Institution: University of St Andrews

Unit of Assessment: 5 - Biological Sciences

Title of case study: The V5 epitope tag: technology for vaccines, diagnostics and disease treatment.

1. Summary of the impact

Proteins are fundamental to life and to many drugs, vaccines and new types of applied medicine with engineered cells. For this work, it is often essential to tag proteins to enable their identification and purification. The V5 tag, which was developed in St Andrews, is used very widely in this role and has some key advantages over alternatives.
Key impacts are:

- V5 tag used in 112 issued patents since 1/1/2008, focussed on treatment of cancer, Alzheimer's, viral infection etc.
- The reagents for V5 tag detection had sales exceeding £600k and generated royalties for St Andrews of £298k (Jan 2008 to Jul 2013).
- Over 130 different products currently available from commercial suppliers make use of V5 technology.
- Recent vaccine and diagnostics development has relied on V5 technology.

2. Underpinning research

In 1993 the group led by **Prof. R Randall** (in post since 1985), at the University of St Andrews was developing methods of new vaccine production targeted at human and simian immunodeficiency viruses (HIV and SIV). This required the presentation of recombinant proteins to the immune system to induce an immune response and so they developed a method of immunization using solid matrix-antibody-antigen (SMAA) complexes. These were highly immunogenic, and were used to make a vaccine against SIV, to help inform how a vaccine to HIV could be produced. However, a method to capture and immobilise the SIV proteins in the SMAA complex was not available. The Randall lab had the idea of making recombinant SIV proteins with a peptide tag attached for purification and capture purposes to generate SMAA complexes using a tag-specific monoclonal antibody. At the time no such system was commercially available, but the Randall lab identified a linear epitope of parainfluenza virus type 5 (originally known as the Pk tag, later renamed the V5 tag) for which they produced a highly specific monoclonal antibody (anti-V5). In 1994, they attached the V5 tag to the p27 protein of SIV and showed that it could be used with the anti-V5 antibody to construct p27-containing SMAA complexes **[1]**.

The Randall lab together with the lab of Prof Ron Hay (in post 1985 to 2005) went on to clone the V5 tag onto many other recombinant proteins. In the period 1995-96, they demonstrated the general applicability of the V5 tag/antibody combination for the detection and purification of a wide range of proteins by a variety of immunological techniques. These included immunoblotting, which allows detection of tagged proteins in a cell extract, immunoprecipitation, which allows the purification of tagged proteins from cells, and immunofluorescence, which allows detection of tagged proteins within cells. Early studies in St Andrews focussed on the role of protein:protein interactions in viruses by the Randall lab **[2, 3]** and in the ubiquitin signalling pathway by the Hay lab **[4]**. Together, this research demonstrated the broad utility of the V5-tag/antibody across the spectrum of applications used by both academic and applied scientists working with proteins.

Subsequent collaborations in 1997-98 further established the utility of the V5 tag in a wide range of experimental applications such as yeast cells **[5]** and the antibody was sent to many academic labs around the world before being commercialised in a deal with Serotec. In 1999, a series of new monoclonal antibodies raised against the V5-tag were described, some of which gave less background immunofluorescence than the original **[6]**.

3. References to the research

St Andrews Authors in bold. Their employment dates were: Bermingham 1993-98; Botting 1993-present; Dunn 2003-07; Green 1991-95; Hay 1985-2005; Precious 1985-2013; Randall 1985-present; Ryan 1993-present; Rodriquez 1997-00; Szawlowski 1989-present; Thompson 1993-01; Young 1985-present.

These are all published in international, peer-reviewed journals. Total citations: >460.

[1] Hanke, T., **Botting, C.**, **Green, A.**, **Szawlowski, P.W.**, Rud, E. & **Randall, R.E.** (1994) Expression and purification of non-glycosylated SIV proteins, and their use in the induction and detection of SIV-specific immune responses. *AIDS and Human Retrovirus Research* 10, 653-662. http://www.ncbi.nlm.nih.gov/pubmed/8074930 (11 citations).

