Institution: University of East Anglia

Unit of Assessment: 5 – Biological Sciences

Title of case study:

Increasing the yield of medically important proteins in plants by suppression of RNA silencing

1. Summary of the impact

Genetically engineered plants are increasingly used to over-express foreign genes, including those for pharmaceutically valuable polypeptides. However, expression of transgenes is repressed via RNAi, a system that probably evolved to combat viral pathogens. In response, viruses themselves encode a "silencing suppressor protein" that counteracts this defence response. This was discovered by David Baulcombe and colleagues at the Sainsbury Laboratory at UEA, who exploited this phenomenon by introducing the suppressor gene into plants and improving them as hosts for transgene expression. RNAi Suppression Technology was patented worldwide and licensed for fees >£500k to several companies, including *Medicago*, that use it to generate plants that effectively produce pharmaceuticals.

2. Underpinning research

When plants are infected by viruses, the threatened host may defend itself via a complex post-transcriptional regulatory system involving RNA silencing, which effectively targets the viral RNA genomes and / or mRNAs. However, in the arms race between pathogen and plant, the viruses have their own counter-weapon to combat the host defences, a "silencing suppressor protein" that can block post-transcriptional gene silencing (PTGS; also known as RNA silencing or RNAi), thus allowing the infection to proceed. The discovery and analysis of these phenomena of RNAi and RNAi suppression were made by Prof (now Sir) David Baulcombe and his colleagues, working in the Sainsbury Laboratory at UEA [1-3]. They found that RNA silencing of transgenes, which is basically a defence against a viral infection, can be suppressed in plants with a viral infection and they went on to identify the viral genome-encoded RNAi suppressor proteins 2b, P19, P1 and HCPro [2,3]. Baulcombe and colleagues also demonstrated that suppression of RNAi is widely used as a counter-defence strategy by plant DNA and RNA viruses and that some suppressors are more effective than others in terms of host range and spatial expression within the plant host [3]. Further research from the Baulcombe group identified a viral movement protein called p25, which generates systemic silencing in the host by blocking one of the two branches of the RNAi pathway in plants [4]. They also used natural RNAi suppressors to show that epigenetic conversion of transgenes explains the effects of transgene dosage on PTGS that were previously interpreted in terms of RNA expression thresholds [5]. Taken together, these findings presented a novel way to enhance transgene expression in plants by bypassing the natural PTGS plant defence. The discovery of RNA suppressors was just one of a series of ground-breaking insights into the whole area of RNA-mediated gene expression control in plants by Baulcombe's team at the Sainsbury Laboratory in the period 1997-2000.

Although the phenomenon of RNAi Suppression was of interest and importance from several basic points of view, from the fundamentals of gene regulation to the mechanisms of disease resistance in plants, the Baulcombe lab realised early on that it might also have important commercial applications. This stemmed back to observations by others that introduced transgenes were often expressed very poorly and did not confer the expected phenotype - e.g. for flower colour in Petunia. Baulcombe and colleagues deduced that this was due to RNAi-mediated inhibition, and therefore it would follow that introduction of a viral gene for silencing suppressor proteins should relieve the poor expression of these transgenes. And, so it proved. There is increasing interest in the use of plants as "factories" to produce foreign proteins, especially high-value pharmaceuticals and other bioactives. But this has been hampered by poor expression of the introduced genes. The utility of RNAi suppression technology, patented and marketed by *Plant Bioscience Ltd* on behalf of the Sainsbury Laboratory, is now reflected in the value (> £0.5 million) of the licenses granted to a

range of Pharma and biotechnology companies to exploit this process.

3. References to the research

(UEA authors in bold)

1. **Ratcliff F**, Harrison BD, **Baulcombe DC.** (1997) A similarity between viral defense and gene silencing in plants. *Science* **276**:1558-1560. (425 citations)
   doi: 10.1126/science.276.5318.1558
2. **Brigneti G, Voinnet O**, Li WX, Ji LH, Ding SW, **Baulcombe DC.** (1998) Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. *EMBO J*. **17** :6739-6746. (697 citations)
   doi: 10.1093/emboj/17.22.6739
3. **Voinnet O, Pinto YM, Baulcombe DC**. (1999) Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. *Proc Natl Acad Sci U S A*. **96**:14147-14152. (578 citations)
   doi: 10.1073/pnas.96.24.14147
4. **Voinnet O, Lederer C, Baulcombe DC**. (2000) A viral movement protein prevents spread of the gene silencing signal in *Nicotiana benthamiana*. *Cell*. **103**:157-167 (390 citations)
   doi: 10.1016/S0092-8674(00)00095-7
5. **Dalmay T, Hamilton A, Mueller E, Baulcombe DC**. (2000) Potato virus X amplicons in Arabidopsis mediate genetic and epigenetic gene silencing. *Plant Cell* **12**: 369-379. (113 citations)
   doi: 10.1105/tpc.12.3.369

4. Details of the impact

Plant Bioscience Ltd, as the Sainsbury Laboratory's technology transfer company, has secured 13 granted patents (in Belgium, France, Germany, Netherlands, Switzerland and UK, all as European Patent EP1232274; US Patent 7,217,854, Canadian Patent 2390152, South African Patent 20023388, Australian Patent 782788, New Zealand Patent 518,947 Israel Patent 49388, Japanese Patent 4884628, International Patent Publication No. WO 01/038512 all entitled "*Enhanced Transgene Expression by Co-Expression with a Suppressor of Post-Transcriptional Gene Silencing (PTGS)")* that include the Sainsbury Laboratory's discovery and the practical uses of RNAi suppression. Details of the Sainsbury Laboratory's patent portfolio covering RNAi and RNAi suppression can be found on the *Plant Bioscience Ltd* website (corroborating sources A and B).

