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GENE REGULATION

# Inner control

The complex mammalian transcriptome has just become even more complex. A new study reveals that many intragenic enhancers in the mouse genome can drive the tissue-specific expression of mature, alternatively spliced mRNA products — that is, these enhancers are acting just like promoters.

Investigating the role of intragenic enhancers has been hampered by technical difficulties: in particular, by the need to eliminate the confounding presence of transcription from the promoter of the gene. To study how intragenic enhancers affect host gene expression, the authors turned to a paradigmatic locus — the mouse α-globin locus. Four distal enhancers of the globin genes lie upstream of the locus, and three of them lie within an adjacent, apparently unrelated gene called nitrogen permease regulator-like 3 (*Nprl3*). When the *Nprl3* promoter was deleted by homologous recombination, *Nprl3* expression was abolished in all but erythroid cells, in which transcripts were shown by quantitative real-time PCR (qRT-PCR) to be still abundant. A closer look at the erythroid transcripts revealed a mixture of alternative gene isoforms characterized by alternative first exons (AFEs). These observations suggested that intragenic enhancers behave like alternative erythroid-specific promoters.

This hypothesis was confirmed in several ways. First, RNA sequencing (RNA-seq) showed that the pattern of transcription at each enhancer within *Nprl3* is similar to that seen at canonical transcription start sites (TSSs): it involves short bidirectional poly(A)$^-$ transcripts as well as abundant, full-length poly(A)$^+$ transcripts produced from the sense strand. The latter species of RNA has been named multiexonic RNA derived from enhancers, or meRNA. Second, a search for active, erythroid-specific enhancers identified sense and antisense expression from hundreds of intragenic enhancers in mouse erythroid cells and in human fibroblasts: meRNAs with AFEs were seen at 176 enhancers in erythroid cells. Third, intragenic enhancers acting in a similar way to those of *Nprl3* were characterized in four additional genes that have naturally inactive promoters in erythroid cells. Fourth, many intragenic enhancers in erythroid cells, and in other cell types, have already been annotated as 'active TSSs', despite bearing the chromatin signature of an enhancer.

The function of meRNAs — which account for 50% of mRNA abundance in the absence of the promoter — is unknown. Future studies should reveal whether they are important in enhancer function or whether they are simply a by-product of the process of transcriptional regulation by enhancers.

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**ORIGINAL RESEARCH PAPER** Kowalczyk, M. S. *et al.* Intragenic enhancers act as alternative promoters. *Mol. Cell* **45**, 19 Jan 2012 (doi: 10.1016/j.molcel.2011.12.021)