A look back: bands on the run

Scientists have been trying to understand the nature and importance of protein-DNA interactions since the early 1960s. Such research started with studies of the association of RNA polymerase with DNA, but soon expanded to encompass the characterization of two newly-discovered but equally intriguing DNA-binding proteins: the Lac repressor[1] and the phage λ repressor[2].

One of the most important early techniques for analyzing these complexes arose from the discovery that certain membrane filters will retain DNA-protein complexes, but not free DNA[3]. By quantifying the retention of radiolabeled DNA fragments mixed with varying amounts of a protein of interest, it became possible to determine with reasonable accuracy the stoichiometry and binding affinity of a protein for a given sequence, and the technique was soon being successfully applied for the analysis of RNA polymerase[3] and the Lac repressor[4]. But even though filter binding became relatively popular, it remained impractical for the characterization of less stable complexes, and could only be used to demonstrate the existence of DNA-protein complexes without necessary revealing all of the participating components.

Arnold Revzin and Mark Garner, two researchers at Michigan State University, were among those frustrated by the limitations of existing techniques. "We were trying to characterize… the thermodynamics and general properties of the interactions of proteins with specific DNA sequences," explains Revzin. "We were just looking for some other way to characterize these interactions." One recent study had shown that the ternary transcription elongation complex—DNA bound to RNA polymerase with a nascent RNA chain—was sufficiently stable for visualization by gel electrophoresis[5]. Garner and Revzin built on these findings, combining purified protein with DNA restriction fragments containing appropriate binding sites and then running the mixture on a polyacrylamide gel. Their results were unambiguous, with protein-DNA complexes forming distinctly 'shifted' higher molecular weight bands on the gels, and demonstrated that this technique could be used to study a wide variety of proteins, including the *lac* operon–associated catabolite activator protein, which had proven especially difficult to characterize by filter binding. Thus was born the electrophoretic mobility shift assay (EMSA).

They soon realized that they weren't the only ones to reach this breakthrough. "Mark went to this meeting—I think it was in Albany," says Revzin. "He just went to it and presented what we were doing, and when he came back, he said, 'You know what? We ought to get this published—like, tomorrow!' Because, you know, other people were doing it." Garner and Revzin published their technique in an article in the middle of 1981 (ref. 6); sure enough, it was followed months later by a similar article from the laboratory of Donald Crothers at Yale[7].

Crothers and coauthor Michael Fried also had developed their version of EMSA while studying protein binding at the *lac* operon. Initially, Fried had speculated that only free DNA would be amenable to electrophoresis, and that DNA-protein binding could be quantified by determining how much DNA did not enter the gel. What they saw instead was a variety of shifted bands that appeared to correlate with the number of repressor molecules bound to each DNA fragment. Crothers recalls: "It was a very dramatic picture, where you could discriminate different kinds of protein-DNA complexes on the gels… what I said to Mike at that time was, forget what you're doing—follow this up!" Their paper also offered some important extensions of Garner and Revzin's assay, using radioactive labeling rather than ethidium bromide staining to detect shifted bands, and demonstrating the capabilities of EMSA as a means for measuring the relative binding constants and stoichiometry of protein-DNA interactions.

Between these two articles—which are typically credited alongside each other for introducing this technique—EMSA quickly caught on and remains popular even now. Among other assets, Revzin cites the ease with which it can be used to precisely isolate 'interesting proteins from even crude extracts: "You can simply ask, here's a gemish of stuff from a bunch of broken-up cells, and is there a factor that's going to bind to a specific sequence of DNA?" Little has changed about this technique in two decades, but perhaps that's because the simplicity of the assay is a key to its success. "We still do it," says Crothers. "Whenever we're working on a protein-DNA complex, we like to be sure that we're getting specific binding,… and in terms of specificity, it's probably the most accurate way to go for relative binding constants. It's a work-horse, and a lot of people do it."

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