We employed RNA-protein cross-linking to map the path of the nascent RNA as it emerges from within RNA polymerase II. A UV-cross-linkable uridine analog was incorporated at two positions within the first five nucleotides of the transcript. Only the two largest subunits of RNA polymerase II cross-linked to the transcript in complexes containing 17–24-nucleotide (nt) RNAs. Extension of the RNA to 26 or 28 nt revealed an additional strong cross-link to the splicing factor U2AF65. In U17 complexes, in which the RNA is still contained within the polymerase, U2AF65 is tightly bound. In contrast, U2AF65 is more loosely bound in C28 transcription complexes, in which about 10 nt of transcript have emerged from the RNA polymerase. Cross-linking of U2AF65 to RNA in a C28 complex was eliminated by the addition of an excess of an RNA oligonucleotide containing the consensus U2AF65 binding site, but U2AF65 was not displaced by a nonconsensus RNA. These findings indicate that U2AF65 shifts from protein-protein to protein-RNA interactions as the RNA emerges from the polymerase. During transcription of one particular template at low UTP concentration, RNA polymerase II pauses just after synthesizing a transcript segment that is a U2AF65 binding site. Dwell time of the polymerase at this pause site was significantly and specifically reduced by the addition of recombinant U2AF65 to the transcription reaction. Therefore, the association of U2AF65 with RNA polymerase II may function not only to deliver U2AF65 to the nascent transcript but also to modulate efficient transcript elongation.

It is now appreciated that transcription is regulated during promoter clearance and transcript elongation as well as at transcription complex assembly (recently reviewed in Refs. 1–6). Also, it is becoming increasingly apparent that transcription and RNA processing are both interconnected and interdependent (recent reviews include Refs. 7–9). It is thus of considerable importance to carefully characterize the interaction of the transcription and processing machineries. We now have a detailed picture of RNA polymerase II structure (10, 11), but how factors interact with this structure to modulate elongation and RNA processing is still very poorly understood. In particular, it is not certain how the transcript itself interacts with RNA polymerase II as transcription proceeds.

Immediately upstream of the point of bond formation in RNA polymerase II, nascent RNA remains in hybrid with the DNA template for 8–9 bp. Structural information is available for the transcript in this region (12, 13), but beyond the RNA-DNA hybrid, the location of the RNA within the transcription complex is unknown. A considerable segment of RNA remains inside the polymerase upstream of the point at which the transcript and template strand separate, since 17–19 nt of RNA within the transcription complex are protected from nuclease digestion and oligonucleotide hybridization probes (14–16). Two possible exit paths have been proposed for the RNA (17, 18). These models place the emerging transcript on opposing sides of the dock region of Rpb1, the largest polymerase subunit. On path 1, the RNA passes around the base of the clamp domain toward subunit Rpb7, which contains both a ribonucleoprotein fold and an oligonucleotide binding fold (19, 20). The Rpb4/Rpb7 heterodimer was shown to bind single-stranded DNA and RNA, and it has been suggested that Rpb7 may bind the emerging RNA transcript (10, 11, 13, 19, 20). On the alternative path, path 2, the RNA passes around the opposite face of RNA polymerase toward subunit Rpb8. Westover et al. (13) point out that in bacterial RNA polymerase only path 2 is electrostatically favorable. The simple extension of the RNA in their structure points toward this second groove (13). Several findings suggest that the transcript remains in contact with the polymerase for some time after emerging from within the active center. Our earlier results (16) are consistent with a stabilizing interaction between the 5′-end of the RNA and the RNA polymerase at the point where the nascent transcript is 30–40 nt long. Hanna and Meares (21) showed that the 5′-end of transcripts generated with Escherichia coli RNA polymerase can cross-link to the polymerase up to a chain length of 94 nt.

In order to better understand the exit path of the transcript from RNA polymerase II, we synthesized RNAs 17 nt and longer, which contained both cross-linkable residues and radioactive labels at their 5′-ends. We were surprised to discover that the emerging RNA immediately contacts U2AF65, a protein known to be involved both in splicing (reviewed in Ref. 22) and in nuclear export (23, 24). We show that the presence of U2AF65 can assist RNA polymerase in recovery into transcriptional competence at pause sites, and we suggest that the close association of U2AF65 and polymerase is important in facilitating the participation of U2AF65 in RNA processing events.

