

THE ESACT NEWSLETTER

Published by the

European Society for
Animal Cell Technology



Issue: January 2004

CONTENTS

1. [Editorial](#)
2. [A Word from the Chairman](#)
3. [Singapore: Filling Biopolis](#)
4. [International Stem Cell Conference](#)
5. [6th Conference on Protein Expression in Animal Cells](#)
6. [UK Stem Cell Bank](#)
7. [News from \[www.esact.org\]\(http://www.esact.org\)](#)
8. [New Members](#)
9. [Joke corner](#)
10. [Training Courses](#)
11. [ESACT Secretariat](#)

EDITORIAL

Dear Readers,

Another year has flown by and here we are again in December reflecting whether our year has been well spent. We hope the answer is yes!

In this issue, our Chairman summarises the year's activities, I contribute an edited article from Nature about the new Biopolis in Singapore: a researcher's dream and a summary of the International Stem Cell conference recently held here. There is also a report from the 6th Conference on Protein Expression in Animal Cells, news on the UK's Stem Cell Bank and an update on the ESACT website. I would also like to remind you that **membership fees for 2004** are now due: payment details can be found in the ESACT Secretariat section.

Wishing all of you success and happiness in 2004.



Steve Oh, Chief Editor

A Word from the Chairman

We are finishing the year 2003, another year in which an ESACT Meeting was organized; and those of us who participated in this excellent meeting, which was organized by Francesc “Quico” Godia in Granada in May 2003, will remember it for the science which was presented as well as for the social aspect, which makes a real ESACT Meeting. Again many thanks to Quico for his great efforts.

We are heading now to a “non-ESACT-Meeting” year meaning that we will not meet before June 2005, when the Harrogate Meeting will take place. By the way, Rod Smith is already working hard on it. As there is no ESACT Meeting in 2004, everybody is free to participate in other meetings in the field of animal cell technology, such as the [Cell Culture Engineering IX Meeting](#) (Cancún/Mexico in March 2004), the 3rd meeting on recombinant protein production: a comparative view on host physiology (joint EFB/FEMS/ESACT meeting proposal; our ex-ESACT chairman and good friend Manuel Carrondo is the chairman of this event (Algarve/P, November 2004), or the 17th JAACT Meeting (Nagoya/Japan, November 2004). Some of you might also participate to the [2004 ESGT-Meeting](#) (Tampere/SF, November 2004), at which a joint ESACT-ESGT Session will take place.

You will have realised that these newsletters are now distributed by email and not by normal post. We had two reasons for the change of the distribution of the newsletters: first, the email version comes much faster on the desk of our readers and second, the use of an electronic distribution system considerably reduces the distribution costs which count for a significant percentage of the total ESACT expenses. Every member will now get their copy at the same time and three months later they will also be posted on the [web site](#) and become publicly available. The advantage for members is that they will be informed much earlier than the non-members.

As indicated in the last newsletters, the connection to other societies, such as [JAACT](#), [ESGT](#), [ACTIP](#) or [EFB](#), should only be continued, when this can benefit our members.

This is the case for the [Engineering Foundation Meetings](#) (now: Engineering Conferences International Meetings), JAACT, ESGT, and ACTIP. ESACT had been a member organization of the EFB for many years; however, the executive committee does not see any advantage for ESACT and its members for keeping this membership. Thus ESACT has informed EFB of its decision to not renew its affiliation to this society any longer.

Again there was a joint ESACT-ESGT session during the last ESGT Meeting, which was organized in Edinburgh in November 2003. This session was well received, competed well with the parallel sessions; about 40% of the conference delegates took part in the session.

Our contacts and interactions with ACTIP are as strong as in the past. The most important joint activity is the preparation of a Marie Curie Research Training Network ([Marie Curie Host Fellowships for Early Stage Research Training](#) – EST), entitled “Development and optimisation of industrial (large scale) animal cell technology and downstream processing of biologicals”, aiming in the “production” of Ph.D.s for the industry active in animal cell technology. The formulation and organisation of this network is well advanced and will be submitted at the beginning of February 2004. It was decided to keep this network on an early stage research level indicating that Ph.D. students will do their early stage research in this programme in the frame of an industrial Ph.D. project. If this project will be accepted, it will be organized in that way, each Ph.D. student has to do a part of his Ph.D. project in an industrial – large-scale – environment. This will automatically lead to experts who have, both, a university as well as an industrial “touch”, a profile, which is of high interest for the industry active in animal cell technology.

As the Christmas holidays as well the celebration of the year’s end are not long gone, I wish you all the best for the New Year and further success in your professional and private life.

Otto-Wilhelm Merten

Singapore: Filling Biopolis

Covering 18.5 hectares and encompassing seven buildings, [Biopolis](#) is a testament to the Singapore government's commitment to biological research, its proposed 'fourth pillar' of the economy. Officially open at the end of October 2003, this massive research facility represents one of the largest international scientific recruitment drives ever undertaken in Southeast Asia. Built at a cost of some S\$500 million (US\$290 million), the complex will eventually play host to more than 1,500 scientists.



Singapore is investing heavily in science

But this is just the beginning. Singapore's drive to attract high-calibre researchers from around the world will see it expand its scientific infrastructure over the next few years. [Biopolis](#) is just part of a larger development covering 200 hectares that will also hold Fusionpolis, a media and information-technology tower, as well as affordable housing for scientists working nearby.

Such a level of investment emphasises the government's commitment to science - but is it enough to entice Westerners away from their labs? In its favour, the city-state's official language is English. However, as well as the long distance from 'home', one other factor that may dissuade Western scientists from heading east is the autocratic reputation of Singapore's government. The centralized nature of the administration is a double-edged sword - on the one hand it allows rapid action, such as the construction of [Biopolis](#) in less than two years, but on the other it has the potential to stifle scientific autonomy.

