanes 3 and 10–13). Complex formation was ATP-dependent and occured less readily on Glo RNA (lane 4 and data not shown). All the data presented above was reproduced with several extract preparations and was also observed with extracts prepared from rat-1 cells transformed following expression of the v-*trk* oncogene (data not shown).

65 kD U2AF activity and integrity

The assembly of splicing complex A requires U2 snRNP and U2AF activity (22). U2AF activity co-purifies with two polypeptides, 65 and 35 kD, the larger of which binds to the polypyrimidine tract/3′ splice site region of model pre-mRNAs (19). Since A complexes did not form in extracts from normal and v-*fos*-transformed rat cells, we investigated 65 kD U2AF binding by performing an ultraviolet crosslinking assay with 5-bromouridine ^{32}P-labeled pre-mRNAs (19). The results obtained are shown in Figure 3. The labeling of the 65 kD U2AF protein was detected in a SV40-transformed FR3T3 nuclear extract with both Ad and Glo RNAs (hy2Δ2005/11 in Figure 3, lanes 3 and 4). In contrast, the intensity of the 65 kD band was strongly and specifically reduced in FR3T3 extracts (lanes 1 and 2). Likewise, extracts prepared from RmT and RLT cells displayed strong 65 kD U2AF labeling with Ad RNA (Figure 3, lanes 8–11), whereas rat-1, Rneo-1, 1302-4-1 and FBJ-VL3 extracts did not yield detectable labeling of the 65 kD U2AF protein (lanes 5–7 and 12). In these extracts, ultraviolet crosslinking to other proteins common to SV40- and Py-transformed rat-1 extracts was also strongly reduced. Thus, the binding of 65 kD U2AF paralleled the ability of each rat extract to form complexes with Ad RNA.

Because we observed a reduction in 65 kD U2AF binding in normal and v-*fos*-transformed rat extracts, we wished to assess the integrity of the 65 kD U2AF protein in these extracts. To this end, we performed an immunoblot analysis using anti-65 kD U2AF antibodies (kindly provided by P. Zamore and M. Green; 23). We first observed that 65 kD U2AF was present in similar amounts in FR3T3 and hy2Δ2005/11 extracts (Figure 4, lanes 9 and 10). This result was surprising since the crosslinking assay had indicated that 65 kD U2AF was labeled much more efficiently in hy2Δ2005/11 than in FR3T3 extracts (Figure 3). We also noted that a FR3T3 extract had a greater level of smaller (≈50 kD) species that reacted with anti-U2AF antibodies and that may represent U2AF proteolytic products (see below). On the other hand, 65 kD U2AF was readily detected in RmT and RLT extracts (Figure 4, lanes 6–8), but not in rat-1, Rneo-1, FBJ-VL3 and 1302-1-4 extracts (lanes 2–5 and 13). Instead, the 50 kD polypeptides constituted the major reacting species in the latter extracts suggesting that extensive U2AF degradation occured in spite of the presence of the protease inhibitors PMSF and EDTA. To verify this possibility, we prepared rat-1 and FR3T3 nuclear extracts in the presence of bestatin, leupeptin, pepstatin and APMSF which respectively inhibit the activity of amino-peptidases, cysteine, aspartate and serine proteases. This procedure yielded FR3T3* and rat-1* extracts that readily assembled splicing complexes on Ad RNA (Figure 5A). The binding activity of 65 kD U2AF, as determined by ultraviolet crosslinking, was dramatically improved in these extracts (Figure 5B, compare lane 1 with lane 2, and lane 3 with lane 4). Crosslinking of Ad RNA to other proteins in a rat-1* extract was also greatly enhanced. Finally, immunoblots using anti-65 kD U2AF antibodies indicated that the rat-1* extract contained a much greater level of 65 kD U2AF than a regular rat-1 extract (Figure 5C, lanes 1 and 4) suggesting that the absence of 65 kD U2AF in regular rat-1 extracts was due to proteolysis. In the FR3T3* extract, slightly more 65 kD U2AF and substantially less of the 50 kD species were present than in a regular FR3T3 extract (Figure 5C, lanes 6 and 7) again suggesting that proteolysis was responsible for reduced U2AF level and activity in FR3T3 extracts.