[2] Precious, B., **Young**, **D.F.**, **Bermingham, A.**, Fearns, R., **Ryan, M.** & **Randall, R.E.** (1995). Inducible expression of the P, V and NP genes of the paramyxovirus SV5 in cell-lines and an examination of NP:P and NP:V interactions. *Journal of Virology* 69, 8001-8010. http://www.ncbi.nlm.nih.gov/pubmed/7494313 (46 citations).

[3] Randall, R.E. & **Bermingham, A.** (1996).NP:P and NP:V interactions of the paramyxovirus simian virus 5 examined using a novel protein:protein capture assay. *Virology*, 224,121-138 http://www.ncbi.nlm.nih.gov/pubmed/8862406 (45 citations).

[4] Roff, M., **Thompson, J.**, **Rodriguez, M.S.**, Jacque, J.M., Baleux, F., Arenznan-Seisdedos, F. & **Hay R.T.** (1996) Role of I kappa B alpha ubiquitination in signal-induced activation of NF-kappa B in vivo. *Journal of Biological Chemistry* 271, 7844-7850. (179 citations).

[5] Craven, R.A., Griffiths, D.J.F., Sheldrick, K.S., **Randall, R.E.**, Hagan, I.M. & Carr, A.M. (1998). Vectors for the expression of tagged proteins in *Schizosaccharomyces pombe* . *Gene* 221, 59-68. DOI: 10.1016/j.bbr.2011.03.031 (170 citations).

[6] Dunn, C., O'Dowd, A.M. & **Randall, R.E.** (1999). Fine mapping of the binding sites of monoclonal antibodies raised against the Pk tag. *Journal of Immunological Methods* 224, 141-150. http://www.ncbi.nlm.nih.gov/pubmed/10357214 (10 citations).

4. Details of the impact

The V5 tag and antibody system has become an integral part of the molecular and cellular biologist's toolkit in industrial, healthcare and commercial laboratories. From 01/01/2008 to 31/07/2013, the impact of the underpinning research can be seen in economic terms (>£600k total sales with £298k in Royalties to St Andrews), commercial R&D (>110 patents issued making use of the technology) and public health (new vaccines for emerging multiply-drug resistant bacteria).

Direct economic impact

The V5-tag and anti-V5 antibody were developed for research purposes in the Randall laboratory in the mid-1990s. The excellent specificity and utility of this antibody meant that it was soon in high demand in the research community. In 1995, a royalty agreement was made between the University of St Andrews and the US company, Adb Serotec, under which the Randall lab would provide Serotec with the purified anti-V5 antibody in return for 50% royalties from sales. AbD serotec also produces a variety of directly conjugated antibodies for which the University receives 40% royalties. A subsequent deal with Invitrogen (1996) resulted in that company marketing a variety of V5 antibodies along with an extensive range of vectors to add the V5 tag to proteins. Serotec now purify the antibody directly and supply to Invitrogen under this agreement. Direct sales of the V5 antibody and related products covered by the Royalty agreement amount to £298k

for 2008- 03/2013 **[S3]**. According to the Senior Vice President of the company, the V5 monoclonals are "*one of (Serotec's) most successful products … sold to a large variety of life science based companies*." **[S1]**

Broader impacts on non-academic R&D

Invitrogen is part of Life Technologies Corporation, a company valued at $13.6 billion in its takeover by Thermo Fisher Scientific in April 2013 **[S4]**. The importance of the V5 tag/antibody technology to Invitrogen is evidenced by the fact that the company has developed over 100 products making use of the technology in their highly successful TOPO and Gateway cloning vectors **[S5]**. These are marketed and sold to a wide range of commercial as well as academic laboratories.