So far, Biopolis is seeing an influx of big names to head its research institutes, as well as the beginnings of corporate interest in the complex. [Novartis](#), which plans to move its Institute for Tropical Diseases into one of two [Biopolis](#) buildings designated for private tenants, is fulfilling both counts. The institute's director is Alex Matter, former head of oncology research at Novartis, who spearheaded the development of the cancer drug Gleevec.

STARRING INSTITUTES

Although [Novartis](#) is a major tenant, a significant amount of space at [Biopolis](#) will be taken up by five research institutes run by the Agency for Science, Technology and Research ([A*STAR](#)) - the Genome Institute of Singapore ([GIS](#)), the Institute of Bioengineering and Nanotechnology ([IBN](#)), the Bioinformatics Institute ([BII](#)), the Institute of Molecular and Cell Biology ([IMCB](#)) and the Bioprocessing Technology Institute ([BTI](#)). Philip Yeo, [A*STAR](#)'s Chairman, has spent the past few years securing his own stars to run these divisions. In March 2001, for example, Edison Liu, former head of clinical sciences at the US National Cancer Institute ([NCI](#)) in Bethesda, Maryland took up his post as director of the [GIS](#), and sent out a clear signal that Singapore was serious about becoming internationally competitive.

Yeo's most recent coup was convincing Jackie Ying to leave the Massachusetts Institute of Technology ([MIT](#)) in Cambridge, US for Singapore to head the [IBN](#). Ying, who was the youngest tenured professor ever at [MIT](#), could not resist the chance to build something up from nothing. "I wanted an environment where there's a tremendous opportunity to grow," she says.

Ying has already started to build the institute up with researchers from the United States, Europe, Asia and Australia. The [IBN](#) has expanded from 20 to over 90 staff since she arrived this March. Eventually it will be home to 250 people, of whom 90 will be permanent staff with PhDs, and 90 a mix of postdocs and graduate students.

The [GIS](#) is also set for some significant growth. When the institute launched in 2001 it

had a staff of 20, six of whom were scientists. It is now up to 180, with plans to grow to about 280 once it moves into [Biopolis](#) this year and to 350 by the end of 2005.

Lance Miller, who followed Liu from the [NCI](#), took about six months to make up his mind to join his mentor. But after visiting Singapore, where he talked to a number of scientists, he was convinced. The [GIS](#)'s role in decoding the genome of the virus that causes severe acute respiratory syndrome (SARS) has since validated his decision.

Miller, like many [A*STAR](#) scientists, is excited about the interconnectedness of the [Biopolis](#) institutes. Walkways join the [GIS](#) and three other [A*STAR](#) institutes to one that is central - both literally and figuratively - to them all, the [BII](#), which will provide informatics support to all of the surrounding institutes. Emphasizing the [BII](#)'s importance, its building also houses the [Biopolis](#) cafeteria and lecture halls. "Everyone has to come to the [BII](#) for the seminars and the meals," says Gunaretnam Rajagopal, the institute's acting executive director.

The [BII](#), like the other [A*STAR](#) institutes in [Biopolis](#), is on course to grow when it moves in. It has 82 people now, including staff, postdocs and students, and will expand to 100 once it moves in this year, adding another 25 or so in two years' time.



The magnificent seven: when completed it will be home to around 1,500 scientists

Torsten Exner, a research scientist at the Bioprocessing Technology Institute, was another recruit who initially had doubts about moving to Singapore. "Singapore is not a

nation that has a big history of research," Exner says. But while he was completing a postdoc at [AstraZeneca](#) in Sweden, Exner did an online search for scientists working in his research speciality and came up with a hit on Singapore. So he moved there in 2002 and has been pleased to be part of the institute's growth - their 70-member staff is set to double by 2005.

While other [A*STAR](#) institutes are trying to create their own history of research, the oldest, the [IMCB](#), has a head start and is sharpening its focus on developmental biology, structural biology, cancer biology and infectious diseases. Established 16 years ago, the IMCB has grown from 35 people when it opened, to about 400 scientists now. Moving into [Biopolis](#) will allow it to expand its number of students and postdocs by 50%. Hong Wanjin, the [IMCB](#)'s deputy director, says that he is proud that the institute has trained over 100 PhD students since its inception. Its next step is to grow more Singapore talent. Of the 35 principal investigators now at the [IMCB](#), only six hold Singapore passports.

CENTRALIZED RESERVATIONS

There are signs, too, that Singapore is overcoming its biggest challenge - growing independent science under an autocratic government. Alan Porter, a principal investigator at the [IMCB](#), joined the institute at its inception. He was attracted by promises that he would be totally funded and independent - a promise that the government has largely kept, he adds. At various times, the government has pushed for more applied research at the [IMCB](#), but has so far accepted that basic as well as applied research can generate intellectual property and contribute to the life-sciences push in Singapore, Porter notes.

For Singapore, the [Biopolis](#) infrastructure creates a good foundation. If the country creates a more open scientific culture, the government should have an easy time attracting world-class researchers to its world-class facility.

Article by Paul Smaglik, Editor of [Naturejobs](#).

ISCC Inaugural Meeting, 28-30th October 2003

The International Stem Cell Conference held in Singapore welcomed the 'Who's Who' of the Stem Cell Community to speak at this meeting. In general, the findings can be summarized into the following key points.

1. Adult stem cells are generally much, much harder to expand to therapeutic quantities.
2. However, differentiation to a tissue type is better understood.
3. Embryonic stem cells may be much easier to culture continuously in vitro.
4. But much needs to be learnt about their differentiation pathways.
5. What works in mouse studies does not always translate well to primates or human cells.