Integrity of other nuclear proteins

Addition of purified U2AF to rat-1 and FR3T3 extracts restored U2AF binding but not complex formation (data not shown)

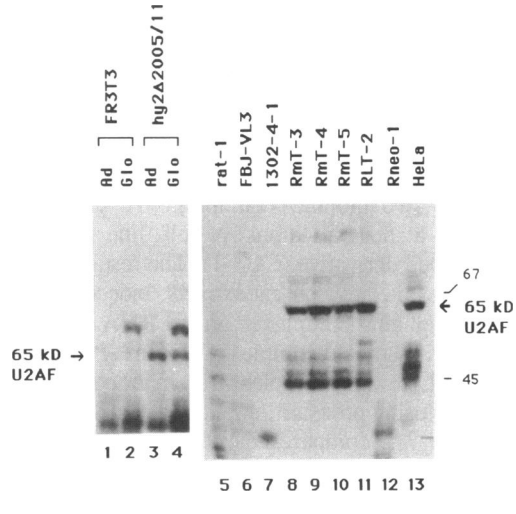

Figure 3. 65 kD U2AF ultraviolet crosslinking assay in rat nuclear extracts. Splicing mixtures containing bromo-uridine (BrUrd) ^{32}P-labeled adenovirus RNA were prepared, except for lanes 2 and 4 which represent binding assays with human β-globin pre-mRNA. Labeled proteins were fractionated on 9% acrylamide/SDS gels. The molecular weight of protein markers are shown and the position of the 65 kD U2AF band is indicated by an arrow.

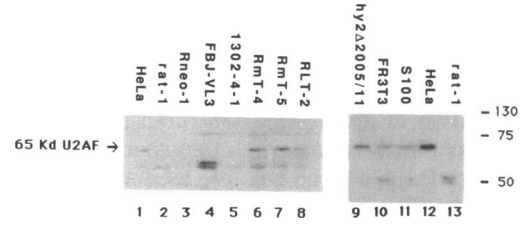

Figure 4. Assessment of the integrity of the 65 kD U2AF protein in rat extracts. 20 μg (total protein content) of various rat and HeLa extracts were run on 9% polyacrylamide/SDS gels and transferred onto nitrocellulose. Filters were probed for 65 kD U2AF by using anti-65 kD U2AF antibodies. Positions of molecular weight markers are shown on the right.

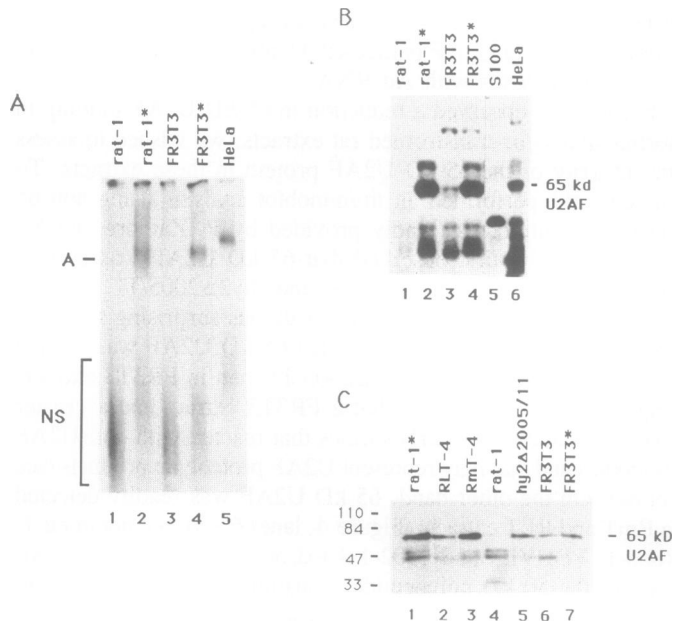

Figure 5. Analysis of rat nuclear extracts prepared in the presence of protease inhibitors. FR3T3* and rat-1* indicate nuclear extracts prepared in the presence of the protease inhibitors leupeptin, bestatin, pepstatin and APMSF (see Materials and Methods). The comparative assays were performed using 20 μg (total protein) of each extract (A) Splicing complex formation on the adenovirus pre-mRNA. The analysis was performed as described in Figure 2 legend. The position of A and NS complexes is indicated. (B) The detection of 65 kD U2AF was performed through ultraviolet crosslinking using BrUrd ^{32}P-labeled adenovirus pre-mRNA as described in Figure 3 legend. (C) Immunoblot analysis using anti-65 kD U2AF antibodies was performed as described in Figure 4 legend.