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The abbreviations used are: Rpb1, Rpb2, etc., the largest, second largest, etc., subunits of RNA polymerase II; U2AF, U2 small nuclear ribonucleoprotein auxiliary splicing factor; U2AF65, the large subunit of U2AF; U2AF35, the small subunit of U2AF; RRM, RNA recognition motif; nt, nucleotide(s); CTD, C-terminal domain.
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MATERIALS AND METHODS

Reagents—We obtained reagents from the following sources: fast protein liquid chromatography-purified NTPs from Amersham Biosciences, 3H-labeled NTPs from PerkinElmer Life Sciences, Bio-Gel A1.5m from Bio-Rad, oligo(dT)-cellulose and DeepVent DNA polymerase from New England Biolabs, 5′-end labeled DNA oligonucleotides from Sigma, DNase I from Roche, Invitrogen, and streptavidin-coated paramagnetic beads from Promega. Cpa dinucleotide was obtained as a custom synthesis from Drakenso. DNA oligonucleotides were synthesized by Integrated DNA Technologies, Inc. The RNA oligonucleotides RNA2 and RNA3 used in Fig. 4C were synthesized with T7 RNA polymerase and subsequently purified on a 15% denaturing polyacrylamide gel. HeLa cells for nuclear extract preparation were obtained from the Babraham Cell Culture Cell Bank. The 8WG16 antibody against RNA polymerase II was obtained from Dr. Margarida Gama-Carvalho.

Transcription Templates—Templates for transcription were all based on the adenovirus major late promoter. The construction of the pML20–42, pML20–40(6G), and pML20–40(17G) plasmids is described elsewhere (25, 26). Plasmid pML220 was constructed by PCR amplifying a 120-bp segment of pGR220 (27) with the following primers: 5′-CCGCTGCTGCAACATTGCGAAGTCGAGGG-3′ and 5′-CCC-AAGCCTGGGATGAACTTCTGCACAGGAAACGTCAGTGGC-3′. The PCR product was cut with restriction enzymes XhoI and HindIII and was cloned into pML20–42 between the XhoI and HindIII sites. This places the sequence CTTTGTTCCTTIT1 base pairs downstream from the transcription start site. To generate the pML222 plasmid, this sequence was altered by changing the underlined G to a T residue through the use of the Stratagene QuickChange™ site-directed mutagenesis kit.

The DNA templates for transcription, which ranged in size from 190–298 bp, were produced by PCR. In all cases, the transcription start site was 96 bp from the upstream end of the fragment. For attached transcription, the upstream promoter fragment, ranging in size from 190–298 bp, was produced by PCR. In all cases, the transcription start site was 96 bp from the upstream end of the fragment.

Protein Purification—Recombinant human U2AF (consisting of U2AF35 and U2AF65) was expressed in and purified from baculovirus-infected High-Five cells (Invitrogen) as described previously (28) with some modifications. The insect cells (0.74 g) were resuspended in 6 ml lysis buffer plus 1% Nonidet P-40 and twice with 10 ml of wash buffer lysis buffer) for 1 h at 4 °C. The resin was washed twice with 10 ml of lysis buffer plus 1% Nonidet P-40 and twice with 10 ml of wash buffer (50 mM Tris, pH 7.9, 500 mM NaCl, 10% glycerol, 5 mM imidazole, and 5 mM β-mercaptoethanol) before loading the sample that was also brought up to a final 400 mM KCl and 1% Sarkosyl in minimal essential medium (MEM) buffer. They were then treated with DNase I (0.3 units/μl) and, where indicated, RNase A (50 μg/ml) for 15 min at 37 °C. An aliquot from each sample was extracted with phenol–chloroform (1:1) and ethanol-precipitated, and resuspended in 50 mM Tris-HCl, pH 7.9, 10 mM β-glycerophosphate, 20 mM MgCl2, 0.5 mM EDTA, and 1 mM dithiothreitol. This Sarkosyl rinsing procedure is described in detail in Ref. 30. The transcripts in Sarkosyl-rinsed complexes were eluted by incubation for 5 min at room temperature with a 50 μM concentration of the appropriate NTPs as indicated in the figure legends.

The experiment in Fig. 5 used templates attached to beads, which were assembled into preinitiation complexes as described (31). A23 complexes were generated by incubation with 100 μM ATP, 10 μM UTP, and 1 μM [α-32P]CTP at 30 °C for 5 min, followed by the addition of 10 μM CTP for an additional 5 min. After Sarkosyl rinsing, the A23 complexes were chased with all four NTPs at 30 °C as indicated in the legend to Fig. 5.