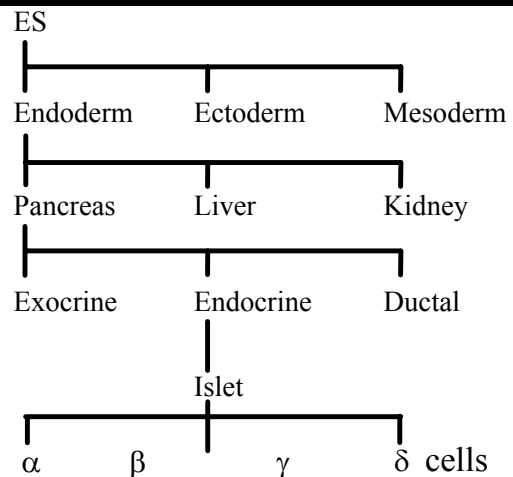
Highlights of some of the key presentations:

Doug Melton, Harvard University

History of embryonic stem cell research followed this time-line: Teratocarcinomas 1954-74, Embryonal carcinomas 1975-82, Mouse embryonic stem cells 1981-84, Human embryonic stem cells 1998-current.

There have been limited studies on how to direct cellular differentiation due to much focus on "gene knockouts" to identify a cell phenotype. Recently the group derived 17 new cell lines from human embryos, which can be passaged by trypsin. All lines have the phenotype of ES cells, such as Oct-4, alkaline phosphatase expression, embryoid body (EBs) and teratoma formation.

Key question: is there a common genetic programme for ES vs. adult stem cells? They have discovered 116 genes unique to ES cells with little or no homologies to other genes and are also investigating formation of beta islet cells from ES cells. A schematic of the process is shown below.



Experiments show that pancreatic beta (β) cells in mouse do NOT self renew. Current theory, is that pancreatic islet are limited in numbers in the lifetime of an animal like ovaries. They are very low in turnover, dividing 4–6 times in their lifetime.

Current limited understanding of β cell development is as follows:

ES \rightarrow Endoderm \rightarrow (Pdx1, Ngn3) \rightarrow β cells

Ariff Bongso, National University of Singapore

He highlighted the importance of deriving new human ES lines in totally serum free or animal free conditions, to eliminate the presence of porcine viruses. Need for continuous karyotyping of cells to ensure normalcy. Mentioned that hES cells over express Annexin 43 and Claudin 3 molecules important in cell-cell communications.

Donald Metcalf, Walter Elisa Hall, Australia

Discoverer of GCSF and LIF (1988). Molecule was purified from a known cell line simultaneously in 2 places and then tested to be supportive in mouse ES cells. LIF in mouse is restricted to embryonic development, whereas LIF is very pleiotropic and has functions in a variety of tissue types in man. Believes that LIF may have a function in human ES cells.

Bing Lim, Genome Institute of Singapore

Transcriptional profiling of hES cells. 3 separate studies of ES cells, only 1 gene found to be common between the 3 groups. Comparison between mouse and human ES specific genes found 20 with similar homology. These are down-regulated in 10 day old EBs, e.g. Oct4, Esg1, Miwi, Nanog. Using MPSS approach identified new genes at very low transcript levels, eg. Esg1, Pou5f1, and Zfp42 at 25-75 copies/million transcripts.

Jonas Frisen, Karolinska Institute

Olfactory bulb and hippocampus are areas where new neurons are generated. Neural crest cells can become mesodermal cells, e.g. bone, cartilage, fibroblasts...etc. Neurogenin 2 promotes neuronal differentiation and neural stem cells induced by this factor perform better in transplants. Effect unknown, but may remove blockage of differentiation.

Jose Cibelli, Michigan State University

Parthenogenesis is the creation of embryos without sperms, but instead by electrical induction of ovum to divide. Embryos do not develop to term but maximum development is 21 – 29 days. Using this as a model to study development. In cells where genes come from the female, brain and endoderm development occurs. In cells where genes come from the male, only muscle develops! Have derived monkey ES cells capable of generating ciliary cells, heart and muscle cells. Mentioned NIH guidelines for cell transplants.

1. Proliferate sufficient quantities of cells
2. Differentiate to progenitor cells
3. Transplant to patient and is not rejected.
4. Function for the lifetime of the patient.
5. Do no harm to the patient.

Ron McKay, NIH

Studies differentiation of ES cells to neural lineage. Differentiates mouse ES cells to EBs and then to precursors of dopaminergic neurons and final neurons, using a series of growth factors such as SHH, FGF8 and Nurr1. Expects embryonic stem cells can target Parkinson's disease, differentiated stem cells in the clinical studies in 3-5 years. Endocrine pancreas differentiation is similar to neuron cell differentiation. ES cells are induced to

differentiate by insulin & somatostatin to EBs. EBs are grown, then measured to be alpha fetoprotein and GATA4 +ve and pancreatic cells are allowed to grow out of EBs.

Hynek Wichterle, Columbia University

Specification of neuronal fates depends on sonic hedgehog, (SHH) which act as a transcriptional repressor. SHH with Nkx6 specifies the ventral and SHH with Pax6 the dorsal spinal cord. Retinoic acid (RA) is a transcriptional activator, sufficient to activate motor neuron genes. FGF2 blocks differentiation. To form motor neurons, RA and SHH are needed together. Key genes expressed are Sox1, Olig2 before neurons are formed.

Patrik Brundin, Wallenberg Neuroscience Centre

100,000 TH +ve cells are needed per side of the brain for treatment of Parkinson's disease, but as only 5 % survive, need 2 million cells to be transplanted. Normally such cells are taken from 3–4 foetal donors. Studies show that as more cells are transplanted (e.g. from 0, 1 and 4 donors), more are present in the graft, but unfortunately functions NOT restored! Perhaps problems due to late disease stage. Showed some successes for Huntington's disease, but need to monitor for 5–10 years. Need to get more cells, as logistically harvesting from donors is difficult and expansion of neuronal phenotypes is not easy.

Fiona Watt, Cancer Research UK

Mammalian epidermal cells express high $\beta 1$ integrin receptors for ECM. Altering integrin receptors controls clustering of cells and their motility! Selecting for high levels of $\beta 1$ integrin gives high no. of colonies. CD8 perturbs E-cadherin binding and Rho GTPases and reduces the adhesiveness of epidermal stem cells. β catenin levels are also important in determining differentiation.

