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Regulation of adenovirus alternative RNA splicing at the level of commitment complex formation

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ABSTRACT

The adenovirus late region 1 (L1) represents an example of an altenatively spliced gene where one 5′ splice site is spliced to two alternative 3′ splice sites, to produce two mRNAs; the 52,55K and IIIa mRNAs, respectively. Accumulation of the L1 mRNAs is temporally regulated during the infectious cycle. Thus, the proximal 3′ splice site (52,55K mRNA) is used at all times during the infectious cycle whereas the distal 3′ splice site (IIIa mRNA) is used exclusively late in infection. Here we show that *in vitro* splicing extracts prepared from late adenovirus-infected cells reproduces the virus-induced temporal shift from proximal to distal 3′ splice site selection in L1 pre-mRNA splicing. Two stable intermediates in spliceosome assembly have been identified; the commitment complex and the prespliceosome (or A complex). We show that the transition in splice site activity in L1 alternative splicing results from an increase in the efficiency of commitment complex formation using the distal 3′ splice site in extracts prepared from late virus-infected cells combined with a reduction of the efficiency of proximal 3′ splice site splicing. The increase in commitment activity on the distal 3′ splice site is paralleled by a virus-induced increase in A complex formation on the distal 3′ splice site. Importantly, the virus-induced shift from proximal to distal L1 3′ splice site usage does not require *cis* competition between the 52,55K and the IIIa 3′ splice sites, but rather results from the intrinsic property of the two 3′ splice sites which make them respond differently to factors in extracts prepared from virus-infected cells.

INTRODUCTION

Alternative pre-mRNA splicing is an important mechanism in the regulation of gene expression in eukaryotic cells. By combining different 5′ and 3′ splice sites (ss) in a pre-mRNA, multiple structurally related, but functionally distinct, proteins can be generated from one gene (1). Of specific interest is that the production of alternatively spliced mRNAs, in many cases, has been shown to be regulated, either in a temporal, developmental or tissue-specific manner. It is known that several *cis*-acting elements are important for alternative splicing. These include the primary sequence of the splice site, the relative position of a splice site, the sequence context and the secondary structure of the pre-mRNA (reviewed in ref. 1).

Pre-mRNA splicing takes place in a large macromolecular ribonucleoprotein particle, the spliceosome. In addition to the pre-mRNA, spliceosomes contain the major small nuclear ribonucleoprotein particles (snRNPs) U1, U2, U4/U6, U5 and many non-snRNP protein factors (2, and refs therein). The exon/intron boundaries, which are loosely conserved in metazoans, are defined, in part, by direct RNA–RNA base pairing. The 5′ ss is recognized through base pairing with U1 snRNA (3) and the 3′ ss is similarly defined by a base pairing between U2 snRNA and the branch site (4–6). Also, U5 snRNA appears to base pair with sequences at both the 5′ and 3′ ss (7). Assembly of the spliceosome has been shown to proceed over two stable intermediate stages, the commitment complex (or E complex) and the A complex (pre-spliceosome). Formation of the commitment complex, which is the earliest detectable stable precursor to the spliceosome, requires the 5′ ss and the polypyrimidine tract at the 3′ ss of the pre-mRNA (8, 9). The recognition and functional interaction of the 5′ ss and 3′ ss in the commitment complex appears to require U1 snRNP, which recognizes the 5′ ss, U2AF, which binds to the polypyrimidine tract at the 3′ ss, and non-snRNP protein factors, such as SC35, ASF/SF2 and/or other SR proteins (10–12, and Garcia-Blanco, personal communication). The commitment complex appears to be converted to the A complex by incorporation of U2 snRNP, in a reaction that requires ATP and other protein factors (13). It has been argued that once the pre-mRNA has been committed to the splicing pathway, its fate is irreversibly determined (8, 9, 12). Thus, formation of the commitment complex is likely to be the key regulatory step at which alternative splicing is regulated. However, so far there are only two examples supporting this hypothesis; *Drosophila* sex-lethal (M.Garcia-Blanco, personal communication) and dsx splicing (11). In this report we present evidence that alternative splicing of the adenovirus 2 late region 1 (L1) is also regulated at the level of commitment complex formation.