Plant Bioscience Ltd has licensed these patents to a number of companies either as stand-alone licences or in combination with other protein expression technologies such as the HT-CPMV expression system. In total, *Plant Bioscience Ltd* has entered into 30 licences for the suppressor technology with a variety of commercial entities. The licensing fee income alone for these is in excess of £0.5 million (corroborating source C), which reflects the commercial importance that several established companies and SMEs, including *Medicago* attach to the RNAi suppression technology (corroborating sources D-F).

The main commercial impact of the silencing suppressor proteins is in "Molecular Pharming" applications in which genetically engineered plants are used as "factories" to produce industrially and medically important proteins. In these applications, maximising the protein yield is paramount, and suppressing gene silencing is therefore an extremely useful tool. *Plant Bioscience Ltd* first licensed the silencing suppressor IP to *Large Scale Biology* (which at the time was a leading Molecular Pharming company) and *CropDesign*, amongst other companies (corroborating sources D and E). *Medicago* has also been using the silencing suppressor P19 discovered by Baulcombe and his colleagues to increase the yield of ectopically expressed proteins about 5-fold in plants. This company combines RNAi suppression with another, in-house, technology, involving the use of Virus-Like Particles (VLP), to produce recombinant vaccine antigens in plants. As *Medicago* state in their letter of support:

> "…..the silencing suppressors developed by Prof. Sir David Baulcombe had a big impact on our activities and are an essential component of our technology platform to produce our lead

influenza vaccines."
(corroborating source G)

We are aware that the VLP-based vaccines that are being produced by *Medicago*, using the RNAi suppression technology, have not yet reached the public market place. However, they are in an advanced stage of development, and are in Phase II clinical trials. Other companies are at a similar stage in the production of other vaccines using the same technology and the investment needed to reach even this stage represents a very substantial commitment, of time, staff and resources.

5. Sources to corroborate the impact

A. Details of the patent portfolio on RNAi and RNAi suppression
Available at:
http://www.pbltechnology.com/cms.php?categoryid=67
http://www.pbltechnology.com/cms.php?pageid=204

B. Press release describing the issuing of a US patent to *Plant Bioscience Ltd.* on silencing suppressors
Available at:
http://www.pbltechnology.com/documents/News%20Documents/SHORT%20RNA%20PRESS%20RELEASE%2099.190%2017.01.12%20for%20PDF.pdf
The press release states: *"Plant Bioscience Limited (PBL) is pleased to announce that the United States Patent and Trademark Office (USPTO) has issued out US Patent No. 8,097,710 with fundamental claims directed to methods of inducing gene silencing using short RNA molecules, or DNA constructs encoding short RNA molecules, in a wide range of organisms, including in plants and humans."*

C. Corroborating statement from the Business Development Manager at *Plant Biosciences Ltd.*

D. News stories about Large Scale Biology Corporation licensing RNA silencing suppressor technology from *Plant Bioscience Ltd*:
Available at:
http://www.thefreelibrary.com/Large+Scale+Biology+Corporation+Exercises+Option+to+Expand+Licensing...-a080526478
http://www.pbltechnology.com/cms.php?pageid=100
The *PBL* press release states: "*Under the terms of the agreement, PBL is granting an exclusive license to certain viral-derived gene silencing and overexpression technologies developed by Professor David Baulcombe and colleagues at The Sainsbury Laboratory, Norwich, UK*."

E. Press release from *CropDesign N.V.*, reporting that they have signed a broad licence agreement concerning the PBL's AMPLICON gene silencing technology developed at the Sainsbury Laboratory (Norwich, UK) by Professor David Baulcombe.
Held on file at UEA.
States: "*CropDesign N.V. (CropDesign) and Plant Bioscience Ltd. (PBL) announced today that they have signed a broad licence agreement concerning the PBL's AMPLICON, AMPLICON-PLUS, and related technologies for application in protein over-expression in plants. Under the terms of the agreement, CropDesign has exclusive worldwide rights to AMPLICON and AMPLICON-PLUS for the production of industrial-type enzymes and proteins in plants. In addition, CropDesign is granted non-exclusive rights to the technologies in the area of production of pharmaceutical products in plants.*

F. Press releases from *Plant Biosciences Ltd* and *Medicago* reporting the license agreement for plant suppressor and other technologies.
Available at: http://www.medicago.com/files/Documents_news/PR_PBLLicense208061.pdf
The *Medicago* press release states: "*Medicago and PBL (Plant Bioscience Limited) announced today the signing of license agreements to give Medicago access to a*

range of technologies for the high level expression of heterologous proteins in plants. The agreements give Medicago access to PBL's bipartite expression system and a wide range of suppressors of gene silencing."

G. Letter of support from the Vice President of Business Development at *Medicago Inc*. Held on file at UEA.

The letter states: "*Medicago has been using the silencing suppressor P19 stemming from the work by Professor Sir David Baulcombe and his colleagues to increase the yield of ectopically expressed proteins. The increase in the yield of protein we can produce - about 5 fold - is significant and therefore using silencing suppressors has been really advantageous for us. The amount of proteins we can produce is a key factor in our business, influencing capacity of production in our facilities in Quebec and North Carolina and cost of goods. The silencing suppressors developed by Prof. Sir David Baulcombe had a big impact on our activities and are an essential component of our technology platform to produce our lead influenza vaccines.*"