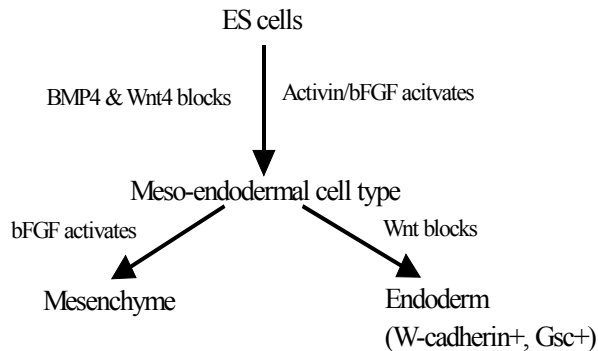
Hiromitsu Nakauchi, University of Tokyo

Lnk -ve knock out mice have much higher numbers of haematopoietic stem cells. Lnk appears to be a negative regulator of haematopoiesis and is involved in stem cell renewal. However, in vitro cultures of cells from these mice did not expand well and

require culture on a stromal cell layer, PO9 cells.

Shin-Ichi Nishikawa, Riken Center for Developmental Biology

Serum free conditions for differentiation of mES cells have been established:-



Gordon Keller, Mt. Sinai School of Medicine

Focus research on mesoderm lineage. Derived mesoderm via EBs with serum. Addition of VEGF forms hemangioblasts which are Flk1 and Brachyury +ve. These cell types are able to form haematopoietic and vascular fates.

Rudolf Jaenisch, Whitehead Institute

Examined the possibility of nuclear reprogramming of somatic nuclei when injected into ovum. One of the functions of the ovum is the demethylation of genes. Is the environment within able to 'reset the clock'. Currently this reprogramming is faulty and not well understood. It appears that male genes are unmethylated and female genes are partially unmethylated.

Christopher Wright, Vanderbilt University

In islet transplants, it is important to determine whether immature or mature β cells are transplanted. It is preferable to transplant a full islet with α , β , γ and δ cells. Key transcription factor is Pdx1 and Ptf1a genes. Pdx1 dosage and timing determines progeny. Inactivation of pdx1 prevents pancreas outgrowth in mice, affects gut, duodenum bile and controls insulin and islet cell production. Endoderm forms foregut, then pancreas, then endocrine and β cells.

Bernat Soria, National University of Singapore

Currently there are 150 million type 1 and 2 diabetic patients which will grow to 200 million by 2010 with 50% of them being in Asia. The aim is to grow islet as a micro-organ consisting of the following proportions: α (20%), β (60%), γ (10%), δ (10%) cells.

Minimal islet size should have 10 β and 20 total cells, of about 150 μ m in size. Formation of ES cells to insulin producing cells occurs at very low efficiency, pancreatic buds produce soluble factors to make insulin producing cells. Blocking by Shh is necessary to get pancreas.

Susan Bonner-Weir, Harvard Medical School

Her estimation is that an islet equivalent is 150-300 μ m in size with 1500 cells. Human islets are formed from ductal progenitors? Pancreas express pdx1 to form ductal cells, which express neurogenin 3 to form islet which express Nkx6 to form β cells. β cell mass in humans is proportional to body cell mass and this mass is regulated by glucose homeostasis. Human islets are smaller than mice and near ducts, growing up from the epithelium. Harvested pancreas were separated and isolate ducts and acinar cells were able to form islet cells. Adult rat after pancreatectomy can form new pancreatic lobes after 4 weeks. With 40% new β cell mass. Replication occurred first, then pdx 1 protein was expressed. Microarray of new islets show 73 new genes .e.g. cytokeratin 19, MMP2, CD24 which are not found in mature islets.

Catherine Verfaillie, University of Minnesota

Took 6-7 years to isolate multipotent adult progenitor cells which are able to form mostly mesodermal tissues, nerve, hepatocytes, muscle, bone, and cartilage. MAPC cells express very low levels of Oct4, Cripto, Nanog. They are not isolated but selected through culture conditions. Cells grow at very low cell densities 1000-4000/cm². Need to feed them daily and cell division is every 48 h for human MAPC. Culture conditions are PDGF-BB, EGF, 2% FCS and LIF. When cultures become confluent, differentiated genes are activated, e.g. TGF β 1,3,BMP4.

Compiled by Steve Oh, November 2003

Report on the 6th Conference on Protein Expression in Animal Cells

This meeting was organized by A. Kamen (Biotechnological Research Institute in Montréal, Québec, Canada) and held at Mont Tremblant between the 7th and 11th of September, 2003 in a holiday village which had activities such as hiking, rafting and horse riding. There were over 200 participants, several companies who exhibited and about 40 posters.

The keynote lecture was by **J.J.M. Bergeron** (Caprion Proteomics, Canada) on proteomic applications for the characterisation of eukaryotic organelle proteins.

Session 1: baculovirus and insect cell technology

A.A. Kamen (Biotechnology Research Institute, Canada) presented a comparison of the metabolism of Sf9, High Five and recombinant High Five cells expressing the pyruvate carboxylase (PYC) gene in an attempt to improve the coupling of glycolysis and the Krebs cycle. However, the recombinant High Five cells showed a 3-fold increase in the Pentose Phosphate Cycle compared to the non-modified High Five cells.

Farrell et al. (Univ. of Calgary, Canada) presented the use of the cassette (pIE1/153A) in lepidopteran cells to express recombinant proteins. The cassette comprises the *Bombyx mori* cytoplasmic actin promoter, the BmNPV hr3 transcriptional enhancer, and the BmNPV ie-1 transcriptional activator. They expressed tissue-type plasminogen activator (tPA) at 160 mg/l by day 12, whereas only 0.45 to 64 mg/l was obtained in a mammalian expression system (day 6). To tackle cell lysis they are developing artificial baculovirus chromosomes which have neither telomeres nor a centromer. By expressing a ts sensitive mutant of the *lef-8* gene in the absence of *lef-8* (or presence of the ts *lef-8* mutant at 33°C), cell lysis is avoided and the viral replication is blocked.

