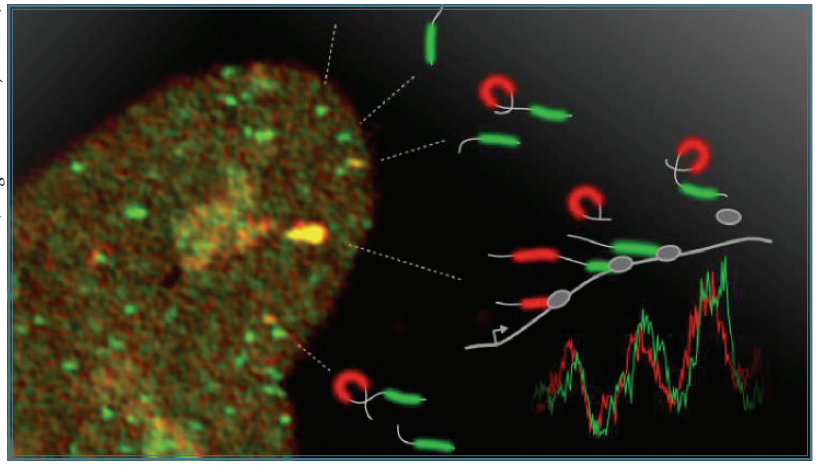
When Timing Matters

Observing mRNA transcription in real time, CCR scientists find a surprising lack of coordination between the timing of synthesis and splicing.

Cancer is a disease of dysregulation. Understanding the normal molecular process in a cell is the first step towards determining what can go wrong and how to fix it. The process of transcribing DNA into mRNA involves multiple macromolecular complexes that initiate RNA synthesis, splice disparate regions of the RNA together, and chemically modify the RNA in preparation for nuclear export. One may assume that these functions occur in a logical, stepwise fashion, amenable to tight regulation; in fact many of them overlap in time, as well as space. To what extent they occur stochastically, i.e., on a first-come-first-serve basis of random molecular interactions (kinetic competition), or are orchestrated by checkpoint molecules, has never been addressed.

Publishing in a recent issue of *eLife*, Daniel Larson, Ph.D., Investigator in CCR's Laboratory of Receptor Biology and Gene Expression, and his colleagues have studied the transcription and splicing of single RNA molecules produced from the human β-globin gene in the nucleus of engineered cell lines, in real time. The gene comprises an upstream intron that is spliced out of the final RNA product and a downstream exon. The team has taken advantage of RNA motifs from bacteriophages that form hairpin structures—PP7 and MS2—recognized by specific binding proteins. By inserting multiple PP7 hairpin-coding sequences in the intron and multiple MS2 hairpin-coding sequences downstream in the exon, the team could observe the transcribed RNA segments through the presence of corresponding fluorescently tagged PP7- (red) and MS2- (green) binding proteins.

(Image: A. Coulon, NIDDK)



Observing single RNA in living cells reveals the kinetics of RNA synthesis and processing.

Monitoring fluorescence in the two channels over time, Larson and his colleagues observed localized fluctuating signals—increasing signals reflecting RNA synthesis and decreasing signals reflecting splicing and/or release of RNA from the site of transcription—for over 1,700 transcripts. By cross-correlation analysis of the intensity signals, they showed the data best fit a model in which the transcript could either be released from the transcription site before splicing (a simultaneous extinguishing of red and green signals) or the intron could be spliced out before transcription was complete (red signal extinguished before green). The data argue for a kinetic competition model in which RNA synthesis and processing occurs at random, rather than in coordinated fashion.

Armed with this knowledge, Larson and his colleagues could ask how a cancer-associated mutation in splicing factor U2 auxiliary factor 1 (U2AF1) affected the balance of competition. U2AF1 is an essential factor involved in splicing of most human transcripts. In the presence of the mutant U2AF1, splicing before release of the transcript was completely abolished, shifting the balance of splicing activity post-transcriptionally. The researchers confirmed this result for an endogenous mRNA, *FXR1*, which is alternatively spliced in the presence of the mutant U2AF1 in cancer.

"Although variations in alternative splicing have been seen in single cells, the mechanism behind such variability has remained elusive," said Larson. "Our studies on the mutant U2AF1 suggest a role for mutations in shifting the kinetic balance to alter gene expression. The kinetic delay may allow for alternate exon pairing during transcription or reconfiguration of the mRNA after release, prior to splicing. This may be one explanation for increased levels of 'noisy splicing' which are observed in cancer."

To learn more about Dr. Larson's research, please visit his CCR website at https://ccr.cancer.gov/daniel-r-larson.