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Single-molecule RNA observation in vivo reveals dynamics of co-transcriptional splicing M.L. FERGUSON, A. COULON, NIH, Bethesda, MD, V. DE TURRIS, Albert Einstein College of Medicine, Bronx, NY, M. PALANGAT, C.C. CHOW, NIH, Bethesda, MD, R.H. SINGER, Albert Einstein College of Medicine, Bronx, NY, D.R. LARSON, NIH, Bethesda, MD — The synthesis of pre-mRNA and the splicing of that pre-mRNA to form completed transcripts requires coordination between two large multi-subunit complexes (the transcription elongation complex and the spliceosome). How this coordination occurs in vivo is unknown. Here we report the first experimental observation of transcription and splicing occurring at the same gene in living cells. By utilizing the PP7/MS2 fluorescent RNA reporter system, we can directly observe two distinct regions of the nascent RNA, allowing us to measure the rise and fall time of the intron and exon of a reporter gene stably integrated into a human cell line. The reporter gene consists of a beta globin gene where we have inserted a 24 RNA hairpin cassette into the intron/exon. Upon synthesis, the RNA hairpins are tightly bound by fluorescently-labeled PP7/MS2 bacteriophage coat proteins. After gene induction, a single locus of active transcription in the nucleus shows fluorescence intensity changes characteristic of the synthesis and excision of the intron/exon. Using fluctuation analysis, we determine the elongation rate to be 1.5 kb/min. From the temporal cross correlation function, we determine that splicing of this gene must be co-transcriptional with a splicing time of $\sim$100 seconds before termination and a $\sim$200 second pause at termination. We propose that dual-color RNA imaging may be extended to investigate other mechanisms of transcription, gene regulation, and RNA processing.

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