R. Kotin (NIH, USA) described the production of recombinant Adeno-associated Virus (AAV) using the Sf9/Baculovirus system: More AAV vector is found in the supernatant than within the cells and a

comparison of AAV produced by insect cells and by triple-transfected 293 cells showed comparable quality during *in vivo* studies. So far, the 1, 2, 4, and 5 AAV serotypes have been produced.

Sessions 2 and 3: gene delivery by viral and non-viral vectors

B. Massie et al. (Biotechnology Research Institute, Canada) presented recent developments in the generation of adenoviral libraries for high throughput functional studies and protein production.

T. Kost & P. Condreay (GlaxoSmithKline, USA) presented their work on recombinant baculoviruses for mammalian cell gene delivery using the BACMAM system (an insect cell-mammalian cell shuttle vector). This system can be used for transient expressions as well as for the development of stable producer cell lines such as CHO cells at a frequency of 1/50-1/100.

R. J. Samulski (University of North Carolina, USA) presented an overview on the biology of AAV and the use of different AAV serotypes as well as modified AAVs for gene therapy purposes. Using a dog model he compared the efficiency of different AAV-serotypes for expressing factor IX: AAV2 produced low levels but AAV1 led to much higher expression levels. Integration was unaffected by the use of a single strand or a dimeric AAV.

Y. Durocher et al. (Biotechnology Research Institute, Canada) presented a reactor scale transfection process for the production of recombinant proteins and AAVs using 293EBNA cells. Although a serum-free process (producing 20 mg/l) is possible, 1% serum and/or peptones led to a 3-fold increase in the product concentration. A process making use of a medium supplemented with 1% serum (triple-transfection of 293EBNA cells with the recombinant AAV vector (ITR-CMV-promoter-GFP-ITR), a plasmid encoding for rep and cap of the AAV, and the helper plasmid encoding for the adenoviral genes VA, ESA, E4) could produce 9×10^7 and 2.8×10^7 infectious particles per ml of crude cell lysate and medium, respectively.

W. Xia et al. (Berlex Biosciences, USA) compared different promoters. Whereas a full length human CMV promoter worked better than rodent CMV promoter and better than the

MPSV promoter in the human 293E cell line for the stable expression of recombinant proteins, the MPSV promoter was the best one for the expression in CHO cells, followed by the rodent CMV promoters. The least efficient promoter in CHO cells was the human CMV promoter. Similar results were found for the transient expression of proteins.

Sessions 4 and 6: cell engineering.

M.S. Kallos et al. (Univ. of Calgary/AB, Canada) presented the large scale amplification and production of neural stem cells, using a serum-free suspension process. These cells have a tendency to form large clumps but can dissociated using pH shocks (alkalinisation->acidification->neutralisation).

G.P. Nolan (Stanford Univ., USA) presented the single cell analysis of multiple intracellular processes by high dimensional flow cytometry.

A.J. van der Eb (Leiden Univ. Medical Center, NL) presented the history of packaging cell lines for adenoviral vectors: from HEK 293 to PER.C6.

H. Hauser et al. (GBF, Braunschweig/D) presented recent developments in the optimisation of the FLP/FRT cassette exchange technology. To tackle the problems of gene silencing, reduced production and the overgrowth of non-producing cells the group has developed an approach based on "stop codon suppression". This occurs when ribosomes read through a stop codon (1 in every 1,000 translation acts). If the in-frame sequence is an antibiotic resistance gene, the producer cell will produce a fusion protein containing this resistance. The advantage is that the cell will always stay resistant against the antibiotic marker and will produce 100-1000 times more protein.

M. Fussenegger (Inst. Biotechnol., CH) presented an overview on the engineering developments of his institute in the field of transcriptional control.

D.S. Conklin (University at Albany, USA) gave an overview on the applications of RNAi, a method for gene silencing and the use of short hairpin RNAs (shRNAs) from DNA vectors. shRNAs can be produced within the cells from a mammalian expression vector in a stable way are long lasting and

- Permit the enrichment of affected cells
- Function in adult animals
- Can generate alleles of varying degrees of severity
- Can be used therapeutically
- Can be used to generate mutant transgenic animals

A. Ishaque et al. (Bayer Corp., USA) presented the effects of the over-expression of the chaperone HSP-70 in recombinant BHK-21 cells on cell viability, apoptosis, and the production of factor VIII. The HSP-70 over-expressing cells expressed factor VIII at 1.5-fold higher levels than the original cell line. These cells showed a prolonged growth phase, longer viability, and higher titers in batch as well as in perfusion cultures and were also resistant to apoptosis inducers.

Session 5: transmembrane proteins and GPCRs

J. Clare (GlaxoSmithKline, U.K.) presented work on ion channel expression for drug discovery.

C.G. Tate (MRC, U.K.) compared seven different protein expression systems for the production of the serotonin transporter (SERT). *Pichia pastoris* produced no active material but a Sf9 system generated high levels although most was mis-folded and inactive. When calnexin was co-expressed, they produced three times more functional SERT. Three inducible systems were compared: decrease of temperature (pCyTS-SERT), addition of DMSO (MEL-SERT) or the addition of tetracycline (T-REx-SERT). The latter was the best for expression of SERT. The MEL-SERT cells produced rather low levels after induction, and the pCyTS-SERT produced a high proportion of non-glycosylated and thus inactive SERT. The T-REx-SERT produced high levels of glycosylated and thus functional SERT.

K. Lundström (BioXtal, CH) presented work using the Semliki Forest Virus expression system. The BHK-based packaging cells can produce between 10^9 and 10^{10} particles per ml, is replication defective, has a broad host range and these viral vectors show a highly efficient infectivity. BioXtal are participating in Mepnet which aims to over-express, purify and crystallize membrane-based G-protein coupled

receptor targets using *E. coli*, *P. pastoris*, and the Semliki Forest Virus expression systems.