L1 represents an example of an alternatively spliced gene where one 5′ ss is spliced to two alternative 3′ ss, producing two

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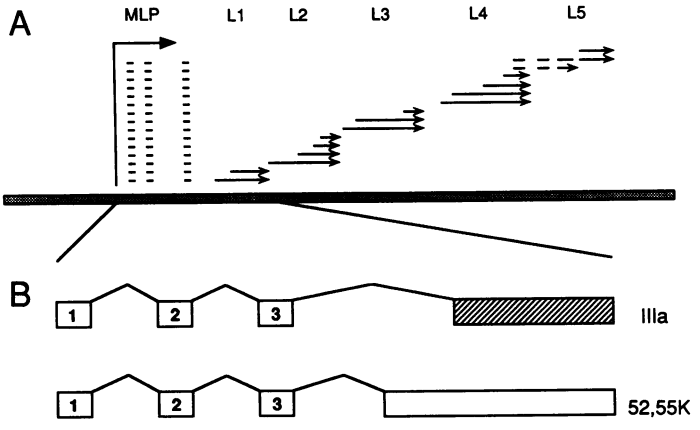


Figure 1. (A) A schematic illustration of mRNAs expressed from the adenovirus 2 major late transcription unit late after infection. The mRNAs are divided into five families (L1–L5) which have co-terminal 3′-ends and a common tripartite leader (1, 2, 3) segment at the 5′-end. (B) Enlargement illustrating the structure of the two major mRNAs expressed from region L1. Boxes represent exons and thin lines the splicing pathways.

mRNAs: the 52,55K (proximal 3′ ss) and the IIIa (distal 3′ ss) mRNAs, respectively (Fig. 1). *In vivo*, the accumulation of L1 mRNA is subjected to temporal regulation. Thus, early after infection all L1 pre-mRNAs are spliced to the 52,55K 3′ ss while the IIIa 3′ ss is activated in late adenovirus infected cells (14–16). The sequence of the regulated, IIIa 3′ ss deviates considerably from the RNA 3′ ss consensus sequence (17), by lacking an extended polypyrimidine tract. Thus, its inefficiency of splicing in nuclear extracts prepared from uninfected HeLa cells can be correlated with an inability to stably bind cellular polypyrimidine tract binding proteins (18). Here we show that the activity of the late specific IIIa 3′ ss significantly increases in nuclear extracts prepared from late adenovirus-infected cells. As a result of this change in ss activity, *in vitro* splicing in extracts from late-infected cells partially reproduces the temporal shift from 52,55K to IIIa mRNA splicing observed during a productive virus infection. Mechanistically the transition to enhanced IIIa splicing results from a virus-induced increase in commitment complex formation on the IIIa 3′ ss, combined with a reduction in the efficiency of 52,55K splicing. Interestingly, this change in ss activity does not require *cis* competition between the 52,55K and the IIIa 3′ ss, suggesting that the two 3′ ss contain sequence elements which makes them responsive to *trans* factors in late virus-infected extracts.

MATERIALS AND METHODS

Plasmids and synthesis of RNA substrates

pGD52Δ3a was constructed by deleting a 127 base pair *Ban*I fragment positioned between the 52,55K and the IIIa 3′ ss in plasmid pGD52,3a (18). pAcc52,55K and pAcc3a were constructed by cloning the *Hin*dIII fragment spanning the respective 3′ ss in pGD52,55K and pGD3a (18) into pGEM3.

The GD52,55K, Acc52,55K, GD52Δ3a-U1, GD3a-U1 and Acc3a-U1 substrate RNAs were generated by PCR synthesis of double-stranded DNA fragments using appropriately designed oligonucleotides, followed by T7 transcription to make a ^{32}P-labeled pre-mRNA (19). A common forward primer (5′-ATTAATACGACTCACTATAGAATACAAGC-TTGGG-3′) beginning with 17 nucleotides corresponding the T7 RNA polymerase promoter (shown in bold), and two reverse primers: GD52,55K and Acc52,55K, 5′-TGCATGTCTGCCGC-TGCTCTTGTCC-3′; GD52Δ3a-U1, GD3a-U1 and Acc3a-U1, 5′-GTACTCACCCCCAGCGCCGCCCGCACC-3′, were used in the PCR reactions. pSP62Δi (20) and pSP64Rchrβ G (21) were linearized with *Sca*I and *Bam*HI, respectively, and used for synthesis of ^{32}P-labeled SP6 transcripts as described by the manufacturer (Promega). The unlabeled competitor transcripts used in the commitment complex assay were uncapped and transcribed according to the Ribomax protocol (Promega).

