Institution: University of East Anglia

Unit of Assessment: 5 – Biological Sciences

Title of case study:

Small interfering RNA – a change in the landscape of biotechnology

1. Summary of the impact

Work by Professor (now Sir) David Baulcombe and colleagues in The Sainsbury Laboratory at UEA was pivotal in discovering small interfering RNAs (siRNAs) and understanding the phenomenon of RNA interference (RNAi). These contributions not only revolutionised understanding of fundamental processes, from cancer to viral infections, but have already underpinned significant economic impacts, including:

- Synthesis, design and sales of high-grade research chemicals, including oligonucleotides and kits, by several multinational Biotech companies.
- Establishment of several companies whose product pipelines are driven by RNAi, and some of which have been acquired for sums > $1 billion.

2. Underpinning research

Some 20 years ago, the concept of antisense RNA seemed rather simple; by using genetic engineering to express the "wrong" strand of a gene, the resulting RNA would bind to its complementary mRNA, blocking (or 'silencing') its translation, and would abolish or diminish its function. Indeed, Petunia flowers with no pigment and tomatoes (*Flavr Savr*) that did not soften were made by expressing antisense RNA of genes for flower colour or for fruit ripening, respectively. However, the reasons for this phenomenon were very different and more complex than envisaged in the original "thought experiments". The underlying mechanism of RNA silencing was discovered by several groups during the period 1997-2007. Several key contributions to understanding how RNA silencing works were made by David Baulcombe, a group leader in The Sainsbury Laboratory in the School of Biological Sciences at UEA, 1988-2008. The influential post-doctoral researchers were: Tamas Dalmay, Andrew Hamilton and Olivier Voinnet. Dalmay worked in the Baulcombe group (1995-2002) and is now Professor of RNA biology in the School of Biological Sciences at UEA.

In a series of seminal papers, Baulcombe and colleagues demonstrated that; silencing can be systemic [1]; characterised the spread of systemic gene silencing [2]; discovered siRNAs [3]; and identified key genes involved in the activity and function of RNAi in plants [4-6]. The importance of this set of phenomena is manifold – not least in the "award" of *Science* magazine's "Breakthrough of the Year" in 2002 to small RNAs: (http://www.sciencemag.org/content/298/5602/2296.summary).

Although all six papers (below) are related to the basic understanding of the RNAi mechanism, the discovery of siRNAs opened up ways to use them as experimental materials, in which individual genes can be switched off, or "silenced", in controlled, targeted ways. This has provided a whole new battery of tools for fundamental and applied biological and biotechnological research that have been adopted by both academic and industrial researchers.

Baulcombe was knighted in 2009 and his contributions to the field of RNAi have been recognised at the very highest levels:

- Lasker Award (2008) (http://www.laskerfoundation.org/awards/2008basic.htm);
- Wiley Prize (2003) (http://eu.wiley.com/WileyCDA/PressRelease/pressReleaseId-49084.html);
- Massry Prize (2005) (http://www.usc.edu/schools/medicine/school/offices/resadv/assets/2010_01_massry_prize_winners.pdf);
- Harvey Prize (2009) (http://harveypz.net.technion.ac.il/prize-winners/#ten);
- Wolf prize in Agriculture (2010) (http://www.wolffund.org.il/index.php?dir=site&page=winners&cs=49&language=eng)

- whose citation reads "*This is of profound importance, not only for agriculture, but also for biology as a whole, including the field of medicine*."

3. References to the research

Publications

(UEA authors in bold)

1. **Voinnet O, Baulcombe DC**. (1997) Systemic signalling in gene silencing. *Nature*. **389**:553. (308 Citations)
   doi: 10.1038/39215
2. **Voinnet O,** Vain P, **Angell S, Baulcombe DC.** (1998) Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. *Cell.* **95**:177-187. (425 Citations)
   doi: 10.1016/S0092-8674(00)81749-3
3. **Hamilton AJ, Baulcombe DC**. (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **286**:950-952. (1481 Citations)
   doi: 10.1126/science.286.5441.950
4. **Dalmay T, Hamilton A, Rudd S, Angell S, Baulcombe DC**. (2000) An RNA-dependent RNA polymerase gene in Arabidopsis is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. *Cell* **101**:543-553. (607 Citations)
   doi: 10.1016/S0092-8674(00)80864-8
5. **Dalmay T, Horsefield R,** Braunstein TH and **Baulcombe DC.** (2001) SDE3 encodes an RNA helicase required for post-transcriptional gene silencing in Arabidopsis. *EMBO J.* **20**:2069-2078. (230 Citations)
   doi: 10.1093/emboj/20.8.2069
6. **Herr AJ, Jensen MB, Dalmay T, Baulcombe DC**. (2005) RNA polymerase IV directs silencing of endogenous DNA. *Science*, **308**:118-120. (318 Citations)
   doi: 10.1126/science.1106910

4. Details of the impact

When a leading article in "*The Economist*" shouts "*Biology's Big Bang: What physics was to the 20th century, biology will be to the 21st - and RNA will be a vital part of it*" - see http://www.economist.com/node/9339752 - then an area of scientific discovery has already transcended the boundaries of the laboratory and reached into a wider consciousness (corroborating sources A-C).