Cross-linking—Samples were placed in microtiter plates in an ice water bath and irradiated for 20 min at 312 nm as described by Bartholomew et al. (32) using a Fisher Biotech transilluminator in the presence of aprozin and leupeptin at 10 μg/ml. They were then treated with RNase I (0.3 units/μl) and, where indicated, RNase A (50 μg/ml) for 15 min at 37 °C. An aliquot from each sample was extracted with phenol–chloroform (1:1) and ethanol-precipitated, and resuspended in 50 mM Tris-HCl, pH 7.9, 10 mM β-glycerophosphate, 20 mM MgCl2, 0.5 mM EDTA, and 1 mM dithiothreitol. This Sarkosyl rinsing procedure is described in detail in Ref. 30. The transcripts in Sarkosyl-rinsed complexes were eluted by incubation for 5 min at room temperature with a 50 μM concentration of the appropriate NTPs as indicated in the figure legends.

RESULTS

Structural studies have shown that the catalytic center of RNA polymerase II resides in a cleft formed by the two largest subunits, Rpb1 and Rpb2 (recently reviewed in Ref. 34). In order to investigate the path of the nascent RNA as it emerges from this central cleft, we have carried out RNA-protein cross-linking experiments in which UV cross-linkable residues were located near the 5′-end of the RNA. An earlier study (32) demonstrated UV cross-linking of 19 of the 26 natural RNA residues containing 4-thiouridine to the two largest subunits of RNA polymerase II.

We found that whereas 4-thio-UTP was an acceptable substrate for extension of relatively long RNAs (~150 nt) by RNA polymerase II, the polymerase would not incorporate 4-thio-UTP in the initial 5 nt of the transcript (data not shown). We therefore employed 5-ido-UTP as our cross-linkable nucleotide. 5-Iodouridine shows specific photoreactivity with aromatic and sulfur-containing residues in proteins (35).

Cross-linking of the 5′ Region of the Transcript to Components of the Transcription Complex—The template employed in
the initial phases of our study, called pML20–40(6G), is derived from the adenovirus major late promoter. The first 30 nt of the transcript from this template are shown in Fig. 1A. RNA polymerase II transcription complexes were assembled by incubating pML20–40(6G) DNA in HeLa nuclear extracts. Transcription proceeded to position +20. These complexes contained two 5-iodouridine residues and two neighboring radiolabeled cytidines at the 5′-end, but no cross-linkable or labeled residues were present beyond the sixth base (see Fig. 1A). We will use the convention of designating transcripts and the associated complexes by the length of the RNA and the final base incorporated; thus, pausing of Cpa-primed transcription at +20 on pML20–40(6G) generates U21 complexes.

U21 complexes were partially purified by Sarkosyl rinsing, which involves transient exposure to the detergent Sarkosyl during gel filtration (see “Materials and Methods”). As shown in Fig. 1B, transcripts in the Sarkosyl-rinsed U21 complexes could be extended very efficiently to generate A24, G26, or C28 complexes. Reactions containing U21, A24, G26, and C28 complexes were exposed to UV light, and the proteins that were labeled by cross-linking to the RNA were visualized after SDS-PAGE (Fig. 1C). Cross-linking of the U21 and A24 reactions resulted in the labeling of two major protein bands, of roughly 220–240 and 135 kDa (Fig. 1C, lanes 2 and 3). These are, as expected, the two largest subunits of RNA polymerase II, Rpb1 and Rpb2, based on their molecular weights and the reactivity of the upper band with anti-Rpb1 antibodies (Fig. 1C) (see also Ref. 32). Reactions in which the transcript was extended only 2 or 4 bases further downstream, to G26 or C28, showed an additional band upon UV cross-linking (Fig. 1C, lanes 4 and 5). After RNase A treatment to truncate the cross-linked RNA, this protein displayed an apparent molecular mass of 65 kDa (Fig. 2, right). There was no significant cross-linking in control reactions, which were not exposed to UV (Fig. 1C, lane 1) or in which no 5-ido-UTP was used (not shown).

Identification of Proteins Cross-linked to the Transcript—Reactions containing U21 or C28 complexes were UV-cross-linked and then immunoprecipitated with the anti-Rpb1 antibody SWG16 (Fig. 2). In control reactions, which were denatured before precipitation (lanes 1–4), only the largest of the three labeled bands was recovered, confirming that this protein is the largest subunit of RNA polymerase II. When the precipitations were done under native conditions, all three bands were recovered (lanes 5–8), indicating that the 65-kDa protein is part of the transcription complex and not simply cross-linked to free RNA that might have been released during the transcription reaction.

