Vol. 203, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1994 Pages 128-132

DIFFERENT MECHANISMS ARE RESPONSIBLE FOR THE LOW ACCUMULATION OF TRANSCRIPTS FROM INTRONLESS AND 3' SPLICE SITE DELETED GENES

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Received July 12, 1994

A large reduction in the accumulation of mouse ß-globin mRNA occurs when both introns are deleted from its gene. This reduction is ameliorated if a sequence from HSV-1 thymidine kinase (TK), a naturally intronless gene, is inserted into the intronless globin construct. A large reduction in globin mRNA accumulation also occurs when just the terminal 3' splice site region is deleted. This reduction is not ameliorated by the insertion of TK sequence. Different mechanisms therefore must be responsible for the reduced accumulation of mRNA from from the intronless and 3' splice site deleted genes. The ability of TK sequence to enhance mRNA accumulation was dependent on its position within the intronless construct. Data are consistent with a model in which pre-mRNAs are co-transcriptionally channelled into intron-dependent or intron-independent metabolic pathways.

A number of eukaryotic genes require at least one intron for their transcripts to accumulate to maximal levels (1-5). To account for this intron-dependent RNA accumulation, it has been suggested that introns contribute to 3' end processing efficiency (4,6,7), nuclear stability (3), nucleocytoplasmic transport (3), or transcriptional enhancement (8). As part of a study to differentiate these possibilites, we monitored the processing and accumulation of mouse $ß^{maj}$-globin (MßG) transcripts after deletion or inactivation of its introns. We observed that deletion of the terminal 3' splice-site region of the MßG gene reduces transcript accumulation to an extent that is similar to that observed for an intronless MBG gene. While a requirement for introns and 3'ss regions may be part of the same intron-dependent phenomenon (2), the structural differences between intronless and the 3'ss-deleted transcripts suggest

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0006-291X/94 $5.00

that different mechanisms may be involved. Most notably, transcripts from a terminal 3'ss deleted MBG gene are not intronless, for intron 1 is intact. An explanation for the decline in mRNA accumulation in 3'ss deleted transcripts is removal of a positive element that stimulates 3' end processing (7,9). An alternative is that the unpaired 5' splice site that remains in these transcripts may serve as a negative element that inhibits 3' end processing (10) or nucleocytoplasmic transport (11).

A sequence from the naturally intronless HSV thymidine kinase (TK) gene has been shown to increase the accumulation of transcripts from an intronless rabbit ß-globin gene (2). If transcripts from terminal 3'ss deleted genes follow a similar pathway of RNA metabolism as those from intronless genes, TK sequence should boost their accumulation to a similar extent. We have used a variety of TK-MßG chimeras to examine this question. In addition, we have determined whether the ability of TK sequence to boost the accumulation of mRNA from an intronless MßG gene is dependent on the relative positioning of the TK and MßG sequences.

Materials and Methods

Mouse ß-globin gene constructs were transiently transfected into COS-1 cells as described previously (12). Total RNA was isolated and analyzed by quantitative RNAse mapping using uniformly labeled riboprobes (13,14). The riboprobes employed in this study contain two regions of complementarity - one for transcripts from the transfected globin gene, and one for transcripts from the SV40 gene that is endogenous to COS-1 cells (14). The globin and SV40 bands within individual lanes were quantified by densitometry (The Discovery Series, Quantity One software, version 2.2; Protein and DNA Imageware, Huntington Station, NY, USA), and the globin/SV40 ratio was used to calculate globin RNA accumulation.