Splicing assay

HeLa spinner cells were grown to a density of approximately 5×10^5 cells per ml and harvested or infected with 10 FFU per ml of adenovirus 2 (wt900; 22). The infection was allowed to proceed for 24 h before the cells were collected. Nuclear extract preparation and *in vitro* splicing conditions were as described earlier (18), except that the nuclear extract concentration was 60% (unless otherwise stated) and the ATP concentration was raised to 2 mM in the splicing assay. Splicing products were resolved on 8% or 12% polyacrylamide/8 M urea gels. Radioactivity was quantitated by phosphorimager scanning.

Analysis of splicing complex formation

At various time points 4.5 μl was removed from a 25 μl splicing reaction, and the complex formation terminated by addition of 1 μl heparin (5 mg/ml). Five microlitres was loaded on a 4% (80:1) native polyacrylamide gel, cast in a buffer consisting 50 mM Tris–glycine and 5% glycerol (23). In the running buffer the glycerol was omitted. The results were quantitated by phosphorimager or densitometer scanning.

Commitment complex assay

The protocol described by Jamison *et al.* (9) was followed. In short, ^{32}P-labeled transcripts were incubated in 6.25 μl splicing reactions at 30°C for 5 min. The reaction conditions were as described above, except that polyvinylic alcohol and ATP were ommited, and that the nuclear extract concentration was 33%. The mixtures were supplemented with a 5000-fold excess of unlabeled homologous transcript as a competitor and incubated further for 5 min. Committed complexes were then chased to A complexes by further incubating extracts for 10 min together with ATP. Reactions were stopped by addition of heparin and splicing complexes resolved by native gel electrophoresis as described above.

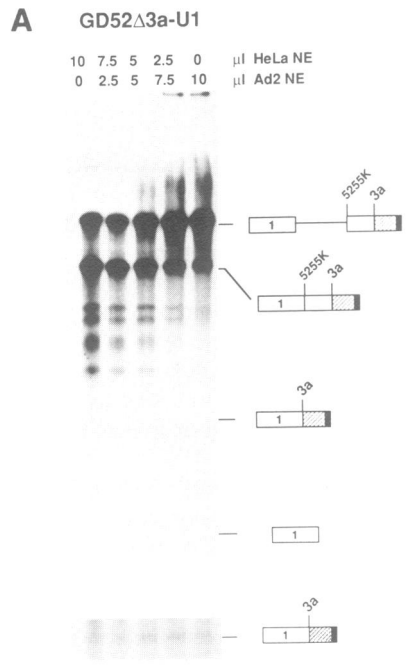
Western blot analysis

Proteins were electrophoresed in an SDS–10% polyacrylamide gel, transferred to a nitrocellulose filter and blocked in non-fat dry milk as described by Zamore and Green (24). Antibodies were detected with an enhanced chemiluminescence detection system (Amersham). The antibody concentration was 0.4 μg/ml (U2AF anti-pepD; 24).

RESULTS

Alternative splicing of the L1 pre-mRNA in nuclear extracts prepared from late adenovirus-infected cells

To study viral factors important for the shift in L1 splicing we compared the splicing efficiency of an L1 substrate containing the 52,55K and the IIIa 3′ ss in tandem in nuclear extracts



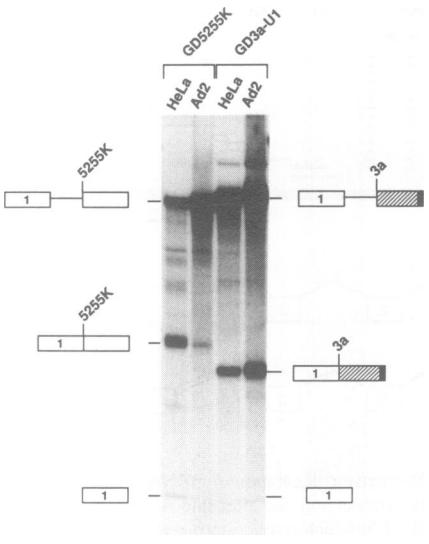


Figure 3. Comparison of GD52,55K and GD3a-U1 splicing efficiency in nuclear extracts prepared from uninfected (HeLa) or adenovirus 2-infected (Ad2) HeLa cells. The position of precursors and products is indicated graphically on both sides of the data lanes. Symbols are as described in the legend to Fig. 2.

