Baculovirus-insect cell interactions: keys for tailoring viral entry, processing and release

Project leaders and principle investigators

- **Dr Just M. Vlak**
- **Dr Monique M. van Oers**
- **Dr Marcel W. Westenberg**
- **Dr Hu Zhihong**

Summary
Baculoviruses are naturally occurring, host-specific insect pathogens and used world-wide as environmental friendly biological control agents of insect pests. For example, in China, 100,000 acres of cotton are sprayed each year with one (HaSNPV) of the 600 baculoviruses against the cotton bollworm Helicoverpa armigera. Each virus isolate contains a number of variant genotypes, collectively determining its biological feature. The role of this diversity in baculovirus evolution and maintenance in crop ecosystems is enigmatic, but it is tempting to assume that the available gene pool allows an adequate response to changes in crop management strategies or environmental changes. Functional analysis of the baculovirus genes in this pool and insight in the genotypic variation is a prerequisite to the understanding of this response of baculovirus isolates towards optimal fitness and survival. The cotton bollworm-baculovirus HaSNPV forms the experimental system. The genome of HaSNPV has been entirely sequenced through collaboration of the Chinese and Dutch partners prior to PSA-1 and 135 open reading frames (ORFs) have been discerned. This already provided new leads for improving insecticidal properties of HaSNPV by genetic engineering. This PSA-1 project aims at understanding the function of these ORFs in virus infection and the interaction of HaSNPV and insects at molecular and cellular level by functional genomic approaches. The nature of the genetic variation in baculovirus isolates will be studied using molecular tools and allows an understanding of the ecological consequences of the introduction of novel (genetically-engineered) baculoviruses in ecosystems. The ultimate aim of the project is to reveal the key virus genes involved in virus-host interaction and genome plasticity in response to virus infection at the molecular level. Both partners as well as the international scientific community will benefit from the increased knowledge of baculoviruses and outreach can be achieved for application of these viruses as biocontrol agent and biotechnological applications.

Genomics of nutritional and developmental traits in Brassica

leaders and principle investigators

- **Dr. Qu Dongyu**
- **Prof . dr. Evert Jacobsen**
- **Dr. ir. A. B. Bonnema**
- **Dr. Xiaowu Wang**
- **Jianjun ZHAO**
- **Ping LOU**

Summary
During the long history of B. rapa breeding and selection, a variety of forms have been selected for use as oilseeds, leafy vegetables and turnips (Zhao et al. 2005) and the genetic basis of this variation is largely unknown. We focus on B. rapa since this genome is being sequenced, it includes both vegetable- and oil crops and can be vernalised at the seedling stage which accelerates the genetic research considerable. The synteny between the rapa, oleracea and napus genomes will facilitate application of B. rapa results to B. oleracea and B. napus improvement.

In this project we follow a genetic approach to genetically dissect complex nutritionally and developmental traits. For this purpose at Wageningen University and CAAS Doubled Haploid (DH) populations, Breeding Inbred Line (BIL) populations and core collections, or Diversity Fixed Foundation Sets (DFFS) were developed all with genotypic and phenotypic data. This allows a QTL mapping approach and an association-mapping approach, which can sample allelic variation.

The traits under study are the glucosinolates, a class of sulfur containing plant metabolites with many forms that have anti-carcinogenic properties and largely responsible for the typical flavour and odour of Brassica

species. We will study the genetic regulation of glucosinolates in the chain, from farm to fork, since various steps in the production chain (processing / preparation) affect the level of potential health-protective glucosinolates. We further study the developmental traits of heading and turnip formation using DH and BILs and exploiting Arabidopsis syntheny. Finally, a biotechnological approach is followed in a project to develop biosensor B. rapa genotypes for monitoring micronutrient deficiency and metal toxicity, a major problem in China. The main aim of this program is to unravel the genetic regulation of nutritional and developmental traits in a fruitful collaboration between Chinese and Dutch research groups.