Results and Discussion

Structures of the constructs employed in this study are outlined in Fig. 1. The wild-type construct contains the three exons and two introns of the MßG gene under the transcriptional control of the SV40 early promoter. The accumulation of transcripts from genes with deletions of both introns (ΔΔ) or of the terminal 3'ss region (Δ3) are compared in Fig. 2. Transcripts from the ΔΔ and Δ3 constructs accumulated poorly - 6.5 and 17%, respectively - compared to those from the wild-type construct. Insertion of a fragment of the HSV-1 thymidine kinase (TK) gene into exon 2 of the ΔΔ construct (ΔΔTK) resulted in a five-fold increase in globin RNA accumulation. This level of accumulation is approximately 30% of that obtained from the wild-type construct. When the TK fragment was inserted in the antisense orientation (ΔΔrTK), no boost in accumulation

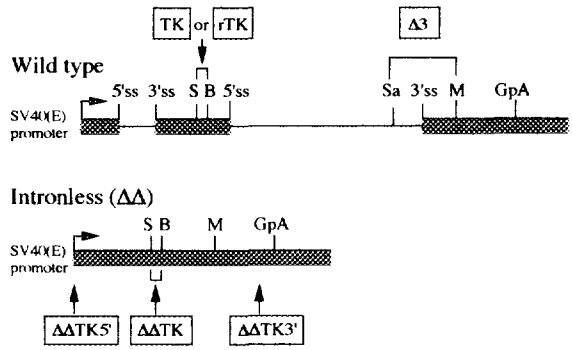

Figure 1. Structures of the mouse ß-globin constructs. Mouse ß-globin (MßG) exons and 3' flanking sequence are represented by shaded boxes, and introns are represented by thin lines. GpA is the MßG poly(A) site. Transcription is driven by the SV40 early promoter. Enzyme sites are BamHI (B), MscI (M), SacI (S), and SalI (Sa). The SalI site was introduced by linker insertion into a PstI site. Precise deletion of both introns (ΔΔ) was accomplished by replacing genomic fragments with cDNA fragments. Deletion of the terminal 3' splice site region (Δ3) was accomplished by removal of the SalI-MscI fragment. The TK and rTK versions of the ΔΔ and Δ3 constructs were made by inserting the 1.1 kb BglII-SmaI fragment of the HSV-1 thymidine kinase gene between the SacI and BamHI sites in sense (TK) or antisense (rTK) orientation. ΔΔTK5' was constructed by placing the TK fragment just downstream from the promoter region of the intronless construct. ΔΔTK3' was constructed by placing the TK fragment 160 bp upstream from the MßG poly(A) site of the intronless construct.

was observed. This demonstrates that the ability of TK sequences to increase the accumulation of transcripts from intronless MßG genes is orientation dependent and not due to transcriptional enhancement or an increase in promoter to polyadenylation site spacing. In contrast, no recovery of transcript accumulation was observed when TK sequences were inserted into exon 2 of the Δ3 construct in sense (Δ3TK) or antisense (Δ3rTK) orientations. These data indicate that the mechanism of mRNA depletion due to deletion of the terminal 3'ss must differ from that due to deletion of both introns.

Why does insertion of thymidine kinase sequence into the middle of the intronless MBG gene increase transcript accumulation? Perhaps separate nuclear pathways exist for intron-dependent (MBG) and intron-independent (TK) transcripts, and specific sequence elements are present in naturally intronless genes that guide and protect transcripts through the intron-independent pathway. The level of restoration seen with transcripts from the ΔΔTK construct suggests that the TK sequences are allowing a fraction of these transcripts to enter the intron-independent pathway. If pathway choice occurs co-transcriptionally, then inserting the TK sequence at the 5' end of the intronless MBG gene may increase the

