

# Recombinant pharmaceutical manufacturing from plants

## The future of molecular farming

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: Friday, October 15, 2010

*After our very successful 'Molecular Farming - plant biologicals' event in 2008  
we are pleased to announce our follow up event*

This meeting has **CPD approval** and a discussion panel session

- 9:00 – 9:45      **Registration**
- 9:45 – 10:00    **Introduction by the Chair:** *Professor Ian A Graham*, CNAP Director & Weston Chair of Biochemical Genetics, The University of York, UK
- 10:00 – 10:40    **Development of a virus-derived system for the co-expression of multiple proteins at defined levels in plant cells**  
*Dr. George Lomonosoff*, John Innes Centre, UK  
A major challenge in the development of plants as bioreactors has been to devise a system that can direct the synthesis of multiple proteins within the same cell at defined and differing levels. Such a system would enable protein complexes containing a variety of polypeptides in different amounts to be produced. It would also enable metabolic pathways which require different amounts of enzyme for each step to be constructed. This talk will concern the development of such a system which is based on sequences derived from Cowpea mosaic virus (CPMV). Though originally intended for transient expression, the system has recently been modified for use in stable transformation, increasing its utility.
- 10:40 – 11:20    **Increasing yields of medicinals by plant molecular breeding**  
*Professor Ian A Graham*, CNAP Director & Weston Chair of Biochemical Genetics, The University of York, UK  
Modern molecular breeding methods will be introduced and used to demonstrate the new timelines that are now possible for the rapid improvement of medicinal plants as robust production systems
- 11:20- 11:25    **Speakers photo**  
11:25 – 12:00    **Mid-morning break**
- 12:00 – 12:40    **Tackling chronic disease through improvements to foods**  
*Professor Cathie Martin*, Norwich Research Park, UK  
A major challenge for society is to reduce the frequency of the chronic diseases; cardiovascular disease, cancer and age-related degenerative diseases. Epidemiological studies have demonstrated the efficacy of diets high in fruit and vegetables in reducing the incidence of chronic disease because they contribute important phytonutrients which serve to promote antioxidant defence mechanisms. Plant biotechnology can make a very significant contribution to dietary improvement through model foods that test the importance of specific bioactives in promoting particular aspects of health, markers that allow molecular breeding for enhanced levels of bioactives and genetic engineering that provides novel, health-promoting (functional) foods.
- 12:40– 12:50    **Expression of human papillomavirus I1 capsomeres in tobacco chloroplasts: a step towards cost-effective second-generation vaccines**  
*Andreas Lössl* University of Natural Resources and Applied Life Sciences, Austria  
Various types of human papillomaviruses (HPV) are causatively associated with cervical carcinoma which is the second most common cancer in women worldwide. Most of the cervical cancer cases occur in developing countries. Due to limitations in the availability of currently used virus-like particle (VLP)-based vaccines against HPV to women of these countries, the development of a cost-effective second-generation vaccine is a necessity. Capsomeres have recently been demonstrated to be highly immunogenic and to have a number of advantages as a potential cost-effective alternative to VLP-based HPV vaccines. We have expressed a modified HPV-16 L1 (L1\_2xCysM) gene that retained the ability to assemble L1 protein to capsomeres in tobacco chloroplasts. The recombinant protein yielded up to 1.5% of total soluble protein. Assembly of capsomeres was examined and verified by cesium chloride density gradient centrifugation and sucrose sedimentation analysis. An antigen capture enzyme-linked immunosorbent assay confirmed the formation of capsomeres by using a conformation specific monoclonal antibody which recognized the conformational epitopes. Transplastomic tobacco plants exhibited normal growth and morphology, but all such lines showed male sterility in the T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> generations. Taken together, these results indicate the possibility of producing a low-cost capsomere-based vaccine by plastids

12:50 – 13:40 **Lunch and Poster Viewing**

13:40 – 14:30 **Discussion panel session**

Please submit questions to Euroscicon staff during the event. These questions will be asked to the panel of speakers at this panel session. Plus you are free to ask additional questions during the session

