

A U1 snRNA binding site improves the efficiency of in vitro pre-mRNA splicing

Jan-Peter Kreivi, Kenn Zefrivitz and Göran Akusjärvi
Department of Microbial Genetics, Karolinska Institute, S-104 01 Stockholm, Sweden

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Most laboratories use in vitro splicing substrates that are generated by run-off transcription of truncated DNA templates. Such substrates usually work well for spliceosome assembly and splicing (1). However, not always. During our studies of adenovirus L1 alternative splicing we have observed that the GD3A transcript, which encode the adenovirus third leader 5′ splice site coupled to the IIIA 3′ splice site, is very inefficiently processed in vitro (2). Frequently nuclear extracts with no detectable IIIa activity were encountered. Here we show that inclusion of a 11 nucleotide 3′ tail on the pre-mRNA, which encode the adenovirus major late first leader 5′ splice site (3) with a nine base pair U1 snRNA/pre-mRNA complementarity, stimulates IIIA splicing significantly (compare GD3A-U1wt and GD3A, Figure 1B). The importance of efficient U1 snRNA binding to the pre-mRNA was suggested by the fact that a transcript where four out of the nine potential U1 snRNA/pre-mRNA base pairs were mutated lost most of its activation potential (pGD3A-U1mut, Figure 1B). The time course of accumulation of intermediates and product suggest that the effect of a U1 end is manifested at the level of efficiency of RNA splicing rather than pre-mRNA stability. The effect of a U1 end is also significant on more efficiently spliced transcripts (not shown). In conclusion, the incorporation of a U1 snRNA binding site in a pre-mRNA may represent a general technique to improve the in vitro RNA efficiency.

The rationale for this experimental approach comes from the observation that U1 snRNP and other splicing factors binding to a 3′ splice site communicate with a downstream 5′ splice site during spliceosome formation, thus resulting in a concerted recognition of the 3′ and 5′ splice sites bordering an exon; the so called exon definition model (4). Here we show that a pre-mRNA with an efficient U1 snRNA binding site downstream of the 3′′ splice site works considerable better than a transcript with a free 3′ end. This observation is presented as a methodological improvement. However, it also provides additional credibility to the exon definition model.

To transform this observation to a simple practical method we used standard PCR and T7 in vitro transcription techniques (5) to synthesize the U1 substrate pre-mRNAs. Appropriately designed oligonucleotides were used in a PCR reaction to generate double-stranded DNA fragments which then were used as templates for synthesis of T7 pre-mRNAs (details on request). This technique eliminates the need for construction of new recombinant plasmids.

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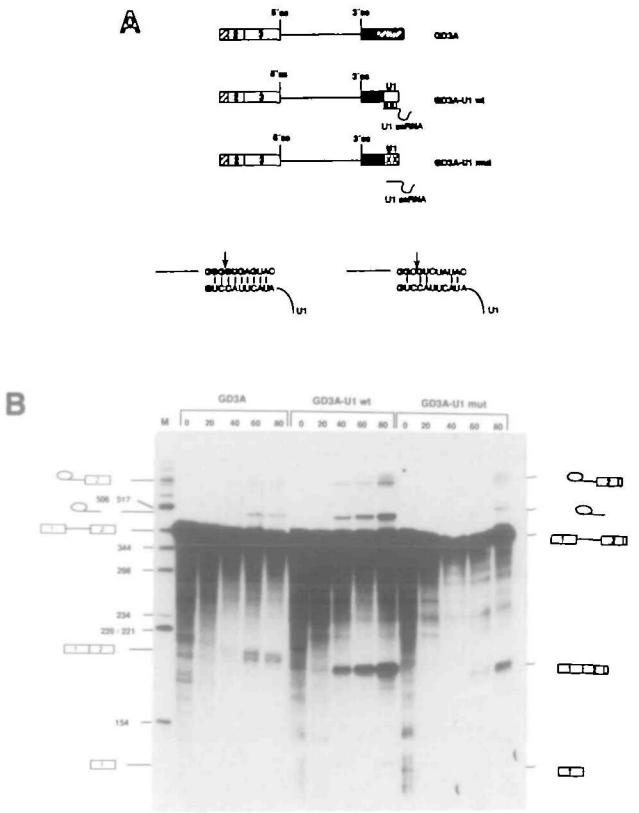

Figure 1. A. Top, Schematic representation of pre-mRNAs used in this study. 5′ss denotes the major late third leader 5′ splice site and 3′ss denotes the IIIa 3′ splice site (2). The distance between the IIIa 3′ splice site and the pre-mRNA 3′ ends are 65 nucleotides (GD3A) and 53 nucleotides (GD3A-U1wt and GD3A-U1mut), respectively. Bottom, possible base pairing between U1 snRNA and the wild type (left) or the mutated (right) late leader 5′ splice site. B. Time course (min) of splicing of GD3A pre-mRNAs with or without efficient U1 snRNA/pre-mRNA base pairing potential. Position of reaction products are indicated; left GD3A and right GD3A-U1wt and GD3A-U1mut. Conditions for in vitro splicing were as previously described (2). M, ^{32}P-labelled pBR marker fragments.