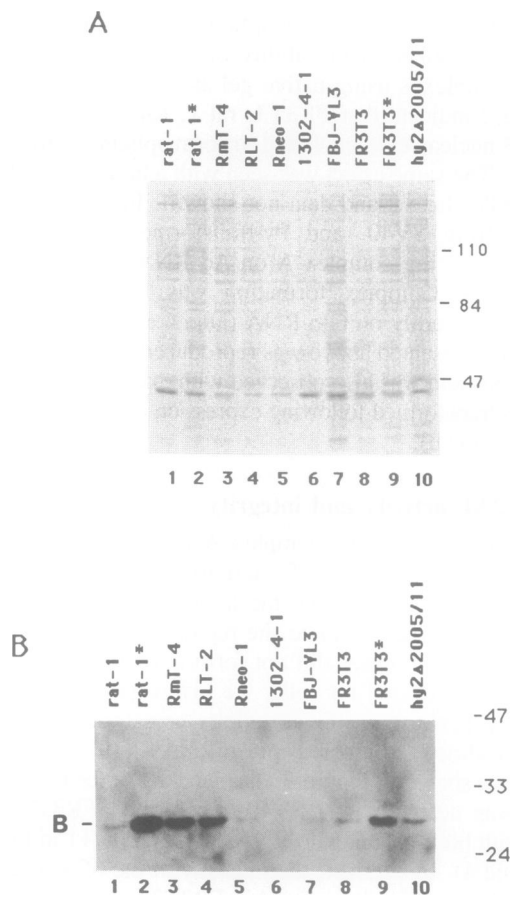

Figure 6. Integrity of other proteins in rat nuclear extracts. (A) The proteins in various rat extracts are displayed through a 9% acrylamide/SDS gel stained with Coomassie blue R-250. Similar profiles were obtained with several extract preparations. In addition, no significant changes in protein profile were detected when freshly-made and several months old extracts were compared. (B) The same amount of each extract (20 μg of total protein) as used in panel A and in Figure 4 was submitted to immunoblot analysis with the Y12 antibody which recognizes the 28 kD snRNP-associated B protein (24).

suggesting that the proteolytic activity associated with fractionation of normal rat cells has affected other proteins important in spliceosome assembly. The dramatic reduction in the labeling of several rat-1 proteins by ultraviolet crosslinking (Figures 3 and 5B) is consistent with this possibility even though total protein profiles looked similar in all extracts (Figure 6A). We assessed the integrity of another spliceosomal protein using the monoclonal antibody Y12 which reacts predominantly with the 28 kD snRNP-associated B protein (24). Immunoblot revealed that the amount of B protein was significantly reduced in normal and v-*fos*-transformed rat-1 extracts (Figure 6B, lanes 1, 5, 6 and 7) compared to SV40 large T- or Py middle T-transformed rat-1 extracts (lanes 3 and 4). The reduction was not as dramatic when FR3T3 and hy2Δ2005/11 extracts were compared (Figure 6B, lanes 8 and 9) but the recovery of B proteins was improved in both rat-1 and FR3T3 extracts prepared in the presence of protease inhibitors (rat-1* and FR3T3*; Figure 6B, lanes 2 and 9, respectively). These results suggest that the B protein also sustained considerable proteolytic degradation during cell fractionation of normal and v-*fos*-transformed rat cells.

We also examined the integrity of another nuclear protein in our rat extracts (data not shown). We used a patient serum that recognizes the La autoantigen, a known transcription termination factor of RNA polymerase III (25). Immunoblots revealed that the amount of La protein in a rat-1 extract was about three-fold inferior to that found in a rat-1* extract. Overall, the results indicate that substantial proteolysis has occured in normal rat extracts suggesting that other proteins such as transcription factors and other splicing factors may also be degraded.