14:30 – 15:10 **Using thin-layer chromatography (TLC) to detect biologically-active compounds in plant extracts**

*Professor Peter Houghton, Emeritus Professor Pharmaceutical sciences Division, Kings College London*

In the last 20 years bioassay-guided isolation of active compounds from plant extracts has been widely used in the discovery of new lead compounds. One disadvantage of the conventional method of testing each fraction is the large number of fractions that have to be tested. The use of TLC together with various spray reagents for activity of biological interest enables the early rejection of fractions showing no activity and also helps to quickly identify the type of phytochemical and its rapid isolation.

15:10 – 15:40 **Afternoon Tea/Coffee and Last Poster Viewing**

15:40 – 16:20 **Containment strategies in biopharming**

*Professor Denis J Murphy, University of Glamorgan, UK*

This paper examines the challenges of segregating biopharmed crops from mainstream crops, particularly those destined for food or feed use. One commercially viable strategy to limit or avoid biopharming intrusion into the human food chain is the rigorous segregation of food and non-food varieties of the same crop species via a range of either physical or biological methods. Even more secure segregation is possible by use of non-food crops, non-crop plants, or *in vitro* plant cultures as production platforms for biopharming. Such platforms already under development range from outdoor-grown *Nicotiana* spp. to glasshouse-grown *Arabidopsis*, lotus and moss. Even more effective methods for secure biocontainment include plastid expression of transgenes, inducible and transient expression systems, and physical containment of plants or cell cultures. In the current atmosphere of heightened concerns over food safety and biosecurity, the future of biopharming may be largely determined by the extent to which the sector is able to maintain public confidence via a more considered approach to containment and security of its plant production systems.

16:20 - 17:00 **Metabolic engineering of high-value and nutritional isoprenoids in plants**

*Dr Paul Frazer, Royal Holloway University London, UK*

Over the past decade genetic/metabolic engineering of isoprenoid biosynthesis and accumulation has resulted in the generation of transgenic varieties containing enhanced or altered isoprenoids. In achieving this important goal many fundamental lessons have been learnt. Most notably is the observation that the endogenous pathways in higher plants appear to resist engineered changes. Typically, this resistance manifests itself through intrinsic regulatory mechanisms that are "silent" until manipulation of the pathway is initiated. In the present presentation the progress made in the genetic engineering of isoprenoids in tomato fruit and other Solanaceae will be reviewed.

17:00 **Chairman's summing up.**

*This meeting was **organised by Euroscicon** ([www.euroscicon.com](http://www.euroscicon.com)), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry. The event was hosted by **BioPark** ([www.biopark.co.uk](http://www.biopark.co.uk)), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies*

## About the Chair

*Ian A Graham*, graduated from the Queen's University of Belfast with a first class honours degree in Botany and Genetics in 1986. He obtained his PhD in Plant Molecular Biology from the University of Edinburgh in 1989, after which he went on to do postdoctoral research at the University of Oxford and The Carnegie Institution Plant Biology Laboratory at Stanford University. He took up his first faculty position at The University of Glasgow in 1993 and moved to the University of York in 1999 where he holds the Weston Chair of Biochemical Genetics and is current Director of CNAP (<http://www.york.ac.uk/org/cnap/>)

## About the Speakers

*Denis J Murphy*; PhD at Univ of York, UK; Fulbright postdoc fellowship at Univ California Davis; Royal Society postdoc fellowship at Australian National Univ, Canberra; Lecturer in Molecular Biology at Univ Durham; Head of Oilseeds Research Dept at John Innes Centre UK. Currently Head of Biotechnology Unit at University of Glamorgan, UK. Also Biotechnology Advisor to United Nations Food and Agriculture Organization and Chair, Biology Advisory Committee, Malaysian Palm Oil Board.

*Professor Houghton* retired as Professor in Pharmacognosy at KCL in 2008, after over 30 years working there. He has published over 250 research papers on the chemistry and biological activity of plants and their constituents. His research areas include substances from plants potentially useful in wound healing, cancer and neurodegenerative disease.