The secondary impacts arising from the use of the V5 antibody in non-academic research are more difficult to qualify in absolute terms but there is clear evidence of the reach and significance of the V5-antibody in these non-academic spheres:

800 patent applications filed and over 110 patents issued in the period 2008-July 2013 made explicit use of the V5-epitope for the science underpinning the patent **[S6]**. Examples from patents issued in 2012-13 include:

- the inhibition of virus infection (patent US8263570)
- molecular markers for characterization of human cancer states (patent US8268568)
- engineered organisms with enhanced fermentation activity to improve chemical product yields (patent US8114974)
- treatment of cancer with novel monoclonal antibodies (patent US8318160)
- therapeutic treatments for Alzheimer's disease (patent US8398981)

Example of an Invitrogen product using V5 technology

Vaccine Research

The development of vaccination is one of the biggest public health successes of the past 100 years. New vaccines are required urgently to treat emerging and newly drug resistant diseases. The V5 tag and antibody "*have been invaluable tools in the development of subunit vaccines against HIV-1 and TB (tuberculosis)*" **[S2]**. A vaccine against tuberculosis, developed using the V5 tag, is currently undergoing phase IIb clinical efficacy trials in South Africa. The vaccine, MVA85A, has already been shown to be "*safe and well tolerated*" **[S7]**. This is the first of a new generation of vaccines against TB and has already completed 15 clinical trials (the current vaccine, BCG, is not 100% effective and TB causes an estimated 1.4 million deaths a year according to the WHO).

Diagnostics – advantages of the V5 tag over alternatives

West Nile virus is a serious emerging disease and rapid new diagnostic methods are needed urgently. The virus can cause fatal neurological disease, but 80% of infected humans don't show any symptoms **[S8]**. Therefore it is important to develop methods to detect the virus in blood samples. In 2011 a new diagnostic reagent based on the prM antigen was reported, which is suitable for the detection of antibodies against West Nile virus in serum samples. This requires expression of the prM antigen in tagged form so that it can be immobilised. Use of a polyhistidine tag "*disrupted*" the protein so that it was not useful as a diagnostic antigen. Use of the V5-tag however "*allowed formation of the authentic antigenic structure and the proper presentation of the V5 epitope"*, allowing the development of a "*useful diagnostic agent*" **[S9]**. Thus, although a variety of tags are available, the V5 tag has clear advantages in some circumstances.

5. Sources to corroborate the impact

[S1] Email from the Senior vice president, Serotec. Corroborates success of V5 products.

[S2] Email from an independent scientific expert, The Jenner Institute, University of Oxford. Corroborates utility of V5 technology in vaccine design.

[S3] Audited financial statement showing royalties accruing to St Andrews University for V5 antibody sales in period 2008- July 2013 of £298,367.

[S4] Widely reported, e.g. http://www.cnbc.com/id/100641197. Corroborates value of the Invitrogen group.

[S5] Invitrogen products with V5 tag. Invitrogen website reports 105 products with V5 tag (correct as of 29 Oct 2013).
http://www.lifetechnologies.com/search/global/searchAction.action?modifier=Invitrogen%26trade%3B&show_taxonomynavigator=true&show_sedocumenttypenavigator=true&navigator=brandnavigator&show_productcategorynavigator=true&resultsPerPage=15&query=v5+tag&show_=&resultPage=1&personaFilterTerm=Product+Catalog

[S6] Patent search using Google, corroborates number of patent applications and awards using this technology in the REF period.
Search patents issued or filed in period 2008-July 2013 with search term “V5 epitope”.

[S7] Details of MVA85A vaccine incorporating V5 tag. Corroborates use of V5 tag in TB vaccine under late stage clinical trial.
http://www.who.int/vaccine_research/QandA_TB_vaccines_MVA85A_feb13.pdf

[S8] Corroborates importance of development of new diagnostics for West Nile virus.
http://www.who.int/mediacentre/factsheets/fs354/en/

[S9] Expression of recombinant West Nile virus prM protein fused to an affinity tag for use as a diagnostic antigen. Setoh Y.X. et al., (2011), Journal of Virological Methods 175, 20-27.
Corroborates the claim that the V5 tag has key advantages over other tags in some circumstances.