But this is just one aspect of the scale of the Impact (in the broad sense of the word) that has been generated by the discovery of RNAi. There have also been substantial Impacts in the form of significant economic activities, which stem from Baulcombe's research at UEA, and whose contribution can be encapsulated in the crucial US Patent 8,097,710 B (inventors, Baulcombe and Hamilton), whose Claim 1 starts:

> *"We claim a method of silencing a gene in cells by post-transcriptional gene silencing which method comprises introducing into said cells a composition that contains short RNA molecules."*

In fact, the most immediate economic Impact of the Baulcombe *et al* work was (and is) the design, production and sale of synthetic siRNAs, which are used as reagents in many biological Research and Development laboratories worldwide and have undoubted importance in industrial R&D. Several multi-national (e.g. *Sigma, Qiagen, Dharmacon, Life Technologies*) and more specialised (*Bioneer, IBA Gmbh, Midland, etc*.) companies sell synthetic siRNAs that target genes in many different organisms. The very fact that this class of compounds has been recognised is directly attributable to their discovery by Baulcombe *et al,*. In addition to their direct syntheses, various other technologies to make and market siRNAs by enzymatic technology, through RNAseIII (*New England Biolabs*) or by transcription (*Selleck Chemicals, Promega, Invitrogen*), have been developed and are commercially available. Furthermore, siRNA-expressing plasmids (*BioVision,*

Promega, EMD Millipore, InvivoGen) or virus-based kits (*Imgenex, Life Technology, Santa Cruz Biotechnology*) are widely marketed and transfection reagents to specifically deliver siRNAs into cultured cells have been developed by (for example) *5 PRIME, Bulldog Bio, Mirus Bio and Lonza Cologne*.

In addition to these current products, it is widely believed that gene silencing will likely bring further economic and other benefit in the form of (e.g.) anti-cancer therapies in medicine and in the generation of improved plant varieties that are (e.g.) disease-resistant. Although these are future impacts and therefore outside the REF2014 timeframe, the scale of the perceived future benefits is such as to have stimulated the formation of several new biotech companies whose pipelines comprise materials, techniques and know-how that depend on RNA silencing. An indication of the perceived values of these companies is seen by the prices paid by "Big Pharma" companies for their acquisition. For example, *Sirna Therapeutics* was bought by *Merck & Co* for $1.1 billion and *Devgen* (a pioneer in research for RNAi-based crop protection applications) by *Syngenta* for $522m in 2012 (corroborating sources D-F).

Currently, there are two publicly announced licence agreements that involve the Baulcombe siRNA patent. One is with *Alnylam Pharmaceuticals*, a leader in the development of siRNA therapeutics, who aim to provide siRNA-based drugs against haemophilia and other blood disorders, with some agents having reached Phase I and II clinical trials. The other licence, to *Dicerna*, is used in their programme with undruggable oncogenes, such as those involved in Hepatocellular Carcinoma.

5. Sources to corroborate the impact

A.(i) US Patents, in which Baulcombe was the named inventor:
Patent Nos. 8,258,285; 8,263,569; 8,299,235; 8,349,607; 8,097,710; 7,704,688 and 6,753,139.

(ii) Details of the Baulcombe siRNA portfolio
Held on file at UEA and available at: http://www.pbltechnology.com/cms.php?categoryid=67

B. BBSRC news story (2012) detailing the non-exclusive licensing of U.S. Patent No. 8,097,710 to *Alnylam Pharmaceuticals*
Available at: http://www.bbsrc.ac.uk/news/research-technologies/2012/120523-n-rnai-licensing-agreement.aspx
"*PBL has granted Alnylam a world-wide, non-exclusive license to the Baulcombe patent (U.S. Patent No. 8,097,710) to enable the development of RNA interference (RNAi) therapeutics*."

C. Media release about licensing the Baulcombe siRNA patent by *Dicerna*
Available at:
http://www.pbltechnology.com/documents/News%20Documents/Dicerna%20Press%20Release%20RN Ai%2099.190%2009.04.13.pdf

D. *Roche* media release revealing an upfront payment of $331M USD for the acquisition of *Alnylam's* research facility
Available at: http://www.**roche**.com/media/media_releases/med-cor-2007-07-09.htm
"In 2007 we struck a major deal with US-based Alnylam Pharmaceuticals that gives us access to Nobel Prize-winning RNAi technology. The accompanying acquisition of Alnylam's research facility in Kulmbach, Germany – including a team of more than 40 world-class scientists – creates Roche's own RNAi centre of excellence.

Also evidenced in the *Roche* Annual Report 2007, available at:
http://www.roche.com/investors/annual_reports/annual_reports_archive.htm
"In 2007 Roche purchased non-exclusive licensing rights to Alnylam technology for developing RNAi therapeutics (Alnylam received $331m up-front)".

E. News release stating that *Syngenta* announces public offer for Devgen
Available at: http://www.syngenta.com/global/corporate/en/news-center/news-releases/Pages/120921.aspx

"The intended takeover bid is for all outstanding shares and warrants issued by Devgen, representing a total consideration of around €403 million."

F. Merck report of acquisition of Sirna for $1.1bn
Available at: http://media.corporate-ir.net/media_files/irol/73/73184/10k/022807_MERCKCOINC10K.pdf

G. Report analysing the worldwide markets for RNA Interference (RNAi) in US$ Million by the following Application Areas: Drug Discovery and Target Validation, Reagents, and siRNA Synthesis
Available at: www.strategyr.com/RNA_interference_RNAi_Market_Report.asp?gclid=COm_x8GEibgCFajKtAodLT4A7A,