In the 1960s, Oscar Miller and his colleagues at Oak Ridge National Laboratory (and later at the University of Virginia) perfected a technique for direct visualization of transcription from eukaryotic chromosomes in situ by transmission electron microscopy. This method, sometimes referred to as “Miller spreads,” was then applied to questions about transcription in bacterial cells (1). Several of these studies of “genes in action,” in which RNA polymerase can be seen traversing the chromosome and transcripts can be visualized emanating from the chromatin, appeared in the Journal of Bacteriology.

In the late 1970s, Miller spreads were used to test the hypothesis that RNase III was responsible for the cotranscriptional processing of rRNA transcripts in Escherichia coli. rRNA operons are transcribed much more actively than most other genes in fast-growing bacterial cells, and because the 16S and 23S rRNA genes are so highly transcribed, rRNA transcription units observed in Miller spreads could be distinguished from other transcription units. Hofmann and Miller (2) showed that in wild-type cells, rRNA operons display a distinctive double gradient of transcripts, sometimes described as a double Christmas tree morphology. They then confirmed that RNase III cleaves the primary rRNA transcript as it is being synthesized by showing that, unlike the situation in wild-type cells, rRNA operons in RNase III-defective strains display a single gradient of rRNA transcripts that extends the entire length of the rRNA operon.

Later, the technique was used to address other transcription questions that were difficult to approach in cell populations. For example, French and Miller (3) showed that multiple rRNA operons from a single E. coli cell are transcribed at similar levels. Furthermore, long before the advent of “omics” technologies and superresolution microscopy, they were able to map patterns of transcription activity over a significant fraction (13%) of the E. coli chromosome and to address questions about coordinate expression of different operons. Finally, the technique was used to measure the actual movement of RNA polymerase along DNA templates (4), i.e., to estimate the transcription elongation rate in growing cells, rather than to estimate elongation rates by extrapolation from purified transcripts. To this day, the Miller spread technique continues to make a unique contribution to studies of prokaryotic and eukaryotic gene expression.

REFERENCES