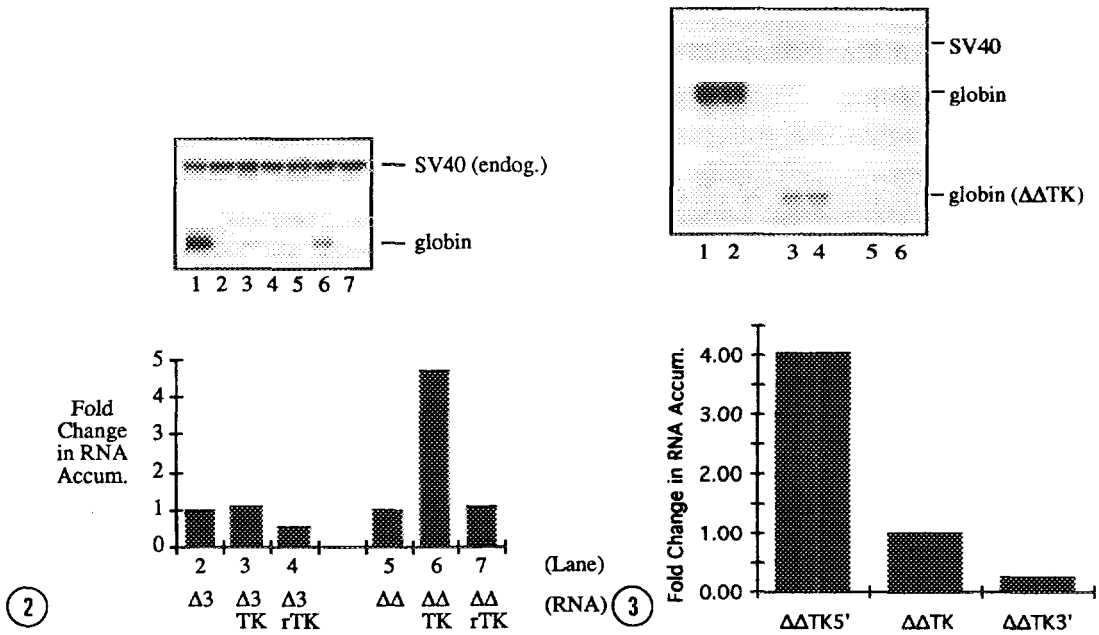

Figure 2. Effect of thymidine kinase sequence insertion on the accumulation of transcripts from intronless and splice site deleted mouse ß-globin genes. Data were obtained with a probe that is specific for globin exon 3 and SV40 sequences. The globin and SV40 bands from individual lanes are shown. For each sample, the level of globin mRNA accumulation was normalized to the level of SV40 mRNA that is endogenous to COS-1 cells. Changes in globin mRNA levels due to the TK sequence insertions are expressed as fold-change in accumulation. For reference, the accumulation of ΔΔTK transcripts (lane 6) is approximately 30% of that of wild-type transcripts (lane 1).

Figure 3. The site of thymidine kinase sequence insertion influences the accumulation of transcripts from the intronless mouse ß-globin gene. RNAs from duplicate transfections were analyzed with a probe that is specific for globin exon 2 and SV40 sequences. ΔΔTK5' (lanes 1 and 2), ΔΔTK (lanes 3 and 4), ΔΔTK3' (lanes 5 and 6). The globin and SV40 bands from individual lanes are shown. The shortened length of the globin band for ΔΔTK transcripts (lanes 3 and 4) is due to the insertion of TK sequence into exon 2 of these constructs. Levels of globin RNA were normalized to those of SV40 RNA; corrections for the length of the globin band were included in the calculations.

fraction of transcripts entering the intron-independent pathway, thus increasing transcript accumulation. On the other hand, inserting the TK sequence near the 3' end of the intronless MBG gene may decrease the fraction of transcripts entering the intron-independent pathway, thus decreasing transcript accumulation. As shown in Fig. 3, shifting the insertion site from the middle (ΔΔTK) to the 5' end (ΔΔTK5') of the intronless MßG gene resulted in a 4-fold increase in transcript

accumulation. In contrast, shifting the insertion to a site near the 3' end (ΔΔTK3') caused transcripts to accumulate only one-fourth as well as ΔΔTK transcripts. Therefore, the position of the thymidine kinase sequence within the intronless MßG gene greatly influences transcript accumulation.

TK sequences may confer intron-independence by channeling transcripts into a pathway that bypasses spliceosomes or spliceosome components. This view is consistent with the experiments of Greenspan and Weissman, who showed that TK sequences inhibit splicing when placed upstream, but not downstream, of human ß-globin introns (16). The enhanced accumulation of transcripts observed in our experiments when TK sequences were positioned in the 5' region of intronless globin constructs supports the idea that the selection of this pathway is co-transcriptional.

Acknowledgments

This work was supported by NIH grants GM33410 and CA55995 and by the Department of Biological Sciences, Fordham University.

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