*Cathie Martin* is a group leader at the John Innes Centre, the leading plant research institute in Europe. She is Professor at the University of East Anglia and Niels Bohr Professor in the Faculty of Life Science, University of Copenhagen, Denmark. Recently she has been co-ordinating research into how diet can help to maintain health and reduce the risk of chronic disease, and how crops can be fortified to improve diets. Her interests span from fundamental to applied plant science. She is Editor-in-Chief of *The Plant Cell*, and the first woman and the first non-American to hold this post.

*Paul Frazer* has over 20 year's experience working both in academia and industry within the UK and abroad. During this period Dr Fraser has worked on the analysis, biosynthesis, regulation and metabolic engineering of carotenoids and isoprenoids, both in plant and microbial systems.

*Andreas Lössl* graduated at the University Giessen in 1991. He worked at the Universities of Munich (TUM: 1995-2000) and LMU (2000-2004). From 2004 to 2010 he developed a research group at the University of Natural Resources and Life Sciences, Vienna. He is focussed on plant organellar genomes and their value for biotechnological use. His group was the first who managed:

1. Synthesis of polyesters in chloroplasts transformed with a bacterial 3-gene operon.
2. Regulation of chloroplast-harboured transgene expression by an inducible T7-RNA polymerase.
3. Expression of novel antigen-vaccines against HPV virus in chloroplasts

*George Lomonosoff* graduated from the University of Cambridge in 1976. His Ph.D. studies, at MRC Laboratory of Molecular Biology (LMB), Cambridge concerned the assembly of Tobacco mosaic virus and were followed by an MRC Post-doctoral fellowship at LMB Cambridge to determine sequence of TMV RNA. He moved to the John Innes Centre Norwich in 1980 and has continued to work there since apart from two periods: as a Fulbright scholar, at Cornell University, USA (1987-1988) and as a Visiting Researcher, at The Scripps Research Institute, La Jolla, USA, (1998). George's research has focused on the molecular biology of RNA plant viruses and their use for the production of foreign proteins in plants. He is an honorary professor and UEA and has co-ordinated several EU FP consortia concerned with the production of plant-derived vaccines.

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### MOLECULAR FARMING IN DUNALIELLA SALINA

Barzegari, A.,<sup>1</sup> Hejazi, M. A.,<sup>1</sup> Rustayi, M.<sup>2</sup>

<sup>1</sup>North and North-West Agricultural Biotechnology Research Institute of Iran, <sup>2</sup>Bu Ali Sina University, Agricultural Faculty, Hamadan

At present, several heterologous protein expression systems are available for the production of recombinant proteins for using in human and animal healthcare. Each of these expression hosts offers distinct advantages consisting soluted protein enhancement, ease of manipulation, and cost of production. Bacteria are the expression host systems that are often used to attempt to produce recombinant proteins. However, the bacterial protein expression system is incapable of reproducing the complexity of eukaryotic proteins which often require extensive folding and post-translational modification. Plant cells carry out many of the post-translational modifications required for optimal biological activity of mammalian proteins. However, there are several problems to use them to produce therapeutic proteins including long period from the initial transformation event to the delivery of usable quantities of antibody that may take even in year scale. The second problem in plants is the potential for genflow (via pollen) surrounding crops. The last problem is herbicide resistance genes. The production of pharmaceutical substances in animal cell cultures is often associated with the risk of product contamination with pathogens dangerous to human health. Therefore, *D. salina* has many advantages compared with traditional systems for the molecular farming of pharmaceutical proteins.

- 1.lacking of cell wall, therefore being easy to manipulate.
2. the single, large, cup-shaped chloroplast present in the cell. The chloroplast transformation is harmless to the environments and does not induce gene silencing.
3. The low cost of their culture. *D. Salina* is an autotrophic organism in media containing inorganic salts.
4. Lack of proteins and pathogenes similar to human's in *D. salina*.
5. post-translational processing for proteins.
6. The availability of a wide variety of promoters regulated by factors such as light or specific nutrient levels in the medium.
7. The ability to produce secreted proteins
8. High growth rate in shorter time, which allows it easy to screen transformed cells.