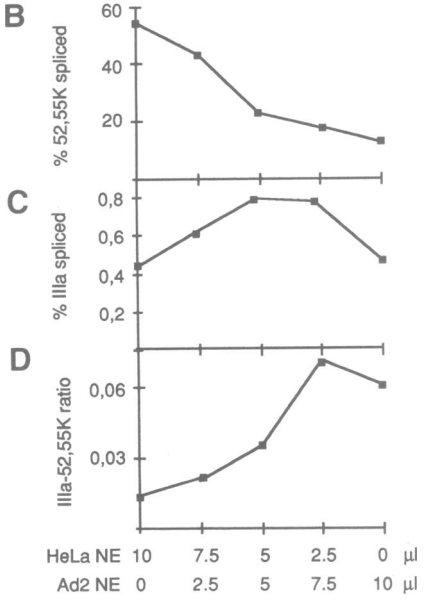


Figure 2. Activation of L1 alternative splicing *in vitro*. (**A**) Nuclear extracts prepared from uninfected (HeLa) or adenovirus 2-infected (Ad2) HeLa cells were mixed in various proportions (10 µl total nuclear extract in a reaction volume of 25 µl) and used to splice the GD52Δ3a-U1 pre-mRNA. Bottom panel shows a longer exposure of the region of the gel corresponding to IIIa mRNA. Splicing products in panel (A) were quantitated and used to calculate the efficiency of 52,55K (**B**) and IIIa (**C**) splicing (expressed as the percentage mRNA of the sum of pre-mRNA plus the 52,55K mRNA and the IIIa mRNA). (**D**) The relative usage of 52,55K and IIIa 3′ ss is presented as the ratio between IIIa and 52,55K mRNA, after correction for the unequal distribution of radioactive nucleotides in the two transcripts. The position of the precursor and the products is indicated graphically to the right. The white box labelled with letter one denotes exon 1. White box, 52,55K exon sequences. Hatched box, IIIa exon sequences. The black box designates the U1 snRNA binding site that was appended downstream of the IIIa 3′ ss (19).

prepared from uninfected or late (24 h post-infection) adenovirus-infected HeLa cells. Since the L1 IIIa 3′ ss is weak compared to the 52,55K 3′ ss (18), the GD52Δ3A substrate was made with a U1 snRNA binding site downstream of the IIIa 3′ ss. We have previously shown that this tag significantly enhances IIIa splicing *in vitro* (19). As shown in Fig. 2A, and quantitated in Fig. 2B–D, maximum 52,55K mRNA splicing was observed in the uninfected extract. Mixing of uninfected HeLa cell extracts and late adenovirus-infected extracts resulted in a decrease in 52,55K splicing. In contrast, the efficiency of IIIa splicing increased as a result of addition of the late extracts (Fig. 2C). It is worth noting that maximum IIIa splicing was consistently observed in assays containing small amounts of uninfected extract, suggesting that late extracts may be limiting in a general splicing factor. As a consequence of the opposite effects on L1 splicing, the ratio of IIIa to 52,55K splicing increased around 5-fold in the late extract (Fig. 2D). This result suggest that the virus-induced temporal shift from exclusive 52,55K mRNA splicing early after infection to both 52,55K and IIIa splicing in late-infected cells is both positively and negatively regulated by a *trans*-acting factor(s) present in the late extract.

The transition from 52,55K to IIIa splicing *in vitro* was not as efficient as that observed during a virus infection. However, this failure to fully reproduce the *in vivo* shift in L1 splicing was not unexpected. For example, *in vitro* splicing is very length dependent and decreases significantly with the distance separating the 5′ and 3′ ss. Thus, placing a weak 3′ ss, like the IIIa, downstream of the strong 52,55K 3′ ss would probably not be expected to result in a strong activation of the weak IIIa 3′ ss. This conclusion is supported by our previous observation that the the weak IIIa 3′ ss is able to efficiently compete with the 52,55K 3′ ss when placed first in a tandem construct (18).