We were surprised at the size of the 65-kDa cross-linked protein. We had expected from our detergent rinsing protocol that only core subunits of RNA polymerase II would be present in the transcript elongation complexes. However, no subunit of RNA polymerase II has a molecular mass close to the observed size. (Human Rpb2 and Rpb3 are 134 and 31 kDa, respectively; see Ref. 36). We considered the possibility that, contrary to expectation, general transcription factors might remain in our complexes. The 62-kDa subunit of TFIIF and the largest subunit (58 kDa) of TFIIF have roughly the correct molecular weight, but we could find no evidence that either protein is responsible for the 65-kDa band (data not shown). A critical clue was provided by the observations of Robert et al. (33), who showed that the splicing factor U2AF65 copurifies with RNA polymerase II through a TFIIS affinity column. The identity of the 65-kDa band was established by the fact that it could be recovered from cross-linking reactions using a monoclonal an-
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U2AF65 Is Tightly Associated with the Early Transcription Complex but Switches from Protein-Protein to Protein-RNA Interactions as the Transcript Emerges—Coulombe and co-workers (33) demonstrated that an anti-U2AF65 antibody will co-precipitate U2AF65 and RNA polymerase II from a HeLa cell extract that was extensively RNase-treated. This provides evidence for a direct protein-protein interaction between U2AF65 and free RNA polymerase II. We wanted to determine how strongly U2AF65 is bound in our transcription complexes and whether this interaction depends on the length of the transcript. We were initially surprised to find U2AF65 in our transcription reactions, because the Sarkosyl rinsing procedure was expected to remove all proteins other than RNA polymerase subunits. However, our usual protocol involves a rapid gel filtration step performed at relatively low resolution. We therefore decided to test whether U2AF65 remained in association with our complexes after transient exposure to detergent and high salt concentrations followed by high resolution gel filtration. We used a variant of our original template, pML20–40(17G), for this experiment (Fig. 4A). Transcription of pML20–40(17G) in the presence of CpaA, radiolabeled CTP, and 5-iodo-UTP resulted in the synthesis of a 7-nucleotide RNA. Subsequent chase with ATP, UTP, and nonlabeled CTP generated a 17-nucleotide transcript. As noted above, this RNA is not long enough to be accessible to external probes. The U17 complexes were Sarkosyl-rinsed by the usual rapid protocol. We set aside 20% of this reaction as a control. The remainder was brought to 1% Sarkosyl and 400 mM KCl, followed by fractionation on a high-resolution 40-ml gel filtration column (see “Materials and Methods”). The excluded volume containing the transcription complexes was incubated with GTP, CTP, and UTP to generate U32 complexes, followed by UV cross-linking. The control sample was divided into two parts and then treated similarly, except that half of the reaction was diluted 7.5-fold to achieve similar dilution conditions to the gel filtration sample. All of the cross-linked reactions were concentrated by precipitation before analysis by SDS-PAGE. The results (Fig. 4A) show that a significant amount of U2AF65 remained with the transcription complex during gel filtration, even after exposure to 1% Sarkosyl and 400 mM KCl. (Note that we confirmed that free U2AF65 was well separated from the void volume on this column; data not shown.) Since U2AF65 cannot be interacting with RNA in the U17 complex, this indicates a strong protein-protein interaction between U2AF65 and RNA polymerase II in this ternary transcription complex.

We repeated the gel filtration experiment with C28 complexes, in which the RNA is accessible to U2AF65. In this case, the gel filtration was performed under native conditions, without the addition of Sarkosyl or 400 mM salt. As shown in Fig. 4B, in this case, all of the U2AF65 was removed from the complex by the gel filtration column. Based on the results of the gel filtration experiment with C28 complexes, U2AF65 is tightly associated with the early transcription complex but switches from a protein-protein to a protein-RNA interaction as the transcript emerges.
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In all panels, samples were resolved on 4–20% SDS-polyacrylamide gels. A, the initial transcript sequence from the pML20–40(17G) template is shown. In the initial step of the transcription reaction, RNA polymerase II incorporated 5-iodouridine residues (rectangles) and 32P-labeled cytidine residues (asterisks). U17 complexes were assembled on this template and processed as shown in the flow diagram (described in detail under “Materials and Methods”). Half of the control reaction was diluted 7.5-fold to achieve a dilution similar to that which occurred during gel filtration. All samples were walked to U32 with the addition of the appropriate NTPs, cross-linked, treated with DNase I and RNase A, and trichloroacetic acid-precipitated. B, Sarkosyl-rinsed U21 complexes were made on template pML20–40(6G) as described in the legend to Fig. 1, walked to C28, and then processed as shown in the flow diagram (described in detail under “Materials and Methods”). Cross-linked samples were DNase I-digested and trichloroacetic acid-precipitated. C, C28 complexes on the pm20–40(6G) template were incubated with RNA2, RNA3, or no RNA before cross-linking (RNAs were added to a final concentration of 3 μM). The sequence of RNA3 is GACACUUUCCCUUUUUUCC, which contains the consensus U2AF65 binding site (42). The sequence of RNA2 is GGACAAAAAAACCCAAAAAAACC.

