A look back: westward expansion

After reflecting on the events that led to the development of the immunoblot some 25 years ago, Harry Towbin has a simple explanation: "It [was] all driven, actually, by the mistrust of antibodies."

The ancestry of modern immunochemical techniques can be directly traced back to the work of pathologist Albert Coons, who first conceived of conjugating specific antibodies to a fluorescent tag over sixty years ago while trying to develop an effective strategy for detecting *Streptococcus* in tissue samples from rheumatic fever patients. This technique, developed in collaboration with chemists Hugh Creech and Norman Jones, proved highly effective[1] and laid the groundwork for other methods that exploit the exquisite specificity of the antibody for detection and quantification of target molecules. The methods used by Coons and his colleagues would subsequently be advanced in the 1960s, when two research groups independently developed systems for conjugating antibodies to enzymes such as horseradish peroxidase, which can in turn catalyze the processing of dye precursors into detectable precipitates[2,3].

These detection techniques would soon be complemented by improvements in the cultivation of antibodies. Key among these was the development of the hybridoma fusion method by Köhler and Milstein, a Nobel-winning breakthrough that allowed researchers to generate large quantities of single-target 'monoclonal' antibodies with identical binding kinetics[4], a considerable step beyond whole-serum mixtures of antibodies, whose properties and quality can vary widely. Even with this innovation, however, determining the effectiveness of a given monoclonal still remained a problem, as hybridoma fusions routinely yield clones that produce promiscuous or useless antibodies.

Enter Towbin, who arrived at Julian Gordon's laboratory at the Friedrich-Miescher Institute in Basel, Switzerland in the late 1970s, hoping to expand his immunochemistry skills by applying monoclonal antibodies to the analysis of ribosomal proteins. Working with Theophil Staehelin, a researcher from Hoffman–La Roche, Towbin generated antibodies against a mixture of ribosomal proteins, only to find himself at a loss for how to characterize them. "We tried to isolate the proteins from the gel by elution," recalls Towbin, "but there were always doubts whether you get a pure preparation in the end. Then one idea was to let the antibodies react with the gel, but the antibodies couldn't penetrate the gel because of the high density of the polyacrylamide. And then somebody had the idea at this stage to make a copy of the proteins onto a membrane." The Gordon lab had an electrophoretic destainer, designed for drawing charged dye molecules out of a polyacrylamide gel positioned perpendicular to the current; knowing that nitrocellulose has a natural affinity for protein, Towbin and Staehelin built the now-familiar immunoblotting sandwich, with a polyacrylamide gel apposed to a nitrocellulose membrane, and applied current via the destainer to transfer their proteins[5]. Using either radiolabeled antibodies or the above-mentioned enzyme-conjugated antibodies as a detection reagent, they were able to readily observe the specific binding of their antisera to ribosomal proteins.

Around the same time, W. Neal Burnette at the Fred Hutchinson Cancer Research Center, developed a similar technique with some adaptions to optimize for use with SDS-PAGE gels[6]. It is arguable, however, that Burnette's most significant contribution to immunoblotting came when he christened the technique 'western blotting', an homage both to the Southern and northern blotting techniques that preceded it, and to the geographical location of his Seattle-based institution. Ironically, this name became a serious bone of contention when Burnette submitted his manuscript to *Analytical Biochemistry*[7]—in the end, however, the journal would accept the article a few years later, by which time immunoblotting had already become a popular method and Burnette's moniker had achieved widespread use.

For the most part, nothing significant has changed about the methodology of western blotting since the early 1980s—the technique remains a hands-on, manual process, with most modifications involving subtler optimizations, such as the use of polyvinylidene difluoride (PVDF) membranes as a more durable and efficient alternative to nitrocellulose[8]—although the latter still are widely used. In the introduction to their seminal paper, Towbin *et al.* would remark, "[This] procedure brings to the analysis of proteins the power that the Southern technique has brought to the analysis of DNA,"[5] and as with Southern's method, subsequent years have demonstrated that the key to this power is in the technique's simplicity.

Michael Eisenstein

1. Coons, A.H. *et al. Proc. Soc. Exp. Biol. Med.* **47**, 200–202 (1941).
2. Nakane, P.K. & Pierce, G.B. *J. Histochem. Cytochem.* **92**, 929–931 (1966).
3. Avrameas, S. & Uriel, J. *C.R. hebd. Seanc. Acad. Sci. D* **262**, 2543–2545 (1966).
4. Köhler, G. & Milstein, C. *Nature* **256**, 495–497 (1975).
5. Towbin, H. *et al. Proc. Natl. Acad. Sci. USA* **76**, 4350–4354 (1979).
6. Burnette, W.N. *Anal. Biochem.* **112**, 195–203 (1981).
7. Burnette, W.N. *Curr. Contents* **44**, 8 (1991).
8. Pluskal, M.G. *et al. Biotechniques* **4**, 272–282 (1986).