Positive and negative control of 3′ ss activity in late extracts

To be able to further characterize the effects of late viral factors on L1 alternative splicing we subsequently compared the activity

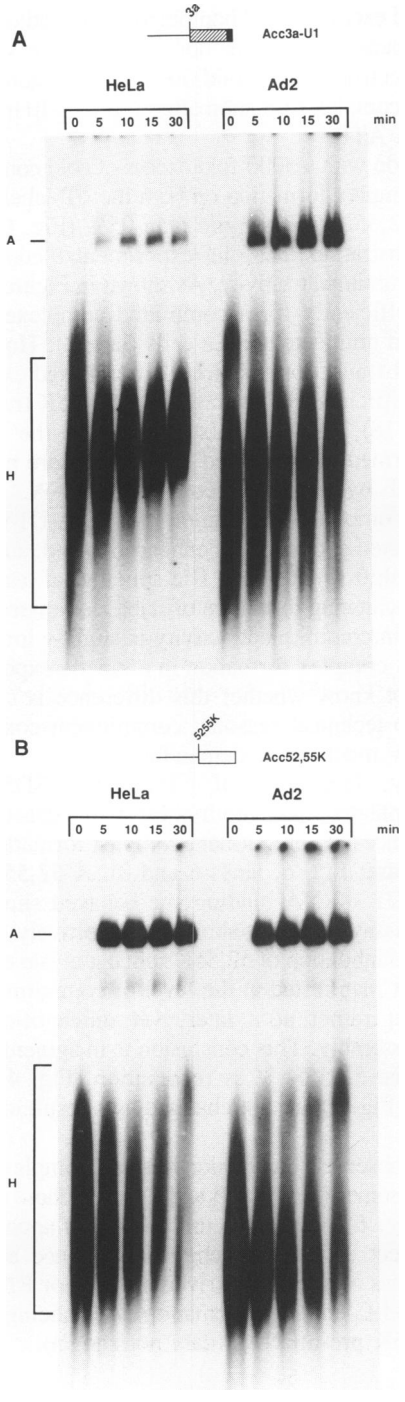


Figure 4. Increase in pre-spliceosome complex formation on the IIIa 3′-splice site in extracts prepared from adenovirus infected cells. Kinetics of A complex assembly on RNA substrates Acc3a-U1 (**A**) and Acc52,55K (**B**) in nuclear extracts prepared from uninfected (HeLa) or adenovirus 2 (Ad2)-infected HeLa cells. At indicated time points, an aliquot of the splicing reaction was removed and resolved by native polyacrylamide gel electrophoresis. The RNA substrates used are schematically illustrated above each panel. H, designates an unspecific complex. Symbols are as described in the legend to Fig. 2.

of the two L1 3′ ss on separate transcripts in late adenovirus-infected versus uninfected HeLa cell nuclear extracts. As shown in Figure 3 the efficiency of 52,55K splicing was repressed around 4-fold (GD52,55K) in late extracts. In contrast, IIIa splicing was stimulated around 3-fold (GD3a-U1) in late extracts.

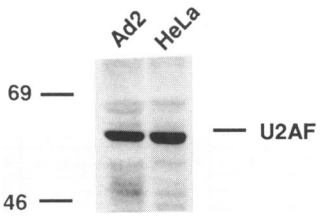


Figure 5. Immunoblot analysis of U2AF levels in nuclear extracts prepared from uninfected (lane HeLa) or wild-type adenovirus (lane Ad2)-infected cells. The positions of molecular weight markers are indicated on the left.

Thus, the opposite effects on 52,55K and IIIa mRNA splicing observed with the GD52Δ3A transcript (Fig. 2) were also reproduced in constructs encoding the individual 3′ ss. It is noteworthy that splicing of a IIIa pre-mRNA lacking a U1 snRNA binding site was also activated in late extracts and that a 52,55K pre-mRNA containing a U1 snRNA binding site downstream of the 52,55K 3′ ss was repressed in late extracts (data not shown). Thus, the positive effect on IIIa pre-mRNA splicing in late extracts was not the consequence of the artificial U1 snRNA binding tag. Collectively these results suggest that the temporal shift from exclusive 52,55K mRNA splicing to both 52,55K and IIIa splicing in late extracts is accomplished by both an inhibition of 52,55K splicing and an increase in IIIa splicing.