#### References

- 1- Armin, H. 2007. Algal Transgenics and Biotechnology, Transgenic Plant Journal 1(1), 81-98
- 2- Geng, D.G., Y.Q. Wang, W.B. Li, and Y.R. Sun. 2002. Transient expression of GUS gene in *Dunaliella salina*. High Tech. Lett. 12, 35-39.
- 3- Scott E F. and Mayfield, S. P. 2004. Prospects for molecular farming in the green alga *Chlamydomonas reinhardtii*, Plant Biology, 7:159–165
- 4- Mewett, O., Hilary, J. and Ruth, H. 2007. Plant molecular farming in Australia, [www.ag.gov.au/cca](http://www.ag.gov.au/cca)

### TRANSPLASTOMIC EXPRESSION OF A MODIFIED HUMAN PAPILLOMAVIRUS L1 GENE FUSED WITH *ESCHERICHIA COLI* HEAT-LABILE ENTEROTOXIN SUBUNIT B (LTB) AS ADJUVANT.

MT Waheed<sup>1</sup>; N Thönes<sup>2</sup>, M Müller<sup>2</sup>, SW Hassan<sup>1</sup>, J Gottschamel<sup>1</sup>, E Lössl<sup>1</sup>, HP Kaul<sup>1</sup>. AG Lössl<sup>1\*</sup>

<sup>1</sup> Department of Applied Plant Sciences and Plant Biotechnology (DAPP) University of Natural Resources and Applied Life Sciences (BOKU), Gregor-Mendel-Strasse 33, 1180 Vienna, Austria

<sup>2</sup> Deutsches Krebsforschungszentrum, F035, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Attempts have been made to develop a cost effective vaccine against human papillomavirus (HPV). Various types of HPV can cause cervical cancer in women worldwide. Adjuvants have been reported to increase immunogenicity against many antigens. Our study includes the expression of modified HPV-16 L1 protein fused with the *Escherichia coli* heat labile enterotoxin subunit B (LTB) in tobacco chloroplasts. Stable transgene integration was confirmed by polymerase chain reaction (PCR). Recombinant protein was under detection limit in western blot analysis; however, ELISA (enzyme linked immunosorbent assay) provided evidence for the production of L1 protein. The correct assembly of protein was confirmed by using conformation specific antibody. All transplastomic plants showed severe pleiotropic effects such as chlorosis, stunted growth and male sterility. Flowers were produced, but either they were shed or did not produce seeds. Seeds were obtained by pollinating T<sub>0</sub> generation with pollen from wild type plants, and later T<sub>2</sub> generation was obtained from T<sub>1</sub> seeds in the same way. These detrimental effects persisted in both T<sub>1</sub> and T<sub>2</sub> progenies. Further detailed studies are needed to investigate these phenomena.

## **EXPRESSION OF HUMAN PAPILLOMAVIRUS L1 CAPSOMERES IN TOBACCO CHLOROPLASTS: A STEP TOWARDS COST-EFFECTIVE SECOND-GENERATION VACCINES**

Lössl A<sup>1\*</sup>, Waheed MT<sup>1</sup>; Thönes N<sup>2</sup>, Müller M<sup>2</sup>, Hassan SW<sup>1</sup>, Gottschamel J<sup>1</sup>, Lössl E<sup>1</sup>, Kaul HP<sup>1</sup>