The first phase of the PSA program was used to develop genetic material (populations, collections), genetic maps and their application to genetically analyse glucosinolates, phytate, morphological traits and mineral accumulation. For this purpose we applied Quantitative Trait Locus (QTL) mapping and developed association mapping for B. rapa. WU-PB developed a core collection and two Doubled Haploid populations from wide crosses within Brassica rapa. Integrated genetic maps were constructed based on AFLP and SSR markers, the latter were used to assign linkage groups and to connect the maps to reference maps. Using these populations, the genetic regulation of glucosinolate composition was studied, and several QTL's were identified. For aliphatic glucosinolates, AOP and MAM were suggested as candidate genes (Lou et al., submitted). A fine mapping population of 6000 F2 was developed to zoom into the QTL. Simultaneously, the glucosinolate degradation during cooking, frying and industrial heating of Chinese cabbage and PakChoi were studied (papers in preparation). These DH populations are also useful to study the genetics of morphological traits (Lou and Zhao et al., 2007), with interesting data on regulation of flowering time. Simultaneously at IVF-CAAS a genetic map was constructed of a large Chinese cabbage DH population (in collaboration with the Henan Academy of Agricultural Sciences). This population was used to identify QTL for morphological traits and for mineral accumulation and tolerance. At IVF-CAAS a number of recombinant Inbred Line populations are developed to study morphological traits but also metabolite composition in future projects. Populations were generated by selfing a number of BC2 plants until BC2S5. Populations are established with turnip, Caixin L58, Chinese cabbage, Yellow Sarson as donors and recurrent parents. These BILs together with the DH populations and core collections represent excellent materials to study the genetics of phytonutrients and morphological traits (heading, flowering, turnip formation).

Effects of human activities on the eco-morphological evolution of rivers and estuaries

Project leaders and principle investigators
Prof. dr. Hubrecht J. de Vriend
Prof. dr. Peter H. J. Herman
Prof. dr. M. J. F. Stive
Prof. dr. C. H. R. Heip
Dr. Tom Ysebaert
Prof. dr. Jack J. Middelburg
Dr. Tj. Bouma
Dr. Johan C. Winterterp
Dr. D. S. van Maren
Dr. Zheng Bing Wang
Dr. Pingxing Ding
Prof. dr. Qing He
Prof . dr. Yunxuan Zhou
Prof . dr. Daoji Li
Prof . dr. Shilun Yang
Prof. dr. Liquan Zhang
Dr .Kelin Hu
Prof. dr. Jianjun Zhou
Prof . dr. Zhao-Yin Wang
Prof. dr. Baosheng Wu
Dr. Junqiang Xia
Dr. Xudong Fu

Summary

River deltas are inhabited by over 60% of the world population, and are, consequently, of paramount agricultural and economical importance. They constitute unique wetland environments which gives river deltas ecological importance as well. The high population density that characterizes these deltas implies a large pressure on the natural resources, resulting in a decrease in water and sediment supply (dam construction, irrigation works), water quality problems (e.g. eutrophication), subsidence (lowering of the groundwater table, oil and gas extraction), loss of the natural sea defense, modified downstream supply of pollutants and nutrients, and loss of biodiversity. All these effects may affect the biogeochemical and ecological functioning of the coastal zone. However, the nature of human-biogeochemical and human-biophysical couplings is still poorly understood.

The equilibrium between fresh water discharge from the river basin and the tidally driven import of sea water is one of the most fundamental forcing functions that determine the ecology and biogeochemistry of estuarine systems. We hypothesize that human activities, both in the watershed and the coastal zone, will strongly influence this balance and as a result the whole food web structure and the benthic-pelagic coupling of estuaries and deltas. Simultaneously, the ensuing pressure on space makes land protection and reclamation a key issue, thus sediment a valuable resource. In order to asses the effect of these human influences on delta systems and estuaries we focus on the morphological response of deltas and estuaries to decreasing sediment supply, and on the ecological effects of a modified downstream supply of water, sediment, pollutants and nutrients. The morphologic behavior influences ecological processes, and vice versa, and therefore both ecological and morphological effects should be analyzed simultaneously. Global climate change may provide additional threats through accelerated sea-level rise and changing weather patterns (IPCC 2007) and as a direct result, floods, droughts and storms are very likely to become increasingly common.

The project aims at identifying and analyzing these effects, formulating large-scale model concepts to describe them, embedding these concepts into predictive simulation models, and validating these models against measured data. Although the work is meant to be generic, the first test sites will be the Yellow River and its delta (China), the Yangtze River and its estuary (China), and the Scheldt estuary (the Netherlands). Knowledge gathered from Northwest European rivers and estuaries will be confronted with Chinese knowledge and experience, jointly improved, consolidated and tested for generality in these three systems.