Enhanced IIIa 3′ ss recognition in late virus-infected extracts

The IIIa 3′ ss deviates from the RNA 3′ ss consensus sequence by lacking an extended polypyrimidine tract (18). Consequently the inefficiency of IIIa splicing in uninfected extracts has been shown to correlate with a reduced capacity of the IIIa 3′ ss to bind cellular polypyrimidine tract binding factors and form stable pre-splicing complexes (18). To determine whether the change in 52,55K and IIIa mRNA splicing was a result of virus-induced changes in 3′ ss recognition we compared pre-splicing complex formation on transcripts Acc52,55K and AccIIIa-U1 (Fig. 4) in uninfected versus late extracts.

As shown in Figure 4A, complex A formation on Acc3a-U1 was accelerated around 7-fold in late compared to uninfected extracts suggesting that the increase in IIIa splicing was accomplished by an increased IIIa 3′ ss recognition. Unexpectedly, the reduction in 52,55K splicing in late extracts (Figs 2 and 3) was not reproduced at the level of A complex formation on the isolated 52,55K 3′ ss (Acc52,55K, Fig. 4B). This contrasts with what has been observed in the regulation of *Drosopholia* sex determination genes where the sex-lethal protein inhibits proximal 3′ ss usage by competing with U2AF binding to the polypyrimidine tract of the proximal 3′ ss (25). Since U2AF has been shown to be a key protein regulating 3′ ss activity by recruiting U2 snRNP to the pre-mRNA (24, 26, 27) we compared the steady-state concentration of U2AF in uninfected and late virus-infected extracts by immunoblot analysis. As shown in Figure 5 the concentration of U2AF remains unchanged in uninfected compared to late extract. This observation is in agreement with our observation that A complex formation on the Acc-52,55K transcript was unchanged in the two extract types. The 52,55K 3′ ss is preceded by an extensive polypyrimidine tract (18 out of 19 nucleotides) and binds U2AF efficiently (18, and data not shown).

Collectively our data suggests that the increase in IIIa splicing observed in late extracts (Fig. 3) results from an enhanced

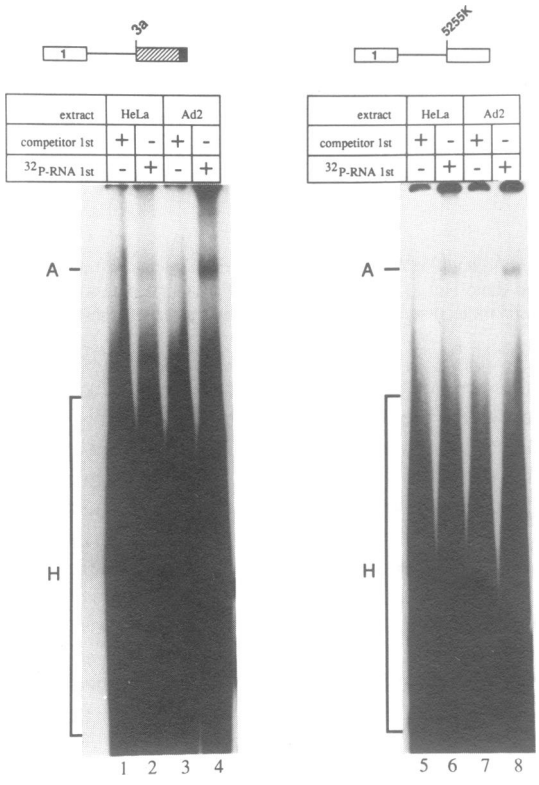


Figure 6. IIIa splicing is activated at the level of commitment complex formation. RNA substrates GD3A-U1 and GD52,55K were preincubated for 5 min with nuclear extracts prepared from uninfected (HeLa) or late adenovirus-infected cells (Ad2) under splicing conditions in the absence of ATP (lanes 2, 4, 6 and 8). After addition of a large excess of cold competitor RNA and ATP, the mixtures were further incubated for 10 min at 37°C. As a control for commitment activity, the cold competitor RNA was preincubated with the extract before addition of the labeled RNA substrates (lanes 1, 3, 5 and 7). Thereafter the formation of spliceosome related complexes were analyzed by separation of complexes by native polyacrylamide gel electrophoresis. H, designates an unspecific complex. Symbols are as described in the legend to Fig. 2.

capacity of the IIIa 3′ ss to form stable pre-spliceosomal complexes.

