Expressions of interest

Professor George Lomonossoff discusses his latest project using transient expression technologies to develop novel vaccines for viruses such as Avian influenza and Bluetongue

What are the core objectives of the 'Plant Production of Vaccines' (PLAPROVA) project?

The major objective of PLAPROVA was to use transient expression technologies to rapidly produce vaccine candidates in plants in sufficient quantity to enable pharmacological studies to be undertaken. A particular advantage of the transient approach is that yields can reach 10-30 per cent of total soluble protein (TSP) within a few days. Using this approach, the consortium has been able to proceed from cloning of relevant genes to the expression of milligramme amounts of candidate vaccines within two to three weeks. Thus the consortium has been able to evaluate potential vaccines against a number of diseases of importance to the EU, Russia and South Africa.

To what extent are plant protein extraction techniques now seen to be market-competitive with more established methods, such as mammalian cell culture? What are the challenges or limitations with extracting plant proteins, and how is PLAPROVA working to bridge these gaps?

Methods for the extraction of active proteins from plants are improving all the time. Though plants have been used as a source of medicinal products since time immemorial, these have usually been small molecules such as opiates. Thus, until recently, little information was available regarding the optimum methods for extracting proteins in a biologically active form. A particular challenge has been the development of methods to prevent the clogging of purification equipment with cellulose fibres. PLAPROVA has made use of methods developed by others for the extraction of candidate vaccines.

Transient expression is a technique whereby foreign genes are introduced into fully developed normal plants where they are expressed to high levels within a matter of days. In contrast to stable genetic transformation, the foreign genes are not inherited by subsequent generations of plants.

Over the past five years, what advances have we seen in the technologies used for expressing and extracting proteins in an active form from plants?

Methods for the transient expression of proteins in plants have come a very long way in the past five years. These methods are based on the infiltration of leaf tissue with suspensions of *Agrobacterium tumefaciens* harbouring plasmids containing the gene(s) to be expressed. Initially the technique was limited to small-scale production using individual leaves but, more recently, methods for the vacuum infiltration of whole plants on an industrial scale have been developed. In addition, through the work of the FP6 'Pharma-Planta' consortium, of which I was also a member, enormous advances have been made in methods of protein extraction.

Can you provide insight into the range of viruses for which you are developing vaccines? What progress have you made so far?

PLAPROVA has focused on diseases which are particularly relevant to the EU, Russia and South Africa, and for which there is an ongoing need for new or improved vaccines. The viruses studied include Avian influenza virus, Human and Bovine papillomaviruses, Bluetongue virus, Hepatitis B virus, Porcine respiratory and reproductive syndrome virus, and Foot and Mouth Disease virus. Many are diseases of veterinary rather than medical importance. This choice was deliberate since immunological testing in target animals is more straightforward in this case. However, the principles established with animal vaccines will also be applicable to the production of vaccines for human use.

As you have hinted, PLAPROVA is a collaborative initiative between the EU and Russia with participation from South Africa. How central has this cooperative aspect been to the fulfilment of the project, and what has each partner offered in terms of expertise and resources?

The collaborative aspect of the project has been critical to its success. For the work to proceed efficiently, it was essential to assemble a consortium with expertise in plant expression systems, animal virology, protein purification and immunology. No single institution has this range of skills. Furthermore, if there was to be take-up of the results of the project, it was essential to involve those countries where the diseases pose a particular challenge. Finally, one should not underestimate the contribution of PLAPROVA towards creating an 'EU-Russia Common Space for Education and Research' and an EU-South African dialogue on the knowledge-based bioeconomy.

What would you highlight as your key achievements to date?

The main achievements to date include the successful expression, in plants, of virus-like particles (VLPs) against Human and Bovine papillomaviruses, Hepatitis B virus and Bluetongue virus using the CPMV-*HT* expression system. Of particular note is the successful production of Bluetongue virus VLPs, which involved the co-expression of four different viral proteins, and the demonstration that such VLPs are able to raise antibodies against Bluetongue virus when injected into sheep. The ability to produce such complex VLPs has pointed the way for the production of a whole range of novel vaccines in plants.