The Lower Yellow River (LYR) is carrying very large amounts of fine sediment from the Löss Plateau to the Bohai Sea. The sediment concentrations that can be reached under flood conditions are so high (up to 911kg/m3 was measured on September 7, 1977 at Sanmenxia station near the entrance of the LYR), that the water-sediment mixture behaves like mudflow. This makes the LYR unique in the world, and it explains why none of the sediment transport models developed for other rivers applies here. In recent years, the problems have increased by man's interference with the discharge regime of the river. Irrigation, drinking water supply and industrial water intake have reduced the base flow to virtually nil (in 1998, the LYR was dry during more than 200 days). As a consequence, the river's main channel has degenerated, whence the flood conveyance capacity is drastically reduced. For example, the maximum water stage at Huayuankou station reached 94.73m in 1996 flood with peak discharge of 7,860m3/s, which is higher than the maximum water stage of 94.42m in 1958 flood with peak discharge of 22,300m3/s.

High expectations are now put on the recently completed Xiaolangdi Project, a high dam with a large reservoir, built as a facility for (among other things) flood and sediment management. Appropriate storage and release strategies of water and sediment should help to solve the problems in the LYR, and to increase the longevity of the reservoir. Prediction of the effects and the effectiveness of these strategies, however, has hardly been possible, so far, by lack of an adequate sediment transport model. Especially the mud flow mode remains to be understood and modelled. The focus within the project phase of the PSA program was on increasing fundamental knowledge of the important sediment transport processes in the Yellow River, and the adaptation and application of existing numerical models. A conceptual model was developed to provide a framework of typical hydraulic and sedimentary conditions in the Yellow River, and the applicability of numerical models. Existing sediment transport formulas were tested for applicability in the Yellow River, and the complex vertical structure of flow velocity and sediment concentration investigated. Several numerical models were developed to simulate hyper concentrated floods and sediment release strategies from the Xiaolangdi dam. Although these model applications successfully simulated some aspects of the complex morphodynamics of the Yellow River, they also revealed some processes that require further research. This is envisaged for the project phase of the PSA program.

Sustainable resource use in rural China: Institutions, policies and markets

Leading researchers
Arthur Mol (Dutch project leader)
QU Futian (Chinese project leader)
ZHANG Tianzhu
Nico Heerink
ZHANG Lei
SHI Xiaoping
TAN Shuhao

Summary
In the coming decades, sustainable resource use will rank high on the policy agenda in China. Efforts aimed at promoting sustainable resource use in rural China will be confronted with two major changes. First, the gradual transition from a state-dominated towards a market-oriented economy implies that massive central state regulation decreases and that economic, decentral and informal resource management institutions are gaining importance. Second, China's domestic economic liberalisation and entry into global and regional trade agreements greatly affect domestic agricultural prices and hence the use of resources, and bring home international environmental regimes, particularly issues of eco-labelling, standardisation, and food safety.

Institutions play a key role in both natural resource degradation and preservation. In studying the role of institutions in such a dynamic socio-economic environment, the research project focuses on two changing institutions: the system of land institutions, with a focus on land tenure and eco-restoration programs, and the system of water institutions, related to both water quantity and quality tenure. The purpose of the project is to contribute to an improved understanding of
the impact of both types of institutions on the sustainability of land and water use in rural areas, and
how these institutions are affected by globalisation, market liberalisation and diminishing central planning.
The research is carried out by one full-time and five part-time post-doc researchers. Research locations include Northeast Jiangxi Province (land tenure and land degradation), Ningxia Province (ecological restoration program), Qinghai Province (grassland degradation), Dianchi Lake in Yunnan Province (water pollution), Miyun reservoir in Beijing / Hebei province (payment for water services), and Zhangye City in Gansu Province (water rights trading). The very rapid economic changes that China is undergoing, and the resulting pressure on rural resource use and on the relevant institutions, make China a very interesting case for analysing such institutional changes. The insights gained from the project are highly relevant for future policy making on promoting sustainable rural resource use in China as well as in other countries undergoing similar transition processes.

The scope of the project has widened during its implementation from a focus on land tenure in Northeast Jiangxi Province and agriculture-based water pollution in Dianchi Lake in East Yunnan Province (as initially planned) to other institutions and to other regions. A start has been made with examining property rights and grassland degradation in Qinghai Province, sustainability of the sloping land conversion program in Ningxia Province, payments for water services in Miyun reservoir in Beijing / Hebei Provinces, and market development and water rights trading in Zhangye City, Gansu Province. This widening was made possible through additional funding and research capacity secured by the project partners.