Regulation of IIIa splicing at the level of commitment complex formation

The earliest detected step that specifically targets the pre-mRNA to the splicing pathway *in vitro* is the commitment complex. Formation of this complex occurs in the absence of ATP, and has been suggested to be the key step by which alternative 5′ and 3′ ss are selected for spliceosome assembly and splicing (8, 9, 28). This prompted us to determine whether the increase in IIIa splicing observed in late extracts could be explained by an increase in commitment activity using the IIIa 3′ ss. This possibility seemed very likely since we observe that the efficiency of IIIa 3′ ss recognition was significantly enhanced in late extracts (Fig. 4A).

To assay for commitment activity we essentially followed the protocol described by Jamison *et al.* (9). In short, ^{32}P-labeled GD3a-U1 or GD52,55K pre-mRNAs were incubated in extracts prepared from uninfected or late virus-infected cells for 5 min in the absence of ATP to allow for commitment complex formation. Further commitment activity was blocked by addition of a 5000-fold excess of cold homologous competitor RNA. Since the commitment complex is not stable under standard non-denaturing electrophoresis conditions, commitment activity was assayed as A complex formation after a further 10 min incubation together with ATP.

Preincubation with a 5000-fold excess of cold competitor RNA reduced A complex formation on both the ^{32}P-labeled GD3a-U1 and the GD52,55K RNAs more than 95% (Fig. 6, lanes 1, 3, 5, 7), demonstrating that our experimental conditions were measuring commitment activity. As shown in Figure 6 the GD3a-U1 did not efficiently form committed complexes in extracts prepared from uninfected HeLa cells (lane 2). However, since the GD3A-U1 transcript is spliced in uninfected extracts, albeit with a lower efficiency compared to the 52,55K transcript (Fig. 3, and ref. 18), this result suggests that the commitment complexes formed in uninfected cell extracts are not stable and out competed by the cold competitor RNA. In contrast, commitment complex formation on the GD3a-U1 transcript, in late virus-infected extracts, was enhanced 4-fold, suggesting that the temporal shift to enhanced IIIa splicing, at least in part, is achieved by facilitating formation of stable committed complexes. The increase in commitment activity is slightly lower than that observed in A complex formation in a kinetic experiment (Fig. 4). We do not know whether this difference is significant or merely due to technical reasons; commitment complex assays are technically much more demanding.

Interestingly, incubation of ^{32}P-labelled GD52,55K pre-mRNA in uninfected and late virus-infected extracts showed no drastic differences in commitment complex formation (less than 1.5-fold increase; Fig. 6, lanes 6 and 8). A 52,55K transcript containing a U1 snRNA binding site behaved similarly in the commitment assay (data not shown). Collectively these results suggest that the inhibition of 52,55K splicing in late extracts (Figs 2 and 3) is not manifested at the level of commitment complex formation, but rather at a later, yet unidentified, stage of spliceosome assembly. This conclusion is in agreement with our observation that 52,55K 3′ ss recognition (Fig. 4B) and total U2AF levels (Fig. 5) are not changed as a result of adenovirus infection.

We did not observe a conversion of the A complex to the more mature spliceosomes (complex B and C) under these experimental conditions (Fig. 6). However, this inhibition appears to be an unspecific effect of the competitor RNA since addition of a 1000-fold excess of a vector-derived competitor RNA similarly inhibited B and C complex formation and splicing of both the IIIa and 52,55K pre-mRNAs (data not shown).

DISCUSSION

It has been suggested that alternative RNA splicing is regulated to a large extent at the level of commitment complex formation (9, 10, 29). However, there are so far only a few examples supporting this hypothesis (11, and Garcia-Blanco *et al.*, personal communication). Here we have provided additional evidence for this hypothesis by demonstrating that adenovirus L1 alternative splicing is also regulated at the level of commitment complex formation (Fig. 6A).