shown in Fig. 4, A and B, it appears that once RNA has emerged from within the polymerase, U2AF65 binds more loosely to the transcription complex, perhaps because its binding is now divided between RNA and protein sites. To address the question of whether any RNA can dissociate U2AF65 from the transcription complex, we incubated C28 complexes prior to cross-linking with an excess of either an RNA oligonucleotide containing the consensus binding site of U2AF65 (RNA3) (see Ref. 42) or a control RNA, RNA2, whose sequence does not resemble a U2AF65 binding site. As shown in Fig. 4C, RNA3 eliminated the cross-link to U2AF65 in C28 complexes, but there was no effect of RNA2. This indicates that the interaction between the 5'-end of the transcript and U2AF65 can be competed away by a consensus RNA but not by any RNA sequence.

U2AF65 Can Assist RNA Polymerase II in Crossing Pause Sites—The results in Fig. 4C indicate that U2AF65 should bind strongly to a consensus U2AF65 site within the nascent RNA as soon as that site emerges from within the RNA polymerase. This could have important functional consequences for transcript elongation, since the reverse threading of the transcript that accompanies extended pausing and arrest by RNA polymerase (14, 43, 44) should be prevented by a tight protein-RNA interaction at the mouth of the RNA exit channel (see Ref. 15).

During the course of our studies, we were made aware of unpublished results from the Conaway laboratory,2 which suggested such a function for U2AF65. These workers showed that RNA polymerase II pauses extensively at low UTP concentrations within a pyrimidine-rich region (notemplate strand) on the pGR220 template (27). This pause was relieved by a chromatographic fraction enriched for U2AF65. We constructed a new template with a 120-bp region around the pGR220 pausing site cloned downstream of the adenovirus major late promoter. This sequence encodes a possible U2AF65 binding site, starting at +81 (CTTTGGTTCCCTTT) in our construct. In preliminary tests, we observed pausing of RNA polymerase II just downstream of this sequence under limiting UTP conditions. The pausing was partially relieved in a dose-dependent fashion by the addition of recombinant U2AF65 (data not shown). To further investigate this effect, we have modified the original template with a single base change to create an uninterrupted pyrimidine stretch on the notemplate strand; the corresponding RNA transcript of this sequence more closely resembles a consensus U2AF65 binding site (see Fig. 5A). A U2AF-depleted nuclear extract was used to assemble radiolabeled A23 transcription complexes. After Sarkosyl rinsing, these complexes could be chased to the end of the template with 1 mM NTPs (Fig. 5A, lane 1). If the UTP concentration in the chase was lowered to 10 μM, complexes paused before the addition of each U residue (lanes 2–6 and 7–11). In the presence of recombinant U2AF, complexes generally proceeded through these pauses slightly faster than in its absence. The most significant effect of the U2AF addition was observed at positions 106–108 (marked by the dashed rectangle in Fig. 5A), which is just downstream of the U2AF65 binding site. The intensities of the bands corresponding to these pauses were quantitated and plotted as a function of time (Fig. 5B). The presence of U2AF does not prevent the initial pausing event (10-min points), but it does allow the polymerase to escape the pause much more rapidly than in its absence (20- and 30-min points). This is consistent with a model in which U2AF65 scans the RNA as it is being synthesized by the polymerase. U2AF65 should bind with high affinity to its consensus RNA binding site. If this binding is competitive with backtracking by the polymerase, it would prevent prolonged reverse threading of the transcript and facilitate resumption of transcription. For the template used in Fig. 5, a polymerase paused at the central base of the pause site would protect about 17 bases upstream, which would place just over half of the polyuridine-segment of the RNA outside of the polymerase. U2AF binding to this emerging consensus site should block backtracking by the polymerase.