Proteolysis during fractionation of monkey cells

To investigate whether some of the observations made above could be generalized to cell lines of other species, we extended our analysis to two monkey cell lines. We prepared nuclear extracts from a normal monkey cell line (CV-1) and a SV40-transformed derivative (COS-1). The results exactly match those obtained with equivalent rat extracts. Indeed, CV-1 extracts failed to form splicing complexes on Ad RNA whereas COS-1 extracts readily assembled complex A (Figure 7A, compare lane 1 with lane 2). Likewise, complex A formation in CV-1 extracts was restored by using protease inhibitors during cell fractionation (CV-1*; Figure 7A, compare lane 9 with lane 10). Moreover, complex formation in monkey extracts correlated with U2AF binding (Figure 7B, lanes 1 and 2) and immunoblots revealed intact 65 kD U2AF in COS-1 extracts whereas only a ≈50 kD species was detected in CV-1 extracts (Figure 7C, lanes 2 and 3). These results suggest that proteolysis was responsible for the failure of CV-1 extracts to function in complex formation.

We wished to determine whether the proteolytic activity associated with normal extract preparation was transient or

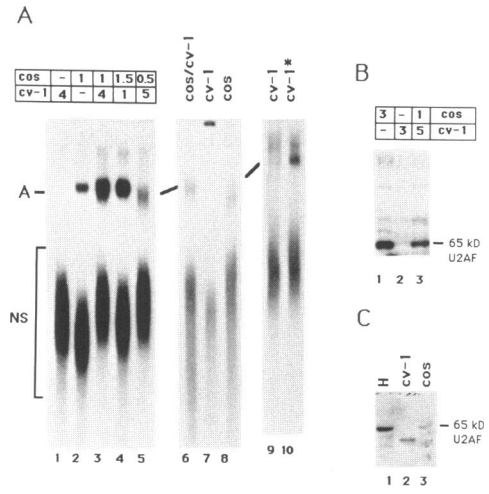

Figure 7. Analysis of extracts prepared from monkey CV-1 and COS-1 cells. (A) Complex formation with Ad RNA and gel analysis were performed as described in Figure 2 legend. Reactions corresponding to lanes 1 to 5 were performed in total volume of 12.5 μl in the presence of the indicate amounts (above numbers in μl) of monkey extracts. CV-1 and COS-1 extracts respectively contained 2 and 8 μg of total protein/μl. Reactions (12.5 μl) corresponding to lanes 6 to 10 contained 20 μg total protein of monkey extracts. The COS-1/CV-1 extract (lane 6) was prepared by mixing the content of 15 plates (100 mm) of each cell line before starting the extraction procedure. CV-1* (lane 10) represents a CV-1 extract prepared in the presence of a mixture of protease inhibitors. (B) A 65 kD U2AF binding assay was performed by UV crosslinking as described in Figure 3 legend. The numbers above the lanes refer to the volume of CV-1 (2 μg/μl) and COS-1 (8 μg/μl) extracts used in a 12.5 μl total reaction. (C) Immunoblot analysis with anti-65 kD U2AF antibodies. Twenty μg of total protein from monkey CV-1, COS-1 and a human WI38VA13 (H, lane 1) extract were used for this assay.

whether it was still present in inactive extracts. Mixing equal amounts of inactive CV-1 with active COS-1 extracts did not reduce complex A formation (Figure 7A, lanes 3–5) nor U2AF binding (Figure 7B, lane 3). Identical results were obtained upon mixing rat-1 and RmT extracts (data not shown). Thus, CV-1 and rat-1 extracts did not inhibit complex formation when mixed to active extracts suggesting the absence of proteolytic activity in final extract preparations. This conclusion was supported by several observations. First, a HeLa S100 fraction rescued splicing in FR3T3, rat-1 and CV-1 extracts (Figure 1 and data not shown). Second, splicing in a HeLa extract is not affected by adding inactive normal rat or monkey extracts (data not shown). Third, purified U2AF molecules added to FR3T3 or rat-1 extracts were still as active as when added to a HeLa S100 fraction (not shown). Fourth, when equal numbers of CV-1 and COS-1 cells were mixed prior to cell disruption, complex formation in the resulting CV-1/COS-1 extract was as efficient as in a COS-1 extract (Figure 7A, lanes 6–8). This result further suggests either that the presence of COS-1 cells inhibit proteolysis during CV-1 cell fractionation or that proteases may remain tightly associated with the nucleus during CV-1 extraction such that a CV-1 protease would be unable to digest COS-1 splicing factors.