L. Swevers et al. (National Centre for Scientific Research "Demokritos", GR) presented lepidopteran cell-based high throughput screens for the identification of ligand mimetics for insect and mammalian receptors.

Session 7: antibodies and other secreted proteins

C. Zhang et al. (Bayer Corp., USA) presented their development and optimisation of cell culture processes for the perfusion production of recombinant biologicals. They monitor pH, pO₂, pCO₂, OUR, CER and perform in situ microscopy, on-line quantification of cellular physiology and metabolism and elicit control through computation of metabolic fluxes using an expert system.

G.W. Lee (Wyeth BioPharma, USA) presented the production of novel recombinant soluble receptor Fc fusion proteins using recombinant CHO cells. In earlier studies Lee has observed that the expression levels were very low in transient as well as in stable expression systems and most of the protein was aggregated. Reducing the production temperature to 31°C improved the transfer of the receptor fusion precursor protein from the ER to the Golgi and reduced aggregation.

J.M. Piret (University of British Columbia, Canada) presented an optimisation study for the high level expression of two recombinant proteins: activated protein C (APC) and tPA. Differences for both proteins were observed for intracellular protein concentrations versus cell specific expression rates.

C. Yallop et al. (Crucell NV, NL) presented the PER.C6 cell line as a production platform for monoclonal antibodies. The use of an optimized fed-batch production system can yield antibody concentrations of up to 1600 mg/ml, with the advantage that these antibodies show a human-like glycosylation pattern. The choice of the production mode (fed batch or batch) had no significant influence on the glycosylation pattern.

Session 8: protein quality

H. Sassenfeld & R. McCoy (Amgen, USA) presented a lecture on misfolding of expressed heterologous proteins. The degree of

misfolding was found to be independent of the cell specific protein expression rate in recombinant CHO cells. Misfolded proteins could be partially reconstituted by using glutathione and that cultivation of recombinant CHO cells at lower temperature (28°C vs. 37°C) could reduce misfolding by at least 90%. Longer cultivation times also increased misfolding. Chaperone analysis found that levels of Erp72 and PDI were 2-3 times higher in cells grown at 28°C but GRP94 and BIP were unchanged. Misfolding could be due to incorrect pairing of S-S bounds, which may be buried or not accessible to the chaperons for repair.

S. Weikert et al. (Genentech, USA) spoke on the modulation of effector functions by deleting the disulfide bonds in the hinge region of monoclonal antibodies. By replacing CYS with SER the physicochemical behaviour and the Fc's N-glycosylation were affected as well as expression which was significantly enhanced in *E. coli*, but not in CHO cells. The data indicates that removal of hinge region CYS may provide a route for high expression of full length antibodies with appropriate target binding, but poor effector function, from both *E. coli* and CHO and demonstrate that unrelated modifications impacting the structure of the hinge region can result in similar deletions of antibody effector functions.

M. Butler (University of Manitoba, Canada) presented an overview on parameters that cause perturbations in the glycosylation of proteins secreted by mammalian cells.

Session 9: high throughput strategies

J. Worley discussed cell-based assays in the microfluidic environment"

R.C. Stevens spoke on the design of high-throughput protein production methods for structural biology

S.K. Chanda presented work on genome-scale functional annotation through modulation of gene expression

Some selected posters:

J. Meghrou et al. (Biotechnological Research Institute in Montréal/Québec, Canada) presented a poster on the production of AAV using the Sf9/baculovirus insect cell expression system. About 60% of the AAV

vector was cell associated, signifying that for an optimal production, both cell lysate and supernatant have to be purified. 2.2×10^{12} IVPs were achievable.

G. St-Laurent et al. (Biotechnological Research Institute, Canada) developed a reactor scale production system for AAV vectors based on the cultivation of 293-EBNA cells in suspension culture and triple transfection of these cells in suspension. 72 h post-transfection, the cells were collected and lysed by freeze/thaw and 76% of the rAAV was still cell associated. Using a 3 l reactor, in total 3.5×10^{11} infectious particles could be produced per culture ($= 1.2 \times 10^8$ IVPs/ml). This signifies that 63 IVPs were produced per cell, and by using QC-PCR the authors determined that the ratio between vector genome and infectious particles was 36/1.

S. Nakowitsch et al. (IAM, University of Agriculture, A) presented a novel method for the generation of influenza B virus particles using a baculovirus gene delivery system. Production uses 293 cells and 5 recombinant baculoviruses to deliver the genes (PA, PB1, PB2, NP (RNP-complex), and (-)GFP(NCR-NS-GFP)).

L. Gagnon et al. (Qbiogene Inc., Canada) presented a new adenovirus packaging cell line (BMAde1) devoid of homologous sequences with the adenoviral vector constructed to avoid the production of RCAs. E1A expression was under the control of the strong constitutive human β -actin promoter and E1B expression under the control of its own promoter. In addition, the authors presented an RCA detection method based on the use of a highly sensitive nested PCR (1 RCA/3 \times 1010 recombinant adenoviruses), using two successive rounds of PCR amplification.

H. Henke et al. (Novartis Institute for Biomedical Research, CH) presented a large scale method for the transient production of recombinant proteins using 293-EBNA and PEI.

A. Burgener et al. (University of Manitoba, Canada) demonstrated that the intracellular ATP concentration had a considerable impact on the production of retroviruses by Vero cells. Levels lower than $4 \text{ nmol}/10^6$ cells at the time of infection led to a >95% reduction in the overall viral titer compared to the control.

