A look back: making mapping easy to digest

In the pre-genomics era, mapping gene expression was a daunting task; remarkably little was known about gene structure and transcriptional regulation, and there was only a limited palette of techniques of which early molecular biologists could avail themselves. Help arrived in the late 1970s in the form of the nuclease protection assay, a technique that saw its debut in the laboratory of Massachusetts Institute of Technology investigator Phillip Sharp, whose research group was already on the verge of making a tremendous splash in the RNA world.

"We were trying to understand transcriptional control using adenovirus as a model system," explains Arnold Berk, a former post-doc from Sharp's lab. "Earlier work from [his] lab had very approximately mapped the positions of early messenger RNAs along the length of the genome, and I was trying to think of more precise ways to map them." The age of hybridization experiments had just recently dawned, and investigators were still in the process of identifying optimal conditions under which different types of nucleic acids could best be brought together; however, Sharp's group had already conducted some experiments that combined RNA-DNA hybridization with nuclease digestion, and Berk saw strong potential in this approach for the fine mapping of genes.

Norman Davidson and his colleagues had recently shown that by conducting experiments in high concentrations of the solvent formamide, one could favor the assembly of complementary DNA-RNA hybrids over DNA-DNA duplexes[1]. Building on this, Berk and Sharp performed a series of hybridization experiments that brought together radioactively labeled fragments of restriction-digested adenovirus DNA with purified RNA, favoring the formation of RNA-DNA hybrids; these hybrids were then digested with S1 nuclease, which chewed away the non-hybridized stretches of single-stranded DNA. The resulting 'hot' DNA fragments could subsequently be analyzed by electrophoresis to identify transcribed genomic sequences, and this data in turn could be applied to the viral genomic restriction map to establish gene position[2]. Fortunately, the technique worked like a charm: "From the very first time, we got bands on the gel," says Berk, "and we were very excited!"

Berk's work with transcript mapping would go hand in hand with the efforts of Sue Berget, another Sharp lab investigator who was working to unravel the mystery of why RNA-DNA hybrids formed from the transcript encoding the adenoviral protein hexon consistently resulted in an unhybridized 5′ tail of RNA, which didn't seem to recognize the DNA sequences contiguous with the rest of the gene[3]. At the same time, other studies had indicated that several 'late' adenoviral transcripts seemed to somehow share a common 5′ leader sequence[4]. With this and other evidence, Sharp's lab ultimately arrived at the conclusion that some sort of transcript splicing process was taking place, continues Berk, "and the question became, was there an RNA transcript initially made that was collinear with the DNA and was then spliced, or did the polymerase jump along the template? And I realized that we could answer that by looking at the late nuclear RNA by S1 mapping." The group's findings would be presented at the Cold Spring Harbor Symposium[5], setting the wheels in motion for the vigorous investigation of the splicing phenomenon, a process that ultimately led to Sharp's sharing the Nobel Prize in Medicine with fellow RNA processing pioneer Richard J. Roberts in 1993.

In the ensuing years, nuclease protection assays would render themselves invaluable both for the mapping of genes and as a means for detecting and quantifying changes in transcription; in particular, the technique has benefited from its effectiveness as a sensitive assay for even relatively scarce mRNA transcripts. In many ways, however, RNase protection studies have recently fallen by the wayside. As the collective store of genomics knowledge has grown over the past decade, so too has the hunger for methods that dramatically accelerate the rate and expand the breadth of gene expression analysis. This is true even in Berk's own lab at Berkeley, which is currently involved in the study of mammalian transcriptional regulation. "We use mostly current techniques of RT-PCR and quantitative PCR," he says. All the same, there remain some occasions when the old ways are best: "[W]e do use nuclease protection when we're mapping the 5 end of an RNA for the first time, especially a low-abundance RNA... the combination of doing both the nuclease protection and the primer extension assay gives you consistent results, and you can be pretty confident that you've mapped the 5′ end of the RNA to within 5 nucleotides or so of the template, and that you haven't been fooled by a short 5′ exon."

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