Institution:
University of Cambridge

Unit of Assessment:
UoA5

Title of case study:
A new process for producing biologically active growth factors: commercial uses for stem cell applications

1. Summary of the impact (indicative maximum 100 words)

Stem cells play an important role in drug discovery and development of therapeutic interventions. Differentiation (and maintenance) of stem cells into specialised cells is achieved by controlled application of specific, expensive growth factors.

Dr Hyvönen has developed an efficient method for producing highly purified, bioactive human growth factors from *E.coli*, reducing costs by up to 10-fold. The technology has been licensed to a major international manufacturer of growth factors (PeproTech Inc.), and to a UK-based specialist stem cell company (CellGS Ltd), enabling them to implement new products and business strategies. Through a departmental facility, material is also being sold to external companies and Cambridge Stem Cell Consortium members. In addition, Dr Hyvönen has made his expertise available to biotech companies through consultancy.

2. Underpinning research (indicative maximum 500 words)

The research in the group of Dr Marko Hyvönen (2008-present Lecturer, 2006-07 Senior Research Fellow, 2001-05 BBSRC David Phillips Fellow, all in the Department of Biochemistry) is focused on understanding the molecular interactions of TGFbeta family growth factors, using structural and biophysical techniques. An underpinning requirement for this work is the ability to produce highly pure proteins in large quantities, as protein crystallography in particular requires large quantities of homogeneous proteins. However, TGFbeta family growth factors have a complicated disulfide structure with complex covalent linkages, making them very difficult to produce in sufficient amounts using recombinant expression technologies.

Research by Hyvönen has focussed on the key target proteins activin A and B, both of which were previously made in eukaryotic expression systems with low yields (typically up to 1mg/l) and requiring extensive purification procedures. Between 2001 and 2008, Hyvönen sought to overcome the inherent limitations of eukaryotic expression systems for large scale structural work, and developed a novel, highly efficient method to make both activin A and B (Refs 1&2, Section 3) from bacterially (*E. coli* BL21(DE3)) expressed protein by refolding (which faithfully reproduces post-translational modifications such as disulphide bridges, and produces yields of 6-25mg/l depending on construct). Purification of the resulting proteins was achieved with ion exchange and reverse phase chromatographies. The resultant activin A has been crystallised, indicating the high quality and homogeneity of the protein produced using the technique (Ref. 1, Section 3).

Many of the TGFbeta family growth factors are involved in very early embryonic development and are used in stem cell research to drive cells towards distinct differentiation pathways. Activin A, itself, is used both in defined media to maintain pluripotency of embryonic stem cells and to differentiate stem cells to endoderm. At the time Hyvönen was developing his method, stem cell researchers were sourcing activin A commercially (expressed in animal cells), at considerable expense, and with the added risk of introducing potential impurities from animal proteins and pathogens into the stem cell culture.

As Hyvönen was refining his technique for producing activin via bacterial expression, Roger Pedersen (Professor at the Anne McLaren Laboratory for Regenerative Medicine, University of Cambridge, 2001-present) and his team developed a defined culture medium for human embryonic stem cells, one of the key components of which was human activin A. This prompted the two teams in February 2006 to test Hyvönen's bacterially expressed activin A in Professor Pedersen's stem cell culture; the data (unpublished) showed the protein to be highly active and indistinguishable from protein made in animal cells (pers. comm. L Vallier).

Between 2008 and 2009 Hyvönen made further refinements to the method, which is now highly

reproducible and has since proved transferable to other laboratories. His lab produced an engineered version of activin A with equal functionality to wild type which increased yields from 6 to 25 mg/l (unpublished due to commercial sensitivity). It is the first efficient method for making activin A from non-animal sources. Similar methodology has since been developed by Hyvönen's group for expression and purification (and therefore production) of other members of the TGFbeta superfamily, including activin B and bone morphogenetic proteins 2 and 4 (BMP-2 / BMP-4). The proteins are expressed in *E. coli* as inclusion bodies, refolded to native form and purified by a combination of reverse phase and ion exchange chromatographies; the method is published for activin B (Ref. 2, Section 3) and BMP-2 (Ref. 3, Section 3), but so far has remained unpublished for BMP-4.