C.A. Yandell et al. (GroPep Ltd., Australia) compared insulin and its analog the human

insulin like growth factor 1: LongTMR³IGF-1. This analog stimulated both the type 1 IGF-1 receptor and the insulin receptor at 200-fold lower concentrations than insulin. It stimulates the anti-apoptotic signaling proteins Akt and MapK to a greater degree than insulin in CHO K1, DG44 and DXB11 cells. It maintains the cell viability of CHO cells in serum-free conditions at concentrations 200-fold lower than insulin and media containing LongTMR³IGF-1 increased recombinant protein expression by 58%.

J. R. Miller (Lilly Research Laboratories, Indianapolis, USA) presented a new bacmid based on the pFastBacTM system in which the GFP as reporter function is integrated in a non essential gene DA26 of the Baculovirus genome.

C. Wittische (Genentech Inc., USA) presented a method to characterise antibody expression in CHO cells using real time quantitative PCR. After cloning, the highest producing cells are screened by TaqMan to determine mRNA/genomic DNA copy number ratio. The stability of each clone is measured by mRNA quantitation.

O.-W. Merten/C. Gény-Fiamma

Establishment of the UK Stem Cell Bank

The UK Stem Cell Bank project was funded in January 2004 by the UK Government to provide well characterised and quality controlled stocks of human stem cell lines for basic research and the development of new therapeutic applications. The Bank is being established at the National Institute for Biological Standards and Control; a Government laboratory involved in work on the quality and safety of biological medicines. The bank is expected to handle stem cell lines of adult and foetal origin as well as embryonic stem cell lines. The requirement to provide cell lines as starting materials for therapeutic applications demands high grade facilities and clinical grade cell lines will be prepared and stored in a purpose built laboratory set up according to EU GMP guidelines. These facilities were completed within twelve months from the start of the project and are now undergoing validation prior to inspection later in 2004.

Applications to deposit research grade cell lines in the Bank were initiated in 2003 through the Steering Committee for the Bank. The first lines in the Bank should be announced shortly and will be publicised on the dedicated website for the Bank that is now live at www.ukstemcellbank.org.uk. The website gives background information on the bank, how it will be run, progress updates and also contains video sequences of the facilities and their construction.

Close liaison between the Bank and the stem cell research community is vital and during the inception of the Bank considerable effort has been put into developing close relations with the various expert centres and national and international stem cell research networks. In addition the Bank contributed to the running of a practical course in the culture of ES cell lines in July 2003 organised by Professors Peter Andrews and Harry Moore at the [University of Sheffield](http://www.universityofsheffield.ac.uk) (it is hoped to repeat this event in 2004).

The bank is keen to expand its list of contacts in the stem cell field and would be happy to communicate with ESACT members. If you would like to be added to the UK Stem Cell Bank mailing list for future events please contact Glyn Stacey at the address below.

Glyn Stacey

Director of the UK Stem Cell Bank
Head of the Division of Cell Biology and Imaging
National Institute for Biological Standards and Control
Blanche lane, South Mimms, EN6 3QG, UK.
Email: gstacey@nibsc.ac.uk
Fax +44-1707-641578

News from www.esact.org

Our [site](http://www.esact.org) has migrated to a new provider between Dec 25 and Dec 26. This migration was probably unnoticed by most of our visitors but we apologize if any of you have encountered any problems on the site or with the emails during this period.

We have taken the opportunity to delete some of the emails which were receiving too much spam or were unused and created a number of new ones. The emails available are:

webmanager@esact.org (replaces webster@esact.org) for any communication to the webmaster.

office@esact.org (unchanged): to contact Bryan Griffiths at our office.

membership@esact.org (unchanged): for all communications concerning your member information.

jin@esact.org (unchanged): for all communications concerning the Job Information Network.

newsletter@esact.org (new): contacts both editors

In addition, each member of the executive committee now has an ESACT email in the form of name@esact.org (for example merten@esact.org)

New Discussion board

We have launched a new [forum](http://www.esact.org/forum) for discussions and questions concerning Animal Cell Technology. It is open for any sort of topic, from a basic technical question to an open discussion on a publication or a presentation in any field of ACT. See www.esact.org/forum.

At the moment this forum is completely public, which means it is not necessary to register to be able to open topics and post messages. But registering brings some benefits, such as:

- Create polls and vote.
- Send private messages (pm) to other registered users.
- Subscribe to user groups and eventually participate to private forums.

It is completely safe to register, your password is fully encrypted in the database and the forum administrator himself can not see it. Also, you can hide your email completely to other users (registered or not) by leaving the "Always show my email address" on NO (which is default). This will completely protect it from being stolen and added to spam distribution lists.

Please help us promote this forum by spreading the word to all your colleagues and students. It can only be useful if it reaches a

minimum number of regular visitors and active participants.

Please address any concerns or questions to bbadmin@esact.org (**Christophe Losberger**).

Future plans for www.esact.org/jin

In the next months, [JIN](http://www.jin.org), the Job Information Network will move to its own site and the system will be completely upgraded. Thanks to the generous support of Professor Alain Miller and CIL biotech (<http://www.cilbiotech.be/>) we are currently developing a complete new system which will be much more attractive, with new or enhanced features such as:

- Better interface for employers to make their positions easier to manage.
- Job seekers will be able to post their resumes.
- Easier and quicker administration: jobs will be online in hours instead of days.
- Better search engine.
- And of course it will remain a free service.

The project is quite advanced at the moment and we hope to be able to inform you very soon by email of the launch.

Christophe Losberger, ESACT website manager.

New Members

ESACT would like to welcome the following new members:

Carol Bullivant (BioDynamics Research Ltd., UK); Veronique Chotteau (Biovitrum AB, Sweden); Eija-Riitta Hamalainen (FibroGen Europe, Dinland); Vikas Lamba (Pfizer Ltd., Canada); Anette Amstrup Pedersen (Novo Nordisk A/S, Denmark); Vicky Pope (BioDynamics Research Ltd., UK); Sabin Geisse (Novartis Pharma Research, Switzerland); Wilfried Weber (ETH Honggerberg, Switzerland).