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Vaccines at velocity

The PLAPROVA project is using newly developed plant protein expression techniques to produce plant vaccine candidates for a number of important pathogens at a speed never before achieved

THE USE OF PLANTS for the production of pharmaceutical proteins has been actively investigated over the last 20 years. There are a number of potential advantages for using plants to produce vaccines: first, there is an inherent safety in that the production system – the plants – cannot become contaminated with an external animal pathogen as can occur in mammalian cell culture; second, there is the potential to administer plant-produced vaccines orally; and third, there are potential benefits in terms of costs of production. Several plant-made products have been assessed for safety and efficacy with favourable results, and these have culminated in the demonstration that plant-produced vaccines can protect target animals against challenge.

However, this area of research now stands at a crossroads. On the one hand, the past five years has seen considerable advances in technologies for expressing proteins and extracting them in an active form from plants. On the other hand, most of the recent successes have concerned the production of well-characterised antigens and antibodies which have already been produced using previously established methods, such as mammalian cell culture. One downside of this is that the plant-expressed proteins which are most highly developed, and in some cases undergoing clinical testing, will be in direct competition with existing products.

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Until recently, a major disadvantage of the use of plants to produce proteins has been the time lag associated with the production of lines of stably transformed plants, as methods for the rapid production of plant-expressed proteins in large quantities were not available. Rapid production is particularly important for cases where it is not currently possible or practical to produce the potential vaccine by other means. In these cases, there is likely to be far less pre-existing information available about the efficacy of the final product and it will be essential that the properties of a number of variants can be quickly assessed to determine which are the most suitable for further development. Stable genetic transformation (either nuclear or plastid) is unsuitable for these studies in view of the time taken to obtain expression. For plants to fulfil their potential as a means of producing vaccines, it is imperative that methods are developed for the rapid production and characterisation of a large number of vaccine candidates.

TACKLING TIME LAGS

The 'Plant Production of Vaccines' (PLAPROVA) project is aiming to address this challenge and is developing a rapid plant-based system to produce and assess the capacity of different proteins to act as vaccines against important diseases of livestock. PLAPROVA is a collaborative project between the EU and Russia with participation from South Africa. It is funded under FP7 and coordinated from the John Innes Centre in Norwich, UK, by Professor George Lomonossoff, a member of the previous 'Pharma-Planta' consortium which succeeded in making huge advances in the development of methods for protein extraction from plants.

One of PLAPROVA's major aims is to refine transient expression technologies so that they can produce sufficient material in a short time frame to enable pharmacological studies of a large number of vaccine candidate variants to be undertaken, hence eliminating the time lags associated with the production of stably transformed lines of plants. This speed means that a wide range of vaccine candidates can be rapidly screened. Thus the consortium is able to evaluate potential vaccines against a number of diseases of great and increasing importance to both the EU and Russia. These include Avian influenza virus, Bluetongue virus and Porcine respiratory and reproductive syndrome virus.

PLAPROVA has concentrated on vaccine candidate proteins that are capable of either forming or being incorporated into protein complexes or virus-like particles (VLPs). "Because they contain multiple copies of antigenic sequences, such particulate structures are potent stimulators of the immune system and are hence excellent vaccine candidates," Lomonossoff explains. Furthermore, they can be used as carriers of additional immunogenic sequences for the development of novel

PLAPROVA

PLANT PRODUCTION OF VACCINES

OBJECTIVES

PLAPROVA is a specific international cooperation action (SICA) project, jointly funded by the EU and Russia with participation from South Africa, that will develop a rapid plant-based system to produce and assess the capacity of different proteins to act as vaccines against important diseases of livestock such as Avian influenza and Bluetongue.

KEY COLLABORATORS

Professor G P Lomonossoff, John Innes Centre, Norwich, UK • **Dr M E Taliansky**, James Hutton Institute, Dundee, UK • **Dr R Kormelink**, Wageningen University (WU), Wageningen, The Netherlands • **Professor L Enjuanes**, CNB CSIC, Madrid, Spain • **Dr E Noris**, Istituto di Virologia Vegetale, CNR, Torino, Italy • **Professor I N Minkov**, University of Plodiv, Plodiv, Bulgaria • **Professor E P Rybicki**, University of Cape Town, South Africa • **Professor K Skryabin**, Russian Academy of Sciences, Moscow, Russia • **Professor J G Atabekov**, Moscow State University (MSU), Moscow, Russia • **Dr O I Kislev**, Russian Academy of Medical Sciences (RAMS) • **Dr V V Borisov**, Federal Centre for Animal Health (FGI ARRIAH), Vladimir, Russia • **Dr N Ravin**, Russian Academy of Sciences, Moscow, Russia

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GEORGE LOMONOSSOFF completed his PhD at the MRC Laboratory of Molecular Biology, Cambridge in 1980 and moved to the John Innes Centre, Norwich later that year. He is currently a project leader in the Department of Biological Chemistry and is an Honorary Professor at the University of East Anglia.