DISCUSSION

In the course of mapping the path of the 5'-end of the RNA during its exit from RNA polymerase II, we had expected to detect interactions of the transcript with subunits other than Rpb1 and Rpb2, which contain the polymerase active site and enclose the RNA-DNA hybrid (12, 13). However, we discovered that as the nascent RNA emerges from within RNA polymerase II, it immediately encounters U2AF65, a factor involved in splicing and in nuclear export. In HeLa whole cell extracts, U2AF65 is primarily associated with the hypophosphorylated form of RNA polymerase II (33), which is the form competent to assemble into preinitiation complexes. In contrast, RNA polymerase in the transcription complexes we studied should be phosphorylated in the C-terminal domain (CTD) of the largest

2 J. Conaway, and R. Conaway, personal communication.
subunit. In order to explore the possible relationship of CTD phosphorylation and U2AF65 association with the transcription complex, we treated our transcription reactions prior to cross-linking with protein phosphatase-1, which will dephosphorylate the polymerase II CTD (45). To ensure that the CTDs of transcriptionally active RNA polymerases were substantially dephosphorylated, we checked the mobility of the labeled Rpb1 subunit after cross-linking. As expected, the mobility of this band increased after phosphatase-1 treatment. An initial titration allowed us to determine the amount of phosphatase sufficient to complete the shift in mobility of the Rpb-1 band. When reactions were treated with this level of phosphatase-1 and then cross-linked, there was no change in the intensity of the U2AF65 band (data not shown). This suggests that U2AF65 is not retained within the transcription complex via the phosphorylated CTD.

It is important to note that the Rpb7 subunit of RNA polymerase II is located near the mouth of proposed RNA exit channel 1 (10, 11). This subunit contains potential RNA binding domains (19, 20). Also, the association of yeast Rpb7 with RNA polymerase II can be completely disrupted by rinsing with a mild denaturant (46). One might therefore suspect that Rpb7 was removed from our complexes by the Sarkosyl rinsing procedure, thereby allowing access of U2AF65 to the transcript. To guarantee the presence of Rpb7 in our experiments, we supplemented our reactions after the Sarkosyl rinsing step with an excess of recombinant human Rpb4 and Rpb7 (gifts from Finn Werner). We observed the same level of cross-linking of U2AF65 to the nascent RNA regardless of whether Rpb4 and Rpb7 were added (data not shown). Thus, it seems unlikely that Rpb7 competes with U2AF65 for association with the polymerase or binding to the RNA.

We demonstrated (Fig. 4A) that U2AF65 is present in early transcription complexes when the RNA is still contained within the polymerase. This indicates that U2AF65 can initially interact with the transcription complex through protein-protein interactions. Note that U2AF contains two protein interaction regions, one of which (the third RRM domain) belongs to a novel class of protein recognition motifs (39). All of our results, taken together with those of Robert et al. (33), suggest that U2AF65 is recruited to RNA polymerase II before the start of transcription. This positions U2AF65 to interact immediately with the nascent transcript.
with the emerging RNA, even if the transcript sequence is not a close match to the U2AF65 consensus binding site. For example, we demonstrated that U2AF65 in C28 complexes can be cross-linked to an RNA with the initial transcript sequence of 5’-CAGUGUGUU (data not shown). We propose that after transcript initiation, U2AF65 retains its interaction with the RNA polymerase, allowing it to scan the newly synthesized RNA for the presence of polypuridine tracts without the requirement for high affinity for all RNA sequences. Retention of U2AF65 in the transcription complex may also be important for those transcription units that lack introns, since U2AF65 is involved in nuclear export of both intron-containing and intronless transcripts (24).

It would be particularly disadvantageous for splicing if RNA polymerase II paused near the 3’-intron-exon junction, which in the vast majority of cases is located just downstream of a polypuridine tract. A paused RNA polymerase would obstruct recognition of this junction and assembly of the spliceosome. Zhang et al. (44) have shown that extended pausing by RNA polymerase II results in some backtracking of the polymerase along the template, which must be reversed for transcription to resume. The interaction of an RNA-binding protein with the transcript at the point of exit from the polymerase could play a role in facilitating splice site recognition of this junction and assembly of the spliceosome. Zhang et al. (44) have shown that extended pausing by RNA polymerase II results in some backtracking of the polymerase along the template, which must be reversed for transcription to resume. 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