A look back: getting the message

RNA has never been 'easy' to work with—compared to DNA, it's a biochemical primadonna, requiring the cleanest conditions and the utmost care in handling. This has been known for decades, but even with kid-glove treatment, RNA research initially suffered from an absence of simple and effective analytical methods. A major turning point came in 1977, however, when Stanford investigator George Stark developed a method that dramatically increased the amount of information that researchers could extract from cellular RNA.

According to Stark, the technique was born of pure and simple expediency—the need to quantitatively detect RNA species as painlessly as possible. "The current method was experimentally horrible," explains Stark. "It involved isolating RNAs and running them out on a tube gel, and then freezing the gel and slicing it into 100 or 200 slices using something that looked like an egg slicer!" These slices would be subjected to hybridization with a probe, then individually analyzed via scintillation counter to reveal the amount of signal localizing to specific gel fractions. "We did this experiment once," he says, "and I thought it was pretty horrible, and that something better needed to be done."

Edwin Southern had recently changed the face of molecular biology by introducing his eponymous DNA transfer and probing technique[1], a method that was already in widespread use. Unfortunately, no analogous technique had yet been established for RNA, which had proven far less amenable to direct transfer to nitrocellulose than DNA. At that time, Stark's team was also playing around with strategies for cross-linking nucleic acids to various matrices—work that centered on the use of cellulose powder that had been chemically activated through a process of nitration and diazotization, permitting the direct covalent linkage of nucleic acids to the modified cellulose particles[2]. Stark reasoned that this same approach should also work with paper, and he and colleagues James Alwine and David Kemp now replicated the configuration of Southern's transfer apparatus in an effort to transfer RNA from a methylmercury agarose gel to cellulose paper that had been chemically activated via their previously established protocol[3]. "It was one of those 'whoopee!' experiments, where it actually worked the very first time," recalls Stark. "And I don't think anybody ever ran a tube gel after that."

The technique became known as 'northern blotting'—a pun on Southern's name, says Stark, "for which he has since forgiven me"—and caught on quickly, offering the ability to detect and analyze RNA transcripts, including splice variants, with astonishing sensitivity. The one drawback was the substrate; the process for generating activated, diazotized paper is a chore, and the quality of individual preparations can vary considerably. Many scientists were therefore relieved by the subsequent development of modified northern protocols using blotting conditions that made possible the rapid and efficient transfer of RNAs onto nitrocellulose[4], or using more rugged nylon-based membranes[5], which offer higher binding capacity for nucleic acids and are better capable of withstanding repeated cycles of washing, stripping and reprobing.

Although transfer and hybridization conditions may have been optimized considerably over time, the northern blot has since its conception demonstrated such profound sensitivity that even with the emergence of high-tech solutions such as quantitative PCR and microarray analysis, blotting remains the gold standard for the confirmation of virtually all gene expression data. "We worked out that you could actually detect one RNA molecule per cell by northern blot," says Stark. "And you don't want any more sensitivity than that, I don't think!"

In subsequent years, Stark would further extend his blotting acumen to develop a membrane transfer and an antibody-based detection system for proteins—the earliest incarnation of the western blot. As with the northern, Stark's protein methodology relied on overnight capillary transfer from gel to activated cellulose paper[6]—which most likely contributed to the rapid supplantation of his technique by Harry Towbin's faster and simpler method for electroblotting proteins to nitrocellulose membranes[7], published later the same year. Today, Towbin's western method is clearly the preferred approach, but Stark's early work in protein detection is notable nonetheless, and his pioneering contributions to the canon of research methods are undisputed—as is clearly evidenced by the fact that 30 years later, even the mightiest of microarrays can be brought low by the absence of corroborating data from a simple, well-prepared northern.

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