Draft papers on the various research sites have been presented at a special session of the 8th meeting of the European Conference for Agriculture and Rural Development in China (ECARDC) in Yiwu, Zhejiang Province in August 2006, at an international seminar on 'Transition towards Sustainable Resource Use in Rural China' in Kunming, Yunnan Province in October 2006, and at various other international conferences. A selection of these papers will be published in a special issue of a renowned international journal (World Development), edited by three senior researchers of the project. Other papers have been published in edited volumes (also edited by project participants) and as separate articles in scientific journals.

Some of the most noteworthy results (some of them preliminary) obtained thus far are:
Under the prevailing system of rangeland property rights, herd households behaviour contributes to rangeland degradation in Qinghai Province.

Due to legal imperfections, lack of clarity of water rights and unclear division of tasks between relevant institutes, the cost of implementing a system of payments for water services is too high at present.
Agricultural market reforms in the 1990s have greatly increased the profitability of fertilizer application, and thereby aggravated the problem of soil compaction in rice-producing areas in Jiangxi Province.
The high degree of land fragmentation that results from the current system of land distribution among households contributes to soil quality deterioration.
Contrary to common opinions, migration and (to a lesser extent) local off-farm employment has benign effects on agriculture-based environmental pollution.
General trust enhances people's willingness to contribute to public goods (such as conversion of sloping land); kinship trust, on the other hand, reduces such willingness.
Policies that aim to protect the ecological environment in poor areas will not succeed in the long term unless capacity building focuses on future economic development. Social capital development needs to be part of the policy strategy and design in order to enable such future economic development.

Novel catalytic materials for chemicals and renewable energy

Project leaders and principle investigators Prof. Rutger A. van Santen
Prof. Can Li
Prof .Bert Weckhuysen
Dr. Zhaochi Feng
Dr. Emile Hensen
Dr. Andrew Beale
Prof. Qihua Yang
Dr. Pieter Magusin
Dr. Tong Ren
Dr. Weixue Li
Ms. Guanna Li

Summary
Worldwide concerns about energy safety and global warming spur research towards replacement of conventional resources as crude oil and natural gas by renewable biomass feedstocks. To convert such new feedstocks into a wide portfolio of chemicals and energy carriers new catalytic processes need to be developed. For the longer term, direct utilization of solar energy for the production of renewable energy and chemicals production is desired. In both cases, complex novel reactive materials are quintessential towards the sustainable production of chemicals and energy from alternative feedstocks.

Here we propose an integrated molecular approach to the tailored design and controlled synthesis of novel catalysts: we will develop generic methods to introduce well-defined reaction sites in an optimized environment. Advanced in situ characterization techniques and molecular simulations will be combined to study synthesis mechanisms of complex catalytic materials in detail. Examples of such materials are hierarchically structured solid acids, catalytic nanoreactors, hybrid inorganic-organic catalysts and semi conducting materials for photo catalytic and photovoltaic applications. While applications are sought in challenging areas of bio diesel production, heterogeneous catalysts for fine chemicals production and photo catalytic water splitting, the generated knowledge can also be applied to improve current catalyst technology.

The consortium consists of excellent catalysis groups in Eindhoven, Utrecht and Dalian involving all relevant expertises and related infrastructure. An advisory panel member by Chinese and Dutch industries closely follows progress and possible utilization of results.

The main research topics in the Project phase were centred on the theme 'Chemistry and reactivity of catalytic sites in microporous and mesoporous materials'. Focus has been on the preparation of well-defined catalytic sites in microporous zeolites and mesoporous materials. Optimization of the reaction environment has also been studied by experimental and computational approaches. Highlights are the synthesis of a mesoporous Fe-based oxidation catalyst, a mesoporous hydrophobic Ti-based oxidation catalyst, improved Au catalysts for environmental applications and understanding of the performance of immobilized oxidation complexes. The high scientific quality of the work may be judged from the large number of articles appearing in high-quality

peer-reviewed international journals. An important driver for this success has been the complementary nature of the expertises in Eindhoven (experimental reactivity studies, computational chemistry) and Dalian (synthesis, spectroscopic characterization). Two workshops, one in Dalian in 2005 and one in Maastricht (The Netherlands) in 2006 were organized to assess research progress and disseminate the scientific results to a wider scientific audience interested in catalysis in China and the Netherlands.

