A look back: bound to succeed

For over fifty years, scientists have recognized the power of immunochemical reagents as a tool for analyzing biological molecules of interest.

One investigator at the vanguard of early immunoassay development was Rosalyn Yalow, whose work with Solomon Berson led to the development of the radioimmunoassay—a powerful diagnostic tool that helped make Yalow the second woman to receive the Nobel Prize in Medicine and the first to win the Lasker Award. As early as 1960, this assay—which can be seen as a precursor to the modern immunoprecipitation—gave investigators the power to quantify surprisingly small amounts of such important biological molecules as insulin¹ and growth hormone².

The basic premise of this technique involves first measuring the extent to which an antibody's binding to radiolabelled antigen can be inhibited by competition with unlabelled antigen; once a standard curve has been generated, subsequent experiments can reveal the concentration of unlabelled antigen present in a sample of interest. To measure binding, the antigen-antibody complexes must be precipitated, and the preferred way of doing this was by binding with a secondary antibody specific for immunoglobulin. Although this technique could be highly effective, it was also grueling, requiring painstaking initial titration of the antibodies to maximize the efficiency of precipitation.

Things would literally change overnight in 1975, when UCLA graduate student Steven Kessler, deeply frustrated by his efforts to get reproducible results from the double-antibody strategy, sat down one day to do some reading. The article he read, work by the Swedish microbiologist Göran Kronvall, opened his eyes: Kronvall had found that a protein from the surface of the bacteria *Staphylococcus aureus*, protein A, has a strong affinity for mammalian IgG³. "The realization that protein A-bearing staphylococci might substitute for my second antibody came immediately and seemed so logical that it simply had to work," Kessler would later recall. He tested his approach with fixed, heat-killed bacteria⁴, and the outcome was clear: "My first experiment, designed purely empirically, gave the cleanest polyacrylamide gel patterns of lymphocyte immunoglobulins I had ever seen⁵."

By removing one of the more tiresome elements of the immunoprecipitation process, Kessler's work opened the door for a considerably wider audience of scientists to apply the method, and it wasn't long before the technique entered its next evolutionary stage, as a means to detect and monitor protein-protein interactions. Some of the most important early work along these lines was being done by

virologists, who were just starting to recognize the potential significance of interactions between viral and host cell proteins. Key among these was a landmark 1979 *Nature* paper by David Lane and Lionel Crawford, in which they demonstrated that antisera directed against the SV40 T antigen were apparently coprecipitating a 53-kDa host protein: the soon-to-be-infamous oncoprotein p53 (ref. 6). Ed Harlow, another Crawford lab alumnus and coauthor of the widely-used *Antibodies* manual, looks at this work as a significant turning point—although at the time there was still some confusion about how to interpret these early studies. He cites a 1975 article from Peter Tegtmeier's lab, in which they first characterized the T antigen and unintentionally coimmunoprecipitated p53 without recognizing the significance of the association⁷. "They thought it must be a breakdown product," explains Harlow, "and that was the big confusion when this process began, trying to figure out whether some of these things were associated or not. There were two possibilities that make it a more boring result: either it's a breakdown product of the protein that you're actually looking at... or alternatively, it's a cross-reaction."

Lane and Crawford's findings withstood close scrutiny, however, and were bolstered by the work of other virologists, who used co-immunoprecipitation to demonstrate other host-viral protein-protein interactions and to dissect their functional relevance. Harlow's work showing the interaction between the adenoviral E1A protein and numerous host proteins⁸ is among the more notable of these early efforts and, as with p53, would yield invaluable insights into cell cycle regulation and tumor biology.

"I suspect that the work with viral-host interactions was influential in getting everyone to think of coimmunoprecipitation as a way to look at functional relationships," says Harlow, and indeed, the method soon spread throughout the molecular biology research community as a simple and potent means for examining protein associations. And even today, thanks to the advent of technologies such as monoclonal antibodies, protein A-conjugated beads, epitope tagging, and mass spectrometry, coimmunoprecipitation still is among the most powerful and practical experimental tools available.

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