PLAPROVA Plant Production of Vaccines

John Innes Centre

PLAPROVA CONSORTIUM MEETING, MOSCOW, JULY 2009

vaccines. Another advantage of choosing to work with VLPs is that because of their relatively large size, they are easy to purify from other cellular components.

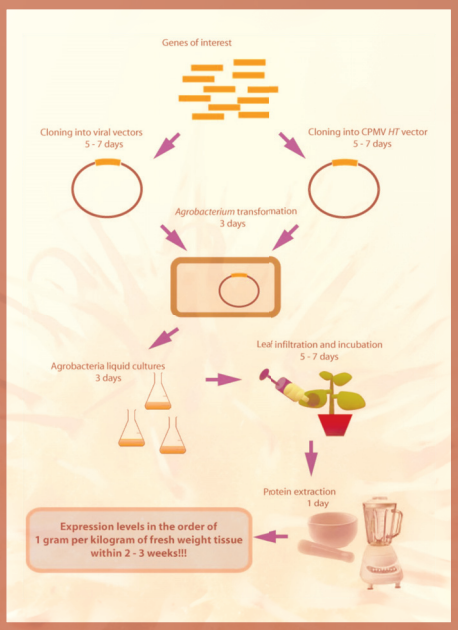
TRANSIENT EXPRESSION TECHNOLOGIES

PLAPROVA is exploiting recent developments in transient expression technologies to screen these vaccine candidates in plants, and the consortium has used systems based on replicating plant virus vectors and non-replicating systems based on hyper-translatable RNA molecules, such as the CPMV-*HT* system. The CPMV-*HT* expression system involves flanking the foreign gene of interest with sequences derived from the 5' and 3' untranslated regions of cowpea mosaic virus RNA-2. These sequences make the inserted gene 'hypertranslatable' leading to high levels of protein synthesis.

Both the replicating and non-replicating systems are suitable for the rapid expression of proteins. In both cases the sequence to be expressed is introduced by infiltrating leaves with suspensions of *Agrobacterium tumefaciens* harbouring appropriate plasmids. In the case of replicating systems, high level expression is achieved through the amplification of RNA molecules encoding the gene of interests, while non-replicating systems rely on the production of hyper-translatable mRNA molecules.

Using these systems, the team has been able to go from cloning of constructs to the expression of milligramme amounts of candidate proteins sufficient for immunological characterisation in a matter of days using only small amounts of plant tissue: "This is of huge benefit when rapid production of a new vaccine is required, such as when combating a newly emerging influenza strain," Lomonossoff affirms.

This contrasts with the months or years required for the production of stably transformed plants. However, Lomonossoff does not feel that the latter method should be overlooked: "Transient and stable expression can be seen as complementary: transient expression allowing the screening of a large number of vaccine candidates with stable transformation being used for the subsequent large-scale production of selected candidates," he acknowledges.



OVERALL TIMESCALE FOR THE EXPRESSION OF CANDIDATE VACCINES USING TRANSIENT EXPRESSION IN PLANTS

DEVELOPING VACCINES

Whatever its origin, any new vaccine must undergo rigorous tests for safety and efficacy before it can penetrate the market, and plant-produced vaccines are no exception. However, the rigour of the testing depends on whether the potential vaccine is destined for veterinary or human use. One particular issue regarding plant-produced vaccines is that because of their novelty, there is less background knowledge available regarding potential contaminants as compared with the situation with mammalian cell systems.

However, the CPMV-*HT* transient expression system developed under PLAPROVA has already been licensed to Medicago Inc (Quebec, Canada) for the production of vaccines against influenza viruses. Medicago's plant-expressed influenza vaccine has recently successfully completed phase II clinical trials, opening up the possibility of the deployment of a plant-based vaccine within the next five years.

PLAPROVA's ability to screen many candidate VLPs will eventually result in the development of novel vaccines against a variety of important pathogens. At the same time as the screening is carried out, the consortium will develop methods to allow the rapid translation of the information gained through the transient studies into larger scale production systems for the most promising candidates. This will enable low cost vaccines to be developed for use in livestock and, ultimately, humans.