We have shown that nuclear extracts prepared from late adenovirus-infected cells partially reproduces the temporal shift from 52,55K mRNA production (early type) to increased IIIa mRNA splicing (late type) *in vitro*. Three important findings emerge from this study to explain the temporal shift in L1 3′

ss usage. First, the shift to increased IIIa splicing observed in late-infected extracts (Figs 2 and 3) results from an increase in commitment complex formation using the distal, IIIa, 3′ ss (Fig. 6A). Second, splicing of the proximal, 52,55K mRNA, is repressed in extracts prepared from late extracts (Figs 2 and 3). Third, the shift in L1 3′ ss usage does not require *cis* competition between ss since the upregulation of IIIa splicing and inhibition of 52,55K splicing can be observed on transcripts encoding the individual 3′ ss.

Activation of IIIa splicing during virus infection requires late viral protein synthesis (30, and refs therein). Thus, the increase in IIIa 3′ ss recognition observed in late extracts may be accomplished by synthesis of a virus-encoded splicing factor(s) which shows a specificity for non-consensus 3′ ss. Alternatively, virus infection may lead to an increase or decrease, or post-translational modification, of a cellular 3′ ss factor. Such a hypothetical virus-induced modulation would probably not involve U2AF, since the steady-state concentration of U2AF is not altered as a result of virus infection (Fig. 5). Furthermore, the activity of U2AF in late extracts does not appear to be changed since A complex formation on the 52,55K 3′ ss, which efficiently binds U2AF (data not shown), is identical in virus-infected compared to uninfected extracts (Fig. 4B). Thus, a regulation of IIIa splicing through U2AF would require a modification of that protein such that its specificity is extended to include also short polypyrimidine tracts. It is also possible that adenovirus induces the synthesis, or post-transcriptionally modifies, a second cellular 3′ ss factor which stimulates the activity of non-consensus 3′ ss, like the IIIa 3′ ss. The existence of such a hypothetical activity is supported by our observation that uninfected HeLa cell nuclear extracts contain a distinct *trans*-acting factor that is required for IIIa splicing but does not appear to have a significant effect on 52,55K or β-globin splicing (31). The IIIa stimulatory activity in late virus-infected extracts is currently being purified.

Little is known about how alternative 3′ ss selection is regulated in eucaryotic splicing. The most detailed studies have been carried out with the genes involved in somatic sex determination in *Drosophila*. These studies have shown that the sex-lethal protein regulates Tra splicing and autoregulates its own splicing (reviewed in ref. 32). Alternative splicing of the adenovirus L1 and the *Drosophila* sex-lethal and Tra pre-mRNAs are similar in that they all represent examples of unequal usage of two alternative 3′ ss under different physiological conditions. In both systems the default pathway is an exclusive usage of the proximal 3′ ss. However, they appear to use different mechanisms to achieve the shift in 3′ ss selection. The sex-lethal protein binds with a higher affinity than U2AF to the polypyrimidine tract of the proximal, male specific, Tra 3′ ss. Since sex-lethal lacks the effector domain of U2AF (25) its binding results in an inhibition of commitment complex formation on the male specific 3′ ss (M.Garcia-Blanco, personal communication) and an activation of the distal female-specific 3′ ss. This results in an on−off switch in sex-lethal and Tra 3′ ss usage. Similar to the sex-lethal system, the increase in IIIa splicing correlates with an increase in distal, IIIa, 3′ ss recognition and commitment complex formation using this ss (Fig. 6A). In contrast to the sex-lethal system, the decrease in proximal splicing (52,55K 3′ ss; Figs 2 and 3) does not appear to result from less efficient commitment complex formation at the proximal 3′ ss, but instead appears to be manifested at the level of conversion of committed complexes to the pre-spliceosome and mature spliceosomes. This difference in mechanism between sex-lethal and L1 pre-mRNA splicing is important since it allows for substantial 52,55K production also during the late phase of the infectious cycle. Since the 52,55K protein is necessary for virus assembly (33) the consequences of extinguishing 52,55K mRNA production during late infection would be deleterious to virus production.

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