3. References to the research (indicative maximum of six references)
Publications:

1. Harrington AE, Morris-Triggs SA, Ruotolo BT, Robinson CV, Ohnuma S and Hyvönen M. Structural basis for the inhibition of activin signalling by follistatin *EMBO J*, 25:1035-1045, 2006. DOI: 10.1038/sj.emboj.7601000
2. Ludlow H, Muttukrishna S, Hyvönen M, Groome NP. Development of a new antibody to the human inhibin/activin □B subunit and its application to improved inhibin B ELISAs. *J Immunol Methods*, 329:102-111, 2008. DOI:10.1016/j.jim.2007.09.013
3. Sharma A, Meyer F, Hyvonen M, Best SM, Cameron RE, Rushton N. Osteoinduction by combining bone morphogenetic protein (BMP)-2 with a bioactive novel nanocomposite. *Bone Joint Res.* 1:145-51, 2012. DOI: 10.1302/2046-3758.17.2000082

Grants (Hyvönen as PI):
BBSRC Follow-on Fund: "Production of recombinant activins and other TGFβ family growth factors for stem cell applications" 2008-2009. Amount awarded: £88k
BBSRC David Phillips Fellowship: "Structural studies of TGFβ and CCN family growth factors", 2001-2005. Amount awarded: £150k

4. Details of the impact (indicative maximum 750 words)
Industry has adopted a new technology or process, cost of production has decreased:
In Nov 2008 a technology licensing agreement was signed between the University's technology transfer office and **PeproTech Inc.**, one of the main international producers of growth factors. The license comprises both the expression plasmids and the detailed protocol for activin A production using Hyvönen's method, and enables the company to produce activin A more efficiently than alternative, eukaryotic methods. Consequently they are able to sell this protein significantly cheaper than eukaryotically expressed activin A (initially the price was half, since then the price for the eukaryotically expressed protein has been reduced as a consequence of the new competition; as of June 2013 the eukaryotic protein is sold for £4250/mg, that of bacterial origin for £3150/mg). Their Director testifies: "..there is no doubt that having access to the *E.Coli* derived material has enhanced our existing
Activin business. Activin A is a protein component in several popular embryonic stem cell media formulations, and has consequently become one of our better selling recombinant protein products. […] our group of stem cell products, of which Activin A is a core component, has allowed us to develop and enhance our approach towards the Stem Cell Market, and its related targets. […] The increased availability and decreased price of *E.Coli* derived material has aided stem cell research, and these substantial sales have indirectly helped PeproTech's business, and created, or saved, jobs." (Ref. 1, Section 5)
Hyvönen has acted as a consultant to **AnshLabs** (a US-based developer and manufacturer of immunoassay reagent test kits), advising on the development of diagnostic kits against activins and related proteins. Their CEO testifies: "Dr. Hyvönen's consultation has helped us with new a concept design of immunogens, screening protocols and purification of biomolecules. [..] His new strategies [..] have been instrumental in achieving enhanced immunogenicity, which translated to [..] improved antibodies. Dr. Hyvönen's consultation and expertise [..] has been valuable to our sales and marketing efforts for the preparation and presentation of scientifically accurate product

information related to our TGF-beta superfamily hormone assays. Marko helped increase the quality/yield of protein purification [..]. He also spent time optimizing our techniques so that we can purify more antibody in a shorter period of time, and trained Ansh Labs' scientists to novel techniques which have significantly improved the production process and quality of our products. In addition thereto, his consultancy is highly valued in our development of world class immunodiagnostic kits that are being evaluated for important clinical applications as well as several products currently under commercial development. His methods have definitely helped us reduce production costs and increase productivity." (Ref. 2, Section 5)

The strategy of a business has changed; business performance has improved; employment has been generated:

Cell Guidance Systems Ltd, (**CellGS**, Cambridge, UK; Ref. 3, Section 5), a research reagents company established in 2010 and focusing on stem cell science, validated the efficacy of activin A from Hyvönen's lab in 2011. In 2012 they took a licence for the production of engineered activin A and BMP-4 and have established an in-house protein production facility. CellGS plans to use Hyvönen's activin A in their novel multivalent "STAR" (Serial Tethered ARray) growth factors and as a component of their stem cell culture media. The company represents a first of its kind for UK plc (research reagent companies tend to be head-quartered in the US), in an area in which the UK has aspirations to be a world leader, and CellGS's success will at least in part depend on activin A and BMP-4 produced using Hyvönen's method. Their CEO testifies: "Our interaction with Marko has been very beneficial for the business. Marko has provided valuable guidance which helped us with our strategy for the development of STAR [..]. The license for Activin [..] has helped us generate significant revenues. This was the first growth factor we made, so I would agree that Marko's input has been influential in the direction the business has taken. He has also been very helpful providing stock whilst we got our own production up and running which allowed us to go to market earlier than would have been possible otherwise. [..] One job has been created."

