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> failure to terminate transcription can result in circRNA production from downstream genes

Circular RNAs (circRNAs) are covalently closed RNAs that can specifically control gene expression. CircRNAs are produced at low levels from many protein-coding genes by pre-mRNA 'back-splicing' of exons, but the mechanisms that modulate their production are poorly understood. Liang *et al.* now show that steady-state circRNA levels increase when co-transcriptional mRNA processing is diminished.

The authors introduced into fruit fly DL1 cells a three-exon minigene derived from the laccase2 gene, which is known to produce circRNA. Depletion of several spliceosome proteins largely eliminated the expression of the minigene linear mRNA. By contrast, expression of the minigene circRNA in these cells was only slightly decreased or even increased. Importantly, the splicing patterns of the endogenous laccase2 gene and of two other circRNA-producing genes were similarly affected. Furthermore, the depletion of 25 other core spliceosome proteins resulted in increased circRNA expression from various endogenous genes. These data suggest that the relative efficiency of back-splicing compared with canonical splicing increases when the quantity of spliceosome proteins is limited.

Next, the authors examined the effect of mRNA 3′-end processing on circRNA production. Depletion of the cleavage and polyadenylation factors Cpsf73 (cleavage and polyadenylation specificity factor 73) and Symplekin greatly increased the expression of the minigene circRNA. Gene expression analyses revealed that depleting these factors reduced transcription termination of an upstream gene, which resulted in readthrough transcription into the minigene and back-splicing.

The torpedo model of transcription termination posits that cleavage by endonucleases such as Cpsf73 provides an entry site for a 5′–3′ exonuclease that degrades the cleaved downstream RNA, catches up with the RNA polymerase and terminates transcription. Consistent with the torpedo model and with increase of back-splicing by readthrough transcription, depletion of 5′–3′ exoribonuclease 2 homologue (also known as Rat1) caused constitutive expression of the minigene circRNA.

In human carcinoma PA1 cells, the genes *MATR3* and *PAIP2* are expressed in the same orientation, and the downstream *PAIP2* generates a circRNA (circPAIP2). Considerable amounts of nascent RNAs were detected throughout the region between *MATR3* and *PAIP2*, and depletion of human CPSF3 increased their expression as well as the expression of circPAIP2, suggesting that failure to terminate transcription can result in circRNA production from downstream genes. This was confirmed using an antisense oligonucleotide that targeted the intergenic region, which considerably reduced intergenic RNA levels and simultaneously decreased circPAIP2 levels.

Thus, circRNA levels increase when the canonical splicing of the parent genes is diminished, or in conditions of reduced transcription termination at upstream genes. The authors propose that when spliceosome activity is limited, cross-exon interactions are not easily replaced with cross-intron interactions, as required for canonical splicing. Instead, the full spliceosome assembles across exons, which results in back-splicing and circRNA production.

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FURTHER READING Szabo, L. & Salzman, J. Detecting circular RNAs: bioinformatic and experimental challenges. *Nat. Rev. Genet.* **17**, 679–692 (2016)