A look back: Fateful attraction

The strong parasitology research being done at the Walter and Eliza Hall Institute (WEHI) in Melbourne in the late 1980s led a young Scottish investigator named Donald Smith to make the long journey to Australia, where he joined an ongoing effort to identify antigens involved in resistance to *Schistosoma* infection.

Previous studies with a partially-resistant mouse strain had revealed a 26-kDa parasitic antigen, Sj26 (ref. 1), for which subsequent analysis by Smith's group identified significant homology to mammalian glutathione-S-tranferases (GSTs), an enzyme family involved in detoxification of xenobiotic compounds through the covalent addition of glutathione. The identity of Sj26 was confirmed after it was readily purified from parasite-derived extract through a column of immobilized glutathione[2], and Smith's group wondered whether GST might be a suitable clinical target.

Ultimately, their findings did not lead to a vaccine, but they caught the attention of fellow postdoc Kevin Johnson, whose studies with the parasite *Taenia ovis* had been frustrated by the lack of an effective protein expression and purification strategy.

Outside of immunochemical techniques, only limited options existed. One common approach was to make and purify insoluble β-galactosidase fusions and attempt to renature them[3]—often a painstaking and counterproductive process. "You'd generate a protein," explains Smith, "and then you spent a lot of time trying to renature it, so you'd often destroy the very thing that you're trying to make in the process of purifying it." Johnson was impressed by the efficiency of Smith's glutathione-based purification strategy for GST isolation, and the two spent Smith's last few months in Australia working on a GST-based purification strategy[4]. "I wasn't very interested in developing methods *per se*," says Smith, "it was just that I could see the advantage for prokaryotic expression work."

Their 1988 article presented the first pGex vectors, in which DNA sequences could be cloned into constructs driven by an inducible promoter, such that the resulting construct has GST fused in frame to the N terminus of the cloned sequence, separated by a protease cleavage site. Following culture and induction of bacteria transformed with pGex constructs, fusion proteins can be rapidly purified from lysates with glutathione-conjugated beads. The bound fusion proteins can then be mixed with cell or tissue lysates to 'pull down' interacting partners; alternately, the moiety of interest can be further purified through elution with glutathione or protease cleavage.

AMRAD, a company formed by a group of Australian research institutes, recognized this technology's potential and made the pGex vectors their first product, patenting the system with Smith in 1997. It proved a huge commercial success, and by April 2002, when Chemicon International acquired the patent from AMRAD, there were over thirty licenses for GST-based technology.

Smith and Johnson both subsequently returned to the UK to pursue different lines of research, but other investigators continued working to expand the use of this system. Smith credits WEHI colleague Lynne Corcoran with introducing pGex to America; her pull-down and purification studies at the Whitehead Institute with transcription factor Oct-2 broadened awareness of this new system[3]. Harvard oncologist William Kaelin also gave his work a considerable boost with GST pull-downs, using the system to identify key interaction domains and partners for the retinoblastoma tumor-suppressor protein[5,6]. Kaelin appreciated that the system eliminated the need for antibodies and the attendant limitations of using immunochemistry to study interactions, noting, "the advantage of the GST pulldown assay is that it is fast, sensitive and simple [and] it is fairly easy to engineer a number of negative controls."

In the years since Smith and Johnson's initial publication in 1988, over 4,000 research articles have cited the use of this system, with efforts by researchers and industry leading to further innovations, including the engineering of more specialized cleavage sites and additional affinity tags, and the creation of eukaryotic GST systems[3].

Smith is hard to track down lately, having spent the last several years away from the laboratory, focusing on more relaxing pursuits, such as spending time with his family, gardening and beekeeping—"I'm kind of a house-husband at the moment, really," he confesses. Nonetheless, he intends to return to research before too long and is clearly gratified by the persistent prominence of this technique. Above all, Smith credits the power of researchers' 'open source' approach to protocol development: "It's much more powerful for something to be out there and, if it works, people make their own modifications, and you let it fly," he says. "You get much more back than if you just tried to do it all yourself."

Michael Eisenstein

1. Beall, J.A. and Mitchell, G.F. J. *Immunol. Methods* 86, 217 (1986).
2. Smith, D.B. *et al*. *Proc. Natl. Acad. Sci.* U.S.A. 83, 8703–8707 (1986).
3. Smith, D.B. Meth. *Enzymol*. 326, 254–270 (2000).
4. Smith, D.B. & Johnson, K.S. *Gene* 67, 31 (1988).
5. Kaelin, W.G. Jr. et al. *Cell* 70, 351–364 (1992).
6. *Chittenden, T. et al. Cell 65, 1073–1082 (1991).*