Commercial income, employment, savings and a spin-out company in the University through services provided:

Income through protein sales

The Hyvönen lab operates a small Research Facility, which produces and distributes growth factors such as activins A and B, BMP-2 and BMP-4 (see Ref. 4, Section 5 for full list). Companies who have been provided with growth factors include CellGS/UK, Stemgent/US, AbCys/France, enabling them to evaluate the market before considering taking out a technology licence. Since its establishment in 2008 over £120k of income has been generated through the sale of the above proteins by this facility to academic partners, and £34k through material supply to companies (Ref. 5, Section 5). These proteins would otherwise have been sourced from the US, the main supplier of such reagents, and the revenue would have been lost to UK plc.

Income through licences

Since 2008, the University has received royalty income of £116k from licences to PeproTech and CellGS for activin A and BMP-4 expression plasmids and know-how (Ref. 5, Section 3).

In addition, Hyvönen has supplied *Xenopus* activin B to Oxford Brookes University (Prof Nigel Groome) in 2006, to use as an antigen for the development of activin B specific antibodies (Ref. 2, Section 3). This was done successfully, and as a result a more sensitive ELISA assay for inhibin B was developed by the Groome group in 2006-08. The new ELISA assay has been licenced by Oxford Brookes to BeckmanCoulter, who in 2009 launched this product commercially. *Xenopus* activin B was crucial to the success of this development, and as a result the University of Cambridge receives its share (0.25%) of the royalty income from BeckmanCoulter; within the eligible period this has amounted to £10k (Ref. 5, Section 5).

Income through consultancy

Hyvönen has acted as a consultant to CellGS/UK and Ansh Labs/US. In the eligible period these services have overall resulted in £30k income (Ref. 5, Section 5).

Savings and employment

The proteins are provided by the Research Facility at cost to members of the University or of the Cambridge Stem Cell Institute. Since 2009 this has resulted in savings of tens of thousands of

pounds on R&D budgets when compared with the cost of purchasing from commercial sources. As at June 2013, the cost of material produced using Hyvönen's method is between 1/5th and 1/10th of the bulk purchase commercial price depending on the protein. Prior to Hyvönen's work, the cost of activin A was often a limiting step in such R&D work. Since 2009 protein sales have fully funded a 0.5FTE technician in Hyvönen's lab.

Spin-out company

The lab of Dr Ludivic Vallier, who has collaborated with Hyvönen to show the bacterially expressed activin A to be indistinguishable from protein made in animal cells, has formed a spin-out company in 2011 (DefiniGen Ltd, Company No. 07595566). Their CEO writes (Ref. 6, Section 5): "The company provides human liver cells for preclinical drug development and disease modelling applications, using human Induced Pluripotent Stem Cell hIPSC technology. Provision of material by Marko Hyvönen's lab made more proof-of-concept research feasible in the Vallier lab […], which then enabled the company to be formed. The knowledge that highly active bacterially expressed activin A for the stem cell growth media (without potential contaminants of animal-derived protein or pathogens) is available at lower cost compared to mammalian derived activin A via the methodology developed in the Hyvönen lab has been helpful in building the business case for the formation of the company. From the list of impact categories […], the following apply:

- *Industry has invested in research and development.*
- *The performance of an existing business has been improved.*
- *A business or sector has adopted a new technology or process.*
- *A new product or service is in production or has been commercialised.*
- *The strategy, operations or management practices of a business have changed.*
- *Jobs have been created or protected.*
- *Production, yields or quality have increased or level of waste has been reduced.*
- *Costs of production have been reduced."*

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. Letter from Director of PeproTech Inc.
2. Letter from CEO of Ansh Labs LLC
3. Letter from CEO of Cell Guidance Systems Ltd
4. http://www.stemcells.cam.ac.uk/about-us/facilities/tissue-culture-facility/sci-services
5. Income spreadsheet for protein sales, licenses and consultancy
6. Letter from CEO of DefiniGEN Ltd