Nanoscale materials for bone regeneration

Project leaders and principle investigators
John A. Jansen
Li Yubao
Joop G. C. Wolke
Fang Yang
Liao Hongbing
Zuo Yi
Wang Xuejiang

Summary
There are a huge number of patients each year with skeletal defects that require bone-grafting procedures to achieve healing or augmentation. Currently, such defects are generally restored by bone grafts taken from the patient and cadaver bone from bone banks. However, both approaches are associated with various problems. An additional challenge appears when the size of the defect is very big and the reconstruction of this defect requires a bone graft capable of supplying similar physical properties and behaviour to the bone being substituted. Unfortunately, at the moment no synthetic materials (scaffolds) are available that meet all the requirements from a biological point of view, i.e:
Able to promote new bone formation in order to reduce the time of bone healing;
Mechanical and structural properties that provide adequate support until natural healing has occurred;
Able to control specific bone cell activation;
Easy to apply and 'tuneable' to the size and shape of the skeletal defect.
In view of the above mentioned, the overall aim of the current project is the development of the ideal scaffold for bone repair and regeneration that elicits a specific and designed biological response, including angiogenicity, osteoconductivity and osteoinductivity. A super hybrid composite, based on calcium phosphate as the inorganic phase, was proposed. The advantage of such strategy is that this composite can be further developed along the following directions in later program phase and strategic alliance phase by:
Combination with the current nano-technology trend, the skeleton of the inorganic phase will be downsized to nanoscale;
Further modification with biomolecules and cells;
Further strengthening of the material.
During the starting project phase, several processing methods were tried, and finally two Ca-P based composite material were selected (one in Nijmegen, The Netherlands and one in Chengdu, China), which were used for further in vitro and in vivo evaluation. Special attention was given to the relationship between biological response, material composition and the inclusion of a bone growth stimulating factor.

The goal of the project is to develop a super composite (calcium phosphate and biocompatible organic matrix), which will show unique biological properties. In Nijmegen, the studies have focused on the development of calcium phosphate cement that could be used as main component for the composite. The strategy focused on the development of an alpha-tricalcium phosphate ("A"-TCP) based cement. The raw "A"-TCP powder, as used in the cement, was prepared by two approaches:
solids reaction (by Nijmegen), and
wet chemistry (by CAM Implants, Leiden).
After manufacturing, various physicochemical and mechanical tests were done with the prepared cement. On basis of various assays, it was decided that the wet chemistry method with additional ball milling provided cement with the appropriate characteristics in terms of setting time and mechanical strength.

In subsequent studies the cytocompatibility of the developed "A"-TCP based cement was investigated. Also, studies were initiated to include organic components (microspheres of poly-lactic acid and gelatine) into the

cement. The advantage is that these microspheres can be provided with bioactive and osseoinductive drugs in order to favour the ingrowth of bone tissue into the cement.

On basis of the obtained in vitro results, different experimental animal studies were initiated. In these studies, we investigated:
the biocompatibility and biodegradation behaviour of polymeric/CaP cement composites,
the kinetics and biological activity of bone growth factor released of CaP cement composites.
The results showed that the new CaP cement and polymeric/CaP formulations are clinically easy to handle and that they are biocompatible and osteoconductive as well as can be used as delivery vehicle for osseoinductive drugs.

Also studies were initiated with the production of biodegradable polymeric nanofibers, which can either be used in combination with the Ca/P cement or can be provided with a CaP coating and osseoinductive drugs using a biomimetic co-precipitation process. Currently performed cell culture studies proved the potential of the chosen approach, but the composition of the coating should be modified to obtain better results with an as-prepared material.

In Chengdu, the studies also focused on the development of CaP based compounds. In their studies, the studies were dealing with nano-hydroxyapatite (n-HA) reinforced inorganic and organic composites. Various approaches were applied like coprecipitation of n-HA, chitosan and konjac glucomannan, and direct precipitation of n-HA and Zinc Oxide (ZNO). Some of the investigated experimental materials were provided with drugs. In vitro and in vivo studies were performed with the prepared materials. The obtained results confirmed that n-HA-based systems hold potential for application as a controlled drug delivery vehicle for bone application.