DISCUSSION

Splicing activity in extracts from normal cells

Nuclear extracts from normal rat-1 fibroblasts completely fail to assemble splicing complexes on a model Ad pre-mRNA. This defect correlates with reduced levels of at least two spliceosomal proteins: 65 kD U2AF and the snRNP-associated B protein. Given that the use of protease inhibitors during rat-1 cell fractionation strikingly improves the recovery of 65 kD U2AF and B protein as well as complex formation, we think that the inactivity of rat-1 extracts is most likely due to proteolysis. Our results also suggest that proteolysis is responsible for the inability of monkey CV-1 and rat FR3T3 extracts to form splicing complexes. Intriguingly, inactive FR3T3 extracts still contain substantial amounts of 65 kD U2AF (Figures 4 and 5C), but these U2AF molecules apparently cannot bind to Ad RNA (Figure 3 and 5B). A possible interpretation of these results is that a large fraction of the 65 kD U2AF present in FR3T3 cells is not available, perhaps associated with endogenous RNP structures. During FR3T3 cell fractionation, this sequestered population of U2AF would escape proteolytic attack but would also remain unavailable for binding to exogenous pre-mRNAs. In that respect, FR3T3 extracts behave like our HeLa S100 which contains 65 kD U2AF (Figure 4, lane 11) unable to associate with Ad pre-mRNA (Figure 5B, lane 5).

Splicing activity in normal extracts is restored by the addition of a HeLa S100 fraction. This result suggests that the 65 kD U2AF proteins present in the S100 (and, possibly, in FR3T3 extracts) can be recirculated into an active pool upon mixed incubation. Indeed, we detect 65 kD U2AF binding to Ad RNA in S100-supplemented rat-1 extracts (data not shown). These observations combined with the results of mixing experiments further indicate that normal extracts are devoided of proteolytic activity and suggest that proteolysis occurs transiently during the fractionation procedure.

Unfortunately, normal rat and monkey extracts prepared in the presence of protease inhibitors still fail to splice Ad pre-mRNA (data not shown), indicating that additional splicing factors are lost or remain inactive. Nevertheless, nuclear extracts prepared in this fashion are considerably more active in complex formation than regular extracts and therefore represent useful tools for examining early interactions of splicing factors with pre-mRNA molecules.

Splicing activity in extracts from transformed cells

In contrast to normal extracts, extracts from rat and monkey cells transformed with SV40 large T or Py middle T (RmT, RLT, hy2Δ2005/11 and COS-1) efficiently promote the assembly of splicing complexes on Ad RNA and display strong 65 kD U2AF binding activity. These results correlate with high levels of 65 kD U2AF and snRNP-associated B protein as well as reduced levels of the putative 50 kD U2AF proteolytic products. Thus, transformation of rat and monkey cells by the SV40 or Py T antigen abrogates the need to use protease inhibitors to yield extracts competent in complex formation. The simplest interpretation of these results is that transformation with SV40 large T or the Py middle T antigen reduce proteolysis during cell fractionation. In support of this explanation is the observation that reduced rates of proteolysis are associated with SV40-transformed mouse 3T3 fibroblasts and rat hepatoma when compared with their respective normal counterparts (26). An alternate possibility is that proteolysis occurs to the same extent during normal and transformed cell fractionation but that SV40-transformed cells simply produce more splicing factors. However, we failed to detect such differences when we analyzed U2AF levels immediately after cell lysis (data not shown).

We find that SV40-transformed FR3T3 (hy2Δ2005/11) extracts are the only extracts that readily splice Ad pre-mRNA. We do

not know why full splicing activity is limited to these extracts. It may be that proteolysis during hy2Δ2005/11 cell fractionation is even more repressed, thus improving the recovery of additional splicing factors. Lastly, not all oncogenes restore complex formation in rat-1 extracts. Two independently derived v-*fos*-transformed rat-1 cell lines (1302-4-1 and FBJ-VL3) fail to assemble splicing complexes, lack U2AF binding activity and contain reduced amounts of U2AF and B proteins. Interestingly, revertant clones of one of the v-*fos*-transformed cell line have been isolated that are resistant to transformation by v-*fos*, v-H-*ras*, v-*mos* or v-*abl*, but can be transformed by v-*trk* or Py middle T antigen (7), two oncogenes which dramatically improve the recovery of splicing factors during rat-1 cell fractionation. Our results raise questions about the differential effects specific oncogenes have on the expression and/or stability of nuclear proteins (including splicing factors) as well as the contribution of these effects to cell transformation.

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