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Various types of human papillomaviruses (HPV) are causatively associated with cervical carcinoma which is the second most common cancer in women worldwide. Most of the cervical cancer cases occur in developing countries. Due to limitations in the availability of currently used virus-like particle (VLP)-based vaccines against HPV to women of these countries, the development of a cost-effective second-generation vaccine is a necessity. Capsomeres have recently been demonstrated to be highly immunogenic and to have a number of advantages as a potential cost-effective alternative to VLP-based HPV vaccines. We have expressed a modified HPV-16 L1 (L1\_2xCysM) gene that retained the ability to assemble L1 protein to capsomeres in tobacco chloroplasts. The recombinant protein yielded up to 1.5% of total soluble protein. Assembly of capsomeres was examined and verified by cesium chloride density gradient centrifugation and sucrose sedimentation analysis. An antigen capture enzyme-linked immunosorbent assay confirmed the formation of capsomeres by using a conformation specific monoclonal antibody which recognized the conformational epitopes. Transplastomic tobacco plants exhibited normal growth and morphology, but all such lines showed male sterility in the T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> generations. Taken together, these results indicate the possibility of producing a low-cost capsomere-based vaccine by plastids (Waheed et al. 2010).

**References:** Waheed MT, Thönes N, Müller M, Hassan SW, Lössl E, Kaul HP, Lössl A (2010) Transplastomic expression of a modified human papillomavirus L1 protein leading to the assembly of capsomeres in tobacco: A step towards cost effective second generation vaccines. DOI: 10.1007/s11248-010-9415-4 Transgenic Research 2010

## **EXPRESSION OF A FUNCTIONAL HUMAN ADENOSINE DEAMINASE IN TOBACCO PLANT CELL SUSPENSIONS AND WHOLE PLANTS**

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An inherited disorder ADA deficiency is a form of severe combined immunodeficiency, which is ultimately caused by an absence of adenosine deaminase (ADA), a key enzyme of the purine salvage pathway. The absence of ADA activity in sufferers eventually results in a dysfunctional immune system due to the build up of toxic metabolites and to date this has been treated, with mixed success, using PEG-ADA, made from purified bovine ADA coupled to polyethylene glycol. It is likely however, that an enzyme replacement therapy protocol based on recombinant human ADA would be a more effective treatment for this disease. Therefore, as a preliminary step to produce biologically active human ADA in transgenic tobacco plants and cell suspensions a human cDNA has been cloned into a plant expression vector under the control of the CaMV 35S promoter and terminator. ADA-specific activities of between 0.001-0.03 units per mg protein were measured in crude extracts isolated from both transformed tobacco plant leaves and calli. Plasmid constructs aimed at targeting ADA to the apoplast have also been made using PR1a and extensin 5' apoplast-targeting sequences. Suspension cultures and calli transformed with these constructs exhibited a five to six-fold increase in ADA activity compared to cultures transformed with the cytosolic-directed construct. In addition, ADA constructs targeted to the ER by a C-terminal KDEL sequence also exhibited an increase in ADA activity of up to six-fold compared to cytosolic-directed constructs. Efforts to increase transgenic ADA expression further are underway using various TMV translational enhancers and chimaeric glycoprotein constructs.

## **EXPRESSING ANTIGENS AGAINST TUBERCULOSIS IN PLANTS THROUGH PLASTID TRANSFORMATION**

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*Institute of Agronomy and Plant Breeding (IPP), University of Natural Resources and Applied Sciences Vienna, Gregor Mendel strasse 33, 1180 Vienna Austria*

Tuberculosis is the widest spread disease with an average of 9.4 million new cases each year. Only in year 2008 more than 1.7 million people died of it making 4500 deaths a day. We investigated the feasibility to produce antigen subunit vaccines against Mycobacterium in inexpensive production facilities by plant transformation. Conventional transformation techniques are connected with some major disadvantages like the risk of transgenic pollen flow and the permanent expression of the antigen. These problems can be reduced drastically by the plastid transformation method. Since plastids are inherited maternally, pollen-mediated gene flow is reduced to a very small probability. Thus a much higher security can be achieved and additionally the plastid genome transformation system is expected to mediate increased foreign protein expression. Along with highly immuno protective antigens like *Ag85A*, *Esat6* and *fbpA* a membrane protein *mmp1* was also tested which characterized immune response to Tuberculosis and confer cross-immunization for further mycobacterial pathogens.