Training Courses

SPI USA, Inc. is running a cGMP Training Course for the Biotechnology and Pharmaceutical Industries in Europe on April 22nd and 23rd, 2004 in London, United Kingdom. SPI USA can be contacted at 10025 Governor Warfield Parkway Suite 214 Columbia, MD 21044 Fax: +1 410.997.3554 or by email: spiusa@usaspi.com. There are further details of courses of interest on the [ESACT website](http://www.esact.org).

Joke Corner

These all have a dating theme:

I've been single-stranded too long! Lonely ATGCATG would like to pair up with congenial TACGTAC.

Some dates have called me a promotor. Others have referred to me as a real operator. Personally, I think I'm just a cute piece of DNA who is still looking for that special transcription factor to help me unwind.

Mature cell seeks same who still enjoys cycling and won't go apoptotic on me. Let's fight senescence together!

Gene therapy graduate. After years of producing nothing but gibberish, I've shed my exons and am ready to express my introns. All I need is a cute vector to introduce me to the right host.

Naked DNA with sticky ends seeks kanamycin-resistant plasmid. EcoRI sites preferred.

I'm a prolific progenitor with great potential for growth and self-renewal. Call me if you're a potent hematopoietic factor who still believes in endless nights of colony stimulation.

I don't always express myself on the surface, but I'm looking for a signal that you appreciate my complexity. Send me the right message that will penetrate my membranes, turn on my protein expression and release my potential energy.

ESACT SECRETARIAT

A reminder that **2004** subscription fees are now due and that following the rationalisation of membership (abolishing Associate Membership in favour of Full membership status) the annual Membership Fee is now the same for everyone

20 Euros, £13 or US\$25

Please remember when paying by credit card to include the **security number** (3 digit on the reverse on the card usually on signature strip; 4 digits for Amex) and **post/zip code** of billing address. Please note the **ESACT OFFICE** email address is office@esact.org. Also, as courier services etc. do not recognise PO Box addresses the following ESACT Office address should be used **PO BOX 1723, 5 Bourne Gardens, Porton, Salisbury, Wiltshire, SP4 0PL, UK. Bryan Griffiths**

ESACT CONTACT INFORMATION

EXECUTIVE COMMITTEE

Chairman: **Otto-Wilhelm MERTEN**
Généthon III
Gene Therapy Programme
1 rue de l'Internationale, BP 60
F-91002 Evry Cedex 2
France
Tel: (+33) 1 6947 259
Fax: (+33)1 6947 2838
omerten@genethon.fr

Secretary: **Alain BERNARD**
Serono Biotech Center
Route de FENIL Z1B
1804 CORSIER-sur-VEVEY
Switzerland
Tel.: (+41) 21 923 23 57
Fax: (+41) 21 923 20 13
alain.bernard@serono.com

Meeting Secretary: **Rod SMITH**
CTM BioTech
Babraham Research Campus
Babraham
CB2 4AT
Tel: (+44) 1223 496 070
Mob: 077 666 07805
rod.smith@esact.org

Treasurer: **Martin FUSSENEGGER**
Institute of Biotechnology
ETH Hönggerberg, HPT D 74
CH-8093 Zürich
Switzerland
Tel: (+ 41) 1 633 34 48
Fax: (+ 41) 1 633 10 51
martin.fussenegger@biotech.biol.ethz.ch

COMMITTEE MEMBERS

Francesc GODIA
Universitat Autònoma de Barcelona,
Dept. d'Enginyeria Química,
Edifici C,
E-08193 Bellaterra (Barcelona)
Spain
Tel: (+34) 93 5812692
Fax: (+34) 93 5812013
francesc.godia@uab.es

Hansjorg HAUSER
GBF
Mascheroder Weg 1
D-38124 Braunschweig
Germany
Tel.: (+49) 531 6181 250
Fax: (+49) 531 6181 262
hha@gbf.de

Stefanos GRAMMATIKOS
Boehringer Ingelheim Pharm.
84 397 Biberach an der Riss
Germany
Tel.: (+49) 7351 544022
Fax: (+49) 7351 844022
stefanos.grammatikos@bc.boehringer-ingenelheim.com

Florian WURM
EPFL
Dept. de Chimie
Centre de Biotechnologie
CH-1015 Lausanne
Switzerland
Tel: (+41) 21 693 6141
Fax: (+41) 21 693 6140
Florian.Wurm@epfl.ch

ESACT OFFICE

Bryan GRIFFITHS
PO Box 1723
5 Bourne Gardens
Porton, Salisbury
Wilts SP4 0PL, UK
Tel.: (+44) 1 980 610 405
Fax: (+44) 1 980 610 405
office@esact.org

Web Site Manager
Serono Pharmaceutical
Research Institute
14 Chemin des Aulx
CH-1228 Plan-des-Ouates
Geneva, Switzerland
Tel: (+41) 22 70 69637
Fax: (+41) 22 79 46965
webmanager@esact.org

Newsletter Editor: **Steve OH**
Tel: +65 6478 9561
newsletter@esact.org

Newsletter Co-Editor: **Merlin GOLDMAN**
Tel: +44 131 472 4704
merlin@magnetical.com

MEMBERSHIP SUBSCRIPTIONS

We advocate the following payment methods:

Master, Visa, Access, Delta Eurocard or Amex

1. 20 Euro per year paid by cheque drawn on a UK bank*.
2. USD 25.00 per year if drawn on any other bank*.

NOTE

We recommend you consider payment of more than one year's subscription at a time to minimise local bank charges.

NEWSLETTER CORRESPONDENCE

Contributions for inclusion in the ESACT Newsletter e.g. meeting reports and comments can be sent direct to the Newsletter team.

PLEASE NOTIFY THE ESACT OFFICE OF ANY CHANGES TO YOUR ADDRESS, TEL, FAX OR EMAIL. CONTACT US WITH CHANGES BY EMAIL TO members@esact.org or online at <http://www.esact.org/forms/membermodif.html>.