GENE EXPRESSION

Catching Pol II in the act

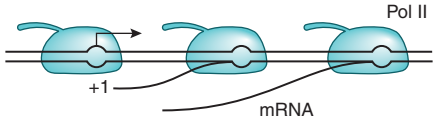
Two methods capture the pausing behavior of RNA polymerase II as it generates messages from mammalian genomes.

The process of transcribing a gene involves a lot of stuttering, thanks to the peculiar properties of RNA polymerase II (Pol II), the principal scribe of messenger RNA in eukaryotes. Following these pauses can reveal the complex regulatory dynamics of transcriptional elongation and coprocessing. Two recent efforts, one led by Stirling Churchman of Harvard University and another by Nicholas Proudfoot of the University of Oxford and Maria Carmo-Fonseca of the University of Lisbon, have independently yielded methods to track Pol II occupancy along nascent human transcripts at single-base resolution.

Currently, chromatin immunoprecipitation using antibodies against Pol II can be used to report polymerase occupancy, but at low resolution. Transcription can also be halted chemically in nuclei and then reconstituted with labeled nucleotides for genomic run-on sequencing. Precision nuclear run-on and sequencing (PRO-seq) provides single-base resolution, but its use has yet to be demonstrated in mammalian cells.

With Jonathan Weissman at the University of California, San Francisco, Churchman developed native elongating transcript sequencing (NET-seq), which consists of immunoprecipitating Pol II and sequencing the 3′ end of RNA protected within its active site, yielding strand-specific nucleotide-resolution maps of Pol II density in yeast. "That's a very appealing experiment," says Proudfoot. "To start with, you're not doing a run-on. Also, because the RNA inside the polymerase is pretty stable, you don't have to cross-link." He believes that these features minimize the potential for artifacts.

To attempt NET-seq on mammalian cells, Proudfoot's and Carmo-Fonseca's teams began with high-affinity homemade antibodies against native Pol II (Nojima *et al.*, 2015). As a twist, two of the antibodies were directed against different forms of Pol II on the basis of phosphorylated residues in the C-terminal regulatory domain. The researchers isolated chromatin from HeLa cells to enrich for nascent RNA and then released transcriptional complexes through treatment with micrococcal nuclease. The different antibodies generated unique and reproducible profiles. For example, a phosphoserine 5 isoform pauses over spliced exons, suggesting that Pol II phosphorylation is highly dynamic, and a phosphoserine 2 isoform typically found at the 3′ end of transcripts becomes associated with the promoter when polyadenylation factors are knocked out.



RNA polymerase II pauses reproducibly at locations along nascent transcripts, as observed by two new methods in mammalian cells.

The approach also recovers RNA present in processing complexes that coprecipitate with Pol II. The researchers found that the phosphoserine 5 isoform is associated with the intron 5′ splice site, indicating that the upstream exon is held by the polymerase in order to make it available for the downstream exon during processing.

Proudfoot says that their experiments have been repeated using commercial antibodies, and they have now profiled additional Pol II C-terminal–domain isoforms. Given the potential for many combinatorial modifications, "I suspect that the C-terminal–domain code is even more complex than the histone-tail code," he says. They have also found a way to remove cotranscriptional complexes to focus on polymerase density.

Churchman had a similar interest in moving NET-seq beyond yeast. "I find splicing to be really fascinating, and one of my main motivations was to look at pausing around exons in mammalian cells," she says. Her group's strategy was to remove the need for immunoprecipitation. "The plan was to make this a very straightforward approach…that would be a really great tool for the community," she says.

To accomplish this, they heavily optimized chromatin fractionation, including urea purification and washing steps, isolating over 99% of engaged polymerase (Mayer *et al.*, 2015). Other critical improvements were the depletion of small and ribosomal RNA, use of short molecular barcodes (allowing the computational removal of library biases) and more efficient ligation during library construction. Together, these provided a very clean signal.

The high resolution revealed interesting Pol II interactions, such as direction-dependent pausing at occupied transcription factor sites. Pol II also pauses preferentially at the boundaries of exons that are retained during splicing. "Somehow it is detecting that exon, and then it is able to detect the processing fate of that exon, which is pretty remarkable," says Churchman. The method also found that convergent, antisense transcription in promoter-proximal regions is a feature of some low-expressed genes. Churchman's group is now applying their approach to many human cell types and developing a protocol that requires far fewer cells.

The new strategies for keeping a close eye on Pol II behavior will help to unravel long-standing questions in mammalian transcription.

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RESEARCH PAPERS

Mayer, A. *et al.* Native elongating transcript sequencing reveals human transcriptional activity at nucleotide resolution. *Cell* **161**, 541–554 (2015).

Nojima, T. *et al.* Mammalian NET-seq reveals genome-wide nascent transcription coupled to RNA processing. *Cell* **161**, 526–540 (2015).